Protective Effects of *Bixa orellana* Seed Oil on Carbon tetrachloride Induced Liver Damage in Rats

Wilson Obidah[1], Grace K. Garba[1], Joseph Z. Fate[2]

[1]Department of Biochemistry, Federal University of Technology Yola P.M.B.2076 Yola, Nigeria.
[2]Department of Science Laboratory Technology, Federal Polytechnic Mubi P.M.B. 35 Mubi, Nigeria.
domwam@yahoo.com

Abstract: The effects of pretreatment with *Bixa orellana* seed oil on CCl_4-induced liver damage were determined in male Wister rats (150±10g). Rats were pretreated with *Bixa orellana* seed oil at 0, 1, 5 or 10% (w/w) through dietary exposure for 4 weeks before a single intrapretoneal injection of CCl_4. Serum biochemical parameters, liver lipid peroxidation and relative organ weights were determined. Pretreatment with *Bixa orellana* seed oil (10%) resulted in significant ($p<0.05$) reduction in serum liver marker enzymes activities, total bilirubin concentration, lipid peroxidation and relative liver weights induced by CCl_4 administration. These results show that dietary exposure to *Bixa orellana* seed oil exhibited moderate protection against CCl_4-induced hepatotoxicity in rats.
[Wilson Obidah, Grace K. Garba, Joseph Z. Fate. **Protective Effects of *Bixa orellana* Seed Oil on Carbon tetrachloride Induced Liver Damage in Rats**. Report and Opinion 2011;3(1):92-95]. (ISSN: 1553-9873). http://www.sciencepub.net.

Key words: *Bixa orellana*; seed oil; hepatoprotection; CCl_4; rat

1.0 Introduction

Hepatotoxicity resulting from exposure to environmental chemicals is a global major public health problem. A number of hepatotoxicants of different types have been documented (Timbrell, 1991).Carbon tetrachloride (CCl_4) is used commonly as a chemical model for the induction of hepatic injury in experimental animals. CCl_4 is activated in the liver by liver cytochrome P_{450} enzymes to form reactive toxic metabolites which can cause liver injury in animals and humans (Uchleke and Werner, 1975; Gonzalez, 1988). Liver damage is associated with membrane lipid peroxidation and cell necrosis (Rush *et al.*,1986; Recknagel *et al.*, 1989;Williams and Burk, 1990).

Bixa orellana is a plant of the family *Bixaceae*. The colored seeds coat yields a carotenoid dye used in coloring foods, drugs and cosmetics. The dye has been shown to have antimicrobial activity against Gram positive bacteria including, *Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis* and reduced activity against *Escherichia coli, Serratia marcesens, Candida utilis and Aspergillus niger* (Irobi *et al.*, 1996). The oil obtained from *Bixa orellana* seeds has been shown to contain fatty acids such as capric, palmate, stearic, oleic and linoleic acid; the unsaturated oleic and linoleic fatty acids being the most abundant (Silva *et al.*, 2008).

Although the seed coat dye is the most widely used in foods, in some countries including Nigeria, the whole seed is crushed and the powder used in food preparations to enhance food color. Though several studies have been carried out on the dye (Preston and Richard, 1980; Irobi *et al.*, 1996; Paumgartten *et al.*, 2002), little is known about the seed oil especially concerning its effects against chemically induced cell injury. In this study, we report data on the effects of *Bixa orellana* seed oil on CCl_4-induced hepatotoxicity in rats.

2.0 Materials and Methods

2.1 Sample collection and Extraction of *Bixa orellana* seed oil.

The *Bixa orellana* seeds were purchased from Jimeta main market Yola, Adamawa state, Nigeria.The seeds were transferred into a lidded plastic container, moistened and shaken vigorously to remove the seed coat. The mixture was diluted with tap water and the seeds were sieved out and further washed with tap water. The decoated seeds were sun dried and later dried at constant weight in the oven at 60°C.The dried samples were first crushed into coarse particles by using mortar and pestle before being ground to fine powder using an electric blender. Oil in the powdered sample was extracted using petroleum ether (60% fraction) using soxhlet apparatus with a yield of about 4.2%. The oil obtained was stored in a brown bottle at room temperature until the commencement of the study.

2.2 Animals

Male Wister rats were purchased from the animal house of University of Jos, Jos, Nigeria. The animals were acclimatized for one week before the start of the study. The animals were housed in plastic

cages at room temperature and were fed pelleted diet (Grand cereals Ltd., Jos, Nigeria).

2.3 Chemicals

The kits for the determination of serum alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) activities were products of Randox Laboratories Co. (Atrium, UK). Thiobarbituric acid (TBA) and carbon tetrachloride (CCl_4) were products of Aldrich chemical company, Milwaukee, USA. Other chemicals and reagents utilized were of highest purity and were purchased from local firms, Nigeria.

2.4 Experimental Design

Twenty five male Wister rats weighing 150 ± 10g were allocated to 5 groups of 5 rats each (Group I – V). Group I (control) and Group II were fed the normal diet only. Group III, Group IV and Group V, were fed diet mixed with *Bixa orellana* seed oil at 1, 5, and 10% (w/w) respectively, for four weeks. On the last day of the pretreatment, hepatotoxicity was induced using a single dose of CCl_4 dissolved in olive oil(1:1), administered intraperitoneally at 2ml/kg body weight (Vanitha *et al.*, 2007) to animals of Group II , III, IV and V. After 24 hours of CCl_4 administration, the rats were sacrificed under ether anaesthesia and blood collected by cardiac puncture. Serum was prepared and used for the determination of serum biochemical parameters. Serum AST, ALT, ALP and total bilirubin levels were assayed using commercial kits (Randox laboratories Co.Atrium, UK). Liver from control and treated animals were removed, weighed and utilized for the determination of lipid peroxidation. The hepatic lipid peroxidation was determined as thiobarbituric acid reactive substance (TBARS) and expressed as the amount of malondialdehyde (MDA) (Uchiyama and Mihara, 1978).

2.5 Statistical Analysis

The data are expressed as mean ± standard error (S.E.M) for five replicates and evaluated by one way ANOVA followed by Dunnett test (Dunnett, 1955). All results were compared with respect to control and as well as the group treated with CCl_4 only (Group II). The level of significance was set at $p< 0.05$.

3.0 Results

Rats treated with CCl_4 showed a significant ($p< 0.05$) increase in serum liver enzymes activities (*Table 1).* These included ALT, AST and ALP. The elevated levels of the liver enzymes were also accompanied with significant ($p< 0.05$) increases in serum total bilirubin concentrations. Pretreatment with *Bixa orellana* seed oil caused significant ($p< 0.05$) dose dependent decreases in the CCl_4-induced elevation of serum liver enzymes and total bilirubin concentrations. Administration of the *Bixa orellana* seed oil at 10% dietary level restored the plasma ALP and total bilirubin levels to normal levels.

The effect of pretreatment with *Bixa orellana* seed oil on CCl_4-induced lipid peroxidation is shown in *Table 2.* The levels of malondialdehyde (MDA) in the group treated with CCl_4 alone increased significantly ($p< 0.05$) when compared with the control group. Conversely, pretreatment with *Bixa orellana* seed oil (10%) significantly ($p< 0.05$) decreased CCl_4-induced hepatic lipid peroxidation in a dose dependent manner.

Treatment with CCl_4 resulted in significant ($p<0.05$) increase in rat relative liver weights when compared with the control group (*Table 3).* Mean final body weights were not affected by either CCl_4 or *Bixa orellana* seed oil administration. Pretreatment with *Bixa orellana* seed oil (10%) resulted in significant ($p < 0.05$) reduction in the mean relative liver weight of rats. Relative liver weights in the 10% group were comparable to those of the control.

Table 1: Effects of pretreatment with *Bixa orellana* seed oil on CCl_4-induced increases in serum ALT, AST, ALP and Total bilirubin levels.

Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bil. (mg/dL)
Group I: control	66.25±4.51	45.41±3.82	42.56±3.19	0.61±0.12
Group II: CCl_4	192.63±4.76[a]	88.34±4.05[a]	58.27±2.37[a]	0.93±0.12[a]
Group III: 1% oil + CCl_4	188.38±5.98[a]	80.62±3.68[a]	54.66±2.36[a]	0.91±0.11[a]
Group IV: 5% oil + CCl_4	173.67±4.02[a]	76.86±5.77[a]	46.97±3.88[a]	0.83±0.13[a]
Group V: 10% oil + CCl_4	156.50±5.58[a,b]	68.84±5.62[a,b]	43.63±3.26[b]	0.75±0.14[b]

Tabulated values are mean±S.E.M, n=6

ALT, Alanine transaminase; AST, Aspartate transaminase; ALP, Alkaline phosphatase; Total Bil., Total bilirubin

a:Significantly different from control group ($p<0.05$)

b:Significantly different from the group treated with CCl_4 only ($p<0.05$)

Table 2: Effects of pretreatment with *Bixa orellana* seed oil on CCl_4-induced increases in liver lipid peroxidation.

Treatment	MDA (mmoles/mg protein)
Group I: control	33.55 ± 4.22
Group II: CCl_4	58.61 ± 3.68[a]
Group III: 1%	56.22 ± 3.72[a]
Group IV: 5% + CCl_4	57.53 ± 4.15[a]
Group V: 10% +CCl_4	44.63 ± 4.39[a,b]

Values are expressed as mean ± S.E.M, n=5; MDA, malondialdehyde
a:Significantly different from control group ($p<0.05$)
b:Significantly different from the group treated with CCl_4 only ($p<0.05$)

Table 3: Effects of pretreatment with *Bixa orellana* seed oil on CCl_4-induced changes in relative liver weight.

Treatment	Mean final body weight (g)	Relative liver weight (g/100g body weight)
Group I: control	264.47±5.71	1.55±0.12
Group II: CCl_4	259.24±6.11	2.25±0.11[a]
Group III: 1% oil + CCl_4	268.65±4.98	2.27±0.14[a]
Group IV: 5% oil + CCl_4	260.53±5.92	2.18±0.12[a]
Group V: 10% oil + CCl_4	262.16±6.34	1.81±0.13[a,b]

Values are expressed as mean ± S.E.M, n=5.
a:Significantly different from control group ($p<0.05$)
b:Significantly different from the group treated with CCl_4 only ($p<0.05$)

4.0 Discussion

Hepatotoxicity was observed in rats treated with CCl_4 alone as shown by increased serum ALT and AST which may suggest liver damage (Chawla, 1999) following CCl_4 administration. The observed significant increases in serum ALP activity and total bilirubin concentration further demonstrate hepatotoxicity in rats arising from cholestatic liver injury (Chawla, 1999; Somchit *et al.*, 2004). Pretreatment with *Bixa orellana* seed oil protected against CCl_4-induced hepatotoxicity as demonstrated by significant decreases in serum liver marker enzyme levels and other biochemical indices.

The biochemical marker of lipid peroxidation, malondialdehyde (MDA) increased significantly in all animals treated with CCl_4 when compared with the control. CCl_4 is known to increase lipid peroxidation (Recknagel *et al.*,1989; Mansour, 2000; Glei *et al.*, 2002). The elevated levels of MDA in the CCl_4-treated groups indicate lipid peroxidation elicited by administration of CCl_4. Pretreatment with *Bixa orellana* seed oil protected against CCl_4-induced liver lipid peroxidation, as shown by reduction in the level of MDA. Consumption of *Bixa orellana* seed oil may decrease liver organ susceptibility to lipid peroxidation and invariably oxidative stress. Pretreatment with *Bixa orellana* seed oil also protected the liver against any change in relative organ weight which normally reflects the pathological state of the organ. The relative liver weights were consistent with serum ALT and AST activities, and liver tissue MDA levels which further demonstrate the hepatoprotective potentials of *Bixa orellana* seed oil. Earlier reports suggested that natural compounds of plant origin can protect against CCl_4-induced toxicity by quenching free radicals and inhibiting lipid peroxidation caused by CCl_4 metabolism (Biasi *et al.*, 1991; Chidambara *et al.*, 2002). Such antioxidant activities are usually attributed to carotenoids and allied substances which could be important in the observed hepatoprotective effects of *Bixa orellana* seed oil. Interestingly, the seed coat has been shown to contain carotenoids (Trimanna, 1981; Mercadante *et al.*, 1997). It is therefore possible that the seed oil also contain carotenoids or other substances with antioxidant potential. The administration of *Bixa orellana* seed oil to rats provided moderate dose dependent protection against CCl_4-induced hepatotoxicity since meaningful antihepatotoxic effects were observed in the high dose (10%) group only.

5.0 Conclusion

Inclusion of *Bixa orellana* seed oil in the animal diet protected against CCl_4-induced liver injury. Thus, consumption of the seed oil may protect against hepatotoxicity caused by environmental chemicals and other prooxidants by inhibiting liver tissue lipid peroxidation.

Aknowledgements

We are grateful to Prof. M.A.Madusolumuo and Mrs. M.S.Nadro of Federal University of Technology Yola, Nigeria, for their assistance in preparing the manuscript and providing some of the chemicals used in this study.

Corresponding author;
Wilson Obidah,
Department of Biochemistry,
Federal University of Technology Yola,
Nigeria.
Email : domwam@yahoo.com

References

1. Biasi F., Albano E., Chiarpotto F.,Corongiu F. (1991).In vivo and in vitro evidence concerning the role of lipid peroxidation in the mechanism of hepatocyte death due to carbon tetrachloride. Cell Biochem. Funct. 9:111-118
2. Chawla R.(1999).Practical Clinical Biochemistry (Methods and Interpretations). Second edition. Jaypee Brothers. Medical Publishers, New Delhi, India. pp110 -118.
3. Chidambara Murthy K.N., Singh P.R., Jayaprakasha K.G. (2002).Studies on antioxidant activity of Pomegranate peels and seeds extract using in vivo systems. J.Agric.Food Chem. 50:4791- 4795.
4. Dunnett CW. (1955).A multiple comparison procedure for comparing several treatments with control. J. Am. Stat.Assoc. 12:1096 -1121.
5. Clarke E.G.C and Clarke L.M. (1977).Veterinary toxicology. Cassel and Collier Macmilan, London. pp268-277.
6. Glei M., Liegibel M.U., Ebert M.N., Bohm V., Pool-Zobel(2002).B-Carotene reduces bleomycin -induced genetic damage in human lymphocytes. Toxicol.Appl. Pharmacol. 179:65-73.
7. Gonzalez, F.J. (1988).The molecular biology of cytochrome P450s. Pharmacol. Rev.40: 243-288.
8. Irobi N.O., Moo-Young M., Anderson A.W (1996).Antimicrobial activity of Annatto(Bixa orellana) Extract. Pharm. Biol. 34(2):87-90.
9. Lorke D. (1983). A new approach to practical Acute toxicity testing. Arch Toxicol.54:275-287.
10. Mansour M.A. (2000). Protective effects of thymoquinone and desferrioxamine against hepatotoxicity of carbon tetrachloride in mice. Life Sci. 66:2583-2591.
11. Mercadante A.Z., Steck A., Pfander H. (1997). Isolation and identification of new apocarotenoids from Annatto (Bixa orellanna) seeds. J. Agric. Food Chem. 45(4):1050–1054.
12. Paumgartten R.J. F., De-Carvalho., Araujo B.I., Pinto M.F., Borges O.O., Souza C.A.M., Kuriyama N.S.(2002).Evaluation of the developmental toxicity of annatto in rat. Food and Chem. Toxicol. 40(11):1595-1601.
13. Preston D.H and Rickard D.H. (1980). Extraction and chemistry of annatto. Food Chem. 5(1): 47 -56.
14. Recknagel R.O., Glende E.A. Jr., Dolak J.A.,Waller R.A. (1989).Mechanisms of carbon tetrachloride toxicity. Pharm.Ther. 43:139-154.
15. Rush J.R., Klaunig E.J., Schultz E.N., Askari B.A., Lacher A.D., Pereira A.M., Goldblatt. (1988).Mechanisms of chloroform and carbon tetrachloride toxicity in primary cultured mouse hepatocytes.Environ.Health Perspect.69:301-305
16. Silva F.G., Gamara C.M.F.,Oliveira L.A., and Cabral A.F. (2008).Extraction of bixin from annatto seeds using supercritical carbon dioxide. Braz. J. Chem. Eng. 25(2):419- 426
17. Somchit N., Norshahida R.A., Hasiah H.A., Zuraini A., Sulaiman R.M., and Noordin M.M. (2004). Hepatotoxicity induced by antifungal drugs intraconazole and fluconazole in rats: a comparative in vivo study.Hum. Exp. Toxicol. 23:519-525.
18. Timbrell J. (1991).Principles of biochemical toxicology. Second edition. Taylor and Francis, Washington DC. pp296-312.
19. Trimanna A.S.L. (1981). Study of the carotenoid pigments of Bixa orellana L.seeds by thin layer chromatography. Microchim. Act. 76(2) :11-16.
20. Uchiyama M. and Mihara M. (1978). Determination of malonaldehyde precursor in tissues by the thiobarbituric test. Anal.Biochem. 86:271-278
21. Uchleke H. and Werner Th. (1975). A compare ative study and on the irreversible binding of labeled halothane, trichlorofluoromethane, chloroform and carbon tetrachloride to hepatic protein and lipids in vitro and in vivo. Arch. Toxicol. 34:289-308.
22. Vanitha A., Chidambara Murthy N.K., Kumar V., Sakthivelu G., Veigas M.J., Saibaba P and Ravishankar A.G. (2007).Effect of the carotenoid-producing Alga, *Dunaliella bardawil*, on CCl4 induced toxicity in rats. Int. J. Toxicol. 26:159-167.
23. Williams A.T. and Burk R. (1990). Carbon tetrachloride hepatotoxicity an example of free radical–mediated injury. Liver Dis. 10:279-284.

7/24/2010