Potency of Aqueous White Grubs Extract Against CCl_4 Induced Liver Diseases in Rats

[1]M. S. Sule, [2]A. J. Alhassan, [3]Y. Abdulmumuni

[1]Hussaini Adamu Federal Polytechnic Kazaure, Jigawa State- Nigeria.
[2]Department of Biochemistry, Faculty of Science, Bayero University, Kano, PMB 3011 Kano - Nigeria
[3]Department of science Laboratory Technology, College of science ,Engineering And Technology

yabdulmumin@yahoo.com

Abstract: The potency of aqueous white grubs extract was assessed in the cure of carbon tetrachloride (CCl_4) – induced lipoperoxidation in rats. The three different dosages were administered (1g/kg, 2g/kg and 8g/kg) daily to different groups of rats for up to 9 days after induced with lipoperoxidation using CCl_4 at a dose of 120mg/kg. the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities as well as levels of serum malondialdehyde (MDA), total protein (TP) and albumin (ALB) of the rats after 9 days of white grubs administration were found to be similar to those of control rats (not CCl_4 – treated). This shows possible curative effects of the white grubs extract, which was found to be depend on both the dose administered and the duration of treatment.

Key words: White grubs, Potency, carbon tetrachloride and liver disease.

Introduction

Unlike insects and related species, the uses of herbs to treat disease is almost universal among non industrialized societies and in most part of the world 70 - 90% of the people rely on plant for medication (Farnsworth and Soejarto, 1985; Hostettmann et al., 2000). The values of medicinal plants to mankind is very well proven and form the basis of health care world wide, and have roles in international trade (Ahmed et al., 2006). The use of, and research for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural product chemists are perfecting phytochemicals for treatment of various diseases. At least 7,000 medical compounds in the modern pharmacopoeia are derived from plant (Tapsell, 2006). It is timely to venture into search for insects that have medicinal properties in addition to their nutritional roles.

White grubs are the larval stage of beetles' metamorphosis commonly found in dump refuse and animal dung, feeding on plants and animal remains (Alhassan et al., 2009a). In Africa, the species is widely distributed, it is found in Nigeria, Niger Republic, Uganda, etc. Though, white grub is seen and / or presented in the world's field of science as more or less a pest, with less or no positive economic importance, recent research and discoveries indicates white grubs are rich in protein, fats and mineral elements (Alhassan et al., 2009a). It is used among communities as food and as medicine among the Hausa/ Fulani in Northern Nigerian (Alhassan et al., 2009a). In South Western Nigeria, edible insects are conceived as food and source of nutrients and among the traditions and the customs that persist, are the consumption of various insects and usage of insects for rituals and medicinal purposes. From the foregoing discussion, white grub, like any other food item and medicinal specimen, may contain one or more nutrients, elements or compounds necessary for body system up-keeping.

Liver is a discrete largest organ in human body that has many interrelated functions and it may be damaged due to one or more of the following: injury from metabolic disturbances, injury from toxins, drugs, chemicals and poisons, lesion of biliary tract, certain viral' infections, hypoxia, tumours (MacSwean, 1980; Roderick et al., 1998). Carbon tetrachloride (CCl_4) induces lipid peroxidation and liver damage (Robbins and Cotran, 2006) and high dose of CCl_4 generates an ideal hepatotoxicity model organism that allows for evaluating the curative effects of medicinal rather than reporting natural healing (Alhassan et al., 2009b).

In a survey carried out, seven out of ten individuals contacted around Kano and its environs are in one way or the other aware of using white grubs to cure jaundice i.e. "shawara". Hausa/ Fulani perception of jaundice "shawara" includes among others, general body weakness, tiredness, general body pain and even lose of appetite, without obvious sign of jaundice. This reported work was carried out to investigate the potency of white grubs extract against CCl_4 induced liver damage.

Materials and Methods

White grubs (WG) found in public wastes of Darmanawa quarters, Tarauni Local Government, Kano State were collected in the months of July and August, 2008. The WG were cleaned of dirt and blot, and was squeezed to release the extract (grubs' extract). The extract was filtered using cheesecloth and adjusted to concentration of 1.0g/ cm^3. Commercially prepared reagent kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST) alkaline phosphatase (ALP) and total bilirubin (TB) obtained from Randox Laboratories, Antrim, UK, were used to assay the enzymes. Thiobarbiturate, biuret and bromocresol green were used for serum malondialdehyde (MDA), total protein and albumin determination respectively.

Experimental Animals

Eighty five (85) rats were obtained and divided into five (5) groups of twenty (20) rats each for groups I and II, and fifteen (15) rats each for groups III, IV and V. The rats in the first group (group I) were not induced with lipid peroxidation and liver damage, they served as positive control, whereas rats in groups II, III, IV and V were induced with lipid peroxidation and liver damage using 120mg/Kg CCl_4 according to Alhassan et al. (2009b). Rats in group II were not administered with white grub extract, they served as negative control. Rats in groups III, IV and V were administered with a daily dose of 1.0, 2.0 and 8.0g/kg body weight of white grub extract. Five (5) rats were removed from each group after 3, 6 and 9 days of white grub extract treatment respectively and sacrificed by decapitation.

Biochemical Analysis

Serum was separated and analyzed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities by the method of Reitman and Frankel (1957), serum alkaline phosphatase (ALP) activity by the method of Rec (1972), serum malondialdehyde (MDA) concentration by the method of Hunter et al (1963) modified by Gutteridge and Wilkins (1982), total bilirubin (TB) (Malloy and Evolyn, 1937) and serum total protein and albumin by the method of Chawla (1999).

Results

Table 1 summarizes the results for serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and levels of total bilirubin (TB), malondialdehyde (MDA), total protein (TP) and albumin (ALB) for a group of rats 48hrs after intramuscularly injected with 120mg/Kg CCl_4. Tables 2, 3 and 4 present the results for groups of rats injected with CCl_4 and administered with various doses of aqueous extract of white grubs daily for 3, 6 and 9 days respectively. There was significant difference ($p<0.05$) between group I and II in the parameters analyzed except for TP. The groups

that received the white grubs' aqueous extract showed significant decrease ($p<0.05$) in ALT, AST and ALP activities compared to group II.

Table 1: Serum ALT, AST and ALP activities and TB, MDA, TP and ALB levels in rats 48 hours after intramuscular administration of CCl_4

GROUP	ALT (U/L)	AST (U/L)	ALP (U/L)	TB (µmol/L)	MDA (µM)	TP (g/L)	ALB (g/L)
I No CCl_4 administered	8.39 ± 0.48 [a]	21.06 ± 1.23 [b]	32.50 ± 2.13 [c]	4.86 ± 0.71 [d]	0.08 ± 0.01	59.99 ± 1.30	32.39 ± 1.84 [f]
II 120mg/Kg CCl_4 administered	36.00 ± 4.41 [a]	110.01 ± 4.39 [b]	68.17 ± 3.01 [c]	10.40 ± 1.12 [d]	0.52 ± 0.02	60.41 ± 2.47	34.87 ± 1.32 [f]

Values in the same column bearing similar superscript are significantly different at $p < 0.05$, $n = 5$

Table 2: Serum ALT, AST and ALP activities and TB, MDA, TP and ALB levels in rats after oral administration of white grub extract for 3 days.

GROUP	ALT (U/L)	AST (U/L)	ALP (U/L)	TB (µmol/L)	MDA (µM)	TP (g/L)	ALB (g/L)
I No CCl_4 administered	8.21 ± 0.53	2.16 ± 1.32	30.89 ± 2.25	4.85 ± 0.80	0.091 ± 0.012	57.36 ± 1.32	32.13 ± 1.44
II No white grub extract administered	30.45 ± 2.05 [a, b, c]	72.81 ± 5.17 [d, e, f]	66.18 ± 2.27 [g, h, i]	10.37 ± 0.90 [j, k, l]	0.37 ± 0.01 [m, n, o]	64.46 ± 0.07 [p, q, r]	33.24 ± 0.86 [s, t, u]
III 1.0g/Kg white grub extract administered	29.86 ± 1.06 [a]	70.56 ± 4.44 [d]	67.46 ± 0.49 [g]	11.46 ± 0.65 [j]	0.33 ± 0.01 [m]	56.62 ± 0.71 [p]	30.95 ± 0.29 [s]
IV 2.0g/Kg white grub extract administered	25.29 ± 1.54 [b]	52.21 ± 1.32 [e]	58.20 ± 1.24 [h]	9.71 ± 1.66 [k]	0.29 ± 0.01 [n]	63.97 ± 1.72 [q]	31.85 ± 1.82 [t]
V 4.0g/Kg white grub extract administered	21.57 ± 2.04 [c]	51.13 ± 0.85 [f]	63.79 ± 3.34 [i]	10.50 ± 0.98 [l]	0.26 ± 0.01 [o]	64.82 ± 1.55 [r]	32.21 ± 0.27 [u]

Values in the same column bearing similar superscript are significantly different at $p < 0.05$, n = 5

Table 3: Serum ALT, AST and ALP activities and TB, MDA, TP and ALB levels in rats after oral administration of white grub extract for 6 days.

GROUP	ALT (U/L)	AST (U/L)	ALP (U/L)	TB (µmol/L)	MDA (µM)	TP (g/L)	ALB (g/L)
I No CCl_4 administered	8.19 ± 0.48	2.06 ± 1.23	32.50 ± 2.13	4.96 ± 0.70	0.082 ± 0.011	58.36 ± 1.31	33.18 ± 1.54
II No white grub extract administered	28.08 ± 0.07 a, b, c	71.31 ± 2.12 d, e, f	63.51 ± 1.86 g, h, i	14.06 ± 1.73 j, k, l	0.28 ± 0.01 m, n, o	62.54 ± 1.44 p, q, r	30.91 ± 0.01 s, t, u
III 1.0g/Kg white grub extract administered	20.29 ± 1.90 [a]	56.16 ± 6.42 [d]	71.03± 0.76 [g]	13.01 ± 0.51 [j]	0.21 ± 0.01 [m]	63.86 ± 1.09 [p]	31.57 ± 0.23 [s]
IV 2.0g/Kg white grub extract administered	16.47 ± 1.81 [b]	31.28 ± 0.81 [e]	58.90± 2.01 [h]	9.01 ± 0.06 [k]	0.18 ± 0.01 [n]	65.13 ± 0.31 [q]	30.99 ± 0.39 [t]
V 4.0g/Kg white grub extract administered	15.28 ± 0.22 [c]	28.94 ± 0.57 [f]	66.81± 0.41 [i]	11.94 ± 0.46 [l]	0.09 ± 0.02 [o]	62.32 ± 0.75 [r]	30.08 ± 0.42 [u]

Values in the same column bearing similar superscript are significantly different at $p < 0.05$, n = 5

Table 4: Serum ALT, AST and ALP activities and TB, MDA, TP and ALB levels in rats after oral administration of white grub extract for 9 days

GROUP	ALT (U/L)	AST (U/L)	ALP (U/L)	TB (µmol/L)	MDA (µM)	TP (g/L)	ALB (g/L)
I No CCl_4 administered	8.16 ± 0.59	2.16 ± 1.33	32.60 ± 4.13	4.96 ± 0.90	0.082 ± 0.011	59.56 ± 1.42	34.18 ± 1.53
II No white grub extract administered	25.88 ± 1.59 a, b, c	62.04 ± 0.77 d, e, f	60.72± 0.56 g, h, i	10.26 ± 1.36 j, k, l	0.18 ± 0.03 m, n, o	58.66 ± 0.72 p, q, r	28.67 ± 0.67 s, t, u

III 1.0g/Kg white grub extract administered	18.87 ± 0.36 [a]	49.84 ± 1.56 [d]	61.72± 1.93 [g]	12.07 ± 0.07 [j]	0.09 ± 0.021 [m]	63.92 ± 0.97 [p]	31.91 ± 0.59 [s]
IV 2.0g/Kg white grub extract administered	14.76 ± 0.29 [b]	27.62 ± 0.48 [e]	60.95± 1.16 [h]	10.15 ± 1.04 [k]	0.08 ± 0.01 [n]	64.03 ± 0.31 [q]	32.41 ± 0.36 [t]
V 4.0g/Kg white grub extract administered	14.36 ± 1.74 [c]	25.94 ± 0.75 [f]	62.11± 1.36 [i]	11.09 ± 0.01 [l]	0.03 ± 0.02 [o]	60.05 ± 0.05 [r]	31.37 ± 0.41 [u]

Values in the same column bearing similar superscript are significantly different at $p < 0.05$, $n = 5$.

Discussion

Rats injected with 120 mg/kg CCl_4 had significantly higher ($p<0.05$) serum AST, ALT, ALP, TB MDA and ALB than the normal rats (Table1). It is therefore, indicating inducement of acute liver toxicity in the injected rats, it agrees with the report by Price and Stevens (2003) and work of Alhassan et al (2009b).

In phase I of this work (Table 2) rats treated with daily dose of 1g/kg, 2g/kg and 8g/kg aqueous white grubs extract for 3 days showed serum ALT, AST, MDA and ALB not significantly higher ($p>0.05$) than the control group. This is an indication of possible fibrosis, the initial repair mechanism of liver injury, as indicated by Burtis et al. (2001) that all cellular damage induces fibrosis as a healing response. However, the healing effect is more pronounced in groups that received daily doses of 8g/kg and 2g/kg for 3 days compared to group III rats which received a daily dose of 1g/kg; this may be possibly due to low serum bioavailability of the extract. It may be inferred that the extract facilitates the fibrosis.

In phase II (Table 3) rats in group V, IV and III, treated with different concentration of the white grubs' extract for six days had mean serum activities of ALT and AST and levels of TB and MDA significantly lower ($p<0.05$) than the control. This shows possible hepatocytes curative effect of the white grubs' extract on the damaged due to CCl_4. possibly by influencing fibrosis and collagen synthesis as indicated by Keith and Robert (2001) that the liver response to injury is hepatocytes regeneration and collagen formation. The observed hepatocurative effect of the white grubs' extract could be associated with proteins, fats, and mineral elements especially iron (Fe) and copper (Cu) (Alhassan et al., 2009a) and humic substances contents of white grubs (Alhassan, 2010).

From Table 4 (phase III), rats treated with daily doses of 1.0g/kg, 2.0g/kg and 8.0g/kg for nine days, had serum activity of ALT and AST and levels of MDA not significantly higher ($P<0.05$) than the control, and the improvement in the liver was more in group IV and V compared to group III.

The possible hepatocurative effects of white grubs extract could be attributed to the chemical composition of white grub. White grub (WG) is rich in fats, protein, some mineral elements (Alhassan et al., 2009a) and humic substances (fulvic and humic acids) (Alhassan, 2010). WG fats may contain phosphatidyl choline, vitamins A and E which are good natural antioxidants, which may role in hepatocurative effect of the grubs. The protein of white grub may contain some amino acids (glycine, lysine, proline and methionine) that favour collagen biosynthesis which is critical in healing response by the hepatocytes. Collagen consists of a dextrorotatory triple helix made up of three polypeptides (α-chains). The triplet Gly-X-Y is constantly repeated in the sequence of the triple-helical regions— i. e., every third amino acid in such sequences is a glycine. Proline (Pro) is frequently found in positions X or Y; the Y position is often occupied by 4-hydroxyproline (4Hyp), although 3-hydroxyproline (3Hyp) and 5-hydroxylysine (5Hyl) also occur. These

hydroxylated amino acids are characteristic components of collagen. The hydroxyproline residues stabilize the triple helix by forming hydrogen bonds between the α-chains, while the hydroxyl groups of hydroxylysine are partly glycosylated with a disaccharide (–Glc–Gal) (Koolman and Roehm. 2005). Choline dihydrogen citrate and DL- methionine are used in pharmaceutical preparation indicated for hepatitis, alcoholic hepatitis and drug induced hepatotoxicity. The presence of Fe and Cu also have roles in hepatic healing being required by lysine and proline hydroxylase respectively, the activities of these enzymes are required for maturation of collagen.

Humic acids seem to accelerate cell metabolism, the rate of breakdown of glucose, leucine and uridine. Humic acids seem to retard the rate of incorporation of these organic molecules into the liver, but once they are absorbed, humic acids appear to accelerate their metabolism (Visser, 1973), thereby reducing metabolic burden on the hepatocytes. That may encourage healing of the injured hepatocytes as it has been part of management of hepatitis to reduce fatty and protein rich diet. Humic acids can apparently stimulate respiration and increase the efficiency of oxidative phosphorylation in rat liver mitochondria (Visser, 1987). Cellular respiration, occurring only in the presence of oxygen, results in the breakdown of nutrient molecules to generate ATP. Cells such as the liver and muscle use this ATP for energy to fuel various processes like stimulating the uptake of nutrients, repair of dead of damage tissue (Nelson and Cox 2006)). This may be due to their acidic functional groups, primarily carboxylic acid and phenolic hydroxyl groups, which give them the capacity to react with various species such as free radicals, minerals and biological enzyme systems (Frimmel and Christman, 1988; Aiken et al., 1985; Shils and Shike, 1994). Both non-enzymatic and enzymatic antioxidants serve as defense to cope with oxygen-free radicals. The former includes a wide variety of such compounds as alfa-tocopherol (vitamin E), betacarotene, and ascorbic acid (vitamin C), whereas the latter includes the scavenging enzymes; superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). They form an important defense system to ensure that cytotoxic oxygen species are degraded to less harmful compounds so that extensive cell damage does not occur (De La Torre et al., 1999). White grubs being rich in fats and proteins, may contain some of these antioxidants and its hepatocurative effects may be via some of them.

Conclusion

The study has shown that white grubs extract has hepato curative effect even at the dose of 1.0 g/Kg body weight in six days of administration. This may be attributed to chemical composition of white grubs, especially the high fats, protein (rich in proline and lysine), humic acids, fulvic acids, and Fe and Cu contents. The presence of proline and lysine that are very important components of collagen may have played significant role. The contents of humic and fulvic acid may also have roles in the hepato curative effects of white grubs extract, because of their antioxidant property, metabolic roles, decreasing loads on hepatocytes.

References:

Ahmed, I., Agil, F. and Owais M. (2006). Modern Phytomedicine. *Turning Medicinal Plant into Drugs.* John Wiley and Sons, West – Sussex England. Pp. 2-24.

Aiken, G., McKnight, D. and Wershaw, R. (1985). *Humic Substances in Soil, Sediment, and Water*. Vol. 1.Jon Wiley: New York.

Alhassan, A. J., Sule, M. S., Hassan, J. A., Baba, B. A. and Aliyu, M. D. (2009a). Proximate and elemental composition of white grubs. *Bayero Journal of Pure and Applied Sciences* 2(2): 188 – 190.

Alhassan, A. J., Sule, M. S., Hassan, J. A., Baba, B. A. and Aliyu, M. D. (2009b). Ideal Hepathotoxicity Model in Rats using Carbon tetrachloride (CCl_4). *Bayero Journal of Pure and Applied Sciences* 2(2): 185 – 187.

Alhassan, A. J. (2010). Medicinal and Toxicological Effect of Aqueous of White Grubs and Characterization of its Protein and Lipid Contents. Unpublished manuscript, PhD Thesis Department of Biochemistry, Bayero University, Kano.

Burtis C. A., Ashwood E.R. and Boder B. G., (2001). *Tietz Fundamentals of Clinical*

Chemistry 5th ed. W.B Saunders Company. Pp 352 -770.
Chawla, R. (1999). *Practical Clinical Biochemistry* (Methods and Interpretations).Second edition. Jaypee Brothers Medical Publishers, New Delhi, India. Pp .106 - 118.
De La Torre, R., Casado, A. Lo´ Pez-Ferna´ Ndez, M.E., Carrascosa, D. and Venarucci D. (1999). Superoxide Dismutase Activity Levels in a Spanish Population 50–93 Years *Am. J. Hu. Biol*. 11:45–47.
Fansworth N. R., and Soejarto, D.D. (1985). Potential Consequence of Plants Extinction in the United State on the Current and Future Availability of Prescribed Drugs. *Econ. Bol.* 39(2): 231 – 240.
Frimmel, F.H. and Christman, R. F. (1988). Humin Substances and Their Role in the Environment. 1st ed. Vol.1., Wiley-Interscience Publication, New York. Pp 60 – 123.
Gutteridge, J. M. C. and Wilkins, C. (1982). Copper – dependent hydroxyl radical damage to ascorbic acid. Formation of a thiobarbituric acid reactive products. *FEBS lett.* 137: 137 – 327.
Hostettmann, K., Marston, A., Ndjoko, K. and Wolffender, J. (2000). The Potential of African Plants as a Source of Drug. *Curr. Org. Chem.* 4: 973 – 1010.
Hunter, F.E., Gebicki, J.M., Hoffstein, P.E., Weinstein, J. and Scott, A. (1963). Swelling and lysis of rat liver mitochondria induced by ferrous ions. *J. Biol. Chem.* 238:828-835.
Keith, G. T. and Robert, R. E. J. (2001). Liver Function In: *Tietz Fundamentals of Clinical Chemistry.* 5th ed. W.B Saunders Company. Pp. 747 -770.
Koolman, J. and Roehm, K.H. (2005) Collagen In: *Color Atlas of Biochemistry* 2nd ed. Thieme Stuttgart, New York. P 344.
MacSwean, R.N.M. (1980). Liver, Biliary Tract and Exocrine phancrease. In: *Murs textbook of pathology.* (Ed J.R. Anderson). Edword Arnold publishers Ltd., London. Pp. 663- 654
Malloy H.T., and Evolyn K.A. (1937). Recommendation on a uniform Bilibirun standard *J. Biol. Chem.,* 119: 481.
Nelson, D. and Cox, M. (2006). *Lehninger Principles of Biochemistry*. 4nd ed, Worth Publishers. New York: Pp.481 - 488.
Price, N. C. and Stevens, L. (2003). *Fundamental of Enzymology*, 3rd ed. Oxford University Press, Oxford. Pp. 404 – 406.
Rec, G.S.C.C. (1972). Colorimetric method for serum Alkaline Phosphatase Determination. *J. Clin. Biochem*. 10(2): 182.
Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum Glutamic Oxaloacetate and Glutamic Pyruvic transaminases. *Amer.J.Clin.Path*. 28: 56 – 61.
Robbins, S. L. and Cotran, R. S. (2006). Cellular Adaptations, Cell Injury, and Cell Death In: R.N. Mitchell, V. Kumar, A.K. Abbas and N. Fausto (Eds.), *Robbins and Cotran Pathologic Basis of Disease. 7th* ed. Saunders, Philadelphia. Pp. 34-36, 48.
Roderick, H., Macseen, M. and Keith W. (1998) In: *Muins Text Book of Pathology*, 13th ed. ESPBS, London Pp 750-763.
Shils, O., and Shike, E. D. (1994). *Modern Nutrition in Health and Disease*, 8th ed., Vol. 2. Williams and Wilkin, Baltimore. Pp 30 – 65.
Sule, M. S., Shehu, D. and Aminu, B. M. (2006). Efficacy of *Guiera seneglensis* leaf Extract against liver diseases in Guinea pigs. *Biol. Environ. Sci*. J. Tropics 3(3): 169 – 171.
Tapsell, I. (2006): *Health Benefits of Herbs and Spices*. Pp. 141-160.
Visser, S.A. (1973), some biological effects of humic acids in the rat. *Acta Biologica et Medica Germanica*, 31: 569-581.
Visser, S.A. (1987). Effect of humic substances on mitochondiral respiration and oxidative phosphorylation. *The Science of the Total Environment*. 62: 347-354.