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Cover Page, Introduction, Contents, Call for Papers, All in one file

CONTENTS

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Knowledge, Attitude and Practices of HIV/AIDS in Selected Fishing Communities of Kainji Lake Basin

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Abstract: The paper examined the knowledge, attitude and practices of HIV/AIDS in the fisheries sector of Yauri emirate of the Kainji Lake Basin where ten fishing communities were selected for data collection through the use of questionnaires and further subjected to descriptive analysis. The findings revealed 98.4% of the respondents were aware of but lacked knowledge on mode of transmission and prevention of HIV/AIDS, while 41.7% of the respondents said they will avoid contact with people living with HIV/AIDS This imply that there may be high level of stigmatization if a relation or member of the community was found living with the virus. 28.9% of respondents' rate access to HIV/AIDS information as high in these fishing communities. The study made recommendations to addressing HIV/AIDS in the area.[Nature and Science 2009:7(10):1-9](ISSN1545-0740)

Keywords: Knowledge, Attitude, HIV/AIDS & Fisheries

Introduction

One of the growing epidemics in the world today is HIV/AIDS and it has been widely acknowledged to be the most dreaded and severe health crisis in this millennium. The Human Immunodeficiency Virus (HIV), the pathogen that results in Acquired Immunodeficiency Syndrome (AIDS) has been the most significant emerging infectious agent of the last century and threat developmental projects. Since the report of HIV/AIDS in 1986, HIV/AIDS is spreading across all geo – political zones and among all segments of the society in Nigeria. According to geographical zone, (FMOH, 2002) the prevalence rates were North Central (5.5%), South South (7.7%), South East (5.8%), North East (5.4%), South West (4.0%) and North West (3.3%). Nigeria has gone through various phases of response. The sensitization awareness and mobilization activities have concentrated in the cities and towns neglecting the many fisher folks. HIV/AIDS prevalence is not only restricted to urban areas; rural areas, especially fishing communities are often among the highest risk groups with overall rates of HIV/AIDS. It is now known that the prevalence of STD/HIV/AIDS in fishing communities in countries like Uganda, Kenya, Thailand and Indonesia is 5 - 10% (Gordon, 2005; Allison and Seeley, 2004) and sometimes 4 - 14% (Kissling *et al*, 2005) higher than the national average. The peculiar nature of this sector requires a unique service package to step up the fight against HIV/AIDS. A project is presently on - going on fisheries and HIV/AIDS in the Chad Basin which has provided little information on the above subject in the fisheries sector on the susceptibility and vulnerability factors in the area. This study is therefore aimed at determining the Knowledge, Attitude and Practices on HIV/AIDS in the fisheries sector of Kainji Lake Basin.

Methodology

Kainji lake basin comprises of Niger and Kebbi States with the following neighbouring emirates Kontagora, Borgu and Yauri . For this study, the sample was taken from Yauri emirates from the following communities: Wara, Wawu, Tunga Mairuwa, Zamare, Rukubalo, Yauri, Rashe Salkawa, Hella, Barashi Tunga Alhaji Sharo. The selections of these communities were based on accessibility, level of fisheries activities and traditional institutions. A total of 187 questionnaires and 20 interview guides for key informants were administered in the communities and further subjected for statistical analysis.

Result and Discussion

On the socio – economic characteristics as shown in Table 1, on sex, 63.6% of the respondents were males while 36.4% were females. The variation may be as a result women restriction to their household that is; they are in Purdah, which buttresses the findings of gender studies carried out by Yahaya, 1999. It can be assumed that the men are more likely to be aware of this deadly disease. The higher number of males in the study agrees with findings of experts that almost twice as many men as women were aware of HIV/AIDS. (UNAIDS, 1998)

From the study, 76.0% of the respondents were still in their active (reproductive) age, that is, 15 – 45 years. 24% were above 46 years. These ages are the active and productive years in agricultural production and they are crucial to agricultural development. The respondents were mainly young people implying that they were in sexually active ages. This study confirmed that majority of those who contract the HIV/AIDS virus fall under the age of 30 years (NDHS, 2003). Thus, they are the very people who are vital to the economic future of the rural communities where poverty is dominant.

Majority of the respondents (78.1%) were married, 21.4% were single while a negligible percent (0.5%) were widow. None of the respondent was divorced neither separated in the study area. This is an indication of a tendency for sexual continuation, particularly among the married people of the fishing communities. On religion, the respondents (84.5%) were Muslim faithful, only 15.5% practiced Christianity and 0.5% claimed to be idol worshipper. With this finding Men are permitted to have more than one wife, it is more acceptable for them to have multiple relationships than for women. Majority (58.7%) were into polygamy, 2.1% were monogamous and 49.2% could not response. This is not surprising because some of the unmarried respondents may constitute to the high percentage.

On educational background, less than half of the respondents sampled had formal education although some of the fishing communities selected lacked the facility. Only 18.7% had primary education and the same percent for respondents who had secondary school education. More than half of the respondents (57.2%) had no formal education. This is a reflection of the areas in which the study was carried out and also the fact that the many of the people are not interested in the western education. Some of the fishing communities are more interested in sending their children to Quaranic School within and outside the community than attending western education. This has made them not see the need for at least primary school in their immediate environment. Therefore, the low level of western education may affect the knowledge of devastating HIV/AIDS that is ravaging globally.

Fisheries sector provides livelihood strategies to its dwellers. The study revealed that 84.5% of the respondents had their primary occupation in fisheries related activities and only 15.5% were into skill labour (such as welding, carpentry) and trading in other products. 27.8% of the respondents had secondary occupation such as firewood cutting, food hawking and haulage. The result confirms the high mobility in labour among fisherfolk. The finding corroborates Neiland et al, 2005 that combination of activities ranging from catching, processing, trading and transportation are important occupation in the fishing communities.

Characteristic of Respondent

Characteristics	**frequency**	**Percent (%)**
Sex		
Male	119	63.6
Female	68	36.4
	187	**100**
Age		
15-25	45	24.1
26-35	55	29.4
36-45	42	22.5
46-55	28	15.5
Above 55	17	9.1
	187	**100**

Marital Status		
Single	40	21.4
Married	146	78.1
Widow	1	0.5
Separated	-	-
Divorced	-	-
	187	**100**
Number of wife		
One	4	2.1
Two	59	31.6
Three	27	14.4
More than three	5	2.7
No response	92	49.2
	187	**100**
Religion		
Islam	157	84.5
Christianity	29	15.5
Idol	1	0.5
	187	**100**
Education		
Primary	35	18.7
Secondary	35	18.7
Tertiary	5	2.7
Adult Education	5	2.7
No formal education	107	57.2
	187	**100**
Primary Occupation		
Fishing	23	12.3
Farming-fishing	23	12.3
Trading in fish	15	8.0
Processing of fish	40	21.4
Boat construction	27	14.4
Craft/gear making	7	3.7
Skilled labour	5	2.7
Others	29	5.5
	187	**100**
Secondary Occupation		
Skilled labour	1	0.5
Firewood cutting	2	1.1
Food vendor	45	24.1
Transporting	4	2.1
No response	135	72.0
	187	**100**

On the awareness of HIV/AIDS, 98.4% of the respondents at one time or the other had heard about the disease but did not know much about the organism responsible for HIV/AIDS pandemic (locally known as Kajanmu in Hausa). Only 30% was able to mention the virus, though they had an idea of what it means as many of them gave different interpretations of AIDS in their local language. Those who had heard of AIDS heard mostly from the radio. This corroborates previous findings by Orubuloye et al, (1995) which reported that prisoners heard most of the information on AIDS from the radio. From observation the result does reflect the true situation and with low level of education many of the respondents might not be able to mention the virus responsible for this disease of poverty.

70% said they don't know name of the responsible for disease. Someone looking healthy is not free from infection of HIV/AIDS. This was confirmed in this study where 71.1% of the respondents agreed that someone looking healthy can harbor the virus like any other disease which may take time before manifestation of symptoms. 10.2% and 18.7% of the respondents disagreed with the statement and could not ascertain whether is true or false. The findings from fishing communities followed the trend of the result obtained by Yahaya (2000). The spread of HIV/AIDS is on the increase due ignorance. This study revealed that 57.8% of the respondents knew that abstinence premarital sex reduce the infection, 16.6% said faithfulness to one's partner should be emphasized. Only 10.2% believed the use of condom while 4.8% don't know. 10.2% of the respondents knew non sharing of sharp object and sterilized any sharp object can reduce infection.

It is imperative that the fishing communities be generally educated on family planning and reproductive health to redeem the loose lifestyle established in the literature on HIV/AID in the fishing communities. Going to hospital for diagnosis is necessary for health problems in which HIV/AIDS is not an exception while 4.8% don't know. Many of the communities lack primary health centre and the available ones were some kilometres away from their reach. On confirmation of the disease on victim, 42.8% believed if someone has many health problems, 12.8% said by establishing the number of sexual partners and 33.6% said they don't know. This is worrisome considering the various programme going on the subject. Although, only 42.2% agreed that they are at risk and 45.5% said that they are not risk in any form while 12.3% don't know whether at risk or not. But from observation, sharing of sharp objects are common habit in the fishing communities selected and it is important to discourage the use sharing of sharp for manicure which is a common activity in the study area. 6.4% and 10.7% said the risks were at average and high risk of HIV/AIDS respectively. The high ignorance of the people who did not know the implication of someone sharing the same razor in cutting their nails corroborates the finding of an earlier study by Iwoh (2004), who reported that there was low knowledge of HIV/AIDS/STIs among prison staff in Nigeria.

The study also revealed that most of the respondents' knowledge of HIV/AIDS is limited to sexual intercourse with the opposite sex. Interestingly, many of them were unaware that homosexual acts, unscreened blood transfusion, sharing of sharp instruments as well other risky practices of AIDS are as risky as sexual intercourse. More so, the fact that such acts as tattooing and sharing of blades are common practices in the fishing communities, which may expose them to HIV/AIDS. However, out of all the means of contracting HIV/AIDS virus, sexual intercourse was the most commonly known to the people. The result support the finding of Isibor and Ajuwon (2004), in their study on journalists' knowledge of AIDS and attitude toward people living with HIV, found a number of misconceptions amongst people concerning HIV/AIDS-related issues. 50.2% of the respondents met /know people living with the virus or have died from the infection while 49.8% said they have met /know one that has HIV/AIDS. 31% of the respondents they were from the village. 47.6% no response. The situation in fishing communities calls for urgent attention. It is surprising to know that large number of respondents (66%) could assess or determine their risk level of HIV/AIDS pandemic. The perception of the respondents on HIV/AIDS is high, 90.4% believed that it is a serious deadly disease but lack the information that could help them to live dignified life. Only 5.3% saw it as an imaginary disease.

Table 3: Knowledge of HIV/AIDS in the fishing communities

Variables	Frequency (F)	Percent (%)
Heard of HIV/AIDS		
Yes	184	98.4
No	3	1.6
	187	**100**
Name of microbe		
HIV	56	30
I don't Know	131	70
	187	**100**

Can someone harbour the virus and look healthy		
Yes	133	71.1
No	19	10.2
I don't know	35	18.7
	187	**100**
Avoiding AIDS infection		
Abstinence from sex	108	57.8
Use of condom	19	10.2
Having only one partner	31	16.6
Use blade, razor& syringe only once	19	10.2
Clean any sharp object before use	1	0.5
I don't know	9	4.8
	187	**100**
Confirmation of HIV/AIDS infection		
By asking person if he has health Problems	80	42.8
By asking person if he/she has many sexual partners	24	12.8
By asking person if he has ever had sex with prostitutes	9	4.8
By asking person if he/ she blood Transfusion	11	5.9
I don't know	63	33.6
	187	**100**
Prone HIV/AIDS Risk		
Yes	79	42.2
No	85	45.5
I don't know	23	12.3
	187	**100**
Assessment of risk Perception		
Low	31	16.6
Average	12	6.4
Very high	20	10.7
I don't know	26	13.9
No response	98	52.4
	187	**100**
Perception of AIDS		
A serious deadly disease	169	90.4
An imaginary disease	10	5.3
A disease caused by witches	1	0.5
No response	7	3.7
	187	**100**

In the study area, the attitude towards the people living with the virus, 38.5% of the respondents said they will stop all sexual relation if their partner tested positive while only 24.1% will go for screening to find out their serological status. The percentages for divorce and stopping sexual relation with partners is high and such person may decide to remarry or have other sexual partners in turn spread the disease to the innocent members of the communities causing more havoc in their immediate environment.

41.7% of the respondents said that they will avoid any contact with people living with HIV/AIDS while 39% were willing to provide moral and material support, this result revealed that there will be high level of stigmatization in a situation a member of the community found living with the virus. From the study it is like a taboo for a spouse to faithful to his/her, 22.5% of the respondents said it is impossible to keep to a partner while 35.8% said they have nothing to say. The finding confirmed the statement that one of the ways to express one social status in our society is in the number of partners he has, so the result is not surprising.

Table 4 : Attitude towards people living with HIV/AIDS virus

Variables	Frequency (F)	Percent(%)
Do you know people living with HIV/AIDS virus		
Yes	94	50.2
No	93	49.8
	187	**100**
Are they from the village		
Yes	58	31.0
No	40	21.4
No response	89	47.6
	187	**100**
If partner tested positive		
Divorced	56	29.9
Stop all sexual relations with him/her	72	38.5
Demand for protected sex	3	1.6
Go for a screening to find out Serological status	45	24.1
Go and look for a traditional medicine man	1	0.5
I don't Know	10	5.4
	187	**100**
Support for people living with HIV/AIDS virus		
Provide moral & material support	73	39.0
Ask for his expulsion from the village	5	2.7
Avoid any contact with him/her	78	41.7
Warn other colleagues in the village	13	7.0
I don't know what my attitude will be	18	9.6
	187	**100**
Faithfulness to Partners		
It is impossible	42	22.5
It is just hypocritical	13	7.0
It is not a way of preventing AIDS	31	16.6
It exposes to AIDS	34	18.2
Nothing to say	67	35.8
	187	**100**

On the sexually transmitted disease(s), 87.7% was aware and could mention at least one of the diseases. Only 12.3% claimed ignorance sexually transmitted diseases. 64.2% of the respondents could mentioned gonorrhoea while 24.6% syphilis. Only 10.2% could not mention any sexually related diseases in the study area. On symptoms associated with sexually transmitted diseases 35.8% recognized burning sensation when urinating while 28.3% said abdominal pain. 12.3% and 11.2% had no idea and no response respectively. The findings revealed little knowledge on sex education and is a common phenomenon in our society the reasons been that sex issues of such are openly discuss in homes and our society.

Table 6: Other sexual diseases in fishing communities

Variables	Frequency (F)	Percent(%)
Do you know any other sexual Diseases?		
Yes	164	87.7
No	23	12.3
	187	**100**
Names of sexually transmitted Diseases		
Gonorrhoea	120	64.2
Chancre	1	0.5
Syphilis	46	24.6
Herpes	1	0.5
No response	19	10.2
	187	**100**
Symptoms of diseases		
Abdominal pain	53	28.3
Vaginal discharge	9	4.8
Burning sensation when urinating	67	35.8
Sore on private part	8	4.3
I don't know	23	12.3
No response	21	11.2
	187	**100**

On seminar/workshop, 77% of the respondents revealed no seminar/ workshop had taken place in the communities to sensitize the people. It implied that knowledge of HIV/AIDS is low among the fisherfolk. Also, the study revealed that 62.6% of the respondents agreed that HIV/AIDS is not openly discussed in the communities while 5.3% didn't response to the question. On rate of access to HIV/AIDS information, 59.9% of the respondent could not answer the question while 28.9% said it is high. The result is against Jimoh (2002) who did content analysis of 2,156 articles and found that newspaper reports were often coverage of workshops and conferences and government policies and pronouncements and corroborates Adesomoye (2002) and Komolafe (1999) findings that coverage of the disease is minimal with inadequacy in the coverage that does exist. The result is reflection of the situation in the fishing communities which is contrary to the major programme on HIV/AIDS that are concentrated in the cities and town and not rural area where 70% people live. This is dangerous for rural economy and other developmental projects in some of the rural areas.

Table7 Information on HIV/AIDS in fishing communities

Variables	Frequency (F)	Percent(%)
HIV/AIDS seminar/ workshop		
Yes	39	20.9
No	144	77.0
No response	4	2.1
	187	**100**
Talk openly about HIV/AIDS in the community		
Yes	60	32.1
No	117	62.6
No response	10	5.3
	187	**100**

Rate of access to HIV/AIDS information		
Low	21	11.2
High	54	28.9
No response	112	59.9
	187	**100**

CONCLUSION

This paper has highlighted the knowledge, attitude and practices of the HIV/AIDS in the Yauri emirate in some selected fishing communities of Kainji Lake Basin of Nigeria . It was discovered from the study the people still lack basic information on HIV/AIDS pandemic. It is unfortunately fishing communities have not benefited much from lectures, seminars and workshops on HIV/AIDS, it is imperative for government and other community based organizations to give fisheries sector attention on HIV/AIDS education and prevention to carry along the population in the struggle against the pandemic being the most vulnerable group given all sorts of experimentations (sexual, drug, gangsterism) . It is to ensure that the impact of HIV/AIDS is properly taken into account in the attempts of government, donor organization and NGOs to manage fisheries and assist fisher folks to find ways out of poverty and vulnerability. It is time to help those in fishing communities who are already living with HIV/AIDS to continue to enjoy productive and dignified lives. Urgent mobilization on HIV/AIDS information and education be organized in the fishing communities to reduce the burden of HIV/AIDS on national economics, loss of labour as highlighted as one of the main economic impacts (Gillespic, 1989, Lisk.2002) and guarding against the prediction of total number of lost workdays in the agricultural sector because of HIV/AIDS by year 2020 (FAO, 2002).

However, the following recommendations will assist the fishing communities to fight against health related problems, especially HIV/AIDS;

- Provision of health facilities and health personnel in fishing communities
- The establishments of HIV/AIDS support Organization in enlightening the people on a broad - based community approach.
- Enlightenment campaigns on HIV/AIDS and education programme on safe sex and behavior change remain key responses to the epidemic
- Encouraging know your status campaign in the fisheries sector.

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Comparative Assessment of Potassium Sorbate and Sodium Metabisulphite on the Safety and Shelf Life of Smoked Catfish

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ABSTRACT

Forty-four sample of Catfish (*Clarias gariepinus*) were obtained from a fish pond in NIFFR divided into 11 portions of 4 each where 5 portions was treated with 1-5% Potassium sorbate respectively, the next 5 portions was treated with 1-5% Sodium metabisulphite (both are antimicrobial agents) prior to smoking and the last portion was not treated (it serve as control). They were later smoked and stored for 8-weeks at room temperature. Smoked samples were drawn after 0, 2, 4, 6, and 8 weeks for microbial, moisture contents and proximate analysis. All treated smoked samples were dominated with *Bacillus coagulans and Klebsiella ozanae* but negative for *E. coli and Streptococcus sp*. Unlike the Sodium metabisulphite 3% Potassium sorbate reduced the *Staphylococcus count* to 0 throughout the 8th week of storage. Potassium sorbate proved to be more efficient in controlling microbial quality and extending shelf life of smoked catfish.
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Key words: Potassium sorbate, sodium metabisulphite, Catfish, Quality and Safety.

INTRODUCTION

Fish is becoming increasingly important in the diet of the Nigerian as there is an increase awareness that regular red meat intake in adult above 40 years of age is not healthy. Fish constitutes 40% of animal protein intake in Nigeria at present (Olatunde, 1989). This is because fish are a cheap source of animal protein with little or no religious rejection of it, which gives it an advantage over pork or beef. Fish are a very perishable commodity, more than cattle, sheep, and poultry, and get spoiled very easily even in temperate climates. So unless it is disposed of quickly after capture, it must be preserved in some way. World fish production was estimated at 100 million tons in 1989, 15% of which was cured in one or another way. One third of the cured fish was smoked and about 20% of the smoked fish goes into international trade (Ward, 1995). Increasing consumer awareness of the nutritional value of seafood especially smoked fish has stimulated a strong demand for this product (Pigott and Tucker, 1990). To satisfy the consumer demand, it is necessary to produce good quality and safe smoked fish. Smoked fish and shellfish products can be a source of microbial hazards. Human infections may be caused by bacteria endogenous to fish. Bacterial pathogens, which may be transferred from fish to human beings include: *A. hydrophila* (septicemia, diarrhea), *Campylobacter jejuni* (gastroenteritis), *Clostridium botulinum* type E (botulism), *Edwardsiella tarda* (diarrhea), *Leptospira interrogans* (leptospirosis), *Mycobacterium fortuitom marinum* (mycobacteriosis), *Plesiomonas shigelloides* (gastroenteritis), *Pseudomonas aeruginosa* (wound infections), *Salmonella sp*.(food poisoning), and *vibrio parahaemolyticus* (food poisoning) (Austin and Austin, 1989). Delay or prevention of microbial spoilage of fish may be achieved by different preservative methods that include the use of smoking and chemical preservatives like sorbates and sulphites. Sorbates are the most effective preservatives against a wide spectrum of food spoilage microorganisms; they include sorbic acid and potassium sorbate. They are among the safest, most efficient and versatile preservatives used in the food industry today. Sorbates are tasteless and odourless. Because they are non-toxic, they are used in a wide variety of foods, including cheese, yogurt, sour cream, bread, cakes, baking mixes, icing, beverages, margarine, fermented vegetables, fruit products, salad dressing, smoked and salted fish and mayonnaise. The antimicrobial activity of sorbates against molds, bacteria and fungi has been reported by researchers Sofos and Busta, 1993; Sofos, 2000). Also sulphites may be used as potassium bisulphite, sodium or potassium metabisulphite, sodium sulphite or sulphur dioxide on food. They are often used as preservatives in wines (to prevent spoilage and oxidation), dried fruits and dried potato products.

Sulphites also occur naturally in almost all wines. Sodium metabisulphite have been used in the preservation of fresh and frozen crustacean up to 150mg/kg in edible parts. (US FDA, 1978).

Considering the antimicrobial activity of Sorbates and Sodium metabisulphite this study was carried out to determine the microbial, organoleptic and nutritional quality changes of smoked catfish and to evaluate the effect of these antimicrobial agents at different concentration on the quality of smoked catfish during storage at room temperature since there are scanty information on this looking at smoked fish in Nigeria.

MATERIAL AND METHODS

Fresh catfish (*Clarias gariepinus)* were obtained from a private Fish pond in National Institute for Freshwater Fisheries Research (NIFFR) Housing Estate, New Bussa, Niger State. The fish samples measuring 17-28cm in length and weighing 180-250g were transferred within 30 minutes to the laboratory in a sterile polythene bags and then killed by severing the spinal cord with a sterile scalpel and aseptically eviscerated, washed and rinsed in sterile water. The fish samples were randomly chosen and divided into 11 groups of 4 fish for each of the Catfish subjected to treatments. The treatments were as follows; (1) control (untreated samples); (2, 3, 4, 5 and 6) are treated with 1, 2, 3, 4 and 5% Potassium sorbate and 7, 8, 9, 10 and 11 are treated with 1, 2, 3, 4 and 5% Sodium metabisulphite for 5 minutes, A sample from each group were separated from each treatment and smoked. Smoking was done according to the methods by Omojowo and Ibitoye (2005). After smoking and the fish were allowed to cool down and stored in different boxes. This was done to mimic commercial practices. The samples were drawn after two, four, six and eight weeks of storage; then subjected to analysis.

Microbiological Analysis

A 25g representative sample (excluding the head and tail) of each fish sample was obtained aseptically to prepare serial dilution using 0.1% peptone water as diluents. Total bacteria counts and coliform counts were determined according to the method of Sneath *et. al*.(1986). *Faecal streptococci* and *E. coli* in samples were determined employing the methods described by speak (1984). *Staphylococcus aureus* counts in samples were determined by employing the method of Bennett (1984). Moisture contents, fat and Crude protein were estimated as per AOAC (1980). All samples were done in duplicates. Sensory evaluation was carried out according to the method of Afolabi et. al. (1984). Statistical analysis was according to SAS, Institute, Inc, (1992) at $P < 0.05$.

RESULTS AND DISCUSSION

A study for the absence and presence of the target food borne pathogens such as *Salmonella, Staphylococcus, and E. coli* is required to evaluate microbial safety of smoked Catfish. The range of specified microbiological limits recommended by ICMSF (1986) for fish and fishery products is as follows: for the TPC, the maximum recommended bacterial counts for good quality products (m) is $5x10^5$ (5.7 log10 CFU/g) and the maximum recommended bacterial counts for marginally acceptable quality products (M) is 10^7 (7 log10 CFU/g). For *E. coli*, the m value is 11 (1.0 $\log^{10}$ CFU/g) and the M value is 500 (2.7 log10 CFU/g), and for *Staphylococcus*, m value is 10^3 (3 log10 CFU/g) (ICMSF, 1986). For all fish, the *Staphylococcus aureus* safety level is equal to or greater than 10^4 /g. In many cases, these levels represent the point at or above which the agency will take legal action to remove products from the market (FDA, 2001, Fish and Fishery Products Hazards & Controls Guidance manual).

Total Viable count (TVC), Coliform, Staphylococci and Fungi count in log CFU/g of fresh and smoked Catfish samples are shown in Tables 1 and 2. TVC of the fresh the control catfish was 6.60 log CFU/g but after the sample were subjected to treatments with 1-5% Sodium metabisulphite and 1-5% Potassium sorbate the TVC, Coliform, Staphylococcus and fungi count were reduced however, the reduction was higher in the treatment with Potassium sorbate also as the concentration is increases..

Smoking sharply reduced the total viable count (Table 1 and 2) in all samples, but the sample treated with 5% Potassium sorbate showed the greatest reduction and maintained a low level throughout 8 weeks of storage, especially on day 0 2.13 log CFU/g as shown in Table 2 while after 8-week storage the TVC was 4.60 log CFU/g. The TVC of the control samples were the highest throughout the period of storage where the sample were completely covered by mold after the 6^{th} week of storage; therefore, no further microbial analysis was conducted. The results obtained were similar to those reported by Efiuvwevwere and Ajiboye (1996), where the samples treated with 0.4% potassium sorbate showed the lowest microbial load and maximum shelf stability. Similar to TVC, the coliform count (of the smoked samples treated with 5% Potassium sorbate had the highest reduction of 0.93 log CFU/g on day 0 and remain the lowest of the treatments throughout the period of storage. Significant increases in coliform population of all samples occurred after 4 weeks of storage. Coliform

count of all treated samples was less than 3.0 log CFU/g throughout the 8-week storage except for the sample treated with 1-2% Sodium metabisulphite which were above 3.0 log CFU/g in the eighth-week.

Table 1: Microbial Load of Catfish Treated With Sodium Metabisulphite (Log10)

	Microbial group	Control	1%	2%	3%	4%	5%
B/4 Smoking	TVC	6.60 ^{a}	5.95 ^{b}	5.48 ^{c}	5.46 ^{c}	5.24 ^{d}	5.10 ^{d}
After „	TVC	4.59 ^{b}	4.10 ^{cd}	4.16 ^{cd}	4.21 ^{d}	4.12 ^{cd}	4.01 ^{c}
2nd week	TVC	6.04 ^{c}	4.48 ^{c}	4.56 ^{cd}	4.70 ^{d}	4.68 ^{d}	4.21 ^{e}
4th „	TVC	6.52 ^{a}	5.20 ^{b}	5.17 ^{b}	5.11 ^{b}	5.06 ^{b}	5.00 ^{b}
6th „	TVC	7.35 ^{b}	6.69 ^{c}	6.68 ^{c}	6.60 ^{c}	6.51 ^{c}	6.32 ^{d}
8th „	TVC	Mouldy	7.79 ^{b}	7.66 ^{bc}	7.61 ^{c}	7.63 ^{c}	7.67 ^{bc}
B/4 smoking	Coliform	4.60 ^{a}	4.46 ^{b}	4.44 ^{b}	4.40 ^{b}	4.43 ^{b}	4.39 ^{b}
After „	Coliform	3.54 ^{b}	2.24 ^{c}	2.19 ^{c}	2.20 ^{c}	2.18 ^{cd}	2.06 ^{d}
2nd week	Coliform	4.10 ^{c}	2.55 ^{d}	2.43 ^{d}	2.20 ^{e}	2.04 ^{f}	2.10 ^{ef}
4th „	Coliform	4.43 ^{a}	2.60 ^{b}	2.48 ^{b}	2.30 ^{c}	2.33 ^{bc}	2.28 ^{c}
6th „	Coliform	5.17 ^{b}	2.98 ^{c}	2.84 ^{cd}	2.76 ^{d}	2.76 ^{d}	2.59 ^{d}
8th „	Coliform	Mouldy	3.51 ^{b}	3.50 ^{b}	3.47 ^{b}	3.39 ^{bc}	3.22 ^{c}
B/4 smoking	Staph.	4.55 ^{a}	4.55 ^{b}	4.26 ^{c}	4.31 ^{c}	4.34 ^{c}	4.20 ^{c}
After „	Staph.	3.17 ^{b}	2.10 ^{c}	1.80 ^{d}	1.25 ^{e}	0.38 ^{f}	0.0 ^{f}
2nd week	Staph.	5.06 ^{c}	1.75 ^{d}	1.68 ^{d}	1.60 ^{d}	0.41 ^{e}	0.0 ^{f}
4th „	Staph.	5.32 ^{c}	1.95 ^{d}	1.73 ^{e}	1.48 ^{f}	0.80 ^{g}	0.0 ^{h}
6th „	Staph.	5.52 ^{c}	2.70 ^{d}	2.49 ^{e}	2.03 ^{f}	1.10 ^{g}	0.0 ^{h}
8th „	Staph.	Mouldy	3.73 ^{a}	3.56 ^{b}	2.74 ^{c}	1.06 ^{d}	0.0 ^{e}
B/4 smoking	Fungi	4.52 ^{a}	4.00 ^{b}	3.92 ^{b}	3.55 ^{c}	3.46 ^{c}	3.30 ^{d}
After „	Fungi	3.11 ^{b}	2.04 ^{c}	2.10 ^{cd}	2.15 ^{cd}	2.20 ^{d}	2.18 ^{d}
2nd week	Fungi	5.28 ^{c}	3.21 ^{d}	3.19 ^{d}	3.19 ^{d}	3.14 ^{de}	3.05 ^{e}
4th „	Fungi	5.41 ^{c}	3.73 ^{d}	4.00 ^{e}	3.70 ^{d}	3.65 ^{de}	3.54 ^{e}
6th „	Fungi	5.70 ^{a}	4.43 ^{b}	4.36 ^{bc}	4.24 ^{c}	4.18 ^{cd}	4.04 ^{d}
8th „	Fungi	Mouldy	6.10 ^{a}	6.09 ^{a}	5.96 ^{ab}	5.88 ^{b}	5.80 ^{b}

Means in the same rows with different superscript are significantly different ($p < 0.05$).

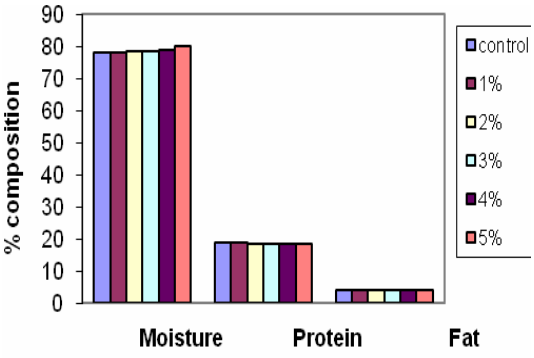

Figure1. Proximate Composition of Fresh Catfish Treated with Sodium metabisulphite

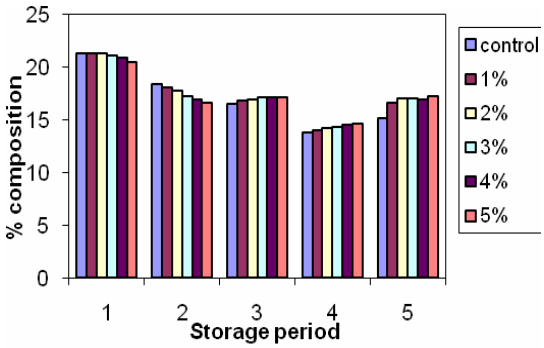

Figure 2. Moisture contents of Smoked Catfish Preserved with Sodium metabisulphite

Note, in x-axis 1= Day 1, 2= 2$^{n\,d}$ Wk, 3 = 4th Wk, 4= 6th Wk and 5= 8th Wk

Table 2: Microbial Load Of Catfish Treated With Potassium Sorbate (Log10)

	Microbial group	Control	1%	2%	3%	4%	5%
B/4 Smoking	**TVC**	6.60 [a]	5.48 [b]	5.46 [b]	5.42 [b]	5.12 [d]	5.07 [e]
After „	**TVC**	4.59 [b]	3.61 [c]	3.50 [c]	3.47 [c]	3.10 [d]	2.04 [e]
2nd week	**TVC**	6.04 [c]	4.14 [d]	4.06 [d]	3.98 [d]	3.65 [e]	2.72 [f]
4th „	**TVC**	6.52 [a]	5.00 [b]	5.01 [b]	4.84 [c]	4.30 [d]	3.43 [e]
6th „	**TVC**	7.35 [b]	5.71 [c]	5.68 [c]	5.50 [d]	4.71 [e]	3.90 [f]
8th „	**TVC**	Mouldy	6.72 [b]	6.64 [b]	6.35 [c]	6.21 [c]	4.54 [d]
B/4 smoking	**Coliform**	4.60 [a]	3.95 [b]	3.76 [c]	3.74 [cd]	3.61 [cd]	3.58 [d]
After „	**Coliform**	3.54 [b]	1.55 [c]	1.40 [cd]	1.32 [d]	1.24 [d]	0.93 [e]
2nd week	**Coliform**	4.10 [b]	1.72 [cd]	1.88 [d]	1.61 [c]	1.55 [c]	1.10 [e]
4th „	**Coliform**	4.43 [c]	2.08 [d]	2.00 [de]	1.76 [ef]	1.62 [f]	1.27 [g]
6th „	**Coliform**	5.17 [a]	2.50 [b]	2.42 [b]	2.23 [c]	2.11 [c]	1.92 [d]
8th „	**Coliform**	Mouldy	2.81 [b]	2.42 [c]	2.54 [c]	2.50 [c]	2.20 [d]
B/4 smoking	**Staph.**	4.55 [a]	3.88 [b]	3.74 [bc]	3.71 [c]	3.74 [bc]	3.65 [c]
After „	**Staph.**	3.17 [b]	0.40 [c]	0.32 [c]	0.0 [d]	0.0 [d]	0.0 [d]
2nd week	**Staph.**	5.06 [a]	0.60 [b]	0.45 [b]	0.0 [c]	0.0 [c]	0.0 [c]
4th „	**Staph.**	5.32 [b]	1.0 [c]	0.84 [c]	0.0 [d]	0.0 [d]	0.0 [d]
6th „	**Staph.**	5.52 [c]	1.60 [d]	1.25 [d]	0.0 [e]	0.0 [e]	0.0 [e]
8th „	**Staph.**	Mouldy	2.10 [a]	1.80 [b]	0.0 [a]	0.0 [a]	0.0 [a]
B/4 smoking	**Fungi**	4.52 [a]	4.12 [b]	4.02 [b]	4.03 [b]	3.71 [c]	3.28 [d]
After „	**Fungi**	3.11 [b]	1.21 [c]	1.22 [c]	1.05 [d]	0.46 [e]	0.0 [f]
2nd week	**Fungi**	5.28 [c]	1.73 [d]	1.84 [d]	1.55 [e]	0.54 [f]	0.0 [g]
4th „	**Fungi**	5.41 [c]	2.59 [d]	2.61 [d]	1.92 [e]	0.62 [f]	0.0 [g]
6th „	**Fungi**	5.70 [d]	3.36 [e]	3.25 [ef]	2.14 [f]	1.26 [g]	0.22 [h]
8th „	**Fungi**	Mouldy	3.78 [a]	3.61 [b]	2.57 [c]	1.42 [d]	0.36 [e]

Means in the same rows with different superscript are significantly different ($p < 0.05$).

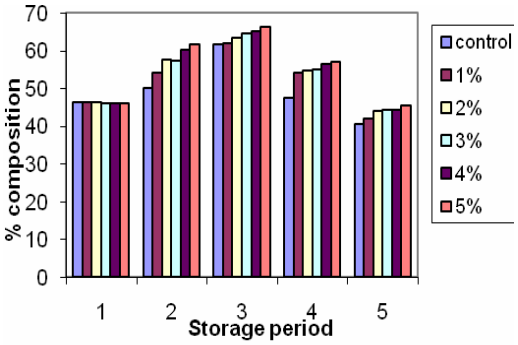

Figure 3. Protein composition of Smoked Catfish Preserved with Sodium metabisulphite

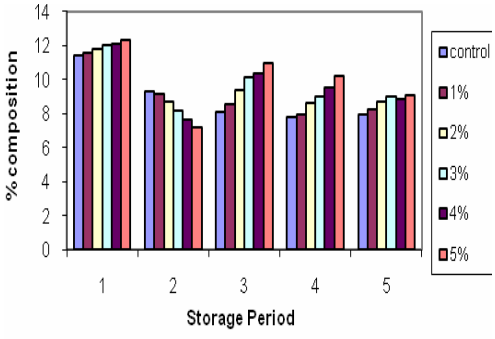

Figure 4. Fat composition of Smoked Catfish Preserved with Citric Acid

Note, in x-axis 1= Day 1, 2= 2n d Wk, 3 = 4th Wk, 4= 6th Wk and 5= 8th Wk

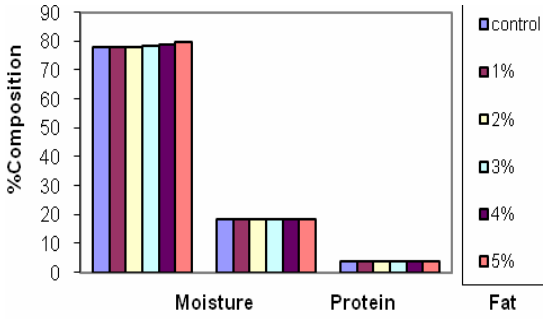

Figure 5. Proximate Analysis of Fresh Catfish Treated with Potassium sorbate

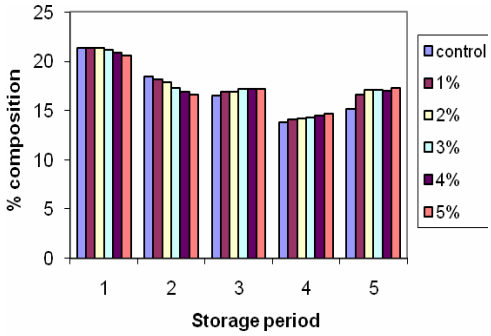

Figure 6. Moisture Contents of Smoked Catfish Preserved with Potassium sorbate

Note, in x-axis 1= Day 1, 2= 2n d Wk, 3 = 4th Wk, 4= 6th Wk and 5= 8th Wk

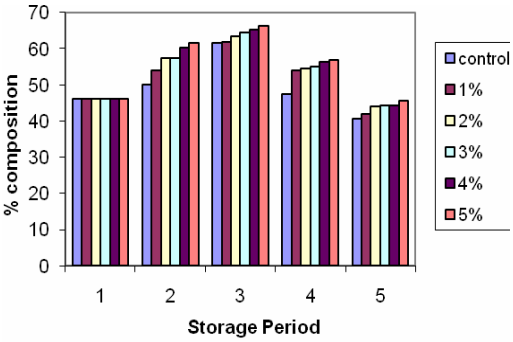

Figure 7. Protein Composition of Smoked Catfish Preserved with Potassium sorbate

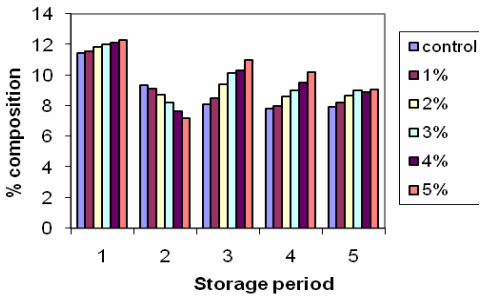

Figure 8. Fat composition of Smoked Catfish Preserved with Potassium sorbate

Note, in x-axis 1= Day 1, 2= 2n d Wk, 3 = 4th Wk, 4= 6th Wk and 5= 8th Wk

In the control samples, the Coliform population was 5.17 log CFU/g on the 6th week while the sample was completely covered by mold on the 8th week of storage. This result was similar to that reported by Virginia, (2002) where the Coliform in the control sample showed 2.6 log CFU/g on the 4th week and the sample was completely covered by mold on the 6th week of storage. The high coliform count recorded in this report may be due to contamination from the animal manure used in fertilizing the ponds at one time or the other. Furthermore, the smoked sample treated with 3-5% Potassium sorbate had no Staphylococcus count throughout the period of storage while only 5% Sodium metabisulphite was able to reduce the Staphylococcus count to 0 and remained 0 until the end of 8th week storage. Generally, Potassium sorbate showed the lowest count throughout the 8th week of storage.

The isolation of *Staphylococcus* in smoked samples on day 0 may be attributed to post processing contamination. However, *Staphylococcus* was killed by the treatments 3-5% Potassium sorbate. Fungi counts were also reduced in all the treatments and at the end of the 8-week storage time; however, the sample treated with 5% Potassium sorbate showed 0 counts till the 4th and 6th weeks of storage. The control samples were high throughout the period of storage and the sample was even completely covered by mould at the end of the 8-week storage. This result were similar to those reported by Efiuvwevwere and Ajiboye (1996), where the samples treated with 0.4% potassium sorbate showed the minimum fungal load during storage and presence of profuse mould growth after day 8 in the control.

It is of interest to observe that in spite of the slightly reduced moisture contents (from 2nd to 6th week) in almost all the samples microbial load still

increases dramatically. This suggests that one single factor may not account for these microbial changes. Cross contamination, pH, purity of preservatives are among other factors that can influence microbial changes. The bacterial contamination of hot smoked fish just out of the smokehouse is usually below 10^3 per gram (Doe, 1998). The TVC of the most of the treated samples were all below $5x10^5$ CFU/g to the 6th week which is below m in a three-class attribute plan and signifies good quality. Low levels of coliform bacteria were detected and the pathogens *S. aureus* counts were below 10^3 in all the treated samples till the 8th week except samples treated with (1-2% Sodium metabisulphite). The control however, has TVC higher than $5x10^5$ CFU/g in the second week and higher than the recommended limit 7.0 log CFU/g (ICMSF, 1986) after the 4th week. In addition the Coliform count already exceeded 10^3 even immediately after smoking. This finding is of concern as a result of the associated public health implications. For example, generally, hot smoked fish are consumed in the tropics with little or no further processing, thus, they fall into the high-risk category of foods (ICMSF, 1986). Hence there is a need for the use of appropriate percentage of choice antimicrobial agent.

BACTERIAL ISOLATES

All treated smoked sample were negative for *E. coli and Streptococcus sp*. However, the control and the fresh fish treated samples showed the following bacteria flora *Bacillus coagulans, B. cereus, Klebsiella ozanae, Proteus vulgaris, Escherichia coli, Staphylococcus aureus, and Streptococcus sp,* while the fungi isolated include *Penicillium verrucosum, Aspergillus niger, A. candidus, A. flavus and A. nidulan* while the smoked untreated sample (control) were dominated by the following organisms *B. coagulans,* (about 70% of the isolates) while the remaining being *S. aureus,* and *Streptococcus sp*. The treated sample showed the microbial load in the following pattern; 1% and 2% potassium sorbate of the fish samples contains the following spp *B. coagulans, S. aureus, K. ozanae, A. candidus and A. nidulan* while in 3% and 4% potassium sorbate treated samples have the following isolates *B. coagulans, K. ozanae and A. nidulan while* 5% treatment have only *B. coagulans.* While 1-4% Sodium metabisulphite treated samples *have* following isolates *B. coagulans, S. aureus, , A. candidus, A. nidulan and A. flavus while* 5% treated sample have all except *S. aureus and A. flavus.*

Proximate Analysis

The proximate analysis of raw and Smoked catfish are presented in Figure 1 to 4 There were no significant ($p \leq 0.05$) differences in Protein (18.3 – 20.2% and 17.8 - 18.6%), Fat (2.6 – 3.0% and 3.9 – 4.30%), and Moisture contents (73.4 - 77.0% and 78.2 - 79.4%) of the samples subjected to different treatments. The moisture content of fresh sample was 78.2%. In the treatments the moisture contents ranged from 78.2 - 79.4%. Moisture content of catfish decreased sharply after the smoking process and this decrease was due to loss of water during smoking (Asiedu et al., 1991). Also the study reveals that the average protein content increases after smoking, and increases till the 4th week and later decreases till the end of the 8th week of storage. There was an inverse relationship between the moisture and protein content in the smoked samples. The initial increase in protein content in smoked fish and till the 4th week may be due an increase in the dry matter content per unit of weight following sample dehydration during smoking and reduction in the moisture contents during the early part of the storage before autolysis becomes pronounced.

These results shows that storage time causes a decrease in the protein content of smoked catfish which agreed with earlier work of Ufodike and Obureke (1989) where there was decrease in crude protein of preserved *Oreochromis niloticus.* These workers attributed the decrease to hydrolysis of protein during the process of autolysis in the fish muscle. However, the treated samples show some corresponding higher value of protein more than the control especially as the concentration of the preservatives increases from 1-5%. This increase may be due to the effects of the preservatives which slow down autolysis in the fish muscles and consequently slow down the protein break down.

CONCLUSION AND RECOMMENDATION

This study has reveals that the samples treated with Potassium sorbate before smoking showed the greatest reduction and maintained a low level throughout the 8th weeks of storage. Hence, Potassium sorbate can be used as a choice preservative in smoked catfish without adversely affecting quality in terms of lipid oxidation, color, microbial and nutritional quality. The use of 3% Potassium sorbate as a choice antimicrobial agent is hereby recommended since it has been found to keep smoked fish in wholesome state for 8th week, reducing the TVC to 6.35 log CFU/g, the Coliform to 2.64 log CFU/g, Staphylococcus count to 0.0s and Fungi to 2.57 log CFU/g at the end of 8th week storage. This will ensure prolonged shelf life and safe consumption of smoked fish of ICMSF standard of smoked fish quality.

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Adaptation And Improvement Of A Simple Solar Tent Dryer To Enhance Fish Drying

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Abstract: Kainji Solar Tent Dryer (KSTD) was constructed as an improvement of Doe's tent and the two solar tent dryers were used to dry fish. The fish used for the study was *Bagrus bayad* of high quality which was divided into two portions and each of the solar tent dryers was used to dry each portion. After drying the indices for comparison were based on the performance of the two dryers, materials used for construction and conditions for fish drying such as temperature, relative humidity as well as the number of occurrences of flies in the tent. Results from each dryer was computed and analysed using one-way analysis of variance followed by the least significant difference (LSD) for comparisons among means. KSTD was better in operation; it recorded the least number of flies that gained access to the dryer, Temperature recorded for both dry and rainy seasons were higher and consequently a lower humidity. Finally for the KSTD, organoleptic study show that the output of fish dried in KSTD was far better than in Doe's tent. [Nature and Science. 2009;7(10):18-24]. (ISSN: 1545-0740).

Key words: KSTD, Doe's tent, *Bagrus bayad*, Temperature, Humidity and fish drying.

Introduction

Drying or dehydration is used to describe any process involving the removal of water from fish or fish product by evaporation (Eyo, 2001). Fish drying is presumably the oldest method of fish preservation using heat from the sun and atmospheric air although it has been limited to certain climatic areas and seasons. In the Lake Chad area where wood is relatively scarce and air temperatures remarkably high for prolonged periods, sun drying is a tradition (Ogali and Eyo, 1996).

Sun drying is fraught with problems such as contamination by dust and insect infestation because the fishes are dried on mats spread on bare ground (BOSTID), 1988 due to spoilage. To arrest these problems, many designs of solar dryers have been developed for the preservation of fish. One of such dryers is the one designed by Doe *et al.* (1977) in Bangladesh.

The Solar tent dryer is made up of a polythene sheet worn over a wooden frame. It works through evaporative drying using the green house principle. When set up in the sun, solar energy passes through the transparent polythene but gets trapped within it thereby raising the internal temperature. Cool air flowing in through an opening gets heated up and moves out moisture from fish laid on racks in the dryer. Solar dryer speeds up the drying process considerably, resulting in a high quality product with extended shelf life. Even under high humid conditions, solar dryers could have other advantages such as: (i) it is rain-proof and hence can be kept in continuous operation even in bad weather. (ii) Drying in an enclosed environment protects the products from dust, dirt, attack by birds, rodents and insect infestation.

This study shows results of trials and modification of Doe's tent and its comparison with the Kainji Solar tent dryer.

Materials and Methods

Materials used for the construction of Doe's (1977) tent include: polythene tent, wooden frames, PVC (black) polythene spread out on the based of the tent and a drying rack. Materials used for Kainji Solar tent dryer are: Transparent polythene tent, wooden frames, mosquito net, black igneous rocks, zip and drying rack. In constructing the Doe type, sticks were simply dug into the ground, tied together and the polythene sheet fastened around the sticks using stapling pins. A flap was left under the tent to serve as access to the fish and also as air inlet. The frame work of Doe's Tent is shown in Figure 2.

The KSTD (Figure 3) was constructed by obtaining five pieces of straight wooden poles each measuring 180 cm. Two of the poles were tied or nailed together at one end and the two other ends were dug 10cm into the ground at 160 cm apart. The same was done for the third and fourth wooden frames which are dug in an opposite direction at a distance of 194 cm apart. The fifth wooden frame

was placed across the two pairs of wooden poles and fastened at both ends to form a tent-like structure. About 10 yards (50 µ thickness) transparent polythene was sewn into shape of the wooden framework above. The polythene tent was sewn to a height of 180 cm, top length of 184 cm and bottom length of 227 cm to fit and worn over the wooden framework . On one of the longer sides of the polythene cover, an opening was cut into the tent and screened with mosquito net. This opening serves as air inlet into the solar tent. At the extreme narrow tops of the triangular part of the tent, openings of 15 cm x 15 cm were made and screened with mosquito net to serve as outlet of the hot air from the dryer. On the opposite side of the net with the air inlet, a ½ meter zip length was sewn to serve as an access opening into the tent for the processor to handle and inspect the fish on the drying rack during the drying process. A fiber rope is passed around the base of the tent for tying the polythene tent firmly on the wooden structure, to prevent wind and insects from escaping into the tent. About 30 pieces of rocks with an average weight of 10 kg each were stacked within the base of the wooden framework. The rocks are painted black hence they serve as capacitor by absorbing, retaining and releasing radiant energy needed for the fish drying. The drying rack is a wire mesh framed with wood (70 x 150 cm) and suspended by two ropes of 150 cm each from the wooden frame above.

Setting-up the Dryers

The two dryers (Doe's Tent and KSTD) were set-up side by side. The dryers were exposed to the sun from sunrise to sunset. The tents were positioned facing the direction of the prevailing wind, to allow air readily into the tents, since the drying process is a combination of air movement and heat. The dryers were set-up 30 minutes before fish were put inside.

Preparing the Fish for Drying

Fish used for this study was *Bagrus bayad* of high quality. This fish was selected because of its semblance to the imported stock fish. The fish was gutted and washed thoroughly in portable water. It was then split open from the dorsal region, then salted and allowed to drain before it was laid on the drying racks. Indices for comparison were based on the performance of the two dryers, materials used for construction and conditions for fish drying such as temperature, relative humidity as well as the number of occurrences of flies in the tent. Results from each dryer was computed and analysed using one-way analysis of variance followed by the least significant difference (LSD) for comparisons among means.

Results and Discussion

Table 1 shows comparison of Doe tent (Doe, 1977) and Kainji Solar tent designs. Components of the two dryers were used to compare the structural make up of the tents. Transparent polythene tent was not sewn but wrapped around the wooden frame in Doe's tent while it was sewn to shape in Kainji Solar tent dryer with rope hemmed around the tent to protect it against the wind. Wooden frames that hold the tent were dug into the ground in both tents. In the Kainji Solar tent dryers, the outlet was screened against flies with mosquito net, while in Doe's tent; the outlet was not screened but was wrapped up, thus allowing flies and pest to have access into the tent (Doe, 2002).

Air exit of the former was screened. In the latter, the tent had no screened exit for air but cut-out openings. One major characteristics of Kainji Solar tent dryer is the use of black igneous rocks that generate heat while in the Doe's tent, PVC black polythene was spread out on the base of the tent. In the former, a zip was attached to serve as access into the tent while in the latter a section of the tent has to be carried up to gain access into the tent to check the fish. Drying rack is fixed rigidly into the ground in Doe's tent, while in Kainji Solar tent dryer, drying rack is adjustable and removable.

Figure 1. Shows the occurrence of flies in Doe and Kainji Solar tent dryers in March and August 1999 and 2000 respectively. Results show that an average of 14 flies was counted in Doe tent while 3 flies were counted in Kainji Solar tent dryer in August 1999. Similarly, by March an average of 11 flies were seen in Doe's tent while in Kainji Solar tent dryer, an average of 1 fly was seen.

Table 1: Comparison of Doe (1977) and the Kainji (2003) Solar Tent design

Doe (1977) Solar Tent	Kainji (2003) Solar Tent
Polyethylene tent not sewn but wrapped around the wooden frames.	Polyethylene tent sewn to shape with roped hemmed around the tent to protect it against wind
Wooden frames are dug into the ground and not movable	Wooden frames are dug into the ground as with Doe's design
Outlet of Polyethylene tent not screened but wrapped up, thus allows in flies and pests.	Outlet screened against flies with mosquito net
Tent has no screened exit for air but cut out openings.	Tent has screened exit for air sewn
Tent has no black rocks but PVC (black) Polyethylene spread out on the base of the tent.	Tent has black rocks to generate heat
A section of the tent has to be carried up to gain access into the tent to check fish.	Tent has zip attached to serve as access into the tent
Drying rack is fixed rigidly to the ground	Drying rack is adjustable and removable

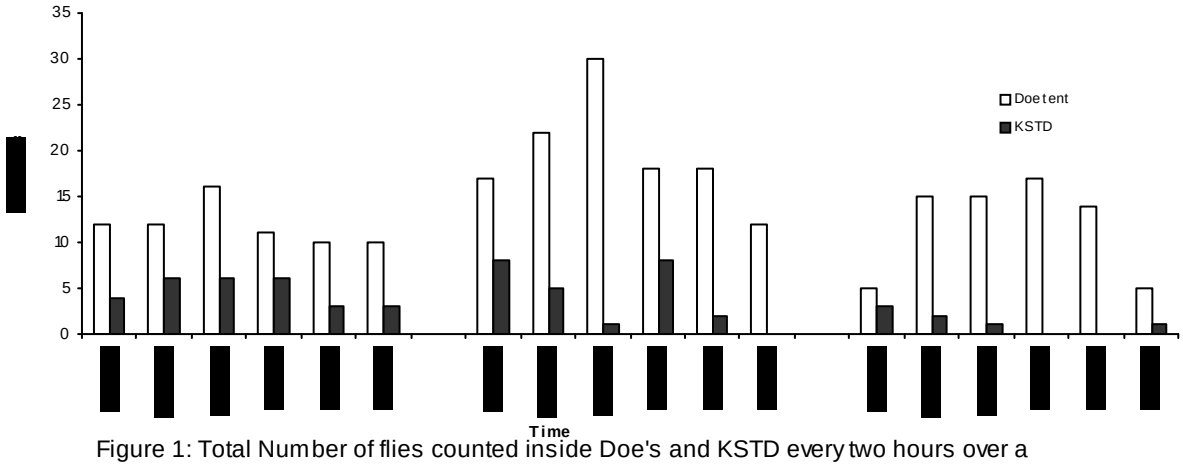

Figure 1: Total Number of flies counted inside Doe's and KSTD every two hours over a 3-day period in August 1999

Table 2 shows the mean temperatures inside Kainji Solar tent dryer (KSTD) and Doe's dryer and outside within the trial period. Temperatures for dry season (March) and rainy season (August) were recorded respectively. Results show that temperature in KSTD was 49°C and 38°C for March and August 1999 and 47°C and 38.8°C for March and August 2000 while 43.9°C 34.4°C, 42.8°C and 35.1°C was recorded for Doe's tent for the same months and years respectively. Similarly, temperature recorded for the ambient was 37.7°C, and 38°C and 31.3°C for the same months and years respectively.

Table 2. Mean temperatures inside and outside solar dryers within trial periods

	March 1999	August 1999	March 2000	August 2000
Kainji Solar Tent	49.8°C	38.5°C	47.3°C	38.8°C
Doe Tent	43.9°C	34.4°C	42.8°C	35.1°C
Outside	37.7°C	30.5°C	38°C	31.3°C

Table 2 shows that KSTD had high temperatures than both Doe and the ambient conditions. Analysis of the data shows that KSTD had temperatures significantly different at 5% significant level from those of Doe. The relative humidity taken at intervals of 2 hours during the trials of March and August 1999 and 2000 are shown in Table 3. The lowest readings were obtained in KSTD followed by Doe tent and outside conditions. March and August have contracting humidity conditions in the study area because of the influence of rain in August and complete absence of rain in March, coupled with the prevalence of harmattan winds. The lowest humidity of 10.5% was obtained inside KSTD dryer in March 2000.

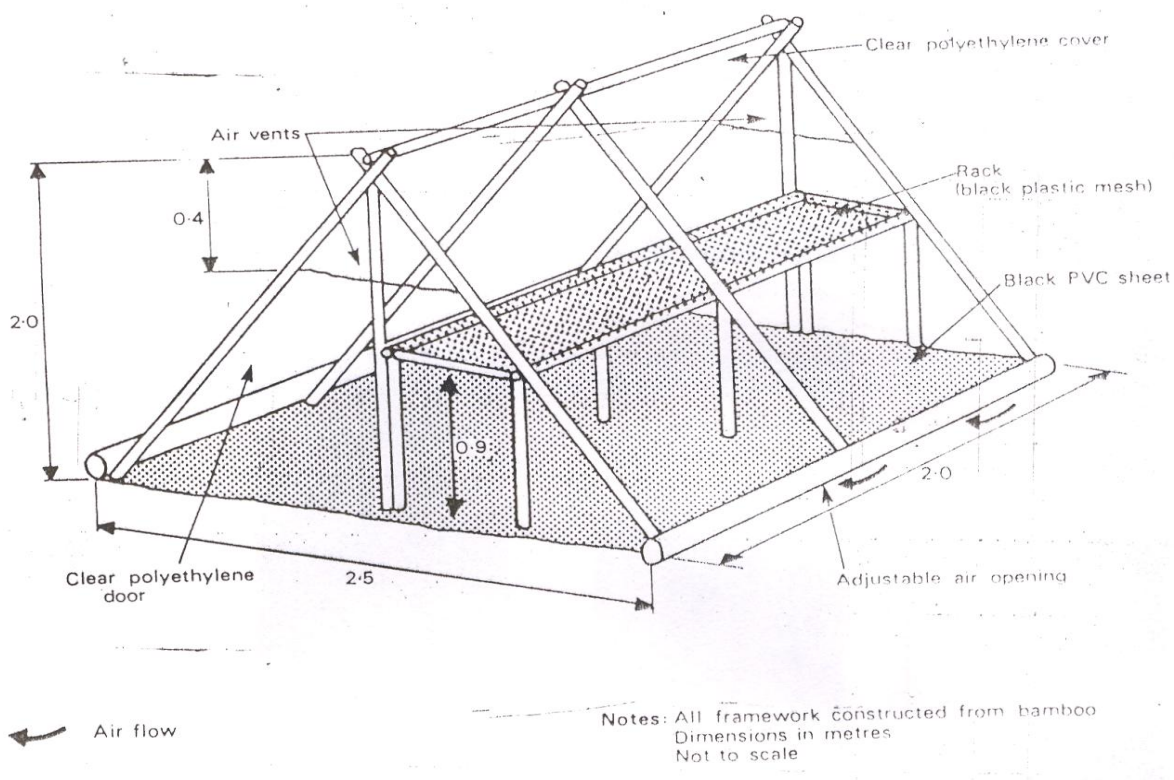

Figure 2. Schematic Diagram of the Framework of Doe's Tent

Ambient conditions were significantly high in August but relatively lower in March. This explains why artisanal fish processors engage in fish drying more at this time.

Table 3. Mean Relative Humidity over a 3-day period inside and outside solar dryers

	March 1999	August 1999	March 2000	August 2000
Kainji Solar Tent	13.5%	27.9%	10.5%	25.3%
Doe Tent	18.9%	38.9%	17.7%	35.8%
Outside	26.1%	72%	24.8%	70.8%

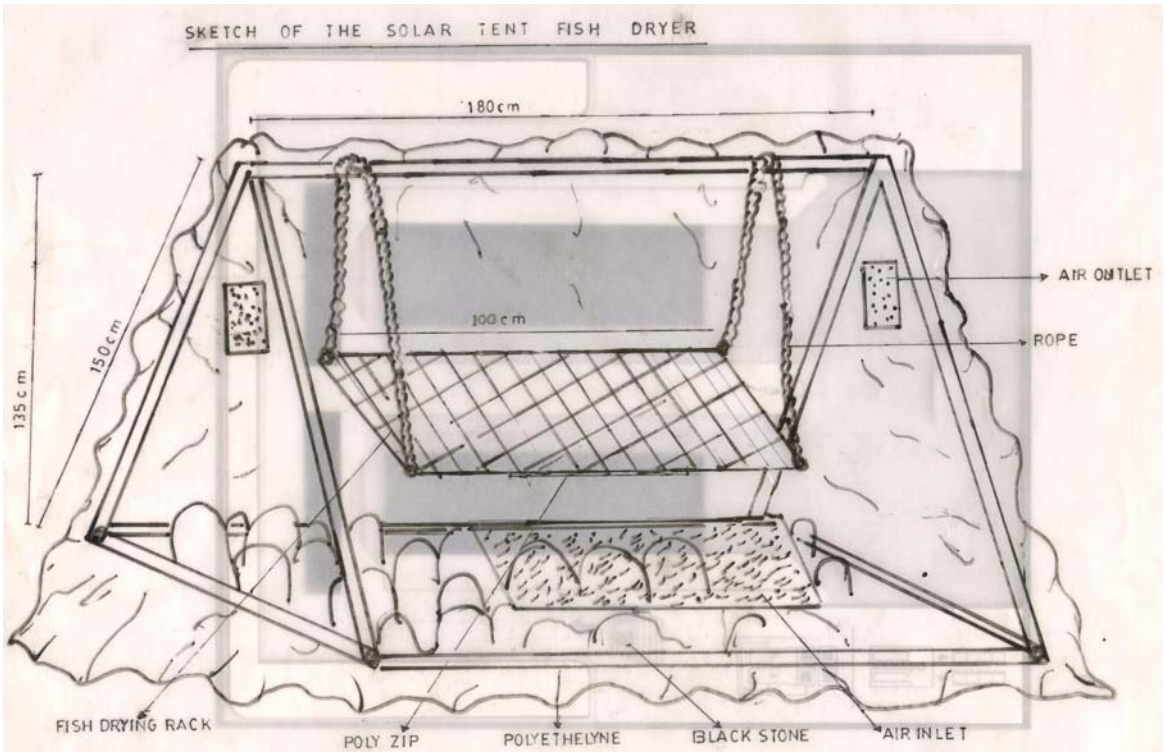

Figure 3. The Sketch of Kainji Solar Tent Dryer

The low incidence of flies in the KSTD was due to the fact that both the inlet and outlets of the polythene were screened which prevented flies from entering the dryer, unlike the Doe's design, which had no screens and flies were observed to move in and out freely. The implication of this is that the flies lay their eggs on the fish during the drying process (Okaeme, 1986) thereby facilitating spoilage.

The internal temperature in KSTD was higher and consistent than Doe's and the outside conditions because of the black igneous rocks, which absorbs and retain solar energy despite hourly fluctuation due to cloud cover prevalent at this time (Olokor, *et. al.*, 1997), while the Doe's design is more responsive to changes in ambient conditions. This result confirms that it is better to use black rocks as solar absorbers instead of black polythene sheet used in the Doe's design. The black rocks intensify the green house effect within the dryer through its capability to store solar energy (Charney, 1975).

The low humidity recorded in KSTD was because of its consistent high temperature of 49.8°C and 38°C for March and August 1999 (Barnett, 1988). Humidity is the strongest factor for fish drying either within or outside a Solar dryer (Ajisegiri, 2001). This is because it determines the rate and speed of drying. Thus, it can be seen that humidity varied throughout the day, generally higher in the mornings and evenings and lowest late afternoons when ambient temperatures is highest. As temperature rises, relative humidity decreases.

SUMMARY AND CONCLUSION

The study revealed that Kainji Solar Tent Dryer which was constructed as an improvement of Doe's tent and the two solar tent Dryers were used to dry fish. The fish used for the study was *Bagrus bayad* of high quality which was divided into two portions and each of the Solar Tent Dryer was used to dry each portion. One major characteristics of Kainji Solar tent dryer is the use of black igneous rocks that generate heat while in the Doe's tent, PVC black polythene was spread out on the base of the tent. After drying the indices for comparison were based on the performance of the two dryers, materials used for construction and conditions for fish drying such as temperature, relative humidity as well as the number of occurrences of flies in the tent. Results show that an average of 14 flies was counted in Doe tent while 3 flies were counted in Kainji Solar tent dryer in August 1999. Similarly, by March an average of 11 flies were seen in Doe's tent while in Kainji Solar tent dryer, an average of 1 fly was seen. Temperature recorded for both dry and rainy seasons were higher and consequently a lower humidity. Finally the study revealed that Kainji Solar Tent dryer was better in terms of structural make up, quality of the products and it dried faster than the Doe's tent. It is therefore recommended for artisanal fish processors in Kainji Lake and Lake Chad areas.

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Change Detection Analysis By Using Ikonos And Quick Bird Imageries

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Abstract The application of urban satellite using for monitoring of changes specially in rapidly growing metropolitan areas not only sensible but utterly necessary. Arguments in favour of the use of satellite system are certainly the fast and accurate data access, the quick visual interpretation, the good representation on a planar surface and their great integrity of a map after the process of geometrical classification . In this paper we used maximum likelihood classification algorithm to attempt and monitor the land cover change .In this work we considered a test area, the Chenggong city in Yunnan province in the south of China. AquickBirds multi-spectral images taken on May 4, 2004 and Ikonos multi-spectral images taken on April 7, 2002, were used in this work. The two images were orthorectified and a first classification produced a map with 7 strata: water, forest, pasture & grass land, cultivated land, transportation, built up areas and unused land. The over all classification accuracy was 97% and the kappa coefficient was 0.92 (i.e. 0.92 more accurate than a random classification).The overall accuracy of land cover change map ,generated from post classification change detection methods and evaluated using several approaches ,ranged from 80 % to 90%.The results of change detection between two dates images were as follows : transportation has increased from 7.6% to 18.3% with change rate of 57.75 $km^2.yr^{-1}$, pasture & grass land has decreased from 26.3% to 8.9% with change rate of 217.5 $km^2.yr^{-1}$, built up areas has increased from 6.7% to 22.3% with change rate of 156 $km^2.yr^{-1}$, cultivated land has increased from 15.3% to 32.4% with change rate of 128.25 $km^2.yr^{-1}$, forest has decreased from 38.8% to 18.2% with change rate of 309 $km^2.yr^{-1}$,un used land has decreased from 25.7% to 9.5% with change rate of 145.8 $km^2.yr^{-1}$,and water have no changed mentioned. The results quantify the land cover change patterns in the metropolitan or urban areas and demonstrate the potential of multi temporal Quick Birds and Ikonos data to provide an accurate, economic means to map and analyze changes in land cover over time that can be used as inputs to land management and policy decisions.

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Keywords: change detection, highmultispectral images, maximumlikelihood classification

1. Introduction

The importance of accurate and timely information describing the nature and extent of land resources and changes over time is increasing, especially in rapidly growing metropolitan areas. Change detection is a remote sensing techniques used to monitor and map land cover change between two or more periods and is now an essential tool in growing urban areas management activities[6] .Urban growth , particularly the movement of residential and commercial land use to rural areas, was, commonly referred to as urban sprawl.

Accurate and timely information on land use and land use change at a national scale is crucial for long term economic development planning and for short-term land management. Remote sensing technology as an efficient surface investigation method was introduced in China for such purpose three decades ago. In the end of1980s, CSLA sponsored the program to analyze land use status in Northwest China using Landsat TM imagery. Later in 1996, time series of Landsat TMdata were analyzed to monitor urban expansion in 17 metropolitan areas including Beijing. Many cases of misuse of cultivated land and illegal constructions were exposed through this investigation, which urged the China government to implement a strict protect policy for cultivated land. The technique to monitor land use transitions using remote sensing imagery was tested and improved in the following years. In 1999, the newly founded MLR launched the Program of National Land Use Change monitoring through remote sensing. The objective of the Program was to investigate the transition from cultivated land to construction land in66 metropolitan areas around China

using Landsat TM and SPOT imagery between 1998 and 1999. Since then, the Program has been carried out continuously for seven years and provides fundamental information on land use change at the national scale for Central Government policy making. The success of this Program demonstrates that remote sensing can act as an operational technology serving land management in China.

The very high ground resolution of Quick Birds and Ikonos data is a new step towards a detailed image of land cover, close to an aerial photograph but with the geometric quality, the homogeneity and periodicity proper to satellite imagery, and they provide a level of detail compatible with urban mapping, i.e. from 4 to 2.5 meters spatial resolution. In this research we used supervised classification which is based on comparison between the classifications maps obtain by classifying the two consider images independently.

2. Study area

The research in this paper addresses the Chenggong city in Yunnan province in the south of China, as shown in Fig .1 below. A Quick Bird images taken in May 4, 2004 and Ikonos images taken in April 7, 2002 for this area were used in this work, the area lies on longitude about 24 00 00 to 25 00 00N and latitude about 101 00 00 to 102 00 00 E. This area includes a diversity of land cover classes interspersed with large areas of cultivated and farm land. Both high and law density urban development are found in the central portion while several rural lands cover types of cultivated crops land ,pasture & grass land and forest characterize the surrounding landscape.

3. Materials and Methods

The spatial resolution of the satellite sensors can be characterized by the ability of defining the object boundaries [1and 8] .It is also possible to define the spatial resolution as, the area of a representative pixel on the ground.

Data and Pre-processing

Remote Sensing images used in this study include two satellite images. The first image was a Ikonos images taken in April 7, 2002. The second one was a Quick Bird images taken in May 4, 2004. The two satellite images characteristics and its centre wavelengths were shown in Table 1, below. The pre-processing of this dataset included geometric corrections .All images were geometrically corrected not only to eliminate geometric distortions present in the images but also to register the satellite images to ground data. Ground Control Points (GCPs) were extracted from vector files for the same region, using geographic features such as big and small rivers .The resampling method chosen was nearest-neighbor, which preserved original reflectance value. Fifty ground control points were chosen on the images, the points were spread quite evenly through out the image, allowing for good control. Image software allowed for easy zooming to assist in point selection. The points were registered in the header files of the image for later rectification .Once all ground control points were compiled, error checking was used to gauge the efficiency of the points used. The RMS errors for all a linear method of rectification were examined with varying accuracies, all approximately 0.5 m in displacement error. The nearest-neighbor resampling method was used in datum WGS 84 and projection UTM (49N). In order to remove or normalize the reflectance variation between images acquired at different times, relative radiometric correction was performed to yield normalize radiometric data on a common scale [9].Here, the histogram normalization, a simpler and more effective technique, was used to carry out the relative radiometric correction.For the analysis of Landsat satellite images, ERDAS Imagine 9.1software was used.

Table 1:Quick Birds and Ikonos characteristics

	Quick Birds	**Ikonos**
Panchromatic band	725.0 nm	727.5 nm
Band 1 (blue)	479.5 nm	480.5 nm
Band 2 (green)	546.5 nm	550.5 nm
Band 3 (red)	654.0 nm	665.0 nm
Band 4 (nir)	814.5 nm	805.0 nm
Resolution (pan)	0.61 m	1.0 m
Resolution (multispectral)	2.44 m	4.0 m

and its centre wavelength

4. Methodology

There are various ways of approaching the use

of satellite imagery for determining land use change in urban environments. [2] divide the methods for change detection and classification into pre-classification and post- classification techniques. The pre- classification technique apply various a algorithms , including image differencing and image rationing , to single or multiple spectral bands, vegetation indices ,principal components , directly to multiple dates of satellite imagery to generate “change” vs. “no- change” maps[7]. These techniques locate changes but do not provide information on the nature of change [10].On the other hand, the Maximum Likelihood classification (MLC) method was chosen to carry out this work .Maximum likelihood classification assumes that the statistics for each class in each band are normally distributed and calculates the probability that a given pixel belongs to a specific class. Unless a probability threshold is selected, all pixels are classified .Each pixel is assigned to the class that has the highest probability (i.e. the “maximum likelihood”). There are several studies in which this supervised classification method has been utilised successfully, either directly or in combination with other methods [3,4and5]. In this type of classification, the user selects the spectral signatures defined from recognized locations in the image or “training sample.” The computer system then identifies the pixels with similar characteristics and assigns them to a class based on specific criteria. For the initial training operation of the classification methods , (100) samples as learning data set of each class , and (1300) samples as “ground truth” for each class were defined with help of ortophotos (scale 1: 10.000) and available maps. These tools helped classify a seven-class legend: water, forest, pasture & grass land, cultivated land, transportation, built up areas and unused land ,as shown in Table 2 below ,which was based on the land cover land use classification system developed by National Land use Change Program. Supervised classification was then performed using the maximum likelihood method, in which a pixel with the maximum likelihood is classified to its corresponding class. The over all classification accuracy was 97% and the kappa coefficient was 0.92 (i.e. 0.92 more accurate than a random classification). The existence of mixed pixels (pixels having more than one class in their footprint), in particular among vegetation classes, would require an analysis at a higher geometric resolution or a comparison with multitemporal data to exploit the phonological selected, the maximum likelihood classification results for Quick Bird and Ikonos images were shown in Figure. 2 and figure. 3 respectively.

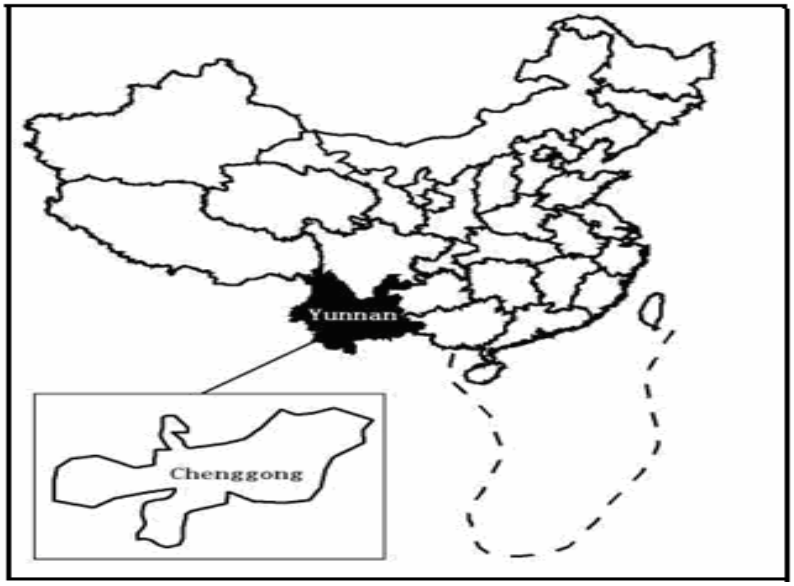

Figure1: The boundary map of China and Chenggong city

Table 2: Classes of training data

Land use class	**Description**
Cultivated land	including crop fields
Pasture & grass	including pasture ,natural and artificial grass ,planted and improved pasture land
Transportation	including railway ,high way ,air port ,port
Water body	including river , lake , reservoir , beach , canal , breeding plot
Unused land	including sandy land , desert ,saline land, bare land , glaciers , permanent snow
Built up area	including urban , rural ,residences ,industry, mining ,salt pan , specially used land
Forest	including forestry land , timber , fuel wood , shelter , economic forests , sparse wood lands and shrubs

Table 3: Change rate of the 7 landscape patterns from 2002 to 2004

Land cover type	**2002**		**2004**		**2002-2004**		**Change rate (+Gain,-Loss) 2002-2004**
	(km^2)	**(%)**	**(km^2)**	**(%)**	**(km^2)**	**(%)**	**($km^2.yr^{-1}$)**
Transportation	82.1	7.6	197.6	18.3	-115.5	-10.7	+57.75
Cultivated land	229.5	15.3	486.0	32.4	-256.5	-17.1	+128.25
Un used land	462.6	25.7	171.0	9.5	+291.6	16.2	-145.8
Built up areas	134.0	6.7	446.0	22.3	312.0	15.6	-156
Forest	1164.0	38.8	546.0	18.2	+618.0	20.6	-309
Water body	76.0	9.5	56.8	7.1	+19.2	2.4	-9.6
Pasture& grass	657.5	26.3	222.5	8.9	+435.0	17.4	-217.5

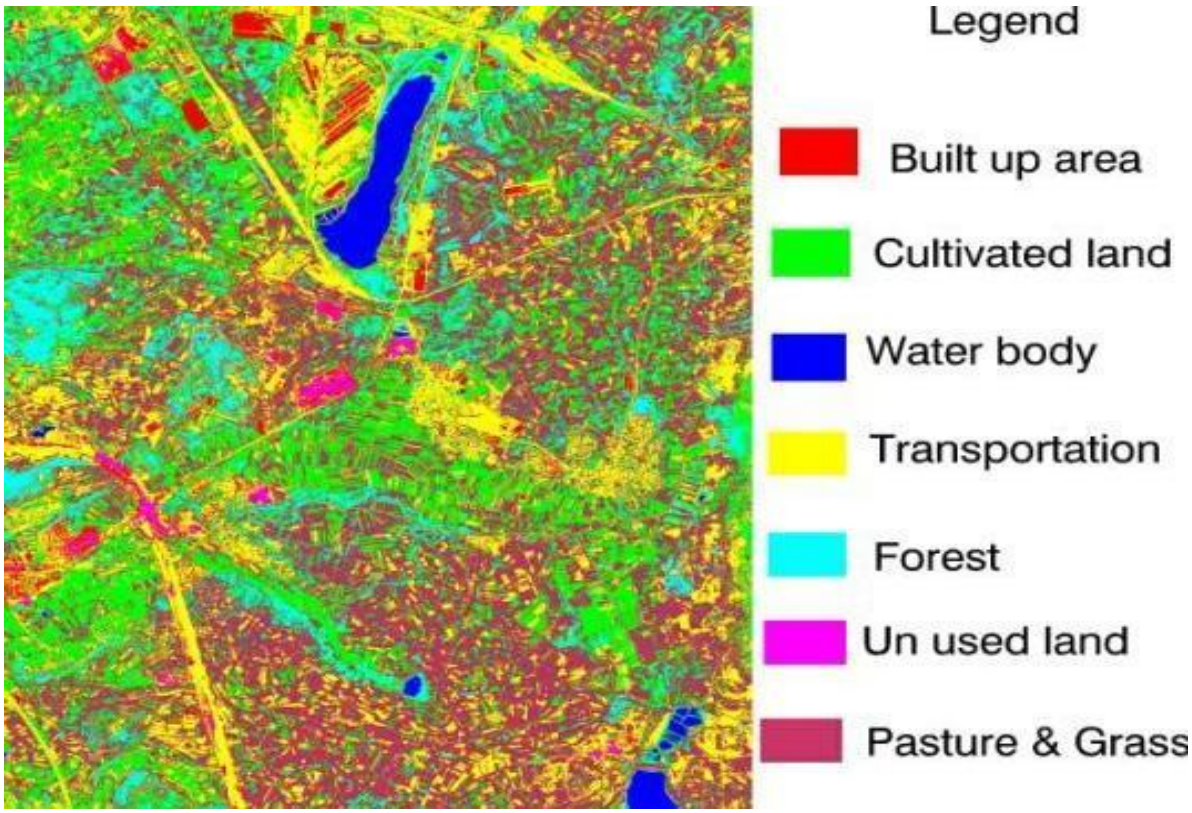

Figure 2: Maximum Likelihood classification for Quickbird image (2004).

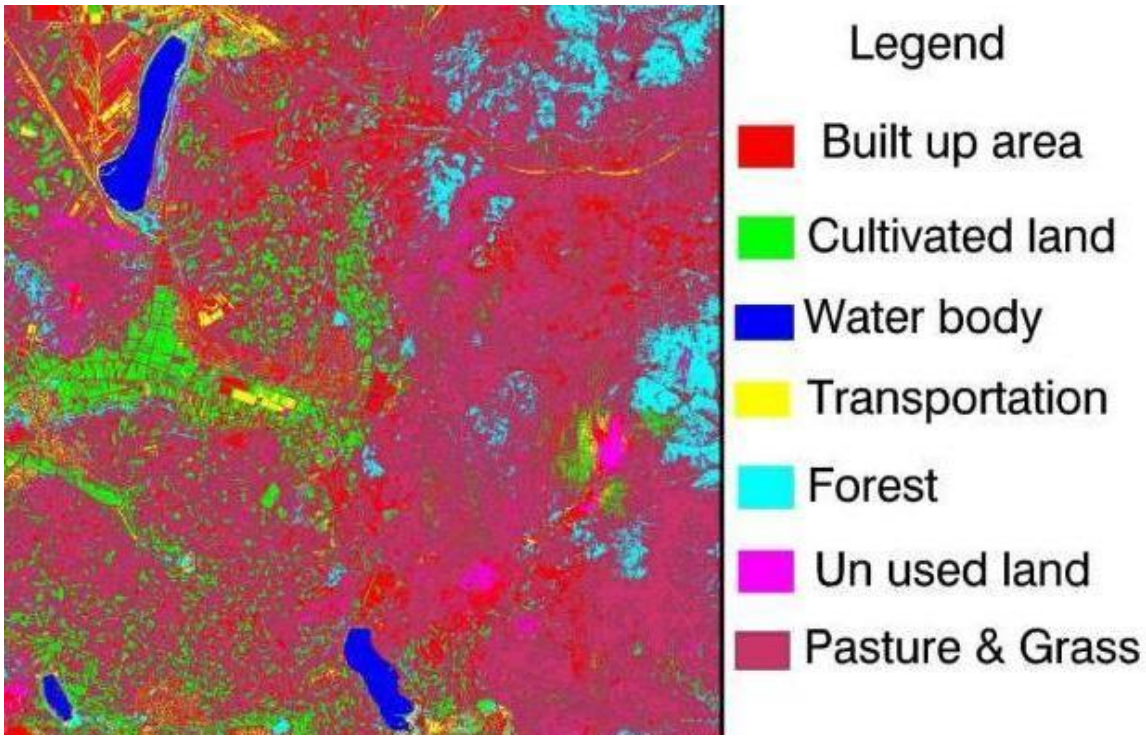

Figure 3: Maximum Likelihood classification for Ikonos image (2002).

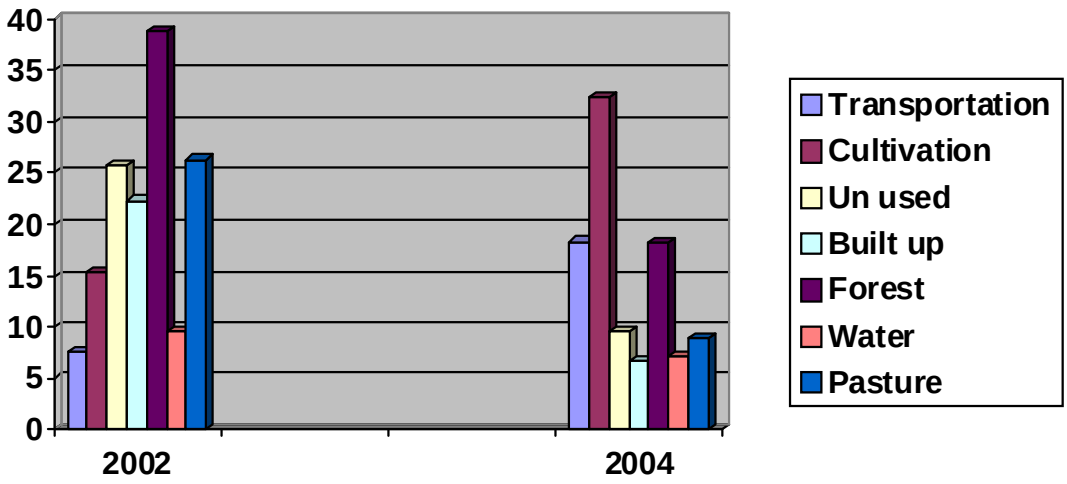

Figure 4: The percentage diagram of land covers change from 2002 to 2004

5. Result

Table 3and Figure 4 show the values of land cover change obtained after applying the supervised classification methods according to the "ground truth" samples.The results of change detection between two dates images were as follows : transportation has increased from 7.6% to 18.3% with change rate of 57.75 $km^2.yr^{-1}$, pasture & grass land has decreased from 26.3% to 8.9% with change rate of 217.5 $km^2.yr^{-1}$, built up areas has increased from 6.7% to 22.3% with change rate of 156 $km^2.yr^{-1}$, cultivated land has increased from 15.3% to 32.4% with change rate of 128.25 $km^2.yr^{-1}$, forest has decreased from 38.8% to 18.2% with change rate of 309 $km^2.yr^{-1}$,un used land has decreased from 25.7% to 9.5% with change rate of 145.8 $km^2.yr^{-1}$, and water has decreased from 9.5% to 7.1% with change rate of 9.6 $km^2.yr^{-1}$ during the study period.

6. Conclusion

In this work two very high resolution images were classified with the purpose to detect the land change. In this paper we found the big changed that occurred in the study area in this limited period of time, just two years, especially from pasture & grass land to cultivated land , built up area and transportation, so I would like to say that it must be diurnal researches for cities every year if it is possible because of high and accelerating rate of urban expanding, in particular in the developing countries like China

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Object-based land use/cover extraction from QuickBird image using Decision tree

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Abstract The traditional pixel-wise statistical and mono-scale based classification approaches do not lead to satisfactory results for neglecting the shape and context aspects of the image information, which are among the main clues for information extraction at very-high spatial resolutions like QuickBird image. This paper extracts land use/cover information from occurrence filters texture features that were derived from the grey-level occurrence matrix from QuickBird image using CART Decision tree, because, this method have substantial advantages for remote sensing classification problems due to their nonparametric nature, simplicity, robustness with respect to non-linear and noisy relations among input features and class labels, and their computational efficiency. CART has a simple form which can be compactly stored and that efficiently classifies new data ,also it can recursively partitions a data set into smaller subdivisions on the basis of tests applied to one or more features at each node of the tree. Overall accuracy of texture features using CART Decision tree is higher than other methods. It concluded that texture features can be used to improve classification accuracy. [Nature and Science. 2009;7(10):32-36]. (ISSN: 1545-0740).

Keywords object-based, land use / cover, classification, decision tree,QuickBird

1. Introduction

The automatic analysis of remotely sensed data has become an increasingly important topic over the last decades. Especially land use/cover and land change information is useful for city development. The segmentation of satellite images into regions of different land cover is of major interest: given data from several spectral bands, one wants to determine for each pixel of the image which type of land cover is present at the corresponding area on the surface(Keuchel et al., 2003, Carlson and Arthur, 2000, Le Hegarat-Mascle et al., 2005, Fan et al., 2007).In land cover classification of remote sensing data, it is desirable to use multisource data in order to extract as much information as possible about the area being classified.

However, classification of multisource remote sensing and geographic data is a challenging problem, especially since a convenient multivariate statistical model is in general not available for such data(Gislason et al., 2006). The traditional pixel-wise statistical and mono-scale based classification approaches do not lead to satisfactory results for high spatial resolution remote sensing data like QuickBird image.

The main drawback of these methods is that they neglect the shape and context aspects of the image information, which are among the main clues for information extraction at very-high spatial resolutions. The successful launch of very-high spatial resolution panchromatic and multi-spectral satellites renders the potential to carry out thematic mapping at large scales in urban areas.

Unfortunately, the high spatial resolution of these advanced sensors increases the spectral within field variability and, therefore, may decrease the classification accuracy results.

This is because most classification techniques are based on spectral homogeneities only (Cushnie, 1987), and do not take into account the textural attributes of the mapped image's features. Due to the more heterogeneous spectral-radiometric characteristics within the land-use/cover units portrayed in high resolution images, applications of traditional single resolution classification methods have led to unsatisfactory results. This paper extracts land use/cover information from texture features that were derived from the grey-level occurrence matrix using CART Decision tree.

2. Study area

The study area covers Chenggong districts in Yunnan province in southwest of China (fig. 1). The centre is latitude 24°55’43″N and longitude102°50’10″E. The remote sensing data

consisted of QuickBird multispectral and panchromatic images that were acquired simultaneously on 4 May, 2004. The QuickBird radiances were not atmospherically corrected as time series analysis of consecutive image data was not required for this study, and detailed information on the atmospheric conditions at the time of overpass was not available.

3. Methods

Within the last 10 years ,there has been increasing interest in the use of classification and regression tree (CART) analysis .CART analysis is a tree–building technique which is unlike traditional data analysis methods .Because CART analysis is unlike other analysis methods it has been accepted relatively slowly .Furthermore ,the vast majority of statisticians have little or no experience with the technique . Other factors which limit CART analysis general acceptability are the complexity of the analysis and, until recently, the software required to perform CART analysis was difficult to use. Luckily, it is now possible to perform a CART analysis without a deep understanding of each of the multiple steps being completed by the software .In a addition ,CART is often able to uncover complex interactions between predictors which may be difficult or impossible to uncover using traditional multivariate techniques.

CART analysis has a number of advantages over other classification methods, including multivariate logistic regression, first, it is inherently non-parametric .In other words ,no assumptions are made regarding the underlying distribution of values of the predictor variables. Thus, CART can handle numerical data that are highly skewed or multi-model , as well as categorical predictors with either ordinal structure(Quinlan, 1993) .This is an important feature ,as it eliminates analyst time which would otherwise be spent determining whether variables are normally distributed, and making transformation if they are not.

As discussed bellow ,CART identifies “splitting” variables based on an exhaustive search of all possibilities .Since efficient algorithms are used, CART is able to search all possible variables as splitters ,even in problems with many hundreds of possible predictors.
Finally, another advantage of CART analysis is that it is a relatively automatic” machine learning” method .In other words, compare to the complexity of the analysis, relatively little input is required from the analyst .This is in marked contrast to other multivariate modeling methods, in which extensive input from the analyst, analysis of interim result, and subsequent modification of the method are required.

Despite its many advantages, there are a number of disadvantages of CART which should be kept in mind .First, CART analysis is relatively new and somewhat unknown. Thus, there may be some resistance to accept CART analysis by traditional statisticians .In addition, there is some well-founded skepticism regarding tree methodologies in general, based on unrealistic claims and poor performance of earlier techniques.Thus,some statisticians have a generalized distrust of this approach. Because of its relative novelty, it is difficult to find statisticians with significant expertise in CART.Thus, it may be difficult to find someone to help you use CART analysis at your own institution .Because CART is not a standard analysis technique ,it is not included in many major statistical software packages(e.g., SAS).

This paper extracts land use/cover information using texture features that were derived from the grey-level occurrence matrix. Occurrence Measures can output five different texture filters. The occurrence filters available are data range, mean, variance, entropy, and skewness. Occurrence measures use the number of occurrences of each gray level within the processing window for the texture calculations. In this paper, 3×3, 5×5, 7×7, 9×9, 11×11 processing windows size were selected. In every processing window, all 4 bands can render 20 layers gray level images (one band has 5 layers). Adding the original 4 bands, Total 104 image layers were used in classification.

In this paper, CART (Classification and Regression Tree) algorithm was used. CART was suggested by Breiman et al. in 1984 (Brieman et al., 1984). The decision trees produced by CART are strictly binary, containing exactly two branches for each decision node. It recursively partitions the records in the training data set into subsets of records with similar values for the target (Steinberg et al., 1997, ManojKumar et al., 2002, Bittencourt et al., 2003). CART is able to search all possible variables as splitters, and it is inherently non-parametric ,the non-parametric property means that non-normal,non-homogenous and noisy data sets can be handled, s well as non-linear reations between features and classes.missing values and both numeric and categorical inputs(Friedl et. al., 1997). CART trees are

relatively simple for nonstatisticians to interpret. Another advantage of CART analysis is that it is a relatively automatic "machine learning". It analysis has a number of advantages over other classification methods. In this paper, inputting all 104 layers into CART algorithm, the final decision tree is shown in fig. 2.

4. Results and disscusion

The classification map constructed by CART Decision tree is shown in fig. 3. In order to verify classification accuracy, the result classified by different classification methods and data were compared. Overall accuracy of original bands using Maximum likelihood, texture features using Maximum likelihood, original bands using CART Decision tree and texture features using CART Decision tree are 93.5%, 97.3%, 92.6% and 98.5% respectively. Furthermore, the CART algorithm is more transparent compared to the other algorithm, because in the former the classification sequence that is followed is controlled by the analyst. Classification and Regression Tree (CART) analysis is a powerful technique with significant potential classification utility.Nontheless,a substantial investment in time and effort is required to use the software, select the correct options, and interpret the result.Nontheless, the use of CART has been increasing and is likely to increase in the future, largely because of the substantial number of important problems for which it is the best available solution. From the Decision tree (fig. 2), some main results can be concluded:

1) Overall accuracy of texture features that were derived from the grey-level occurrence matrix is higher than the original data. Texture features can be used to improve classification accuracy.

2) Among all occurrence filters included data range, mean, variance, entropy, and skewness, mean is more effective in classification than others.

3) Different processing windows size can enhance different land use/cover information. Band1 when processing windows size is 9×9 or 11×11 can distinguish different land use/cover type.

4) Due to low spatial resolution or other reasons, some band like band4 is not suitable for occurrence filters.

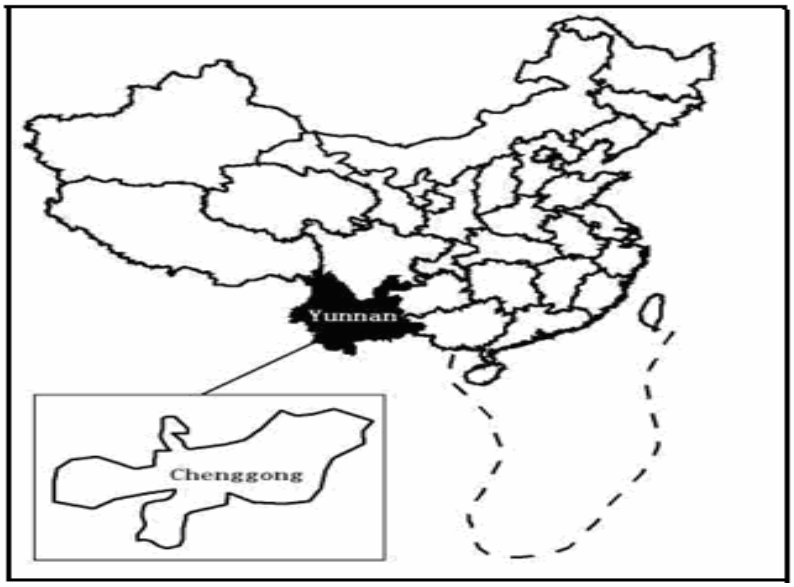

Figure1: The boundary map of China and Chenggong city

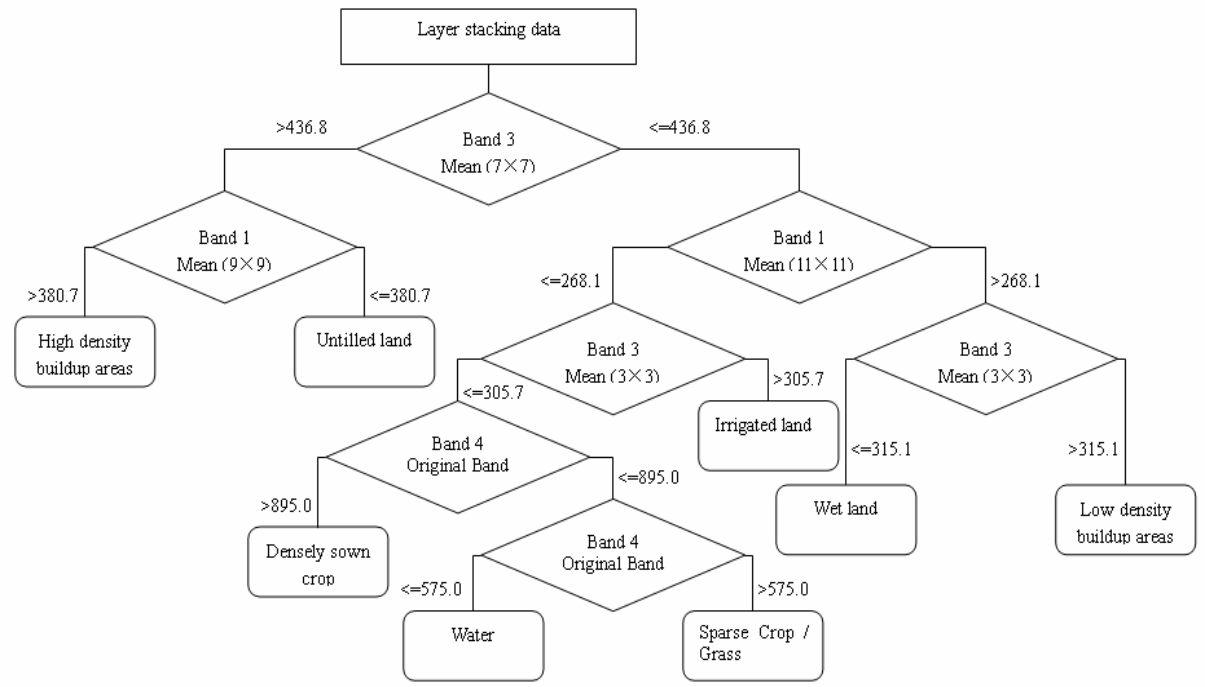

Figure 2: The decision tree constructed by CART algorithm.

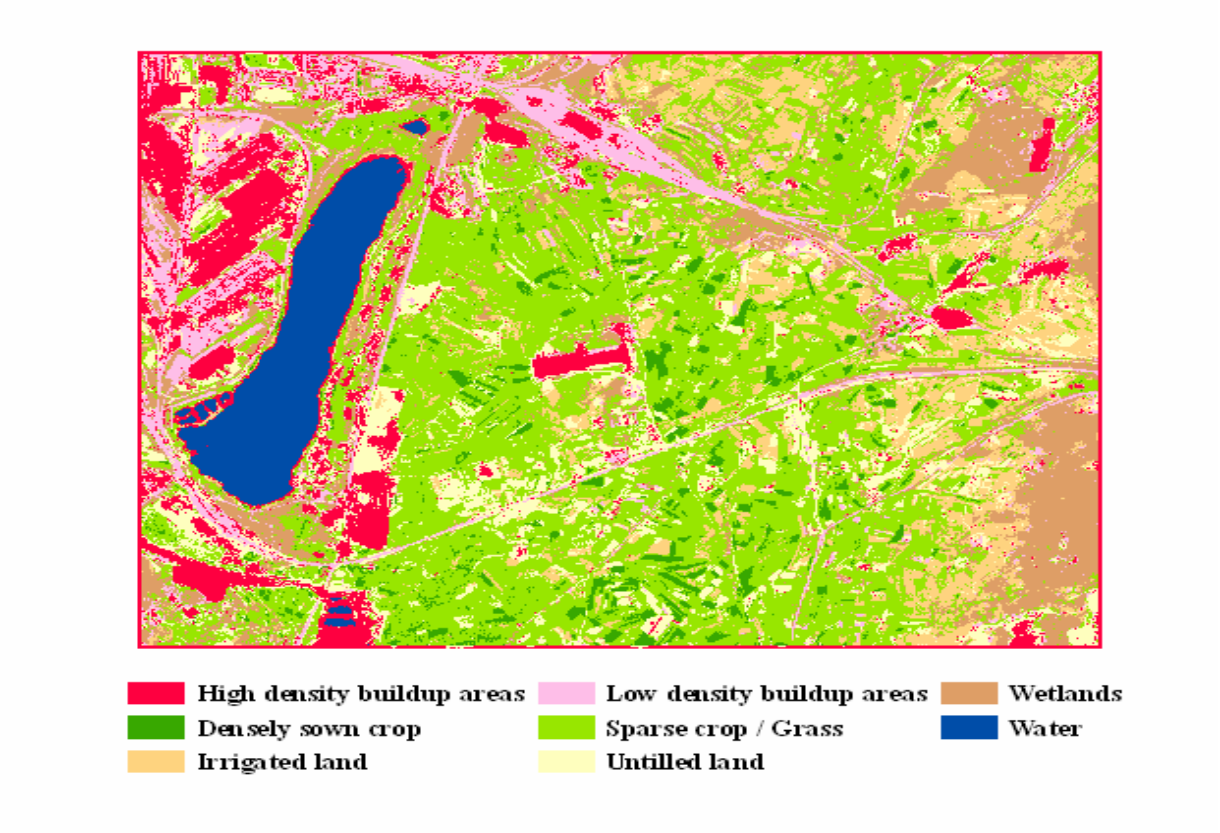

Figure 3**:** the classification map using CART decision tree.

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Protective Effect of Taurine and Bismuth Subnitrate Against Cyclosporine and NSAID Induced Neurotoxicity in rats: a study of drug interaction.

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ABSTRACT

Coadminisration of an immunosuppressive cyclosporine A (CSA) and nonesteriodal antiinfilammatory drug (NSAID), sodium diclofenac (SD) increases the efficacy for relief pain for patients with rheumatoid arthritis. However, clinical studies showed enhancement of cyclosporine toxicity upon this combination. Neurotoxicity is one of the most significant side effects of CSA toxicity. To characterized biochemical parameters of neurotoxicity, the study was assessed the effect of CSA (10mg/kg) alone or in combination with SD (10 mg/kg) for 6weeks on energy metabolism (ATP), glucose metabolism, oxidative stress { MDA (end product of lipid peroxides, nitric oxides (NO, as total nitrate), reduced glutathione (GSH) lactic dehydrogenase enzyme LDH}, and neuroamines {dopamine (DA), noradrenalin (NA) and serotonin (5-HT)} in different brain areas (cerebral cortex, cerebellum, Striatum, pons, and thalamus & hypothalamus) of adult albino rats. CSA alone inhibited energy production (ATP and glucose metabolism), alternated oxidative stress through increasing levels of MDA and LDH, and decreasing levels of GSH and NO in blood and tested brain areas tissues as well as it inhibited neurotransmitters releases. When SD combined with CSA, it enhanced CSA-induced inhibition of mitochondrial energy, inhibition of neuroamines release and increase oxidative stress alternations. The study also extended to evaluate and compare the protective effect of taurine (a major intracellular free ß-amino acid and potent endogenous antioxidant) with **B**ithmus subnitrate (BSN), an antiulcer drug and a specific inducer of metalothionine (MT) in infected tissues, against neurotoxicity induced by concurrent administration of CSA and SD. BSN co-administration could somewhat reduced CSA-induced neurotoxicity only through ameliorated oxidative stress state by showing significant increase in the level of GSH, and significant reduction in the level of MDA and LDH activity. Whereas the co-administration of the potent cytoprotective antioxidant, taurine, antagonized all CSA negative effects, by ameliorating CSA-induced mitochondrial dysfunction, improvement oxidative stress and modulated neurotransmitters. [Nature and Science. 2009;7(10):37-48]. (ISSN: 1545-0740).

Key words; cyclosporine A, NSAID, sodium diclofenac, neurotoxicity, taurine, **B**ithmus subnitrate, oxidative stress, neurotransmitters, energy metabolism (ATP).

INTRODUCTION

CSA is an immunosuppressive undecapeptide drug, which is most frequently used in transplant surgery and in the treatment of autoimmune disease (Allison, 2000). The adverse effects of CSA include acute and chronic nephrotoxicity, neurotoxicity, hypertension, and new-onset diabetes (Kozlowska et al, 2006). Cyclosporine is a calcineurin inhibitor that inhibit nuclear factor of activated T cells (NFAT) results in inhibition of interleukin 2 production in the T cells. The most limiting side effects of calcineurin inhibitors is nephrotoxicity (Dunn et al, 2001) followed by neurotoxicity (Wijdreks, 2001). Even though nephrotoxicity remain the biggest problem with CSA treatment, CSA dependent neurotoxicity occurs up to 60% of organ transplant patients and also can lead to serious life-threatening condition and to withdrawal of CSA from patient's regimen (Helderman et al, 2003). CSA-induced neurotoxicity was reported in stem cell transplant recipient (Raza et al, 2007), in hematopoeitic malignancies after allogenic bone marrow transplantation (Bartynski et al, 2005). Brain is the organ with the highest demand for oxygen and oxidative energy production. The central intermediate substance of oxidative metabolism is acetyl–coenzyme A (acetyl-CoA), which can originate from carbohydrates, fatty acids, or amino acids. Brain, in contrast to other organs, utilizes only glucose to produce ATP and lacks the enzyme for ß-oxidation of fatty acids. (Serkova et al, 2004). Mitochondrial encephalopathies (ME) are a heterogeneous group of metabolic diseases characterized by mitochondrial malfunction that leads to cellular

energy failure and cells damage (Serkova et al, 2004).

Although CSA has been shown in a series of controlled trails to be of benefit, patients continue to require NSAID, as SD, for relief of joint pain and stiffness (Rossi, 2006). Sodium diclofenac (SD) is a non steroidal anti-inflammatory drugs (NSAID), taken to reduce inflammation and an analgesic reducing pain in condition such as in arthritis or acute injury (Rossi, 2006).The adverse effects of SD include, gastrointestinal complaints, liver damages, acute and chronic nephrotoxicity, heart attack, bone marrow depression (Brater, 2002; Rossi 2006 and Solomon et al, 2006)), and develop neurological side effects such as; confusion, depression, dizziness, headache, sedation, sleep disturbance, somnolence (Slagle, 2001). In experimental animals, SD was found to decrease neuron number of the rat hippocampus (Gokcimen et al, 2007). Long term treatment of NSAID leaded to ulceration and gastrointestinal bleeding. Hence most patients must receive ulcer-protection drugs as bithmus subnitrate (BSN) during long term treatment with SD. Many studies showed that BSN can protect tissues against toxicity by inducing metalothionine in these tissues (Kondo et al, 2004).

Taurine is sulfur containing ß-amino acid; it was found to play an important role in the field of cytoprotective through its antioxidant effect (Erdem et al, 2000). Taurine was found to have a modulated action against neurotoxicity (Louzada, 2004).

Based on these observations, the study aimed to study the positive or negative effects upon drug interaction for rheumatoid arthritis patients receiving CSA, SD. and BSN upon neurotoxicity induced by CSA. Also the study extended to through light on the possible ameliorative effect of the potent cytoprotective antioxidant taurine, against neurotoxicity induced by concurrent administration of CSA and SD.

MATERIALS AND METHODS

Animals

A total number of 56 female albino rats weighing 120±20g B.wt were used. The animals were brought from laboratory animal breeding of National Organization of Drug Control and Research (NODCAR), Giza, Egypt. They were kept under strictly hygienic conditions. They were put on a standard basal diet and allowed free access to drinking water.

Materials

-Bismuth subnitrate, taurine were purchased from Sigma Co. USA.

-Cyclosporine A and Sodium Diclofinac were purchased from Egyptian market pharmacy.

Drug doses were freshly prepared before administration dissolved in water and given orally.

Experimental design

Rats were classified into 7 equal groups each comprises 8 rats and treated for 6 weeks, as follow:

G1; -ve control group (CN), fed on basal diet daily.

G2; Taurine control group (T), orally administrated 500 mg/kg of taurine daily.

G3; BSN control group (B), orally administrated daily 15 mg/kg of BSN.

G4; Cyclosporine group (CSA), orally administrated 10 mg/kg of CSA daily.

G5; Combined treated group (CSA+SD), orally administrated 10 mg/kg of CSA plus 10 mg/kg of diclofenac daily.

G6; Taurine treated group (T+C), treated as in G5 and supplemented with 500 mg/kg of taurine.

G7; Bismuth treated group (B+C), treated as in G5 and supplemented with 15 mg/kg of BSN dissolved in citrate solution.

At the end of the treatment schedule, blood samples were taken from each rat and then they were sacrificed, brain tissue was removed some subjected to histopathological examinations as described by Bancrofet et al. (1996), the others were homogenated in 4 different areas (cerebral cortex, cerebellum, pons, and thalamus & hypothalamus) in iced 10% KOH. Separated serum and supernatant of homogenated tissues were processed for the biochemical analysis; ATP were determined by the method of (Zhang et al, 2000), blood glucose by (Trinder 1969), oxidative stress (MDA, NO, as total nitrate and GSH were determined by HPLC methods of Karatepe (2004); Everett et al, (1995); Jayatilleke & Shaw (1993) respectively. LDH, were determined by the commercial kits of (Buhl and Jackson, 1978).

Neuroamines; DA, NA and 5-HT were measured by HPLC chromatography according to the methods of Pagel et al, (2000).

Statistical analysis were done using SPSS ANOVA test, version 11.5.

RESULTS AND DISCUSSION

Neurotoxicity is one of the most significant clinical side effects of the immunosuppressive undecapeptide cyclosporine CSA, occurring at some degree in up to 60% of transplant patients. The clinical mechanisms of CSA-induced neurotoxicity remain controversial and poorly understood. It was found that the clinical symptoms of CSA neurotoxicity mimic those of mitochondrial encephalopathy (ME) (Beal, 1998; Serkova et al, 2004)). ME are a heterogeneous group of metabolic diseases characterized by mitochondrial malfunction that leads to cellular energy failure with increased lactate production through increasing activity of LDH release (Serkova et al, 2004).

Table (1): The effect of taurine and BSN against CSA and SD on the levels of ATP (nmol/gm) in different brain areas after 6 weeks of treatment.

Groups (n=8)	Cerebral cortex	Cerebellum	Striatum	Pons	Thalamus& hypothalamus
CN	1.494 ± 0.047	1.460 ± 0.058	1.391± 0.0428	1.354 ± 0.027	1.250 ± 0.047
T	1.515 ± 0.073	1.467 ± 0.063	1.414± 0.0651	1.346 ± 0.040	1.247 ± 0.044
B	1.482 ± 0.052	1.499 ± 0.073	1.380 ± 0.047	1.349 ± 0.031	1.269 ± 0.070
CSA	1.316 ± 0.031*	1.392 ± 0.066	1.278 ± 0.039*	1.310 ± 0.044	1.135 ± 0.031*
CSA+SD	1.233 ± 0.032*,a	1.417 ± 0.034	1.158± 0.044*,a	1.266 ± 0.017*	0.934 ± 0.045*,a
T+C	1.379 ± 0.038*,b,c	1.426 ± 0.035	1.348 ± 0.033b,c	1.350 ± 0.029,b	1.166 ± 0.034b,c
B+C	1.231 ± 0.051*,a	1.428 ± 0.053	1.190 ± 0.063*	1.335 ± 0.022	1.00 ± 0..056

Confirming with the recent study of Leu et al, (2008) who demonstrated sever CSA neurotoxicity including chondoriod encephalopathy, seizures, paralysis, coma, and cerebella ataxia. The brain, in contrast to the other organ utilizes only glucose to produce ATP and lack the enzyme for B-oxidation of fatty acids, Metabolism of one molecule of glucose produces thirty-six molecules ATP: Two ATP through cytosolic glycolysis, two ATP via the mitochondrial Krebs cycle, and thirty-two molecules through mitochondrial oxidative phosphorylation. It should be obvious that disturbances in mitochondrial glucose metabolism would lead to a considerable decrease in energy production, which, in the brain, cannot be compensated by ß-oxidation of fatty acids (Serkova et al, 2004). These later observations were in good keeping with the present study that showed treatment rats with CSA (10 mg/kg/day for 6 weeks), exhibit a significant ($P<0.05$) decrease in ATP concentrations (Table1), accompanied by significant increase ($P<0.05$) in the levels of blood glucose concentrations (Table & Figure 3).

Table (2.I): The effect of taurine and BSN against CSA and SD on the levels of MDA (nmol/gm) in different brain areas after 6 weeks of treatment.

Groups	Cerebral cortex	Cerebellum	Striatum	Pons	Thalamus& Hypothalamus
CN	39.43 ± 0.92	40.87 ±1.12	41.18 ±11.14	51.60 ±0.97	44.18 ±0.97
T	37.21 ± 1.11	39.43 ± 0.37	40.22 ± 13.01	52.90 ± 0.90	42.19 ± 1.12
B	38.80 ± 0.89	38.60 ± 1.00	42.17 ± 1.06	49.09 ±1.27	42.50 ± 0.72
CSA	39.37 ± 0.63	50.04 ± 0.82*	61.49 ± 1.13*	63.09 ± 0.37*	43.01 ± 1.06
CSA+SD	47.27 ± 1.09*,a	48.95 ± 0.76*	59.58± 0.72*	63.01 ± 0.92*	45.39 ± 0.67
T+C	30.68 ± 0.97b,c	35.11 ± 1.09b,c	41.08 ± 0.86b,c	52.94 ± 1.01b	42.39 ± 0.80
B+C	40.58 ± 1.15b	42.40 ± 1.23b	47.60 ± 1.10b	54.91 ± 1.27b	43.84 ± 0.68

- Sign. difference *vs.* CN: *P<0.05. -Sign. difference *vs.* CSA+SD: ^{b}P<0.05.
- Sign. difference bet. T+C & B+C: P<0.05. - Sign. difference *vs.* CSA: ^{a}P<0.05.

This interpreted the finding of earlier investigators who demonstrated that the cerebral energy metabolism is the most sensitive indicator of CSA neurotoxicity in vitro, even 100 ng/ml CSA added to incubation medium or perfusate reduces high-energy phosphate (ATP) concentrations by 20% (Serkova et al, 1999). Inhibition of mitochondrial glucose metabolism (the Krebs cycle and oxidative phosphorylation) was accompanied by increased reactive oxygen species (ROS) production (Christian, 2004) that it considered to be another responsible factor for CSA-induced neurotoxicty. The same pattern of metabolic changes was found in the present study, that showed significant increasing (P<0.05) in the level of serum and brain MDA (end product of lipid peroxides), accompanied by significant decrease (P<0.05) in the level of non enzymatic antioxidant reduced glutathione (Table2$_{I, II}$ & 3, *vs.* non treated control)

Table (2.II): The effect of taurine and BSN against CSA and SD on the levels of GSH (umol/gm) in different brain areas after 6 weeks of treatment.

Groups (n=8)	Cerebral cortex	Cerebellum	Striatum	Pons	Thalamus& Hypothalamus
CN	3.94 ± 0.09	4.07 ± 0.06	3.82 ± 0.07	4.60 ± 0.07	4.84 ±0.094
T	3.97 ± 1.11	4.14 ± 0.37	4.01 ± 0.14	4.59 ± 0.09	4.90 ± 0.11
B	3.88 ± 0.089	3.86 ± 0.14	3.89 ± 0.06	4.79 ± 1.27	4.69 ± 0.072
CSA	3.67 ± 0.053*	3.21 ± 0.08*	3.04 ± 0.09*	3.09 ± 0.17*	4.41 ± 0.06*
CSA+SD	3.07 ± 0.09*,a	3.12 ± 0.07*	2.88 ± 0.22*	3.01 ± 0.09*	4.33 ± 0.07*
T+C	3.68 ± .07*,b,c	3.59 ± 0.09*,b	4.08 ± 0.086b,c	3.94 ± 0.06*,b,c	4.79 ± 0.098b
B+C	3.41 ± 0.05*,b	3.43 ± 0.11*,b	3.55 ± 0.099*,b	3.45 ± 0.07*,b	4.58 ± 0.086b

Table (2.III): The effect of taurine and BSN against CSA and SD on the levels of NO as total nitrate (nmol/gm) in different brain areas after 6 weeks of treatment.

Groups (n=8)	Cerebral cortex	Cerebellum	Striatum	Pons	Thalamus& hypothalamus
CN	51.75 ± 1.66	45.50 ± 1.02	62.11 ± 1.22	55.41 ± 2.11	43.45± 0.77
T	47.89 ± 1.93	45.87± 1.04	65.22 ± 1.91	57.85 ± 1.98	41.74 ± 0.79
B	53.74 ± 2.20	44.78 ±1.11	61.52 ± 2.12	59.00 ± 2.22	45.98 ± 0.020
CSA	43.14 ± 1.64*	36.37 ± 1.41*	51.11 ± 0.99*	50.87 ± 1.14*	36.74 ± 1.01*
CSA+SD	32.04 ± 1.79*,a	34.99 ± 1.41*	49.74± 0.88*	46.46 ± .11*,a	37.00 ± 0.87*
T+C	41.88 ± .12*,b,c	42.18 ± 1.71b,c	56.15 ± 1.94b,c	53.22 ± 0.95b	44.41 ±1.21b
B+C	34.11± 1.15*,a	37.01 ± 1.54*	47.33 ± 1.01*	48.73 ± 1.17*	39.69 ± 1.94

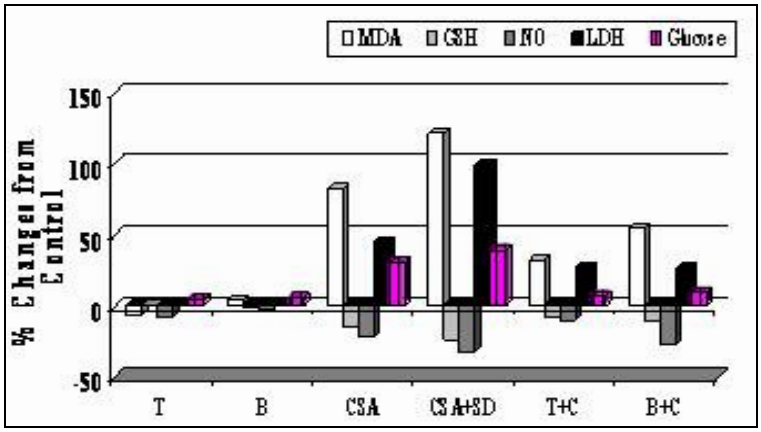
-Sign. difference *vs*. CN: *P<0.05.
- Sign. difference *vs*. T+C & B+C: ^{c}P<0.05.
- Sign. difference *vs*. CSA+SD: ^{b}P<0.05.
- Sign. difference *vs*. CSA: ^{a}P<0.05

Treatment with CSA has been shown to increase O^{-}_2, H_2O_2 and OH^{-} radicals production (Hagar, 2004). The main detoxifying system for lipid peroxides is GSH. The decrease in GSH following CSA observed in this study greatly supported the role of ROS in CSA neurotoxicity. Cyclosporine is a calcineurin inhibitor, the most limiting side effects of calcineurin inhibitors is inhibition of NO production, through a calcinurin-regulating eNOS dephosphorylation (Kou et al, 2002)., which lead to vasoconstriction (Gitanbeek et al, 1999). So it was postulated that CSA can induce neurotoxicity indirectly through its vasoconstriction (Serkova et al, 2004). Another side effect of calcineurin inhibitors is increased activity of LDH and lactate accumulation (Higginson et al, 2002).

Table & Figure (3): The effect of taurine and BSN against CSA and SD on the levels of on the levels of serum MDA (umol/ml), GSH (nmol/dL), NO (nmol/ml), LDH (umol/ml) and glucose (mg/dL) after 6 weeks of treatment.

Groups (n=8)	MDA (umol/ml)	GSH (nmol/dL)	Nitric oxide (NO2&NO3) (nmol/ml)	LDH (umol/ml)	Glucose (mg/dl)
CN	0.73 ± 0.0142	85.50 ± 3.42	32.11 ±1.52	168.25 ±1.54	87.33 ± 2.87
T	0.68 ± 0.018	85.87± 2.04	29.82 ±1.41	170.88 ±1.69	91.22 ± 1.98
B	0.76 ± 0.04	84.18 ± 4.11	31.52 ±1.12	171.33± 1.79	92.11 ± 2.72
CSA	1.33 ± 0.0 3*	73.87 ± 4.41*	25.21 ±1.99*	243.88 ± 1.87*	114.13 ± 3.08*
CSA+SD	1.62 ± 0.047*,a	64.99 ± 2.41*,a	21.87±1.18*	335.88 ± 0.047*,a	121.78 ± .11*,a
T+C	0.97 ± 0.049b,c	79.18 ± 2.77b	29.15 ± 1.44b	214.13 ± 1.76*,b	93.78 ± 3.04b
B+C	1.13 ± 0.057b	77.01 ± 4.54b	23.33 ±1.81	212.13± 1.56*,b	95.56 ± 3.17b

- Sign. difference *vs.* CN: *P<0.05. **- Sign. difference *vs.* CSA+SD: ^{b}P<0.05.**
- Sign. difference bet. T+C & B+C: ^{c}P<0.05. **- Sign. difference *vs.* CSA: ^{a}P<0.05.**



These results are in agreement with the data of the present study that revealed a significant decrease in the level NO as well as increase in activity of LDH release (Table3, P<0.05 *vs.* to untreated control group). In agreement with the earlier studies of Serkova et al, (2001) who found a decrease in ATP concentrations accompanied by increased lactate concentration in blood and rat brain after six day of CSA treatment. Another study in the skeletal muscle cells showed increased activity of LDH after CSA treatment, which was related to calcineurin inhibition by CSA (Higginson et al, 2002). The data depicted in Table ($4_{I, II, III}$) showed significant inhibition (P<0.05) of the neurotransmitters (AD, DP & serotonin) generation. In agreement, it was found that CSA-induced inhibition of energy production was accompanied by reduction of glutamate and γ-amino butyric acid (GABA), neurotransmitters generated from Krebs cycle which identified by MRS in rat brain extract (Serkova et al 2001). This result suggests that CSA induced neurotoxicity through its exictotoxic damage of neuron by inhibiting GABAnergic transmission. The histopathological examination made on the brain of CSA-treated rats, showed structural changes include congestion in choroids, in med brain, in blood capillaries of cerebral cortex, edema of the meninges and hemorrhage in the fissure between the two hemisphere (Figure $2_{a, b}$)

Table (4.I): The effect of taurine and BSN against CSA and SD on the levels of NA (ug/gm) in different brain areas after 6 weeks of treatment.

Group (n=8)	Cerebral cortex	Cerebellum	Striatum	Pons	Thalamus& hypothalamus
CN	0.483 ± 0.026	0.643 ± 0.021	1.450 ± 0.056	1.127± .041	1.311 ±0.048
T	0.465 ± 0.031	0.639 ± 0.020	1.425 ± 0.070	1.136 ± 0.045	1.305 ±0.040
B	0.458 ± 0.041	0.658 ± 0.023	1.471 ± 0.046	1.193 ± 0.035	1.278 ±0.087
CSA	0.427. ± 0.025	0.570 ± 0.029*	1.275 ± 0.067*	0.919 ± 0.059*	1.166 ±0.052*
CSA+SD	0.372 ± 0.030*	0.498 ± 0.032*,a	1.198± 0.087*	0.876 ± 0.046*	1.145 ±0.054*
T+C	0.458 ± 0.031b,c	0.589 ± 0.022b,c	1.363 ± 0.071b	1.006 ± 0.039b,c	1.249 ±0.059
B+C	0.380 ± 0.042*	0.518 ± 0.020*	1.213 ± 0.058*	0.889 ± 0.064*	1.197 ±0.073

Table (4.II): The effect of taurine and BSN against CSA and SD on the levels of DP ((ug/gm)) in different brain areas after 6 weeks of treatment.

Group (n=8)	Cerebral cortex	Cerebellum	Striatum	Pons	Thalamus& hypothalamus
CN	0.574 ± 0.014	0.994± 0.028	0.925 ± 0.030	0.789 ± 0.017	0.855 ± 0.026
T	0.549 ± 0.021	1.007 ± 0.021	0.918 ± 0.035	0.778 ± 0.025	0.903 ± 0.015
B	0.557 ± 0.022	0.992 ± 0.025	0.911 ± 0.031	0.792 ± 0.035	0.836 ± 0.030
CSA	0.437 ± 0.014*	0.721 ± 0.037*	0.816 ± 0.013*	0.559 ± 0.036*	0.797 ± 0.031
CSA+SD	0.415 ± 0.018*	0.682 ± 0.043*,a	0.732 ± 0.023*,a	0.594 ± 0.035*	0.785 ± 0.022*
T+C	0.590 ± 0.012b,c	0.976 ± 0.022b,c	0.819 ± 0.016*,b,c	0.651 ± 0.024*,b	0.891 ± 0.019b,c
B+C	0.436 ± 0.014*	0.715 ± 10.021*	0.712 ± 0.020*	0.617 ± 0.024*	0.769 ± 0.030*

- Sign. difference *vs*. CN: *P<0.05.
- Sign. difference bet. T+C & B+C: ^{c}P<0.05.
- Sign. difference *vs*. CSA+SD: ^{b}P<0.05.
- Sign. difference *vs*. CSA: ^{a}P<0.05.

Table (4.III): The effect of taurine and BSN against CSA and SD on the levels of Serotonin ((ug/gm)) in different brain areas after 6 weeks of treatment.

Group (n=8)	Cerebral cortex	Cerebellum	Striatum	Pons	Thalamus& hypothalamus
CN	0.394 ± 0.017	0.660 ± 0.018	0.511 ± 0.018	0.352 ± 0.011	0.850 ± 0.027
T	0.365 ± 0.013	0.647 ± 0.023	0.534 ± 0.021	0.348 ± 0.010	0.887 ± 0.020
B	0.382 ± 0.022	0.639 ± 0.023	0.495 ± 0.007	0.349 ± 0.013	0.889 ± 0.020
CSA	0.356 ± 0.021	0.592 ± 0.016*	0.487 ± 0.009*	0.350 ± 0.014	0.855 ± 0.021
CSA+SD	0.283 ± 0.012*,a	0.627 ± 0.024*	0.454± 0.014*,a	0.333 ± 0.010	0.834 ± 0.025
T+C	0.379 ± 0.008b,c	0.666 ± 0.025b,c	0.488 ±0.009b,c	0.350 ±0.009	0.861 ± 0.024
B+C	0.301 ± 0.011*	0.608 ± 0.023*	0.450 ± 0.013*	0.330 ± 0.009	0.815 ± 0.016

- Sign. difference *vs*. CN: *P<0.05.
- Sign. difference bet. T+C & B+C: ^{c}P<0.05.
- Sign. difference *vs*. CSA+SD: ^{b}P<0.05.
- Sign. difference *vs*. CSA: ^{a}P<0.05.

Studies demonstrated a correlation between clinical symptoms of CSA-dependent neurotoxicity and morphological changes in the brain, such as hypodensity of white matter, cerebral edema, metabolic encephalopathy, and hypoxic damage (Gopal et al, 1999; Shah, 1999).

Another principal mechanism for CSA neurotoxicity is that CSA-mediated neurotoxicity caused by a direct effect of CSA on nervous tissue, earlier reports demonstrated that CSA was present in cerebrospinal fluids of patients treated with the drug (Wijdiks, 2001).

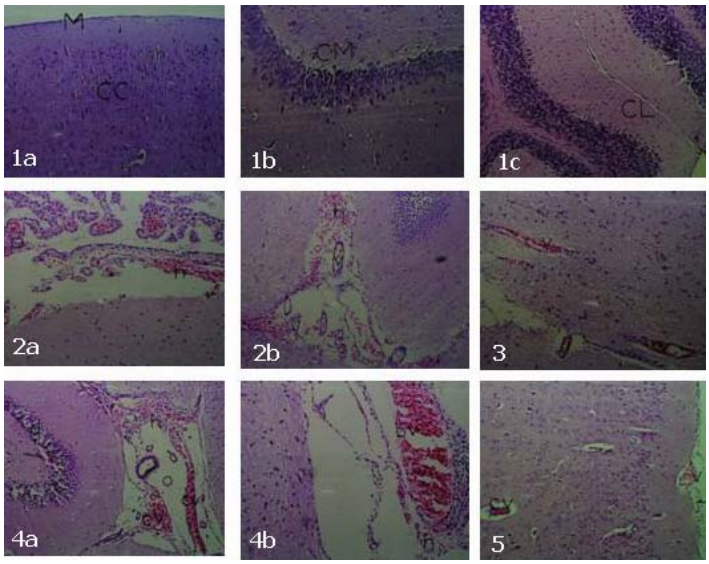


Fig. (1a): Brain of CN group showing the normal histological structure of meninges (M) and the cerebral cortex (CC), H&E X42.
Fig. (1b): Brain of CN group showing the normal histological structure of the cerebellum (CL).) (H&E X24)
Fig. (1c):Brain of CN group showing the normal histological structure of the cerebellum (CL).
Fig. (2a): Brain of CSA group showing congestion in choroids plexus (p) and hemorrhage in the fissure between the two hemisphere (h), H&E X40.
Fig.(2b): Brain of CSA group showing capillaries con-estion with hemorrhage (h) in the two cerebral hemis-phere, H&E X64
Fig.(3): Brain of T+C group showing congestion in blood vessel of med brain (v).

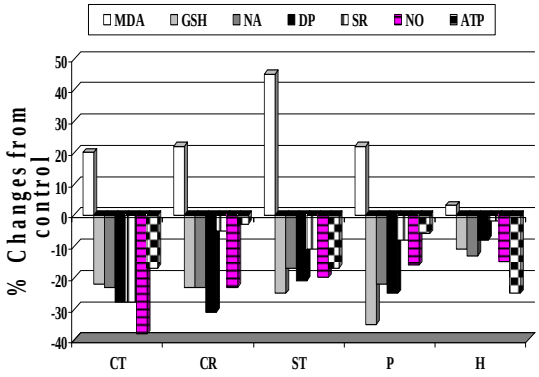
Fig. (4a): Brain of CSA+SD group showing focal hemorrhage in bet. degenerated cells of corpus callosum as well as hemorrhage (h) oedema (o) and congested blood capill.(CV)in the fissure between corpus callosum and cerebral cortex**.**
Figure (4b: Brain of CSA+SD group showing severs dilatation and congestion of the blood vessels (bv) with hemorrhage in the fissure between the cerebral tissue and med brain, H&E X64
Fig. (5): Brain of B+C group showing congestion in blood capillaries (v) and oedema (arrow) of the meninges as well as congestion in blood capillaries (cv) of cerebral cortex, H&E X64

In the brain of lung-transplanted monkeys treated with CSA, low CSA concentrations were found in the brainstem (tissue-to-blood partition coefficient: 0.3), cerebellum (0.2), and cerebrum (0.3) (Serkova et al, 2004). Even though distribution of CSA into the brain was less than its distribution into other target organs of CSA toxicity (i.e., kidney; kidney-to-blood distribution coefficient: 4.3), CSA was detected in the brain of each study animal. Finally, studies of Sercova et al, (2002*)* have shown that in the rat, CSA not only penetrates brain tissue, but the drug is also found in mitochondria isolated from CSA treated brain, in vivo as well as in vitro. These results were in good keeping with the present study which showed that all tested brain areas affected by CSA toxicity and exhibited marked inhibition of mitochondrial energy, inhibition of neuroamines release and generation of free radicals result in increase oxidative stress alternations. The potency of affected brain area were arranged of ordered; cerebral cortex >

striatum > cerebellum = pons > thalamus &hypothalamus (Table & Figure 5).

Table & Figure (5): The percentage differences from the control values to show the potent effected brain areas against CSA&SD-induced neurotoxicity.

Parameters	CT	CR	ST	P	H
MDA	20%	22%	45%	22%	3%
GSH	-22%	-23%	-25%	-35%	-11%
NA	-23%	-23%	-17%	-22%	-13%
DP	-28%	-31%	-21%	-25%	-8%
SR	-28%	-5%	-11%	-8%	-2%
NO	-38%	-23%	-20%	-16%	-15%
ATP	-17%	-3%	-17%	-6%	-25%
(X)	25	19	22	19	11



Coadminisration of CSA with the nonesteriodal antiinfilammatory drug (NSAID) sodium diclofenac (SD) increases the incidence of neurotoxicity. The data recorded in the combined treated group (CSA+SD), depicted in Table (1, $2_{I, II, III}$ & 3), showed significant decrease in ATP, GSH, and NO production in blood and brain tissue ($P<0.05$ *vs.* CSA treated alone), increment of both serum and brain MDA, LDH release and increase in blood glucose concentration ($P<0.05$ *vs.* CSA treated alone). Earlier evidences revealed that SD induced toxicity may involve production of ROS leading to oxidative stress and massive genomic DNA fragmentation and apoptotic cell death (Hickey et al, 2001). In addition, significant increase of brain MDA production accompanied with significant reduction in GSH levels that reflected by significant inhibition of dopanergic neurotransmitter in mice treated with NSAIDs (Salwa et al, 2002). Furthermore, it was found that co-administration of NSAIDs enhanced the excitatory effect of ofloxacin by decreasing the inhibitory amino acid GABA, inhibiting the release of monoamines including NA, DP, and serotonin in rat brain tissue (Nadia et al 2004). It was found that most NSAIDs penetrate poorly into the CNS. However, the COX enzymes are expressed constitutively in some areas of the CNS, meaning that even limited penetration may cause adverse effects such as somnolence and dizziness (Rossi, 2006). Confirming the biochemical results in present study, histopathological observations in brain tissues of combined treatment showed structural changes in brain more than observed in CSA alone, showing severs dilatation and congestion of the blood vessels with sever hemorrhage in the two cerebral hemisphere, cerebellum, cerebral tissue, med brain and in the fissure between them in plus to edema of the meninges observed in CSA treatment alone (Figure$4_{a,b}$). The primary mechanism responsible for its anti-inflammatory /analgesic/antipyretic action is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (Slagle, 2001) lead to reducing formation of leukotrienes that it might be responsible for its neurotoxicity. It was fount that the decrease in the level of prostaglandin in different tissue might be responsible in the dysfunction of this organ. Decrease prostaglandin in epithelium of stomach cause gastrointestinal bleeding (Rossi 2006). In kidney cause nephrotoxicity (Brater, 2002; Rossi, 2006), in heart cause heart attacks (Solomon et al 2006)). Long term treatment of NSAID develop neurological side effects; confusion, depression, dizziness, headache, sedation, sleep disturbance, somnolence (Slagle, 2001).These neurological

side effects might be due to the inhibition of prostaglandin synthesis in neuron tissue. This postulation can confirmed the result of Huang 2000 and Whelton (1999) who demonstrated that neurotoxicity of NSAIDs is due to renal insufficiency that caused by these drugs and lead with the study of Mignat (1997), which described an increased risk of central nervous system following the combined use of NSAID and immunosuppressive drugs. To interpret the latter observations, the present data strongly explored potential drug interactions when CSA co-administered with SD that cause deleterious effects on brain tissue that enhancement inhibition of mitochondrial energy, inhibition of neuroamines release and increase oxidative stress.

On other hand, BSN co-administration antagonized to a lesser extends some of CSA & SD-induced neurotoxicity. It has been shown to attenuate the level of GSH, decreased MDA production on both blood and tested brain areas (Table$2_{I, II}$) as well as reduces serum LDH activity (Table 3). On contrast, BSN have no effect on NO production and could not succeeded to ameliorate CSA-induced inhibition of both mitochondrial energy and neurotransmitters release (Table1 & $4_{I, II, III}$). Furthermore the histopathological changes that induced by CSA & SD treatment were still observed and could not be attenuated by BSN treatment (Figure 5). It was found that Bi can produce neurotoxic effects in both humans and animals under certain dosing condition (a single 2500 mg/ kg i.p.), the pattern of regions and cells with the highest Bi accumulation is very similar to pattern reports for xenobiotic metals (i.e. mercury, silver, gold) and supports the hypothesis that these metals may share some mechanisms for entry, distribution and storage in the brain (Ross et al, 1996) on other hand, Noach, et al, (1995) concluded that the normal use of BSN and bismuth subcitrate does not exhibit clinical neurotoxicity. This later finding in agreement with the present results, which does not exhibit any neurotoxic symptoms, when rats treated with 15 mg/kg of BSN alone. BSN an antiulcer, used to protect against ulceration produced with long treatment of SD. Many studies showed that BSN can protect renal tissues against drug toxicity by inducing Metallothionine (MT) in renal tissues (Kondo, et al, 2004). MT is a thiol rich protein contain sulfur group that bind with radical peroxide to protect cell from radicals to development of CNS toxicity. On the other hand drug interaction between immunosuppressive and NSAIDs increase its level in blood that might responsible in increasing incidence of neurotoxicity caused by CSA. This postulation was in good keeping induced damage. Furthermore BSN found to bind to and induce GSH as similar as MT, as seen by the extended x-ray absorption (Sun et al, (1999). To interpret the latter observations, the result showed that BSN markedly increase ($P<0.05$) the lower level of GSH induced by CSA (Table 2_I). GSH is an efficient endogenous antioxidant defense system operates to compact free radicals and plays a vital role in protection of cells against oxidative stress and detoxification of xenobiotes including CSA. Thus induction for brain GSH by BSN might be the major role of BSN in, somewhat, protecting brain tissue against CSA-induced oxidative damage and neurotoxicity.

In contrast to SD and BSN, the co-administration of the potent cytoprotective antioxidant, taurine, antagonized ($P<0.05$) all CSA negative effects, by antagonizing CSA & SD-induced mitochondrial dysfunction showed significant increase in serum and brain ATP, and increase in blood glucose concentration ($P<0.05$ *vs.* CSA & SD treated group), improvement oxidative stress by increasing the level of serum and brain GSH & NO and decline of serum and brain MDA product, and decrease activity of LDH release ($P<0.05$ vs. CSA & SD treated group), as well as modulated neurotransmitters release by increasing the level of NA, DP & serotonin as regarding to the level recorded in CSA&SD treated group (Table1, $2_{I, II, III}$, 3 & $4_{I\ II, III}$) Taurine, a ß-amino acid found at high concentrations in the brain. It was postulated that neuroprotective action of taurine may be through several mechanisms, include ; 1) activation of GABA receptors that decreases neural vulnerability to exictotoxic damage (Louzada, 2004) in agreement it was found that CSA-induced exictotoxic damage to neuron through inhibition of glutamate and GABAergic transmission (Serkova et al 2001) which accompanied by inhibition the release of monoamins (NA, DP & serotonin). 2) It can interfere with the activity of the renin-angiotensin-aldosterone system and minimized the elevation in serum cytokine, endothelin, and thromboxane B_2 and inhibited the proliferation of vascular smooth muscle cells (Hu, et al, 2009), in

addition it increased serum levels of nitric oxide and nitric oxide synthase (Fennessy et al, 2003; Hager et al, 2006), which all lead to inhibit vasoconstriction induced by CSA and attenuated its neurotoxicity. 3) taurine is a potent antioxidant and may attenuate tissue lipid peroxication either by scavenging a wide variety of oxygen derivative free radicals or by binding Fe^{2+} like a chelator or forming chloramines with HOCl and HOCl-metalloproteins, or by binding to or complixing the sulfonic acid group (SO3-) to free metal ion species such as Fe^{2+}, Cu^{+} or oxidant metalloprotein (Erdem et al, 2000). Treatment of rats with taurine attenuated CSA-induced depletion of GSH. It was being found that taurine has the ability to increase intracellular and extracellular GSH; this might be a crucial factor in protecting brain tissue in CSA-induced oxidative damage. Finally, the data presented here suggest that concomitant use of antioxidant such as taurine might be useful in reducing CSA-mediated neurotoxicity that postulated that taurine has a protective action against a variety of toxins where cellular damage is a consequence of ROS. Confirming the biochemical results, histopathological changes that induced by CSA & SD treatment showed marked improvement except some congestion in blood vessel of med brain was observed (Figure 3).

In conclusions, this study indicated that; there was a negative relationship when CSA co-administered with SD and a positive one when co-administered with taurine and a neutral one when combined with BSN against CSA-induced neurotoxicity. 2) Reactive oxygen species (ROS) is one of the most key roles in mediating the negative effects of CSA. 3) Taurine has more potent effect than BSN. 4) Taurine exhibited protective effect against a variety of CSA side effects, the potent of which exist where cellular damage is a consequence of ROS. Histopathological examination done on brain sections reinforced the results obtained.

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Assessment the Ameliorative Effect of Pomegranate and Rutin on Chlorpyrifos-ethyl-Induced Oxidative Stress in Rats

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Abstract: Oxidative stress is one of the possible mechanisms resulted from organophosphate toxicity. Therefore, the aim of this study is to evaluate the *in vivo* effects of chlorpyrifos-ethyl (CE; 16.4 mg / kg / day body weight), on the serum and tissues antioxidant system of male albino rat and the efficacy of pomegranate peel extract (P; 500 mg/ kg/ day body weight) and rutin (R; 50 mg/ kg/ day body weight) as polyphenols to antagonize this response. The parameters were cholinesterase (ChE), acid phosphatase (ACP) and protein thiol (PrTh) in serum. Levels of malondialdehyde (MDA) as a marker of lipid peroxidation (LPO), reduced glutathione (GSH), glutathione-S-transferase (GST), and catalase (CAT) were estimated in liver, brain and kidney tissues. In addition, the activities of lysosomal enzymes (acid phosphatase, cathepsin D and RNase) in the liver were measured as early apoptosis marker. Administration of CE orally by gavage for two weeks induced a significant increase in serum ACP activity, LPO levels and liver lysosomal enzymes. Associated inhibitions in serum ChE activity and PrTh level were detected to CE exposure. Also, results showed significant decreases in GSH content, GST and CAT activities in liver, brain and kidney. Supplementation with P or R to treated animals was significantly ($P < 0.01$) attenuated the toxicity and oxidative stress evoked by CE. [Nature and Science. 2009;7(10):49-61]. (ISSN: 1545-0740).

Keywords: Oxidative stress, chlorpyrifos-ethyl, antioxidant system, pomegranate peel extract, rutin, acid phosphatase, cathepsin D, RNase

Introduction

Organophosphates (OPs) act mainly as acetylcholinestrase inhibitors (AChE) and can be an indicator of chronic toxicity of Ops (Tinoco and Halperine, 1998). Oxidative stress is one of the possible mechanisms that could be involved in the OPs toxicity. Oxidative stress is known to be a key factor in several diseases and was reported as a result of OPs exposure in human and experimental animals (Abou-Donia, 2003; Abdollahi et al., 2004; Milatovic et al., 2006; Dettbarn et al., 2006). Chlorpyrifos is a member of the most commonly used organophosphorus insecticide. As a result of widespread use, residues of chlorpyrifos have been detected in the air (Cattani et al., 2001) and in the crops (Sun et al., 2006; Atif Randhawa et al., 2007) which considered a risk for living organisms (Zhao et al., 2006). Chlorpyrifos, in particular chlorpyrifos-ethyl (CE), resulted in deleterious effects including hepatoxicity, genotoxicity, teratogenecity, immunotoxicity as well as neurochemical and neurobehavioural alterations (Thrasher et al., 1993; Bagchi et al., 1995; Song et al., 1998; Dam et al., 1999; Gomes et al., 1999; Hunter et al., 1999). Previous studies has been shown that there is a correlation between acetylcholine inhibition and lipid peroxidation level following subchronic exposure to OPs (Ranjbar et al., 2002; Akhgari et al., 2003). Exposure to chlorpyrifos increased levels of lipid peroxides in the rat liver, kidney, brain, and erythrocytes (Bagchi et al., 1995; Gultekin *et al.,* 2001; Verma and Srivastava, 2001; Oncu et al., 2002 ; Tuzmen et al., 2008) and altered antioxidant enzymes in rat blood, liver, and lung (Bebe and Panemangalore, 2003). Moreover, administration of CE to pregnant rats induced oxidative stress and altered antioxidant system in liver, kidney, brain, and fetus (Zama et al., 2007).

Lysosomes are membrane bound structures that contain hydrolytic enzymes capable of degrading most of the cellular constituents. They are essential for controlled intracellular pathways such as autophagy, heterophagy and endocytosis. Apoptosis or programmed cell death which follows from moderate oxidative stress is preceded by partial lysosomal rupture and such lysosomal destabilization seems to be an initial event in apoptosis caused by a variety of other agents (Brunk et al., 2001). Exposure to organophosphorus insecticides has been shown to inhibit all cytoplasmic proteases and some of the lysosomal proteases in the liver tissue; the major site of insecticides metabolism (Mantle, 1997).

Polyphenols are one of the most abundant groups in plant which have a high antioxidant activity. Phenolic compounds are a biologically active group of phytochemicals. They are classified according to their chemical structure into flavonoids, phenolic acids, coumarins, and tannins (Tapiero et al., 2002; Mennen et al., 2005). Because of its potent anti-oxidant activity, pomegranate considers one of the commonly used natural anti-oxidants. Pomegranate fruit, juice, and peel extracts is a rich source of polyphenols and hence posses a potent antioxidant properties (Gil et al., 2000; Noda et al., 2002; Murthy et al., 2002; Singh et al., 2002). The effectiveness and safety of its isolated antioxidants have been tested (Cedra et al., 2003a, b). Murthy et al., 2002 added that methanolic extract of the peel has shown a higher anti-oxidant potential than that of seeds and could prevent CCl_4-induced hepatotoxicity. Recently, study by Elhalwagy et al., 2008 shown that supplementation with (60 mg/animal) green tea polyphenols, partially attenuate oxidative stress resulted from the toxic effect of fenitrothion insecticide, on the liver and kidney of rats. Also, tea polyphenols have been shown to protect against liver injury in animals intoxicated with chlorpyrifos insecticide (Khan and Kour 2007).

Flavonoids are a family of phenloic compounds (Harborne, 1986). It has become increasingly popular in terms of health protection because they possess a remarkable spectrum of biochemical and pharmalogical activities. Flavonoids affect basic cell function such as growth, differentiation and apoptosis. Also, they were shows to be potent antioxidant because of their radical-scavenging activity; ability to complex heavy metal ion and to antagonize a broad spectrum of enzymes such as tyrosine protein kinase (Akiyama et al., 1987; Hollman et al., 1996; Knekt et al., 2002; Mira et al., 2002). Rutin, a flavonoid, has shown pharmacological benefits including anti-tumor (Deschner et al., 1991), anti-inflammatory (Aleksandrov et al., 1986), anti-diarrhoeal (Di Carlo et al., 1993), anti-mutagenic (Bear and Teel, 2000), myocardial protecting (Pozin et al., 1996), immunomodulator (Chen et al., 2000) and hepatoprtoctive activities (Janbaz et al., 2002). Literatures revealed that rutin increase of antioxidant capacity in the kidney of normal rats (Gao et al., 2002) as well as in a liposomal model (Nagasawa et al., 2003). Moreover, treatments of diabetic rats with rutin inhibit lipid peroxides while total protein and reduced glutathione were increased (Kamalkannan and Stanely, 2006). Based on our knowledge on free radicals and their involvement in several diseases (Hogg, 1998) and the dependence on traditional medicine to replace ineffective medications, in this context, more research should be conducted to investigate the effectiveness of natural antioxidants (Madhavi and Salumkhe, 1995). The aim of this study was to investigate the ameliorative effect of pomegranate peel extract and rutin on toxicity, oxidative stress and apoptosis induced by chlorpyrifos-ethyl intoxication in male rats.

Material and methods

Plant Extraction

Pomegranate fruit peel was purchased from local market, dried and powdered. Amount of 500 g of the powdered plant material was extracted three times with ethanol (80%). The extracts were filtered, concentrated and freeze dried. The residue yielded was stored at 4^{o}C for further analysis.

Chemicals

Chlorpyrifos-ethyl (CE) [O,O-diethyl-O-(3,5,6-trichloro-2-pyridal) phosphorothioate] 40% CE emulsion concentrate (Caribo^{TM}, Egychem) was purchased from local market and reconstituted to 1% solution in disilled water. Rutin, reduced glutathione, 1-chloro-2,4-dinitrobenzene (CDNB), bovine serum albumin and 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), were purchased from Sigma (St Louis, MO,USA). All solvents used were HPLC grade (Merck, Darmstadt, Germany).

Animal

Sprague Dawily rats (200±30g) were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR), Egypt. The animals were kept under standard laboratory conditions of light/dark cycle (12h /12h) and temperature (25 ± 2^{o}C). They were provided with a nutritionally adequate standard laboratory diet.

Experimental Design

Sixty rats were treated according to the standard procedures laid down by OECD guidelines 407 (1992) repeated dose 28 days oral toxicity study in rodents .They are randomly allocated to six groups of six rats each as follow:
Group1 received distilled water by gavage orally and acts as control.
Group 2 received CE (16.4 mg / kg / day bw) orally by gavage.

Group 3 received P (500 mg / kg / day bw) and CE (16.4 mg / Kg / day bw) orally by gavage
Group 4 received P (500 mg / kg / day bw) orally by gavage.
Group 5 received R (50 mg / kg / day bw) and CE (16.4 mg / Kg / day bw) orally by gavage.
Group 6 received R (50 mg / kg / day bw) orally by gavage.

Sampling

The animals were sacrificed by cervical decapitation after one and two weeks of exposure. Blood was collected and the separated serum was used for the estimation of ACP, ChE activities and PrTh content. Liver, brain and kidney tissues were removed quickly, washed in cold isotonic saline and homogenized in 50 mM phosphate buffer (pH 7) using an electronic homogenizer to prepare 10 % W/V homogenate. The homogenate was centrifuged at 3000 rpm for 10min at 4 ^{o}C by cooling centrifuge (Sigma 3K 30) to separate the nuclear debris. The supernatant were used for biochemical analysis.

Isolation of liver lysosomal fraction

A portion of liver homogenate was centrifuged at 4^{o}C and 1000 xg for 10 min. The supernatant was centrifuged at 16.000 xg for 20 min to obtain lysosomal fraction. The residual pellets were then resuspended in phosphate buffer, pH 7.4 (Galvin Jr. et al., 1980).

Biochemical Analysis

The procedure used for the determination of cholinesterase activity in serum is a modification of Ellman et al. (1961) method as described by Gorun et al. (1978). Enzymatic activity of serum ACP was determined according to Moss (1984) using ready made kits by QCA, Spain. While serum protein-SH was measured by spectrophotometric method using 5'-5'-dithio-bis-2-nitrobenzoic acid according to Motchink et al., 1994. Lipid peroxidation (LPO) was measured by estimation of thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa et al. (1979). Reduced glutathione (GSH) and glutathione –S-transferase (GST) contents in tissues were measured by the methods of Ellman (1959) and Habig et al. (1974), respectively. Catalase (CAT) activity was estimated by the method of Takahara et al. (1960). Protein was assayed by the method of Lowry et al (1951) using bovine serum albumin as standard. The activities of lysosomal enzymes (acid phosphatase, cathepsin D and RNase) were measured according to the method of Barrett and Heath (1977) and Gianetto and De Duve (1955).

Statistical Analysis

Data were expressed as mean ± standard deviation (SE). Differences in experimental groups were determined by one-way analysis of variance (ANOVA) followed by Dunnett's *t* test. $P< 0.01$ was considered to be statistically significant.

Results

Serum parameters

The present data revealed that chloropyrifos ethyl-treated rats showed a significant ($P<0.01$) increase in the activity of Acid phosphatase (ACP) when compared with the normal rats, while associated inhibition in ChE activity and Prth level were detected. Supplementation with pomegranate peel extract significantly countered this effect; while supplementation with R significantly ($P<0.01$) restored ACP activity near the control values (Table 1).

LPO level

Exposures to 16.4 mg/kg chlorpyrifos–ethyl up to two weeks elicited a significant increase in lipid peroxidation of liver, brain and kidney of rat as measured by the estimation of thiobarbituric acid reactive substances (TBRAS). After one week, the increase in LPO levels ranged from 256.3%, 167.6% and 219.6%, in the liver, brain and kidney respectively. While after two weeks, the levels estimated as 391.9%, 317.1% and 252.1%, in the same tested tissues. Co-administration of pomegranate or rutin significantly ($P<0.01$) modulated this increase. However, treatment with P and R showed a noticeably decrease in LPO levels comparing to the CE-treated group. Thus this study shows that both of the P and R protected cells from oxidative damage by decreasing LPO levels elevated as a result of CE treatment as showed in Figures 1, 2 and Table 2.

Table (1): Effect of pomegranate peel extract or rutin on serum activities of some enzyme as well as protein-thiol level induced by chloropyrifos ethyl intoxication.

		Parameters		
Groups	**Time**	**ACP** (U/L)	**ChE** (µmol SH/hr/ml)	**Prth** (µmol/L)
C	W1	41.05±2.07	247.02±15.12	926.47±12.18
	W2	39.98±1.41	308.76±17.34	955.59±40.58
CE	W1	50.70±1.12a	174.84±7.45a	494.41±35.18a
	W2	49.27±1.72a	191.52±7.27a	535.00±17.92a
P+CE	W1	34.60±1.79b	176.82±15.58	836.47±67.00b
	W2	36.54±2.13b	250.2±19.51b	836.76±66.99b
P	W1	37.43±1.58b	212.34±10.16b	1044.62±78.53b
	W2	40.32±1.51b	256.68±14.14b	1025.59±55.6b
R+CE	W1	38.64±2.19b	176.88±5.22	824.12±32.61b
	W2	36.20±0.59b	187.8±10.24	544.41±31.01
R	W1	37.41±1.22b	225.96±11.11b	922.06±10.43b
	W2	37.94±2.24b	253.44±10.38b	836.18±46.88b

Table (2): Effect of pomegranate peel extract or rutin on some oxidative stress parameters induced by chloropyrifos ethyl intoxication in kidney.

		Parameters			
Groups	**Time**	**LPO**	**GSH**	**CAT**	**GST**
C	W1	1.40±0.09	4.73±.015	172.10±1.92	199.80±3.30
	W2	1.20±0.10	4.86±0.16	175.20±2.60	200.30±2.01
CE	W1	5.10±0.12a	2.40±0.06a	109.70±3.61a	115.00±3.62a
	W2	6.10±0.23a	1.96±0.10a	98.20±2.21a	97.60±4.83a
P+CE	W1	2.70±0.13b	3.94±0.08b	138.10±1.34b	156.90±5.13b
	W2	2.50±0.13b	4.12±0.21b	145.20±2.61b	176.20±5.98 b
P	W1	0.96±0.05b	5.00±0.07b	169.80±2.70b	197.40±4.65b
	W2	0.90±0.06b	5.28±0.11b	172.30±4.91b	201.10±3.12b
R+CE	W1	2.10±0.09b	3.62±0.17b	129.70±2.75b	172.40±1.84b
	W2	1.90±0.11b	3.92±0.15b	132.40±4.27b	184.10±3.24b
R	W1	0.94±0.09b	5.24±0.12b	170.50±1.87b	190.80±5.66b
	W2	0.80±0.06b	5.44±0.15b	175.30±5.30b	200.80±4.93b

***** Significant difference is indicated by ^{a}P <0.01 when compared with control group (C). ^{b}P <0.01 indicates significant difference when compared with chlorpyrifos-methyl-treated group(CE).

n=6; values are expressed as mean±SE

a indicates significant difference against control group at P <0.01

b indicates significant difference against chlorpyrifos-ethyl treated group (CE) at P <0.01

GSH (nmol/ mg protein)

GST (nmol/min/ mg protein)

LPO (nmol/ mg protein)

CAT (nmol/min/ mg protein**)**

Table (3): Effect of pomegranate peel extract or rutin on some lysosomal enzymes induced by chloropyrifos ethyl intoxication in liver.

Groups	Time	Parameter		
		ACP (nmol/min/ mg protein)	**Cathepsin D** (U/ mg protein)	**RNase II** (U/ mg protein)
C	W1	0.48±0.01	35.6±1.14	0.36±0.02
	W2	0.51±0.02	36.9±0.89	0.38±0.01
CE	W1	0.98±0.04a	74.0±1.75a	1.07±0.04a
	W2	1.34±014a	82.1±2.22a	1.42±0.08a
P+CE	W1	0.68±0.02b	52.1±1.33b	0.72±0.01b
	W2	0.63±0.02b	49.7±0.93b	0.61±0.01b
P	W1	0.45±0.02b	35.4±1.86b	0.38±0.02b
	W2	0.53±0.01b	36.1±1.49b	0.39±0.01b
R+CE	W1	0.64±0.02b	53.1±0.83b	0.71±0.01b
	W2	0.53±0.03b	48.4±2.3b	0.61±0.02b
R	W1	0.45±0.02b	33.8±1.47b	0.38±0.01b
	W2	0.50±0.01b	35.5±1.38b	0.37±0.02b

n=6; values are expressed as mean±SE
a indicates significant difference against control group at P <0.01
b indicates significant difference against chlorpyrifos-ethyl treated group (CE) at P <0.0

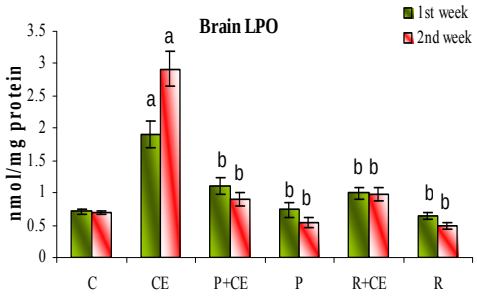

Figure 1: Effect of pomegranate peel extract (P) or rutin (R) on LPO content in liver of rats-treated with chlorpyrifos-ethyl (CE).

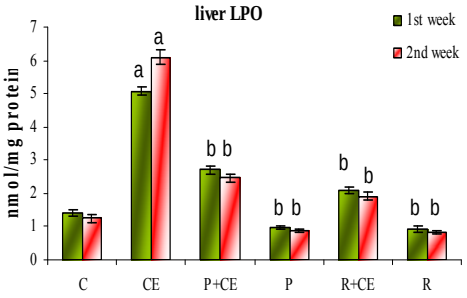

Figure 2: Effect of pomegranate peel extract (P) or rutin (R) on LPO content in brain of rats-treated with chlorpyrifos-ethyl (CE).

GSH Level

In order to evaluate endogenous antioxidant system, glutathione levels were examined. Figures3, 4 and table 2 showed the decrease in GSH levels in the same previous tissues after treatment with CE recording its

maximum value at the second week. While administration of P extract or R along with CE significantly increase GSH levels ($p<0.01$) compared to CE-group.

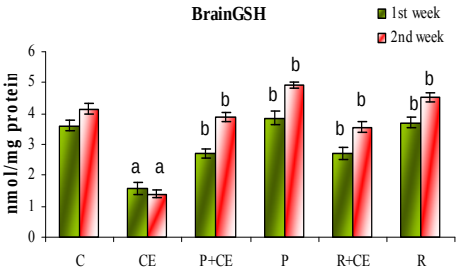

Figure 3: Effect of pomegranate peel extract (P) or rutin (R) on GSH level in liver of rats-treated with chlorpyrifos-ethyl (CE).

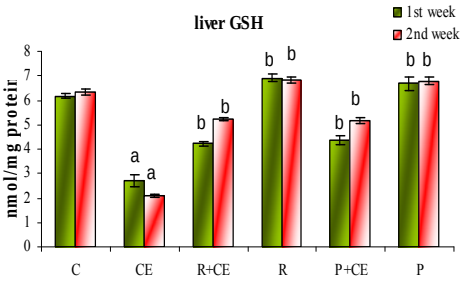

Figure 4: Effect of pomegranate peel extract (P) or rutin (R) on GSH level in brain of rats-treated with chlorpyrifos-ethyl (CE).

* Significant difference is indicated by ^{a}P <0.01 when compared with control group (C). ^{b}P <0.01 indicates significant difference when compared with chlorpyrifos-methyl-treated group(CE).

CAT activity

Highlights the activity of the scavenger enzyme (CAT) in the same tissues, it was clearly demonstrated that tissues CAT inhibited significantly ($P<0.01$) as a result of CE treatment when compared to the controls. Administration of P or R (500 mg/ kg , 50 mg/ kg respectively) along with CE caused a significant increase ($P<0.01$) in CAT activity as compared to CE-treated rats and restored its activity near that of the control (Figures 5, 6 and table 2).

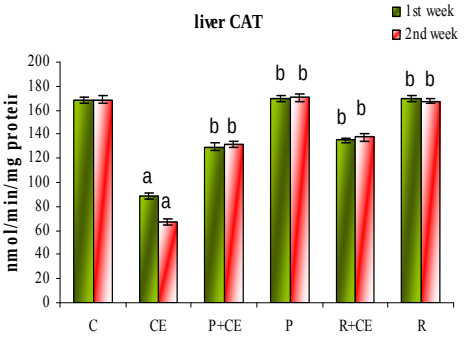

Figure 5: Effect of pomegranate peel extract (P) or rutin (R) on CAT activity in liver of rats-treated with chlorpyrifos-ethyl (CE).

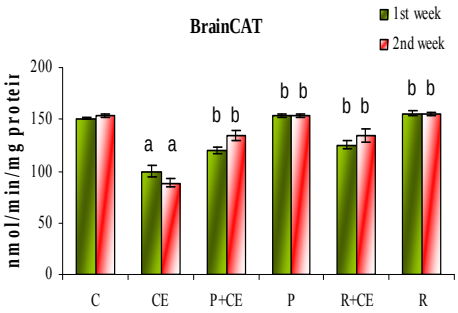

Figure 6: Effect of pomegranate peel extract (P) or rutin (R) on CAT activity in brain of rats-treated with chlorpyrifos-ethyl (CE).

* Significant difference is indicated by ^{a}P <0.01 when compared with control group (C). ^{b}P <0.01 indicates significant difference when compared with chlorpyrifos-methyl-treated group(CE).

GST activity

A consistent and significant decrease was observed in GST activity (GSH dependent antioxidant enzyme) in the tissues of rats treated with CE. Supplementation of P or R significantly countered this decrease which restored GST values close to the control level as shown in Figures 7, 8 and table 2.

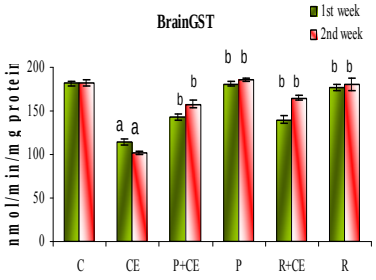

Figure 7: Effect of pomegranate peel extract (P) or rutin (R) on GST activity in liver of rats-treated with chlorpyrifos-ethyl (CE).

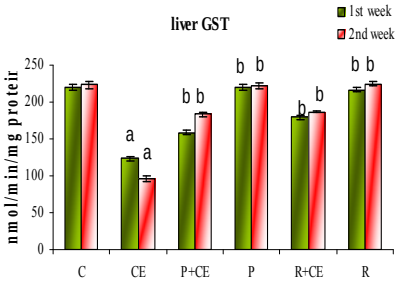

Figure 8: Effect of pomegranate peel extract (P) or rutin (R) on GST activity in brain of rats-treated with chlorpyrifos-ethyl (CE).

* Significant difference is indicated by ^{a}P <0.01 when compared with control group (C). ^{b}P <0.01 indicates significant difference when compared with chlorpyrifos-methyl-treated group(CE).

Lysosomal Enzymes activities

Effect of CE treatment on liver lysosomal enzymes (acid phosphatase, cathepsin D, and RNase II) was presented in Table (3). It concluded that CE treatment increase the activities of the three enzymes significantly ($P<0.01$) compared to control group. Administration of P or R along with CE significantly ($P<0.01$) ameliorated the effect of CE when compared to CE-group.

Discussion

Accumulated data from in *vitro* and in *vivo* studies shown that the primary mechanism of action and most acutely life threaten effect of OPs insecticides are related to accumulation of acetylcholine within the cholinergic synapses resulting inhibition of acetyl cholinesterase by active oxon metabolites (Milesson et al., 1998; Karanth et al., 2006; Gokcimen et al., 2007; Elhalwagy and Zaki, 2009). The present study conform the pervious finding, CE treatment reduced ChE activity as a marker of Ops toxicity. Pomegranate peel extract and rutin combined treatments restored ChE activity near to control level indicating their ameliorating effect. Oxidative stress biomarker is apparent in blood in association with AChE inhibition (Banerjee et al., 1999; Akhgari et al., 2003; Abdollahi et al., 2004; Shadnia et al., 2007). In line with the pervious studies we found decrease in protein thiol due to CE administration, which may be due to increased degradation of protein or increased consumption of this antioxidant in stress environment which confirm the role of OPs in disruption of body total antioxidant capacity (Ranjbar et al., 2002; Teimouri et al., 2006; Soltaninejad et al., 2007). Our results indicated that supplementations with P or R can reduce both the toxicity and oxidative stress of CE treatment. Recent literature pointed to the role of OPs in interference with metabolism of carbohydrate, biosynthesis of protein as well as respiration of mitochondria. Increased serum activity of ACP was also shown to CE treatment indicated damage to any or all of the organs producing this enzyme. While its activity was significantly lower in the groups co-treated with P or R, indicating their protective effects on the damage induced by CE on organs producing this enzymes, especially liver. This finding agrees with those (Khan et al., 2006; Elhalwagy et al., 2008; Gawish and Elhalwagy, 2009) using phenolic antioxidant against organophosphate toxicity.

In addition, pervious studies have been shown a correlation between inhibition of AChE and lipid peroxidation following subchronic and chronic exposure to OP (Ranjbar et al., 2002; Akhgari et al., 2003). Lipid peroxidation has considered one of the molecular pathways involved in the toxicity of OPs (Datta et al., 1992). Organophosphates have been suggested to induce lipid peroxidation *in vivo* by enhancement of MDA production (Debnath and Mandal, 2000; Gultekin et al., 2001; Altuntas et al., 2002; Altuntas and Delibas, 2002; Oncu et al., 2002; Sharma et al., 2005). The present study shows that CE affected lipid peroxidation and defense mechanisms in rats. The obtained results show that CE may have properties to induce oxidative stress indicated by enhancement of MDA production, decrease in GSH content, GST and CAT activities in rat tissues. The increase of free radicals and lipid peroxidation may result from the inhibition of GSH levels induced by CE toxicity. The present findings are in agreement with other investigations indicating that accumulation of lipid peroxides has been resulted after exposure to acute dose of cholrpyrifos in rat liver (Bagshi et al., 1995), kidney (Oncu et al., 2002), brain (Gultekin et al., 2001), and erythrocytes (Verma and Srivastava, 2001). However, repeated doses increased LPO levels as well as antioxidant enzymes in blood, liver, and lung of rat (Ahmed et al., 2000; Bebe and Panemangalore, 2003; Akhgari et al., 2003). *In vitro* studies have been reported that accumulation of lipid peroxidation in human erythrocytes (Gultekin et al., 2000) and PC12 cells (Qiao et al., 2005). Supplementation with antioxidants effectively suppressed the oxidative damage induced by OPs (Ahmed et al., 2000; Gupta, 2001; Gultekin et al., 2001; Karaöz et al., 2002; Oncu et al., 2002; Camkayali et al., 2005; Elhalwagy et al., 2008). Antioxidant activity of pomegranate is referred to its polyphenolic capacity such as ellagic acid and ellagitannis (Seeram et al., 2005), which may suggest its role as an electron donor in scavenging free radicals (Kaur et al., 2006). Previous investigation revealed the ability of pomegranate fruit extract (Sudheesh and Vijayalakshmi, 2005; Noda et al., 2002) and peel extract (Singh et al., 2002) to suppress lipid peroxidation. Pretreatment with pomegranate flower for a period of one week significantly protected against oxidative damage and hepatic injury induced by Ferric nitrilotriaacetate (Fe-NTA) by modulation of LPO levels and GSH content as well as antioxidant enzymes CAT and GST (Kaur et al., 2006). The same result has been obtained using the peel extract in mice treated with CCl_4 (Murthy et al., 2002). Ingestion of pomegranate juice for 4 weeks reduced hepatic oxidative stress (Faria et al., 2007) and improved the antioxidant enzyme activity in elderly subjects (Guo et al., 2008). Oral administration of pomegranate fruit extract inhibit LPO and increase GSH content as well as CAT activity in liver, kidney, and heart (Sudheesh and Vijayalakshmi, 2005). Previous studies revealed the effect of rutin on the activity on gene expression

of antioxidant enzymes in different experimental models both *in vivo* and *in vitro* (Lores-Arnaiz et al., 1995). In this context, treatment with rutin increased the activity of enzymatic antioxidants and also levels on non-enzymatic antioxidants in liver, kidney and brain of CE intoxicated rats. Inhibition of MDA levels in the group treated with both of CE and rutin was referred to the ability of rutin to transfer electrons and free radicals (Ferrali et al., 1997) in addition to activation of anti-oxidants enzymes (Elliott et al., 1992). However administration of rutin alone did not show any significant effect. The same finding has been obtained after pretreatment with rutin to isoproterenol-treated rats for 42 days (Karthick and Stanely Mainzen, 2006). Also, it has been reported that rutin has effectively reversed the biochemical, behavioral, and neurochemical changes in rat treated with haloperidol (Bishnoi et al., 2007) and improved the antioxidants enzymes system in human hepatoma cell line (Hep G2) by inhibition MDA levels and increasing CAT activity and therefore preventing or delay oxidative damage and its adverse effects (Alia et al., 2006). Moreover the antioxidant enzyme status was increased after rutin feeding in normal liver and in diabetic liver and kidney (Kamalakkannan et al., 2006).

In the present study CE intoxicated rats showed significant increase in the liver lysosomal enzymes activity (acid phosphatase, cathepsin D and RNase II). We suggested that reactive oxygen species (ROS) generated from CE pesticide may be responsible for the release of lysosomal enzymes, as a result decrease in membrane integrity and the leakage of enzymes from the enclosed sacs. These lead to intracellular dysfunction, disruption of potential substrates and organelles such as mitochondria, sarcolema etc. (Kennett and Weglicki, 1978 and Mayanskaya et al., 2000). Also, the present study showed that induction of lysosomal enzymes was associated with a decrease in serum protein thiol level. Early study by Teimouri et al., 2006 confirms our finding and concludes that ROS can induce oxidation of critical sulfhydryl (SH) groups in protein and DNA, which will alter cellular integrity and function. The phospholipids-rich lysosomal membrane is a potential site of free radical attack subsequently causing loss of membrane stability. However, Brunk et al., 2001 added that apoptosis or programmed cell death is preceded by partial lysosomal rupture and such lysosomal destabilization seems to be an initial event in apoptosis. Treatment with pomegranate peel extract or rutin were able to decrease the release of lysosomal enzymes which could be due to the membrane stabilizing affect of P and R on the lysomal membrane. The antioxidant properties of P and R scavenge the oxygen free radicals and preservation of cellular viability serving secondarily to preserve lysosomes, thereby retaining near normal functioning of the lysosomes. Indeed, cathepsin D is lysosomal proteases possibly involved in autophagic of discrete areas of cytoplasm, myofibrillary and mitochondria proteins (Zak et al., 1976). Cell death causing by oxidants may be initiated by lysosomal rupture and that this latter event may involve intralysosomal iron which catalyze Fenton-type chemistry and resultant peroxidative damage to lysosomal membrane. The antioxidant activity of rutin and pomegranate peel extract stopped this reaction by iron chelating activity. According to our results, pomegranate peel extract and rutin have shown abilities to preserve the activity of anti-oxidant enzymes and lysosomal membrane which may be referred to its role in modulating the levels of H_2O_2 and O_2. GST over expression after co-treatment with the extract, and CE may be attributed to the increase in oxidative damage. Furthermore, the contents of the extract have been suggested to induce the de novo synthesis of anti-oxidant enzymes by acting as several loci in the metabolic pathway.

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Conclusion

The present study highlights the protective role of pomegranate and rutin against OPs pesticides but the mechanism involved is still unclear. Further work is required to clarify how the plants extract works to enhance the antioxidant enzymes or their gene expression.

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Rapid Determination of Diuretic Resistant Ascites Using Furosemide Induced Natriuresis Test in Egyptian Cirrhotic Patients

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Abstract: Background: Diagnosis of refractory ascites is a challenging task for physicians. This study aimed to evaluate the value of urinary sodium measurement after a single dose of intravenous furosemide (furosemide natriuresis test) in determination of diuretic resistant ascites. **Methods:** forty five Egyptian cirrhotic patients with massive ascites were included in the present study. All of them were maintained on low sodium diet and were subjected to furosemide natriuresis test then they were given maximum oral diuretic therapy (160 mg furosemide and 400 mg spironolactone) for one week; the response to oral therapy was assessed by the difference in body weight before and after therapy. According to the results of oral therapy the patients were divided into two main groups: Responsive group with decrease in body weight over 4 days >800gms, Resistant group with decrease in body weight over 4 days <800gms. **Results:** No significant difference in liver or renal functions was found between responsive and resistant group. Comparing the results of the furosemide natriuresis test to the results obtained from response to oral diuretic therapy; It was found that all patients resistant to oral therapy (twelve patients) had 8 hours urinary sodium <90mmol/L. In the responsive group (Thirty one patients), twenty eight patients had 8 hours urinary sodium >90mmol/L, three patients had 8 hours urinary sodium <90mmol/L and were found to be responsive to oral therapy. According to these results the sensitivity of the test is 100%, the specificity is 90.3%, the positive predictive value is 80% and the negative predictive value is 100% for detection of diuretic resistant ascites. **Conclusion:** measurement of urinary sodium eight hours after administrating 80 mg of intravenous furosemide is helpful in detection of diuretic resistant ascites patients "[Nature and Science. 2009;7(10):62-67]. (ISSN: 1545-0740).

Key Words: Furosemide natriuresis test; diuretic resistant ascites; single dose of intravenous furosemide.

Introduction:

The diagnosis of refractory ascites carries a poor prognosis with survival rates of 30% to 40% at 1 year. Therefore it is important to identify those patients reliably and rapidly as liver transplantation is urgently needed in this situation ***Spahr et al., 2001.*** For the ascites to be considered as non responsive to diuretic therapy the patient should be on spironolactone 400mg/day and furosemide 160mg/day for at least one week and to be on salt restricted diet of <90mmol/day. Lack of response is defined as a mean weight loss of less than 0.8kg over 4 days and urinary sodium output less than sodium intake. Early recurrence is defined as the reappearance of grade 2 or 3 ascites within 4 weeks of initial mobilization. Diuretic induced complications include diuretic induced hepatic encephalopathy, renal impairment (increase is serum creatinine >100% to a value of >2mg/dL) hyponatremia (decrease in serum sodium >10mmol/L to serum sodium <125mmol/L) and hypo or hyperkalemia (a change in serum potassium <3mmol/L or >6mmol/L respectively despite appropriate measures)**.** These complications increase, as the dosage of diuretics is increased ***Arroyo et al., 1996*** & ***Moore et al., 2003***. In addition it is important to ascertain that the lack of response is not related to dietary noncompliance or inadequate dosage of diuretics. Due to these facts, it is very difficult to apply this definition of refractory ascites to patients in outpatient department; identification of refractory ascites according to the proposed definition often requires prolonged observation, preferably in hospital to optimize the diet and the diuretic treatment. In this study, we tried to determine if ascites is truly diuretic resistant rapidly by measuring urine sodium after the administration of intravenous furosemide. The furosemide natriuresis test can be the standard in early differentiation of the response to diuretics treatment.

Patients and Methods

Forty five patients with massive ascites due to liver cirrhosis participated in the study. A written informed consent was obtained from each patient. Diagnosis of liver cirrhosis was based on clinical & laboratory evidences. Patients with malignant ascites, tuberculous, chylous and pancreatic ascites as well as ascites due to constrictive pericarditis, hepatic venous congestion, ovarian tumors spontaneous bacterial peritonitis and ascites due to bowel perforation were excluded from the study.

All patients were subjected to the following: Liver enzymes (AST and ALT), serum albumin, serum bilirubin (direct and indirect), prothrombin time and Child- pugh scoring was obtained after full clinical evaluation. Urea, creatinine, creatinine clearance, abdominal ultrasound, viral markers, diagnostic abdominal paracentesis, follow up of serum sodium and potassium, urea and creatinine all through the study time.

Patients were subjected to the furosemide test as follows: All diuretics were withdrawn for 3 days. On the fourth day the patient was asked to void his bladder and a bolous of furosemide, 80mg I.V. was injected. The urine was collected for 8 hours for detection of urinary sodium and urinary volume. The blood pressure was measured immediately and after 12 and 24 hours respectively and patients were asked about symptoms of hypotension ***(Spahr et al., 2001).***

Then after two days, All patients were given intensive diuretic therapy in hospital (160mg furosemide and 400mg spironolactone) for one week while being under sodium restriction. <90mmol/day (= less than 5.2gm/day) ***(Moore et al., 2003).*** The body weight was measured over the last 4 days and the average weight was taken. Two patients did not complete the study because of diuretic induced hepatic encephalopathy and were not included in further data analysis.

According to the obtained results from the oral therapy the forty three patients who completed the study were classified into 2 groups without knowing the results of the furosemide test: Diuretic responsive group: in which the loss in body weight was >800gms over the last 4 days (the loss is >200gms/day). b- Diuretic resistant group: in which the loss in body weight was <800gms over the last 4 days (the loss is <200gms/day). Patients of resistant group were subjected to 24 hours urine collection and 24 hours urinary sodium excretion was measured to confirm dietary sodium restriction (Patients who excrete greater than 90 mmol of sodium per day, and who fail to lose ascites are not compliant with their diet) ***Moore et al., 2003***

Statistical methodology

Analysis of data was done by IBM computer using SPSS (statistical program for social science package 16) as follows Description of quantitative variables as mean, SD and range description of qualitative variables as no and %, Chi-square test was used to compare qualitative variables, Unpaired t-test was used to compared two independent groups as regard a quantitative variable, paired t-test was used to compare variables in the same group before and after, correlation co-efficient rank test was used to rank different variables against each other in linear correlation. sensitivity = true +ve /true +ve + false -ve = ability of the test to detect +ve cases, specificity = true -ve/ true -ve + false +ve = ability of the test to exclude negative cases, positive predictive value (PPV) = true +ve/ true +ve + false +ve = % of true +ve cases to all positive cases, NPV = true-/true-ve + false -ve = % of the true -ve to all negative cases. Level of significance; P value >0.05 insignificant, P<0.05 significant, P<0.01 highly significant. ***Altman, 1994.***

Results:

43 Egyptian patients with liver cirrhosis secondary to hepatitis C infection and massive ascites were included in this study. They were 33 males and 10 females, their age range from 42 to 63. 15 patients were Child-pugh class B and the remaining 28 patients were of Child- pugh class C. According to the results of oral diuretic therapy while being on sodium restricted diet; the patients were divided into two groups:

Responsive group: Those with decrease in body weight over 4 days >800gms. Included 31 patients, 24 males and 7 females, mean age was 47.3±8.4. 12 patients were of Child B class and 19 of Child C.

Resistant group: Those with decrease in body weight over 4 days <800gms. Included 12 patients, 9 males and 3 females, mean age was 50.3±7. 3 patients were of Child B class and 9 of Child C.

No significant difference was found between responsive and resistant groups as regard age, sex or Child-pugh class. No statistically significant difference was found between the two groups regarding biochemical variables before the test as shown in Table 1.

Table (1): Comparison between both groups as regard different laboratory data before the test

Variables	Resistant		Responsive		t	P
	Mean	±SD	Mean	±SD		
Serum Na (mEq/L)	136.75	4.883	136.35	5.161	0.22	>0.05
Serum K (mEq/L)	4.267	0.624	4.071	0.445	1.15	>0.05
AST (mg/dl)	65.083	24.942	52.871	13.031	1.72	>0.05
ALT (mg/dl)	35.833	14.345	27.839	10.733	1.999	>0.05
Total bilirubin (mg/dl)	3.067	1.425	2.845	1.456	0.450	>0.05
Direct bilirubin (mg/dl)	1.158	0.793	1.106	0.681	0.213	>0.05
Total protein (g/dl)	7.142	0.699	7.071	0.773	0.275	>0.05
Albumin (g/dl)	2.467	0.481	2.545	0.492	0.471	>0.05
PT (minutes)	16.667	1.702	16.781	1.995	0.174	>0.05
INR	1.446	0.173	1.506	0.331	0.593	>0.05
Urea (mg/dl)	25.0	7.311	30.0	11.855	1.359	>0.05
Creatinine clearance (ml/min)	91.083	13.601	96.355	12.643	1.201	>0.05
Serum creatinine (mg/dl)	1.375	0.480	1.190	0.322	1.461	>0.05

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Following furosemide natriuresis test, it was found that 8 hours urine volume and 8 hours urinary sodium showed highly significant decrease in resistant than responsive group (table 2)

Table (2): Comparison of Results of furosemide natriuresis test between both groups

Variables	Resistant		Responsive		t	P
	Mean	±SD	Mean	±SD		
Urine volume (ml)	612.92	254.85	1062.7	313.21	4.43	**<0.0001****
Urine Na (mEq)	76.583	7.317	115.39	18.422	7.042	**<0.0001****
Serum Na (mEq)	136.42	4.379	134.81	3.719	1.212	>0.05
Serum K (mEq)	4.117	0.525	3.939	0.479	1.064	>0.05

In the responsive group, following furosemide natriuresis test there was highly significant decrease in body weight, significant decrease in serum sodium and non significant decrease in serum potassium (Table 3). While there was no significant change in any of these parameters in the resistant group (Table 4)

Table 3 : Changes in body weight and serum electrolytes before and after the test among responsive group

Variables	Before		After		t	P
	Mean	±SD	Mean	±SD		
Weight (kg)	70.62	9.91	68.33	9.64	3.342	<0.01
Serum Na (mEq/L)	136.35	5.16	134.81	3.71	2.179	<0.05
Serum K (mEq/L)	4.07	0.44	3.93	0.47	3.017	<0.05

Table (4): Changes in body weight and serum electrolytes before and after the test among resistant group

Variables	Before		After		t	P
	Mean	±SD	Mean	±SD		
Weight (kg)	74.20	13.08	74.12	13.15	0.518	>0.05
Serum Na (mEq/L)	136.75	4.88	136.42	4.37	0.741	>0.05
Serum K (mEq/L)	4.26	0.62	4.11	0.52	1.964	>0.05

No significant change in blood pressure was detected in both groups in serial blood pressure follow up after furosemide natriuresis test except for significant decrease in systolic blood pressure 12 hours after the test in the responsive group (110.97±6.38 twelve hours after the test versus 116.13± 7.154 before the test, t= 2.998, p<0.05).

In the responsive group 8 hours urinary sodium after the test were statistically correlated versus all studied variables before the test and no significant correlation could be detected with any one of them. The results were presented in table (5). The same correlation was studied in diuretic resistant group and no significant correlation was found.

Table (5): Correlation between urinary Na 8 hours after the test versus other variables among responsive group

Variables	r	P
Age	-0.288	>0.05
Weight	-0.001	>0.05
Serum Na	0.056	>0.05
Serum K	0.331	>0.05
AST	-0.022	>0.05
ALT	-0.098	>0.05
Total bilirubin	0.020	>0.05
Direct bilirubin	0.101	>0.05
Total protein	0.057	>0.05

Albumin	-0.069	>0.05
PT	0.300	>0.05
INR	0.224	>0.05
Urea	-0.306	>0.05
Creatinine clearance	-0.034	>0.05
Serum creatinine	-0.028	>0.05

The results of the furosemide natriuresis test were compared with the results obtained from response to oral diuretic therapy. According to these results, the sensitivity and specificity of the test as well as its positive and negative predictive value were calculated. The sensitivity was 100% which means that all cases with urinary sodium <90mmol/L were found to be resistant to oral therapy (12 cases) (true +ve cases). The specificity was 90.3% which means that among 31 patients responsive to diuretic therapy, 28 patients only had urinary sodium >90mmol/L (true -ve) and 3 patients had urinary sodium <90mmol/L (false +ve). The PPV of the test was 80% which means that among the fifteen patients with urinary sodium <90mmol/L 12 patients only were truly resistant to oral therapy (true +ve). The NPV was 100%, which means that all patients with urinary sodium >90mmol/L (28 patients) were responsive to oral therapy (true –ve).

Discussion:

The development of ascites in patients with cirrhosis indicates a poor prognosis. The probability of death in cirrhotic patients hospitalized with ascites is nearly 40% at 2 years. The prognosis is worse for those with refractory ascites, ***Moore et al., 2003.*** This study was designed to evaluate the furosemide induced natriuresis test as simple, cheap and rapid method for early determination of the resistant type of refractory ascites**.**

Result of the present study revealed that the number of resistant cases was 28% of total cases while responsive cases represented the remaining 72%. This is in accordance with ***Zervos and Rosemurgy, 2001 and Moreau et al., 2004***; both authors reported that percentage of refractory ascites was less than that of responsive one, and less than 30% of total cases. It was proved that medical treatment based on sodium-restricted diets, anti-mineralocorticoids, and loop diuretics achieves a response rate in up to 90% of patients without renal failure in controlled clinical trials ***Moore et al., 2003***. However the selection of patients for the present study could account for higher percentage of diuretic resistance as we selected patients with massive ascites which reflects more disturbances in salt and water homeostasis. We found non significant difference in almost all studied biochemical parameters between diuretic resistant and responsive patients; it was reported that the course of underlying chronic liver disease and the prognostic factors in relation to the outcome of ascites have not been determined and refractory ascites may occur in the absence of poor liver function ***Moreau et al., 2004***.

The pathophysiological basis of ascites development in cirrhotic patients could explain the highly significant increase in urine sodium 8 hours after the test in the responsive group in comparison with resistant group. The later patients have significant abnormalities in their fluid and electrolyte balance which is manifested mainly by development of ascites and edema ***Cardenas and Arroyo, 2005.*** The response of ascites is usually better in patients with moderate sodium retention than in those with marked sodium retention ***Bernardi et al., 1993.*** With the progression of the disease, patients with severe urinary sodium retention develop refractory ascites ***Cardenas and Arroyo, 2005.*** Accordingly urinary sodium excretion in some cases of severe resistant ascites may approximate to zero ***Cárdenas and Arroyo, 2003.***

Impaired water handling is common in cirrhotic patients as indicated by an impaired ability to eliminate water load ***Cardenas and Arroyo, 2005.*** This explains the highly significant decrease in urine volume in the resistant group in comparison with the responsive group. In addition, ***Arroyo et al., 1996*** reported that when the renal ability to excrete free water is markedly reduced, patients become unable to eliminate excess ingested water and dilutional hyponatraemia develops.

The significant decrease in serum Na after the test among patients of responsive group is most probably the result of the increase in urinary sodium excretion

in this group as a response to the test and the significant decrease in serum K after the test in this group is most probably the side effect to IV furosemide ***Arroyo et al., 1996.***

The furosemide natriuresis test was statistically evaluated as regard its value in diagnosing resistant ascites and its safety for the patient. Firstly, eight hours urinary sodium was statistically correlated with all studied biochemical variables as well as the child classification in both groups. The results revealed non significant correlation of 8 hours urinary sodium with any of the studied variables. This result indicates stability of the test which means that is not changeable in response to any other variable except the degree of response of ascites. Data of the present study supports the findings of other authors who proposed furosemide natriuresis test as a simple test to identify patients with refractory ascites ***Spahr et al., 2001*** .

Conclusion: From this study we can conclude that when urinary sodium excretion following 80mg IV furosemide injection is >90mmol/L (under dietary sodium restriction) the patient can be diagnosed as responsive to diuretics. Meanwhile when it is <90mmol/L (under the same restriction) the patient is 80% resistant to diuretics. Accordingly, the test is highly sensitive but not highly specific.

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Rural Tourism Development through Rural Cooperatives

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Abstract: The concept of rural tourism has become important around the world. It is thought that rural tourism can revitalize the conventional concepts and views on tourism, and bring in a new dimension in the sustainable development. It has been realized that local communities based tourism can play a fundamental role in poverty alleviation in rural areas. This paper used qualitative approaches to illustrated development of rural tourism through rural cooperatives. The study also attempted to highlight the barriers of rural tourism in Iran. This article looks at how rural cooperatives can develop the rural tourism in rural area at the case study Iran This research draws from our scientific experience in a variety of disciplines namely; rural cooperatives, tourism development and community development. [Nature and Science. 2009; 7(10): 68-73]. (ISSN: 1545-0740).

Keywords: Rural tourism, rural cooperatives, tourism development

1. Introduction

World Tourism Organization (WTO) used rural tourism concept for defining that tourism product "that gives to visitors a personalized contact, a taste of physical and human environment of countryside and as far as possible, allow them to participate in the activities, traditions and lifestyles of local people." According to WTO it is considered that take part from rural tourism a wide range of activities like: climbing, riding, adventure tourism, educational travel, sport and health tourism, arts and heritage tourism (Negrusa et al., 2007). Oppermann (1997) saw rural tourism as tourism that occurs in nonurban settings where human activity is present (Beeton, 2006). Negrusa et al (2007) defines rural tourism as that form of tourism offered by people from rural areas, with accommodation on small-scale and with the implication of important components of their rural activities and customs of life Tourism it appears to be developing an elitist bias as broadening of its social base with participation from all sections of the society is clearly not visible. The important role of participatory and community based organizations like cooperatives in promoting tourism has yet to be recognized. As a result, the concepts like "sustainable tourism", "poverty reduction through tourism", ‘community tourism’, etc. which can be best implemented through participatory institutions have yet to be popularized in a big way (Verma, 2008). Rural tourism development has become a top priority of the economic agenda of all the countries. It is not unusual to hear that rural areas of Iran are underdeveloped in text of tourism. Hence this paper suggested development of rural cooperatives for the tourism development in rural areas.

2. Rural Tourism

There are a variety of terms used to describe tourism in rural areas, including farm tourism, agritourism, soft tourism and even ecotourism (Beeton, 2006). According to the Organization of Economic Co-Operation and Development (OECD), rural tourism is defined as tourism taking place in the countryside (Reichel et al., 2000). Rural tourism is located in agricultural landscapes and is characterized by enjoyment of a tamed nature or highly modified landscape. It is about the land uses and human cultures that the interaction between humans and the land have created. It positions agriculture and farms as the foundation upon which the attraction is built (Knowd, 2001). Any form of tourism that showcases the rural life, art, culture and heritage at rural locations, thereby benefiting the local community economically and socially as well as enabling interaction between the tourists and the locals for a more enriching tourism experience can be termed as rural tourism. Rural tourism is essentially an activity which takes place in the countryside. It is multi-faceted and may entail farm/agricultural tourism, cultural tourism, nature tourism, adventure tourism, and eco-tourism. As against conventional tourism, rural tourism has certain typical characteristics like; it is an experience oriented, the locations are sparsely populated, it is predominantly in the natural environment, it meshes with seasonality and local

events and is based on preservation of culture, heritage and traditions. Rural tourism has many potential benefits for rural areas (Frederick, 1992). Rural tourism can be an important source of jobs for local communities. Tourism can be an important force for developing disadvantaged rural areas. In particular, rural communities with few other options for development may perceive that tourism represents a panacea for growth. While tourism can certainly be an important component of a sound development plan, this is not always the case. Bontron and Lasnier (1997) note that the rural tourism impact varies greatly among rural regions and depends on a host of factors including work force characteristics and seasonality issues. Figure 1 presents one way of viewing the complex nature of rural regions and tourism's role by mapping the links between elements and issues. The map serves its purpose in illustrating the relationship between tourism and rural regions. The community is central to this process, and in many ways cannot be separated from any of the elements on the map.

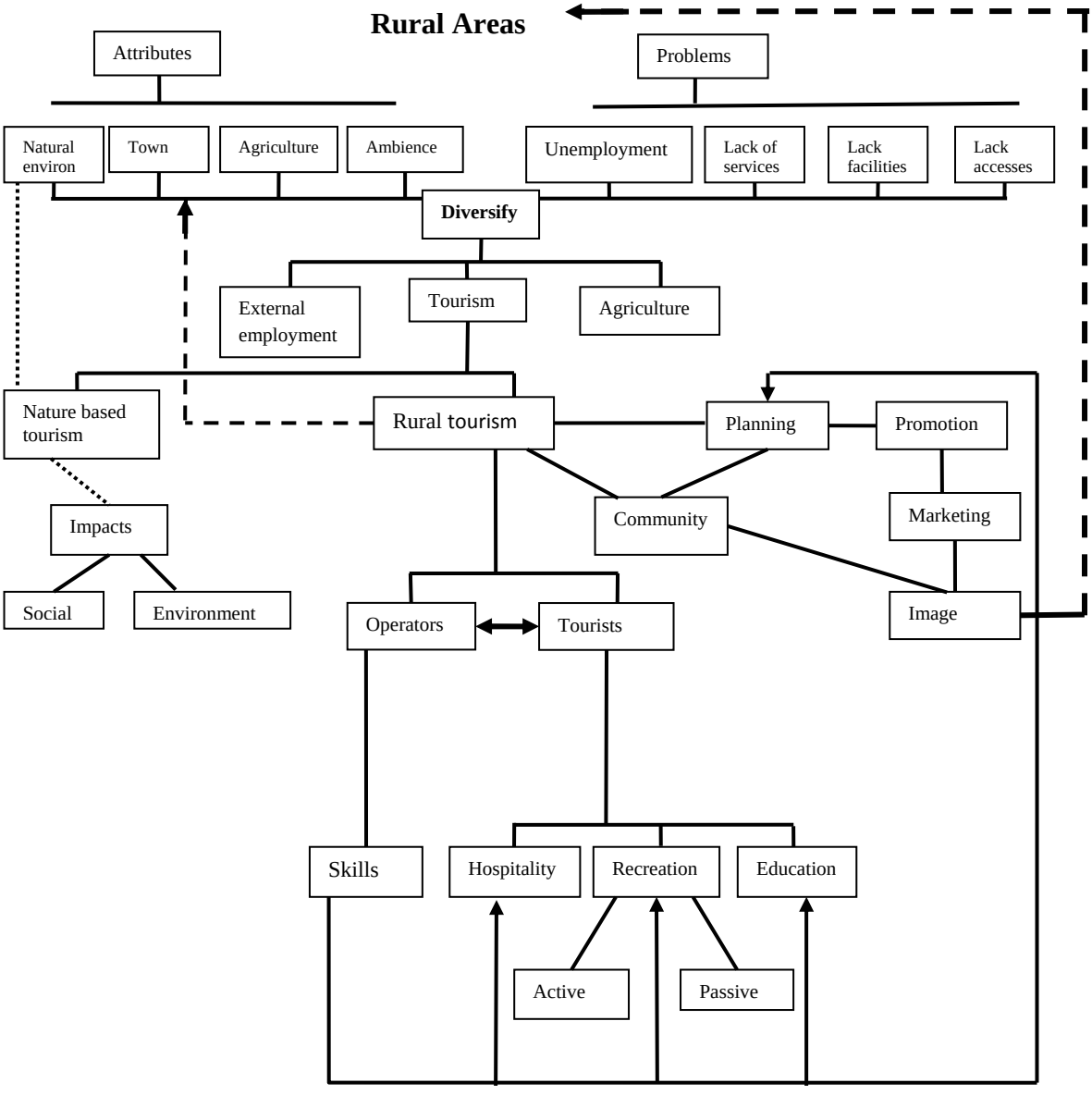

Figure 1: Rural tourism. Adopted from Beeton (2006, p. 143)

3. Methodology

The research was performed as a qualitative library in which the researcher had to refer to relevant and related sources. Sources that we used to collect needed information about Iran in order to write this article were the Cultural Heritage and Tourism Organization, rural cooperatives, State Planning Organization, official websites of tourism Iran, as well as relevant literature and articles about the tourism. Likewise, we have used a number of articles and official websites of the various world known organizations, such as United Nations Environment Programme, World Tourism Organization, and World Trade and Tourism Council.

4. Rural Tourism in Iran

The rural cooperatives in Iran in the recent years have diversified themselves into various areas of socio-economic activities. The failure of the government sector and various limitations of the private sector have compelled the policy-makers to pin their faiths on the cooperative system. For certain activities/areas, the success of which is based on the ability of the grassroots institutions to tackle them with their participatory and people-based approach, the cooperatives are considered to have an advantage over other organizations. For example, in Iran the rural cooperatives are considered most effective organizations in the field of rural Agriculture. Similarly, because of their vast network and reach, the rural cooperatives are considered best promoters for agriculture in Iran. Rural tourism is often considered an economic alternative for rural areas facing decreasing profits and requiring a second or third economic footing (Verma, 2008). However, like other tourism activities, rural tourism results in a full range of environmental impacts (Kuo, 2008). Rural tourism in Iran doesn't have a long history because of insufficient infrastructure and preparation. Iran definitely has the great potential for tourism especially rural and ecotourism. The only problem and difficulty are in attracting the tourists. Unspoilt nature, varied picturesque landscapes, a thousand-year-old cultural and architectural heritage, a profusion of leisure opportunities and recreation, closeness to the urban centres as well as the authentic character and rural charm, all these are the most important factors for development of rural tourism in Iran. However there are some other steps that should be taken, because Iran isn't ready for welcome rural tourists yet: Attractions (for example development of rural tourism around a heritage site), rural infrastructure, accessibility (roads, transportation) and Building rural capacity for tourism development

There is a still more budget needed for rural areas. Rural tourism has some advantages in rural are in Iran, for example, it provides employment for local residents and prevents their immigration to cities. Currently young people leave country-sides and go to big cities to study or work. Usually they never come back to their homelands. Some of the reasons for failure of these efforts are as follows: The role of the rural cooperatives in this industry is not defined, socio-cultural and political barriers, and lack of human and economic resources.

5. Barriers of Rural Tourism

Understanding barriers of rural tourism is important when a community is getting organized for involvement in tourism activates. This understanding can help individuals, community and organizations more effectively impact the tourism policy-making process. Further, it is important for government to understand that rural also face barriers that can hinder its progress in responding and recognizing the priorities of local communities in Iran. Overcoming the barriers to tourism development presents a challenge to both communities and government, and will serve to facilitate the policy making process. There are several literatures that directly deal with the barriers of tourism development through local communities particularly in third world countries. Rural tourism in Iran has several barriers that cannot develop. Roads and accommodation infrastructures were cited as the two main barriers for growing rural tourism in our case study. In the long-term, developing accommodation, sealing the road, and providing other services like cafes and shops are essential to fulfill the tourism potential of Iran, and attract a broader range of visitors to stay in the region overnight. Beside The rural cooperatives in Iran yet have not to recognize the importance of tourism despite the rapid growth of tourism sector in the world. Following are the main barriers:

- Inability to analyze the changing socio-economic dimensions of rural tourism in Iran, and demarcate the areas in which

rural cooperatives have a strategic advantage over other forms of organizations.

- Lack of policy research in this field which can provide definite indicators for future.
- Inability to strategically link the rural cooperatives with the rural tourism in those cities in which tourism is in a boom. For example, in Esfahan and Shiraz, tourism has emerged as a big force. But, the rural cooperatives have not yet to come up in this field.
- Inability of the cooperatives to extend their areas of operations or activities in the field of rural tourism.
- Weak advocacy for rural tourism development is also a big hindrance. Holding of Advocacy conferences by the cooperatives in the area of cooperative tourism can set the ball rolling in a big way and create a conducive atmosphere for rural tourism development (Verma, 2008).

Bushell & Eagles (2007, p. 154) also states tourism as a phenomenon of affluent contemporary societies is a particularly difficult concept in local communities in developing countries to grasp. In this sense tourism development may be more difficult than other activities. Shortcomings are similar to those local communities, but a few factors tend to be more pronounced among local area:

- Lack of formal education and appropriate managerial training
- Lack of foreign language skills
- Different ways of dealing with hygiene, litter, maintenance of infrastructure
- Limited knowledge of food preparation for foreigners, including catering to dietary, nutritional and culinary tastes
- Lack of decision making and planning skills concerning the possible consequences of tourism, coupled with limited ability to control tourism, unpredictable political climates, and long-term funding uncertainty (Bushell (Bushell & Eagles, 2007, p. 154).

As consequence, rural tourism facilities and services may be unacceptable for international tourists. Hence building capacity through rural cooperatives is necessary for stakeholders involved in tourism in local communities (Bushell & Eagles, 2007). However, due to lack of awareness, this is not being done at present. Similarly, lack of development of cooperatives in the field of cooperative tourism is also a sign of weak advocacy. There is also lack of documentation of few successful models of cooperative tourism in the Region.

6. Rural cooperatives for rural tourism

The rural cooperative has worked in Iran After land reform in 1963. Today there are more than 10000 rural cooperative. However, their ability of these cooperatives is limited and the Iran government is still not doing considering rural tourism as one of the development factors for rural area. This paper attempted to outline the concept of rural tourism development in relation to rural cooperatives. Rural cooperatives have been cited as a goal in rural participation for rural development processes. Aref (2009) in his study recommended establishing tourism cooperatives to support the local people for investment in tourism development. According to his findings, the level of organizational capacity in tourism development in Iran is weak. Hence establishment of tourism cooperatives in Iran can boost tourism development. From this analytic perspective, rural cooperatives can be an effect on rural tourism development through three major capacity levels ; community, organizational and individual levels (Daniela, 2002). For rural tourism development the organizational level refers to a tourism organization; community context refers to informal groups bounded geographically; and individual context refers to people. In the organizational level, rural cooperatives can contribute to the rural tourism development through empowering the tourism organizational (Daniela, 2002). The individual level leads to community empowerment in the rural tourism development process through empowerment rural people. The individual level in this process plays out repeatedly, increased individual power toward rural tourism development. In considering the application of rural cooperatives in tourism development, the role of the rural leaders deserves consideration (Aref & Ma'rof, 2009). A leader frequently plays the importance role in these processes. Important goals of leaders with respect to rural cooperatives in tourism development would include facilitating; encourage participants, encouraging learning, and developing local skills in rural areas.

Figure 2 illustrates a conceptual model for how rural cooperatives can be an effect on three levels of community. The figure indicates overall

interaction between rural cooperatives and rural tourism development. Three community capacities level to have a vital role in this process. Through this model rural cooperative must play an active role in promoting tourism in the rural area through establishing strong networks with the tourism organizations, and fostering collaboration local people in this way (Verma, 2008).

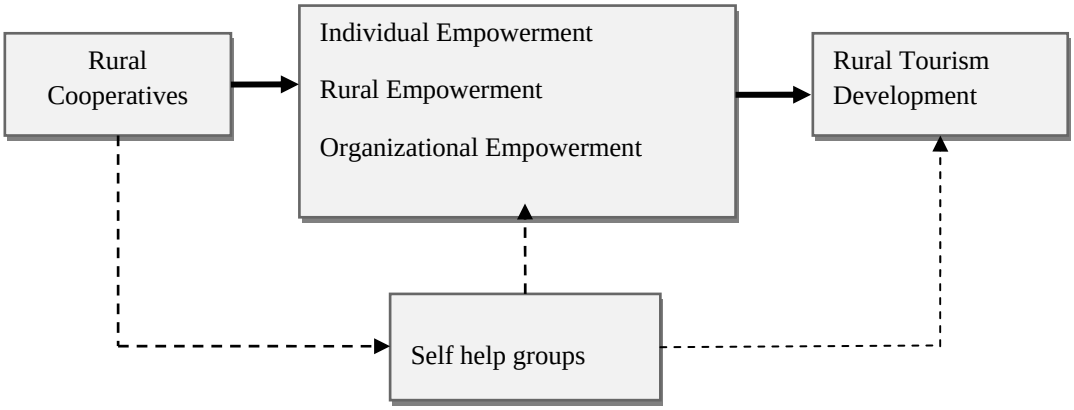

Figure 2: The interactions between rural cooperatives and rural tourism development

7. Conclusion

The main objective of the present paper is to determine status of the rural tourism in Iran. Rural tourism is considered to be a multi-dimensional activity essential to the local area not only rural areas in Iran, but all the nations of the world. However Iran has many potential in development of tourism especially rural tourism but development of rural tourism in Iran is still in its nascent stage. Iran has perfect opportunities to enhance its rural tourism. This paper showed a brief conception of rural tourism and its barriers in the rural areas of Iran. The main importance approaches which suggested in this study were development of rural cooperatives for rural tourism development. Thus rural cooperatives are a major critical success factor in rural tourism. Hence this study can be motivation for futures investigate in rural cooperatives for tourism development in the local areas in Iran.

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Impact of Human Disturbance on Forest Vegetation and Water Resources of Nainital Catchment

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Abstract: Nainital is a prime example of Lake Township that has been severely impacted by human activities owing to expansion of urbanization. The impacts of these pressures are felt in varied ways and from varied sources. During the few decades increasing local population 6903 (1901) to 38559 (2001) and the logarithmic increase in tourist influx into the watershed has effected the water resources and biodiversity of the area. A significant number of water resources have dried up in the past two to three decades. The present study is an attempt to document and relate the population rise and increase in the jungles of concrete in the past 50 years in the Nainital catchments to its impact on the forest cover forest density, biodiversity and water resources. Certain herb and shrub species that were abundant in the oak forests (*Q. leucotrichophora* and *Q. floribunda*) have now disappeared. In Nainital catchment area in un-disturbed sites the tree richness is 11, shrub richness is 19 and herb richness is 51, whereas in disturbed forest the richness is declining and is 7 for tree species, 19 for shrubs and 31 for herb species. The study is important as it indicates the changes that are occurring in forests that are degrading because of relentless biotic pressure. [Nature and Science. 2009; 7(10): 74-78]. (ISSN: 1545-0740).

Key Words- Anthropogenic pressure, Plant Richness, Catchment.

Introduction

Lake Nainital lies in a densely populated valley in the Kumaun Himalaya and is one of the most popular tourist resorts in Northern India. The existence of the lake Nainital was first reported by P. Baron in 1841. Nainital is a prime example of Lake Township that has been severely impacted by human activities owing to expansion of urbanization. During the few decades increasing local population and the logarithmic increase in tourist influx into the watershed has affected the water resources and biodiversity of the area. A key study in Himalayan hydrology is to assess the role of forests in maintaining the hydrological services. Scientific studies from Uttarakhand record many instances of accelerated soil erosion, landslide activities, increasing flood hazards and diminishing discharge in springs and rivers all associated with forest degradation and loss of forest cover (Valdiya, 1987).

In the Uttarakhand, mountains springs emanating from a variety of land use recharge zones are the main sources of fresh water for household consumption. Although spring water yield is largely a function of geological attributes, the land use and land cover are known to influence the spring water yield, water quality and longevity of spring discharge and season discharge patterns (Valdiya and Bartarya, 1991, Negi and Joshi, 1996). Therefore, each spring shows different discharge patterns. Geology rock type and anthropogenic pollution in the recharge area are also known to influence water quality and quantity. In recent decades, gradual drying up of these springs, low discharge during dry months and perennial spring becoming seasonal have been reported all across the region (Singh and Pande, 1989; Singh and Rawat, 1985). It has been observed that land cover change, biotic interference in the fragile watershed have caused soil erosion, depletion in soil organic matter and concomitant loss in the water absorption by soil resulting in a too much and too little water syndrome (Valdiya and Bartarya,1989).

The land use/ land cover of Nainital watershed and adjacent hills indicates that the hill resort is still substantially covered with forest. The total catchments area is approximately 5.70km^2 of which the built-up area is about 20% and is alarmingly increasing. Oaks (*Quercus leucotrichophora* and *Q. floribunda*), which are hardwood evergreen species make most of the forests. Only towards China Peak a conifer (*Cupressus torulosa*) dominates.

The present study is an attempt to document and relate the population rise and increase in the jungles of concrete in the last few decades in the Nainital catchments to its impact on forest biodiversity and water resources.

Material and Methods:

Nainital is located at 20º24´N latitude and 79º29´E longitude near the Main Boundary Thrust (MBT) that separates the Siwaliks from the lesser Himalaya. The elevation at Lake Level is 1938m and encompassing hills (7 in number) rise from 2139 to

2611m above the sea level. The basic pattern of climate is governed by the monsoon. The summer precipitation (June end to September end) brought by the monsoon accounts for 75% to 80% of the annual rainfall, which generally ranges between 200cm and 250cm. The mean temperature at Nainital (at 1938m altitude) ranges between 8ºC in January and 20ºC in June. Winter snowfall is common and some surrounding hills are cooler than areas around the lake.

In the present study the study was divided in two sections. To study the richness and diversity of the disturbed and undisturbed forests analysis was done following Curtis and McIntosh (1950) by placing 10 random quadrats of 10X10m for trees, 5X5m for shrubs and 1x1m for herb species. Data of Upreti (1982) on vegetational parameters collected in 1982 was referred for comparisons.

The data related to different form of anthropogenic pressure were collected from different government agencies and EERC report (2002). Data on spring and water recharge zones of Dr. G.L.Shah (unpublished) was used.

Result:

1. Morphological Features of Lake Nainital:

The morphometric features of lake Nainital are given in Table 1. Presence of 100m wide ridge in the middle divides the lake into two parts with different maximum depth. As a consequence the water of the two parts does not mix during thermal stratification (Rawat, 1987).

The tree layer, richness decline in 03 sites while in Nirmala convent there was no change in species richness (Table 2). There was sharp decline in density of individuals in 3 sites (Government house, St. Xaviers and China Peak). The total basal area was more or less similar in all the sites except St. Xaviers where it declines from 19.90m^2 /ha to 11.61 m^2 /ha. This decline could be due to the heavy construction at this site.

Shrub layer richness increases in government house site whereas in all other sites there was decline in species richness (Table 3). The density of shrubs also declines except at Nirmala convent site where it increases from 89.5indi/ha to 135.4indi/ha (Table 3).

As for herbs, over the period of two decades the species richness declined at three sites whereas in Nirmala convent site there was a slight improvement (Table 4). However the decline was marginal. The density of trees at Nirmala Convent site has increased significantly in the last two decades. Protection afforded to this site because of a boundary wall of the school appears to be the principal reason for this increased tree density.

3. Comparison of Status of Natural Springs and Recharge Zones Over a Period of Seven Decades-

In the Nainital catchments area total 8 water springs are present. A very special feature about these springs is that all these springs are situated in the altitudinal range of about 6100-7000ft. About seven decades before all these springs are perennial with enough water but now the situation is very critical. Of these totals eight springs now only three are perennial and remaining five are completely dry on with low discharge in the summer months. There are total 5 recharge zones of Nainital lake. But now due to heavy construction and development of car parking in these areas the recharge area has decline by 15 to 50%.

4. Effect of different forms of Anthropogenic Pressure on Nainital lake catchments.

There are four major types of anthropogenic pressures on Nainital lake catchment

(a) **Sedimentation and erosion**.- carbonate rock lithology, which is more susceptible to weathering, high precipitation and frequent landslides accounts for a higher sedimentation rate in Nainital lake (0.69cm/year) (EERC Report, 2002). Besides these natural factors heavy anthropogenic pressure like increased construction and construction based activities further increased it. Between 1895 and 1979 the mean depth of Lake Nainital has reduced from 21.43 to 18.55 m.

(b) **Human population**- The census of 2001 has estimated the permanent population in the catchment area of Nainital to be 3984. This indicates that nearly 100people/year was added during last decade. The catchment also hosts a large floating population of about 5000persons during the peak tourist season who mostly works as coolies, boatmen, horsemen etc.

(c) **Vehicular traffic**- Another indicator reflecting the increased tourism activity and anthropogenic pressure in recent past is the number of vehicles entering the town. The EERC (2002) data shows that the number of light vehicle that entered the town during the peak tourism month has increased by about 46% in pat three years (2000-2002). The revenue earned through toll tax in June has increased from less than 6 lakhs in 2000 to close to 8 lakhs in 2002. One of the implications of increased tourist vehicle pollution. This pollution is already being felt and could become a major problem to human health and plants.

Discussion

The lake systems of the region have served as centers of population. They provide a range of ecosystem services: supporting services, provisioning services, regulating services and cultural services. Change in these services affect human well being through impacts on security, the basic material for a good life, health and social and cultural relations (Cruz 2004). The threat posed to the lake region is owing to the eco-disturbances caused in the nucleus of the area i.e. Nainital town and its lake basin. Whatever happens in Nainital, whether it is increase in tourism or construction activity, it triggers a chain reaction else where. It is disturbing that the very existence of Nainital is threatened.

The natural resources in the lake region are being used erratically and ruthlessly due to increasing population pressure and resultant increase in demand for shelter, arable land, grazing area, fodder, fuel wood etc along with growing needs of tourism. It is therefore, increasingly realized that the formulation and implementation of the process of development planning in the region must be consistent with the natural resource base and its ecological productive potential (Rawat & Shah 2005).The goal of ecologically sustainable development with economically viable growth. Since land is the primary and fundamental natural resource and it is the basis of the genesis, management and sustainable development of all other natural resources, land management has acquired critical importance in this fragile region. The region, therefore, deserves specific attention and priority conservation measures for protecting the lake and their environment.

Table1. Morphological Features of Lake Nainital (Source: EERC Final Report 2002)

a. Maximum length (m)-	1423
b. Width (m)-	253-423
c. Maximum depth (m)*-	27.3 in northern half
	25.5 in southern half
d. Mean depth (m)-	18
e. Surface area (ha)-	48
f. Watershed area (km^2)-	5

Note- * the reason for giving maximum depth in the two halves is because the ridge divides the lake into two parts.

Table.2. Tree Layer Changes at four sites on the Northern and North-Eastern aspects of Nainital catchment (Source: Upreti, 1982)

Site	**1982**			**2004-05**		
	Species richness	**Density indi./ha**	**Total basal area m^2/ha**	**Species richness**	**Density indi./ha**	**Total basal area m^2/ha**
Government house	11	1434	48.48	6	1140	48.27
Nirmala Convent	4	348	36.20	4	630	37.79
St. Xaviers	7	605	19.90	5	260	11.61
China Peak	5	500	27.96	4	440	27.05

Table 3. Shrub Layer variation at four sites on the Northern and North-Eastern aspects of Nainital catchment (Source: Upreti, 1982)

Site	**1982**		**2004-05**	
	Species richness	**Density indi./100m^2**	**Species richness**	**Density indi./100m^2**
Government house	22	137.27	23	105.2
Nirmala Convent	10	89.5	7	135.4
St. Xaviers	13	143	7	65
China Peak	10	136.5	8	105

Table 4. Herb Layer variation at four sites on the Northern and North-Eastern aspects of Nainital catchment (Source: Upreti, 1982)

Site	1982	2004-05
	Species richness	Species richness
Government house	89	84
Nirmala Convent	24	25
St. Xaviers	42	36
China Peak	33	29

Table 5. Condition of Springs water in 1936 & 2007

Location of Spring	Altitude (ft)	Status in 1936	Current Status
1. Spring Field	6675	Perennial	Perennial
2. Chuna Dhara	6550	Perennial	Dry in summer
3. Near Spring Field cottage	6550	Perennial	Perennial
4. Rajpura	6525	Perennial	Low discharge (Feb. -June)
5 Near Lake View	6730	Perennial	Low discharge (Feb. -June)
6. Near Mount. Rose	6575	Perennial	Dry in summer
7. Near Bhabar Hall	6375	Perennial	Little water, still alive
8. Parda Dhara	6100	Perennial	Perennial

Table 6. Recharge areas of Nainital Lake in 1936 & their current status-

S.No.	Recharge area	Total area m^2	Current Status
1	Sukhatal	33369	A major car parking and settlement reduced the recharge area by approx 25%
2	Oak Park	13220	Area reduced by approx 35%
3	Sleepy Hollow	10872	Area reduced by approx 35%
4	Near Dalhausi villa	4597	Area reduced by approx 15%
5	Sherwood	4790	Area reduced by approx 50%, because of car parking

Table 7. Urban population and its growth trends in Lake Region
(Source: Census of India, 1991, series 25, Part ix-A& PCA 2001, vol4)

Year	Population	Percent growth
1961	16080	+22.8
1971	25167	+56.51
1981	26093	+3.61
1991	30951	+18.62
2001	39840	+28.72

Table 8 Anthropogenic Pressure in Nainital: A Comparison (Source Shah, 2007)

Indicators	Basedon Survey (1936-37)	Information as 2001
Permanent Population	10673 (1931 census)	39840
Tourist No.	--	3,10,000
Hotels No.	09	120
Shops No.	50 (estimated) in 4 markets	900
Residences No. (Bungalows, states, houses etc)	851	8000
Floating Population	---	5000 (estimated)
Others (Banks, Schools, Offices etc)	95	200

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PHYTOREMEDIATION OF CRUDE OIL CONTAMINATED SOIL: THE EFFECT OF GROWTH OF *Glycine max* ON THE PHYSICO-CHEMISTRY AND CRUDE OIL CONTENTS OF SOIL

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Abstract
The remediation of oil contaminated soils has been a major problem in oil producing countries and recently use of plants to clean such soils has been on investigation. In order to identify plants that can enhance the remediation of crude oil contaminated soil, the effect of the growth of *G. max* on the physico-chemistry and crude oil content of soil contaminated with different concentrations of crude oil was investigated in this study. The results revealed that the pH, moisture and organic matter contents of soils contaminated with crude oil were significantly affected by the growth of *G. max* at differently levels of significance ($P<0.001$, $P<0.01$ and $P< 0.05$). Crude oil loss was enhanced in soil with 25g crude oil in the presence of *G. max*. Although the growth of the *G. max* did not significantly affect the crude oil level in the 50g and 75g treatments, the soils became more favourable for plant growth as weeds sprouted from the contaminated soil vegetated with *G. max*. The implication of the findings of this study is that within 110 days, growth of *G. max* can lead to cleanup of crude oil contaminated soil and the reduction in toxicity of crude oil in soil. The ability of *G. max* to reduce the level of crude oil in oil polluted soil can help to restore polluted soils back for agricultural use. The high acceptability of *G. max* due to its high nutritional value, high adaptability and ease of propagation will make it an easy tool for remediation of soil contaminated with crude oil. [Nature and Science. 2009;7(10):79-87]. (ISSN: 1545-0740)

Keywords: Contamination, Crude oil, *Glycine max*, Cleanup, pH, Moisture, Organic matter

Introduction

Since commercial exploration of petroleum started in Nigeria in 1958 (Okoh, 2003), petroleum has continuously grown to be mainstay of the Nigerian economy. However, the exploration of petroleum has led to the pollution of land and water ways. The agricultural lands have become less productive (Dabbs, 1996) and the creeks and the fishing waters have become more or less dead (Okpokwasili and Odokuma; 1990; Odokuma and Ibo, 2002). Several civil unrests due to environmental degradation due oil exploration have also been witnessed in the Niger Delta region of Nigeria (Inoni *et al*., 2006).

The physical, chemical and thermal processes are the common techniques that have been involved in the cleaning up of oil contaminated sites (Frick *et al*., 1999). These techniques however have some adverse effects on the environment and are also expensive (Frick *et al*., 1999; Lundstedt, 2003). Recently, biological techniques like phytoremediation are being evaluated for the remediation of sites contaminated with petroleum. Phytoremediation is the use of plants and/or associated microorganisms to remove, contain or render harmful material harmless (Cunningham *et al*., 1996; Schwab and Banks, 1999; Merkl, 2005). It has been shown to be effective for different kinds of pollutants (contaminants) like heavy metals, radionuclides and broad range of organic pollutants (Schroder *et al*., 2002; Schnoor, 2002) According to Pivetz (2001), plants for phytoremediation should be appropriate for the climatic and soil conditions of the contaminated sites. Such plants should also have the ability to tolerate conditions of stress (Siciliano and Germida, 1998a). Njoku, *et al.,* (2008a) demonstrated that *G. max* germinates and grows in crude oil polluted soil. Also Frick *et al.* (1999) included *G. max* in the list of plants that can grow and remediate petroleum hydrocarbon contaminated sites. However, no record has shown that *G. max* can remediate crude oil polluted soil. It is therefore important to study the ability of *G. max* to affect the physico-chemistry (pH, moisture and organic matter contents) and the crude oil content of soil polluted with crude oil. The overall goal of this investigation was to evaluate the suitability of *G. max* for use in remediation of crude oil polluted soil.

The study is significant for some reasons. Firstly, phytoremediation has mostly involved the use of weeds (Aprill and Sims, 1990; Lee and Banks, 1993; Schwab and Banks, 1994; Qui *et al*., 1997; Banks *et al*., 2000). The use of food crops will improve the economic value of the technique (Van de Lelie *et al*., 2001). Secondly, although the conditions in the tropics favour phytoremediation, few researches have been carried on this technique in the tropics (Gallegos Martinez *et al*., 2000; Merkl *et al*, 2005a). There is the need therefore to evaluate the potentials of phytoremdiation in the tropics especially in Nigeria where pollution due to oil activities is high. In addition, the high nutritional value of *G. max* makes it acceptable by many and Njoku *et al*., (2008b) reported that *G. max* has the potential of growing in sandy loam soil, a soil type found in Niger Delta region of Nigeria.

Materials and methods

This study was carried out in the Biological garden of the University of Lagos, Akoka Lagos, Nigeria. The crude oil (Wellhead medium) was obtained from the SPDC Port Harcourt while the *G. max* was obtained from the Gene Bank Section of IITA Ibadan, Nigeria. The soil used is sandy loam soil and the treatments included 25g, 50g, and 75g crude oil mixed with 4000g of the soil filled in plastic containers. For each treatment, the control had no *G. max* grown on it. Both the treatments and the control were replicated thrice. Seven seeds of *G. max* were sown into each of the containers at 2cm depth and the containers were moderately watered regularly to keep the soils moist.

Soil samples were collected at the surface and 15cm depth from each container every 21 days (3 weeks) for 105 days (15 weeks). The collected soil samples were used to investigate the effect of *G. max* on the pH, moisture and organic matter contents of crude oil polluted soil. The soils from the surface and 15cm depths were usually mixed together and the mixture used for the study of the above physico-chemical features. The soil samples used in the study of the effect of G. *max* on the crude oil content of the soil were collected on the 110th day of sowing of the seeds of *G. max* in the soils.

The pH of the homogenized soils was determined following the protocols outlined by Eckerts and Sims (1995). The soils were air-dried and sieved to remove large particles and debris. 5g of the sieved soils were mixed with 5mls of distilled water and stirred very well after which mixture was allowed to stand for 30 minutes. The electrode of a pH meter was put into slurry of the soil-water mixture and the pH of the soil was read off. The moisture content of the soil samples was determined according to the method of Schneekloth *et al*. (2002). The procedure of Schulte (1995) was used to determine the organic matter content of the soil samples.

The amount of crude oil the soil samples was determined using air-dried soils that were sieved through 1mm mesh. The crude oil in the soil was first extracted with n-hexane by shaking with a mechanical shaker for 30 minutes as was described by Okolo, Amadi and Odu (2005). The soil-crude oil-n-hexane mixture was filtered into a beaker of known weight through a Whatmann No.1 filter paper. The crude oil content of the filtrate was determined after heating the beaker at 40^{o}C to a constant weight (Merkl, Schutze-Kraft and Infante, 2005b). The amount of crude oil lost from the soil was determined as the amount of crude oil added to the soil minus that in the soil at the time of analysis.

The effect of *G. max* on the pH, moisture, organic matter and crude oil contents of the soils was determined by comparing each parameter in soil with *G. max* with that in soil with *G. max.* Statistical analyses of the data obtained were done using Graphpad Prism 5.0 package using a 2 way ANOVA followed by Bonferroni posttests at 5%, 1% and 0.1% significance levels. Correlation analyses were also carried out.

Result and Discussion

The growth of *G. max* generally reduced the acidity of the crude oil polluted soil. However on days 21 and 42, the growth of *G. max* led to increase in the acidity of crude oil polluted soil (Table 1). On days 21 and 63, the pH of the control differed significantly from those of soil with 50g crude oil and *G. max* ($t = 2.701$ for day 21 and $t = 3.696$ for day 63) and those of the soil with 75g crude oil and *G. max* ($t = 2.985$ for day 21 and $t = 3.838$ for day 63). Negative correlations exist between the pH of soils with *G. max* and soils without *G. max* for each concentration of crude oil ($p = 0.350$, 0.083 and 0.683 for 25g, 50g and 75g crude oil concentrations respectively). A perfect positive correlation exists between the pH of the soil with

50g crude oil and the soil with 75g crude oil (p = 0.017) while no correlation exists the soils with 25g crude oil and *G. max* and 75g crude oil and *G. max*.

The positive correlation between the pH of the soils and the amount of crude oil added to the soil may be an implication that crude oil pollution leads to increase in soil pH. This is similar to the findings of Andrade *et al*. (2004) and Ayotamuno *et al.* (2004) who observed increase in the pH of soils polluted with crude oil. In the opinion of Dibble and Bartha (1979), the higher pH of soils with *G. max* than in soils without *G. max* means that higher degradation of crude oil took place in soil with *G. max* than in soils without G. max. The trend of the pH over the period of studies was against the expectation going by the reports of Ayotamuno *et al.* (2004) and Merkl *et al*. (2005c). These researchers reported that the pH of soils decreased as a result of degradation of crude oil. This decrease in the pH of soil with degradation of crude oil could be due to accumulation of organic acids produced during degradation in the soil (Merkl *et al*., 2005a) or the production of acid radicals through nitrification (Tisdale and Nelson, 1975). However, since soil bacteria thrive better in neutral than in acidic soils (Song *et al*., 1986; Phung, 1988), the increase of the soil pH towards neutral condition means more favourable conditions for soil bacteria. Many researchers have reported that bacteria play good role in the degradation of crude oil (Atlas and Bartha, 1977; Amund and Igiri, 1990; Frick *et al*., 1999; Van Hamme, Singh and Ward, 2003). This means that as observed in this study, growth of *G. max* can enhance the bacteria population in crude oil polluted soil and thereby lead to higher degradation of crude oil in the soil. The continual increase in the soil pH as the period of the study increased means that there was continual increase in favourable conditions soil bacteria and for biodegradation (Dibble and Bartha, 1979).

Table 1: The effect of *G. max* on the pH of crude oil polluted soil. Values are means ± standard error of three replicates

Days of sampling	Control	25g	25g and *G. max*	50g	50g and *G. max*	75g	75g and *G. max*
21	4.73±0.233	5.30± 0.115	5.37±0.067	5.87±0.186c	5.37±0.067a	5.50±0.153b	5.60±0.153a
42	5.03±0.176	5.30±0.173	5.23±0.067	5.57±0.145	5.37± 0.033	5.07±0.120	6.23±0.433□
63	5.07±0.088	5.17±0.067	5.33±0.333	5.37±0.233	5.77±0.176a	4.97±0.088	5.97±0.240a□
84	5.03±0.067	5.13±0.088	5.37± 0.067	5.37±0.203	5.80±0.231	4.97±0.133	5.97±0.186□
105	5.00±0.115	5.13±0.033	5.37±0.120	5.37±0.233	5.87±0.233	4.97±0.088	5.97± 0.203□

Note: a = significant difference between treatment and control at p<0.05 significant difference between treatment and control, b= significant difference between treatment and control at p<0.01, c = significant difference between treatment control at p<0.001, * = significant difference between soil with *G. max* and soil without *G. max* at p<0.05 ,+ = significant difference between soil *G. max* and soil without *G. max* at p<0.01, □= significant difference between soil *G. max* and soil without *G. max* at p<0.001

The growth of *G. max* in soils polluted with 25g crude oil led reduction of the moisture content of the soil. The reverse was the case for the soils with 75 g crude oil. In the case of the soils with 50 g crude oil, the growth of *G. max* led to reduction of the moisture in the first 42 days and afterwards the growth of *G. max* enhanced the moisture content of the soil (Table 2). The

control has positive correlation with the treatments and there is a positive correlation among the treatments.

Crude oil pollution causes among other things low permeability and low infiltration of water into the soil (Hutchinson *et al*., 2001; Andrade *et al*., 2004). These conditions can lead accumulation of water on the soil surface and an artificial drought in the subsurface layer of soil. This can lead to difficulty for the roots to absorb water and nutrients which in the water as the roots usually grow deeper into the soil subsurface layers. The growth of plant root into soil help to create pores in the soil and thereby enhance water penetration and infiltration in soil polluted with crude oil. This increased water penetration and infiltration could be the cause of low moisture contents of soil contaminated with 25g crude oil and that had *G. max* grown on it as observed in this study. This can help to eliminate water logging of crude oil polluted soil and can lead to increased aeration of the soil. The increased aeration can lead to increase in the activities aerobic microbes in the soil and this can lead to increase in the degradation of oil.

Since the phytotoxic effect of crude oil increases with the concentration of the crude (Cullie and Blanchet, 1958), the higher moisture content of the soil with 75g crude oil and *G. max* than in the soil with 75g crude oil and no *G. max* could be due to inhibition of root growth by such amount of crude oil. The inhibition of root growth can lead to low penetration of water and higher accumulation of water on the soil surface. Reduction of transpiration is one of the phytotoxic effects of crude oil (Baker, 1970). The reduction in transpiration also affects the rate at which water absorption and uptake as these are controlled by transpiration pull (Taylor *et al*., 1997; Kent, 2000). Therefore higher moisture content in the soil with 75g crude oil and *G. max* than in soil with 75g crude oil and no *G. max* could be attributed to reduced loss of water due transpiration and subsequent reduction in the rate of water absorption in such soil. A possible cause of the difference between the trend of moisture content in the soil with 25g crude oil and soils with 50g and 75g crude oil is that because better growth of *G. max* in soil with 25g crude oil led to more absorption of water from the soil than from soils with 50g and 75g crude oil.

Table 2: The effect of *G. max* on the percentage moisture content of crude oil polluted soil. Values are means ± standard error of three replicates.

Days of sampling	Control	25g	25g and *G. max*	50g	50g and *G. max*	75g	75g and *G. max*
21	13.04±0.211	13.50±1.381	12.24± 0.701	9.97± 0.573	10.31± 0.693	5.20±1.743	12.59± 0.763□
42	11.06±0.647	13.59±0.935	12.42± 0.739	10.05±0.427	11.12±1.832	3.34±0.975	13.32± 0.978□
63	13.16±0.230	14.03±0.420	13.06± 0.502	12.41±0.290	15.41±0.188	8.19±1.236	15.85± 0.593□
84	13.43±0.578	14.05±0.677	13.12± 0.430	12.74±0.133	15.52±0.133	8.16±1.196	15.97±0.477□
105	15.06±0.920	14.07±0.580	14.40± 0.534	13.66±1.420	16.45±0.423	8.86±0.700	14.84±0.629□

Note: a = significant difference between treatment and control at $p<0.05$, b= significant difference between treatment and control at $p<0.01$, c = significant difference between treatment control at $p<0.001$, * = significant difference between soil with *G. max* and soil without *G. max* at $p<0.05$,+ = significant difference between soil *G. max* and soil without *G. max* at $p<0.01$, □= significant difference between soil *G. max* and soil without *G. max* at $p<0.001$

The organic matter content of the soil was reduced by the growth of *G. max* in the first 42 days (Table 3). This might be due to the use of growth of *G. max* in the first 42 days might be as a result of the use of the organic matter by the *G. max* as it grew. Since the plants were in their early growth stages, they could possibly be absorbing nutrients from the soil and returning

little or none to the soil. Such could have caused lesser accumulation of organic matter in the vegetated soil than in non-vegetated soil. Ayotamuno *et al*. (2004) reported similar observation of lower organic matter contents in vegetated soil.

From day 63, the growth of *G. max* enhanced the accumulation of organic matter in the soils. The observed higher organic matter accumulation in vegetated soil as from day 63 has some interpretations. Firstly, it is possible that *G. max* started shedding its leaves from after the first 42 days and the decomposition of such leaves increased the organic matter content of the vegetated soil more than that of the non-vegetated soil. The release of organic carbon to the soil due to degradation of crude oil possibly led to accumulation of more organic matter in the vegetated soil than in the non-vegetated soil. This is because organic carbon is a major component of organic matter (Okolo *et al*., 2005). Also the fixation activities in the root nodules of the plant also had a possible impact on the amount of organic matter accumulated in the vegetated soil.

The organic matter content of the soils has negative correlation (p = -0.237) with the days of sampling and positive correlation (p = 0.767) with the amounts of crude oil added to the soil. This means that while the organic matter contents of soil polluted with crude oil decreases with time, it increases with the quantity of crude oil added to soil. Apart from the 25g treatment, the soil organic matter was significantly affected by the addition of crude oil and growth of *G. max* at different levels of significance (P<0.001, P<0.01, P<0.05) for the different days of study. The growth of *G. max* however did not produce any significant effect on the organic matter content within each concentration of crude oil. There was negative correlation between the organic matter content of the control and the treatments. The 25g and 50g treatments have a perfect correlation (± 1) and same applies to the 25g and *G. max* and 50g and *G. max* treatments.

Table 3: The effect of *G. max* on the percentage organic matter content of crude oil polluted soil. Values are means ± standard error of three replicates.

Days of sampling	Control	25g	25g and *G. max*	50g	50g and *G. max*	75g	75g and *G. max*
21	0.89±0.118	1.95± 0.529	1.55±0.041	2.47±0.176	2.14 ±0.284	3.16±0.180	2.54± 0.170□
42	1.29±0.651	1.53± 0.073	1.15±0.367	2.22±0.115	1.65± 0.103	2.70±0.306c	2.21± 0.111□
63	0.90±0.096	0.96± 0.060	1.14±0.042	1.67±0.111	1.63± 0 .071	2.05±0.140c	1.97± 0.119□
84	0.91± 0.096	0.99± 0.018	1.29±0.168	1.70±0.124	1.66 ±0.100	1.87±0.204c	1.98± 0.127□
105	0.91± 0.142	1.35±0.066	1.33±0.123	1.88±0.140	1.78 ±0.061	1.91±0.228c	1.95± 0.030□

Note: a = significant difference between treatment and control at p<0.05, b= significant difference between treatment and control at p<0.01, c = significant difference between treatment control at p<0.001, * = significant difference between soil with *G. max* and soil without *G. max* at p<0.05 ,+ = significant difference between soil *G. max* and soil without *G. max* at p<0.01, □= significant difference between soil *G. max* and soil without *G. max* at p<0.001.

The effect of crude oil the pH, moisture and organic matter content of soil observed in this study conforms with the reports of Njoku *et al*., (2008c) that these change with addition of crude oil to soil. Soil pH, soil moisture and soil organic matter contents have influence on the soil

properties. The organic matter content of soil improves the binding processes in the soil. Such binding reduces water drainage and improves water retention ability of soil. Therefore the low organic matter in soil with 25g crude oil could be a cause of the low water accumulation in that soil. Excess binding of the soil particles together reduces root penetration and inhibit the absorption of materials. This can lead to malnourishment of plants even in the presence of abundant nutrients.

The amount of crude oil lost from the soil contaminated with 25g crude oil was enhanced by the growth of *G. max*. However in soils with 50g and 75g crude oil, more crude oil was lost from soils without *G. max* than in soils with *G. max* (figure 1). It is however worthy to note that in this study weeds were observed to have sprouted out from the contaminated soils with *G. max* and none of such was observed in the non-vegetated soil. This shows that even though the growth of *G. max* did not produce any significant effect on the percentage of crude oil lost from the soils the plant can reduce the quantity and toxicity of crude oil in soils. This is shown by the lesser amount of crude oil left in soil with 25g crude and *G. max* than in soil with same amount of crude oil and no *G. max* and the sprouting of weeds from the soils with 50g and 75g crude oil and *G. max*. The sprouting of the weeds indicates that the toxicity of crude oil in the vegetated soils reduced to the extent of allowing for the growth of weeds in such soils. This confirms the findings of Siciliano and Germida (1998a) that plants may not reduce the concentration of contaminants and yet can reduce the toxicity of such contaminants. For example, Siciliano and Gemida (1998a), observed a reduced toxicity of 2,3-dichlorobenzoic acid and 3-chlorobenzoic acid without reduction in the contaminant concentration in vegetated soil. The reduction is also a mechanism of phyoteremediation going by the definition of phytoremediation as a technique of rendering harmful materials harmless using plants and their associated microbes (Cunningham *et al*. 1996; Pivetz, 2001). Conversely, the absence of such weeds from soils without *G. max* indicates that the soil has not reach the level that will enable plants to grow.

The effect of *G. max* on the removal of crude oil from the soil polluted with 25g crude oil is similar to the findings of Aprill and Sims (1990), Lee and Bank (1993), Schwab and Banks, (1994) and Merkl *et al*. (2005b) who reported higher degradation of petroleum hydrocarbon in vegetated soils than in non-vegetated soil. The higher removal of crude oil observed in this study conforms with the reports of Frick *et al*. (1999) who listed *G. max* as one of the plants that can remediate petroleum hydrocarbon (anthracene) polluted soil. It also conforms with the suggestions of Njoku *et al*. (2008b) who suggested that *G. max* can be tried for its efficacy to remediate crude oil polluted soil. The removal of crude oil by *G. max* possibly occurred through one of the several mechanisms of phytoremediation. Such mechanisms include polymerization of the contaminants (Adler *et al*., 2004), interaction of the plant with fungi and bacteria (Siciliano and Germida, 1998) and production of root exudates and plant materials which serve as source of carbon, nitrogen and phosphorus for petroleum degrading microbes (Horvath, 1972; Rajaram and Sethunathan, 1975; Alexander, 1977; Smith, 1990; Burken and Schnoor, 1996). Nitrogen fixed in the soil by legumes reduces plant/microbes competition for nitrogen and thereby increase plant growth exudates production. This increases the ability of plants to increase the degradation of pollutants.

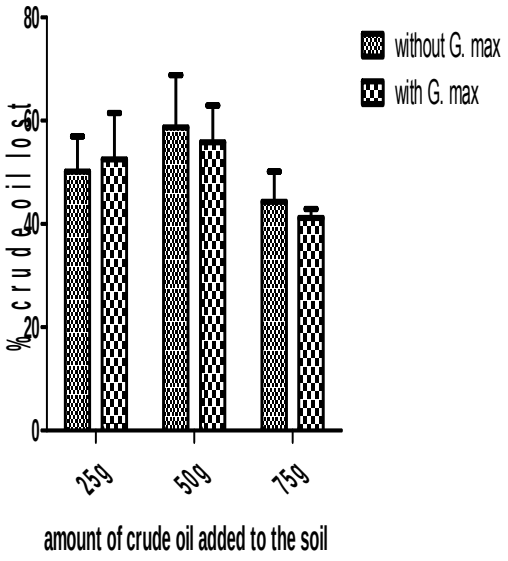

Figure 1: The effect of *G. max* on the removal of crude oil from polluted soil. Values are means ± standard error of three replicates.

Conclusion

The findings of this study indicate that growth of *G. max* in crude oil contaminated soils affects the physico-chemistry of the soil enhancing the degradation of crude oil. For instance, the significant effect that the growth of *G. max* produced on the pH and moisture content of the soil with 75g crude oil indicates that *G. max* affects the physico-chemistry of crude oil contaminated soil. It can also be inferred from the findings of this study that the growth of *G. max* in crude oil contaminated soils reduces the toxicity of crude oil in the soil. This is going by the sprouting of weeds in the soils with *G. max* and none of such soils without *G. max*. We suggest that to soil augments like cow dung should be added to crude oil contaminated soil to enhance the increase the efficacy of using *G. max* in remediating crude oil contaminated soils as Njoku *et al.* (2008a) have reported that addition of cow dung to crude oil contaminated soils enhances the growth of *G. max* in such soil.

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