Journal of Complementary and Alternative Medical Research

8(2): 1-10, 2019; Article no.JOCAMR.52409
ISSN: 2456-6276

Therapeutic Effects of the Anti-diabetic Polyherbal Drug Diawell in Combination with Metformin on Liver and Lipid Parameters in Type 2 Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author ONB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EON and HB managed the analyses of the study. Author KNEA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2019/v8i230118
Editor(s):
(1) Dr. Sahdeo Prasad, Department of Immunotherapeutics and Biotechnology, Texas Tech University Health Sciences Center, USA.
Reviewers:
(1) Yanwen Wang, National Research Council, Canada.
(2) Gayatri C. Gawade, Bharati Vidyapeeth Dental College and Hospital, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/52409

Original Research Article

Received 11 August 2019
Accepted 21 October 2019
Published 28 October 2019

ABSTRACT

Type 2 diabetes is one of the most important diseases worldwide. It affects several organ systems including the liver and lipid metabolism. Many herbal formulations have shown anti-diabetic potential, however, their safety and efficacy remain a debate in the medical community.

Aim: This study evaluates the therapeutic effects of the anti-diabetic polyherbal drug diawell in combination with metformin on liver enzyme and lipid profile in type 2 diabetic rats.

Methodology: A total of 35 male Wistar albino rats weighing between 120-220 g 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

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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 tablet.
Results: The results show no significant differences in mean TC levels in all groups. TG level was significantly higher in the diabetic control when compared to the negative control. There were no significant differences in TG levels in the metformin group, and diawell group when compared to the diabetic control. TG levels in the combination group (metformin + diawell) was significantly lower versus the diabetic control, and showed no significant difference compared to the negative control. HDL-C was significantly higher in the negative control when compared to the diabetic control and the treatment groups. There were no significant differences in HDL-C levels in all the treatment groups, when compared to the diabetic control. LDL-C levels were significantly lower in the negative control 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. The diabetic control had significantly higher ALT, AST and ALP levels compared to the negative control and treatment groups. All the treatment groups showed no significant differences in ALT and AST levels compared to the negative control. 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 45 mg/kg of STZ produced significant diabetes in the Wistar rats with dyslipidaemia and elevated liver enzyme levels. Metformin and the polyherbal tablet diawell had no impact on the lipid levels as it did not correct the dyslipidaema, however, the treatments showed hepatoprotective potentials and restored liver enzyme levels to normal. Lipid lowering drugs should be included in the management of type 2 diabetes, and there should be proper evaluation of anti-diabetic herbal products.

Keywords: Diabetes mellitus; dyslipidaemia; lipid profile; liver enzymes; herbal therapy; diawell; metformin; high fat diet; streptozotocin.

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic syndrome characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. There is altered metabolism of carbohydrates, lipids, and proteins along with an increased risk of complications [1]. It has been predicted that the proportion of adult population with diabetes will increase by 69% for the year 2030 [2]. Type 2 diabetes is associated with decreased anti-oxidant parameters [3], and an increased oxidative stress, contributing to diabetic complications [4]. The complications of diabetes affects several organs including the eyes, brain, kidney, cardiovascular system, and the liver. Effects of diabetes on the liver include a wide range of disorders, from steatosis to cirrhosis, culminating in liver injury and distortion of the liver tissue [5]. The changes in the liver could be detected by the use of liver enzymes, which are useful biochemical markers of liver function and integrity. Lipid abnormalities associated with type 2 diabetes present in a characteristic pattern termed diabetic dyslipidaemia and plays a major role in the onset of cardiovascular disease in diabetes [6].

With the increased burden of diabetes and the unresolved complications, most patients resort to complementary and alternative medicine (CAM), in which they practice polypharmacy, combining orthodox and herbal drugs, in an attempt to improve the outcomes of their illnesses as well as to improve general well-being. The increased prevalence in the use of CAM by diabetic patients in Africa [7], particularly Nigeria, must be matched with efforts to ascertain the safety as well as the therapeutic efficacies of these drugs. Diawell is a well-known anti-diabetic herbal tablet in Nigeria used by many diabetic patients. This study evaluates the therapeutic effects of the anti-diabetic polyherbal drug diawell in combination with metformin on liver function and lipid profile in type 2 diabetic rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of 35 male Wistar albino rats weighing between 120-220 g 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 diawell and metformin. The polyherbal drug diawell, is manufactured by Kedi Healthcare Company Ltd, Hong Kong, China and commercially sold in Nigeria as an anti-diabetic tablet. Metformin, a biguanide is manufactured by by LEK SA, Poland.

2.3 Acute Toxicity Study

Acute toxicity study was done according the fixed dose procedure [8], using 3 rats. 2000 mg/kg body weight of diawell 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 signs of toxicity, hence the herbal drug diawell was deemed safe at a dose up to 2000 mg/kg body weight. Metformin is a standard anti-diabetic drug, and the dosage used in the study was translated from the human dose as described below.

2.4 Dose Calculation of Metformin and Diawell

The dose of each drug was extrapolated from the human dose using the formula by Paget and Barnes [9].

Metformin:

Human daily dose is 1 tablet (500 mg) twice daily, that is, 1000 mg/day.

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

= 90 mg/kg body weight/day.

Diawell:

Human daily dose is 4 tablets (300 mg each) three times daily, that is, 3600 mg/day.

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

= 324 mg/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 a high fat diet (HFD) with 42.1% fat content, 3 weeks prior to the induction of diabetes with streptozotocin (STZ). Diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (45 mg/kg body weight) 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 14 mmol/L (250 mg/dl) [10]. 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 metformin.

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

Group 5: Diabetic rats treated with a combination of metformin and diawell.

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 [11, 12]. The liver was also harvested and preserved in 10% formol saline for histological analysis. 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 the Glucose oxidase method [13], as modified by Randox Laboratories Limited (UK). Total Cholesterol (TC) was determined by enzymatic method [14], as modified by Randox laboratories limited (UK). Triglyceride was determined by enzymatic method [15], as described by Randox laboratories limited (UK). High Density Lipoprotein Cholesterol (HDL-C) was determined by enzymatic method [16], as

modified by Randox laboratories limited (UK). Low Density Lipoprotein Cholesterol (LDL-C) was calculated from the Friedewald's equation [17]. The liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using the Reitman-Frankel method [18], as modified by Randox laboratories limited (UK). Alkaline phosphatase (ALP) was determined using the Colorimetric Phenolphthalein method [19] as modified by Teco Diagnostics (USA). Qualitative and quantitative phytochemical analysis were done on the herbal drug using classical and spectrophotometric methods respectively [20]. Liver sections were stained using the standard haematoxylin and eosin (H&E) staining technique.

2.7 Statistical Analysis

Data 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 ± Standard deviation.

3. RESULTS

Table 1 shows alkaloids and flavonoids present in the herbal drug diawell, with concentrations of 119.27 µg/mg and 89.67 µg/mg respectively. Other phytochemicals such as phenolic acids, saponins, cardiac glycosides, terpenoids, quinones, and tannins were not found.

Table 2 shows the results of serum 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 showed 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. There were no significant differences ($p>0.05$), in TG levels in the metformin group, and diawell group when compared to the diabetic control. TG levels in the combination group (metformin + diawell) was significantly lower ($p<0.05$) than the diabetic control, and did not differ ($p>0.05$) from the negative control.

Table 1. Qualitative and quantitative phytochemical analysis of the herbal drug diawell

Phytochemicals	Diawell	Concentration (µg/mg)
Alkaloids	+ve	119.27
Flavonoids	+ve	89.67
Cardiac glycosides	-ve	
Phenols	-ve	
Phlobatanins	-ve	
Saponins	-ve	
Tanins	-ve	
Terpenoids	-ve	
Quinones	-ve	

+ve – Present, -ve – Not present
Source: Briggs et al. [30]

Table 2. Effects of treatment on the 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 (Met) n = 7	2.42 ± 0.20	0.83 ± 0.15[a]	0.63 ± 0.15[a]	1.42 ± 0.05[a]
Group 4 (Dia) n = 7	2.57 ± 0.24	0.89 ± 0.15[a]	0.60 ± 0.10[a]	1.57 ± 0.22[a]
Group 5 (Met + Dia) n = 7	2.58 ± 0.09	0.69 ± 0.10[b]	0.68 ± 0.16[a]	1.56 ± 0.12[a]
P-value	0.0842	0.0006	< 0.0001	< 0.0001
F-value	2.325	7.008	23.48	24.37

n – Number of samples, Met – Metformin, Dia – Diawell, [a] – Significantly different from the negative control, [b] – Significantly different from the diabetic control. [#]- A rat died in the diabetic group in the course of the study

The results show 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 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.

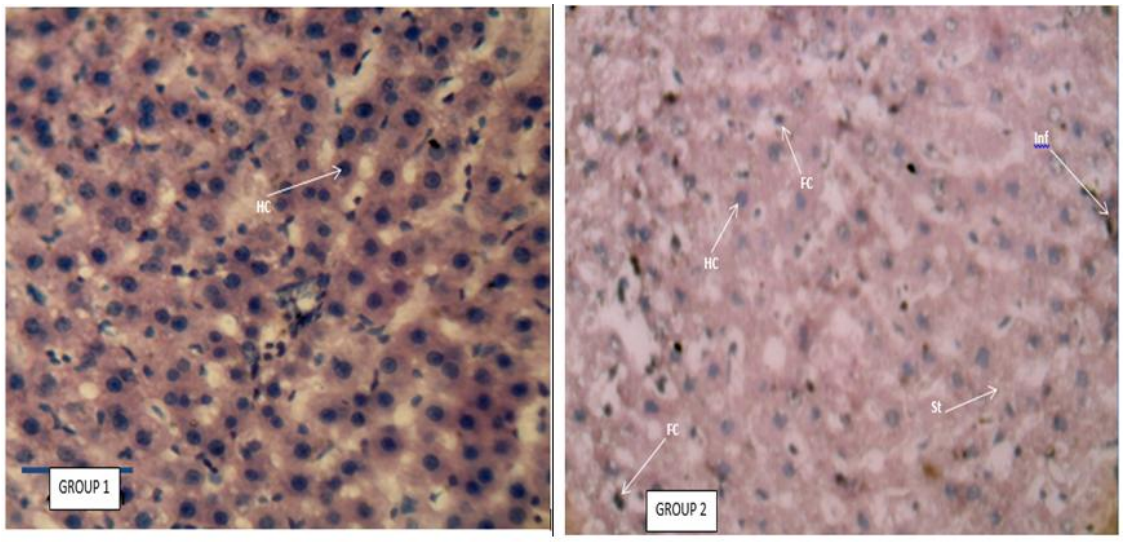
AST levels were significantly higher in the diabetic control ($p<0.05$) compared to the negative control and treatment groups. All the treatment groups showed no significant differences ($p>0.05$) in AST levels when compared to the negative control.

ALP levels were significantly higher in the diabetic control ($p<0.05$) compared to the negative control and treatment groups. ALP levels were significantly higher in the metformin treated group and diawell treated group, but showed no significant difference in the combination group ($p>0.05$) when compared to the negative control.

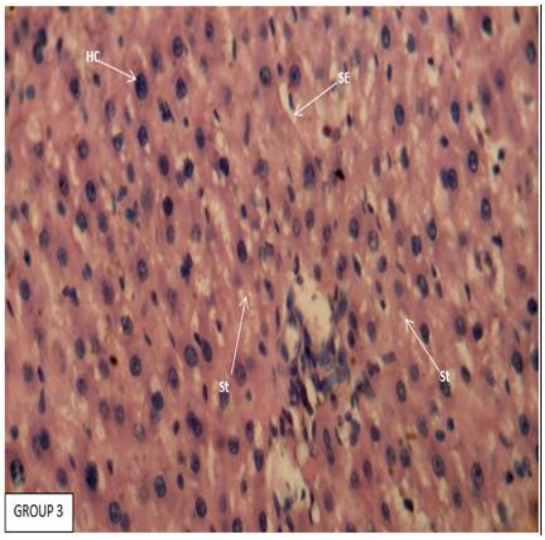
Table 3. Effects of treatment on liver enzymes of the rats

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 (Met) n = 7	19.33 ± 3.67[b]	64.33 ± 10.01[b]	110.50 ± 8.25[a b]
Group 4 (Dia) n = 7	20.33 ± 3.63[b]	63.83 ± 8.75[b]	128.00 ± 22.61[a b]
Group 5 (Met + Dia) n = 7	19.50 ± 2.35[b]	57.17 ± 15.01[b]	90.41 ± 7.80[b]
P-value	< 0.0001	0.0009	< 0.0001
F-value	12.73	6.668	45.78

n – Number of samples, Met – Metformin, Dia – Diawell, [a] – Significantly different from the negative control, [b] – Significantly different from the diabetic control. [#]- A rat died in the diabetic group in the course of the study



(a) (b)



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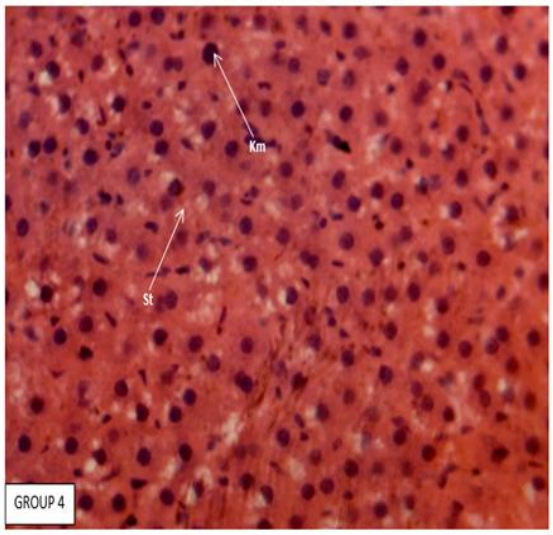

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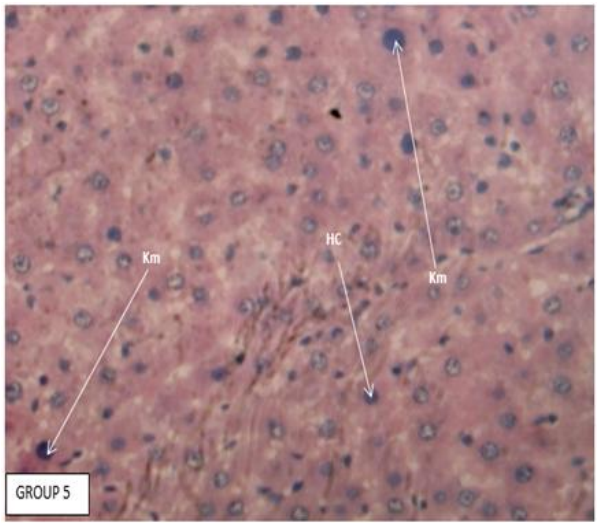

(e)

HC-Hepatocytes, St-Steatosis, Km-Karyomegaly, Inf-Inflammation, Fc- Clear Cell Foci

Fig. 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 metformin treated group (c) showed moderate hepatic steatosis and sinusoidal dilatation. (d) The diawell treated group showed minimal hepatic steatosis, mild perivascular inflammation with a nearly normal histoarchitecture. The combination group (e) showed normal radially arranged hepatocytes around the central vein, with mild fatty deposition

4. DISCUSSION

Phytochemical analysis of the polyherbal drug diawell revealed the presence of alkaloids and flavonoids in variable amounts. Plant products have been shown to contain different bioactive phytochemicals or secondary metabolites which have nutritive value, but also possess the ability

to affect metabolic pathways and produce drug-like responses. This forms the basis for their use and application in medicine [21,22].

The results from this study revealed no significant differences in total cholesterol (TC) levels in all the groups, 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 were significantly higher than that of the negative control, indicating an increase in the rate of lipolysis in the diabetic state. The results showed no significant differences in TG levels in the metformin treated group and diawell treated group, when compared to the diabetic control. This implies individual treatments had no effect on the increased TG levels in the diabetic state. The combination group (metformin + diawell) was significantly lower than the diabetic control, and did not differ from the negative control. This implies the combination therapy was effective in returning TG levels to control values. High density lipoprotein cholesterol (HDL-C) levels in the negative control was significantly higher when compared to the diabetic control and all the treatment groups. The results also revealed no significant differences in HDL-C levels in the treatment groups, when compared to the diabetic control, indicating that metformin and the polyherbal drug diawell and their combination had no effect in improving HDL-C levels in the diabetic state. Low density lipoprotein cholesterol (LDL-C) was significantly lower 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 from the diabetic control, indicating the administration of metformin, diawell, and their combination had no effect on LDL-C values in the diabetic rats.

The results of lipid profile revealed that the diabetic rats were dyslipidaemic, having normal TC levels, hypertriglyceridaemia, reduced HDL-C and a higher LDL-C levels. This abnormality was not corrected by the anti-diabetic treatments administered. Diabetes is known to have not only defective glucose metabolism but also disturbances in lipid metabolism, which mostly presents as the characteristic diabetic dyslipidaemia and is a risk factor for cardiovascular disease [6]. 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 [23].

The findings are in consonance with the works of Gupta et al. [24], in which a combination of HFD and STZ treatment produced significant dyslipidaemia, with elevated total cholesterol and triglyceride levels in diabetic Sprague-dawley rats. Similar research by Gotama et al. [25] corroborates with our findings. In their research, treatment with the herbal extract of *Sargassum hystrix* produced no significant effect on HDL-C and LDL-C levels, when compared to the diabetic control. However, they found significant treatment effect on total cholesterol and triglyceride levels. Mishra et al. [26] 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-C) 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. In the same study, metformin was found not to have any impact on the lipid profile of the diabetic rats. In another research, Gupta et al. [27] reported significant reduction in plasma and hepatic triglyceride and cholesterol levels in STZ-induced diabetic rats treated with a combination of metformin and the ethanolic extract of *Scutellaria baicalesis*. The combination treatment was also found to more effective when compared to metformin alone, suggesting *Scutellaria* enhances the antihyperlidaemic actions of metformin.

The results of liver function in this study revealed significantly elevated enzyme levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the diabetic control, compared to the negative control. Diabetes induced by high fat diet and STZ might have led to the 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 [28]. There are also reports of direct organ toxicity of STZ to the liver apart from the ensuing diabetes [29].

The results revealed significantly lower AST, ALT, 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 from the negative control group. This reveals administration of the orthodox drug metformin, the polyherbal formulation diawell and their combination had hepatoprotective effect on the liver of the diabetic rats. This may be due to the antioxidant potentials of the treatments [30], preventing oxidative damage to liver hepatocytes. From this research, diawell and metformin were not so effective in reducing ALP levels to normal control values when used as single treatments. However, when used together, ALP levels were returned to normal, indicating drug-herb synergy.

The findings are in consonance with the works of Salih et al. [29] and Khajuria et al. [31], in which STZ-induced diabetes significantly altered liver parameters resulting in elevated levels of AST, ALT, ALP and acid phosphatase (ACP) in experimental animals. In a similar study, Balamash et al. [32] observed significantly elevated ALT, AST, and ALP levels in STZ-induced diabetic rats. In their study, ALP levels remained significantly higher in the diabetic rats after treatment with metformin and or olive oil for 6 weeks. Nna et al. [33] in their work reported improved liver enzyme parameters in Malaysian propolis and metformin treated diabetic rats. They also reported that the combination of metformin and Malaysian propolis had a better effect than both treatments administered individually.

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 [30], thus providing hepatoprotective effects on the liver. This is in consonance with the work of Balamash et al. [32], in which STZ-induced diabetic rats had apoptotic hepatocytes with degenerated nuclei. Treatment with metformin, olive oil and their combination had hepatoprotective effects on the liver, restoring the liver histology.

5. CONCLUSION

High fat diet in combination with 45 mg/kg of STZ 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, metformin and the polyherbal tablet diawell had no impact on the lipid levels as it did not correct the dyslipidaema, however, the treatments showed hepatoprotective potentials and restored liver enzyme levels to normal. Although the lobular architecture of the liver was preserved, diabetes is associated with changes in liver histology. The liver histology of the treatment groups showed improvements, with a persistent fatty change. Lipid lowering drugs should be included in the management of type 2 diabetes. There should be proper evaluation of anti-diabetic herbal products and care should be taken in the use of herbal drugs as some may not be effective and treat what they claim. Utmost care should be taken in the combination of herbal drugs with orthodox medications because of the risk of drug-herb interactions, which may pose health risk.

CONSENT

It is not applicable.

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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