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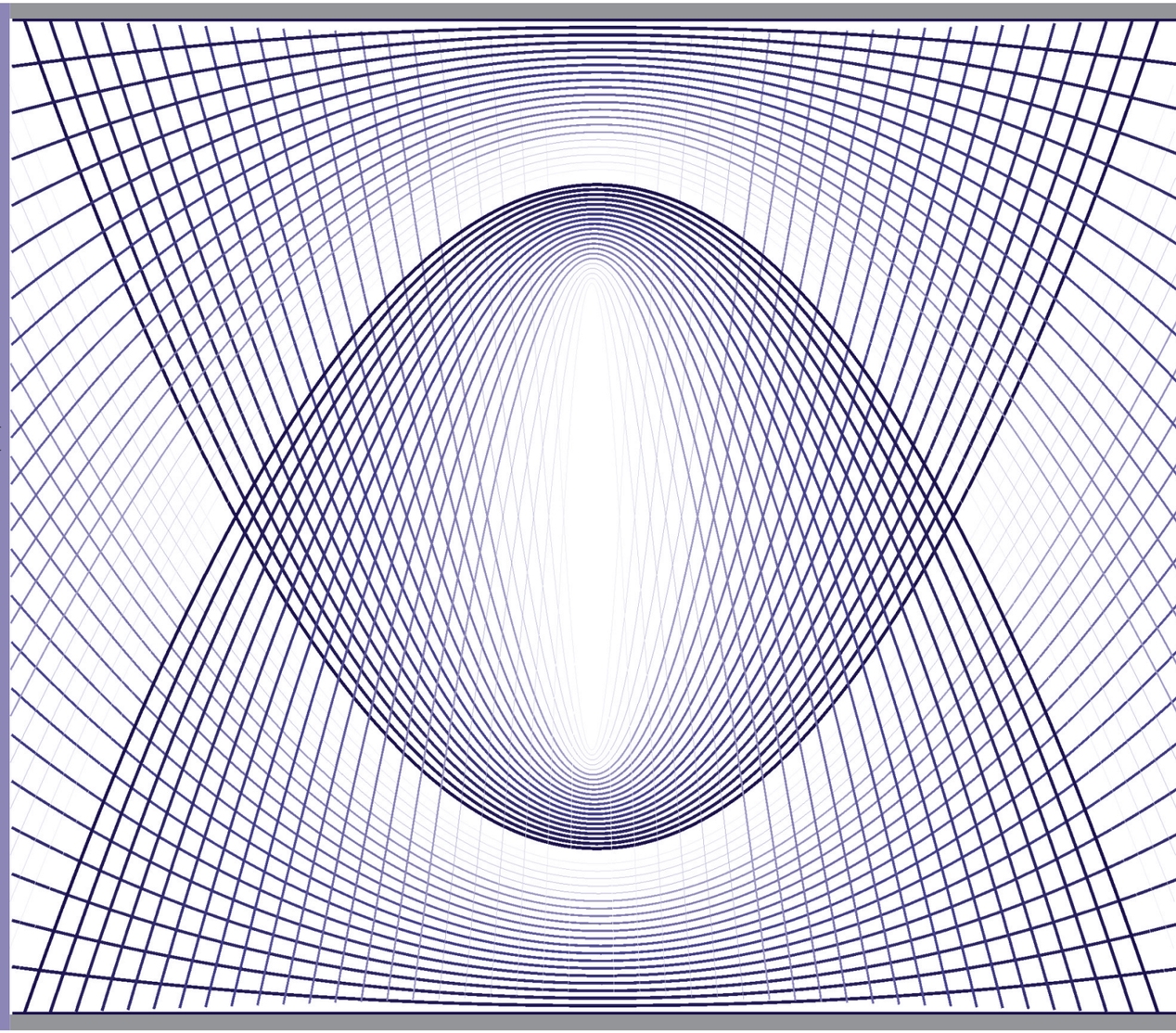

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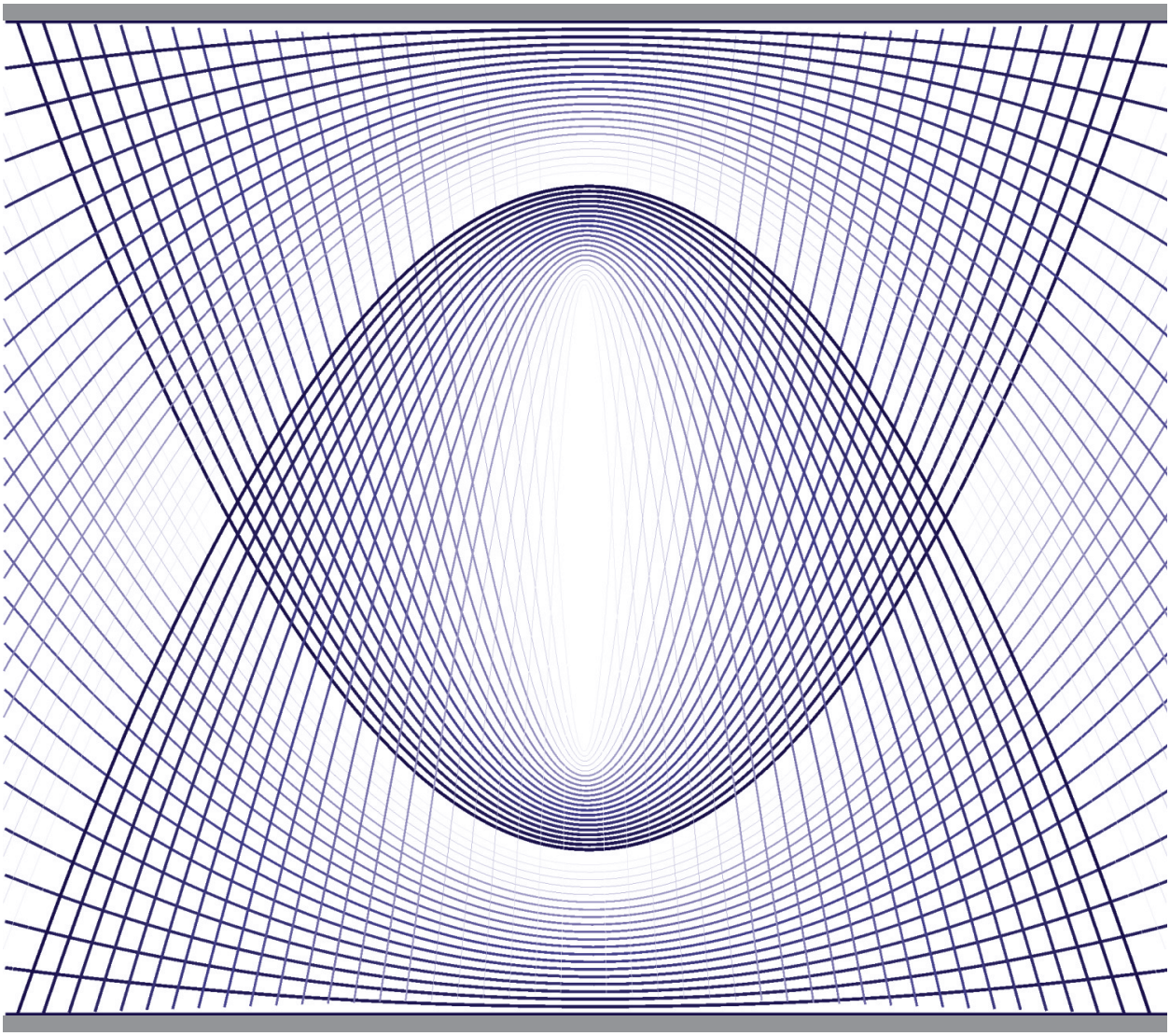

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CONTENTS

The Effects of Time Budget Pressure on the Behavior of Internal Auditors

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Abstract: Every auditing instiute has to regulate and schedule timed budget of auditing operation as one of its current plans. Preparing an unreasonable and unsuitable time budget will lead to failure in achieving the defined goal in the predefined time; and these will give rise to unprofessional behaviors of the auditors. In such a case, the auditors' deviation from the predefined time table will look natural and this can lead to decrease in the quality of auditing. Among the most important unprofessional behaviors one can point to underreporting the real audit time and audit signoff without informing the superior manager. The results of this research show that internal auditors commit unprofessional behaviors against the pressures of time budgeting. Additionally, the pressures of time budgeting cause unsuitable work pressure on internal auditors.
[Behzad Teimouri, Zahra Rahmati, Bahman Gholami. **The Effects of Time Budget Pressure on the Behavior of Internal Auditors.** *Academ Arena* 2012;4(8):1-7] (ISSN 1553-992X). http://www.sciencepub.net/academia. 1

Keywords: Time Budget, Time Budget Pressure, Unprofessional Behaviors, Unsuitable Work Pressure, Underreporting Real Work Time

Operational definition of the terms

Time budget of the work: the anticipated time needed for doing the work;

Time Budget Pressure: the time needed for doing auditing operation is more than the anticipated time in the budget while the auditor has to do the work in scheduled time;

Underreporting real work time: when the time spent for doing auditing operation is more than the time budgeting but the real audit time is being underreported for the sake of preserving time budget;

Audit signoff: when some stages of auditing is not being done without the permission of the superior manager but these steps are being reported in the worksheets (Mehrani, 2000);

Unsuitable work pressure: the pressure that cause unprofessional behaviors in auditors during auditing operation;

Unsuitable time budgeting: the time needed for doing auditing operation but is calculated and assessed in an unsuitable and unusual way (Hashemian, 2009);

Unprofessional behaviors: auditors' feelings and interests may lead to tendencies and actions in order to ignore budget controlling system and reaching scheduled time. Such behaviors that make the auditing plan and its scheduling distorted and cause the deterioration of auditing quality are called unprofessional behaviors.

Internal auditing: is an activity in the organization in order to evaluate other internal controls and offering good suggestions for optimized using the resources (Azad, 2005).

1. Introduction

In auditing profession, using time budget is quite common. Such a use can make the ouditing operation quality and auditing scheduling controlled in order to improve the efficiency and desirable programming for the next projects. If the time budget is defined in a good and suitable way, reaching the mentioned advantages will be accessible and even secured. When the access to budget in the performance appraisal is effective and important on one hand and the time budget is defined in an unsuitable and unusual way at the other hand, then the over-emphasis on the observing the time budget will cause the auditors show reactions and behaviors that are called unprofessional behaviors in auditing. Such unprofessional behaviors will lead to the decrease in the auditing quality. Some unprofessional behaviors are as follow:

1. Deleting some stages of the auditing
2. Doing the work in personal times without reporting the sent time
3. Illogical shortcutting of some auditing instructions
4. Shortage in needed follow-ups
5. Over-reliance on the employers' explanations

Budgeting in its advanced form is the same as programming. Nowadays, budget is the reflection of different programs planned to reach different goals. In today's competitive environment, the life of the organizations depends on the suitable programming and controlling and supervising the performances. Two terms of programming and control encompass a

single concept that is a specified method of doing the tasks. It can be claimed that the most important factor of any organization is programming and quality control In this regard, budgeting and the process of adjusting the budget is the heart of management control systems (Mehrani, 2000).

Organizational complexities and the extension of commercial deals in most countries has caused the managers of beneficiary and non-beneficiary agencies create a separate department for internal auditing in order to reach the organizational goals, and being sure about the optimal conduct of all resources. Internal auditing is an activity that is being done in any organization to evaluate other internal controls and to offer good suggestions for optimally use of the resources. Indeed, internal auditing is a sort of management consultation.

Internal auditing as an inseparable component of the organizational controlling structure, and the internal auditors as the arm of the managers, evaluate internal controls and help the managers of the organizations to do their responsibilities in the most effective and most efficient ways; hence the internal auditing helps in-organizational persons in particular and out-organizational persons in general. The responsibility of this department is to evaluate and assess the financial controls, observational controls, and operational controls. The role of internal auditing in each of these three roles is very important for the organization.

In each of these three positions, internal auditors offer practical suggestions on the process of financial, observational, and operational auditing and thus can fulfill the optimized usage of the organization's resources, both at micro and macro levels, by a controlling deterrent mechanism or by discovering the errors.

In recent years, the profession of internal auditing has been considerably developed in many countries. The theoretical documents show that the activities of this profession in Iran are restricted in comparison to many developed or developing countries.

Using time budget for programming and controlling is quite common in internal auditing. The objectives and the advantages of time budgeting in auditing can be counted as follow:

1. Time scheduling of the work of auditing and auditors
2. Quality control over the auditing operation
3. Increasing the motivation of the auditors to improve the efficiency
4. Desirable programming for doing coming projects
5. Annual professional evaluating the auditors

2. Literature review

On 1974, American Accounting Association (AAA) established a commission to study about the legal responsibilities of the auditors (known as Kohen Commission). The report of the Commission published on 1987 and contained the results of John Road studies in which he wrote that %68 of CPA auditors who have recently joined this profession, and more than %60 of the auditors who have recently left the profession, had some sort of signoff from their auditing program without the permission of their superior manager but had reported doing the program. Additionally, %55 of the respondents had done the auditing operation in their personal times without reporting the spent time.

In his research, he found that the pressure of time budgeting and the belief of the auditor about the lack of importance and necessity of some stages of auditing are the most fundamental reasons of such behaviors. As he states, other reasons include: auditor's lack of experience, weak supervision, and auditor's exhaustion and carelessness. He shows that such behaviors in local and regional auditing companies are more than big companies (quoted by Naeimi, 2003).

In 1982, Alderman and Detrik conducted a research whose results show that the occurrence of such unprofessional behaviors is not unusual for the auditors. They found that despite the results of previous researches, unprofessional behaviors can be found in big companies as well. Besides, the occurrence of such behaviors at the level of auditors and their assistants is more than the other levels. Moreover, they believe that the results of previous researches may underestimated the real unprofessional behaviors of the auditors because the previous researches have not dealt with the behaviors of the non-CPA auditors or the auditors who have less than one year experience (Alderman and Detrik, 1982).

The results of another research by Litner and Liznering (1983) showed that the pressure of time budgeting is one of the most important effective factors on the auditors' behavior. They found that receiving expected rewards and requesting a headman for observing the time budget, are the most important factors in underreporting the real time of doing auditing work (Litner and Liznering, 1983).

Kely and Margim (1987) conducted another research on the occurrence of unprofessional behavior and its relationship with the type of auditing contract (fixed and non-fixed contract). They believe that the highly competitive environment in providing audit services and the relying on tender method will lead to a considerable decrease in the wage for auditing. The auditing institutes thus have to decrease

their own costs at an acceptable level in order to preserve their benefit margin. This is the first reason for reducing the time budget and making the pressure on the auditors. Such pressure will deteriorate the quality of the audit, though such a low quality is not discoverable in short time. The results of the mentioned research showed that the occurrence rate of unprofessional behaviors in fixed contracts is higher than the unfixed ones (Kely and Margim, 1987).

Azad has conducted two researches on the budget pressure and the occurrence of unprofessional behaviors in internal auditing. The results of the both researches showed that the occurrence of unprofessional behaviors has existed in the internal auditing as well; and the main causes of such behaviors was the pressure of time budgeting, lack of necessity of some stages, and lack of sufficient supervision (Azad, 1994).

In Iran, Sasan Mehrani (2000) conducted a yet another research titled "the impact of Time budget pressure on the behavior of independent auditors on the basis of relative justice theory". The results of his research showed that the occurrence of unprofessional behaviors can be found in Iran as well as other countries; and the main reason of the behaviors is the Time budget pressure, the pressure imposed by the project manager, the lack of sufficient supervision, and the lack of paying enough attention some stages of the auditing. Additionally, he found that the highest rate of unprofessional behaviors can be found in behaviors like ignoring some stages of the audit, and the lack of following up some other stages. Besides, the occurrence of unprofessional behaviors in studying the structure and preserving internal controls is more than other cases like inventory and commodity.

Another research by Naeimi (2002) dealt with the impacts of the Time budget pressure on the behavior of independent auditors under the moral theory. The results of the research showed that the auditors invoke different reactions against such phenomenon despite their own belief. Such reactions that are known as unprofessional behaviors deteriorate the quality of the auditing directly or indirectly. The decrease of the quality will inevitably damage the auditing institute. Such damage is especially stronger when considering the time budget is effective on the auditors' evaluation.

Hashemian (2009) conducted another research titled "the effects of Time budget pressure on the behavior of independent auditors under motivational theory in Tehran". The results of this research revealed that independent auditors are affected by the Time budget pressure and consequently they commit unprofessional behaviors and thus the quality of their audit will be reduced.

Jesse C. Rabertson administered a research entitled "the impact of superior authorities and information resources on the judgment of the auditors under the pressure of specific time budgeting". The results showed that the auditors are not affected by the impact of superior authorities. Besides, the auditors are willing to the evaluation of the executive management less than the authorities and managers; and they are not directed by their prejudices for the coming decisions of economic authorities and other authorities. He has suggested that the able and talented auditors have not to show undesirable behaviors at the level of the employees (Jesse C. Rabertson, 2005).

Another research was conducted by Gregory and Shoan (2007) on "the effects of time budgeting on New Zealand auditing". The results showed that bigger companies are under the pressure of time budgeting more than small companies. This research is very useful for learning the budgeting in auditing institutes and the involvement of the employees at different levels and for important decision-makings, and the evaluation of such decisions being operationalized by the employees (Gregory and Shoan, 2007).

In 2008, Shion, Taylor and Gregory conducted a research entitled "the pressure of time budgeting and the behavior of ordinary auditor in comparison to job stress model". The results of this research revealed that time budgets affect the behavior of ordinary auditors. This effectiveness can be due to the specific relationship between the ordinary auditors and their superior auditors because ordinary auditors work for the superior ones. The results also showed that there is a reverse relationship between the benefits of employees and the capability of reaching time budgets of the auditing. Although the capability of reaching the budget by participation has a positive effect on the time budgeting process, but this capability has been spent for the real time of the work in previous year. Moreover, there is a relationship between the capability of reaching time budget and the behavior of ordinary auditors (Shion, Taylor and Gregory, 2008).

Finally, Arnold (2010) conducted a research on "supporting internal auditors in the decision-makings" the results of Arnold's research showed that the independent auditors can rely on internal auditors in analyzing the professional standards of the decision-makings. Additionally, the results of the mentioned research revealed that the companies can rely on the internal auditors in their executive affairs and in doing evaluations. In this research, the management maximally relied on the internal

auditors and strengthened their auditing roles in order to reduce the cost of external auditing. Besides, it got clear that the role of internal auditors is not merely limited to the evaluation, but the management has to follow the auditors' opinions in its decisions. The relative importance of 6 factors of Arnold's research shows that the experience of internal auditors can be very helpful in rejecting false opinions, evaluating audit documents, the importance of changing the amounts of financial lists, or the auditing process itself (Arnold, 2010).

Hypotheses

This research includes 5 main hypotheses as follow:

1. Unsuitable time budget will cause work pressure on the internal auditors.
2. Time budget pressure negatively affects the auditing operation.
3. Time budget pressure will cause ignoring some stages of auditing by the internal auditors.
4. Time budget pressure will cause underreporting real time of the work by the internal auditors.
5. The increase of Time budget pressure will increase the occurrence of unprofessional behaviors.

4. Methodology

This research is an experimental- survey that gain and analyze the opinions of internal auditors and then analyze in order to study the effects of the time budget pressure on the behavior of internal auditors. The method of this research is based on field study, and as mentioned before, it deals with the reactions of internal auditors in facing time budget pressure. To do so, the researcher referred to the standpoints of internal auditors of auditing institutes who are trusted in Tehran Stock Exchange Organization.

In this research, the questionnaires were adjusted on the basis of the research hypotheses. The questionnaires were collected from the internal auditors who worked in auditing institutes.

4.1. Statistical population and sample

The statistical population of the research includes the internal auditors who worked in auditing institutes that were trustworthy in Tehran Stock Exchange Organization. The names of the mentioned auditors were extracted from the local journals, taxation districts, internet websites, etc. According to mentioned list, there were around 100 auditing institutes till the end of 2010. Since the previous researches on the independent auditors under the justice, motivational, and moral theories with statistical sampling, in this research we considered all auditing institutes (trustworthy in Tehran Stock Exchange) as the statistical population. Since there are always difficulties in responding, some institutes were not willing to receive the questionnaire and some of them failed to return back the questionnaires.

Anyway, totally 161 questionnaires were distributed between March to May 2011 among which 141 questionnaires were picked and 121 questionnaires were accepted.

One of the advantages of this research is that around all the statistical population were subjects of the questions. If there were active internal auditors in Iranian companies during the financial period, it would be better to conduct the research among the companies who are officially accepted in Tehran Stock Exchange; but since there is no active internal auditor in Iranian commercial, production, and service companies, this research has been conducted among the auditing institutes that are trustworthy in Stock Exchange.

The percentage of sending back the questionnaires were approximately %88 (141 out of 161) and the percentage of accepted questionnaires for the statistical analysis was approximately %86 (121 out of 141). In order to be sure about the respondents' awareness and knowledge, the criteria of more than one year of experience was regarded as the selection criterion.

To gain the agreement of the respondents to fill the questionnaire, the researcher explained the nature of the research to the subjects, and if they were not willing to fill the questionnaire, immediately some other respondents were being selected.

and validity was tested and confirmed. Then the final version of the questionnaire was prepared. The final questionnaire contains two parts. The first part encompasses general information and the second part relates to the time budget, its applications, its related pressures, and the professional experiences of the respondents in facing with the research subject. Moreover, to give the needed knowledge to the respondents, the operational definitions of the related keywords of the research was attached to the questionnaire. The second part included professional questions. 5 questions were designed for each hypothesis respectively.

4.3. Measurement

The questions of the questionnaire have been design in Likert range. This range includes five equal parts in which the researcher provides some items suitable to the research subject so that the respondent can specify his/ her ideas and tendencies. The mentioned answers are ranged from "completely agree" to "completely disagree". The researcher can assign numbers from 1 to 5 to each parts of the range, e.g. number 5 for "completely agree" and number 1 for "completely disagree", and then she/ he can calculate the numbers accordingly.

Likert range is an interval measure containing some expressions and answer items. Thus the Likert scale is a complex measure. The answer items in this measure usually imply the scale of respondent's agreement or disagreement against a specific concept, whether a positive or negative answer. Using this measure the researcher can determine the sensitivity, attitude, or the belief of the respondents, because the respondent who has a weak or strong emotion against any specific subject will show his/ her sensitivity to the subject by his/ her answer, whether a positive answer or a negative one (Khaki, 2007).

Table 1. A sample of Likert range

Answer items	Numerical value
Completely disagree	1
Disagree	2
No idea	3
Agree	4
Completely agree	5

4.4. Statistical methodology

Most statistical tests are being administered given the normality of data distribution. Such a test is valid when the normality of the measured data distribution is not rejected. Drawing methods (like chart drawing with normal curve, drawing the normal line of the data, etc.) and quantitative methods (like goodness fit, comparison of aggregative frequencies with probability aggregative function, etc.) are available for the assumption of data normality. If the normality of data distribution is rejected, then the tests have to be administered without assuming the normality. Such tests are called free-distributed or nonparametric tests. Nonparametric statistical method is used for one of the following data types:

- Data with nominal measuring scale
- Data with ordinal measuring scale
- Data with relative or interval measuring scale

Since in nonparametric method the data are studied on the basis of the ranks, to rank the data from lower to higher, the ranks of "1, 2, …, n" are assigned.

Like other researches in the field of humanities that use Likert range, the given traits are quantitative and nonparametric. In this research we first used Cronbach's alpha to determine the reliability of the questionnaire, and since the Cronbach's alpha was high (0.776), so the questionnaire is reliable. The summary of the results is shown in table 2.

Table 2. Questionnaire's reliability test

Cronbach's alpha	N of Items
0.766	30

Then to assess the normality, we used Kolmogorov- Smirnov test. The results of the test shows that the variables of hypotheses 1, 3 and four have a normal distribution but the variables of hypotheses 2 and 5 are not. This point is shown in the last row of the table 3, so that each variable that is equal to or higher than %5, it will be normal and each variable that is less than %5 will not be normal. At the other hand, due to the high amount of the data, these two sets of variables have been dealt as normal variables. The results of Kolmogorov- Smirnov test is shown in table 3.

Table 3. Kolmogorov- Smirnov test

		ch1	ch2	ch3	ch4	ch5
N		121	121	121	121	121
Normal Parameters[a, b]	Mean	3.8116	3.8116	3.8397	3.8231	3.6264
	Std. Deviation	.69392	.61281	.58288	.50013	.50790
Most Extreme Differences:	Absolute	.120	.158	.121	.101	.149
	Positive	.064	.114	.078	.094	.094
	Negative	-.120	-.158	-.121	-.101	-.149
Kolmogorov-Smirnov Z		1.315	1.737	1.328	1.111	1.635
Asymp. Sig. (2-tailed)		.063	.005	.059	.169	.010

a. Test distribution is Normal.
b. Calculated from data.

Since all the variables have been assumed normal, to test the hypotheses the researcher has used unilateral T-test with N-1 freedom degree. Data analysis has been done using SPSS software at the confidence level of %95.

5. Data analysis

Hypotheses 1: *Unsuitable time budget will cause work pressure on the internal auditors.*
To study hypotheses 1, the answers of the questions 1 to 5 were analyzed. A summary of the results is shown in table 4.
H_0: ≤ 3
H_1: > 3

Hypotheses 2: *Time budget pressure negatively affects the auditing operation.*
To study hypotheses 2, the answers of the questions 6 to 10 were analyzed. A summary of the results is shown in table 4.
H_0: ≤ 3
H_1: > 3

Hypotheses 3: *Time budget pressure will cause ignoring some stages of auditing by the internal auditors.*
To study hypotheses 3, the answers of the questions 11 to 15 were analyzed. A summary of the results is shown in table 4.
H_0: ≤ 3
H_1: > 3

Hypotheses 4: *Time budget pressure will cause underreporting real time of the work by the internal auditors.*
To study hypotheses 4, the answers of the questions 16 to 20 were analyzed. A summary of the results is shown in table 4.
H_0: ≤ 3
H_1: > 3

Hypotheses 5: *The increase of Time budget pressure will increase the occurrence of unprofessional behaviors.*
To study hypotheses 5, the answers of the questions 21 to 25 were analyzed. A summary of the results is shown in table 4.
H_0: ≤ 3
H_1: > 3

6. Conclusion

According to the obtained results, all 5 hypotheses are confirmed.

Table 4. The results of testing hypotheses 1 to 5

Hypotheses	Mean	T-value	Freedom degree	Sig.	Result
1	3.8116	12.865	120	.000	H_0 was rejected
2	3.8116	14.568	120	.000	H_0 was rejected
3	3.8397	15.846	120	.000	H_0 was rejected
4	3.8231	18.105	120	.000	H_0 was rejected
5	3.6264	13.567	120	.000	H_0 was rejected

As shown in table 4, since all calculated means are higher than 3, thus all hypotheses are confirmed.

6.1. Suggestions

In this research we studied the effects of time budget pressure on the behavior of internal auditors. Like other researches on the independent auditors, the results of this research showed that the internal auditors are affected by the pressure of pre-specified time budgeting. Accordingly, the following suggestions can be offered:

1. The companies have to plan a suitable and usual time budget for the internal auditors.
2. Auditing program that directly affects the quality of auditing operation has to be prepared in a way that prevents unprofessional behaviors.
3. The effects of time budget pressure on the behavior of internal auditors have to be studied under different theories.

6.2. Limitations

Like other field studies, this research has had many limitations among which the followings can be mentioned:

1. Ensuring that the questionnaire is realy perfect and defect-free is extremely hard and even impossible.
2. Regaining the questionnaires always faces difficulties and usually leads to researcher's costs without any outcome.
3. Most Iranian researchers are being administered in the capital, Tehran. This issue causes the decrease in generalization of the research.
4. Many respondents complete the questionnaire without scrutinizing it, but with just a glance at the questionnaire. This way of completing the questionnaire reduces the precision of the questionnaire.
5. Finding the best statistical test for the research is a difficult task because in most researches, the research's field of study is different from Statistics and so the researchers usually are not sufficiently equipped with statistical methodology. In many cases, this shortage will result in using illogical test for his/ her research.

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Proximate analyses, phytochemical screening and antibacterial potentials of bitter cola, cinnamon, ginger and banana peel

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Abstract: The proximate and phytochemical analyses were carried out on the dried and pulverized samples of ginger; (*Zingiber officinale),* and cinnamon (*Cinnamomum verum)*, banana(*Musa acuminata*) and bitter kola(*Garcinia spp*) which were obtained from an open market in Ibadan, Lagos, and Sagamu in south west Nigeria. Carbohydrate content in ginger and cinnamon were 71.315% and 66.69% respectively while for banana peels and bitter kola values ranged from 43.08% to 74.81%. *Proteus vulgaris and Klebsiella pneumoniae* showed susceptibility to extracts from bitter kola and banana peel.
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Keywords: phytochemicals,bitter cola, cinnamon, ginger, banana peel, antibacterial

INTRODUCTION

Incessant reported cases of tissue pathologies arising from metabolic disorders or microbial invasion have invited increasing interest in local herbs as alternative therapeutic focus in Africa. Dental, urinogenital tracts, gastrointestinal and other morbidities which are rampant among the hinterland settlers and uninformed have made bioprospecting of medicinal plant extracts attractive. A near breakthrough had been reported on oral infections (Topsoba and Deschanmps 2006; More *et al*, 2008; Soukos and Godson, 2000), where the significant property was the expression of good antibiofilm activity of the extracts(Silva *et al* 2012) Treatment of prostate gland enlargement using banana peels extracts (Fagbemi*et al* 2009Andrade *et al* , 2008; Akamine *et al* 2009), anti cariogenic activity and hepatoprotection with bitter kola (Uju and Obioma 2011; Oze *et al* 2010); induction of tumor cells and insulin resistance using cinnamon (Wand *et al* 2007; Shan *et al* 2007; Kwon *et al* 2010) and the suppression of osteoarthritis by ginger (Altman and Marcusse 2001; Lantz *et al* 2007, Stewart *et al* 1991; Ekwenye and Elegalam, 2005; Gur *et al* 2006; Malu*et al*. 2009; Poeloengam 2011) are all encouraging advantages of bioprospecting of plant materials for human use.

The aim of the present study was to examine the contents of the various –plant materials with a view to justifying the expected therapeutic functions of the extracted phytochemicals.

MATERIALS AND METHODS

The samples *Garcinia kola, Cinnamon, Ginger* and *Musa acuminata*, obtained from Ilishan , Ibadan and Lagos markets in south west Nigeria, were sun-dried to constant weight to remove the water content and ground in preparation for further analyses.

Proximate analyses were carried out in line with standard AOAC (1984) methods

PHYTOCHEMISTRY

Weight of lipid in sample

Principle

One gram of sample was grinded using pestle and mortar with 10mL of distilled water. The pulp was transferred into a conical flask containing 30mL chloroform-methanol (2:1, v/v) and mixed well. This was kept overnight at room temperature in the dark. The mixture was then centrifuged at 5000 rpm for 10minutes. The upper layer was then discarded and the lower lipid layer was carefully collected into another beaker. The beaker was placed in warm water (50^0C) to enable evaporation of residual chloroform.

Calculation

Weight of lipid in sample, g = (weight of beaker + chloroform extract) – Weight of beaker.

2.4.2 Determination of total phenols by spectrophotometric method:

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15minutes. 5ml of the extract was pipette into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 minutes for colour development. This was measured at 505 nm.

Tannin determination by Van-Burden and Robinson (1981) method:

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered mixture was pipette out into a test tube and mixed with 2 ml of 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes.

CULTURE PREPARATION

The bacteria used in this study included *Staphylococcus aureus* ATCC 6538, *Proteus vulgaris* KZN, *Klebsiellapneumoniae* ATCC 13047, *K. pneumoniae* ATCC 4352, *Micrococcus luteus*, *Bacillus cereus* ATCC 10702, and *Enterobacter cloacae* ATCC 13047. The standard bacteria strains were obtained from the culture collections of the Department of Microbiology and Biochemistry, University of Forte Hare, South Africa and grown on Mueller Hinton agar and incubated at 35^0C for 16hrs prior to use, while slants were maintained at 4^0C.Their susceptibility to the reference antibiotics was investigated.

DETECTION OF INHIBITORY ACTIVITIES AGAINST BACTERIA

The disc diffusion technique was adapted using Whatman s filter paper. The (10^{-3} dilution) were maintained on Mueller Hinton agar. The pure culture of each bacterium was inoculated in peptone water for 18 hrs. and the growth of organisms was observed as turbidity determined by a spectrophotometer The extract was impregnated into the filter paper discs with the use of methanol and distilled water which served as the solvent at concentrations of 5mg/ml inside a Petri dish and placed in the incubator at 35^0C for 2 hours. After drying, a sterile forceps which was regularly flamed was used in picking 10 filter paper discs one at a time into each of the Petri dishes containing the different seeded organisms. The Petri dishes were incubated at 35^0C for 16-18 hours and observed for zones of inhibition.

RESULTS AND DISCUSSION

The result of proximate analysis of the sample presented in (Table1) shows that the crude protein (6.42%), crude fat (2.16%), crude fibre (17.84%), ash(3.16%) of banana peel is higher than that of bitter kola which crude protein (4.32%),crude fat (0.99%), crude fibre (1.26%), ash (1.61%). While the moisture content (17%),and the carbohydrate (74.81%) present in bitter kola is higher than that of banana peel which the moisture content (13.5%)and carbohydrate (43.08%).these values are different from what had previously been reported for bitter kola (Eleyinmi *et al*., 2006) reported a protein content of (3.95%),lipid of (4.33%), ash(1.14%),and crude fibre content of (1.14%).It has been reported that that the moisture and ash contents of banana peels ranged from 78-94% and 1.25-8.80% respectively(Ankrah, (1974, Adewuyi *et al*., 2008).The varying composition reported by researchers reflected the influence of environmental conditions on nutrient composition of these plant materials.

The phytochemical analysis of the two extracts showed that the weight of lipid of bitter kola (0.46g) is higher than that of the weight of lipid of banana peel (0.22g). The total phenols of banana peel (8.86g) is higher than that of the total phenols of bitter kola (6.3g). The tannin present in banana peel (0.32g) is higher than the tannin present in bitter kola (0.23g). The phytochemical compounds in this study are similar to the finding of (Adegboye*et al*., 2008) while investigating *G.kola*. Earlier report has proven that cinnamon can serve as a n antibacterial against *Salmonella*and*Listeria* (Shan *et al.,* 2007) suggesting that it has bioactive compounds that can serve as food preservatives.

PHYTOCHEMICAL ANALYSIS

The weight of lipid of bitter kola (0.46g) is higher than that of the weight of lipid of banana peel (0.22g). The total phenols and tannins in banana peels were higher than those in bitter cola (Table 4,5 and 6).

SPECTRA PROFILE OF PHENOLICS FROM THE OIL SAMPLE OF BANANA PEEL

In bitter cola the compounds detected were tannin, phloroglucinol and gallic acid within the stated wavelengths (Fig 3). Using the standard wavelength characteristics of phenolic compounds found in plants, the phenolics that can be found within this range were gallic acid, purpurogallin and phloroglucinol (Fig. 4).

SENSITIVITY TEST

The agar filter paper disc method showed that *Proteus vulgaris* KZN and*Klebsiellapneumoniae* ATCC 13047 were the most susceptible to the antibacterial activities of bitter kola and banana peel.

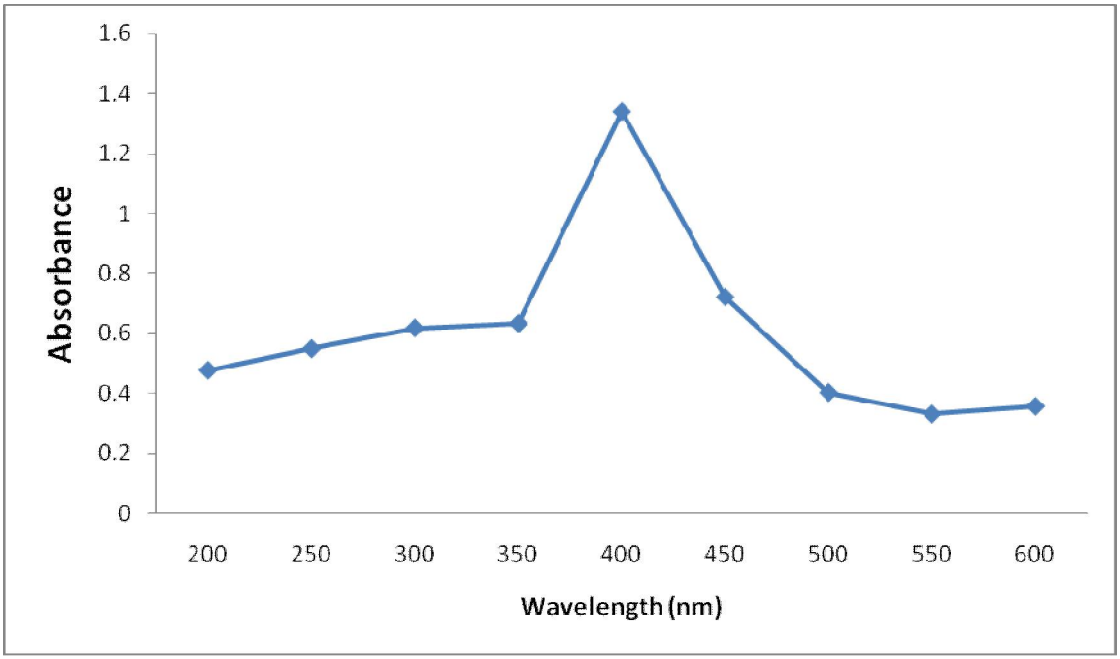

Figure 1: Spectra profile of phenolics of cinnamon

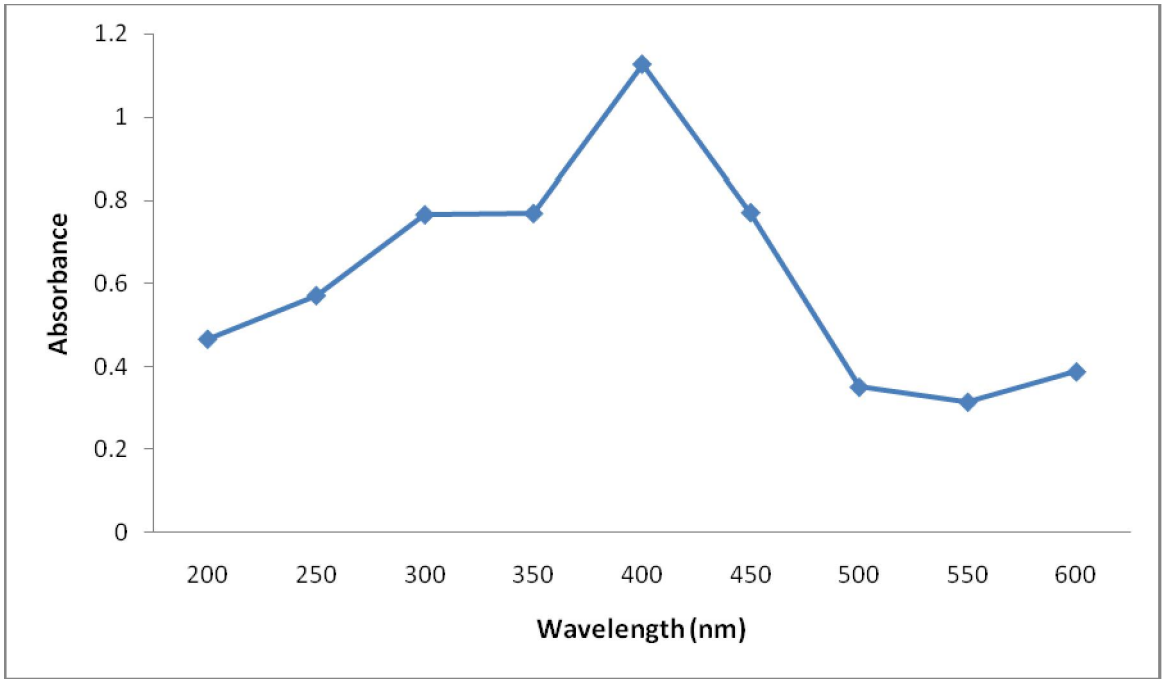

Figure 2: Spectra profile of phenolics of ginger

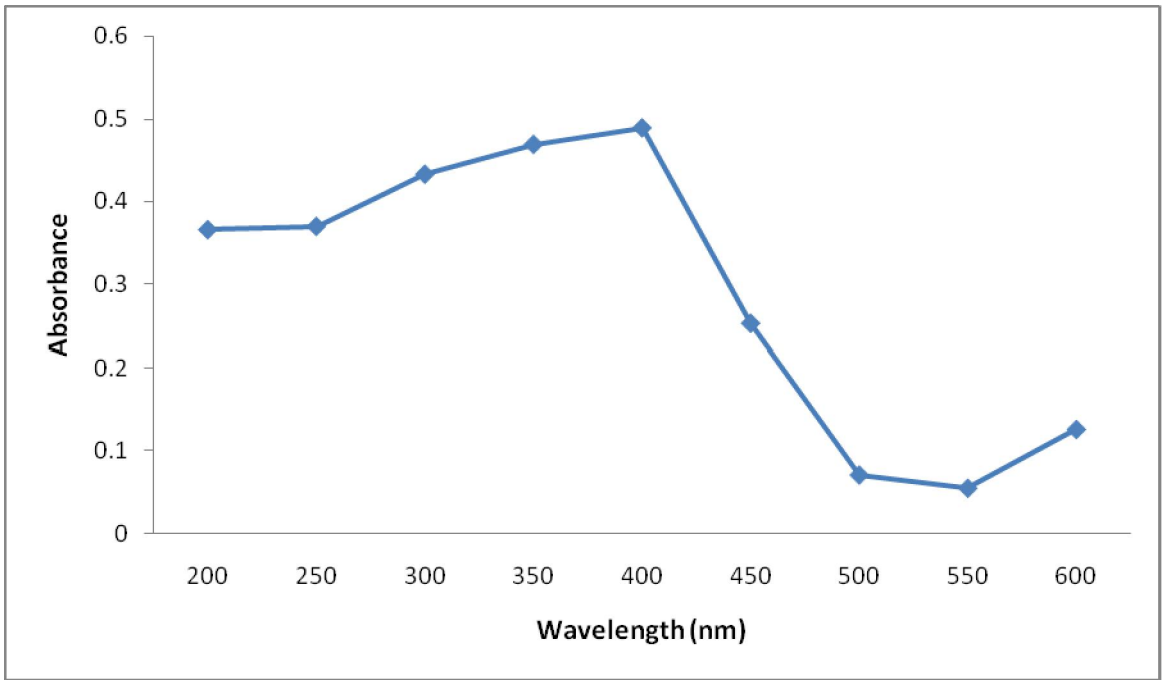

Figure 3: Spectra profile of phenolics of bitter cola

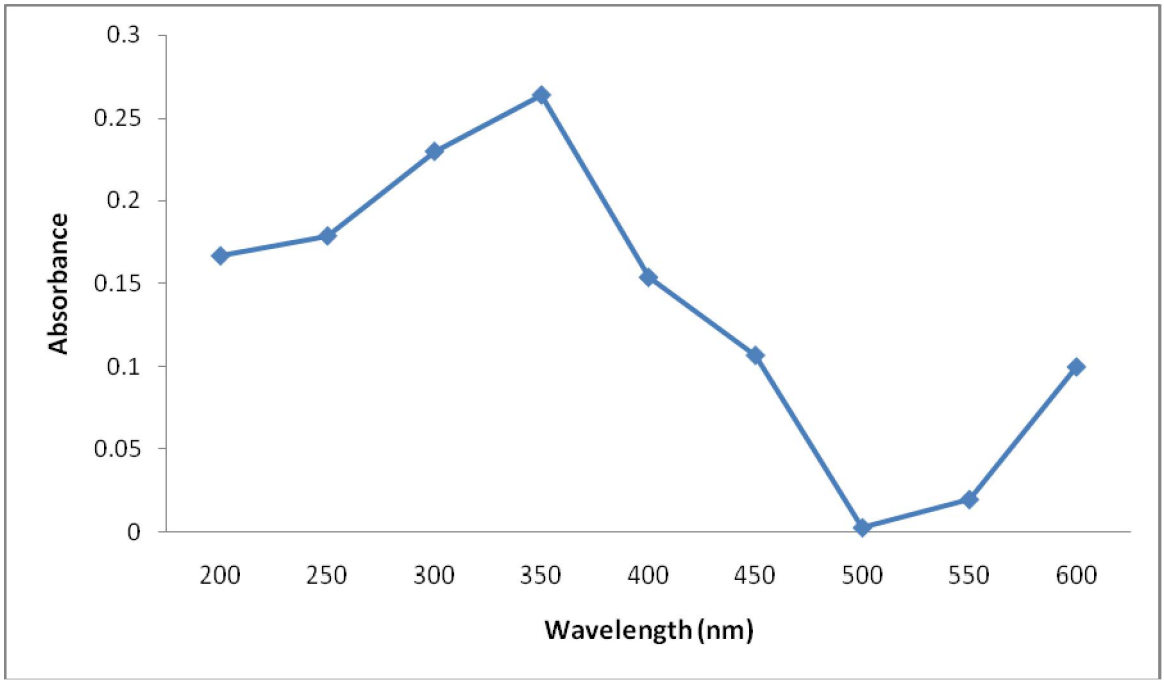

Figure 4: Spectra profile of phenolics of banana peel

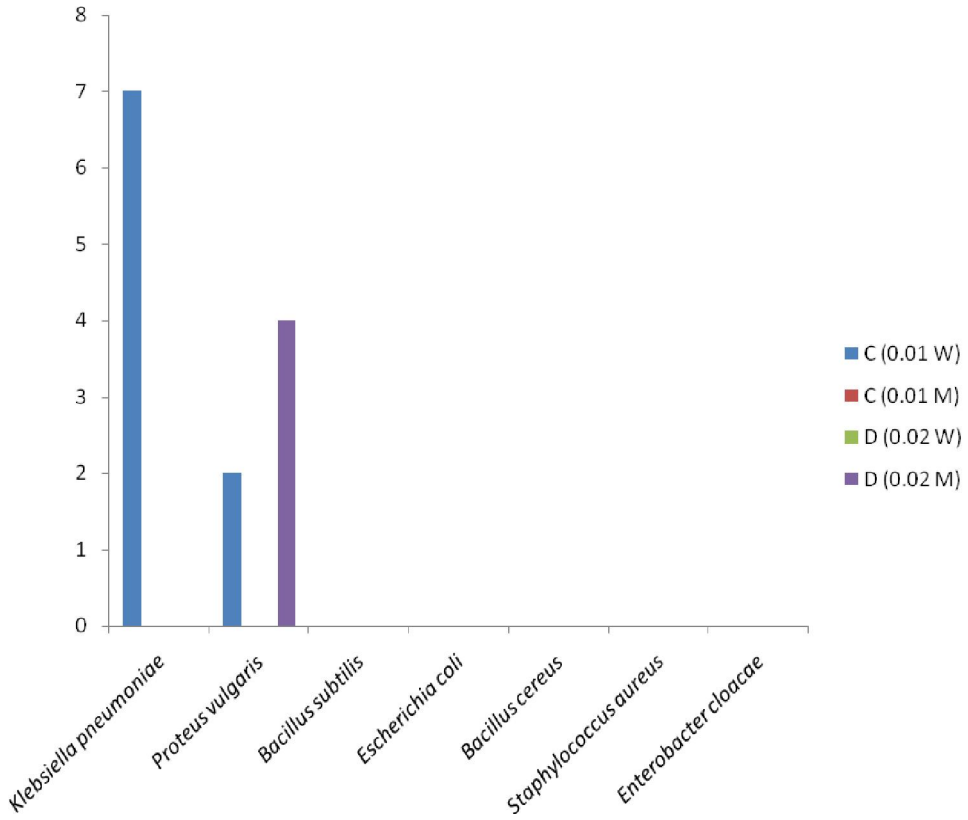

Figure 5: Antibacterial potentials of the methanolic and aqeous extracts of bitter cola (C) and banana peel (D).

TABLE 1: Proximate analyses (%)of the plant samples.

	FAT	FIBRE	ASH	MOISTURE	PROTEIN	CHO
BITTERKOLA	0.99	1.26	1.61	17	4.32	74.8
BANANA PEEL	2.16	17.84	3.16	13.5	6.42	43.08
CINNAMON	14.61	2.24	3.17	5.25	8.03	66.7
GINGER	1.41	4.6	3.60	13.55	5.54	71.3

TABLE 2: Phytochemical screening of the plant samples

	OIL CONTENT(g/g)	PHENOL (g/g)	TANNING/g
BITTERKOLA	6.3±0.36	0.46±0.01	0.236±0.002
BANANA PEEL	8.87±0.47	0.22±0.02	0.327±0.004
CINNAMON	0.45±00.1	0.496±0015	15.53±0.66
GINGER	0.37±0.005	0.447±0002	7.06±0.25

Table 3: ANTIMICROBIAL ACTIVITIES OF GINGER AND CINNAMON

BACTERIA	ZONES OF INHIBITION(mm)	
	Ginger	Cinnamon
- *Enterobacter cloacae* ATCC 13047	2.4	NIL
- *Bacillus subtilis* KZN	3.5	0.8
- *Salmonella typhi* ATCC 13311	NIL	1.2
- *Escherichia coli*	NIL	NIL
- *Klebsiella pneumoniae*	2.5	6.1
- *Staphyllococcus aureus* OK 2b	NIL	4.5
- *Bacillus cereus*	0.5	0.5
- *Proteus vulgaris*	5.8	5.5

The result of antimicrobial sensitivity on the tested organisms shows that *Proteus vulgaris* was the most susceptible to the antibacterial activities of bitter kola and banana peel and *Klebsiella pneumoniae* ATCC 13047 also were susceptible to the antibacterial activities of bitter kola and banana peel. Similar study has also shown that crude extract of *G.kola* exhibited antimicrobial activities in vitro against both Gram-positive and Gram-negative organisms (Adegboye *et al*., 2008).

It can be concluded that the extracts obtained from *Garcinia kola* and *Musa sapientum* displayed a good activity against *Proteus vulgaris* and *Klebsiella pneumonia* These extracts can be applied in antimicrobial treatment of the specific infections. *Zingiber officinale* and *Cinnamomum verum* are nutritionally and medically valuable. They contain extracts that proved effective antimicrobials Although the experiments were carried out *in vitro*, further analysis of the extracts of the 4 plant materials are needed in order to establish a "structure- function" and dose- response relationships.

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7/7/2012

从巴拿马船闸到希格斯王国
---非线性希格斯粒子数学讨论（5）

单炜滕

摘要：希格斯大小不能小于“希格斯船闸”可供进靠的大量子的极限“长度”的悖论，类似**“谷仓内的标枪悖论”**。但这反而能为 ATLAS 和 CMS 两个研究团队接下来该怎么办？提供了一个方向：依据顶夸克的质量打开希格斯粒子质量寻找的判据，是大型强子对撞机将它产生时的速度达到光速的 97%。判据确定，它的质量为 125.9GeV 就一锤定音。

[单炜滕. **从巴拿马船闸到希格斯王国---非线性希格斯粒子数学讨论（5）.** *Academ Arena* 2012;4(8):16-20] (ISSN 1553-992X). http://www.sciencepub.net/academia. 3

关键词：希格斯粒子 大量子论 质量 魔杖

一、用巴拿马船闸模型解释希格斯大质量

1、欧洲核子研究中心（CERN）2012 年 7 月 4 日宣布，CERN 的 ATLASA（超环面仪器）实验和 CMS（紧凑缪子线圈）实验都观测到新粒子。CMS 发现质量为 125.3±0.6 GeV/c^2 的新玻色子。ATLAS 发现质量为 126.5GeV 的玻色子。CMS 实验组的发言人乔·因坎迪拉表示：“这是一个初步的结果，但我们认为这个结果非常有力。”我国的态度如何？有两个人的新闻引起我们的关注，特提出来与大家商榷。

1）陈和生先生是中科院高能物理研究所院士、欧洲核子中心大型强子对撞机实验 CMS 和 ATLAS 物理研究的中方首席科学家，一直致力于相关科研实验和组织协调工作。他说：“这个粒子是否就是希格斯教授提出来的那种，还需要大量的验证。现在有一种模型是‘超对称模型’，该模型中也有一种希格斯玻色子，但其性质与‘标准模型’中的希格斯玻色子的性质并不相同。这两种粒子的性质不同，衰变也不同。因此，对这次发现的新粒子究竟是哪一种粒子还需要多年的验证。”“目前需要增加统计性，看到更多的粒子。预计在今年年底，这一结果可以出来。至于究竟是什么粒子，还需要更长时间，甚至于还要再造一个加速器。”（2012-07-22《人民日报 海外版》张保淑、李理）

2）陈国明先生是中科院高能物理研究所研究员、参与寻找希格斯玻色子的欧核中心 CMS 项目中国组成员及负责人，他说：虽然这次发现新粒子的一些特征，比如产率（出现几率）、衰变模型等与之前预言的希格斯粒子相吻合，但现在统计性太少，还不能确定这个新粒子的各种特性，因此这次也可能发现的是另一种新粒子。以目前取得的数据，要最终确认希格斯粒子的存在恐怕还远远不够，仍然需要更多的实验数据积累。可能还需要再建一个高能量的直线正负电子对撞机，才能更仔细、准确地验证这个结果。（2012-07-11《科技日报》，董子凡）“99.99994%的置信度并非意味着是希格斯玻色子的可能性，而是指这是一种新发现粒子的可能性。”陈国明说：“要最终证实这个新粒子是不是‘上帝粒子’，还需要验证它的自旋宇称，衰变道，衰变分支比以及与其他粒子的相互作用等特性。”这些特性都得到验证后，如果仍然与彼得·希格斯的预言相符，才能说找到了“上帝粒子”。（2012-07-13《光明日报》詹媛）

2、被认为与科学界寻求已久的希格斯玻色子一致的新粒子，质量已经确定，而且在统计学上有极高的确定性，但为何却不敢确认这就是最终的希格斯玻色子？陈和生院士和陈国明研究员的解释都不错，但可能都没有说到要害。要害是希格斯粒子的单位质量为什么是从大到小，希格斯以及标准模型和超对称模型都还没有一个理论上，预言它的较准确的质量值。

1）希格斯物理是一个神秘的王国，陈和生和陈国明说得对，但不是关键，这不奇怪。例如，1964 年希格斯提出的 $E=M^2h^2+Ah^4$ 希格斯场公式，是对的，但他和合作者这只是理论上预言它能解释其他粒子的质量起源。

2）物理学标准模型和大爆炸理论是对的，但大爆炸理论并不能计算出夸克、轻子等费米子和除希格斯粒子以外的规范玻色子等 24 种基本粒子的质量。杨振宁—米尔斯规范场理论是对的，得到了实验的验证，但它无论应用到弱还是强相互作用，都禁止规范玻色子带有任何质量，却与实验不符。即标准模型也没有预测希格斯粒子较准确的质量。

3）希格斯之后，科学家中所有关于希格斯场自发对称破缺产生质量机制的科普模型解释，都是对的，但也都没有留下希格斯玻色子的质量较准确的预测。

4）霍金等科学家支持的弦论，是对的。“舶来品”弦论能把包括引力在内的 4 种自然基本作用力统一起来，但它也没有预言希格斯粒子的质量。按

汉语“弦”的词意，弦有多种模具，可如魔杖，西方超弦并非金科玉律。

3、中国科学殿堂之外的希格斯之梦，已经做了数十年。中国在科学理论方面不都是照抄照搬西方的理论，没有自己的原始创新理论体系。2012 年第 7 期《环球科学》杂志发表陈超先生的文章说：“2006 年，借助于俄罗斯数学家佩雷尔曼证明的庞加莱猜想外定理的----空心圆球内外表面翻转熵流，人们把时间和热力学、量子论、相对论、超弦论等联系了起来，点燃了第三次超弦革命”。这事还得从川大流出的数学说起。

1958 年量子中国走到了大跃进年代“超英赶美”的向科学进军，四川大学数学系有教授带领少数大学生，开出研究类似拓扑数学“灵魂猜想、灵魂定理”的 Alexandrov 空间（亚历山德罗夫空间）课题。这是苏联著名数学家亚历山大·丹尼洛维奇·亚历山德罗夫在 20 世纪 50 年代便放弃了的研究。“灵魂”按汉语词意被解释为：“迷信的人，认为附在人的躯体上作为主宰的一种非物质的东西”。但毛泽东同志却有“政治是灵魂，政治是统帅”的论断。这是我们中国人内心的乱麻吗？不！

论断符合数学对“灵魂”性质的定义：“针对某类特定的数学对象，可从这类数学对象的一些小区域将性质推广到整体。这些小区域称之为数学对象的灵魂”。前苏联对外公开称为 Alexandrov 空间。也许这与被一批中国高层的从哲学家到物理学家组织有“无神论研究会”打击“伪科学”的对象类似，川大的数学家们为避嫌，对外公开就改为一道难题：“不撕破和不跳跃粘贴，把空心圆球内表面翻转成外表面，把它证明出来”。但最终因三年自然灾害还是偃旗息鼓，川大数学随着学生毕业流落到了民间。

4、川大流出的数学能否再流回川大，我们不管。川大流出的数学与科学殿堂之外的三旋理论结合，在庞加莱猜想的基础上对原先的弦论扩容，我们发现有六种内部结构的特定弦图，类似“魔杖”或“变形金刚”：

1）弦图像孤子链组成的弦线。可用来描绘基因双螺旋结构，微观波粒二象性以及粒子的产生、湮灭、虚发射、虚吸收、电磁波的传播；因孤子链中每个圈子体旋为 1/2 的自旋，可对应费米子和反费米子的自旋等。

2）弦图像《羊过河》寓言中的独木桥变形“魔杖”的弦线。针对萨斯坎德的《黑洞战争》书中的“持球跑进”和特霍夫特的全息信息守恒的疑难，“魔杖”类似空心圆球内表面翻转成外表面，两只羊在桥中间碰头的“转点”，有类圈体三旋式的自旋能化解矛盾。

3）弦图像“泰勒桶”、里奇流、傅里叶变换结构的弦线。这对宇宙总质量(100%)≌重子和轻子(4.4%)+热暗物质(≤2%)+冷暗物质(≈20%)+暗能量(73%)方程，可用类似幺正性概率守恒的办法，作出准确计量的解释。

4）弦图像道路交通网络的公路线、立交桥和车库、城市及各种汽车组成的弦线。解释对应费曼图中的树图、圈图进行的对撞、衰变等更好。

5）弦图像长江及其三峡大坝船闸模型组成的弦线。我们称为“大量子论”，可为求证夸克、轻子和规范玻色子等基本粒子的质量谱计算公式提供说明。例如希格斯粒子的单位质量，为什么反比除顶夸克质量外的所有的基本粒子的质量还大？就是它能解答的难题。

6）第 6 种有内部结构的特定弦图，我们在最后说。

在近百年的粒子物理学史上，多数的事实说明，实验发现的新未知粒子，理论上早先有质量预测值的，实验都能一锤定音。对新发现的疑似希格斯粒子，类似船闸模型的大量子论能一锤定音吗？我们试着来讨论。

5、我们生活在中国，但对长江三峡大坝船闸的数据并不了解，只是用作大量子论的科普，长江大家熟悉。现换为巴拿马运河船闸，更为恰当。

据《南方周末》2012 年 6 月 21 日发表的《巴拿马运河》一文报道：巴拿马运河是沟通近在咫尺而又相隔千万里的太平洋与大西洋的闸门式运河工程，两端的三级闸门，围起巴拿马地峡的热带雨水，形成一条高高的悬河，让轮船在其中来来往往。船闸可供进靠的船舶极限为长 292 米、宽 32.2 米、吃水 12.04 米。事实上，船闸的尺码极大地改变了造船业，业界把 32.2 米宽且 292 米长的船称为巴拿马极限型，成为造船工程师的首选。

2）这是一幅生动的希格斯场、希格斯机制、希格斯粒子和其他基本粒子质量起源的写照。这里对撞机寻求证明的希格斯王国不再神秘，这并不是说希格斯粒子可有可无，而是说类似巴拿马的船闸每级闸门至少要修多宽？多长？才是巴拿马极限型类似的基本粒子大质量。因为基本粒子中的庞然大物，与被精确地塞进为它特制的容器是一致的。我们把所有 24 种的夸克、轻子和除希格斯玻色子以外的规范玻色子等基本粒子，类似对应船只，修的大坝的船闸闸门才合适，就可知希格斯船闸的极限型。

3）众所周知，在希格斯物理的理论中，有它预测的最大质量的基本粒子。而这个预测竟获得证实，并已通过重要的实验检验，这就是发现顶夸克的质量为 175GeV，它极大地增强了超对称希格斯物理的理论分量。

二、谷仓内的标枪悖论修正希格斯大质量

现在我们可以把巴拿马比作希格斯王国，巴拿马运河的船闸限定大船的机制与希格斯王国生成大量子的机制连接。因为根据物理学标准模型和大爆炸理论，我们的宇宙起始于一次大爆炸。大爆炸刚发生时，无数的正反粒子同时产生，轻子和夸克通过与希格斯场的相互作用获得了质量。这些粒子凝聚成物质，通过长时间的演化形成了星系。

那么我们可以这样来设想希格斯王国，它出现在 137 亿年前的宇宙大爆炸初始，说来它的使命已经大部分完成。这就是希格斯王国有条闸门式工程的“运河”，沟通唯物的点外真空与唯物的点内空间。这类似巴拿马那条高高的悬河，我们的世界就生活在这片热带雨水的“地峡”。

希格斯运河的两端同样是三级闸门，船闸可供进靠的大量子的极限“长度”为 175GeV 类似的质量；这个“船闸”的尺码，极大地打造了基本粒子物理，被称为希格斯场机制，成为造“上帝粒子”的首选。目前欧洲粒子物理研究所的希格斯王国模拟实验，125.3±0.6 GeV/c^2 为 CMS 发现的质量，而 ATLAS 发现的质量为 126.5GeV。取他们各自发现的概率为 50%，那么希格斯粒子的质量准确值为：

（125.3 +126.5）×50%=125.9（GeV） （1）

ATLAS 和 CMS 已经取得了重大研究进展，大多数科学家都认为这种粒子应该就是捉摸不定的希格斯-玻色子，但为什么又不能一锤定音吗？

如今，大型强子对撞机的这些实验仍在继续，两个研究团队希望能够拿出更权威的证据来证明他们所“看到”的粒子就是希格斯-玻色子。但是，接下来该怎么办呢？我们说，不能一锤定音，是因为这个 125.9GeV/c^2 的希格斯粒子质量，与顶夸克的实验质量为 175GeV/c^2 是矛盾的。

这是一个类似“**谷仓内的标枪悖论”，**即希格斯大小不能小于“希格斯船闸”可供进靠的大量子的极限“长度”的悖论。但解决这个悖论反而能为 ATLAS 和 CMS 两个研究团队接下来该怎么办？提供了一个方向：依据顶夸克的质量打开希格斯粒子质量寻找的判据，是大型强子对撞机将它产生时的速度达到光速的 97%。判据确定，质量为 125.9GeV 就可一锤定音。

1、上海科技教育出版社 2010 年 4 月出版的查尔斯·塞费的《解码宇宙》一书介绍的“**谷仓内的标枪悖论”，**是个早已闻名和已经研究解决了的悖论。它的关键点类似塞费的分析是，希格斯王国的“宪法”，测量或观察执行的是爱因斯坦相对论两个假设知识的密码。虽然这个希格斯王国在 137 亿年前的宇宙大爆炸初始，就已完成了它的使命，但质量“宪法”没变。

塞费说，相对性原理和光速不变原理两个假设有许多离奇的结果，但该理论却有着完美的对称性。观察者或许对长度、时间、质量以及许多其他基本实物各抒己见，但与此同时，所有的观测者都是正确的。塞费用具体数据解说了**“谷仓内的标枪悖论”：**想象有一名短跑运动员能以光速 80%的速度快跑，他是手持一根 15 米长的标枪，向着一座 15 米长的谷仓跑去。

这座谷仓有一个前门和一个后门。一开始，谷仓前门开着，后门关着。观测者原地不动，坐在屋顶椽架上测量，由于奔跑者米尺的相对论性效应，他实际测量到这根 15 米长的标枪缩短了，只有 9 米。而固定不动的谷仓，仍然保持它原来的 15 米的长度。塞费说：“正如爱因斯坦的理论所说，信息即实在。如果我们的精确测量仪器获取了关于标枪的信息，这些信息显示标枪是 9 米长，那么它就是 9 米长----不必考虑一开始时它有 15 米长”。

我们不想重复塞费在书中从各个角度论证他的这个正确结论。丹尼尔·肯尼菲克出版的《传播，以思想的速度》一书中，也重复了对类似**“谷仓内的标枪悖论”**塞费得出的分析：短跑运动员与屋顶椽架上的观测者对事件的顺序意见不一致，解决这个悖论与时间有关。肯尼菲克说，我们习惯于独立地在空间或在时间中测量，但实际存在一个描述两扇门关闭之间信息传播需要时间的时空区域，它兼有空间的和时间的两个方面。

2、具体联系到 ATLAS 和 CMS 两个研究团队，他们是要在人工实验室里重新“复活”大爆炸时期的希格斯王国和希格斯运河的船闸，以寻获希格斯粒子的踪迹。这里时间顺序是被颠倒了，但爱因斯坦的理论告诉这却有着完美的对称性。我们用类似巴拿马运河船闸模型的大量子理论，解释希格斯粒子是一种理论上预言的能解释其他粒子质量起源的新粒子，先是在 1996 年推证出 24 种的夸克、轻子和除希格斯玻色子以外的规范玻色子等基本粒子的质量谱公式。这类似从薛定谔猫到彭罗斯的薛定谔团块，假设宇宙大爆炸的撕裂，质量变化有类似轮船在船闸的位移在不同落差的分段的数学分析来解释的；当然还有类似射影几何的投射锥和取截景等交织基于撕裂的质量谱公式，理论上才算出顶夸克的质量为 175GeV 的。

1）但我们说 125.9GeV/c^2 为今天希格斯粒子的质量，不是把它比作大爆炸时期的希格斯运河的船闸，而是与顶夸克调换了一个角色，成了希格斯巨轮，顶夸克的质量成了船闸的长度。而且根据前面塞费的**谷仓内的标枪悖论**分析，还应把希格斯运河的船闸与谷仓调换，成为“希格斯谷仓”，那么顶夸克的质量成了谷仓的长度，希格斯粒子也被再调换为短跑运动员和标枪的组合。设希格斯粒子在对

撞机里“跑”的速度为 v_x，质子速度为 v_z。虽然大型强子对撞机有能力将质子流加速到光速的99.99%，但已知顶夸克的质量是约质子质量的200倍，希格斯粒子也比质子的质量大，且由质子生成，希格斯粒子速度 v_x 自然比质子速度 v_z 是光速的99.99%还小。

2）希格斯粒子的速度 v_x 是光速的多少？根据塞费对**谷仓内的标枪悖论**提供的数据：短跑运动员以光速80%的速度向着一座15米长的谷仓跑去，他手持的15米长的标枪缩短为只有9米。如果塞费说的准确，因相对性原理和光速不变原理的信息真实效应适用于“希格斯谷仓”，其对应比例是：

（标枪的测量长度/谷仓长度）：运动员速度=

等于（希格斯粒子质量/顶夸克质量）：希格斯粒子的速度 v_x，即：

（9/15）: 0.80=（125.9/175）: v_x （2）

v_x=（0.80×0.72）÷0.60=0.58÷0.60=0.97（光速）

3）这个希格斯粒子速度 v_x 为光速的97%，是已知实验数据的理论反推。实验“重演”的过程是欧洲核子研究中心在建造的能量强大的大型强子对撞机设备里面，有能力将质子流加速到光速的99.99%，使两束高能质子流进行加速、对撞。每 10^{12} 次的质子对撞，才可能产生一次希格斯粒子。困难的是它一旦产生，就转瞬即逝，衰变成光子和强子等其他粒子。

目前ATLAS和CMS寻找该粒子最主要的过程，只是“抓住”希格斯粒子衰变产生的光子，反推它们会不会是希格斯粒子产生后又衰变出来的。遗憾的是，他们没有反推希格斯粒子的速度 v_x。希格斯粒子没有自旋，即没有内在的角动量，是一个标量场。如果质量为125.9 GeV，则标准模型的能量等级可以有效直到普朗克尺度（10^{16} TeV）。如果对撞机实验能测出希格斯粒子的速度 v_x，与我们理论预测的 v_x 为光速的97%数据，进行对比吻合，应该说新粒子是希格斯粒子和质量为125.9 GeV能定下来。

三、从实验分辩希格斯粒子幺正方法讨论

2012年7月份，ATLAS和CMS这两个团队宣布发现了可能是难以捉摸的希格斯玻色子，8月份他们发表的论文分别有39页和59页，详细描述了新发现粒子衰变成γ射线、W和Z玻色子等粒子的过程。这过程只是证明类似**“谷仓内的标枪悖论”**手持缩短为9米标枪的短跑运动员来过，但还需是否类似以光速80%的速度向着谷仓跑去，才能最终确定他的身份。

这是我们提出的一个最简便、快速证明希格斯粒子的第二步程序。即寻找希格斯粒子的第二个方向，最方便、直观的判据是检查大型强子对撞机，将它产生时的速度是否达到光速的97%。判据确定，125.9GeV是它的质量也一锤定音。但有人说，ATLAS和CMS在统计和系统误差范围内，在不同的搜索渠道中得到的结果与标准模型希格斯玻色子的预期一致；然而还需要更多的数据去测量该粒子的特性，如不同衰变道(γγ，ZZ，WW，bb和ττ)中的衰变率，和最终该粒子的自旋和宇称，从而认定它确实是标准模型希格斯玻色子？还是超出标准模型的新物理的产物？

1、这是一个无稽之谈：实验已经反推出疑似希格斯子的质量，各人用的哪种理论模型自然知道，难道还需要再问吗？寻找认定希格斯粒子之难，众所周知。如果第二步的方向仍是进一步探索如不同衰变道中的衰变率，和最终该粒子的自旋和宇称的数据，难道这就一定是希格斯粒子的本性吗？可想实验科学家队伍中一定“混进”了不少“中国南郭先生”。

实话实说，兹维•伯恩（Zvi.Bern）、兰斯•J•狄克逊（Lance.J.Dixion）和戴维•A•科索维尔（David.A.Kosower）等三位科学家在2012年第7期《环球科学》杂志撰文《粒子物理学迎来革命时刻》，就一针见血地类似指出：要破译这个数学关联中蕴涵的物理内涵，还需要一些时间。其实，无论从哪方面看，探寻基本粒子散射的奥秘，与在既定的地铁路线上穿梭完全不同。可能需要一个更深刻的理论来处理它们，也许就是弦论。

2、当然这个弦论不可能是那种“舶来品”的西方僵化了弦论，而是按汉语“弦”的词意扩容，包括还有前面没说的第六种弦图像利用干涉方法得到测得的磁重联结构的弦线。我们曾在《刘路与西塔潘猜想和大亚湾中微子实验》一文中说过，张英伯教授的《对称中的数学》书中的“格点”理论，与粒子对撞的散射和宇宙大爆炸后的星云分布等图像信息联系，可见格点成为扩容弦论统一提取大量有关量子物质结构分辨率信息的又一分支方法。具体说到宏观的“磁重联”弦图，也类似粒子碰撞概率的散射。

1）这种散射的碰撞，也分为两个部分的方向：如来自太阳风和地球磁场两个部分的作用。对撞的结果会造成地磁场由于压缩拉伸甚至交叉而发生重联过程，导致磁场拓扑结构的改变，使太阳冕区物质抛射及耀斑等活动，以高能粒子与射线的形式释放出巨大能量。这是一种自然王国里的现象，与希格斯王国的运河船闸现象，都属自然格点弦论同构。

这不同于在人工实验室，对磁场重联物理过程的模拟，和对希格斯粒子物理过程的模拟。这种人

工自然实验室里的研究，所取得的有关数据、图像照片等信息，具有极大的偶然性。但这也有别于人们在室外，对自然王国被动性较强的观测。因为人工实验格点弦论的物理观察，仍使得可以在条件参数可控的情形下，重复地、全过程地研究相关的一些物理现象。

2）例如，中科院物理研究所/北京凝聚态物理国家实验室（筹）光物理实验室的李玉同、上海交通大学的张杰和中科院国家天文台的赵刚研究团队，在上海高功率激光联合实验室神光 II 实验平台上，利用激光等离子体实验构造相似的磁重联结构研究，条件是不够的，但他们想了很多办法。

一方面他们利用别人的卫星恰好在地磁重联发生的短暂时间内，在现场观察到的不同时间地点的地磁重联现象的不一致信息，如 2003 年欧洲太空总署的 Cluster－1 卫星在地磁场的一个重联区中心位置，测量到一个细长的电子扩散区（EDR）的记录，与 2005 年发现的 19 个 EDRs 全部分布在磁重联区两侧的观测记录，就存在极大的差异。

另一方面是他们自己做模拟实验来研究重联过程中 EDRs 的特征。他们的实验捕捉到了激光等离子体重联区产生的一个运动的“磁岛”，以及其运动导致的二阶电流层及明亮的尖状结构。同时，他们还发现了磁重联区中心与两侧边缘一共三个 EDRs，其中中心 EDR 的出现时间要略晚于两侧 EDRs，但其速度明显要高得多。这一发现也为揭示检查大型强子对撞机产生的希格斯粒子的速度达到光速的 97%，是否过大，提供了解释参照。

3、再回过头来看《粒子物理学迎来革命时刻》一文，他们已经直接挑明，大型强子对撞机及其 ATLAS 实验小组，使用过他们发现的目前全世界最先进的幺正方法，计量过不同衰变道中的衰变率。他们的预测与 ATLAS 的实验数据进行过对比，结果两者非常吻合，为寻找梦寐以求的希格斯粒子已经出了一把大力。但问题是，即使做过，却并没有使全世界所有这类专业物理学家相信发现的新粒子能一锤定音，就是希格斯粒子。

1）他们这类专业物理学家说的理由是：一些与希格斯粒子无关的粒子，也能产生这样的结果。幺正方法的初次使用，就是精确计算这些容易让人混淆物理反应的出现概率。

2）但可笑的是，他们这类专业物理学家仍然坚持，“实验人员接下来还会用这些结果探究新的物理现象”。具体到寻找希格斯粒子，陈和生和陈国明先生的解释，也没有脱离他们的这种思路。这是一个循环的悖论啊！

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Enzyme profile and haematology as indices of morbidity in broilers fed dietary aflatoxin

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Abstract: An experiment was conducted to evaluate the toxic potentials of aflatoxin contaminated feed on vital organs and tissues in broilers using various 'marker' like enzymes, kidney function indices and haematological parameters. Fifteen birds were randomly distributed on three dietary treatments comprising of five birds per treatment. Treatment A (control) received a diet containing less than 20ppb, treatment B (90ppb) and treatment C (180ppb) aflatoxin level respectively throughout the 5-week study period. Using analysis of variance (ANOVA) and Duncan's Multiple Range Test, the result showed that the concentration of Liver alkaline phosphatase and liver alanine transaminase and aspartate transaminase reduced progressively with an increase in dietary aflatoxin concentration. However, there was an increase in serum alkaline phosphatase and alanine transaminase as the toxin load increased in the feed.. The overall results of the haematological parameters indicate that the birds are not affected by the varying aflatoxin levels.
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INTRODUCTION

Mycotoxins are a group of extremely toxic and biologically active substances. Among them are the aflatoxins (AF) which are produced consistently by strains of by the fungi *Aspergillus flavus* and *A. parasiticus* (Wilson and Payne, 1994). A. flavus is the most common contaminant of many grains used in human and animal nutrition (Abarca *et al*., 1994). AFs have been detected in the pre-harvest, post-harvest, transport, storage and after processing and packing of grains. Under appropriate humidity and temperature conditions, A. flavus produces four toxins: aflatoxin B1 (AFB1) and three compounds with similar structures (AFB2, AFG1, and AFG2). AFB1 is considered to be one of the most potent hepatotoxins and well-known hepatocarcinogens (Wilson and Payne, 1994). Aflatoxins are the most common contaminants in the feed of domesticated animals, including birds (Jindal *et al*., 1993). One report on contamination of grains with aflatoxins showed 77% was due to B1 while the rest were contaminated with other aflatoxin types (Wilson and Payne, 1994). Some important characteristics of these toxins are their capacity for bioconcentration and bioaccumulation as well as their great stability in different biotic and abiotic environments (Penla and Duran – de Bazua, 1990). Aflatoxins are potent carcinogens and cause growth depression (Umesh *et al*., 1990) and reduced disease resistance in poultry, other livestocks (Giambrone *et al.,* 1978). The Aflatoxin contamination of feed stuff has been reported to range from 10-1500 ppb in commercially used feed ingredients and 34-115 ppb in mixed feed samples (Devegowda *et al*., 1993). The high level of contamination though not resulting in better utilization of available ingredients and severe outbreaks of aflatoxicosis causes heavy economic loss in terms of health and production. The toxicity with aflatoxin followed by contamination of feed with fungi in chickens is characterized by mortality, listlessness, anorexia, decreased growth rates, negative feed conversions, fatty liver, decreased egg production, poor pigmentation and increased susceptibility to other diseases (Arafa *et al*., 1981; Doerr *et al*., 1983) and cause of economic losses in broiler production. Furthermore, a small amount of aflatoxin and its metabolites can be found in several edible tissues (Micco *et al*., 1998) and risks for public health. The toxin may also serve as an antimicrobial(Praveena and Padmini, 2011).

The aim of this study was to evaluate the toxic potentials of aflatoxin contaminated feed on vital organs and tissues in broilers with particular reference to enzymes, kidney function indices and haematological parameters.

MATERIALS AND METHODS:

The experimental diets used were formulated in the Biochemistry and Nutrition unit of the Fountain University Osogbo, Osun state, where the study was

carried out. Common to all of the formulations were 23% crude protein (CP), 0.6% methionine, and 1.2% lysine. Three diets were prepared.(Table 1) Control diet contained 20%ppb aflatoxins level. Diets 2 and 3 (experimental) had 90 and 180% respectively as shown in table 1. The aflatoxin assay was carried out by the ELISA method using AgraQuant test kit

A total of 15 day-old broiler chicks (Arbor Acre strain, CHI Ltd, Ajanla Farms, Ibadan) were wing banded, weighed and randomly allocated to the 3 dietary treatments.. The birds were housed in a well illuminated and ventilated poultry house. Feed and water were provided *ad libitum*. They were vaccinated against Newcastle disease virus (lasota vaccine) at day 8 of age, and against Infectious Bursal Disease (IBD) virus at day 10 of age via drinking water. Second Newcastle disease virus vaccine (booster vaccine) was given at the 16th day. There were 5 birds for each treatment

Serology and preparations of tissue homogenate:

Blood samples were collected from the five birds in each treatment through the jugular vein at the end of the experiment. Serum was separated by centrifugation (8,000 rpm for 5 minutes) and was kept frozen until needed. The liver and kidney were removed, cleaned, weighed and were homogenized in 0.25M ice - cold sucrose solution which was later frozen till required.

Haematological study:

Blood samples were collected using EDTA treated bottles from five chicks per treatment through the jugular vein at the 35th day (last day) of the experiment and was analysed for the following haematological parameters : white blood cell, red blood cell, lymphocyte, platelets (Mitruka and Rawnsley 1981), so as to assess the health status of the birds .

Chemical analysis / Statistical analysis :

The aflatoxins level in the feed was determined using Enzyme linked immunoassay kit according to the methods of AgraQuant test kit.

The group mean (n=5) + S.D (Standard Deviation) was subjected to analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT).

Table 1. Gross composition (g/100gDM) of the experimental diets.

	DIET A	DIET B	DIET C
Crude protein (%)	23	23	23
Methionine (%)	0.6	0.6	0.6
Lysine (%)	1.2	1.2	1.2
Afflatoxins level	<20% ppb	90% ppb	180% ppb
Ingredients			
Maize	36.00	36.00	36.00
Soybean meal	41.00	41.00	41.00
Wheat Offal	13.00	13.00	13.00
Palm oil	5.00	5.00	5.00
Dicalcium phosphate	2.14	2.14	2.14
Limestone	2.09	2.09	2.09
Vitamin-mineral premix	0.25	0.25	0.25
Salt	0.25	0.25	0.25
Met	0.27	0.27	0.27
Lysine	-	-	-
Total	100.00	100.00	100.00

CP-crude protein **Premix supplied the following information kg of diet: Vitamin A (12,500,000 I.U), Vit D3 (2,500,000 I.U), Vit E (40,000mg) Vitamin K3 (2,000mg), Vit B, (3,000mg), Vit B2 (5,500mg), Naicin (55,000mg), calcium panthothenate (11,500mg) Vit B6 (5000mg) Vit B12 (25mg), choline chloride (500, 000mg), folic acid (1,000mg), Biotin (80mg), Mn (120,000,mg), Fe (100,000mg), Zn (80,000mg), Cu (8,500mg), I (1,500mg) Co (300mg), Se (120mg)*

RESULTS AND DISCUSSION:

At 20ppb, there was a significant reduction in alkaline phosphatase activity in the liver from 36.630±1.722 to 28.992±1.376(Table 2) in birds fed with 180ppb aflatoxin based diet while there was a corresponding significant increase in serum and kidney. The cellular enzyme increased in the serum. This means that the enzymes could have leaked into extracellular fluids as a result of loss of membrane components , the loss in ALP activity in liver may adversely affect the transfer of metabolite or required ions across the cell membrane, which may result in insufficient ions and metabolite to renal cells. This was earlier suggested by Akanji *et al*; (1993).

Table2: Results of Enzyme studies of birds fed varying levels of aflatoxins based diets

		Dietary treatments	
Parameters	<20ppb	90ppb	180ppb
Serum Alkaline Phosphatase	3.202±0.170[a]	3.752±0.206[ab]	11.80±2.138[b]
Kidney Alkaline Phosphatase	34.260±0.477[a]	39.300±2.217[a]	69.760±6.492[b]
Liver Alkaline Phosphatase	36.630±1.722[a]	36.528±1.818[a]	28.992±1.376[b]
Serum Alanine Transaminase	1.328±0.122[a]	4.540±0.493[b]	4.880±0.432[b]
Kidney Alanine Transaminase	0.316±0.150[a]	1.580±0.912[b]	3.640±0.344[c]
Liver Alanine Transaminase	1.926±0.161[a]	1.738±0.152[b]	1.232±0.188[bc]
Serum Aspartate Transaminase	76.064±4.174[a]	32.722±1.221[b]	23.646±1.036[c]
Liver Aspartate Transaminase	68.572±6.510[a]	38.294±4.352[b]	34.958±3.744[b]
Serum Creatinin	4894.4±73.531[a]	5046±57.678[b]	5094.8±63.291[b]
Serum urea	95.964±4.704[a]	107.280±18.821[b]	142.920±16.463[c]

[*abc] means within the rows with different superscripts are significantly different. ($p<0.05$)

Table3: Haematological Parameters of birds fed varying levels of aflatoxins based diets

		Dietary Treatments	
Parameters	<20ppb	90ppb	180ppb
Red blood cell	2.66±0.198[a]	2.59±0.193[a]	2.32±0.197[ab]
White blood cell	257.80±22.538	245.26±17.296	264.82±11.389
Haemoglobin	7.84±1.435[a]	10.46±0.826[b]	11.00±0.803[b]
Haematocrit	36.66±2.978	34.44±2.131	35.40±2.691
MCV	140.38±5.126	139.56±4.250	136.96±0.508
MCH	38.68±4.101	41.56±1.201	41.92±1.184
MCHC	29.08±1.648	29.80±1.336	30.58±0.909
Platelets	0.80±0.837	1.20±0.837	1.20±0.837
Lymphocytes	95.58±0.581	95.48±2.701	95.17±2.273
RDW	16.46±1.643	16.56±0.546	17.28±1.737

[*ab] means within the rows with different superscripts are significantly different. ($p<0.05$)

The aminotransfererases occupy a central position in amino acid metabolism and are active in both the cytoplasm and mitochondria of cells where they linked protein metabolism to carbohydrates metabolism (Rafelson *et al*., 1980). They are present in the liver, heart, kidney, skeletal muscle and other tissues. (Tietz, 1987). Both enzymes are 'markers' of liver damage caused by exposure to chemicals (Nelson and Cox, 2000) with alanine transaminase been more liver specific (Tietz, 1987). Aspartate transaminase levels are elevated when there is liver damage, leading to possible heart attack. (Tietz, 1987). Increase in serum enzyme may also be due to cell proliferation, increase cell turnover, or reduced clearance from plasma (Mayne, 2005).

The loss in alanine transaminase activity in liver from 1.926±0.161 in birds fed less than 20ppb to 1.232±0.188 in birds fed 180ppb aflatoxin based diet is consistent with its increase in the serum and the kidney. It may be due to leakage of this enzyme into extra cellular fluid caused by altered endothelial permeability (Wroblewski and La Due, 1955; 1956) leading to escape of abnormal quantities of the enzyme into the extracellular space. The loss in alanine transaminase activity would adversely affect the liver since pyruvate is a source of carbon for glucose synthesis and the

enzyme is also involved in deamination of alanine to pyruvate, providing amino groups for the urea cycle.

The consistent decrease in activity observed in the serum and liver aspartate transaminase in birds fed from less than 20ppb to180ppb aflatoxin based diet could be due to inhibition of the enzyme activity, inactivation of the enzyme *in situ* or depletion of important molecules required for their activities.

Kidney Functions Indices:

The kidney excretes urea and also reabsorbs electrolytes back into the blood thereby regulating their excretion. Filtration occurs in the glomeruli and reabsorption occurs at the renal tubules (Mayne, 2005). As glomerular function deteriorates, substances normally cleared by the kidneys accumulate in the plasma, e.g. urea and creatinine. Urea is formed in the liver from amino acids and is excreted by the kidneys. Creatinine is mostly derived from endogenous sources by tissue creatine breakdown and its concentration in blood is related to body mass (Mayne, 2005). The significant increase in serum Creatinine concentration from 4894.4±73.531 in birds fed less than 20ppb to 5094.8±63.291 in birds fed 180ppb aflatoxin based diet revealed a low glomerular filtration rate caused by impaired glomerular function.

Concentration of serum urea increases significantly from 95.964±4.704 in birds on 20ppbb to 142.920±16.463in birds on 180ppb aflatoxin based diet possibly due to the fact that its rate of production exceeds rate of clearance.

Haematological Parameters:

Marked difference among the treatments was noticed on the red blood cells and haemoglobin levels(Table 3) Red blood cell count, haemoglobin and mean corpuscular haemoglobin concentration are all indices of red blood cell and their reduction can indicate anaemia just as an increase can indicate increased rate of erythropoiesis (Ganong, 2001). Platelets activate the blood clotting mechanism (Pasternak, 1979) and function mainly in the formation of mechanical plugs during the normal haemostatic response to vascular injury (Hoffbrand *et al*., 2004). Haematological analyses(Table 3) did not also show a marked departure from the normal which indicated that the birds were not affected by the varying aflatoxin levels, even though there was a likely compromise in the rate of oxygen transport, which is a major function of rbc. In an experiment with chicken and ducks, Ostwoski-Meissner,(1984) earlier reported no significant reduction in body weight .Haematology, body weight gain may therefore be linked in avian aflatoxicology

Conclusion:

The results obtained in this study showed that birds could still survive at less than 20ppb and 90ppb in most cases but not at 180ppb aflatoxin level. This report could not confirm that consumption of chicken poults fed above 90ppb aflatoxin based diet could be injurious to the health even when Gregory *et al*, (1983) earlier reported that muscles (not of the liver) tissues of chicken fed up to 500ppb aflatoxin B1may not be a source of serious danger to a human consumer of such birds' infected muscles

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Investigating the relationship between finance index and effective factors on determining the capital structure of accepted companies in Tehran stock exchange.

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Abstrac: In the present study , the main issue is the finance index and effective factors on determining capital index in Tehran stock exchange .The sample study includes 162 companies in the time period from 2005-2006. The results of this study show that finance indexes are affected by effective factors on capital structure of accepted companies in Tehran stock exchange and basically dependent on free cash flow ,fixed assets of company ,profitability and investment opportunities variables . Although the findings of this study does not envy the prediction of finance options hierarchy theory and .information asymmetry hypothesis, is seems that companies at Tehran stock exchange practically pass finance options to provide their required financial resources.
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Key words: finance index, effective factors on capital structure, finance index options hierarchy theory, fixed balance theory

Introduction

One of the major decision making area of the corporation financial managers is capital budgeting and financing (Barely and Mayerze, 2004).

Capital cost is one of the important factors in capital budgeting decisions because capital cost is used as cash flow discount rate resulted from capital projects. Therefore, the companies choose the best rate to reject or accept the investment projects. Now the major question is that "Is there a favorite structure in which capital cost of company minimized and firm value become maximum?"And if so, what are influential factors in determining it?

Modigiani_Miller theorem about financial leverage and capital cost

In 1958, Modigiani_Miller in their famous article rejected traditional theory and expressed that the firm value in all usage levels of leverage remains fixed.

In other words, any change in the financial leverage level has on influence on capital cost of company .This theory suggested on the basis of the following assumptions:

1. Market capital is complete and assesses to the information has no cost.
2. There is on tax in corporations (this view was modified because of criticisms)
3. Investors can use of personal leverage instead of firm leverage.
4. All cash flows are permanent, that is the firm has zero growth ratio and revenue is predictable before tax and return.
5. Firms are exposed to the same risk level and trading risk can be measured through revenue stand arid deviation before return and tax.

On the basis of the above mentioned assumptions, particularly based on the second assumption the Modigiani_Miller theorem is presented in two ways irrespective of tax and in regarding to the tax.

Modigiani_Miller theorem first theory which does not consider tax forms the basis for modern thinking on capital structure. The basic theorem states that ,under a certain market price process , in the absence of taxes of taxes , bankruptcy costs, agency cost and asymmetric information ,and in an efficient market ,the value of a firm is unaffected by how that firm is financed . It does not matter if the firm's capital is raised by issuing stock or selling debt .It does not matter what the firm's dividend policy is. A firm that sells bounds and common stock , in fact , presents actual revenue in the form of collection of investors .In doing so, they selected two grows of firms (levered and unlevered firms) and finally they concluded that value of two firms is the same.

Modigiani_Miller also that (second theorem considering tax) expected return ratio for common stock of levered firm increases as debt ratio increases and it's become of increasing capital risk.

Therefore, expected return ration for common shareholders in levered firm equals common stock cost of unlevered firm in the same trading risk level plus the risk, as much the difference between common stock cost and loan cost for a levered firm.

On the basis of this, return ratio for unlevered firm, a firm that has no any debt, equals total return. That is: key=kef

Key=common stock return kef=unlevered firm return (total capital cost)

And if the firm uses debt, the firm cost does not change, but common stocks capital cost (common stock return) will be as follow:

Ke=expected return (capital/cost) for unlevered firm, kf=the required rate of return (cost) on common stocks, ki=cost of debt rate

Modigiani_Miller theorem of levered firm and capital cost with tax

Modigiani_Miller considered tax in their new theorem and expressed the firm value a follow:

T=tax ratio of revenue

In this way the capital cost of firm remains and as debt increases become tax saving, the firm value increases and capital cost of firm decreases. In other words , capital cost of levered firm depends on capital cost of unlevered at the same level and with the same trading risk level and depends on the difference between common stocks cost and debt cost of levered firm , leverage level degree and tax rate .

Modigiani_Miller considered the "with tax" assumption and recommended that the firms should use of too percent of debt to maximize their value and benefit from tax advantages (tax saving).

We tried to recognize the capital structure pattern of the firms accepted in Tehran stock exchange and determine the most important effective factors which influence this pattern.

Research background

Different theories have been presented to justify not reflecting 100 percent of firms for borrowing to obtain their financial resources.

These are as follow:

1. **Information asymmetry hypothesis** :managers have more and better information about firms in comparison to market in the world of information asymmetry .Managers know more about the firms because they have more private and secret information ,that is , they access to particular type of more firm information before the market become aware of it .For example , Meyers and Major (1984) suggest that if investors have less information about the actual value of firm, they may misprice the shares of the firm . If the firm has to financial the new projects through stocks selling, the pricing may be less that market value and new investors gain more that the net present value of the project and the previous stockholders face with losing .The rare, in such a situation the firm has to ignore the new project investment with positive net present value (Harris and Rio, 1991).
2. **Fixed or stable balance theory**: This theory says that tax debt advantage increases the value of a firm which has debt.
   On the other hand, bankruptcy and financial crisis costs resulted from not doing obligations on time decreases the firm value. So we can consider the capital structure of the firm as the balance between tax debt advantage and probable bankruptcy and financial crisis cost resulted from debt (Braila and Mayors, 2004).
3. **Financing options hierarchy theory**: On the basis of this theory the firms pass the determined hierarchy to gain required finance .the forming of this hierarchy is the result for consequence of asymmetry .According this theory, when there is information asymmetry between managers and external investor, managers prefer financing from internal sources of the firm to the external resources, that is, they first finance through accumulated profit or finance savings.
4. **Cost agency theory**: this theory was presented by Jenson and Cackling in 1976 for the first time .the capital structure of the firm was determined via agency costs resulted from interest conflict between different stakeholders of the firm. Jenson and Macklin recognized two types of interest conflict in enterprise framework a) interest conflict between managers and stockholders b) interest conflict between stockholders and firm debt securities holders.
5. **Free cash flow theory:** is another theory which explains the capital structure and has a suitable back ground .studies which was introduced in 1986 by Michel Jenson .They theory has important reactions for capital structure .According this hypothesis paying dividends to the shareholders increases the free cash flow of the firm. therefore , it is expected that increasing the payable dividends with reduction managers ability to follow the goals or activities which are in conflict with stockholders interest , the interest of stockholders increases .looking at other researchers conducted in other countries:

The low cost rate in comparison with other capital resources and tax saving resulted from debt interest that is considered as an acceptable tax cost , the financial experts believe that the proper combination of shares and debt in financial structure of firms can be an influential factor in increasing market value of firm and shareholders .since paying attention to the firm value

increasing to help the combination of capital structure ,different studies conducted to investigate the effective factors on capital structure form and how the finance is done .Is this regard the famous theory of Modigiani_Miller in 1958 expressed that capital structure does not have effect on firm value and this was a start for conducting researches in this field . A year later in 1959 David Durand published an article and criticized Modigiani_Miller theory and in 1963 Furrow stone in 1965 Brow Rojacob and between 1977-1979 Morton and Jack Bicker strongly criticized this theory. So that these two persons had to defend their theory via publishing articles in the years of 1957, 1963, 1965 and finally in 1966 and they also modified their theory .including considering tax saving of borrowing finance cost and finance method on firm value.

In 1977 a person called Varner emphasized on finance effect through borrowing on firm value and in 1973 Block pointed that the issue of tax saving through borrowing has effect on firm value, although is not shown high.

On the other hand scot and Martin in the U.S.A concluded that the industry type is a determinant and effective factor in capital structure of firms. In 1990 found out this point that capital firms have higher debt ratio and this shows the relationship between capital structure of firms and their technology.

The findings of Bent Stuart and David Galls research in relation to the interest resulted from renewing capital structure showed that the use of financial leverage is the best method of renewing capital structure and pointed out that financing through debt causes tax saving and this is because of finance cost payment . Moreover, with accrued loan the installment will be resulted which reduces the improper reinvesting surplus.

Rimerz in a research in relation to industry type and the extent of its relation to capital structure pointed out this issue that the capital structure type in countries like Japan, France is significant in different industries while this is not true about some countries like Netherland and Norway.

Free and Johns investigated the relationship between firm size, business risk, industry type and return, operational leverage of firms and debt leverage .In this research 233 firms were investigated during 5 years and the findings suggest that firm size, industry type, the operational leverage degree of the firm have effect on applying debt risk in the firm while business risk has no relationship with the degree and applying financial leverage in the firm.

Charles Kim and Badly investigated 851 firms in service industries (electricity, telephone, gas and airline) and concluded that leverage ratio fluctuating ratio in the earning of firms have opposite relationship.

Research Hypotheses:

1. Thereis a significant relationship between debt ratio (financial leverage) and sale volume
2. There is a significant relationship between debt ratio (financial leverage) free cash flow.
3. There is a significant relationship between debt ratio (financial leverage) and fixed tangible properties ratio.
4. There is a significant relationship between debt ratio (financial leverage) and investment opportunities.
5. There is a significant relationship between debt ration and abnormal return.
6. There is a significant relationship between debt ration and profitability.

Research Variables:

Debt ratio (financial leverage):four criteria for measuring financial leverage or debt ratio have been used in this that everyone measured based on two book value (BV)and market value (MV) criteria .the market value and market value of equity to debt are as follow:

$$(BV1)=\frac{\text{total long-term liabilies +business credits}}{\text{Total assets}}$$

$$(MV1)=\frac{\text{Total long-term liabilities +business credits}}{\text{Total assets-book value of equity +market value of equity}}$$

$$(BV1)=\frac{\text{Total liability}}{\text{Total assets}}$$

$$(MV2)=\frac{\text{Total liability}}{\text{Total assets -book value of equity +market value of equity}}$$

Total debt to total assets

$$(BV3)=\frac{\text{Total liability}}{\text{Total liability + book value of equity}}$$

$$(MV3)=\frac{\text{Total liability}}{\text{Total liability +market value of equity}}$$

Adjusted debt to adjusted capital

$$(BV4)=\frac{\text{Total liability –cash – readily marketable securities}}{\text{Total liability –owners equity – cash – readily marketable securities}}$$

$$(MV4)=\frac{\text{Total liability – cash _ readily marketable securities}}{\text{Total liability– market value of equity–cash– readily marketable securities}}$$

Investment opportunity

$$(MB\ Asset)=\frac{\text{Sum of book value of assets –book value of common stocks +sum market value of common stocks}}{\text{Sum of book value of common stocks}}$$

$$(MBEQUII)=\frac{\text{Sum of market value of common stocks}}{\text{Sum of book value of common stocks}}$$

$$EP=\frac{\text{Each share profit}}{\text{Each share price}}$$

Sale Volume:

The nature logarithm (LN) of firm annual net sale volume is used to measure this variable .In regression model; LNS symbol has been used for sale variable. Abnormal return of this variable is written from USB.

Free cash flow is as follow:

Fixed tangible assets ratio which for measuring it the book value of fixed tangible assets to total assets has been used.

$$\frac{\text{Book value of total fixed tangible assets}}{\text{Total assets value}}$$

In regression model TANG has been used as the symbol of fixed tangible assets ratio.

Profitability:

$$(EBITD)=\frac{profit\ before\ tax\ and\ depreciation}{book\ value\ of\ total\ assets}$$

Methodology

The required information for the present study were obtained from information in annual financial statements (balance sheet, accumulated income statement and statement of cash flow)of nonfinancial firms selected in the time period of research and the market value of share of every firms the end of the year. time period of this research was four years which began from the end of 2005 to the 2009.the subject are all nonfinancial firms accepted in Tehran stock exchange and include 120 firm in which the "criteria –filtering technique was used .

The first step was generally knowing about debt ratio and consequently capital structure of firms , and every of debt ratio was calculated on the basis of book value and market value to total investigated subjects .the results of calculations are as follow:

Table 1 .Summary of descriptive information of 162 investigated firms form 2005-2009.

Variable	Ratio	Mean	Median	Observation
Book values				
Noncapital debt to total assets	BV1	%46	%44	162
Total debt to total assets	BV2	%31	%31	162
Total debt to total capital	BV3	%23	%22	162
Adjusted debt to adjusted capital	BV4	%19	%12	162

Market values				
Noncapital debt to total assets	MV1	%35	%32	162
Total debt to total assets	MV2	%22	%20	162
Total debt to total capital	MV3	%20	%19	162
Adjusted debt to adjusted capital	MV4	%16	%12	162

Then, to verify or reject the hypothesis, on the basis of the provided information, regression model was used and the result is in the following table2. As table 2 shows , approximately all coefficients (except few) are significant at %1 level .it also shows that when dependent variable (debt ratio)is measured on the basis of market value , it keeps all its expected coefficients.

Table2. Results of time analysis to debt at the end of 205

D.M	CONSTANT	IOS	AR	FCF	TANG	EIT	LNS	ADJ	F-STAT
BV_1	0/48	0/02	0/09	0/11	0/27	0/69	0/06	0/17	46/15
BV_2	0/20	0/06	0/08	0/18	0/16	0/49	0/08	0/14	24/04
BV_3	0/18	0/03	0/09	0/12	0/12	0/50	0/11	0/14	32/12
BV_4	0/22	0/09	0/12	0/19	0/19	0/62	0/12	0/11	18/40
MV_1	0/61	0/06	0/08	0/10	0/30	0/62	0/04	0/16	47/03
MV_2	0/39	0/12	0/06	0/19	0/16	0/40	0/07	0/19	20/01
MV_3	0/48	0/04	0/13	0/13	0/11	0/56	0/12	0/20	28/16
MV_4	0/36	0/12	0/15	0/16	0/20	0/59	0/12	0/12	20/20

Firm sale volume (LNS):

As it is seen in the table2 , sale volume has positive relationship with debt ratio , this relationship confirms the hypothesis 4.In spite of positive relationship between sale value of the firm and debt ratio , it is not consistent with nonce of theories or capital structure hypothec thesis.

Logically, we can argue that, in big firms in comparison to small firms, information asymmetry of firm managers and market is lower. Therefore, it's expected that big firms are not faced with serious problems in publishing the stocks and consequently use less debt.

As it was in table2, the research finding, in spite of expectations the prediction of finance options hierarchy theory was not verified. To investigate and known more about the issue , the information of board activities reported to the general assembly of stockholders was used and firms that increased the capital along with the financial resources were identified .The results are reported in the relationship between financial leverage and cash flow has been investigated in hypothesis 2 and the findings show that there is a relationship between free cash flow and debt ratio and it confirms Jensen theory in 1968 , Biking and

Ferdinand theory in 1999 and Ferdinand and Tipsy theory in 1998.

Fixed tangible assets ratio (TANG): As expected, there is a positive relationship between fixed tangible assets ratio and debt ratio. The logical reason is that, fixed tangible assets of the firm has mortgage value and consequently the borrowing agency cost decreases .so , it's expected that the firms which have fixed tangible assets and mortgage value , prefer borrowing to the stock publishing to provide the required financial resources .therefore , the third hypothesis is verified .

The relationship between financial leverage and investing opportunities has been investigated in hypothesis. Since the measurement of investment opportunities the daily values are used and the market value is used for financial leverage, the financial of this hypothesis, like researches conducted in abroad (Ferdinand parch, 1999), are confirmed.

The confirmed financial background is that those firms that have higher market value to book value, their financial crisis cost is also higher .therefore, it's expected that there is a negative relationship between market value to firm assets and its debt ratio . of course , it may be for other reasons .for example , the shares of the firms that face with financial crisis are reduced with the higher expected rate by investors(Fame and French , 1992).if this reason be valid , it's expected that this negative relationship bestiality found in the firms which has the lower market value ratio to book value ratio. But it seems that there is a negative coefficient between market value to book value and debt ratio in the firms that has higher market value ratio to book value ratio. Anyhow, the financial crisis is not the only reason for this coefficient.

On the basis of research financial, there is a significant relationship between debt ratio (financial leverage) and abnormal return ratio which is consistent with Tesangarlkiss.

Profitability of the firm (EBITD):

Both financial options hierarchy hypothesis and information asymmetry hypothesis predict that those firms that have higher profitability are less dependent on the borrowing. The sixed hypothesis is based on this idea .if spite of the prediction, the negative relationship between firm profitability and its debt ratio was observed. So on the basis of the finding the hypothesis is not verified. The findings the hypothesis confirms prediction of finance options hierarchy theory and information asymmetry theory.

The finding of the study report a positive relationship between debt ratio and firm profitability in most cases, these findings are consistent with the finding of Vessel and Titman (1998), Harris and Roy (1991), Raja and Zing les (1995) and Bionand Danbolet (2002).

To justify the positive relationship between debt ratio and profitability of firms in Tehran stock exchange, we can argue that the firms which have move profitability art more able to do their obligations and pay their debt on time and can attract the creditors to invest for long –time. On the other hand creditors do well to credit to these firms or renew credit.

Conclusion and suggestions:

Miller and Modigliani believe that under special assumptions, the economic unit value is independent of its capital structure. In other words, they believe that managers cannot change the value of the firm only through changing in the capital structure form. Miller and Modigliani believe that with the assumption that capital markets do their main duties there would be on any cost on stock exchange, bankruptcy costs and tax, and also with the assumption of complete replanting of internal and external finance resources, the financing method does not have any effect on total value of firm. But, unfortunately in the real world none of Modigliani –Millers assumption is practical .Moreover, the firms are facing some proems to provide their financial resources from outside of the firm and the costs of different external financial resources are different .in these situations, the firms try to chose a suitable level of debt and stock in the financial resources form to reach to a proper capital structure. Therefore, it seems that the capital structure has relationship with firm value.

The findings of this research show that capital structure pattern of the firms accepted in Tehran stock exchange basically dependent directly on variables like assets ratio of firm, sale volume of the firm investment opportunities, abnormal return ratio, free cash flow and its profitability. Keep it another way, in Tehran stock exchange, the firms that have higher investment opportunities from sale volume point of view, are more dependent on debt rather than stock. The main reason may be the easy access to bank resources or potential market capital .In addition, it seems that information asymmetry between big firms and market capital is less than small firms .Moreover, according to the findings of this research, in Tehran stock exchange, those firms which have the mortgage assets are more dependent on debt rather than stocks. The main reason may be the easy access to bank financial resources.

This is suggested to the researchers to investigate the mentioned theories in the research in different industries. Moreover, they study the relationship between short-term financial leverage and long-term in capital structure and firm performance from risk and return point of view. Investigating the relationship between with product type/ firm product and its exclusiveness in the market, and also studying the relationship between managers' ownership level in the

firm and firm financial leverage are other interesting issues in this study which implies further investigation.

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HORMONAL STUDY DURING OVARIAN CYCLE IN THE EMBALLONURIDAE FEMALE BAT *TAPHOZOUS KACHHENSIS* (DOBSON)

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Abstract: The ovarian hormone examined during different stages of reproductive cycle in bat *Taphozous kachhensis* are describe. During estrus there is sharp increase in estrogen level is observed, while the progesterone level is decrease. This sharp increase in the level of estrogen correlates with histological observation during estrus where ovary shows well developed Graffian follicles. During the early pregnancy the concentration of progesterone is increases, while estrogen concentration decreases, this observation correlates with the histological finding of corpus luteum during early pregnancy. As the pregnancy advanced there is further decline in the level of progesterone during mid pregnancy but it is high as compare to estrogen, this decrease in the level of progesterone is due to the regression of corpus luteum during mid pregnancy. The higher level of progesterone is further maintained after the formation of placenta. During the late pregnancy there is sharp increase in the level of estrogen, while the progesterone level again decreases. During lactation sharp increase in the level of estrogen is observed, while the level of progesterone is decreases and attains the low concentration.
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Key words: Ovary, estrogen, progesterone, pregnancy.

INTRODUCTION

The peripheral concentration of a hormone reflects the balance between rates of secretion and metabolic clearance, with the latter influenced by the extent to which the hormone is bound by plasma proteins.

In female bats, the major circulating ovarian hormones are oestradiol, progesterone. The two principal sources of steroid hormones are the ovary (interstitial tissue, thecal cells, granulosa and luteal cells) and placenta. While the steroidogenic activity of the mammalian ovary has been and remains an active field of research, very few studies have examined ovarian steroidogenesis bats. This is in spite of the fact that the structure of the ovary of several species poses interesting questions about ovarian steroidogenesis.

The two major sources for progesterone in bats are the corpus luteum and the placenta with the relative importance of these two organs differing both temporally within a species and between species. Additional sources of progesterone are the ovarian interstitial tissue and the adrenal gland. The most complete data are available for *Myotis lucifugus* and *Miniopterus schreibersii* and clearly illustrate these points. In both species the placenta takes over progesterone production from the corpus luteum in the final third of pregnancy (Buchanan and Younglai, 1988 and van Aarde *et al.*, 1994 respectively). Less complete data, indicating that the placenta takes over progesterone production from the corpus luteum are available for many other species. For example, the corpus luteum of a range of species undergoes luteolysis in late pregnancy (e.g. Kayanja and Mutere, 1975, *Otomops martiensseni*; Kitchener and Halse, 1978, *Chalinolobus gouldi* and *Eptesicus regulus*; Kitchener and Coster, 1981, *Chalinolobus morio*; Gopalakrishna, 1969, Gopalakrishna *et al.*, 1986, *Rousettus leschenaulti*; Towers and Martin, 1995).

MATERIALS AND METHODS

Taphozous kachhensis (Dobson) is an exclusive Indian Emballonuridae bat found in caves, tunnels and temples. The bat selected for present study because of unique habits. The gestation length of adult female of the species *Taphozous kachhensis* (Dobson) is about 100 days. The collection of the specimen commenced in February 2006 and the last specimen for the present study was collected in May 2009.The specimen of Taphozous *kachhensis* were collected from Ambai Nimbi, 45 kilometers from Bramhapuri (M.S.). Many collections were made during the breeding season so as to coincide with the time of reproductive cycle and to get an accurate pregnancy record. During day time their roosting places were visited and the specimens were netted at random with the help of a butterfly net. These bats are very

sluggish in nature after collection they were sexed and only the females were brought to the laboratory. Weight recorded with sensitive spring balance before they were sacrificed. After noting the weight the blood is collected from the heart or from the wing vain with the help of disposable syringe for hormonal essay.

The plasma concentration was measured by Radioimmunoassay by using RIAK-5 kit. This method involved the binding between specific antigen and the antibody. The different hormone like progesterone and estrogen, were measured by using assay kit. The method is worked out in biochemical laboratory, Health care immunoassay division, Nagpur.

RESULTS

Hormonal profile during ovarian cycle

The ovarian hormone examined during different stages of reproductive cycle in bat *Taphozous kachhensis* are describe below.

Table-2:-Hormonal concentration during reproductive cycle

Reproductive Period	Concentration of Estrogen pg/ml	Concentration of Progesterone ng/ml
	Estrogen	Progesterone
Anestrus	7.9	6.2
Estrus	39.23	4.7
Early pregnancy	16.73	24.67
Mid-pregnancy	5.49	9.87
Late-pregnancy	13.60	3.2
Lactation	31.24	1.24

During Anestrus period the estrogen concentration is found 7.9pg/ml, while the progesterone is 6.2 ng/ml. During estrus there is sharp increase in estrogen level is observed (39.23pg/ml), while the progesterone level decreases to 4.7 ng/ml. This sharp increase in the level of estrogen correlates with histological observation during estrus where ovary shows well developed Graffian follicles. During the early pregnancy, the concentration of progesterone is increased to 24.67ng/ml., while estrogen concentration decreases to16.73 pg/ml; this observation correlates with the histological finding of corpus luteum during early pregnancy.

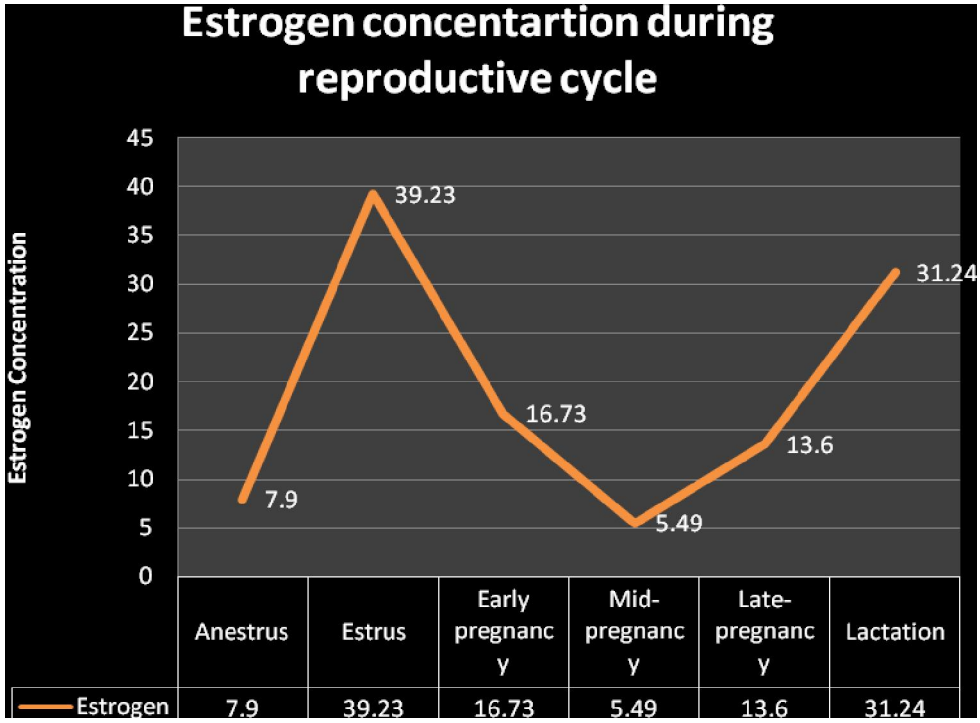
As the pregnancy advances further there is further decline in the level of progesterone (9.87 ng/ml); during mid pregnancy but it is high as compared to estrogen (5.49 pg/ml) this decrease in the level of progesterone is due to the regression of corpus luteum during mid pregnancy. But the higher level of progesterone is maintained due to the formation of placenta. During the late pregnancy sharp increase in the level of estrogen is noticed (13.60 pg/ml) while the progesterone level again decrease to (3.2 ng/ml). During lactation there is a sharp increase in the level of estrogen (31.24 pg/ml) is observed. While the level of progesterone is decreased to (1.24ng/ml).

DISCUSSION

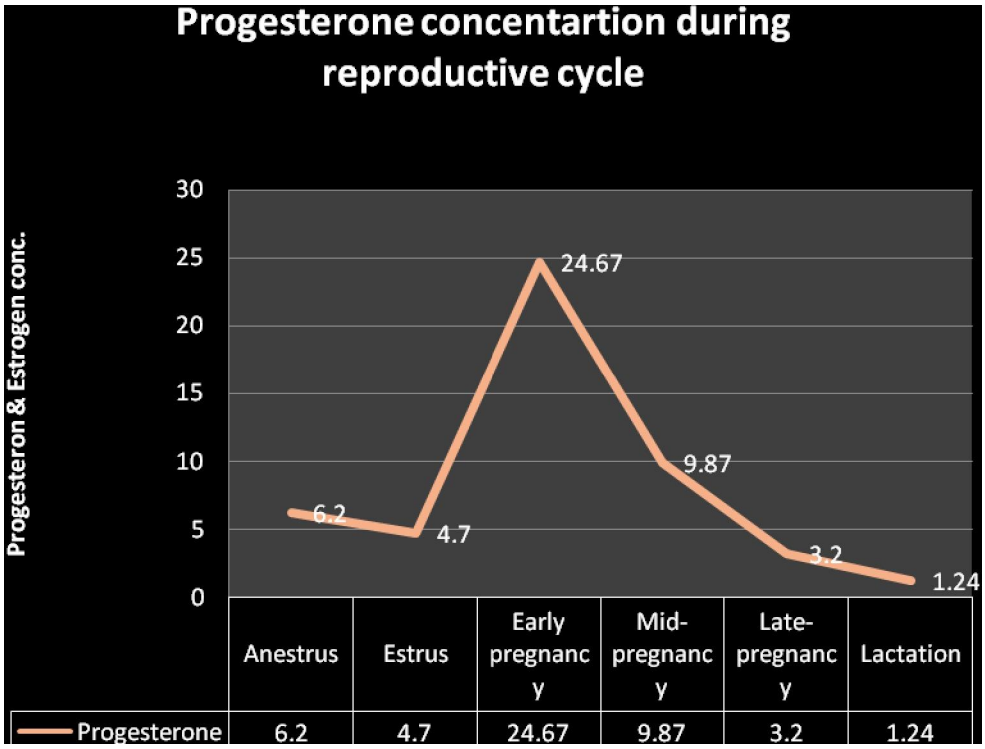
In the female bat, the pattern and levels of increases in progesterone concentration and its decline after the luteal phase is similar to that found in other species of bats. The progesterone concentration remains at basal levels throughout the estrus, as observed by others (Sonwane D.D.2010; Khadiga et.al, 2005).

The cyclic pattern of progesterone concentration in bat plasma found in this study is in agreement with known changes in cl function in the bat that occur during the estrous cycle. The rapid decline of progesterone in the peripheral plasma of the bat towards the end of the cycle as well as the marked rise in concentration during the time of cl development is strong evidence for suggesting cl function can be monitored in peripheral plasma by progesterone determination. That the plasma progesterone levels would decrease rapidly with declining cl function is suggested by other researcher (Sonwane D.D.2010., Imori 1967).

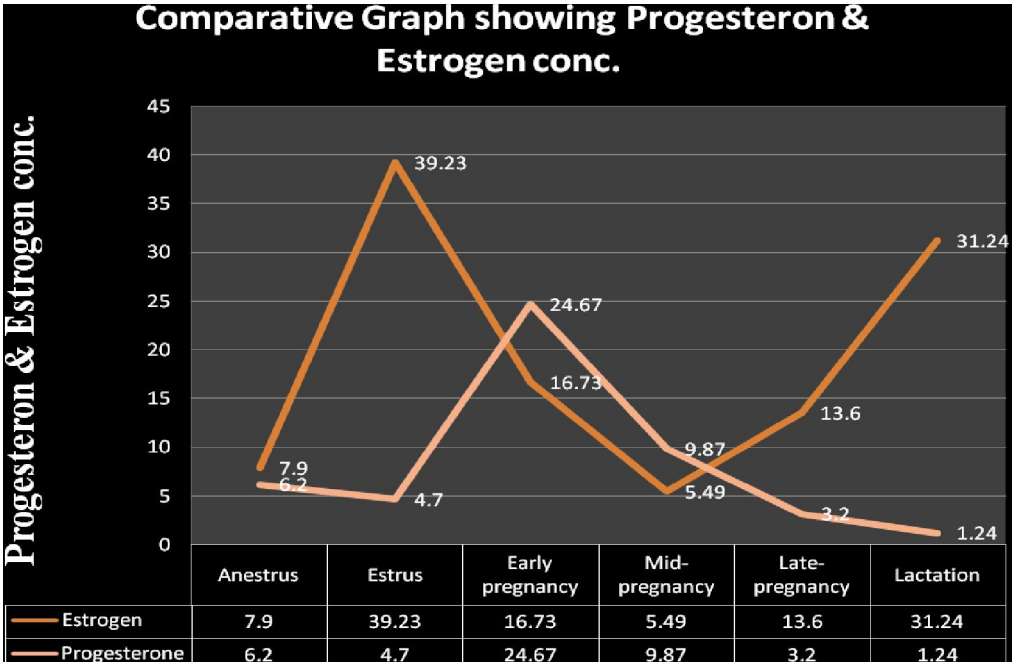
Musaddin *et al*. (1996) reported mean progesterone concentration during the follicular phase of estrous cycle as 0.19 and 0.26 ng/ml in DorsetHorn-Malin (DHM) and Malin ewes, respectively.



Graph 1:- Estrogen concentration during various phases of reproductive cycle.



Graph 2:- Progesterone concentration during various phases of reproductive cycle.



Graph 3:-Comparative Graph showing Progesterone & Estrogen conc.

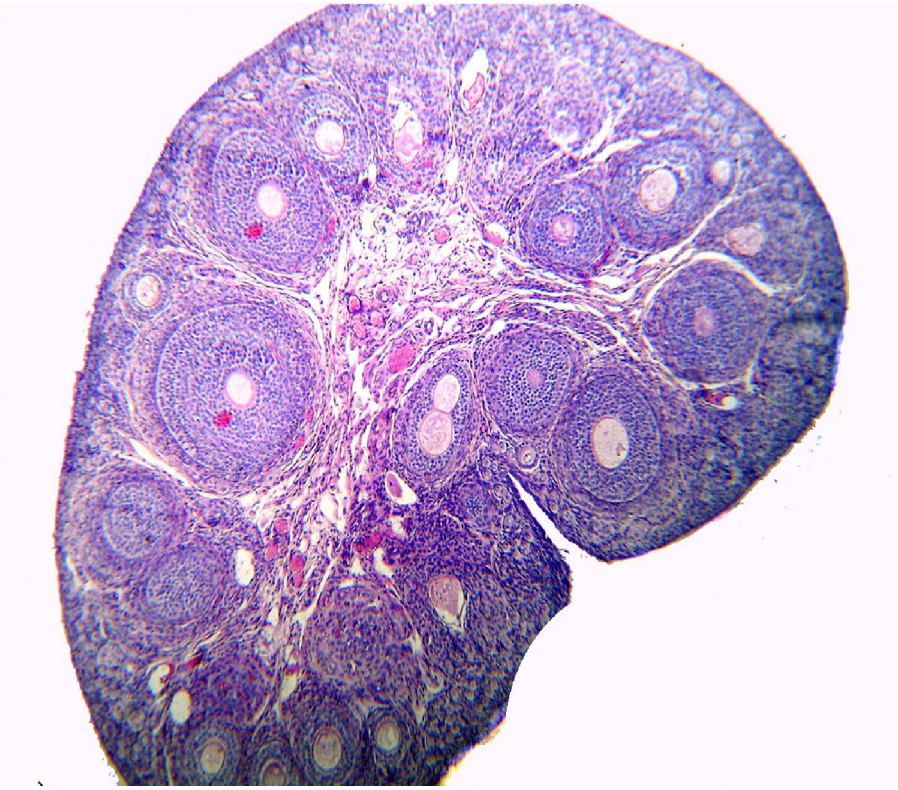


Fig 1.Transverse section of ovary during anestrus showing primordial follicle at peripheral part of ovary while deeper part of ovary shows the presence of multilaminar follicle (100X)

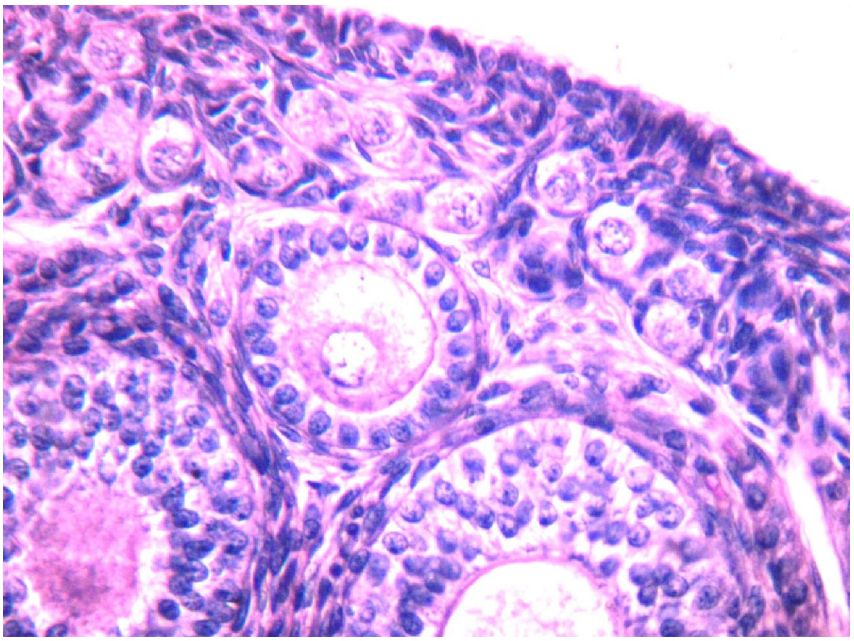


Fig 2.A part of ovary enlarged to show primordial follicle at the peripheral part of ovary. The cortex shows the presence of multilaminar and unilaminar follicle (400X);

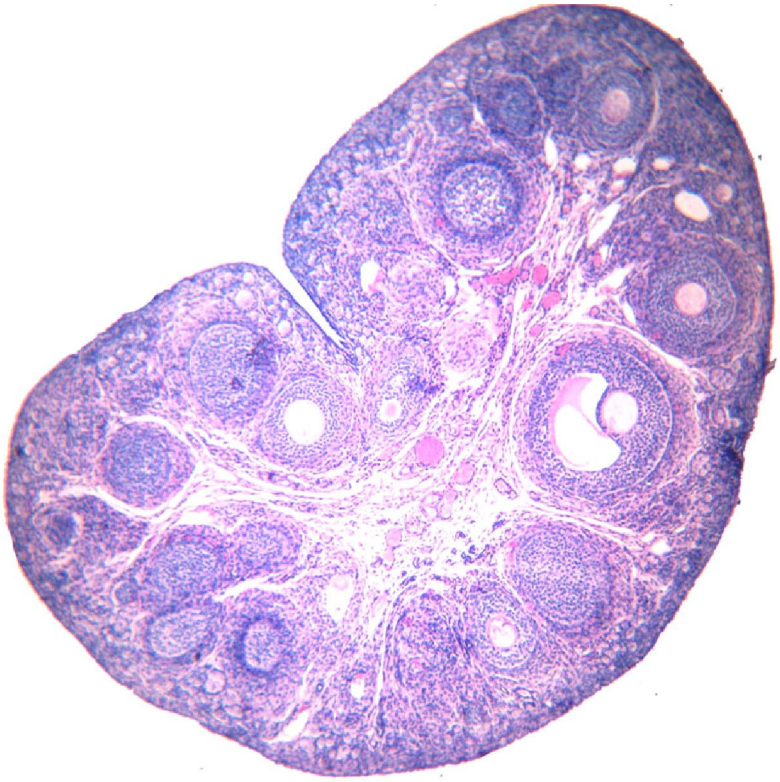


Fig 3. Transverse section of right ovary during estrus showing the presence of primordial follicle (PF), multilaminar follicle and Graffian follicle (GF). Note the antrum (A) filled with liquor folliculi (100X)

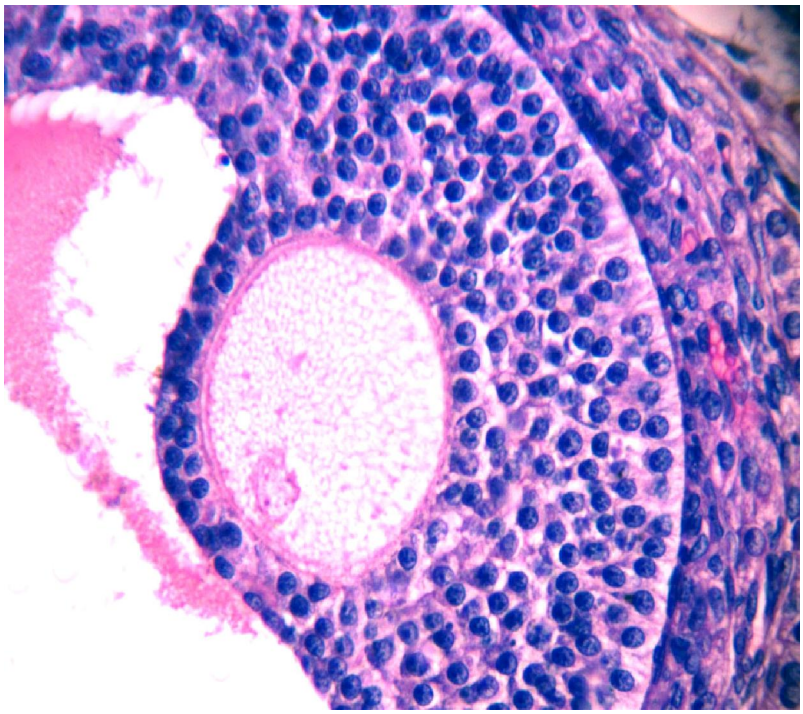


Fig4. A part of ovary during the estrus showing a large graffian follicle at peripheral part of ovary. The antral cavity occupies large part of follicle due to which oocytes pushed to one side (400X)

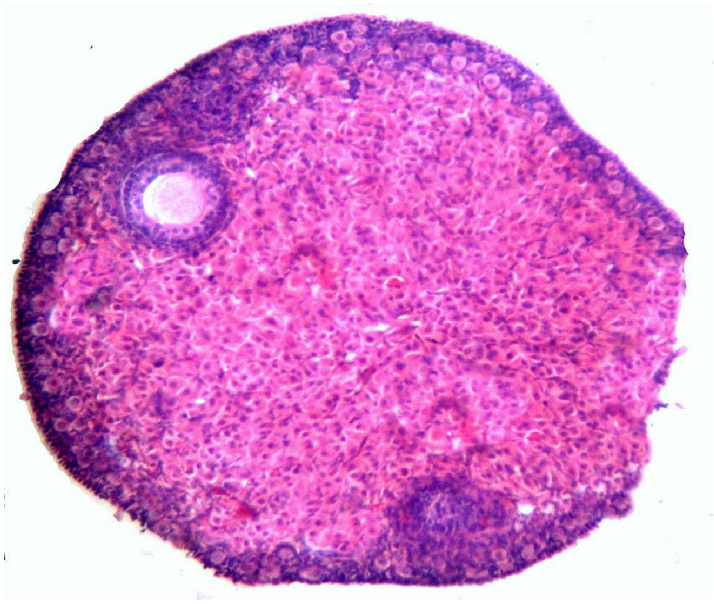


Fig5. Transverse section of ovary during early pregnancy showing the presence of introvert corpus luteum (100X)

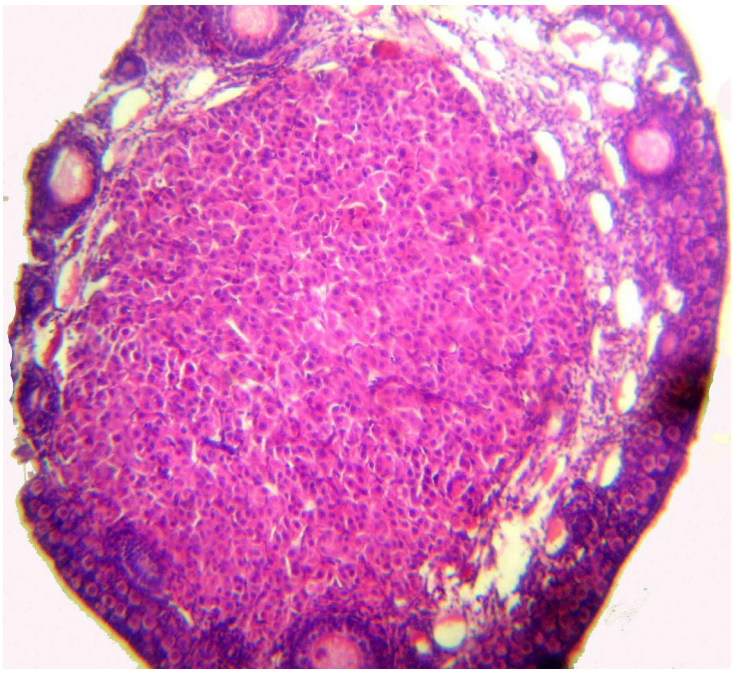


Fig6. Transverse section of ovary during mid-pregnancy showing the presence of regressed corpus luteum, few primordial follicles, and one double layered follicle (100X

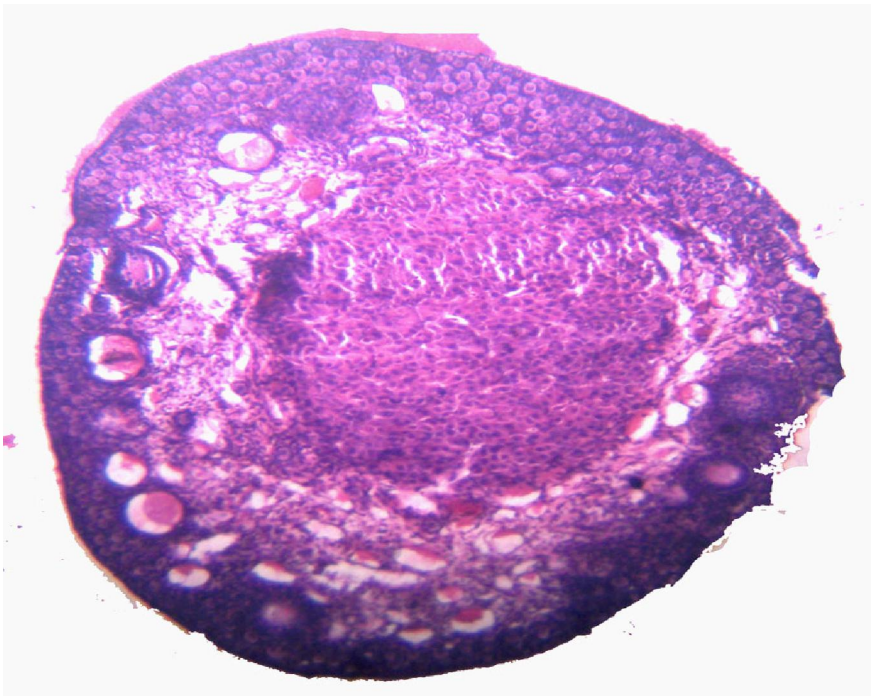


Fig7. Transverse section of ovary during late-pregnancy showing the presence of regressed corpus luteum

During the luteal phase, the concentrations were 2.33and 2.94 ng/ml, respectively. But in female bat *Taphozous kachhensis* the mean estrogen and progesterone concentration during the follicular phase of estrus cycle were 39.23pg/ml and 4.7ng/ml respectively. In Damascus goats, the progesterone level during luteal phase ranged from 2.6 to 5.4 ng/ml (Khadiga *et al*., 2005*),* which is comparable to the levels in *Taphozous kachhensis* during mid pregnancy.

Blaszczyk *et al.* (2004) reported the estradiol levels in Anglo-Nubian does at the time of estrus as 15.3 ± 5.0 and 12.2 ± 3.8 pg/ml in and outside the breeding season, respectively. While the estradiol level in *Taphozous kachhensis* dose at the time of estrus as 39.23pg/ml and 16.73pg/ml in outside the breeding season, respectively.

In *Taphozous kachhensis* during the gestation the higher level of progesterone were maintained with the wide variations. In this bat the mean plasma progesterone and estrogen concentration ranged from 6.2ng/ml to 9.87ng/ml and 7.9pg/ml to 5.49pg/ml from anestrus to mid pregnancy. The overall increase in progesterone levels during gestation and a decline towards the prepartum and parturition, observed in the *Taphozous kachhensis* and also resembles with the *Megaderma lyra lyar* (Sonwane D.D.2010)., Dwarf goat (Khanum et al., 2008).

Prepartum decline in the progesterone levels was correlated with the onset of parturition (Laura et al., 2004).

CONCLUSION

We conclude that estrogen is essential for normal folliculogenesis beyond the antral stage.

Estrogen is the first significant hormone of the estrous cycle. Rising estrogen levels result in the clinical signs of estrous. Progesterone produce by the corpus luteum prepare the uterus for the entry of the fertilized egg and quite the uterus to maintain the pregnancy.

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Evaluation of some biochemical, microbiological and organoleptic characteristics of some honey samples in Nigeria.

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Abstract: This work evaluated honey samples for their nutritive value, wholesomeness and effect of sucrose and honey on food functional properties. Honey samples obtained from Saki (A), Minna (B) and Maiduguri (C) exhibited the following characteristic features: 18 - 24% moisture content, 1.43 - 2.72% protein content, 0.49 - 0.86% ash, 73.7 - 78.6% carbohydrate, mainly sugars. No fibre or fat was detected. The pH values were between 3.2 - 3.6 which signify the honeys to be classified as acidic food. The most predominant minerals are Sodium (Na) (6.30 - 7.02), Potassium (K) (5.6 - 7.6), Calcium (Ca) (2.14 -3.40), Magnesium (Mg) (0.21 - 1.90) and Phosphorus (P) (2.40 -3.60) ppm. The mean viable microbial population counts are 0.5 x 10^7 - 1.15 x 10^7 cfu / ml at 10^{-6} dilution and 0.2 x 10^8 - 0.8 x 10^8 cfu/ml at 10^{-7} dilution. At 10^{-6} and 10^{-7} dilution, no mould growth was found except in Minna (B) honey. Honey samples from different parts of Nigeria are shown to be rich in nutrients and endowed with organoleptic properties. Some spoilage organisms were also isolated from the honey samples: *Xanthomonas campestris, Micrococcus roseus, Staphylococcus saprophyticus, Lactobacilllus fructivorans, Serratia marcescens and Aerococcus viridans.*

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Key words: honey**;** organoleptic; microbial organisms; biochemical properties, proximate analysis

Introduction

Honey is a natural substance produced by bees , consisting basically of a complex mixture of carbohydrates, especially glucose and fructose, organic acids, amino acids, minerals, vitamins, enzymes, pollens, and pigments (Crane 1987, Fallico et al. 2004) Its nutritional quality, medicinal, and sensory properties have attracted thousands of consumers(Carlos et al, 2009**)**.It is a mixture of concentrated aqueous solution of inverted sugars and complex mixture of other saccharides, amino acids, proteins, organic acids, vitamins, minerals, Maillard reaction products and both enzymatic and non-enzymatic antioxidants, including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids and carotenoid derivatives. (Smith 1960,Almamary ,2002,Gheldof 2002)

Bees obtain all their nutritional components from nectar, pollen and water. Nectar is reduced to honey containing predominantly carbohydrates with a very little protein, vitamins and minerals. Fully ripened honey consists of levulose / fructose (41%) and dextrose / glucose 35% and 22 others which are more complex than the monosaccharides present in quite minute quantities.(White *et al,*). Of the 22 complex sugars, the oligosaccharides identified are maltose, isomaltose, maltulose, nigerose, turanose, kojibiose, laminarihiose, α,β- trehalose and gsentibiose. Ten trisaccharides are present: melezitose, maltotriose, 3 - α - isomaltosylglucose, 1-kestose, panose, isomaltotriose and isomaltopentaose. Most of these sugars do not occur in nectar but may arise from enzymes added by honey bee during honey ripening or by chemical action in the concentrated acid sugar mixture of honey. (Gheldof *et al, 2002*)

The presence of phytochemicals such as flavonoids and phenolic acids that suggests the role of honey, along with fruits and vegetables, as a nutritional source of natural antioxidants responsible for protecting human health has been reported. *Gheldof et al 2003,,* McKibben 2002 , Schramm *et al 2003,* Tonks 2001,Tonks 2003) Its antibacterial, anti-inflammatory, antioxidant and anticancer properties have been extensively discussed.(Orsolic 2005,Swellam 2003,Blasa, 2006,Board 1972). Vitamin C and most of the Vitamin B complex are present in variable amounts. (Oszmianski ,1990).

Apart from being a high energy substance, honey has high digestibility, high acidity as well as a high taste appeal. By this characteristic antioxidant property, honey when applied at 10% has been found to inhibit enzymatic browning in apple slices and grape juice.(Khan 1985) It has been demonstrated that it is the proteins, peptides and amino acids in honey that exert an inhibitory effect on polyphenol

oxidase activity by chelating the essential Copper (Cu) at the active site of polyphenol oxidase thus forming stable complexes with Cu^{2+}.(Chen, 1998) It has also been revealed that the antioxidant content and the efficacy of honeys in inhibiting polyphenol oxidase activity vary in accordance with the type of honey. Agunbiade 1996

Honey has been used in the treatment of necrotic pressure sores, ulcers, burns and wounds, thereby eliminating characteristic odours of wounds. It is used also in treating external eye infections and diabetic foot(Aljadi , 2004)

Materials and Methods

The honey samples used in this study were obtained from Saki, Oyo state, South West of Nigeria, Minna, Niger state, North central of Nigeria and Maiduguri, Borno state, North east of Nigeria. Collection was by the conventional method of a beekeeper and each was kept in sterile bottle and covered with a lid.

Analytical Procedures

Proximate Analysis

The Proximate concentrations of the honey samples was determined by the Standard AOAC (1990) methods for estimating moisture. Ash, crude fibre, crude oil, crude protein calculated as Nitrogen (N x 6.25) by the Kjeidahl method.

pH Determination

pH of each honey sample was determined by the method of Ojeleye 1991.

Viscosity Determination

Samples were run through Standard burettes and allowed to train 1ml at a time. The time taken for 1ml sample to flow was recorded with the aid of a stopwatch.

Refractive Index

Abbey Refractometer was used to determine the refractive index of each honey sample. From a reference sugar table, the refractive index and brix of the honeys could be convertibly estimated.

Mineral concentrations of Honey samples

Samples were wet washed, using AOAC method and analysed for Calcium, Magnesium, Potassium and Sodium, using Atomic Absorption Spectrophotometer.(AOAC,1990)

Phosphorus was estimated by a reaction between Phosphorus and Molybdovanate forming a Phosphomolybdovanate complex measurable colorimetrically at 420nm.

Microbial Assay of Honey Samples

Viable Quantitative and Fungal Population counts The methods of Miles and Misra, described by Collins and Lyne were used.[23] Serial dilutions of 10^{-6} and 10^{-7} were made and 1ml each was pipetted into Petri dishes and Nutrient Agar and Potato Dextrose Agar were separately aseptically dispensed and carefully mixed. The organisms in Nutrient agar mixture were incubated at 37^0C for 24 hours while that of organism in Potato dextrose agar mixture was incubated at 22^0C for 5 days. The colonies formed were counted using the colony forming units/ml sample. Organisms found in honey samples were characterized.

Results and Discussion

Table 1 shows the proximate composition of the three honey samples. Ash content was between 0.49 - 0.86%. Moisture contents of the three honeys ranges from 18-24%. Honey Regulation 197651 No 180 Council Directive 74/4009/EEC stipulated honey moisture content should not be more than 21%. (Egan,1978) it is apparent that the water content varies greatly and may range widely. The amount of moisture is a function of factors involved in ripening, including, among others, the original moisture of the nectar. According to the United States Standards, extracted honey may not contain more than 18.6% moisture. Moisture level of about 17% has been found to be optimum. When honey is not hermetically sealed, because of its hygroscopic nature, it absorbs moisture. Honey with less than 17.1% water will not ferment in a year, irrespective of the yeast count. Between 17.1 and 18% moisture, honey with 1000 yeast spores or less per gram will be safe for a year. However when the moisture is above 19% honey can ferment even with only one spore per gram. This study shows that honey is a high energy carbohydrate food. Honey is a supersaturated sugar solution, with more than 95% of its dry mass consisting of sugar and water, although different valuable nutrients such as vitamins, minerals, enzymes, flavoring organic compounds, free amino acids and numerous volatile compounds constitute minor components (Baroni et al) .The carbohydrate in honey of about 98% obtained in this study is similar to the figure range of 95-99.9% of White and Doner.(25) The crude protein content of 1.43 -2.72% obtained in this study shows that honey is not an adequate source of dietary protein

Table 1. Proximate Composition of Honey Samples in %

Honey sample	Dry matter	Crude protein	Ash	Fat	Sugars
A. Saki	76.00±2.4	1.43±0.1	0.86±0.1	Nil	97.71±2.5
B. Minna	81.80±2.0	2.72±0.1	0.49±0.1	Nil	97.80±2.2
C.Maiduguri	77.80±2.2	1.83±0.1	0.64±0.1	Nil	97.53±2.4

Values are expressed as means of duplicate determinations ± standard deviation.

Table 1 shows the Proximate composition of Honey samples in %

Table 2. Physicochemical characteristics of Honey samples

Sample	pH	Flow rate	Refractive Index
A Saki	3.6	1.65	1.4765
B Minna	3.5	0.30	1.4910
C Maiduguri	3.2	0.45	1.4810

Table 2 reports the physico-chemical characteristics of the three honey samples. The pH values of 3.2-3.6 recorded in this work is lower than 4.3 - 6.0 pH range reported by Adebiyi et al, 2004. Low honey pH shows the three samples to be acidic. . Glucose oxidase in honey has been implicated in the conversion of dextrose to gluconolactone which in turn forms gluconic acid, the principal acid in honey. Glucose oxidase also forms hydrogen peroxide during its action on dextrose. This end product (hydrogen peroxide) is well known for its antiseptic property even in a diluted honey.(Wahdan,, 1998) In addition to gluconic acid, other acids, including lactic acid are said to be present in honey. High acidity of honey in combination with high sugar content therefore confers on honey high antimicrobial property. The refractive indices of the three honeys ranging from 1.4765 – 1.4910 are similar to 1.460 – 1.488 values of Adebiyi et al, 2004. When refractive index is extrapolated on a reference standard table, it may serve as a rapid and simple measure of the % of total soluble sugar solid in honey at 20^0C

Table 3. Mineral constituents of honey in mg/kg

Sample	Na	K	P	Ca	Mg
A Saki	6.84±0.2	6.08±0.15	3.60±0.16	3.39±0.2	1.90±0.15
B Minna	7.02±0.2	7.60±0.14	2.40±0.17	2.44±0.12	0.26±0.01
C Maiduguri	6.30±0.2	5.61±0.20	3.45±0.12	2.14±0.18	0.21±0.17

Values are expressed as means of duplicate determinations ± Standard Deviation.

Table 3 shows the mineral composition in mg/kg of the three honey samples. The major minerals are Na, K, P and Ca while Mg constitutes a very minute proportion especially in Minna and Maiduguri honey samples. All these minerals had their origin from the soil .The mineral levels obtained in the present work are quite lower than those that have been reported.(23)This wide disparity may be due to variation in the vegetations and soil composition of minerals at the different locations from which the honeys were produced.

Table 4. Fungi and Coliform counts in Honey

Sample	Dilution factor	Mean mould count (cfu/ml)	Coliform count (cfu/ml)
A Saki	10^{-6}	0.5×10^7	Nil
	10^{-7}	0.2×10^8	Nil
B Minna	10^{-6}	0.8×10^7	0.1×10^7
	10^{-7}	0.35×10^8	Nil
C Maiduguri	10^{-6}	1.15×10^7	Nil
	10^{-7}	0.8×10^8	Nil

cfu/ml = Colony forming unit per ml sample

Table 4 reports the total bacterial and fungal counts in the three honey samples.

Maiduguri sample (C) with the highest microbial counts at dilution 10^{-6} and 10^{-7} produced 1.15 x 10^{7} cfu/ml and 0.8 x 10^{8} cfu/ml respectively. Sample A (Minna honey) on the other hand, with the least microbial counts at dilutions 10^{-6} and 10^{-7} produced 0.5 and 0.2 cfu/ml respectively. It was only in Minna Sample B that fungi were detected at 10^{-6} dilution. No coliform was however isolated in any of the honey samples. The presence of fungi, presumably yeast, in B may explain the possibility of fermentation of honey under extreme acidity and high osmolarity.

Table 5. Microbial Isolates in Honey Samples

Sample	Isolate	Organisms
A & B	a	*Lactobacillus fructivorans*
A & C	b	*Staphylococcus saprophyticus*
B & C	c	*Xanthomonas campestris*
ABC	d	*Micrococcus roseus*
A	e	*Serratis marcescens*
B	f	Unidentified
C	g	*Acrococcus viridans*

Table 5 shows the isolates from the three honey samples (A, B & C). All the organisms were either osmophilic or they survived only in the resting spore form (Wahdan H ,1998). They were also acid fermenters. The spoilage organisms apparently attacked honey sugars fermentatively at low pH of 3.2 – 3.6 under which the proteolytic and lipophilic organisms may be incapacitated. It should be noted, however, that lactic acid bacteria are only weakly proteolytic and lipolytic.

Conclusion

This study has confirmed honey to be a high acid and high sugar food. These two characteristics also show that it can harbour spoilage organisms (fermenters) only sparingly especially if bee-keepers can maintain a high degree of cleanliness in terms of personal hygiene, equipment cleaning, careful process control and good packaging, distribution and good storage etc. Its usefulness as an antimicrobial, anti-oxidant agent has been highlighted by some other workers. By its osmotic effect due to high sugar content and its acidity, honey may be used as a dressing for wounds, inflammations and diabetic sores. This application makes honey unsuitable for pathogens and it thus hastens healing. As an anti-oxidant it counteracts free radicals, destructive chemical agents which have been linked to many diseases. In addition to the above, honey is an energy giver and therefore it is recommended for all and sundry consumption and especially for the diabetics as an alternative to sucrose.

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评李子丰教授竞聘中科院理论物理研究所所长

单炜滕

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Abstract: 罗斯说爱因斯坦广义相对论方程的核心发展是度规里奇张量。我们知道，里奇张量的核心是向心加速度，杨振宁教授说圆周运动的向心加速度，与平移运动有根本区别。这也是里奇张量和韦尔张量正是区别。而且在这一点上，爱因斯坦建立了广义相对论方程“协变”或“缩并”的基础。联系牛顿力学的惯性定律和反作用力定律，可成为“光速”和“超光速”推导的基础。万有理论即所谓终极理论，在温伯格的《终极理论之梦》一书看来，就是指一组简单的最具必然性的物理原理，原则上我们所知的关于物理学的一切都可以从这些原理推导出来。温伯格说，“哲学并不能对科学研究提供什么正确的概念科学哲学也不能指导科学家如何工作……我只能认为它的目的是去感动那些混淆晦涩与深刻的人。”终极还原，不是研究纲领的指南，而是对自然本身的态度。我们似乎只能由“简单”来理解“复杂”而无法反其道而行之。不论我们从基本粒子那里学会什么，化学、热力学、浑沌和生物学仍将继续说自己的语言，但这些不同层次的科学原理之所以如此，是因为在它们背后都存在着更深层次的原理（以及某种历史事件），而所有那些原理的解释箭头都能追溯或汇聚到一组简单的定律上来，这就是所谓的终极理论。

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Keywords: 罗斯; 爱因斯坦; 广义相对论; 里奇张量; 向心加速度

燕山大学李子丰教授，要与吴岳良等竞聘中科院理论物理研究所的所长一职，完成反相大业。弦论本是连接牛顿力学和相对论及量子力学的一门学问，李子丰教授的优势是在石油工程技术方面，对牛顿力学仅是一知半解，也要“造反有理”。是什么机制在拉这些“公科”下水造反？

彭罗斯说爱因斯坦广义相对论方程的核心发展是度规里奇张量。我们知道，里奇张量的核心是向心加速度，杨振宁教授说圆周运动的向心加速度，与平移运动有根本区别。这也是里奇张量和韦尔张量正是区别。而且在这一点上，爱因斯坦建立了广义相对论方程“协变”或“缩并”的基础。联系牛顿力学的惯性定律和反作用力定律，可成为“光速”和“超光速”推导的基础。

那么看王令隽教授反驳彭罗斯，说彭罗斯犯的低级错误是“里奇=能量”。王令隽说，里奇张量不能等于能动量张量，因为能动量张量的散度为零，而里奇张量的散度不为零，而是等于黎曼曲率的一半。爱因斯坦方程应该是：爱因斯坦张量=能动量张量乘与一个常数。所谓“缩并”，通常叫做张量的指标收缩，是一种最简单的张量运算，指标收缩的结果使得张量的阶数降了二阶。一个四阶张量收缩一次就变成二阶张量，再收缩一次就变成零阶张量（常数）。爱因斯坦在试图建立他的引力场方程时，将空间的曲率和能动量张量直接联系到一起，认为能动量张量造成了空间的弯曲。描述空间弯曲的几何量是黎曼张量。可是黎曼张量是四阶张量，有 256 个原素；而能动量张量是二阶张量，只有 16 个元素。这两个张量不可能相等。如果将黎曼张量收缩，就成了一个二阶的里奇张量，至少在原素的个数上和能动量张量相同，有可能放在方程式的两边。这就是爱因斯坦为什么要将黎曼张量收缩成里奇张量的原因。由此可见，张量的收缩，仅仅是一种缩小张量阶数的代数运算，和“力”扯不上任何关系。这种代数运算是离散的操作，不是连续的操作，因为张量的阶数是整数，不可能是分数。比如说，没有 1.2 阶的张量。可是，力是一个连续量，可以取整数之间的任何小数，也可以是负数。力是一个矢量，而里奇张量是一个二阶张量。一个二阶张量能够等于一个矢量吗？力的量纲是牛顿，里奇张量的量纲是曲率，量纲就不对。力是变化的，能动的。指标收缩是固定的，不变的。一旦从四阶的黎曼张量收缩成二阶的里奇张量就不动了，如何产生“缩并力”？是黎曼张量从四阶收缩到二阶的里奇张量产生了“缩并力”呢，还是从里奇张量收缩到曲率常数产生了“缩并力”？还是两者都产生“缩并力”？哪一种“缩并”的力量更大？我们现在知道的有四种自然作用力。每一种作用力都是一种物理过程。而彭罗斯的“缩并力”是一种纯数学操作，没有任何物理过程与之对应。

圆周运动的向心加速度，王令隽说仅是一种最简单的数学计算原因；是一种纯数学操作，没有任何物理过程与之对应。是一种缩小张量阶数的代数运算，和“力”扯不上任何关系。王令隽真理直气壮？彭罗斯和杨振宁教授都错了？王令隽就在

美国，为什么不当面与彭罗斯和杨振宁辩论？为什么在美国不用英文发表论文，与彭罗斯和杨振宁辩论，而要用中文送回国内来忽悠？

美国社会与我国香港社会制度相同，王令隽当然明白科学框架是“专政”与“自由”并存的。

从天津一所大学迁居香港的张亚鹏先生现身说法讲：他在香港主编的《新科技》杂志，旨在建立新科学基础理论新体系和发现的新定律，指出类似西方科学大师牛顿错了、爱因斯坦错了、霍金错了、威滕错了。有一位香港中文大学的教授看了，称赞很好。于是张亚鹏请他作《新科技》杂志的编委，他也答应了。但香港中文大学的校方知道这件事后，对这位教授说，如果他做了《新科技》杂志的编委，就请他自动离开香港中文大学。教授很害怕，给张亚鹏打电话，请不要把他的名字印在《新科技》杂志的编委中，也请不要把《新科技》杂志送给香港中文大学。所以王令隽在美国如果用英文大肆发表类似牛顿错了、爱因斯坦错了、霍金错了、威滕错了的论文，他不可能在大学里捞到“终身资深教授”的头衔。张亚鹏说他的杂志是在法国注册，在香港出版，在中国大陆发行的。而且在大陆能招募到很多发行员。

可见有人也在利用我国的科学框架漏洞，拉“公科”下水。因为像李子丰这种专家，在张亚鹏说的情况里，不应该在燕山大学里教书，应该调到指挥石油工程技术企业作战，同时他也有自由，但他不是代表我国的“公科”而是企业自由业余从事类似牛顿错了、爱因斯坦错了、霍金错了、威滕错了的宣传活动。这是一个有争论的科学事件。李子丰不在大学，大家一起作为“家科”， 平等竞争这些问题的对与错，不是更好吗？但我国还要走漫长的路。因为李子丰先生理直气壮认为他是在宣传唯物主义，反对资产阶级思想，受我国的宪法、党章的保护。但我国的宪法、党章明确具体说了牛顿力学、爱因斯坦相对论、霍金宇宙大爆炸论、威滕弦论就是反唯物主义，就是资产阶级思想了吗？而王令隽教授又说让霍金到北京宣传弦论，是我国制度没有“专政”，只有“自由”。但为什么会出现文革在北京，陈伯达１９７０年４月亲自到北京大学召集会议，鼓动批判爱因斯坦和相对论；在上海张春桥和姚文元指使亲信在复旦大学，组织动员对爱因斯坦和相对论的批判运动呢？陈伯达、张春桥和姚文元曾是当时一些重要的国家领导人，也主动拉“公科”造反科学框架。也造成今天“公科”在职或退休，或到国外的一些人，引导我国“家科”造反科学框架。

温家宝总理有一段话类似能揭开此之谜。他说“历史告诉我们，一切符合人民利益的实践，都要认真吸取历史的经验教训，并且经受住历史和实践的考验。这个道理全国人民懂得。因此，我们对未来抱有信心。”温家宝还说，新中国成立以来，在党和政府的领导下，中国的现代化建设事业取得了巨大的成就，但是也走过弯路，有过教训。党的十一届三中全会，特别是中央作出关于正确处理若干历史问题的决议，做出了改革开放这一决定中国命运和前途的重大抉择。可见一切都事出有因。

3）新中国成立以来，在党和政府的领导下，独立自主研究弦论，中国同样也走过漫长的“家科”阶段的考验。南京大学教授沈骊天先生说：“读罢美国弦理论家 B•格林的《宇宙的琴弦》，尚在赞叹感慨之时，又有幸浏览一部中国作者的奇书《三旋理论初探》，让我知道了：在中国本土，有一位不屈不挠的探索者，经过几十年执着的追求，按自己的方式独立构建了一种不仅不同于经典物理学，不同于量子力学、相对论，而且不同于超弦理论的崭新物理学体系。它所引起的惊喜，犹如在遥望世界科学最高峰的攀登壮举之时，惊奇地发现另一面山坡上竟闪现出中国攀登者的身影”。沈骊天教授对此书的不满意是：“该书把物理学上的讨论随意推广到其他领域、乃至社会领域，是我不太赞成的；该书作者同样有太多的‘万有理论’情结，而追求包罗万象、无所不适用的所谓万有理论往往都是吃力不讨好的”。

万有理论即所谓终极理论，在温伯格的《终极理论之梦》一书看来，就是指一组简单的最具必然性的物理原理，原则上我们所知的关于物理学的一切都可以从这些原理推导出来。温伯格说，“哲学并不能对科学研究提供什么正确的概念科学哲学也不能指导科学家如何工作……我只能认为它的目的是去感动那些混淆晦涩与深刻的人。”终极还原，不是研究纲领的指南，而是对自然本身的态度。我们似乎只能由“简单”来理解“复杂”而无法反其道而行之。不论我们从基本粒子那里学会什么，化学、热力学、浑沌和生物学仍将继续说自己的语言，但这些不同层次的科学原理之所以如此，是因为在它们背后都存在着更深层次的原理（以及某种历史事件），而所有那些原理的解释箭头都能追溯或汇聚到一组简单的定律上来，这就是所谓的终极理论。

《三旋理论初探》可以与李淼教授的《超弦史话》比较。《三旋理论初探》的核心是类圈体的拓扑与自旋研究，国内起源于 1959 年，该书是直到 2002 年以来对此的探索和应用。李淼是我国最先投入超弦理论研究这一领域的年青人之一，1962 年才出生于江苏。1982 年他从北大毕业，考取中科大研究生。1985 年第一次出国，至此开始长达 15 年的留学之旅。1999 年作为中科院“百人

计划”入选者回国，成为中科院理论物理研究所研究员、博士生导师，中科大客座教授。

李淼回国前主要做超弦理论，回国后从 2001 年开始写出一系列“弦论小史”，在同事办的《超弦论坛》网站上每隔几天更新发表。2005 年，《弦论小史》结集成《超弦史话》出版。该书主要是细致介绍西方弦论的缘起、发展、高峰和未来前景，以及此领域的一个个“牛人”们的历史。李淼教授说，在美国，可能只有很大牌的教授才有机会在《纽约时报》这样的媒体上写科普专栏，而不是谁想写就可以写的。例如超弦理论的某项研究，最起码要给全部物理系的人讲明白。相反，出于职称、报酬等功利性因素的考虑，国内科学家会认为科普“得不到好处”。李淼认为，“理论物理研究是一个长期的过程，不会在短期之内就看到效益。提出一个理论，可能要在几十年后才被人验证。”至于他以前的研究，成果能否有历史性的贡献，则似乎还要看看运气。李淼在超弦理论中的研究有一定的国际影响，特别是在两维刘维尔理论、D 膜以及黑洞的量子物理等方面。近年致力于研究超弦中的黑洞物理、超弦宇宙学以及暗能量等。

弦论是科学，不在于王令隽、沈致远、李子丰等教授的反对，最有说服力是目前关于跨世纪预言或将应验“拓扑量子”的新闻，显示出弦论的拓扑应用确实多有成效，并影响着我国。因为拓扑方案，有望比其他类型的量子计算机的容错能力更强。而目前国际上已有多个研究组能生长出高质量拓扑绝缘体薄膜，但由于界面反应和晶格匹配等问题，拓扑绝缘体与超导体之间的高质量的薄膜非常难以制备。

a）拓扑量子的纠错研究。中国科技大学微尺度物质科学国家实验室潘建伟及陈宇翱、刘乃乐等教授，成功制造出并观测到了具有拓扑性质的八光子簇态，并将此簇态作为量子计算的核心资源，实现了拓扑量子纠错。这也许能解决长期困扰量子计算机物理实现的最大问题即量子计算机不可避免地与环境耦合而产生的各种噪声使计算过程产生各种错误的“消相干效应”。

b）拓扑量子的薄膜研究。上海交大低维物理和界面工程实验室贾金锋、钱冬、刘灿华、高春雷等教授，已经制备出最适合探测和操纵 Majorana 费米子的人工薄膜系统。“Majorana 费米子”是意大利科学家学马约拉纳（Majorana）的预测，而被冠名的一类特殊的费米子。上海交大是在拓扑绝缘体与超导体之间，插入一种超薄的过渡层，而形成的一种由拓扑绝缘体材料和超导材料复合而成的特殊人工薄膜，超导的特性能够传递到拓扑绝缘体上，拓扑绝缘体也具有了超导体的“本领”，首次成功实现了超导体和拓扑绝缘体的“珠联璧合”。厚度只有发丝的万分之一的这种薄膜，通过精确控制，将所需材料的原子一层一层垒起来可达到产生 Majorana 费米子的要求。

C）量子自旋霍尔拓扑绝缘体的研究。美国莱斯大学科学家杜瑞瑞、克尼兹等教授研制出的“量子自旋霍尔拓扑绝缘体”的微型设备，也是与超导体结合研制而成。因为在“拓扑量子计算”机的研制竞赛中，各国研究人员采用了许多种制造量子比特的方法，但不管什么方法，一个普遍的问题就是如何确保将信息编码为量子比特而又不会因为量子波动而随时间变化，这就是一个容错问题。量子自旋霍尔拓扑绝缘体被用作“电子高速公路”，是量子计算机中产生量子粒子用来存储和处理数据的关键构件之一。拓扑量子计算在美国得到极大的重视，微软公司在其加州的研究所中网罗了大量理论人才，从事拓扑量子计算方面的开创性研究，并每年投入数百万美元直接支持加州理工学院、芝加哥、哥伦比亚、哈佛等大学相关的分数量子霍耳效应的实验研究。

d）我国拓扑量子计算研讨会活跃。如 2011 年 5 月 21 至 22 日，由上海微系统所蒋寻涯研究员、上海交大刘荧教授和浙大万歆教授联合牵头的“普陀论拓扑”专题研讨会，在浙江舟山举行，全国近 50 名研究人员参加。2011 年 11 月 25 日至 27 日，由理论物理国家重点实验室资助的“理论物理前沿研讨会—凝聚态物理中的拓扑物态和量子计算研究专题研讨”，在北京郁金香温泉花园度假村召开，来自于北大、北师大、人民大学、北京科技大学、中科院研究生院、北京计算科学研究中心、中科院物理研究所、北京应用物理与计算数学研究所和中科院理论物理研究所等国内知名单位 20 余位专家参与。而早在 2006 年的拓扑量子计算研讨会，就汇集了中科院理论物理所、北大、清华大学、北师大、人民大学、 南开大学、南京大学和浙大的学者。其目的就是要推进我国在拓扑量子物态与拓扑量子计算、拓扑绝缘体与相关系统、拓扑超导体方面的研究，交流思考从传统物相理论到今天泛拓扑图像的物理背景、实验、和分类方式，对拓扑量子计算的背景、理论和实验的基础、现状以及前景等作专题讨论。

e）拓扑量子在交叉科学中的应用。如《有机化学中的拓扑量子方法》一书，是湖南科技大学副校长曹晨忠教授 2010 年在科学出版社出版的专著。内容主要包括基团极化效应参数和拓扑立体效应指数的计算；有机分子拓扑量子键连接矩阵的构造以及分子结构特征参数的提取，矩阵特征根、拓扑量子轨道能级、原子电荷、化学键的键级等参数的计算；应用上述分子结构参数，对烷

烃、单取代烷烃、链状烯烃、含 C=0 键和 N＝0 键有机化合物、芳香烃和极性芳香化合物等各类有机物的热力学性能、化学反应性能、光学性能、色谱性能、价电子能量、酸性和生物活性等进行定量的相关研究。又如《非相对论物理学中的拓扑量子数》，是 2000 年由世界图书出版公司出版论述拓扑量子数在非相对论物理系统中作用的专著。与普通由对称性定义的量子数相比，拓扑量子数的特点是对系统中的缺陷不敏感。近年来，拓扑量子数在物理量的精确测量中变得非常重要，并提供了最好的电压和电阻的标准。

4）由上可见弦论已进入交叉科学领域。这不是沈骊天教授式担忧的把弦论从高能物理随意推广到其他领域。但不知李子丰教授对中科院理论物理所、北大、清华大学、北师大、人民大学、南开大学、南京大学和浙大等上述学者，在拓扑量子这种数学、物理和计算机科学的交叉领域内的研究了解否？

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Short communication

Expression analysis of some boiling stable proteins (Hydrophilins) under combined effect of drought stress and heat shock in drought tolerant and susceptible cultivars of *Triticum aestivum*

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Abstract: The combined effect of drought stress and heat shock on the induction of boiling stable proteins viz: WGA, SOD, HSP90,Aquaporin, CyPs, APase and LEA proteins was studied in 3-days old seedlings of drought tolerant and drought susceptible cultivars of wheat. Boiling stable protein profile was outlined via SDS electrophoresis of tissue extracts. The results obtained were confirmed by Immunoblot analysis with anti-WGA, anti-SOD, anti-HSP90, anti-APase, anti-Aqua, anti-Cyp and anti-LEA antibodies. Western blot analysis revealed the induction of boiling stable proteins (SOD, HSP90, Aquaporin, CyPs) during combined drought and heat stress (DH) conditions as compared to separately applied heat (H) and drought treatments (D) in drought tolerant cultivars of wheat, indicating their role in water stress adaptation under simultaneous applied abiotic stress conditions. Alternation in boiling stable protein expression was more pronounced in seeds as compared to shoots of both the cultivars. Based upon these observations the possible role of hydrophilins in water stress tolerance is discussed.
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Key words: APase, Aquaporin, Cyclophilin, SOD, HSP90, LEA, WGA, drought, heat shock, wheat

Introduction

Throughout their life cycle, plants are subjected to many adverse environmental conditions such as drought, heat, cold and flooding etc. that dramatically affect plant survival and limit productivity (Serrano et al. 1999). Some environmental factors (such as temperature) can become stressful in a few minutes, others may take days to weeks (soil water) or even months (mineral nutrients) to become stressful. Most cultivated crop plants are highly sensitive and either die or display reduced productivity after they are exposed to long periods of abiotic stresses. All these stresses are often interconnected and may induce similar cellular damage (Ingram and Bartels, 1996). Under field conditions, plants are often simultaneously exposed to soil drying and high temperature stresses. These two stress factors could create water deficit in plant tissues, which in turn may affect the synthesis of stress-induced proteins. In most of the studies on stress-associated proteins; plants have been exposed to only one environmental stress factor viz: high temperature. Although abiotic stress response has been studied considerably in recent years (Chaves et al. 2003), however, analyzing the effect of single stress on plants can be very different from conditions encountered by plants in the field in which a number of different stresses may occur simultaneously. These can alter plant metabolism in a novel manner that may be different from that caused by each of the different stresses applied individually. It may require a new type of response that would not have been induced by each of the individual stresses. Plants inherently posses various molecular-biochemical mechanisms that are involved in stress tolerance (Ingram and Bartels, 1996). Some of these stress-responsive genes encode regulatory proteins, soluble proteins, appearance of new isozymes; whereas others protect cells by causing the accumulation of metabolic proteins and cellular protectants including sugars (Ingram and Bartels, 1996). These stress-induced responses enable the plant to adapt its physiology and survive. Stress induced proteins play a definite role in protecting plants from possible damage by these conditions. Concomitant to induced stress tolerance, protein metabolism of the cells undergoes changes in terms of acquiring specific stress proteins, which are either not detected or present in low amounts in the un-induced cells (Ingram and Bartels 1996). A growing body of evidence suggest that stress response involves synthesis of one set of proteins and degradation of the other (Serrano and others. 1999). Therefore, stress responsive changes in gene expression in general and protein profiles in particular have been targeted for intensive investigation. One of these mechanisms that may confer stress tolerance is the activation of a large set of genes, which leads to the accumulation of specific cellular proteins. Heat shock proteins (HSPs), dehydrins and late embryogenesis proteins

(LEAs), are the major groups of stress-induced proteins which believed to contribute to the protection of cellular structures and metabolites during water stress (Chaves and others. 2003). In addition, these proteins accumulate to high levels during natural growth of seed development and maturation when a loss of water from the cell occurs (Rurek , 2010). These proteins also seem to respond similarly to the application of ABA (Chaves and others. 2003). Some drought stress-induced proteins (e.g. dehydrins, LEAs) are highly hydrophilic and remain soluble even after boiling (Close et al. 1989), a characteristic that has been termed "boiling stability" (Jacobsen and Shaw 1989). Even some of the proteins detected in total protein extracts, under drought stress, are lost in boiling treated extracts (Pelah and others. 1995). Earlier research also indicated that hydrophilins represent less than 0.2% of the total protein of a given genome (for review see Battaglia et al. 2008). Bioinformatic analyses of hydrophilins from several kingdoms including plant, bacteria and fungi have revealed the conservation of glysine-rich regions in these proteins, thus, suggesting an evolutionary role for these cellular boiling stable proteins during water-deficits (Arroyo and others. 2000). Accordingly, data suggest that hydrophilins have evolved independently in different protein families and in different organisms, but with the similar goal of protecting specific functions under partial dehydration. It is noteworthy that all hydrophilins from different phyla show higher expression under water limiting conditions, imposed by environment. This is not only the case for LEA and non-LEA like hydrophilins from plants, but also for hydrophilins expressed in bacterial and fungal spores. Although the functional role of hydrophilins remains speculative, there is evidence supporting their participation in accilimation and/or in the adaptive response to abiotic stresses. Overexpression analysis of some hydrophilins (LEAs) revealed enhanced salt, cold and drought tolerance in plants, indicating their role in water limiting conditions (Battaglia and others. 2008). It was reported earlier that the large number of more hydrophilic residues like Gly probably confers a very flexible backbone and this is likely responsible for the boiling stability of these proteins. It facilitates the formation of intramolecular hydrogen bonding and thus gives the protein a random coil conformation. This property allows the protein to stretch, bend and expand in all directions, a property that could be useful to protect cellular structures against water stress.

At present hundreds of genes induced under water stress have been identified which may allow plants to adapt to water limiting conditions. Because plant responses to environmental stresses are complex and multigenic, the functions of many of the induced genes and their related products are still a matter of conjuncture (Bray, 2002). Therefore, to better understand the role of these proteins in water stress tolerance, it is a prerequisite to examine their expression not only under water stress, but also after boiling of extracts. Thereafter, the sequencing of the relevant hydrophilic proteins and cloning of the corresponding genes will generate probes for early selection of drought resistant genotypes. Therefore, to assess the role of these proteins in water stress adaptation it is imperative that variability in boiling stable proteins (BSPs) should be studied in stress tolerant and susceptible cultivars of a crops. In the light of these observations, the proposed study was undertaken to investigate the effect of combined drought and heat stresses on the expression of some boiling stable proteins like: SOD, WGA, HSP90, Aquaporins, CyPs, APases, and LEA in the drought tolerant and susceptible cultivars of wheat so as to gain an insight into the physiological role of these proteins in water stress adaptation and the possible implication as a marker for drought stress tolerance. Wheat is one of the most important crops in arid and semi arid areas worldwide and is sensitive to drought and temperature stress. In view of this, we have chosen wheat as an important tropical crop for the present investigation. To facilitate the detection of BSPs, we focused on heat stable (HS) fractions that resists coagulation upon heating at 100°C. By this method, the soluble protein extract containing hydrophilic proteins could enriched with BSPs and devoid of storage proteins.

Materials and Methods

Seed germination and growth conditions

The seeds of *Triticum aestivum* L. cvs. PBW 527 (drought tolerant) and PBW 343 (drought sensitive) were procured from PAU Ludiahana, Punjab, India. Seeds were thoroughly rinsed with deionized water and imbibed for 6 h. After imbibition, seeds were placed in Petri plates containing sterile filter sheets, moistened with water. The plates were incubated at 25 ±1°C in a seed germinator in darkness and allowed to grow for 3 days (Sharma and others. 2012). Stress treatments were performed on 3 M Whatman filter paper. For combined drought stress and heat shock treatment, 3-day old seedlings were exposed to 3-day drought stress followed by 4-h heat shock (42°C). Individual drought stress was imposed to 3-day old seedlings for 3 days. Heat treatment was imposed to 6-day old seedlings for 4-h at 42°C. The tissues (seeds/shoots) from all treatments were harvested and pooled for further analysis.

Extraction of proteins

Tissues (seeds/shoots) were homogenized with chilled mortar and pestle in extraction buffer (50 mM Tris-HCl, pH 7.0). Crude extracts were centrifuged at 10,000 g for 10 min, and total protein content in the supernatant was determined by the Bradford method busing BSA as a standard (Bradford, 1976). Protein samples were resolved on SDS-PAGE on 15% (w/v) polyacrylamide gel and visualized by Coomassie brilliant blue as described in Sambrook and others. (1989).

Western blot analysis

Western blot analysis was carried out with antibodies against WGA (wheat germ aggulitinin), SOD (superoxide dismutase), Aquaporin, HSP90 (heat shock protein 90 kDa), APase (Acid phosphatase) and CyP (Cyclophilin). After electrophoresis, proteins were electroblotted to a nitrocellulose membrane (Hybond C extra, GE Healthcare). Protein blots were reacted with anti-WGA (1:500 dilution), anti-SOD (1:2000 dilution), anti-Aqua (1:3000 dilution), anti-APase (1:2000 dilution), anti-Cyp (1:1000 dilution) and anti-LEA (1:500 dilution) and developed using an alkaline phosphatase-conjugated secondary antibody (1: 500 dilution) and 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt/*p*-nitroblue tetrazolium chloride reagent systems (Sambrook and others. 1989).

Results and discussion

In the present study, effect of combined drought stress and heat shock (DH) was studied on the expression of some boiling stable proteins viz: WGA, SOD, HSP90, Aquaporin, APase, CyP and LEA in drought tolerant (PBW 527) and drought susceptible (PBW 343) cultivars of wheat. Our results strongly suggest that the effect of this combination (DH) on plants is very different from that of drought and heat shock applied individually. Earlier, Mittler and others. (2006) also claimed that simultaneous exposure to different stresses would result in co-activation of various stress response pathways with synergetic or antagonistic effects and that their combination should be regarded as new state of abiotic stress in plants. To examine the role of boiling stable hydrophilins, the BSPs expression was examined in the samples collected, run on a 12% SDS-PAGE followed by immuno-blotting and analyzed. Figure 1 shows the boiling stable protein (BSP) profile of seeds and shoots of drought tolerant cultivar (PBW 527) and drought sensitive cultivar (PBW 343) under drought (D), Heat (H) and combined heat and drought (DH) conditions. As can be seen in both the cultivars, seeds exhibited a more number of protein bands (high mol wt and low mol wt), however, in shoots very few barely detectable BSPs (high mol wt) were observed. Such observation is quite expected, since it is well known that an intense proteins synthesis take place in the reproductive plant structures (Duck et al. 1989). In seeds several groups have proposed that proteins were critical for protection of cellular components during seed development. Although both lines almost had almost similar pattern of synthesis of low mol wt and high mol wt proteins, some quantitative differences in the synthesis of proteins between the two lines might exist, which were further analyzed by immunoblot analysis.

Western blot analysis of BsWGA49 under drought (D) heat (H) and combined DH conditions

Wheat germ agglutinin (WGA) is the best characterized lectin, which supposed to be maximally synthesized in the developing embryos (Triplett and Quatrano, 1982). Enhanced accumulation of WGA in response to abiotic stresses has been reported earlier (Plant and others. 1991), however, its physiological function as boiling stable proteins is still a matter of conjuncture. Anti-WGA immuno-blotting of seed samples of drought tolerant cv. PBW-527, detected a strong cross-reacting protein band at about 49-kDa (BsWGA49) under heat (H) stress only, indicating its role during higher temperature. However, no expression of BsWGA49 was observed under D and DH conditions (Fig 1A). The absence of WGA expression in response to D and DH conditions indicates that the regulation of these proteins differ from that of number of other high-temp induced proteins. It is also plausible that at high temp, BsWGA49 may be involved in seedling development. Earlier studied also documented the role of wheat germin during seed germination (Hurkman and others. 1991). Jaikaran and others., 1990, also speculated that wheat germin could have a role in cell wall expansion during seed germination in cereals.

Immuno-blot analysis of BsSOD under drought (D) heat (H) and combined DH conditions

It was reported earlier that water deficit stress often results in oxidative stress. It arises from the production of free radicals or ROS (reactive oxygen species) which damage proteins by amino acid modifications, fragmentations of aggregation of cross linked reaction products, or increase susceptibility to proteolysis (Bowler, 1992). Plants contain a number of enzymes like SOD, catalase, peroxidase, GST that catalysis the cascade of ROS and convert them into less reactive products (Gazanchian and others. 2007). Superoxide dismutase (SOD, EC 1.15.1.1) is ubiquitous, being widely

distributed among $O2^-$ consuming organisms and is the first line of defense against oxidative stress. Anti-SOD Immuno-blotting of seed samples of both the cultivars, detected BsSOD30 band under combined DH conditions. However, in cv. PBW 527, the relative expression of BsSOD30 was remarkably higher, as compared to sensitive cv. PBW 343 under combined DH conditions (Fig 1B). Consequently higher expression of BsSOD30 may represent a kind of anticipating mechanism for protection of developing seeds against stress-provoking factors. Applied alone, drought stress (D) did not provoke any changes in BsSOD30 levels. However, under heat (H) stress alone, we also detected another differential protein band of 35 kDa (indicated by arrow), having antigenic similarity with SOD, in cv. PBW 527, indicating its important role in response to heat stress. Earlier, abundance of heat stable SOD was shown to be up regulated in chenopodium (Khanna Chopra and Sabarinath, 2004), However, in shoots, no detectable cross-reacting protein bands were observed under all the conditions, suggesting tissue specific induction of BSPs. Differences in the expression of specific gene products between stress-sensitive and stress tolerant cultivars indicate that tolerance is conferred by genetically encoded mechanisms (Bray, 1993) so, it is reasonable to expect the inter- and intra-specific differences in the pattern of protein synthesis between plants which differ in their stress resistance.

Immuno-blot analysis of BsHSP90 under drought (D) heat (H) and combined DH conditions

HSPs represent a large protein family that includes several subfamilies (HSP 90, HSP 70 and HSP60). HSPs are found in all organisms exposed to high temperature stress and many posses molecular chaperone activities, which involve in the proper folding of native polypeptides and in helping damaged proteins to regain their biological active structures (for review see Waters and others. 1996). Accumulating evidence showed that plants HSPs are not only expressed in response to heat shock, but also upon water, salt and oxidative stress and at low temperature (Waters and others. 1996). The antiHsp90 monoclonal antibody that we used for immune-blot detection, recognized protein band of Mr 66 kDA in seeds as well as shoots of both the cultivars, but in different amounts (Fig 1C). During DH conditions, in cv. PBW 527, the BsHSP66 level was substantially higher as compared to drought sensitive cultivar PBW 343, confirmed its important role for survival under combined stress conditions. Common responses to different stress conditions in both the tissues may indicate similar functions of stress-responsive gene products for plants under stress conditions involving water deficit.

Immuno-blot analysis of BsApase under drought (D) heat (H) and combined DH conditions

Acid phosphatases (APases) are widely found in plants having intracellular and extracellular activities. APases are believed to be important for Pi scavenging and remobilization in plants, but role of boiling stable APases has not been critically evaluated under abiotic stresses. Acid Phosphatases (APases; EC 3.1.3.2) largely catalyze the hydrolysis of Pi from small molecules, which are believed to be important for many physiological processes, including regulation of soluble phosphorous (Pi) (Yan et al., 2001). As shown in Fig. 1 D, western blot analysis detected a strong protein band (BsAPase67) being constitutively expressed in both the tissues of cv. PBW 527 and cv. PBW 343. Contrary to cv. PBW 343, the relative concentration of BsAPase67 was rather higher in cv. PBW 527 under heat stress (H) in seeds and droughted (D) shoot samples. From these observations it was suggested BsAPase14 may be playing a significant role in the maintenance of orthophosphate (Pi) levels in the germinated tissues under stress conditions. Earlier studies also reported that APases were implicated in providing Pi during seed germination from stored phytate (for review see Vance and others. 2003). It may also be possible that under conditions of drought, delivery of phosphate (Pi) is impaired, thus, resulting in the activation of the cellular phosphatases that release soluble phosphate from its insoluble compounds thereby modulate osmotic adjustment by free phosphate uptake mechanism. Olmos and Hellin (1997) also observed that acid phosphatases are known to act under salt stress by maintaining a certain level of inorganic phosphate which can be co-transported with H^+ along a gradient of proton motive force.

Immuno-blot analysis of BsAuap under drought (D) heat (H) and combined DH conditions

Aquaporins (Auap's/AQPs) belong to the major intrinsic proteins (MIP family), members which are found in almost all living organisms, are believed to increase water permeability in roots and also maintain the physiology and development of leaves (for review see Heinen and others. 2009). Regulation of their expression and activity has been reported to be modulated by dehydration and ABA. Evidences are accumulating that AQPs play an important role in plant hydraulic relations at the cell, tissue, organ and whole plant level. They facilitate the rapid, passive exchange of water across cell membranes and are responsible for up to 95% of water permeability of plasma membranes (Heinen

and others. 2009). The mechanism by which AQPs synthesis is enhanced and its in vitro remain poorly understood. In this study, the antiAqua5 antibody that we used for immuno-blot detection recognized five distinctive, tissue specific bands. These bands with molecular mases of 60kDa, 48kDa, 34kDa,29kDa and 28kDa were herein designated as BsAqua1, BsAqua 2, BsAqua 3, BsAqua 4, and BsAqua 5, respectively (Fig 1E). Although it is reasonable to suppose that these bands represent distinct AQPs isoforms, the possibility that at least some of them are degradation products of those with higher molecular masses can not be ruled out. As compared to drought susceptible cv. PBW 343, the expression of BsAqua 1 was considerably higher in cv. PBW 527 during combined DH conditions. Interestingly, no other isoform except BsAqua 1 was detected in shoots under all conditions in both the cultivars. The BsAqua 2 was the only AQP that was exclusively present in the seed sample of cv. PBW 527 under H stress. Our study clearly indicated that the relative concentration of the examined AQPs was disproportionately distributed over distinct tissues in both the cultivars. The unequal distribution of AQPs bands in the shoots versus seed tissues under physiological conditions in both studied populations suggest that different species of this protein may serve diverse cellular roles with in different tissues. Different responses of AQPs (up/does-regulated/ no change) to abiotic stresses suggest that AQP isoforms can be divided to different groups which can contribute differently to water transport and regulation, with some being stress responsive. Further, differential responses of AQPs to water stress were found in drought tolerant and susceptible cultivars, indicating that AQPs present in the same species, but in different cultivars can respond differently to water stress depending upon their tolerance to water deficit. High expression of AQPs in seeds may be indicating that cells grow through irrereverseble expansion of cells, a process that requires the continues uptake of water. So it is tempting to believe that they are involved in the growth process under water stress conditions. This suggestion has been strengthen by demonstrations that introduction of aquaporin gene (OsPIP1) in drought-sensitive cultivar of rice resulted in higher leaf water potential and transpiration rare, indicating the role of OsPIP1 in drought resistance (Heinen et al. 2009). Earlier studies also indicated that overexpression of Arabidopsis homolog of MtPM25 in germinating seeds led to improved growth under high NaCl, KCL and sorbitol conditions (Borrell et al. 2002). Liu and Zheng (2005) also reported similar findings by overexpressing PM2 in *E coli*. Recently, Zhu et al. (2007) has reported heat protection in *Arabidopsis thaliana* by overexpressing Aspen sp1.

Immuno-blot analysis of BsCyP under drought (D) heat (H) and combined DH conditions

Cyclophilins are involved in Protein folding *in vivo* and by virtue of their stress-inducibility the different genes have been proposed to play a role in stress adaptation of plants (reviewed in Chou and Gasser, 1997). However, a direct relationship between stress tolerance and expression of BSPs has not been reported as yet. Immuno-blot analysis of seed samples revealed the presence of BsCyp53 in both the cultivars under DH conditions (Fig 1 F), however, the BsCyp53 expression was substantially higher in cv. PBW 527. Under D conditions, scarcely visible bands of BsCyp53 were detected with no substantial quantitative differences in both the cultivars. Applied alone, heat stress (H) did not provoke any BsCyp53 expression in both the cultivars. So, taken together, it can be suggested that by virtue of its hydrophilicity, BsCyp53 belongs to the broad family of boiling-soluble proteins, including those associated with cellular dehydration, either as a result of environmental stress (dehydrins), or during normal seed desiccation (HSPs)(Dure and others. 1989). From these observations it is also suggested that like other stress regulated proteins (HSPs/dehydrins proteins), BsCyp53 may be playing a significant role in water stress tolerance in drought tolerant cv. PBW 527, but not in drought sensitive cv. PBW 343. The specific induction of this protein during a combination of drought and heat shock may suggest that this combination is accompanied by the activation of a unique protein, which is not activated when each of these stresses was applied individually. Thus it may be possible to enhance the tolerance of plants to multiple stresses by manipulating the expression of cyclophilins. It is plausible that BsCyp53 may assist in import, folding and assembly of storage proteins in ER and may be essential for post translational processing of storage proteins. BsCyp53 may be helping other stress induced proteins to maturation besides regulating the expression of other genes imparting stress tolerance. Due to their hydrophilic nature, BsCyP53 may also function specifically in the protection of membranes and proteins against desiccation damage, possibly by binding water tightly or providing hydrophilic interactions in the absence of free water and by preventing the crystallization of cellular components through their ability to act as stabilizing “solvents’ (Close and others. 1989). Another possible role of BsCyp53 may be is to bind with the accumulated ions (ion sequestering) under water stress and to control solute concentration in the cytoplasm. Earlier studies

indicated that at high moisture contents, some BSPs like LEA proteins act as compatible solutes that preferentially exclude chaotropic agents (such as salts) from the surface of macromolecule (Liu and Zheng 2005). Likewise when hydration shell is removed they might exert their protective effects in the dry state by replacing water molecule by hydrogen bonding and/or forming a glass which stabilizes the system in the dried state (Wolkers and others. 2001). The high concentration of BsCyp53 induced by combined DH conditions prompted their consideration as essential factors of the adaption process to this type of environmental insult.

Immuno-blot analysis of BsGST under drought (D) heat (H) and combined DH conditions

Plant glutathione *S*-transferases (GSTs, EC 2.5.1.18) are a family of multifunctional enzymes involved in the intracellular detoxification of xenobiotics and toxic compounds produced endogenously (Edwards et al. 2000). Most of the enzymes are stress-inducible and play a role in the protection of plants from adverse effects of stresses (Marrs and Walbot, 1997). The GSTs have been associated with both normal cellular metabolism as well as in the detoxification of xenobiotics, limiting oxidative damage and other stress responses in plants. In the present study, Anti-GST immuno-blotting resulted only in scarcely visible trace reactions in seeds of both the cultivars under all conditions. In cv. PBW 527, the BsGST55 expression was substantially higher under H treatment. In contrast, no band was recognized by the anti-GST antibodies in seed samples of cv. PBW 343, suggesting specific role of GST proteins in drought tolerant cultivar. Earlier studies also reported enhanced GST expression by 2.12 folds upon drought stress (Gazanchian and others. 2007). Further, no protein band antigenicaly similar to GST was detected in shoots samples of both cvs. PBW 527 and PBW 343 under all the abiotic stress conditions, again indicating tissue specific expression of proteins.

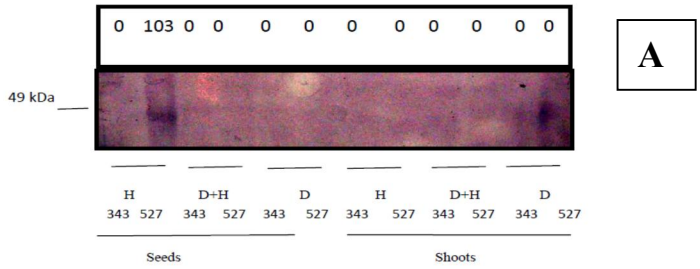
Immuno-blot analysis of BsLEA under drought (D) heat (H) and combined DH conditions

Besides, other hydrophilins (HSPs, CyPs and GSTs), LEA proteins are proposed to protect membranes and protein structures against drought induced damage. LEA proteins accumulate in seeds during the later stages of embryogenesis (Close and others. 1989) and some also accumulate in vegetative tissues in response to osmotic stress. They are supposed to act as solubilizing agents with chaperonic activities, maintaining cellular structural organization and prevent ion crystallization during desiccation. But surprisingly, in our study no specific band was detected in the both the cultivars under all conditions. Earlier also in cereals, it was reported that the expression of high molecular weight heat stable polypeptides was relatively high but did not cross react with antibodies against LEA or RAB (responsive to ABA) (Knight and others. 1995).

Conclusion

To conclude, enhanced expression of BsSOD, BsHSP, BSAQPs and BsCyPs particularly during DH conditions suggested that a combination of drought and heat shock affects plants differently from individual stress. These hydrophilins may be necessary to maintain protein function during this specific type of abiotic streses (Reyes and others. 2005). It is been possible that some hydrophilins particularly BsCyP may target molecular chaperones, and in combination, they could contribute to protect proteins under conditions. Other studies have shown that certain hydrophilins afford protection to other proteins like LDH against freeze-induced inactivation in vitro (Houde and others. 1995). A LTP1 protein, in addition to be responsive to abiotic stresses, has been suggested to be involved in transport of cutin monomers and flowering (Lindorff-Larsen and Winther 2001). Similarities in the conditions under which BsSOD, BsHSP, BsAQPs and BsCyPs proteins expressed, together with their hydrophilic character, may underlie a common function for at least a subset of these proteins, possibly in ameliorating the injurious effects of cellular dehydration. It has been reported earlier in tobacco and maize that several HSPs or transcriptional factors such as a pathogenesis related factor (WRKY) and ethylene responsive transcriptional co-activator (ERCTCA), are induced or accumulate during drought stress and heat shock treatments which supports the presence of key regulators involved in this response (Jacobsen and Shaw, 1989). Further, differences in the expression of specific gene products between stress-sensitive and stress tolerant plants indicate that tolerance is conferred by genetically encoded mechanisms (Bray, 1993). It is reasonable to expect that inter- and intraspecific differences in the pattern of protein synthesis between plants which differ in their stress resistance, however, this has also been some controversial issue.

A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49 kDa
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Seeds
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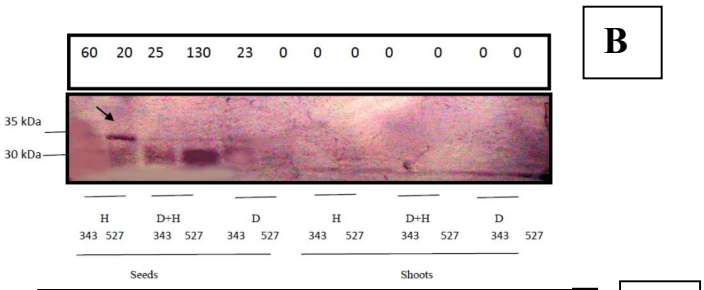
A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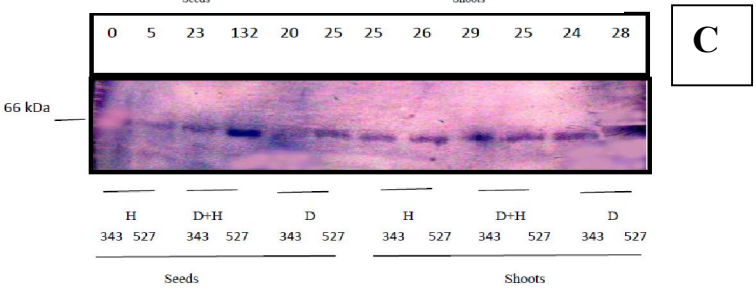
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66 kDa
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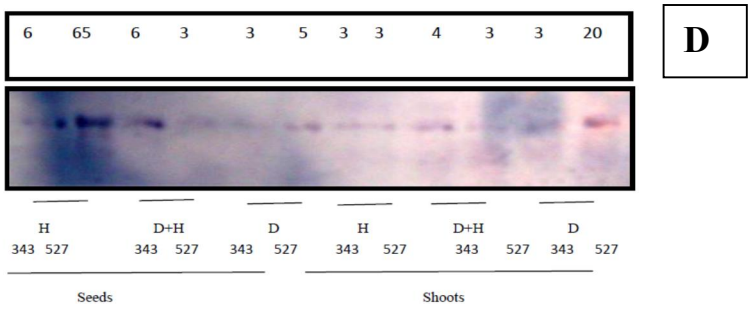
Seeds
Shoots

C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Seeds
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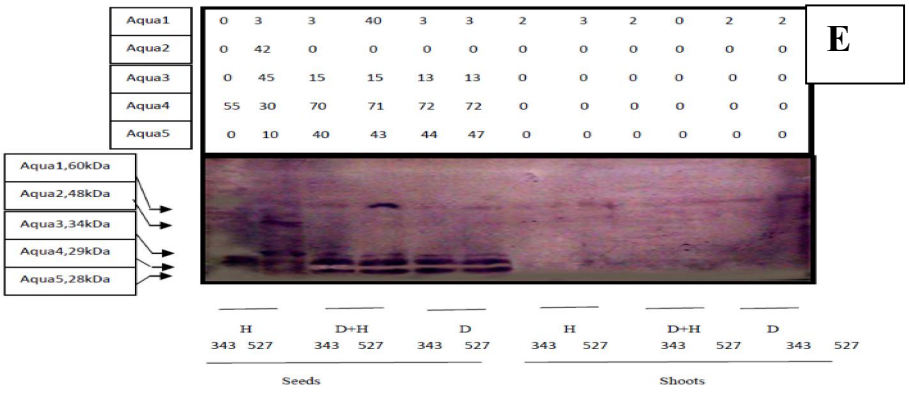
D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Aqua2 0 42 0 0 0 0 0 0 0 0 0 0
Aqua3 0 45 15 15 13 13 0 0 0 0 0 0
Aqua4 55 30 70 71 72 72 0 0 0 0 0 0
Aqua5 0 10 40 43 44 47 0 0 0 0 0 0
Aqua1,60kDa
Aqua2,48kDa
Aqua3,34kDa
Aqua4,29kDa
Aqua5,28kDa
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Seeds
Shoots

E

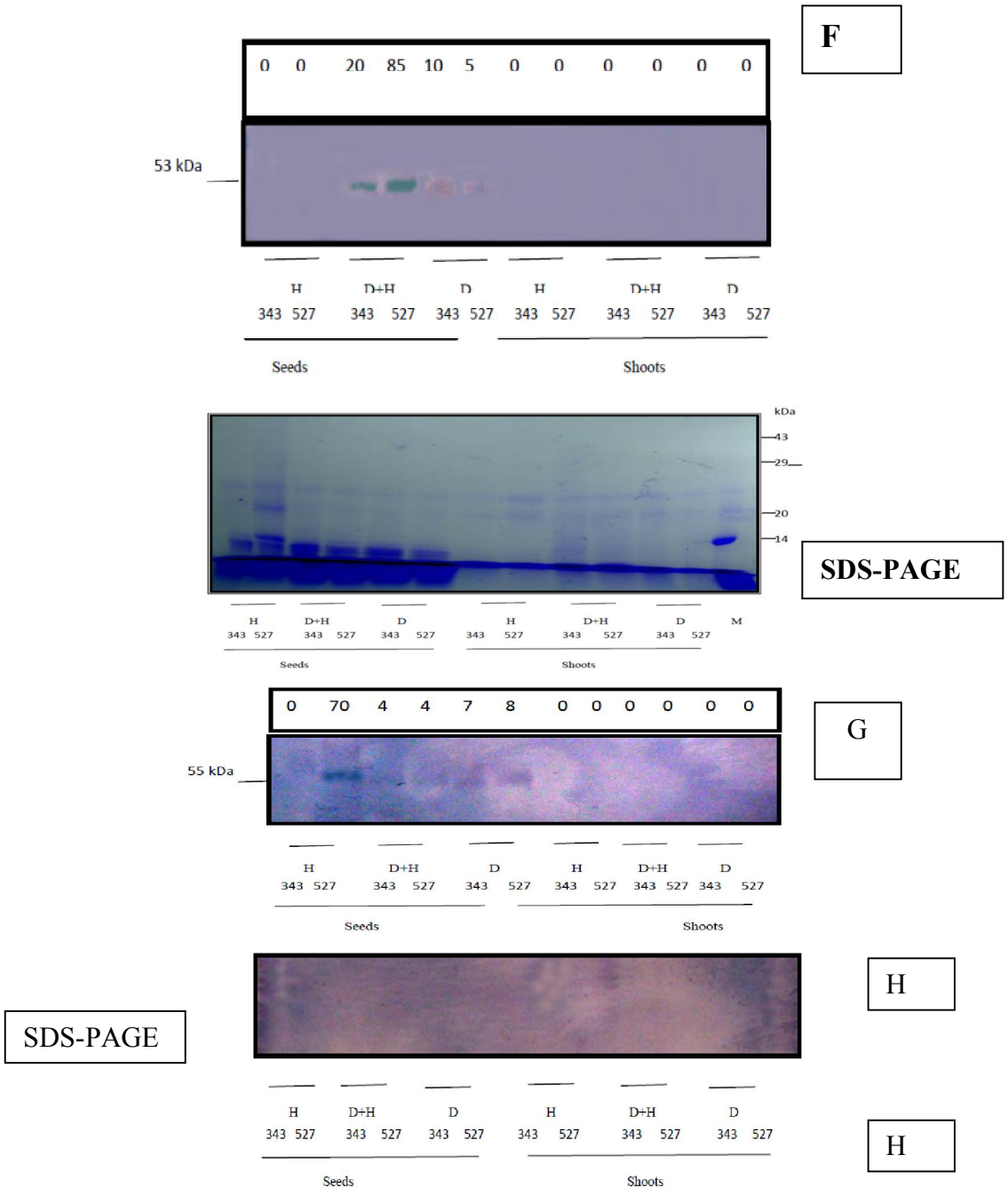

Figure 1: An SDS-PAGE profile of boiling stable proteins of seeds and shoots of drought tolerant (PBW 527) and drought sensitive (PBW 343) cultivars *Triticum aestivum* after stress treatments. Each lane was loaded with 60 µg of boiling stable proteins. D: drought, H: heat, DH: combined drought and heat, M: molecular weight marker.
Immunoblot analysis of BsWGA(A), BsSOD(B), BsHSP90(C), BsAPase(D), BsCyP(E), BsAqua(F), BsGST(G) and BsLEA(H) in seeds and shoots of drought tolerant (PBW 527) and drought sensitive (PBW 343) cultivars *Triticum aestivum* after stress treatments. Numerical values as shown in the top of Panels, indicates relative band intensities, which were determined using Gel Visualization, Documentation and Analysis system (Bio-Rad, USA). Numerical comparisons are only valid within panels and cannot be made between panels. Each lane loaded with 60µg of boiling stable proteins was resolved on 12% SDS-PAGE and transferred to nitrocellulose membrane and probed with different antiserums.

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Antioxidant activity of callus culture of *Vigna unguiculata* (L.) Walp.

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Abstract: Tissue culture of *Vigna unguiculata* (L.) Walp. was done on MS medium supplemented with various concentrations of auxins and cytokinins. Maximum callusing was observed in basal MS medium containing 5 ppm Kn and 1 ppm NAA. Methanolic extract of callus was successively partitioned with n-hexane, chloroform and ethyl acetate. Maximum phenolic content and antioxidant activity using DPPH and FRAP assays was observed in ethyl acetate fraction and minimum potential in n-hexane. The results reveal that *in vitro* culture of *V. unguiculata* as an alternative source of antioxidant.

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Key words: *Vigna unguiculata*, callus, DPPH, FRAP, antioxidant

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1. Introduction

Free radicals are produced in our body as a by product of many reactions. However, improper life style, ill eating habit and stress enhance the production of free radicals. These free radicals initiate a chain reaction which makes other molecules unstable thereby adversely affecting the metabolism. Human system is equipped to combat these free radical through antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase and prevent oxidative stress. Nevertheless, external supplementation of antioxidants is necessary. There are synthetic antioxidants available but search for natural antioxidants is the need of the hour as whole world has now realized the significance of alternative and complementary medicines. Plants and plant products forms one of the best source of natural antioxidants and possess various bioactivities (Mathur *et al*, 2007; Bhatia *et al*, 2008). Several plants have been explored for its antioxidative potential (Miguel, 2010).

Overexploitation plant for therapeutic purpose has led to biodiversity problems. There is need to search for alternative sources for the production of natural antioxidants. Plant tissue culture is an important venture in this regard. There is production of useful compounds under controlled conditions free from environmental constraints. Moreover, production can be more reliable, simpler and rather predictable.

In the present study *Vigna unguiculata* L. (Walp.) was explored for its tissue culture and antioxidant potential. *V. unguiculata* (Family: Fabaceae), commonly called as Cowpea is an annual plant. It is a grain legume which forms an important source of dietary protein in many parts of the world. There are few reports on tissue culture of *Vigna* sps. Micropropagation of cowpea has been successfully attempted (Odutayo *et al*, 2005; Diallo *et al*, 2008). Plant regeneration from cotyledonary node in *Vigna radiata* has been also been reported (Gulati and Jaiwal 1994). There are scanty reports on antioxidant activity of Cowpea (Siddhuraju and Becker, 2006; Nair *et al*, 2007). However, there are probably no reports on antioxidant activity of *in vitro* culture of *V. unguiculata* to the author's knowledge.

2. Materials and Methods

Tissue culture

The seeds of *V. unguiculata* were washed under running water for 20-25 min and then treated with 0.1% $HgCl_2$ (Mercuric chloride) for 6 minutes in laminar flow hood, finally washed with sterile distilled water for 3 times. Hypocotyl was used as the explant from *in vitro* grown seedling. The explants were then inoculated in MS media (Murashige and Skoog 1962) supplemented with various concentrations of auxins viz., 2, 4-D (2, 4-Dichlorophenoxyacetic acid) and NAA (Naphthalene Acetic Acid), and cytokinin Kn (Kinetin) and BAP (Benzylaminopurine). All cultures were maintained at 25±2ºC under light intensity (1200 lux) for photoperiod of 16h light and 70±10 relative humidity. The cultures were maintained for about 6 months by periodic subculturing of 6-8 weeks time interval.

Extraction

Callus was dried in oven at 50°C till constant weight was achieved. It was then powdered and extracted with methanol in shaker at 30°C for 48 h. Methanol was evaporated and redissolved in water to obtain crude extract. This crude extract was then partitioned with hexane (H) and then successively partitioned with chloroform (CF) and ethyl acetate (EA). Every fraction was dried, redissolved in

methanol and filtered for further biochemical analysis.

Total phenolics content

The total phenolics were determined colorimetrically according to the Folin- Ciocalteaeu method (Sharma *et al*, 2009). Briefly, 0.5 mL of water and 0.125 mL of the methanolic extract of various fractions were added to a test tube. Folin-Ciocalteu reagent (0.125 mL), 1.25 mL of the sodium carbonate solution and 3 mL of water was added successively and allowed to stand for 90 minutes. The absorbance was measured at 760nm. Total phenol content was expressed as gallic acid equivalents (GAE) in (mg GAE/g dry weight of sample) dry material. Values are expressed as Mean ± S.D

DPPH free radical scavenging activity

Different fractions (1ml) were mixed with 1ml of 0.3mM. DPPH reagent and allowed to stand at room temperature for 30 minutes in dark. The absorbance was taken at 517nm. Radical scavenging activity was calculated according to the following formula and IC_{50} value was evaluated and expressed as Mean ± S.D.

$$\% \text{ DPPH radical scavenging} = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100$$

FRAP assay

Acetate buffer, 300mmol/l, 10 mmol/l 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mmol/l HCl and 20 mmol/l $FeCl_3$ x $6H_2O$ in distilled water was prepared. 25ml of acetate buffer, 2.5ml TPTZ solution and 2.5ml $FeCl_3$ x $6H_20$ solution was mixed to make working solution. 50µl of sample extract was mixed with 1.5ml of FRAP reagent and monitored up to 5 min at 593nm. Absorbance was compared with calibration curve of aqueous solution of known Fe (II) concentration (µM/l). Values are expressed as Mean ± S.D.

3. Results and discussion

Basal MS medium was used for *in vitro* germination of the seeds of *V. unguiculata*. Germination started after 2-3 days. Hyocotyl was found to be more responsive to initiate callus as compared to other parts of seedling. Initially the cut ends of the explants showed swelling which later turned into callus. The callus was fragile and light brown in colour. Maximum % induction of explant (90%) and callusing was observed in MS + Kn (5ppm) + NAA (1ppm). Similar observations were made, where higher cytokinin concentration induced callus at the basal end of explants (Diallo *et al, 2008*). Minimum % induction of explant (70%) and callusing was observed in MS + 2, 4-D (1 ppm). Other combination of hormones showed moderate callusing (Table-1). The media in which maximum amount of callusing was observed were sub-cultured periodically after 6 weeks and maintained further and analysis (Fig. 1). Callus in Cowpea was observed in MS medium supplemented with 1 µM NAA and BAP (Odutayo *et al*, 2005). Amitha and Reddy (1996) reported smooth nodular callus from zygotic embryos.

The dried and powered callus was extracted in methanol and subjected to fractionation. Percentage yield of fractions in hexane, chloroform and ethyl acetate was 4.2 ± 1.2, 5.6 ± 0.9 and 9.8 ± 1.6, respectively. The EA fraction showed maximum (Fig. 2) phenolic content (60 ± 0.64 mg/g GAE) and minimum content in H fraction (6 ± 0.72 mg/g GAE). This suggests that phenols are mostly soluble in ethyl acetate fraction. EA fraction possessed highest DPPH radical scavenging activity as it had minimum IC_{50} value (80 ± 1.2 µg/ml) whereas in H fraction IC_{50} value was maximum (640 ± 1.9 µg/ml). The FRAP value exhibited similar results like DPPH (Fig. 2). The maximum ferric reduction activity was observed in EA fraction (812 ± 2.1 µM/l) and minimum in H fraction (245 ± 1.8 µM/l). Since EA fraction had maximum phenolic content this might be the reason for highest scavenging activity. Phenols have –OH group which donate H to form DPPH-H leading to higher antioxidant potential. Moreover phenolic compounds are known to inhibit the oxidation activity of free radicals and enhance activity of antioxidative enzymes. Phenolic compounds include flavonoids which have diverse therapeutic potential mainly due to their antioxidant potential (Pietta, 2000; Vats, 2009). Dietary flavonoids are recommended by many dieticians for healthy living.

Conclusion

The results confirm the antioxidant potential of *V. unguiculata* and provide callus culture as an alternative source of natural antioxidant.

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Table 1: Effect of different concentrations of phytohormones on % induction and callusing of *V. unguiculata*

2,4D	Kn	NAA	BAP	Callusing	% induction
1ppm	-	-	-	++	70±1.0
1ppm	0.1ppm	-	-	+++	81±1.2
-	5ppm	1ppm	-	++++	90±0.08
0.1ppm	-	-	1ppm	+++	85±1.4

Values are Mean ± S.D (++++ = very good; +++ = good; ++ = moderate)

Figure 1: Callus induction in MS + Kn (ppm) + NAA (1ppm)

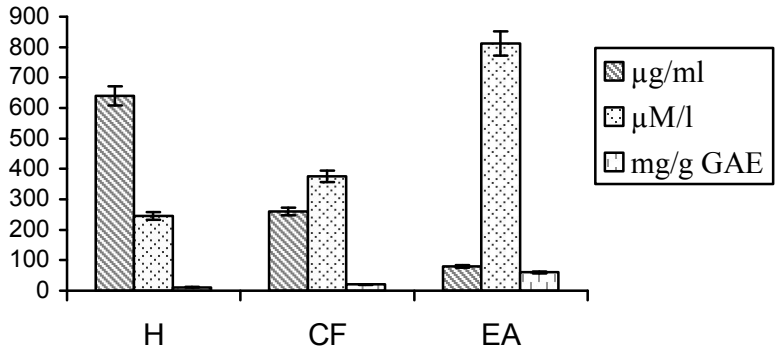

Figure. 2: DPPH (ug/ml), FRAP (uM/l) and total phenol (mg/g GAE) values of fractions.
H- hexane, CF- Chloroform, EA- Ethyl Acetate.

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