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Polyherbal Therapies Attenuated Diabetes Induced Liver Damage

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Abstract
To evaluate glucose, liver function, lipid profile, urea, total protein, albumin, globulin and weight of liver tissues in diabetic rats. Thirteen groups of 6 rats each were used. Groups 1 and 2 Normal and Diabetic Control received 0.5 ml Dimethylsulphoxide; 3 and 4 received 5UI/Kg b.w insulin and 5mg/Kg b.w glibenclamide; 5, 6, 7 and 8 received 500 mg/Kg b.w of *Vernonia amygdalina*, *Moringa oleifera*, *Gongronema latifolium* and *Ocimum gratissimum* extracts respectively; 9, 10 and 11 received 250 mg/ Kg b.w of *M. oleifera*/*V. amygdalina*, *M. oleifera*/ *G. latifolium* and *M. oleifera*/*O. gratissimum* respectively; 12 received 166.66mg/kg b.w of *V. amygdalina*/ *G. latifolium*/ *O. gratissimum* while 13 received 125mg/kg b.w of all extracts. There was reduction in liver weight, glucose, lipid profile and urea in all treated groups. Total protein, albumin, globulin and HDL-cholesterol increased in the treatment groups. Polyherbal treatments are potent at attenuating diabetes induced liver damage.
Keywords: Hepatoprotective, Moringa oleifera, Vernonia amygdalina, Gongronema latifolium, Ocimum gratissimum

1. Introduction

Type 1 diabetes mellitus (DM1) is a group of disorders characterized by the chronic progression of hyperglycaemia which is linked to inhibition of insulin secretion from ß-cells of the pancrease. This results in metabolic defects and tissue damage which is implicated in the pathological process of complications [1]. These includes several macrovascular and microvascular complications. Macrovascular complications araise from defects in insulin secretion [2] due to oxidative stress induced by hyperglycemia. Such complications include peripheral arterial disease, coronary heart disease, and stroke [3]. Microvascular complications include retinopathy, nephropathy, peripheral and autonomic neuropathies as well as lower extremity disease [4].

Hyperlipidemia and liver damage has also been reported in later stages of diabetes due to disorders in lipid metabolism, increased gluconeogenesis and ketogenesis [5]. The liver plays a central role in the maintenance of glucose homeostasis. It collects glucose from the blood which is utilized as fuel. It also stores dietary glucose in the form of glycogen for future use as well as synthesize glucose from non-carbohydrate sources for the maintenance of blood glucose level during fasting [6]. Examples of liver abnormalities in diabetes include abnormal glycogen deposition, acute liver disease, non-alcoholic fatty liver disease (NAFLD), cirrhosis, fibrosis, hepatocellular carcinomas (HCCs), abnormal elevated hepatic enzymes and viral hepatitis [7]. Increased insulin resistance and hyperglycaemia have resulted in fatty liver and subsequent destruction of liver cells. These have contributed to increased morbidity and mortality [8].

Results from previous research have shown that the liver stops oxidising fatty acids and uses them instead to synthesise triglycerides which eventually is accumulated abnormally in the liver [9]. Furthermore, insulin resistance causes lipolysis which results in the increase of circulating fatty acids taken up by the liver as an energy source. The accumulation of these fatty acids, disrupts the β-oxidation system in the hepatic mitochondria leading to further infiltration of fats in the liver [10, 11]. In insulin dependent DM, there is upregulation of hormone-sensitive lipase in the adipose tissues resulting to insulin deficiency which leads to increased lipolysis and increase in the circulation of free fatty acids, which subsequently accumulates in the liver. These processes enhance the hepatic uptake of very-low-density lipoproteins and synthesis of triglycerides [12].

To access how functional the liver is, liver function tests (LFTs) have been used as reliable biochemical assays for hepatic malfunction [13]. Serum concentrations of liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT)] are strongly linked to glycemic status and/or insulin resistance [14, 15].

Increasing antioxidant intake by people with DM-induced liver damage may ameliorate the effects of

oxidative stress and inflammation, thus, reducing the severity of diabetic complications. Phytochemicals and vitamins have been investigated as ways of protecting against and possibly reversing liver damage caused by oxidative stress and inflammation [16, 17]. Far above exploring single herbal therapy in the management of DM, polyherbal therapy have been investigated. Results have shown marked amelioration of damaged tissue with single therapy[18, 19]. Sequel to this, it became imperative to do a polyherbal study on common local herbs in Nigeria that have been used traditionally in the management of DM [20, 21, 22]. *Vernonia amgdalina* [23, 24], *Moringa oleifera* [25, 26], *Gongronema latifolium* [27, 28] and *ocimum gratissimum* [29] have been shown to have tissue protective effects.

2. Materials and methods

2.1 Sample collection and identification

Fresh leaves weighing (1kg) each of *V. amgdalina*, *M. oleifera*, *G. latifolium* and *O. gratissimum* was harvested from the Endocrine Research Farm, Calabar, Cross River State. All plants were identified at the herbarium of the Department of botany, University of Calabar, Calabar.

2.2 Plant materials and preparation of extracts

Fresh leaves of *V. amgdalina*, *M. oleifera*, *G. latifolium* and *O. gratissimum* were collected, macerated and allowed to stand in 80% alcohol for 48 hours at room temperature. The filtrate was later evaporated in a rotary evaporator and allowed to concentrate in a water bath at 36° C. A greenish paste was obtained. The extraction of *V. amgdalina*, *M. oleifera*, *G. latifolium* and *O. gratissimum* leaves was done in the Department of Biochemistry, University of Calabar. The obtained leaf extracts were stored at 4°C.

2.3 Animals

Seventy eight rats weighing between 140 - 180g, were gotten from the Department of Biochemistry animal house, University of Calabar and divided into thirteen groups of 6 rats each. Before the experiment, the rats were allowed to acclimatize to the animal house for 7 days. They were maintained in a controlled environment (12 hours dark/light cycle) and temperature (30 + 2°C). All the animals were fed with standard rat chow and water was allowed ad- libitum under strict hygienic conditions.

2.4 Induction of diabetes

Diabetes was induced in a 12hrs fasted rats with streptozotocin (40mg/kg b.w) dissolved in citrate buffer (0.1 M, pH 4.5) and injected intraperitoneally in a volume of 0.5 ml citrate buffer/rat. After 48hrs of injection, diabetes was confirmed with a fasting blood sugar (FBS) concentration ≥ 200mg/dl. This was estimated using One Touch ® Glucometer (Lifescan, Inc. 1996 Milpas, California, U.S.A) with blood obtained from the tail vein of the rats.

2.5 Experimental design

Table 1. Animal grouping and treatment schedule

S/no	No of Animals	Treatment
1	6	Normal control
2	6	Placebo (Diabetic control)
3	6	Insulin (5 IU/kg b.w s.c.)
4	6	Glibenclamide (0.5ml)
5	6	*V. amygdalina* (500mg/kg b.w)
6	6	*M. oleifera* (500mg/kg b.w)
7	6	*G. latifolium* (500mg/kg b.w)
8	6	*O. gratissimum* (500mg/kg b.w)
9	6	*M. oleifera/ V. amygdalina* (250mg/kg b.w each)
10	6	*M. oleifera/ G. latifolium* (250mg/kg b.w each)
11	6	*M. oleifera/ O. gratissimum* (250mg/kg b.w each)
12	6	*V. amygdalina/ G. latifolium/ O. gratissimum* (166.66mg/kg b.w)
13	6	*V. amygdalina/ G. latifolium/ O. gratissimum/ M. oleifera (125mg/kg b.w)*

2.6 Acute toxicity studies

The oral acute toxicity study of *V. amgdalina*, *M. oleifera*, *G. latifolium* and *O. gratissimum* was determined in mice as described by Lorke, 1983 [30].

2.7 Liver lipid extraction

Liver lipids were extracted as described by [31]. Briefly, 1g of liver tissue from each group was homogenized in

20 ml chloroform/methanol, agitated for 15 to 20 min in an orbital shaker and centrifuged (3500xg) to recover the liquid phase. 0.2 ml of water was added to the recovered solvent vortexed at 2000 rpm for some seconds and centrifuged at 3500xg to separate the two phases. The lower phase containing liver lipids was collected for lipid profile determination.

2.8 Determination of biochemical parameters

The biochemical analysis of liver samples was performed using Randox reagent kits. Biochemical parameters measured were aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, glucose, urea, triacylglycerol, total cholesterol, HDL-cholesterol, total protein, albumin and globulin.

VLDL and LDL are estimated from relationships established by [32] using triglyceride, HDL-cholesterol and cholesterol concentrations, provided these conditions are fulfilled: no chylomicrons are present in the sample; the triglyceride concentration in the sample does not exceed 400mg/dl; the sample does not show signs of any type III hyperlipoproteinaemia.

$$\text{VLDL conc. (mg/dl)} = \frac{\text{Triacylglycerol concentration in sample}}{2.2}$$

$$\text{VLDL conc. (mmol/L)} = \frac{\text{Triacylglycerol concentration in sample}}{5}$$

$$\text{LDL conc. (mg/dl)} = \frac{\text{Total Cholesterol} - \text{Triacylglycerol} - \text{HDL-Cholesterol}}{2.2}$$

$$\text{LDL conc. (mmol/L)} = \frac{\text{Total Cholesterol} - \text{Triacylglycerol} - \text{HDL-Cholesterol}}{5}$$

In brief: LDL conc. = TC – (VLDL + HDL)

2.9 Statistical analysis

The results were analyzed for statistical significance by one way analysis of variance (ANOVA) using the SPSS 17 statistical program and Post Hoc Test (LSD) between groups. All data were expressed as mean ± SD. P values < 0.05 were considered significant.

3. Results & Discussion

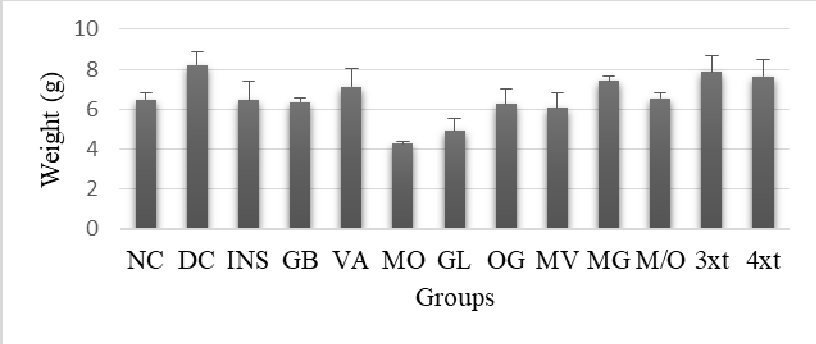

Figure 1. The effect of treatment on liver weight

The result of this investigation indicates clearly that there was an enlargement of the liver tissue in untreated diabetic rats as seen in figure 1. Non-alcoholic fatty liver disease has been linked to hepatomegaly (increase in liver size) in uncontrolled diabetes [33]. This observation is in line with [34] where it was stated that glycogenic hepatopathy is characterized by elevated liver enzymes and hepatomegaly in diabetes. Oral administration of ethanolic leaf-extract of *V. amgdalina*, *M. oleifera*, *G. latifolium* and *O. gratissimum* in single and polyherbal therapies showed a reduction in the liver weight. MO group had the lowest weight when compared with other groups. 3xt and 4xt groups also showed a reduction in liver weight but these results was not significant at ($p>0.05$) when compared with DC. Weight of liver for INS and GB was comparable with NC.

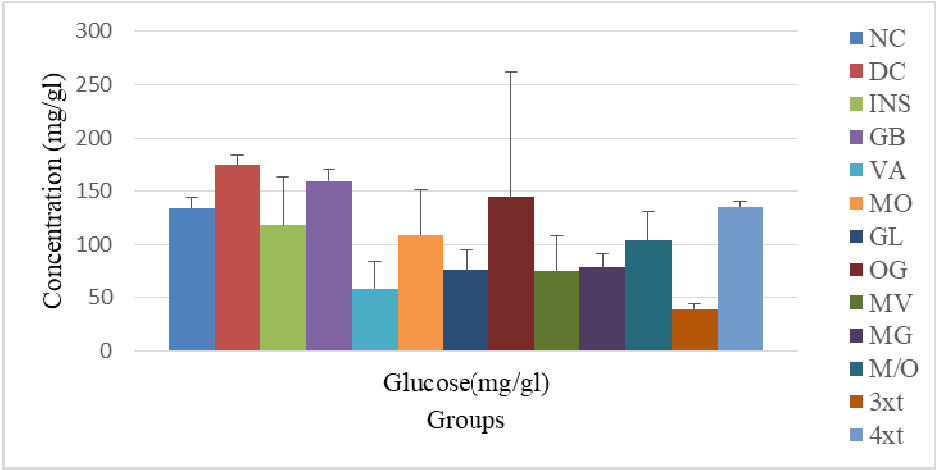

Figure 2. The effect of treatment on liver glucose

From figure 2, glucose levels of DC increased significantly at ($p<0.05$) when compared with NC. This observation is in consonant with that of [6, 35] where glycogenic hepatopathy (GH) is predominantly seen in patients with longstanding type 1 diabetes mellitus. Also [36] reported that fast onset of hyperglycemia resulted in the accumulattion of glycogen leading to GH. Levels of liver glucose in all treatment groups decreased. However, synergistic effect was recorded in all polyherbal groups except 4xt groups. This result agrees with that of [34] .

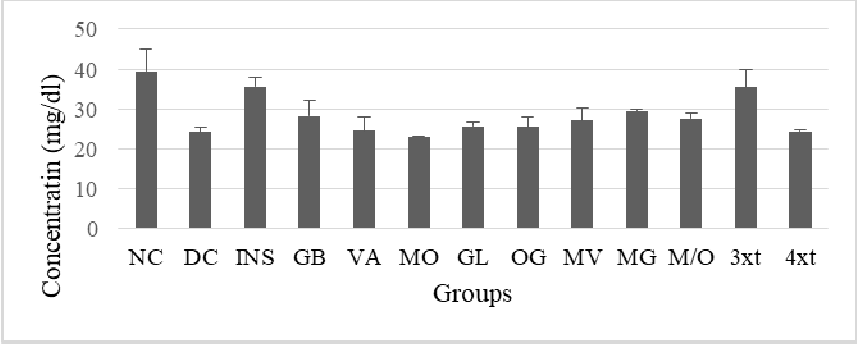

Figure 3. The effect of treatment on liver urea

Urea concentration of liver decreased significantly from figure 3 at ($p<0.05$) in the untreated group when compared with NC. The liver is responsible for the conversion of amino acids generated from muscles and other tissues in uncontrolled diabetes to ammonia and then urea. An elevated urine and serum levels of urea in diabetics is as a result of increased metabolism of amino acids. According to [37] urea level in untreated diabetics declined. From our study, there was a non-significant increase in urea concentration for all treated groups at ($p<0.05$) when compared with DC. INS and 3xt was an exception to this as urea level was significantly increased at ($p<0.05$) when compared with DC. Synergistic effect was not significant ($p>0.05$) for the polyherbal treatment groups except 3xt groups.

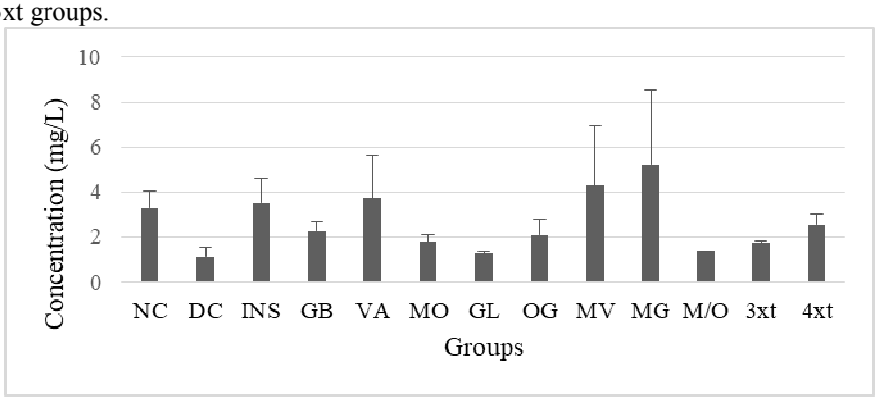

Figure 4. The effect of treatment on liver total protein

Figure 4 represents results for total protein. There was a significant reduction in total protein concentration at ($p<0.05$) for DC when compared with NC. Uncontrrolled diabetes resulted in increased catabolism of protein in the liver which translates to reduced liver total protein content [38]. The reverse was the case across all treatment groups. More significant increase at ($p<0.05$) was observed for MV and MG groups. Synergistic effect

was not significant (p>0.05) for the polyherbal groups except MV and MG groups.

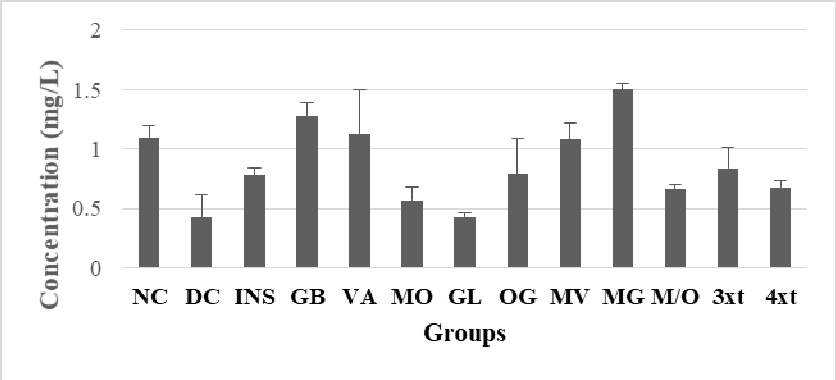

Figure 5. The effect of treatment on liver albumin

Albumin concentration from figure 5 reduced significantly (p<0.05) in DC. Abu-Lebdeh and Nair, 1996 reported that insulin deficiency resulted in protein breakdown as confirmed from fig. 5. The same result showed significant (p<0.05) increase across all treated groups except GL treated group. Synergistic effect was observed in all polyherbal groups but more significant (p<0.05) in MV and MG.

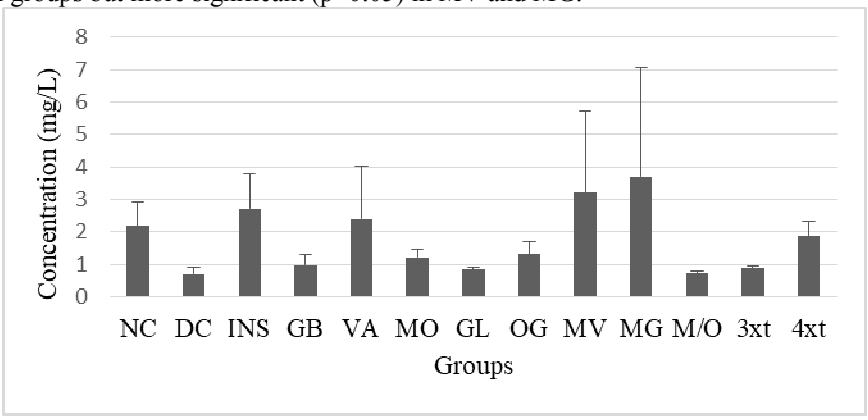

Figure 6. The effect of treatment on liver globulin

The effect of treatment on globulin levels in diabetic animals is represented in figure 6. DC group showed a significant (p<0.05) reduction in globulin concentration when compared with NC. [39] reported that diabetes caused a reduction in concentration of albumin. The reciprocal result was observed for all treated groups GL, MO and 3xt groups that recorded a non-significant (p>0.05) increase when compared with DC. Synergistic effect was noticed only in MV, MG and 4xt groups.

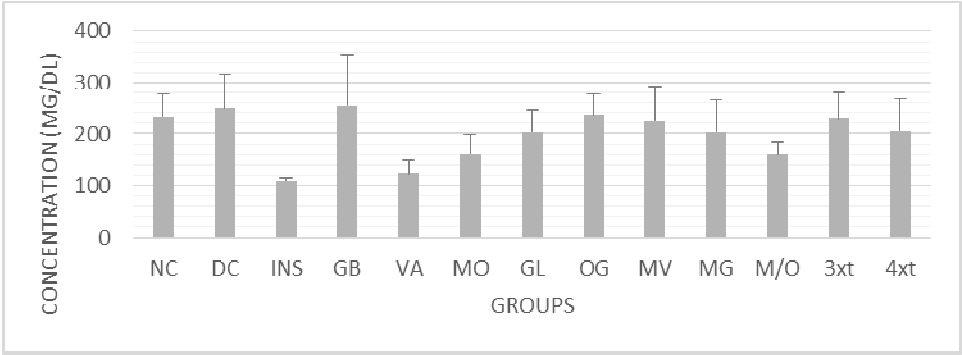

Figure 7. The effect of treatment on liver triacylglyceride

Figure 7 shows the effect of treatment on TG concentration. There was an increase in TG concentration for DC when compared with NC. This result agrees with that of [40]. The reverse was observed for the treated groups except GB whose concentration compared with DC. Synergistic effect was only recorded for M/O treated group.

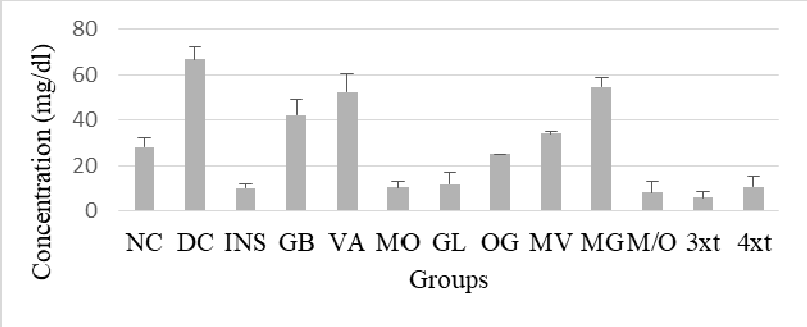

Figure 8. The effect of treatment on liver total cholesterol

Total cholesterol from figure 8 increased significantly ($p<0.05$) in DC but reversed in all treated groups. This was also reported by [41]. A synergistic effect was observed for all combined groups except MG.

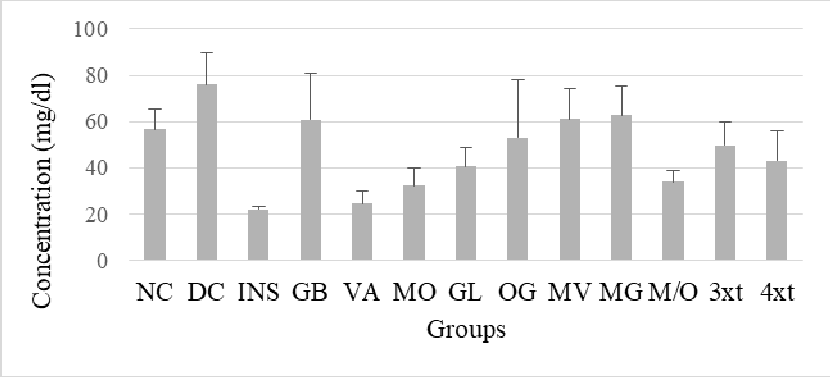

Figure 9. The effect of treatment on liver Very Low Density Lipoprotein cholesterol

Figure 9 represents the concentration of VLDL. Results showed significant ($p<0.05$) increase for DC which was reduced in all treated groups. [41] reported similar results. The results compared well with NC. A synergistic effect was observed for MO, 3xt and 4xt groups.

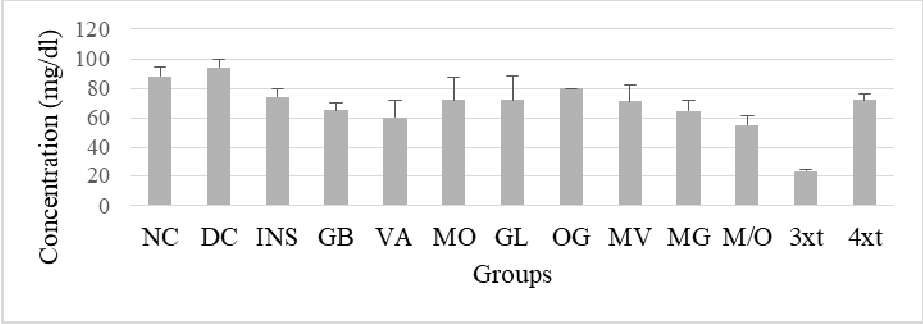

Figure 10. The effect of treatment on liver Low Density Lipoprotein

Figure 10 represents the concentration of LDL- cholesterol. There was a significant ($p<0.05$) increase in the untreated diabetic group. This result was reversed in the treated groups. [40] reported similar result. A more significant ($p<0.05$) decrease was recorded for 3xt group while synergism was observed in MV, MG, M/O and 3xt groups.

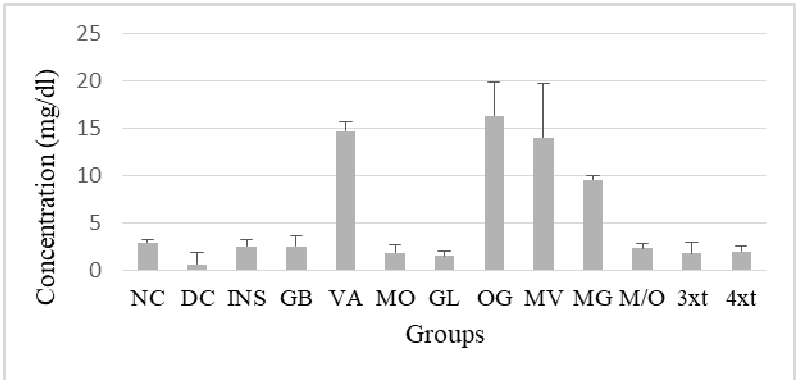

Figure 11. The effect of treatment on liver High Density Lipoprotein

HDL concentration from figure 11 showed a significant ($p<0.05$) reduction for DC when compared with NC. The concentration increased for all groups. [40] reported similar result. A significant ($p<0.05$) increase for HDL was recorded for groups treated with VA, OG, MO and M/G. Although MO level was not significantly ($p<0.05$) elevated, when in combination with GL. There was a synergistic effect which lead to increase in MG concentration. Same result was reported for MV. The combined extracted in M/O, 3xt and 4xt groups didn't show a synergistic effect.

Generally, the hepatoprotective effects of ethanolic extracts of *V. amygdalina, M. oleifera, G. latifolium* and *O. gratissimum* are not unconnected with their active principles such as alkaloids, flavonoids, tannins, glycosides which are documented to have hypoglycaemic, hypolipidemic, hypocholesterolemic and antioxidant properties [19, 23, 26].

4. Conclusions

The treatment groups showed an amelioration of damage to the liver tissues especially the combined groups. Prolonged administration of the extract should possibly completely reverse the insult to the tissues. In general, the combination of *M. oleifera/ V. amygdalina* and M. oleifera/ *G. latifolium* followed by M. oleifera/ O. gratissimum and *V. amygdalina*/ M. oleifera/ *G. latifolium* are more preferred treatment options for the attenuation of diabetes induced liver damage when compared with single extracts and the conventional anti-diabetic drugs.

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