Original Research Article

Lipidaemic and Hepatic Status of Type 2 Diabetic Rats Treated with the Polyherbal Capsule Glucoblock

ABSTRACT

The scourge of diabetes has led to an increase in the use of complementary and alternative medicine. The lack of regulation and control leads to the indiscriminate use of these herbals, with potential risk to the patients.

Aim: This study evaluates the lipidaemic and hepatic status of type 2 diabetic rats treated with the polyherbal capsule glucoblock.

Methodology: A total of 35 male Wistar albino rats weighing between 120-220g were used for this study. The rats were placed on high fat diet, and diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) (45 mg/kg body wt). Fasting plasma glucose (FPG) was determined using the glucose oxidase method. Total Cholesterol (TC), Triglyceride (TG) and High density lipoprotein cholesterol (HDL-C) were determined using enzymatic methods. Low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using Reitman-Frankel method, while alkaline phosphatase (ALP) was determined using the colorimetric phenolphthalein method. Liver sections were stained using haematoxylin and eosin (H&E) staining technique, and phytochemical analysis was also done on the herbal capsule.

Results: The results show no significant differences in TC levels in all groups compared to the negative control. TG level was significantly higher in the diabetic control group when compared to the negative control. TG level in the singular treatment groups were significantly lower, but the combination group (glibenclamide + glucoblock) showed no significant difference compared to the diabetic control. The negative control had significantly higher HDL-C compared to the diabetic control and treatment groups. There were no significant differences in HDL-C levels in all the treatment groups, when compared to the diabetic control. The negative control had significantly lower LDL-C compared to the diabetic control and treatment groups. There were no significant differences in LDL-C levels in all the treatment groups, when compared to the diabetic control. ALT, AST and ALP levels were significantly higher in the diabetic control, but was significantly reduced to normal levels by the treatments. Liver sections of the negative control showed normal histoarchitecture. The diabetic control showed inflammation and fatty deposition. The treatment groups showed a nearly normal histoarchitecture, with fatty deposits.

Conclusion: High fat diet in combination with a sub-diabetic dose of streptozotocin produced significant diabetes in the Wistar rats with dyslipidaemia and elevated liver enzyme levels. The anti-diabetic treatments, glibenclamide and glucoblock did not correct the dyslipidaema caused by diabetes. However, the treatments had equipotent hepatoprotective effect and restored liver enzyme levels to normal as well as improving liver histology.

Keywords: Diabetes mellitus, Dyslipidaemia, Lipid profile, Liver enzymes, Complementary and alternative medicine, Phytochemicals, Glucoblock, Glibenclamide, High fat diet, Streptozotocin.

1. INTRODUCTION

Type 2 diabetes is a heterogeneous disorder characterized by peripheral insulin resistance, impaired regulation of hepatic glucose synthesis, declining beta-cell function, ultimately leading to beta-cell failure [1, 2]. It is one of the most important diseases worldwide, with a huge disease burden on the patients [3]. It is associated with depressed anti-oxidant parameters [4], and increased oxidative stress, leading to diabetic complications [5]. Diabetes affects liver and lipoprotein metabolism leading to distortion of liver tissue and dyslipidaemia [6, 7].

Despite the availability of orthodox medicine, the increased disease burden and multi-organ complications of diabetes has led to the increase in the use of complementary and alternative (CAM), in an attempt to improve disease outcomes, with lesser side effects as well as to improve general well-being [8]. In Africa, especially Nigeria, there are a number of constraints in the control of CAM usage. For instance, there is lack of integration of CAM therapies into African mainstream health care systems. This is despite the World Health Organization (WHO) recommendation to integrate traditional and CAM therapies into national health care systems [9]. Another major concern is the lack of regulation on CAM use, therefore exposing the population to potential harm. This study evaluates liver and lipid profile changes in type 2 diabetic rats treated with the polyherbal drug glucoblock.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of 35 male Wistar albino rats weighing between 120-220g were used for this study. The rats were housed in standard cages at regulated room temperature, with controlled 12 hour light-dark cycles, and allowed access to feed and water *ad libitum*. The animals were allowed to acclimatize for two weeks prior to the commencement of study.

2.2 Drugs

The drugs used for the study were glucoblock, a polyherbal drug manufactured by Green World Group, Michigan, USA, and commercially sold in Nigeria as an anti-diabetic capsule. Glibenclamide, a sulfonylureas was manufactured by Glanil Pharmaceuticals, Nigeria.

2.3 Acute Toxicity Study

Acute Toxicity Study was done by the fixed dose procedure [10], using a group of 3 rats. 2000mg/kg body weight of glucoblock was orally administered to each of the rats. The rats were then observed for signs of toxicity for 48 hours. After observation, there were no observed signs of toxicity, hence the herbal drug glucoblock was deemed safe up to a dose of 2000mg/kg body weight. Glibenclamide is a standard antidiabetic drug, and the dose was translated from the human dose as shown below.

2.4 Dose Calculation

The administered rat dosages were extrapolated from the human dose using the formula by Paget and Barnes [11]

Glibenclamide

Human daily dose is 1 caplet (5mg) twice daily, that is, 10mg/day.

Rat dose (mg/kg) = Human daily dose x 0.018 x 5.

= 0.9mg/kg body weight/day.

Glucoblock

Human daily dose is 2 capsules (500mg each) once daily, that is, 1000mg/day.

Rat dose (mg/kg) = Human daily dose x 0.018 x 5.

= 90mg/kg body weight/day.

2.5 Study Design and Diabetes Induction

The rats were weighed and grouped into 5 groups of 7 rats each. Group 1 (negative control) was placed on a normal chow diet, while groups 2 to 5 were placed on high fat diet (HFD) having 42.1% fat content, 3 weeks prior to induction with streptozotocin (STZ). Diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (45 mg/kg body wt.) dissolved in 0.1 M citrate buffer (pH 4.5), after a 6 hour fast. Diabetes was confirmed after 72 hours in all the rats, with fasting blood glucose levels above 14mmol/L (250 mg/dl) [12]. Treatments (drugs) were administered daily according to the groupings by means of oral gavage for 28 days.

Group 1: Negative control. The animals were only injected citrate buffer intraperitoneally.

Group 2: Diabetic control

Group 3: Diabetic rats treated with glibenclamide.

Group 4: Diabetic rats treated with the polyherbal drug glucoblock.

Group 5: Diabetic rats treated with a combination of glibenclamide and glucoblock.

On the 29th day, the rats were fasted for 6 hours, anaesthetized with chloroform and sacrificed. Blood samples were collected by cardiac puncture. This is in line with the National Institutes of Health (NIH) and the Animal Models of Diabetic Complications Consortium (AMDCC) protocol, on the fasting of laboratory animals [13, 14]. All the animal experiments were conducted according to the ethical norms approved by the Institutional Ethical Committee.

2.6 Reagents and Biochemical Analyses

All reagents were commercially purchased and the manufacturer's standard operating procedures strictly followed. Quality control (QC) samples were run together with the biochemical analysis. STZ was gotten from Sigma-Aldrich, USA. Fasting plasma glucose (FPG) was determined using Glucose oxidase method [15], as modified by Randox Laboratories Limited (UK). Total Cholesterol (TC) was determined by enzymatic method [16], as modified by Randox laboratories limited (UK). Triglyceride was determined by enzymatic method [17], as described by Randox laboratories limited (UK). High Density Lipoprotein Cholesterol (HDL-C) was determined by enzymatic method [18], as modified by Randox laboratories limited (UK). Low Density Lipoprotein Cholesterol (LDL-C) was calculated from the Friedewald's equation [19]. The liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using the Reitman-Frankel method [20], as modified by Randox laboratories limited (UK). Alkaline phosphatase (ALP) was determined using the Colorimetric Phenolphthalein method [21] as modified by Teco Diagnostics (USA). Qualitative and quantitative phytochemical analysis were done on the herbal drug using classical and spectrophotometric methods respectively [22]. Liver sections were stained using the standard haematoxylin and eosin (H&E) staining technique.

2.7 Statistical Analysis

Data generated was analysed using Graph Pad Prism version 5.03. Groups were compared using one way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test. Results were considered statistically significant at 95% confidence interval ($p \leq 0.05$). Values are expressed as Mean ± SD.

3. RESULTS

Table 1: Qualitative and Quantitative Phytochemical Analysis of the Herbal Drug Glucoblock

Phytochemicals	Glucoblock	Concentration (µg/mg)
Alkaloids	**+ve**	100.31
Flavonoids	**+ve**	131.45
Cardiac glycosides	**+ve**	55.93
Phenols	**-ve**	
Phlobatanins	**-ve**	
Saponins	**+ve**	61.47
Tanins	**-ve**	
Terpenoids	**-ve**	
Quinones	**-ve**	

+ve – Present, -ve – Not present

Table 1 shows alkaloids, flavonoids, cardiac glycosides and saponins present in the herbal drug glucoblock, with concentrations of 100.31µg/mg, and 131.45µg/mg, 55.93µg/mg and 61.47µg/mg respectively. Other phytochemicals such as phenolic acids, terpenoids, quinones, and tannins were not found.

Table 2: Effect of Treatment on Lipid Profile of the Rats

Groups	TC (mmol/L)	TG (mmol/L)	HDL (mmol/l)	LDL (mmol/L)
Group 1 (Negative control) n = 7	2.30 ± 0.22	0.59 ± 0.05^{b}	1.22 ± 0.13^{b}	0.82 ± 0.16^{b}
Group 2 (Diabetic control) n = 6$^{\#}$	2.56 ± 0.19	0.82 ± 0.06^{a}	0.71 ± 0.11^{a}	1.47 ± 0.17^{a}
Group 3 (Gli) n = 7	2.35 ± 0.09	0.64 ± 0.10^{b}	0.72 ± 0.08^{a}	1.34 ± 0.15^{a}
Group 4 (Gluco) n = 7	2.54 ± 0.26	0.63 ± 0.10^{b}	0.77 ± 0.11^{a}	1.49 ± 0.14^{a}
Group 5 (Gli + Gluco) n = 7	2.57 ± 0.04	0.82 ± 0.14^{a}	0.63 ± 0.07^{a}	1.57 ± 0.06^{a}
P-value	0.0612	0.0002	< 0.0001	< 0.0001
F-value	3.162	8.436	32.77	27.03

n – Number of samples. Gli – Glibenclamide, Gluco – Glucoblock, [a] – Significantly different from negative control, [b] – Significantly different from diabetic control. [#] - A rat died in the diabetic group in the course of the study.

Table 2 shows the results of the lipid profile parameters, total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) of the rats after treatment. The results show no significant differences ($p>0.05$) in TC levels in all groups compared to the negative control, even though the negative control had a lower value.

TG level was significantly higher ($p<0.05$) in the diabetic control group when compared to the negative control. TG levels in the glibenclamide and glucoblock treatment groups were significantly lower ($p<0.05$) compared to the diabetic control, and also showed no significant difference ($p>0.05$) compared to the negative control. TG level in the combination group (glibenclamide + glucoblock) showed no significant difference ($p>0.05$) compared to the diabetic control.

The negative control had significantly higher ($p<0.05$) HDL-C level, when compared to the diabetic control and the treatment groups. There were no significant differences ($p>0.05$) in HDL-C levels in all the treatment groups, when compared to the diabetic control.

LDL-C levels were significantly lower ($p<0.05$) in the negative control compared to the diabetic control and treatment groups. The results reveal no significant differences ($p>0.05$) in LDL-C levels in all the treatment groups, when compared to the diabetic control.

Table 3: Effect of Treatment on Liver Enzymes of the Rats

Groups	**ALT (IU/L)**	**AST (IU/L)**	**ALP (IU/L)**
Group 1 (Negative control) n = 7	17.67 ± 1.37^{b}	61.17 ± 12.81^{b}	83.95 ± 8.57^{b}
Group 2 (Diabetic control) n = 6[#]	27.83 ± 1.84^{a}	87.17 ± 7.55^{a}	181.70 ± 17.03^{a}
Group 3 (Gli) n = 7	19.50 ± 4.51^{b}	62.33 ± 9.29^{b}	93.86 ± 5.50^{b}
Group 4 (Gluco) n = 7	18.83 ± 3.66^{b}	61.33 ± 6.89^{b}	90.91 ± 9.19^{b}
Group 5 (Gli + Gluco) n = 7	20.33 ± 5.13^{b}	58.33 ± 12.13^{b}	$103.40 \pm 9.70^{a\ b}$

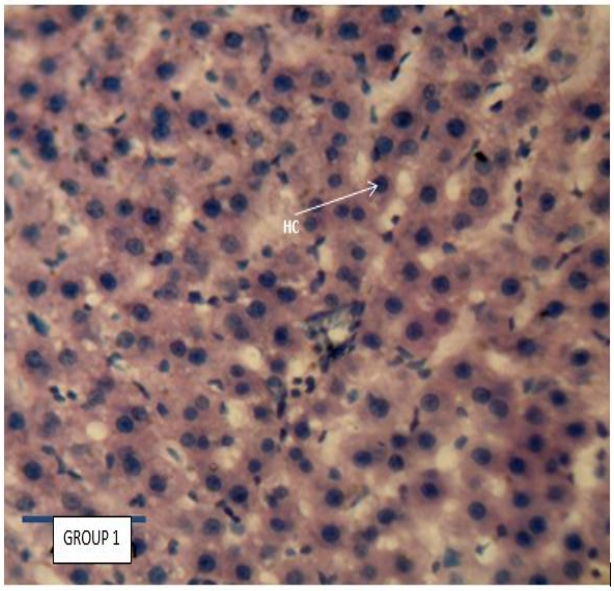
P-value	0.0004	0.0002	< 0.0001
F-value	7.487	8.448	84.95

n – Number of samples. Gli – Glibenclamide, Gluco – Glucoblock, [a] – Significantly different from negative control, [b] – Significantly different from diabetic control. [#] - A rat died in the diabetic group in the course of the study.

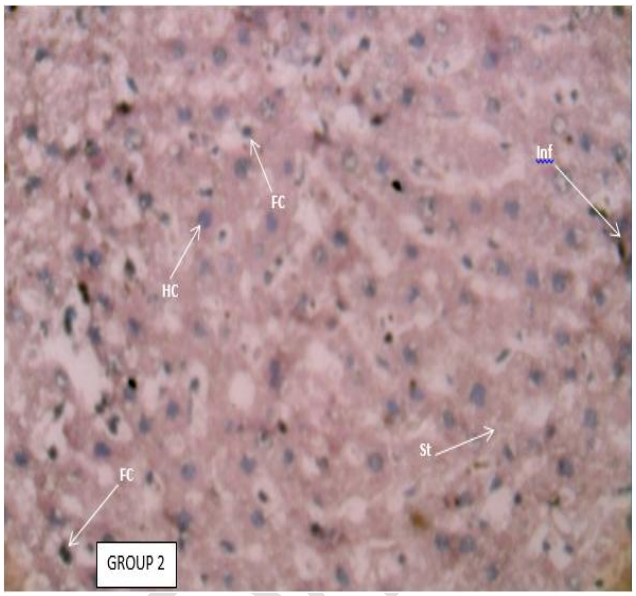
Table 3 shows the results of the liver enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphatase (ALP). The diabetic control had significantly higher ($p<0.05$) ALT levels compared to the negative control and treatment groups. All the treatment groups showed no significant differences ($p>0.05$) in ALT levels compared to the negative control.

AST levels were significantly higher in the diabetic control ($p<0.05$) compared to the negative control and treatment groups. All the treatment groups showed no significant differences ($p>0.05$) in AST levels when compared to the negative control.

ALP levels were significantly higher in the diabetic control ($p<0.05$) compared to the negative control and treatment groups. ALP levels in the glibenclamide and glucoblock treatment groups showed no significant difference ($p>0.05$) compared to the negative control. ALP level in the combination group (glibenclamide + glucoblock) was significantly higher compared to the negative control.


HC
GROUP 1

(a)


FC
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HC
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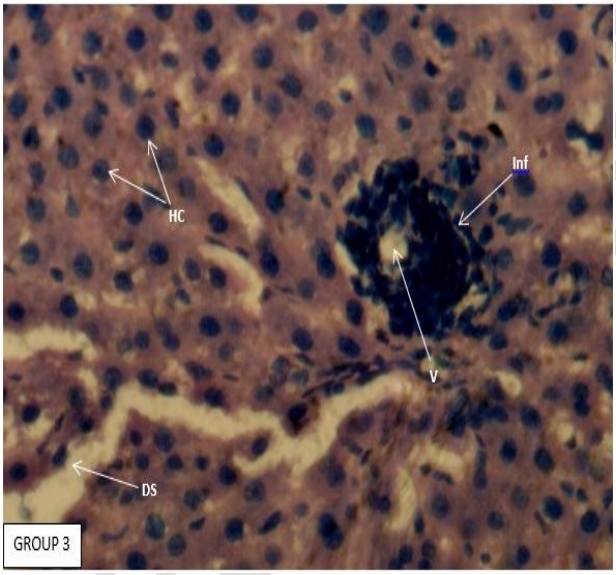
FC
GROUP 2

(b)


HC
Inf
V
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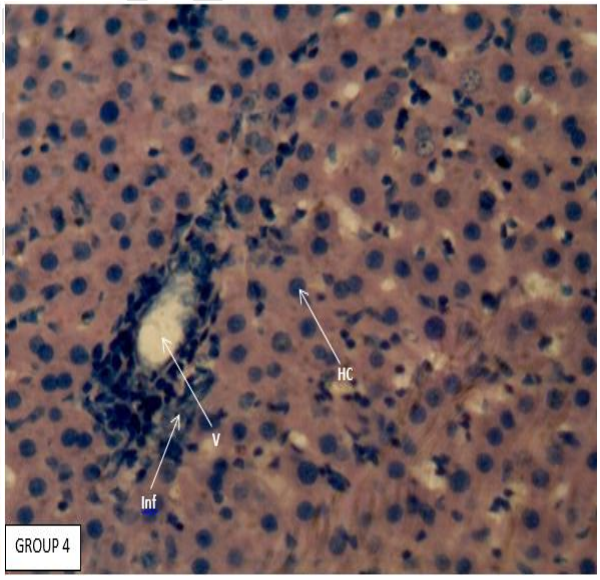
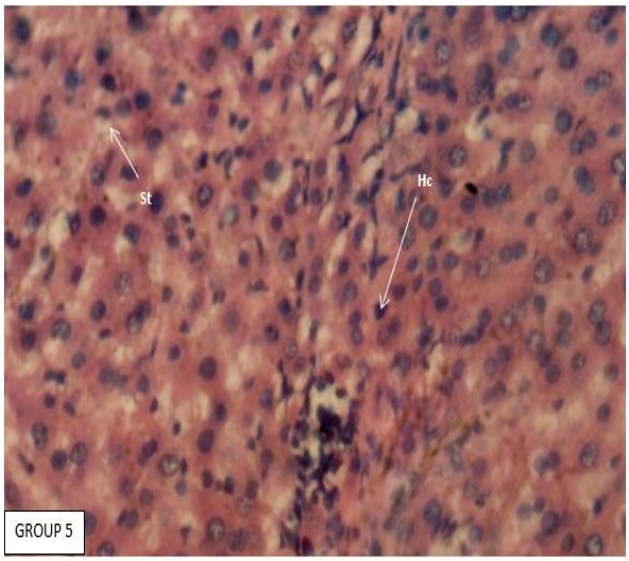
GROUP 3

(c)


HC
V
Inf
GROUP 4

(d)



(e)

HC-Hepatocytes, St-Steatosis, Inf-Inflammation, Fc- Clear Cell Foci, V- Central vein, DS-Dilated Sinusoid

Figure 1: (a), (b), (c), (d) and (e): Shows photomicrograph (X 400) of H&E stained histologic sections of the liver of the rats. The negative control (a) showed normal histoarchitecture (hepatocytes are radially arranged around the central vein) and vascular channels. The diabetic control (b) showed that the lobular architecture was maintained, but there is mild clear cell foci, inflammation and fatty deposition. The glibenclamide treated group (c) showed nearly normal architecture with mild focal perivascular inflammation and steatosis. The glucoblock treated group (d) showed normal radially arranged hepatocytes around the central vein, with mild fatty deposition. The combination group (e) showed reduced inflammation and mild steatosis.

4. DISCUSSION

Phytochemical analysis of the polyherbal drug glucoblock revealed the presence of bioactive phytochemicals like alkaloids, flavonoids, cardiac glycosides, and saponins in variable amounts, which could have contributed to the changes in the biochemical parameters analyzed. The phytochemicals can exert their biological action by modulating molecular targets like enzymes, ion channels etc, to bring about structural and physiological changes, and are thus used in evidence-based medicine [23].

The results from this study revealed no significant differences ($p>0.05$) in total cholesterol (TC) levels in all the groups compared to the negative control, although the levels in the diabetic control and treatment groups were higher than that of the negative control. Triglyceride (TG) levels in the diabetic control was significantly higher ($p<0.05$) than that of the negative control, indicating an increase in the rate of lipolysis in the diabetic state. TG levels in the glibenclamide and glucoblock treatment groups were significantly lower ($p<0.05$) compared to the diabetic control, and also showed no significant difference ($p>0.05$) compared to the negative control. TG level in the combination group (glibenclamide + glucoblock) showed no significant difference ($p>0.05$) compared to the diabetic control. This implies the individual treatments were effective in reducing TG levels to normal control values, but not effective when used together, indicating antagonistic drug-herb reaction.

High density lipoprotein cholesterol (HDL-C) levels in the negative control was significantly higher ($P<0.05$) when compared to the diabetic control and all the treatment groups. The results also revealed no significant differences ($p>0.05$) in HDL-C levels in the treatment groups, when compared to the diabetic control, indicating glibenclamide, the polyherbal glucoblock, and the combination had no effect in improving HDL-C levels in the diabetic state. Low density lipoprotein cholesterol (LDL-C) was significantly lower ($p<0.05$) in the negative control as compared to the diabetic control and treatment groups, which had significantly higher values. LDL-C levels in all the treatment groups were not significantly different ($p>0.05$) from the diabetic control, indicating the administration of glibenclamide, glucoblock, and their combination had no effect on LDL-C values in the diabetic rats.

The results of lipid profile revealed that the diabetic rats present with diabetic dyslipidaemia, having normal TC levels, hypertriglyceridaemia, reduced HDL-C and a higher LDL-C levels. This was not corrected by the anti-diabetic treatments given, and would possibly require a different treatment regimen to correct the dyslipidaemia.

Diabetes is known to have not only defective glucose metabolism but also disturbances in lipid metabolism, which mostly presents the characteristic diabetic dyslipidaemia and is a risk factor for cardiovascular disease [7]. Apart from the changes in the concentration of the different lipoproteins, their content and composition are also affected. Hypertriglyceridaemia and the

presence of triglyceride-rich lipoproteins are thought to play a central role in the disease process and the presentation of diabetic dyslipidaemia [24].

The findings are in consonance with the works of Gupta *et al*. [25], in which a combination of HFD and STZ treatment produced significant dyslipidaemia, with elevated total cholesterol and triglyceride levels in diabetic Sprague-dawley rats. Gupta *et al*. [25] also found that treatment with a polyherbal plant extract and treatment with glibenclamide significantly reduced total cholesterol and triglyceride levels when compared to the diabetic control. Treatment with glibenclamide was more effective in reducing the lipid levels than the polyherbal plant extract. The lipid levels however, did not return to normal control values. Arshadi *et al*. [26] reported significant improvements in lipid profile after treatment with fenugreek extract and glibenclamide in STZ-induced diabetic rats, when compared to the diabetic control.

Similar research by Gotama *et al*. [27] corroborates with our findings. In their study, treatment with the herbal extract of *Sargassum hystrix* produced no significant difference in HDL-C and LDL-C levels, when compared to the diabetic control. They however, found significant differences in total cholesterol and triglyceride levels in the treatment group as against the diabetic control. Mishra *et al*. [28] found that *Ocimum sanctum* extract administered at 100 and 200 mg/kg body weight produced no significant difference in TC, TG, and very low density lipoprotein cholesterol (VLDL) levels in HFD/STZ-induced diabetic rats, when compared to the diabetic control. There were however significant improvements in HDL-C and LDL-C values, against the diabetic control.

The results of liver function in this study revealed significantly elevated ($p<0.05$) enzyme levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the diabetic control, when compared to the negative control. Diabetes induction may have led to physiological and biochemical changes in the liver, resulting in leakage of the liver enzymes. The liver has been associated with diabetes related oxidative stress. Injury to the liver is a common occurrence in patients with uncontrolled diabetes, with biochemical, histopathological and physiological changes [29]. There are also reports of direct organ toxicity of STZ to the liver apart from the ensuing diabetes [30].

The results revealed significantly lower ($p<0.05$) ALT, AST, and ALP levels in the treatment groups, when compared to the diabetic control. Also, the enzyme levels in the treatment groups were not significantly different ($p>0.05$) from the negative control group, with glucoblock having equipotent effects with glibenclamide treatment. This reveals administration of the orthodox drug glibenclamide, the polyherbal formulation glucoblock, and their combination, had hepatoprotective effect on the liver of the diabetic rats. This may be due to the antioxidant potentials of the treatments [31], preventing oxidative damage to liver hepatocytes. From this research, the combination therapy was not effective as the individual treatments in reducing ALP levels.

The findings are in consonance with our previous work [32], in which high fat diet/streptozotocin-induced diabetes significantly elevated the liver enzymes, ALT, AST and ALP in wistar albino rats. Khajuria *et al*. [33], also found significantly altered liver parameters resulting in elevated levels of transaminases (AST and ALT) and phosphatases (ALP and ACP) in STZ-induced diabetes in experimental animals. The results are in agreement with the works of Otunola & Afolayan [34], in which treatment with glibenclamide significantly reduced levels of the liver enzymes ALP, AST. ALT, gamma glutamyl transferase (GGT) and cholinesterase in diabetic rats. Also, administration of a polyherbal mixture brought about equipotent results as compared to glibenclamide treated rats.

Histologic analysis of the liver of the non-diabetic rats showed normal liver histoarchitecture, in which the hepatocytes are radially arranged around the central vein. The hepatocytes had well preserved nucleus, cytoplasm and sinusoids. In the diabetic control group, the lobular architecture of the liver was maintained, but there were some histopathological changes like fatty deposition, inflammation, degeneration and minimal necrosis. These changes in the diabetic rat liver could be due to insulin resistance and inaction leading to intracellular fat accumulation. The histologic analysis of the treatment groups showed nearly normal histoarchitecture, with hepatocytes having minimal necrosis and inflammation. There was however, observed fatty change in the liver. The improved liver histology compared to the diabetic control could be due to the antioxidant potential of the drugs [31], thus providing hepatoprotective effects on the liver. This is in consonance with the work of Balamash *et al*. [35], in which STZ-induced diabetic rats had apoptotic hepatocytes with degenerated nuclei. Also, Otunola & Afolayan [34] found

hepatocytes with nearly normal appearance and reduced necrosis in glibenclamide and aqueous extract of garlic, ginger and cayenne pepper (GCCP) treated diabetic rats.

5. CONCLUSION

High fat diet in combination with a sub-diabetic dose of streptozotocin produced significant diabetes in the Wistar rats with dyslipidaemia and elevated liver enzyme levels, indicating type 2 diabetes is associated with dyslipidaemia and damage to the hepatocytes. The anti-diabetic treatments, glibenclamide and glucoblock did not correct the dyslipidaema caused by diabetes. However, the treatments had equipotent hepatoprotective effect and restored liver enzyme levels to normal as well as improving liver histology. With equipotent effects compared to glibenclamide, glucoblock could be incorporated in the management of diabetes in addition to lipid lowering drugs. Anti-diabetic herbal products should be properly evaluated, and utmost care taken in the combination of herbal and conventional medicines, for the risk of adverse drug-herb reactions.

Sponsorship

None.

Ethical approval

All the animal experiments were conducted according to the ethical norms approved by the Institutional Ethical Committee.

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