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Characterization and Antidiabetic Activity of Aqueous Extract of Whole White Grub and Waste in Alloxan Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, action or both, resulting in impaired glucose, lipids and proteins metabolism. The aim of this study was to investigate the effect of administration of aqueous White Grub and Waste extract on alloxan-induced diabetic rats. Diabetes was induced in rats after 72 hours of fasting intraperitoneally by alloxan (100 mg/kg). A total of 30 rats were used. The rats were divided into five groups (GA-GE) of six rats each. GB-GE was induced with diabetes. GA served as normal control, GB were administered only distilled water and GC were administered the standard anti-diabetic drug, Glibenclamide '5 mg/kg', while GD (WGE, 100 mg/kg), GE (WE, 100 mg/kg) were respectively administered. After eighteen (18) days of oral administration of the extracts, the animals were sacrificed and the serum was collected for analysis of lipid profile (Total Cholesterol, 'TC', Low Density Lipoprotein-Cholesterol, 'LDL-C', High Density Lipoprotein-Cholesterol, 'HDL-C', Very Low Density Lipoprotein, 'VLDL' and Triglycerides, 'TG'). In the first week treatment the extract-treated group showed elevated level of TC, TAG, LDL, HDL (WGE), VLDL (WE) and significant decreased ($p < 0.05$) VLDL (WGE) and HDL (WE) while in the second week treatment the WG extract treated group showed significant decreased ($P<0.05$) serum level of TC, TAG, LDL cholesterol, VLDL and HDL cholesterol normalized significantly while increased levels of TC, TAG, VLDL, LDL, and decreased HDL cholesterol were observed in WE treated group compared to diabetic untreated control group. WGE showed similar effect compared to the reference drug as the blood glucose levels returned to normal after 18 days of oral administration. Percentage increase and decreased

blood glucose (BG) levels and lipid profile were evaluated for 18 days of oral administration of the extracts. The Fourier Transform Infrared (FTIR) analysis of the most active extract showed that the compounds may contain the functional groups like–CO, Nitro, -COOH, an aromatic ring, and other compounds, while its Gas chromatography-Mass spectroscopy (GC-MS) analysis may contain carbonic acid, fatty acids, carbohydrate and other compounds. This result showed a positive effect of the Whole White Grub extracts in the management of Diabetes mellitus (DM) and lipid abnormalities may be associated with the presence of the compounds and functional groups.

KEYWORDS: White Grub, Diabetes mellitus, lipids, Glibenclamide

INTRODUCTION

White grubs (WG) are the larval stage of beetles' metamorphosis commonly found in dump refuse and animal dung, feeding on plants and animal remains [1, 2]. In Africa, the species are widely distributed: it is found in Nigeria, Niger Republic, Uganda, etc. Though, WG is seen and/or presented in the world's field of science as more or less a pest, with little or no positive economic importance, recent researches and discoveries indicated that WG are rich in protein, fats and mineral elements [1]. It is used among communities as food and as medicine among the Hausa/Fulani in Northern Nigeria [1, 2]. In South Western Nigeria, edible insects are consumed as food and source of nutrients. Among the traditions and customs that persist, are the consumption of various insects and usage of insects for rituals and medicinal purposes.

Diabetes mellitus is a combination of heterogeneous disorders characterized by episodes of hyperglycemia and glucose intolerance as a result of lack of insulin, defective insulin action, or both [3]. Diabetes has become a highly problematic and increasingly prevalent disease world-wide. In 2011, there were 366 million people with diabetes, with this number expected to rise to 552 million by 2030 [4]. Diabetes is mainly classified into Types 1 and 2 [2, 5]. It is of interest to note that the intake of white Grub extract reduces blood glucose level [2]. The humic substances of white Grub extract may have achieved hypoglycemic and hypolipidemic properties through increased insulin secretion, increased peripheral utilization of glucose [2].

The aim of this study was to investigate the characterization and antidiabetic activity of aqueous extracts of whole white Grub and waste in alloxan induced diabetic rats.

The objectives of the study are:

1. Determine the serum blood glucose levels and lipid profile parameters of the extracts
2. Evaluate the percentage increase and decrease in lipid profile parameters
3. Characterize the active extract using FTIR and GC-MS analysis

MATERIALS AND METHODS

Sample Collection and Identification

WG used as sample were collected by hand picking at a cow dung site at the Federal University DutsinMa livestock farm, DutsinMa Local Government, Katsina State, Nigeria. It was authenticated at the Department of Biochemistry and Molecular Biology, Federal University DustinMa, Katsina State, by Mr Said Said Sani.

Plate 1: White Grub larvae

Extract preparation

About 60 g of WG extract was weighed using digital balance and poured into 500 ml conical flask in which 250 ml of distilled water was added. The mixture was kept for twelve (12) hours with constant agitation at 30-minute interval. The extract was filtered using cheesecloth into a measuring cylinder. The filtrate was dried in an oven at 80 ºC. The residue was weighed and the difference between the extract weight and the initial weight of the WG gives the weight of the known volume of extract. The semi-solid extract was stored in the refrigerator for further use. The WG was dissected and the waste was removed into a beaker and placed inside a petri dish, which was dried in the oven at 80 ºC. The extraction was repeated using 60 g of the powdered part.

Induction of diabetes by alloxan

Diabetes was induced in rats by a single Intraperitoneal (I.P.) injection of a freshly prepared solution of Alloxan monohydrate (100 mg/kg) after 18 hours of fasting. The blood glucose level was monitored after alloxanization and blood samples collected by tail tipping method using a Glucometer. After seventy-two hours, rats with blood glucose concentrations above 190 mg/dL were considered diabetic [2] and 30 of such rats were used for the study.

Estimation of glucose levels

Serum glucose was estimated by glucose oxidase method using Randox kit [6].

Estimation of serum lipid profile levels

Serum Total Cholesterol (TC) [7], High-Density Lipoprotein Cholesterol (HDL-C) [8] and Triglycerides (TAG) [9] were quantified by an enzymatic method using Randox kit. LDL-C and VLDL-C were calculated using friedewald formula [10],

1. LDL-C (mmol/l) = Total Cholesterol - (HDL-C) – Triglyceride/2.2
2. VLDL-C (mmol/l)=TG/2.2

Percentage increases or decrease were calculated using the relation: % = Xs-Cv/Cv × 100 [2].
Where, Xs = Sample value, Cv = Reference value (Normal control or Diabetic untreated group).

Experimental design and grouping

After the acclimitization period, rats were weighed and randomly divided into five groups comprising six animals in each group. Animals were administered with White Grub and waste (100 mg/kg same dose). The groups are as follows:

Group A: Normal control fed with feed and distilled water

Group B: Diabetic untreated fed with feed and distilled water.

Group C: Diabetic treated fed with distilled water, feed and administered with Glibenclamide (5 mg/kg) orally for 2 weeks.

Group D: Experimental animals administered with white grub extract (100 mg/kg) orally for 2 weeks.

Group E: Experimental animals administered with White Grub waste extract (100 mg/kg) orally for 2 weeks.

Collection of Blood

The animals were sacrificed by cervical dislocation on the 18th day. The blood was collected by cutting the jugular vein of the animals. The whole blood was collected into heparine sample bottles and centrifuged to obtain the serum which was used to for lipid profile assays.

Statistical Analysis

The results were expressed as mean ± standard deviation (S.D) with the results analyzed by using one-way analysis of variance (ANOVA). The Statistical Package for Social Sciences (SPSS) Computer software version 16 was used for data analysis. Post-Hoc Dunnett's-test at 95% level of significance was used to assess the significant difference between the control and treated groups. $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Table 1: Blood glucose level of diabetic rats administered with White Grub, waste extracts and standard drug orally for 18 days

Group Treatment	Before induction	72hrs after induction	7thday after induction and extracts administered	9th day after induction and extracts administered	11th day after induction and extracts administered	14th day after induction and extracts administered	16thday after induction and extract administered	18th after induction and extracts administered
GA-NC	52.5±2.81[a]	83.25±0.59[a]	88.0±1.21[a]	90.00±2.63[a]	90.81±2.71[a]	91.00±4.54[a]	92.17±3.80[a]	102.17±3.03[a]
GB- DC	48.50±3.04[b]	201.50±3.55[b]	212.±1.17[b]	252.17±3.00[b]	274.50±3.55[b]	295.50±3.67[b]	336.00±4.90[b]	342.02±3.55[b]
GC-Diabetic rats administered Glibenclamide (5mg/kg) bw	52.33±1.80[a]	328.83±3.07[c]	291.50±4.89[c]	244.33±4.05[b]	200.17±3.28[b]	185±2.26[c]	128.67±5.84[c]	118.67±5.84[a]
GD-Diabetic rats administered WGE 100mg/kg bw	51.33±1.78[a]	303.17±5.27[c]	254.40±3.30[c]	219.50±4.05[b]	201.33±2.19[b]	151.33±4.67[c]	129.67±0.54[c]	119.27±7.54[a]
GE Diabetic rats administered WE 100mg/kg	60.17±4.10[c]	390.83±4.06[c]	354.10±4.06[c]	300.67±47.22[c]	265.67±41.99[b]	207.50±4.71[b]	200.00±48.03	198.09±29.5[a]

Values are Mean ± SD of 6 determinations. Values with different alphabetical superscript along a column are significantly different at $P<0.05$. n = 6 NC= Normal Control, DC= Diabetic control

Table 2: Effect of administration of white Grub, waste extract and Glibenclamide on lipid profile parameters in alloxan induced diabetic rats for 7 days.

GROUP	T.CHOL	HDL-CHOL	TAG	LDL	VLDL
GA normal control	3.60±0.17[a]	0.93±0.09[a]	0.76±0.02[a]	2..49±0.02[a]	0.34±0.01[a]
GB diabetic control	2.07±0.87[b]	0.66±0.01[b]	1.28±0.30[b]	0.79±0.01[b]	0.58±0.14[b]
GC Glibenclamide 5mg/kg bw	1.81±0.26[c]	0.62±0.06[b]	0.46±0.06[c]	0.86±0.26[b]	0.21±0.03[c]
GD diabetic rats treated with WGE (100mg/kg)	2.73±0.81[b]	0.74±0.07	1.13±0.02[b]	1.77±0.17[c]	0.51±0.09[b]
GE diabetic rats treated with WE (100mg/kg)	2.73±0.65[b]	0.67±0.04[b]	1.80±0.47[b]	1.34±0.06[c]	0.81±0.21

Values are Mean ± standard error of 3 determinations. Values with different alphabetical superscript along a column are significantly different at P<0.05, n=3.

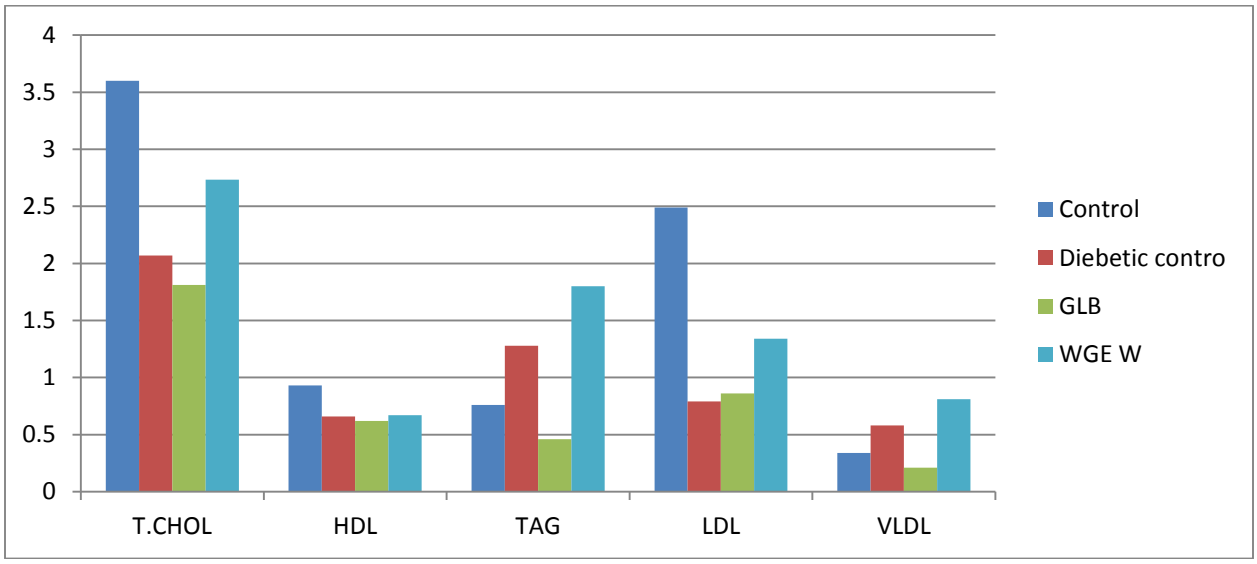


Figure 1**:** Percentage increase and a decrease in lipid profile indices for diabetic rats treated for 7 days.

Table 3: Lipid profile parameters of diabetic rats orally administered with White Grub, waste extracts and Glibenclamide for 14 days.

Group Treatment	T.CHOL (mMol/L)	HDL.CHOL (mMol/L)	TAG (mMol/L)	LDL (mMol/L)	VLDL (mMol/L)
GA Normal control	2.30 ± 0.50^{a}	0.68 ± 0.05^{a}	0.93 ± 0.04^{a}	0.42 ± 0.02^{a}	1.15 ± 0.03^{a}
GB Diabetic control	2.40 ± 0.10^{a}	0.82 ± 0.26^{b}	0.78 ± 0.10^{b}	0.35 ± 0.04^{b}	1.38 ± 0.13^{b}
GC Glibenclamide 5mg/kg b.w	1.24 ± 0.42^{b}	$0.76. \pm 0.13^{c}$	0.65 ± 0.14^{c}	0.29 ± 0.01^{c}	1.24 ± 0.21^{a}
GD WGE 100mg/kg b.w	1.55 ± 0.64^{b}	0.82 ± 0.11^{b}	0.53 ± 0.01	0.24 ± 0.33^{c}	1.27 ± 0.19^{a}
GE WE 100mg/kg b.w	2.50 ± 0.54^{a}	0.68 ± 0.01^{a}	$1.19. \pm 0.74$	0.54 ± 0.01	3.57 ± 0.55^{b}

Values are Mean ± standard error of 3 determinations. Values with different alphabetical superscript along a column are significantly different at $P<0.05$, n=3.

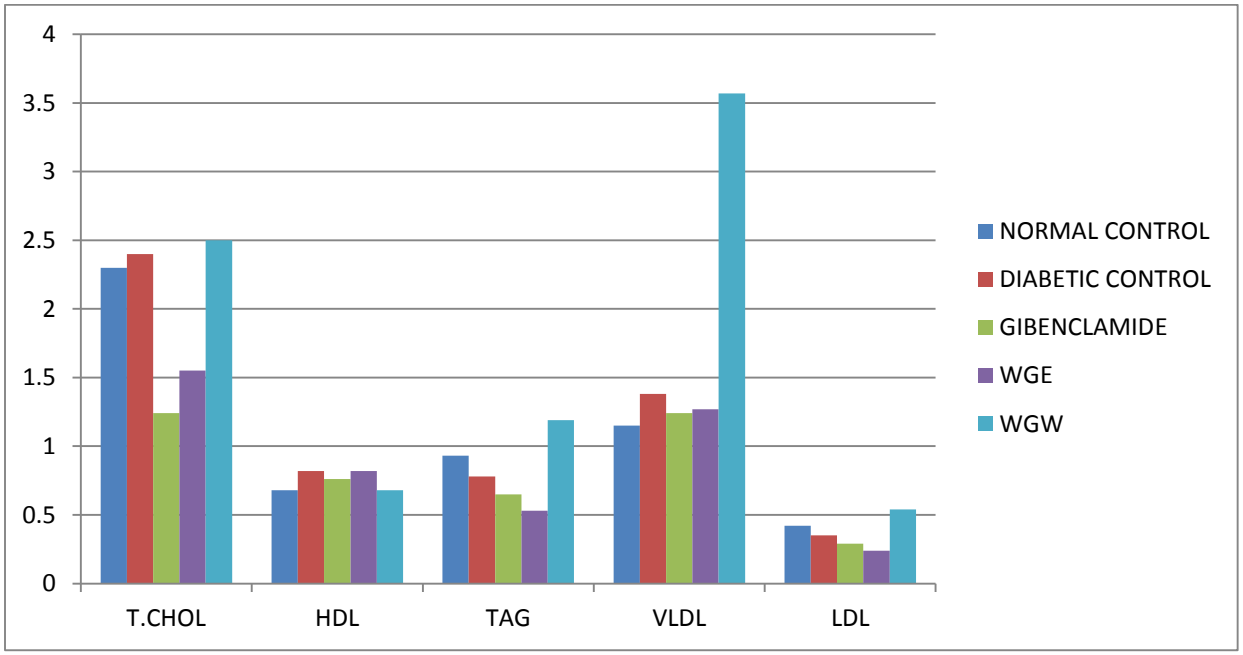
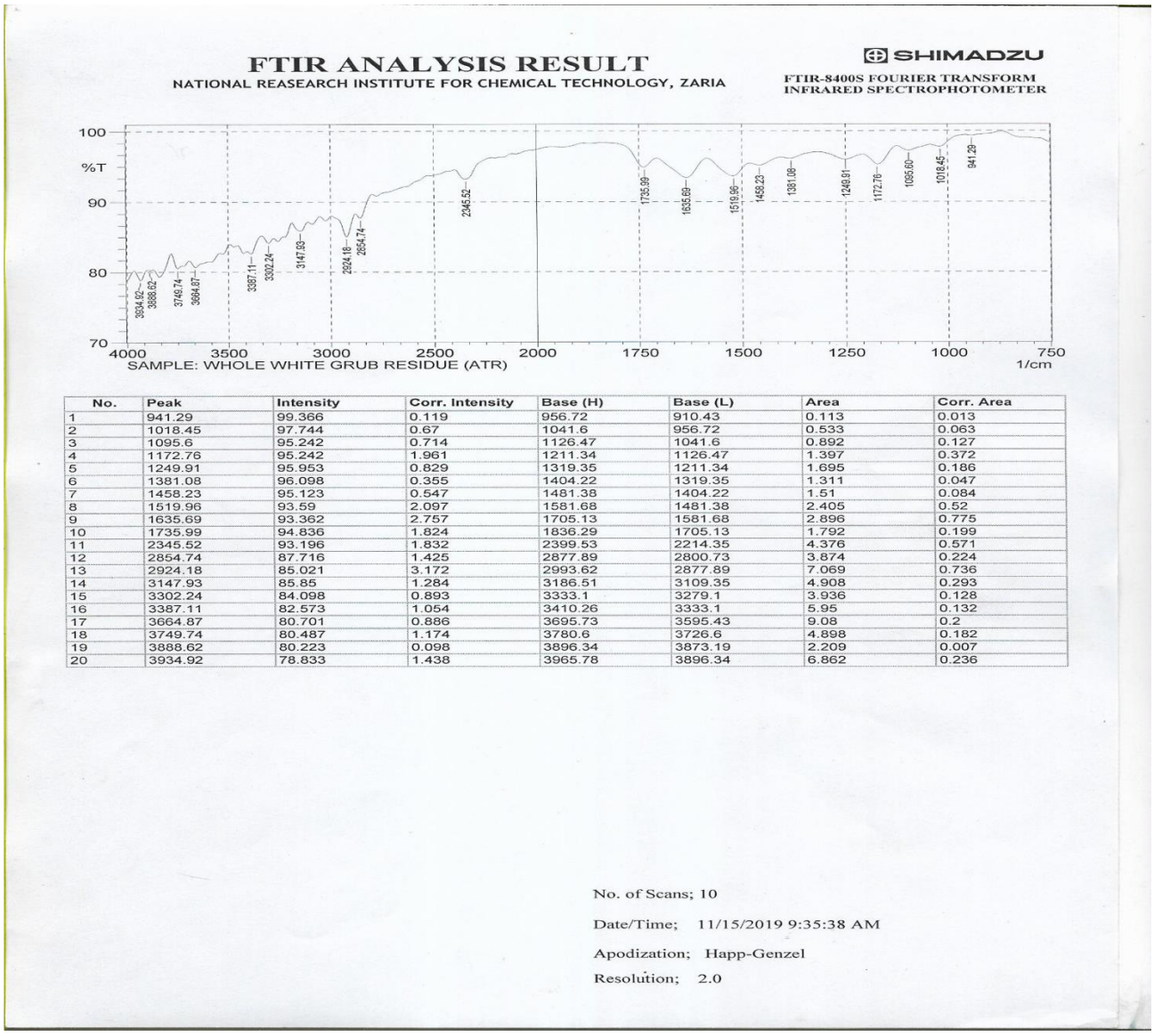


Figure 2: Percentage increase and a decrease in lipid profile indices for diabetic rats treated for 14 days.



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	941.29	99.366	0.119	956.72	910.43	0.113	0.013
2	1018.45	97.744	0.67	1041.6	956.72	0.533	0.063
3	1095.6	95.242	0.714	1126.47	1041.6	0.892	0.127
4	1172.76	95.242	1.961	1211.34	1126.47	1.397	0.372
5	1249.91	95.953	0.829	1319.35	1211.34	1.695	0.186
6	1381.08	96.098	0.355	1404.22	1319.35	1.311	0.047
7	1458.23	95.123	0.547	1481.38	1404.22	1.51	0.084
8	1519.96	93.59	2.097	1581.68	1481.38	2.405	0.52
9	1635.69	93.362	2.757	1705.13	1581.68	2.896	0.775
10	1735.99	94.836	1.824	1836.29	1705.13	1.792	0.199
11	2345.52	93.196	1.832	2399.53	2214.35	4.376	0.571
12	2854.74	87.716	1.425	2877.89	2800.73	3.874	0.224
13	2924.18	85.021	3.172	2993.62	2877.89	7.069	0.736
14	3147.93	85.85	1.284	3186.51	3109.35	4.908	0.293
15	3302.24	84.098	0.893	3333.1	3279.1	3.936	0.128
16	3387.11	82.573	1.054	3410.26	3333.1	5.95	0.132
17	3664.87	80.701	0.886	3695.73	3595.43	9.08	0.2
18	3749.74	80.487	1.174	3780.6	3726.6	4.898	0.182
19	3888.62	80.223	0.098	3896.34	3873.19	2.209	0.007
20	3934.92	78.833	1.438	3965.78	3896.34	6.862	0.236

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Figure 3: Fourier Transform Infrared (FTIR) Spectroscopy of Whole White Grub extract

Table 4: Absorption band in the infrared spectral region

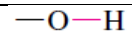
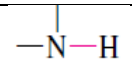
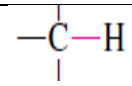
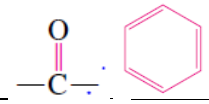
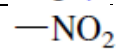
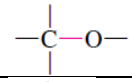
Position (cm^{-1})	Group	Inference
4000-3500		Strong intensity, Very broad band.
3400-2800		Weaker intensity and less broad than O-H; NH_2 shows two bands, NH shows one.
2500-2000		C is Sp^3 hybridized; $3000cm^{-1}$ is a convenient dividing line between this type of C-H bond and the preceding type.
1750-1500		Strong intensity, exact position depends on substituents and four bands of variable intensity.
1400 - 1250		Two strong intensity bands.
1200-1000		Strong intensity.
900-750		Strong intensity.

Table 5: Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of Whole White Grub extract.

No	Retention Time (RT)	Name of compound	Area %	Quality
1	6.129	2-Pentanamine	0.47	43
2	6.262	TATP	0.26	53
3	6.516	Benzeneethanamine	0.46	50
4	6.839	3-Aminopyrrolidine	0.73	50
5	7.212	2-Heptamine	1.48	43
6	7.367	Acetic acid	0.88	47
7	7.712	5-methyl-2(3H)-Furanone	2.13	43
8	7.838	Benzene Methanol	1.01	46
9	8.021	Acetamide	1.24	43
10	8.345	n-butylethylenediamine	2.48	46
11	8.640	Benzene ethanamine	2.23	38

12	8.908	phenylephrine	2.31	38
13	8.992	Carbonic acid	0.94	53
14	9.077	Nonadecylamine	0.86	47
15	9.358	Northianden	3.90	50
16	9.471	1-hexadecansulfonamide	1.65	47
17	9.590	1,3,5-triazine	1.69	50
18	9.949	3-isopropyl-1,2,4-oxadiazol-5-amine	4.18	47
19	10.097	Phosphonoacetic acid	4.13	50
20	10.287	Adipamide	2.08	50
21	10.554	Propanoic acid	4.85	37
22	10.702	Cyanoacetylurea	3.11	27
23	10.976	Tris (2 amino ethyl) amine	5.03	43
24	11.138	2,5 dimethoxy-4 ethyl ampethamine	2.65	46
25	11.251	Dodecan-8-one	6.14	48
26	12.053	Cis-vaccenic acid	4.21	90
27	12.193	Oleic acid	5.03	60
28	13.720	Cis- octadecanoic acid	2.24	97
29	13.931	1- Eicosene	4.97	70
30	14.219	Octadec-9- enoic acid	2.92	97
31	14.810	6-octadecanoic acid	2.68	98
32	15.197	9-octadecanoic acid	2.14	91
33	15.507	9- octadecanoic,(E)	2.21	98
34	16.365	1- Nonadecene	1.47	84
35	16.668	cycloeicosane	2.18	81
36	16.794	E-9-Hexadecenal	1.79	72
37	12.294	Cis-9-Hexadecenal	1.92	53
38	17.048	E-15-Heptadecenal	2.00	55
39	17.582	cyclopentadecanone	3.03	90
40	17.815	11- octadecanoic	3.66	70
41	18.511	2,5- Furandione	0.28	86
42	22.141	Elaidic acid	0.36	78

As observed from the result above WG extract reduces blood glucose level after two weeks treatment with the whole grub in which the blood sugar significantly ($P < 0.05$) returned to

normal level. The WG extract exact its effect similar to the reference drug but the waste extract did not. The extract may have achieved hypoglycemicproperty through increased insulin secretion and, increased peripheral utilization of glucose as reported existing literature [2]. This indicates a positive effect of Grub Humic substances (Fulvic acid, Humic Acid and Humin) on the 18^{th} day of oral administration of white Grubs extract in reducing blood sugar [2]. There is a marked increase in sugar level (hyperglycemia) in diabetic rats. Theresult agrees with already existing literature [2] that alloxan induces diabetes mellitus by selectively destroying the betacells of the pancreas leading to marked increase in blood glucose concentration observed in rats after administration and confirms the development of diabetes mellitus. After first week of treatment the diabetic control rats had elevated Triglyceride (TAG), low density lipoprotein (LDL), very low density lipoprotein cholesterol (VLDL), with decreased Total cholesterol (TC) and High density lipoprotein (HDL) (Table 2).

The extract of WG (WGE) had shown elevated level of TC, HDL, LDL and significant decrease of VLDL compared to diabetic control. The waste extract (WE) showed significant increased ($p < 0.05$) T.chol, TAG, LDL, VLDL and significant decreased ($p < 0.05$) HDL compared to diabetic untreated group. The reference drug treated group showed significant decreased ($p < 0.05$) TC, HDL, TAG, VLDL and elevated level of LDL compared to diabetic untreated group. After second week of treatment the diabetic control rats had elevated levels of TC, HDL, VLDL, and significant decreased ($p < 0.05$) LDL compared to normal control (Table 3). Glibenclamide administered group had shown decreased levels of all the lipid profile parameters compared to diabetic untreated group. The WE treated group showed elevated levels of TC, TAG, VLDL, LDL and decreased HDL cholesterol compared to diabetic control. The WGE administered group showed significant decreased ($p < 0.05$) TC, TAG, VLDL, LDL and normalized HDL. The cholesterol, triglyceride, and LDL lowering effect coupled with HDL elevating effect of an extract may help in reducing complications associated with hyperlipidemia as a result of diabetes mellitus [2, 11, 12]. A higher level of HDL is related to lower risk of heart and blood vessel disease. Triglycerides measures are used in the diagnosis and treatment of diseases involving lipid metabolism and various endocrine disorders such as DM.

Figures 1 and 2 show the percentage increase and a decrease in lipid profile indices for diabetic groups treated with extracts standard drug and diabetic group untreated for the first and second week. Figure 3 explains that the wavelength of CH bonds can be detected in the above spectrum.

The absorption bands as a result of the nitro group: 3400-2800 and 1400-1250 cm^{-1}. The wavelengths are at lower wave numbers than usual because of the conjugation between nitro group and benzene ring. The benzene ring is responsible for the absorptions of the strong intensity (1000 – 750 cm–1). On the basis of these absorptions, it is usually possible to determine the nature of the functional group that is present in the compound whose spectrum is being considered [2]. Many functional groups require the presence of several characteristic absorptions, whereas the absence of a band in a particular region of the spectrum can often be used to eliminate the presence of a particular group.

Result from the GC-MS analysis (figure 4) of whole White Grub conducted at the National Research Institute for Chemical Technology (NARICT) Zaria, Nigeria, showed 42 compounds possessing antihyperlipidemic and antihyperglycemic potentials. Possibly these compounds act mutually, stimulating to protect the pancreatic damage during oxidative stress, by either inhibiting or scavenging free radicals such as superoxide anion radical ($O_2^{\cdot-}$), hydroperoxyl radical ($HOO^{\cdot}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), lipid peroxide radical ($ROO^{\cdot}$) e.t.c. Compounds like acetic acid, propanoic acid, oleic acid, benzene, carbonic acid, octadecanoic acid, phenylephrine, Eicosene and Tris (2 aminoethyl) amine as part of their functional groups and help in satisfying the free radicals by donating the electron.

High level of total cholesterol can develop fatty deposits in the blood vessels. Eventually these deposits grow, making it difficult for enough blood to flow through the arteries. Sometimes, these deposits can break suddenly and form a clot that causes heart attack or stroke. WG is rich in fats, protein, some mineral elements and humic substances (humin, fulvic and humic acids) [13]. 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 or damaged tissue. 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 [2].

CONCLUSION

Results obtained from the studies revealed the antidiabetic potentials of white Grub extract. The Whole WG is more active than the WG waste.

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