

RESEARCH ARTICLE

Effect of Aqueous Administration of White Grub and Waste Extract on the Levels of Liver and Kidney Indices in Diabetic Rats

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ABSTRACT

Introduction: The liver plays a major role in the regulation of carbohydrate metabolism, as it uses glucose as a fuel and kidneys are to excrete metabolic waste products as well as to maintain water, pH, electrolyte balance, production of calcitriol, and hemopoietin. **Aim:** This study aims to investigate the effect of the administration of white grub and waste on liver and kidney indices on diabetic rats. **Materials and Methods:** The rats were induced with diabetes by alloxanization and treated with the extracts of white grub and waste for 2 weeks. A total of 25 rats used, were randomly distributed into five groups (G1-G5) each with five rats. G1 served as normal control. G2-G5 served as diabetic control. At the end of the 1st week of extract administration, two animals from each group were randomly selected and sacrificed. At the end of the 2nd week, the remaining three animals from each group were also sacrificed and serum was collected for the determination of liver function indices (serum alkaline phosphatase [ALP], alanine aminotransferase [ALT], aspartate aminotransferase [AST] total bilirubin [TB], direct bilirubin [DB], total protein [TP], albumin [ALB], and globulin [GLB]) and kidney function parameters (urea, creatinine, and electrolyte [sodium “Na,” potassium “K,” bicarbonate “HCO₃,” and chloride “Cl”]). **Results:** After the 1st week, the extract-treated group (G4 and G5) showed significant reductions of ALP, ALT, AST, TP, GLB, and ALB while TB and DB have normal value compared to diabetic untreated group and for renal function (G4 and G5) showed significantly lower levels of urea, Na, K, HCO₃, creatinine, and Cl. After the 2nd week, the extract-treated group showed significant reductions of ALP, ALT, AST, DB, TP, ALB, and TB with significant increased levels of GLB and TP compared to diabetic untreated group (G2). G4 (extract treated) showed significantly ($P < 0.05$) lower levels of urea, Na, Cl, HCO₃, and creatinine and with significant increased K levels compared to G2. G5 also extract-treated group indicates significant lower levels of urea, Cl, Na, and HCO₃ and higher levels of creatinine and K compared to G2. **Conclusion:** These results suggest that the administration of aqueous extract of white grub and waste did not have any adverse effect on the liver and kidney functions in diabetic rats. The extracts have positive effect which showed that G4 (treated with whole white grub [WG]) is more effective compared to G5 (treated with WG waste).

Keywords: Liver disease, renal failure, waste, white grub

INTRODUCTION

White grub is the larval stage of many species of beetle. Dung beetles play a crucial role by burying

dung in natural savannah and grassland used for cattle grazing in Africa. In addition to their effect on plants, other species biodegrade environmental wastes. 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

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poisons, lesion of biliary tract, certain viral infections, hypoxia, and tumors. The liver plays a major role in the regulation of carbohydrate metabolism, as it uses glucose as a fuel, it has the capability to store glucose as glycogen and also synthesize glucose from non-carbohydrate sources. This key function of liver makes it vulnerable to diseases in subjects with metabolic disorders, particularly diabetes. Increased activities of liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ -glutamyltranspeptidase are indicators of hepatocellular injury. Increased activity of these markers is associated with insulin resistance, metabolic syndrome, and Type 2 diabetes. An association exists between diabetes and liver injury.^[1-8]

In diabetes, the cells do not receive glucose and most of it is accumulated in the blood. Too much sugar in the blood can lead to serious health problems, including heart disease and damage to the nerves and kidneys. Failing to control diabetes can give rise to many complications. Diabetic kidney disease takes many years to develop. The kidneys excrete metabolic waste products and the serum concentrations are regulated from variety of substances. At certain level of renal failure, the following routinely measured substances often become abnormal and the extent of the abnormality generally depends on the severity of the disease. Serum creatinine, urea, and electrolyte concentrations change inversely with changes in chronic renal failure (glomerular filtration rate [GFR]) and are, therefore, useful in gauging the degree of renal dysfunction. The hypoglycemic and hypolipidemic properties of this extract have earlier been shown. It is important to investigate the effects of this grub on liver and kidney functions in diabetic rats so as to ascertain its safety. Therefore, this study aimed to evaluate the liver and kidney functions in diabetic experimental rats.^[9-12]

MATERIALS AND METHODS

Collection and identification of white grub

White grubs were collected by hand picking at dump site in Federal University Dutsin-Ma livestock farm, Dutsin-Ma Local Government, Katsina State. The grubs were identified and then clean thoroughly to remove all the dirt using distilled water. It was

authenticated at the Department of Biochemistry and Molecular Biology, Federal University Dutsin-Ma, Katsina State by Mr. S.S Said.

Preparation of the white grub extract

A 60 g of grub extract were weighed and poured into 500 ml conical flask with 250 ml of distilled water. The mixtures were kept for 12 h with constant agitation at 30 min interval. The extract was filtered using cheesecloth into a measuring cylinder. The filtrate was dried in an oven at 80°C. The residue was weighed and the difference between the extract weight and the initial weight of the grub gives the weight of the known volume of extract. The semi-solid extract was stored in the refrigerator for further use.

Experimental animal

Twenty-five rats weighing 70–120 g were purchased from the Department of Biological Science, Bayero University, Kano State, Nigeria. The rats were allowed to acclimatize for 2 weeks. The rats were fed with starter mesh with full access to pure water. They were kept in a well-ventilated cage at the animal facility in Federal University Dutsin-Ma, Katsina State.

Animal groupings and treatment

The induction of alloxan was done intraperitoneally with 100 mg/kg body weight of the alloxan and diabetes was confirmed after 72 h. Twenty-five Wistar albino male rats were assigned into five different groups with five rats in each of the group. Group 1, normal control fed with feed and distilled water. Group 2, diabetic untreated fed with feed and distilled water. Group 3, diabetic rats orally administered with glibenclamide “5 mg/kg.” Group 4, diabetic orally administered with extract of white grub 100 mg/kg. Group 5, diabetic rats orally administered with white grub waste 100 mg/kg.

The rats were fasted for 18 h and all the rats from each group were anesthetized with chloroform and blood was collected by cutting the jugular vein of the animals, the whole blood was collected into

heparin sample bottles and centrifuged to obtain the serum which was used for analysis.^[13-17]

Biochemical analyses

Serum was separated and analyzed for ALT and AST activities by the method of Reitman and Frankel (1957), serum alkaline phosphatase (ALP) activity by the method of Rec (1972), total bilirubin (TB) (Malloy and Evelyn, 1937), and serum total protein (TP) and albumin (ALB) by the method of Chawla (1999).^[18-20]

Determination of serum creatinine: Principle (Jaffe's method, 1964)

Creatinine reacts with picrate ion in an alkaline medium to give an orange-red color complex. The alkaline medium is 0.75 N NaOH.^[21]

Determination of serum electrolyte

Serum sodium (Na) and potassium (K⁺) were determined using flame photometry method, while chloride (Cl⁻) and bicarbonate (HCO₃⁻) were determined by titration.

Serum chloride: Principle

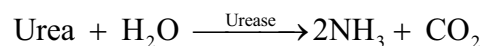
Chloride in serum was precipitated out with standardized mercuric nitrate; the excess mercuric ion reacts with an indicator diphenylcarbazone to produce a violet color.

Serum bicarbonate: Principle

Hydrochloric acid (HCl) reacts with sample bicarbonate and liberates carbon dioxide, the excess hydrochloric acid was then titrated with sodium hydroxide using phenol red as an indicator.

Determination of serum urea: Principle (Urease-Berthelot method, 1977)

Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia was then measured photometrically by Berthelot's reaction.^[22]



NH₃+hypochlorite+phenol→indophenol (blue compound)

Statistical analysis

The experimental results obtained are expressed as mean ± standard deviation. The data were subjected to one-way analysis of variance and differences between samples were determined by Tukey multiple comparison tests using the SPSS version 16.0 program.

RESULTS

After the 1st week, the extract-treated group (G4 and G5) showed significant reductions of ALP, ALT, AST, TP, GLB, and ALB while TB and DB have normal value compared to diabetic untreated group and for renal function (G4 and G5) showed significantly lower levels of urea, Na, K, HCO₃, creatinine, and Cl. After the 2nd week, the extract-treated group showed significant reductions of ALP, ALT, AST, DB, TP, ALB, and TB with significant increased levels of GLB and TP compared to diabetic untreated group (G2). G4 (extract treated) showed significantly ($P < 0.05$) lower levels of urea, Na, Cl, HCO₃, and creatinine and with significant increased K levels compared to G2. G5 also extract-treated group indicates significant lower levels of urea, Cl, Na, and HCO₃ and higher levels of creatinine and K compared to G2.

DISCUSSION

After the 1st week of treatment with standard drug, WG and waste extracts "100 mg/kg" [Table 1] revealed that the alloxan-induced rats had significantly higher ($P < 0.05$) serum ALP, ALT, AST, TP, and ALB and significantly lower ($P < 0.05$) TB, direct bilirubin (DB), and globulin (GLB) when compare to normal control (G1). G3 glibenclamide-treated group showed significant lower levels of ALP, AST, ALT, TB, TP, and ALB and significant higher levels of GLB with normal value of DB compared to diabetic untreated group.

Table 1: Liver function indices of rats administered with same dose of white grub and waste extract after 7 days

Group treatment	Alkaline phosphatase (U/L)	Alanine aminotransferase (U/L)	Aspartate aminotransferase (U/L)	Total bilirubin ($\mu\text{mol/L}$)	Direct bilirubin ($\mu\text{mol/L}$)	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)
G1	120.00 $\pm$ 11.00 ^a	50.00 $\pm$ 2.00 ^a	44.00 $\pm$ 2.00 ^a	5.0 $\pm$ 0.0 ^a	3.0 $\pm$ 0.5 ^a	93.00 $\pm$ 6.00 ^a	35.50 $\pm$ 0.50 ^a	72.50 $\pm$ 8.50 ^a
G2	421.50 $\pm$ 98.50 ^b	94.50 $\pm$ 7.50 ^b	209.50 $\pm$ 5.50 ^b	4.0 $\pm$ 0.0 ^b	2.0 $\pm$ 0.0 ^b	96.50 $\pm$ 6.50 ^a	37.00 $\pm$ 0.00 ^a	52.50 $\pm$ 0.50 ^b
G3	118.00 $\pm$ 18.00 ^c	44.50 $\pm$ 1.50 ^c	59.00 $\pm$ 3.20 ^c	3.0 $\pm$ 0.0 ^c	2.0 $\pm$ 0.0 ^b	90.50 $\pm$ 0.50 ^a	33.00 $\pm$ 2.00 ^a	56.50 $\pm$ 5.50 ^b
G4	209.50 $\pm$ 18.00	74.30 $\pm$ 2.50 ^a	66.00 $\pm$ 3.00 ^c	4.0 $\pm$ 0.0 ^b	2.0 $\pm$ 0.0 ^b	71.00 $\pm$ 15.00 ^b	33.00 $\pm$ 1.00 ^a	43.00 $\pm$ 9.00 ^c
G5	255.50 $\pm$ 10.50	84.50 $\pm$ 0.50 ^b	124.00 $\pm$ 9.00	4.0 $\pm$ 0.0 ^b	2.0 $\pm$ 0.0 ^b	80.00 $\pm$ 0.00 ^b	33.00 $\pm$ 0.00 ^a	47.00 $\pm$ 0.00 ^c

Values are mean $\pm$ standard deviation of two determinations. The different superscript letters mean significant difference ($P < 0.05$) along the column

Table 2: Kidney function indices (in mmol/L) of rats administered with same doses of white grub and waste extract after 7 days

Group treatment	Urea	Na	K	HCO ₃	Cl ⁻	Creatinine
G1	7.1 $\pm$ 0.10 ^a	146.5 $\pm$ 0.50 ^a	10.1 $\pm$ 1.30 ^c	19.0 $\pm$ 1.0 ^a	97.0 $\pm$ 1.0 ^a	71.0 $\pm$ 0.00 ^a
G2	7.35 $\pm$ 0.70 ^a	141.5 $\pm$ 9.50 ^a	9.0 $\pm$ 1.50 ^b	21.5 $\pm$ 0.5 ^b	97.5 $\pm$ 2.5 ^a	72.0 $\pm$ 0.00 ^a
G3	9.0 $\pm$ 2.00 ^c	151.5 $\pm$ 2.50 ^c	6.15 $\pm$ 2.70 ^a	19.5 $\pm$ 1.5 ^a	96.5 $\pm$ 2.5 ^a	68.0 $\pm$ 2.00 ^b
G4	5.7 $\pm$ 0.40 ^b	140.5 $\pm$ 1.50 ^b	7.05 $\pm$ 0.25 ^a	18.5 $\pm$ 1.5 ^a	97.0 $\pm$ 1.0 ^a	60.5 $\pm$ 2.50 ^c
G5	6.65 $\pm$ 0.05 ^a	147.5 $\pm$ 0.50 ^a	9.60 $\pm$ 0.00 ^b	21.5 $\pm$ 0.5 ^b	93.5 $\pm$ 0.5 ^b	69.0 $\pm$ 1.00 ^b

Values are mean $\pm$ standard deviation of two determinations. The different superscript letters mean significant difference ($P < 0.05$) along the column. Na: Sodium, K: Potassium, HCO₃: Bicarbonate, Cl: Chloride

The extract-treated group (G4 and G5) showed significant reductions of ALP, ALT, AST, TP, GLB, and ALB while TB and DB have normal value compared to diabetic untreated group. The renal parameters during the 1st week of treatment [Table 2] had significantly ($P < 0.05$) elevated levels of urea, bicarbonate, creatinine, and chloride with significantly ($P < 0.05$) reduced levels of sodium and potassium compared to normal control group. Reference drug-treated group indicated significantly ($P < 0.05$) higher levels of urea and Na and lower levels of K, HCO₃, Cl, and creatinine compared to diabetic untreated group (G2). G4 (extract treated) showed significantly ($P < 0.05$) lower levels of urea, Na (sodium), K (potassium), HCO₃ (bicarbonate), creatinine, and chloride (Cl) and also extract-treated group (G5) indicated significantly lower levels of urea, Cl, and creatinine and significantly higher levels of Na and K while HCO₃ normalized compared to G2. G4 extract-treated group showed much reduction compared to G5 extract-treated group during the 1st week of treatment.

After the 2nd week of treatment [Table 3], the diabetic rats have significantly elevated levels of ALP, AST, DB, TP, ALB, and GLB while there was a significant ($P < 0.05$) decreased level of ALT and TB showed normal level compared to non-diabetic control group. G3 glibenclamide-

treated group showed significant lower levels of ALP, AST, ALT, DB, TP, ALB, and GLB and with normal value of DB compared to diabetic untreated group (G2). The extract-treated group (G4 and G5) showed significant reductions of ALP, ALT, AST, DB, TP (G4), ALB (G4), and TB (G5) with significant increased levels of GLB, TP (G5), and ALB (G5) while TB (G4) had normal value compared to diabetic untreated group (G2). Furthermore, G4 (treated with whole WG) is more effective compared to G5 (treated with WG waste). The renal function parameters during the 2nd week of treatment [Table 4] had significantly ($P < 0.05$) elevated levels of urea, HCO₃, creatinine, and Cl with significantly ($P < 0.05$) reduced levels of Na and K compared to G1. Reference drug-treated group indicated significantly ($P < 0.05$) lower levels of urea, Na, K, HCO₃, Cl, and creatinine compared to G2. G4 (extract treated) showed significantly ($P < 0.05$) lower levels of urea, Na, Cl, HCO₃, and creatinine and with significant increased K levels compared to G2. G5 also extract-treated group indicated significantly lower levels of urea, Cl, Na, and HCO₃ and significantly higher levels of creatinine and K compared to G2. During the 2nd week of treatment, G4 administered (WG 100 mg/kg b.w) showed much reduction compared to G5 administered (WG waste 100 mg/kg b.w).^[23-25]

Table 3: Liver function indices of rats administered with same dose of white grub and waste extract after 14 days

Group treatment	Alkaline phosphatase (U/L)	Alanine aminotransferase (U/L)	aspartate aminotransferase (U/L)	Total bilirubin (umol/L)	Direct bilirubin (umol/L)	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)
G1	111.00±2.50 ^a	139.00±5.20 ^a	45.00±2.50 ^a	4.0±0.0 ^a	2.0±0.5 ^a	84.67±1.76 ^a	32.00±1.15 ^a	52.00±1.15 ^a
G2	422.50±8.50 ^b	94.50±7.50 ^b	222.50±45.50 ^b	4.0±0.0 ^a	2.5±0.0 ^a	91.66±1.86 ^b	34.67±1.45 ^a	57.67±0.88 ^a
G3	110.00±8.20 ^a	34.50±0.50 ^c	49.00±2.20 ^a	4.0±0.0 ^a	2.0±0.0 ^a	78.00±1.52 ^c	26.00±1.54 ^b	52.33±2.60 ^a
G4	112.50±8.20 ^a	36.50±2.50 ^c	45.00±1.00 ^a	4.0±0.0 ^a	2.0±0.0 ^a	89.67±3.84 ^a	33.33±1.33 ^a	60.00±1.73 ^b
G5	198.50±0.50 ^a	83.50±0.50 ^b	121.00±5.00	3.5±0.0 ^b	2.0±0.0 ^a	94.00±2.31 ^b	35.00±2.32 ^a	65.00±0.57 ^b

Values are mean±standard deviation of three determinations. The different superscript letters mean significant difference ($P<0.05$) along the column

Table 4: Kidney function indices (in mmol/L) of rats administered with same doses of white grub and waste extract after 14 days

Group treatment	Urea	Na	K	HCO ₃	Cl ⁻	Creatinine
G1	6.7±0.05 ^a	140.0±1.15 ^a	13.0±0.29 ^a	25.7±0.33 ^a	96.0±1.15 ^a	77.0±1.15 ^a
G2	12.8±1.03 ^b	138.6±0.33 ^b	11.1±0.53 ^b	26.7±0.89 ^a	97.0±1.15 ^a	73.3±1.76 ^a
G3	5.7±0.20 ^b	122.3±2.02 ^c	9.13±0.03 ^c	22.0±1.15 ^a	88.0±1.15 ^b	63.0±1.15 ^c
G4	9.7±3.03 ^c	130.6±6.06 ^b	12.6±1.78 ^{ac}	20.0±0.00 ^a	96.3±0.33 ^a	70.3±1.45 ^a
G5	11.4±0.47 ^c	132.0±1.15 ^b	11.5±0.55 ^b	22.0±0.58 ^a	96.3±0.88 ^a	78.0±1.52 ^a

Values are mean±standard deviation of two determinations. The different superscript letters mean significant difference ($P<0.05$) along the column

White grubs' extract has hepatocurative effect even at the dose of 1.0 g/kg body weight in 6 days of administration (Alhassan and Sule, 2012). 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 hepatocurative effects of white grubs' extract, because of their antioxidant property, particularly metabolic role thus, decreasing load on hepatocytes.

The fact that the levels of the enzymes were maintained in the liver and kidney in all groups of the rat means that the administered extract has no membrane labilizing effect on these organs. Enzyme activities in the tissues are often used as "marker" to ascertain early toxic effects of administered foreign compounds to experimental animals (Akanji and Ngaha, 1989; Adesokan and Akanji, 2004). ALP is a membrane-bound enzyme while ALT and AST are cytosolic enzymes. These enzymes are highly concentrated in the liver and kidney and are only found in the serum in significant quantities when the cell membrane becomes leaky and even completely ruptured (Cotran *et al.*, 1989; Ngaha, 1981). Urea is formed by the liver as an

end product of protein breakdown and is one marker of the kidney function (Manivannan *et al.*, 2015). An increase in serum urea observed here might be due to impairment in its synthesis as a result of impaired hepatic function and/or due to disturbance in protein metabolism (Manivannan *et al.*, 2015, Manjunatha *et al.*, 2011). Creatinine is a waste product that is normally filtered from the blood and excreted with the urine. Higher creatinine levels in diabetic patients may be related to disturbance of kidney function (Manivannan *et al.*, 2015). In addition, the observed increases in urea and creatinine may be explained on the basis of glomerular hyperfiltration due to increase creatinine clearing from blood (Varghese *et al.*, 2001). Serum creatinine and urea are established markers of GFR. Although serum creatinine is a more sensitive index kidney function compared to urea level. This is because creatinine fulfills most of the requirements for a perfect filtration marker.

CONCLUSION

The result of the study indicated the positive effect of the administration of aqueous white grub and waste extract on liver and kidney functions in experimental rats. Thus, its continued usage as traditional medicine is recommended.

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