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METHANOLIC EXTRACTS OF VERNONIA AMYGDALINA DEL. AND OCIMUM GRATISSIMUM IMPROVEDS LIVER FUNCTION IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

This study investigated the combined effects of *Vernonia amygdalina* and *Ocimum gratissimum* on some liver enzymes; aspartate aminotransferase (ALT) and alanine aminotransferase (AST), serum albumin, bilirubin and total protein in streptozotocin-induced diabetic rats. Experiments were performed in 36 adult male Wistar rats weighing 150-270 g. Thirty rats received 65 mg/kg body weight of streptozotocin (i.p). They were randomly divided into three groups (A, B, C) of 10 each. Six non diabetic rats served as control (CONT). Group A rats received 6 I/U insulin injection per day. Group B received methanolic extract (100mg/dl *Vernonia amygdalina* and 200mg/kg *Ocimum gratissimum)* per day. Group C did not receive treatment. Data showed that AST and ALT were markedly decreased only in insulin treated diabetic rats and methanolic extract treated diabetic rats respectively. Serum albumin decreased significantly in group C rats with increased bilirubin leve. The total protein did not differ significantly among groups. The haematoxylin and eosin-stained section showed a lymphocytic infiltration in the hepatocytes with the presence of haemorrhage, improved hepatocytes plates and absence of necrosis in group B. Results indicate that methanolic extract may restore liver enzymes; AST and ALT, serum albumin, serum bilirubin, total protein; although the hepatocytes plates are altered and marked lymphocytic infiltration, hence effect of the extract may be time-dependent.

Keywords: *vernonia amygdalina***.** *ocimum gratissimum***.** alanine aminotransferase**.** aspartate aminotransferase**.** bilirubin**.** albumin

INTRODUCTION

Diabetes mellitus is a disorder basically characterized by high levels of blood glucose caused by defective insulin production; actions that are often responsible for severe health problems and early death (Leahy, 2005). Streptozotocin is widely used to induce insulin-dependent diabetes mellitus in experimental animals because of its toxic effects on islet beta cells (Ohno *et al*, 2000; Merzouk *et al*, 2000). Herbal medicine has been used for many years by different culture around the world for the treatment of diabetes. Many traditional treatments for diabetes exist, wherein lies a hidden wealth of potentially useful natural products for diabetes control (Gray *et al*, 2000). Nonetheless, a few traditional anti-diabetic plants have received scientific or medical scrutiny, despite recommendations by the WHO (1980). *Vernonia amygdalina* (Del.) is a multipurpose and rapid regenerating soft wooded shrub of 2 to 10m tall with petiolate leaves around 6mm in diameter. This plant has been named differently by different ethnics in Nigeria; Ibo (onugbu, olugbu), Hausa (shiwaka) and Yoruba (Ewuro). *Vernonia amygdalina Del.* is common homestead farming vegetable and fodder tree in Nigeria (Ndaeyo, 2007) and has been used as an ingredient to prepare Nigerian (Ogbono soup) of Cameroon (Ndole) dish after removal of

its bitter taste through soaking in several changes of water or boiling (Onabanjo and Oguntona, 2003).
Many experimental studies of *Vernonia amygdalina Del.* have reported that this plant possess anti-oxidant activity (Ayoola *et al,* 2008), hypolipidemic effect (Adaramoye *et al,* 2009), anti-diabetic activity (Erasto *et al*, 2009; Taiwo *et al,* 2009) and liver protective effect (Arhoghro *et al,* 2009; Adesanoye and Farombi, 2009).

Ocimum gratissimum is a perennial plant that is woody at the base with an average height of 1-3m high. The leaves are broad and narrowly ovate, usually 5-13 cm long and 3-9 cm wide. It is a scented shrub with lime-green fuzzy leaves (Wagner *et al*, 1999). In Nigeria, this plant is called "effinrin-nia" by the Yoruba speaking tribe, "nchumou" in Ibo and "daidoya" in Hausa (Effraim *et al,* 2001). *Ocimum gratissimum* is extensively used throughout West Africa as a febrifuge, anti-malarial and anti-convulsant (Ezekwesili *et al,* 2004). In the savannah areas, decoctions of the leaves are used to treat mental illness (Abdulrahman, 1992). The leaves of *Ocimum gratissimum* are used as a laxative and its infusion serves as a relief for respiratory disorders, headaches, fever, cold, dysentery, pile and convulsion (Danziel, 1980; Idu *et al,* 2005). Administration of aqueous leaf extract caused a statistically significant reduction in plasma glucose level in streptozotocin-induced diabetic rats and appears non-toxic (Egesie *et al*, 2006).

Many studies have shown an association between specific diabetic complication and disturbances in various tissues, such as diabetic nephropathy and peripheral neuropathy, but only limited data is available on the possible association between diabetic complication and liver function (Gu *et al,* 1997; Arkkila *et al*, 2001). The liver is the most important organ for regulating glucose metabolism by assimilating increased blood glucose in the form of glycogen and/or regulating the new synthesis of glucose through gluconeogenesis (Yoon *et al*, 2001). Enzymes directly associated with the conversion of amino acid to keto acids are alanine aminotransferase (ALT) and aspartate aminotransferase (AST). ALT and AST activities are used as the indicators of hepatocyte damage (Whitehead *et al,* 1999).
Therefore, the aim of this present study is to investigate the combined effect of *Vernonia amygdalina* and *Ocimum gratissimum* on some liver enzymes, serum albumin, bilurubin, total protein and the liver (in comparison with insulin treatment) in streptozotocin-induced diabetic Wistar rats.

MATERIALS AND METHODS

Rats and Experimental Protocol

Thirty six adult male Wistar rats (University of Nsukka, Nigeria) weighing 150-270g were used in this study. They were placed individually in cages in a room with constant temperature in the animal house of Faculty of Basic Medical Sciences, Madonna University Elele, Rivers State, and had access to tap water and normal rat food. Experimental procedures involving the animals and care were conducted in conformity with the institutional guidelines that are in compliance with National and International Laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research.

Induction of Diabetes

Streptozotocin (STZ) (Sigma Chemical Co., St Louis, MO, USA), 500 mg/ml was freshly prepared in 0.1 mol of citrate buffer (pH 4.5). After four days of accustomation to cages

and basal measurements (Table 1) thirty rats received a single intraperitoneal (i.p) injection of STZ, 65 mg/kg body weight (BW). Six control rats (CONT) received only citrate buffer. On the first week of STZ injection, the rats were fasted for 6 hours; glucose was measured in blood collected from the tail artery after a small incision with a razor blade. They all exhibited glucose value above 200 mg/dl after four weeks. The thirty diabetic rats were randomly divided into three experimental groups of ten rats each. The group A represents diabetic rats that received insulin (n=10) The group B represents as diabetic rats treated with combined extract of *Vernonia amygdalina* and *Ocimum gratissimum* (n=10) The group C represents diabetic rats without treatment (n=10)
The CONT represents control rats that received citrate buffer (n=6)

Collection of Blood Sample and Organ
At the end of two weeks treatment, the rats were anaesthetized using chloroform following 12 hours fasting blood glucose and then were sacrificed. Blood was collected by direct cardiac puncture with a 5ml syringe and put into a heparinized sample bottles. The livers were dissected out quickly and stored in 10% formyl saline.

Preparation of Extracts
The fresh leaves of the plants (*Vernonia amygdalina,* 400g and *Ocimum gratissimum,* 500g) were separately sundried, pulverized, dissolved in 500 ml of methanol (95%) and filtered. The filtrate was concentrated and evaporated to dryness in vacuo at 40 degree centigrade using rotary evaporator. The yield was calculated and the dry extract was stored in a refrigerator at -4 degree centigrade until use for the experiment. During the two weeks of the experiment, the crude extracts were dissolved in distilled water and administered to the animals orally at 100mg/kg and 200mg/kg for *Vernonia amygdalina* and *Ocimum gratissimum* respectively.

Biochemical measurements, Hormonal Assay and Histopathological studies
Glucose was carried out by the glucose oxidase principle (Beach and Turner, 1958). Serum enzymes for alanine aminotransferase and aspartate aminotransferase were estimated using Randox test kit (Reitman and Frankel, 1957). Serum bilurubin was estimated using the colorimetric method as described by Jendrassik and Grof (1938). Serum albumin was estimated using the photometric colorimetric test (BCG-method) (Doumans, 1971) and the total protein estimated with the Biuret method. The harvested liver were routinely processed and stained with Hematoxylin and Eosin.

Statistical Analysis
Statistical analyses were carried out by SPSS program for windows. Data were expressed as Mean$\pm$SEM. Statistical analysis was performed using One-way analysis of variance (ANOVA) followed by Tukey post hoc test. The criterion for statistical significance was $P < 0.05$

Results
At the end of 6 weeks of the experiment, the results were expressed in mean values. The plasma glucose concentration was markedly elevated in the diabetic rats before treatment; suggesting a diabetic state with statistical significance at $P < 0.05$ in CONT (80.0 ± 1.84) when compared with groups A, B, and C (245.4 ± 5.22; 256.2 ± 5.22 and

230.0 ± 5.22). At the end of the two weeks duration of the treatment, a decrease in the fasting blood glucose was observed in group A (48.0 ± 1.41) and group B (90.0 ± 3.45) respectively and was significant when compared with group C (247.0 ± 5.21) at $P < 0.05$. (Group A was very significant when compared with group C at $P < 0.05$).

Serum AST and ALT levels were markedly decreased in the group A and B (AST, 155.2 ± 4.04 and 174.4 ± 4.79; ALT, 79.4 ± 4.01 and 75.2 ± 2.43) respectively when compared with group C (AST, 224.0 ± 11.11; ALT, 335.6 ± 32.11) at $P < 0.05$. However, no significant difference was observed in serum AST and ALT levels in groups (A and B) when compared to CONT (AST, 198.8 ± 2.47; ALT, 84.6 ± 1.72) at $P > 0.05$. There was a decreased serum albumin level in the group C rats (35.6 ± 1.86) when compared with CONT (56.2 ± 6.04), group A (45.8±1.31) and group B (55.0 ± 3.80) at $P < 0.05$. However, a significant difference was observed between group A and group B at $P < 0.05$.

The serum bilirubin increased markedly in the group C rats (45.74 ± 1.48) when compared with CONT (34.16 ± 1.22), group A (15.54 ± 0.19) and group B (20.44 ± 1.58). There was also significant difference ($P < 0.05$) between the CONT and groups (A and B). There was no significant difference ($P > 0.05$) in the total protein among groups.
Histological sections are showed in Figures 5-8. Figure 5 shows the normal photomicrograph of liver architecture in CONT rats with normal lobular pattern, well-defined central veins and radiating anastomosing plates of hepatocytes and intervening sinusoids. Figure 6 shows the photomicrograph of liver architecture in group A rats with slight distortion of the cellular architecture and nuclear pattern and normal plates of hepatocytes. Figure 7 shows the photomicrograph of liver architecture in group B rats with normal cellular architecture, abnormal plates of hepatocytes and presence of a haemorrhage. Figure 8 shows the photomicrograph of liver architecture in group C rats with accumulation of lipid droplets and lymphocytic infiltration.

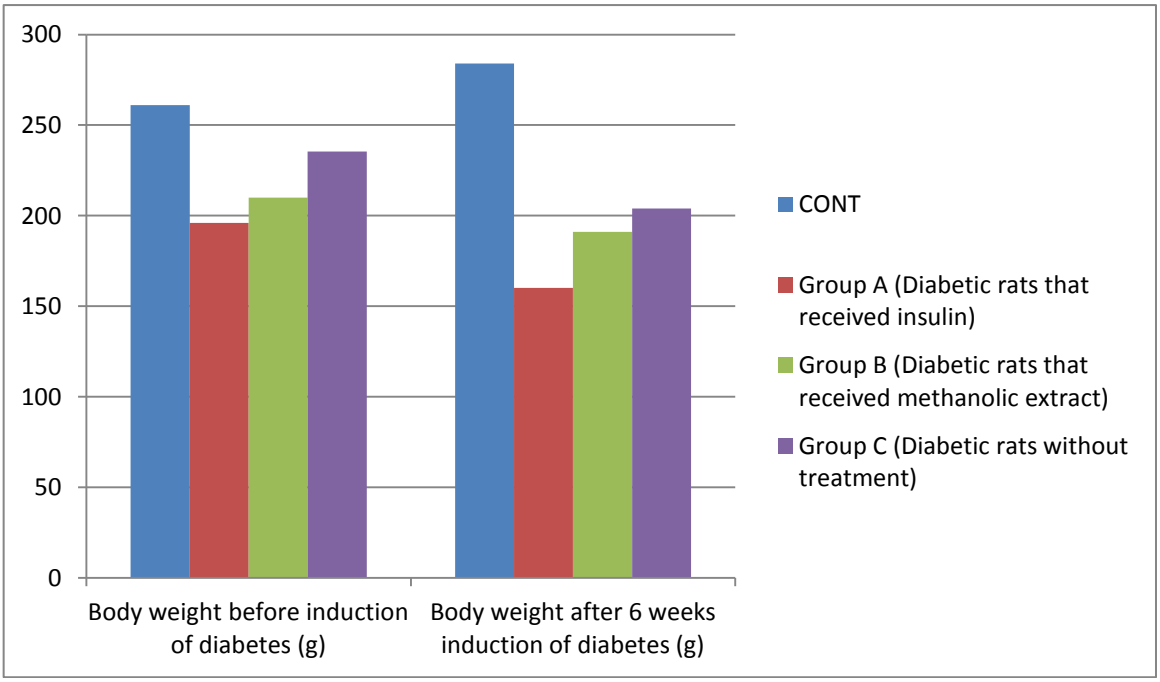

Figure 1. Body weight of the control (CONT) and experimental rats before and 6 weeks after induction of diabetes

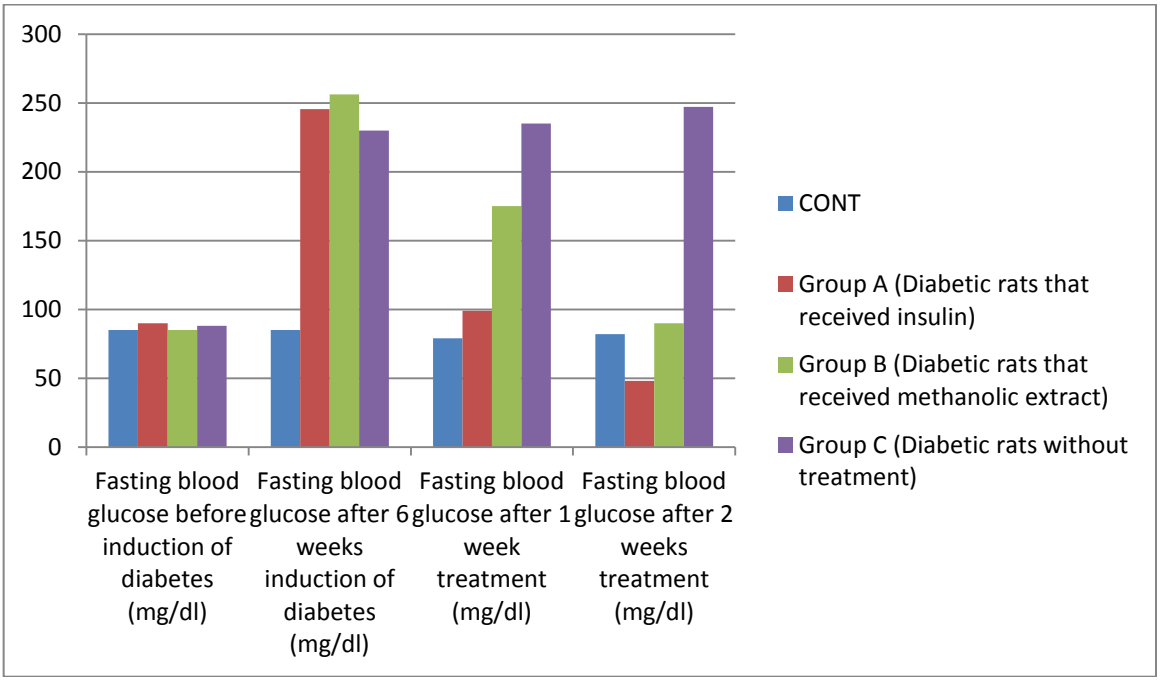

Figure 2. Fasting blood glucose before and after 6 weeks induction of diabetes. Fasting blood glucose level after 1 week and 2 weeks treatment

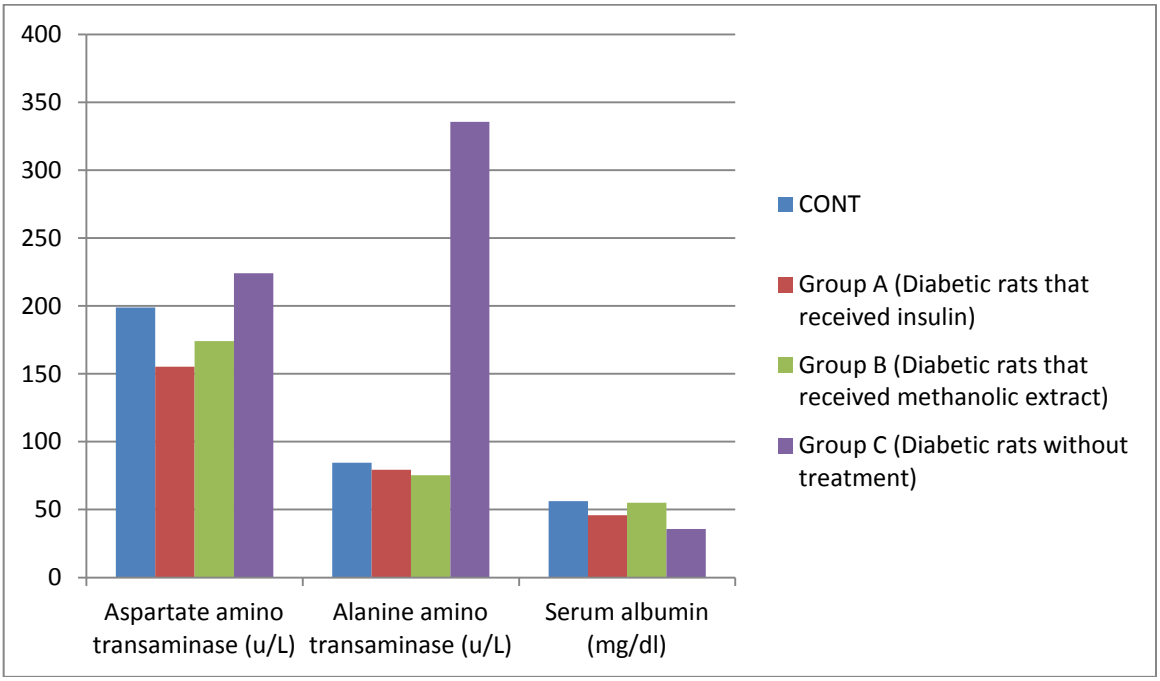

Figure 3. The serum level of aspartate amino transferase (AST), alanine amino transferase (ALT), and serum albumin in the control (CONT) and experimental rats after 2 weeks treatment

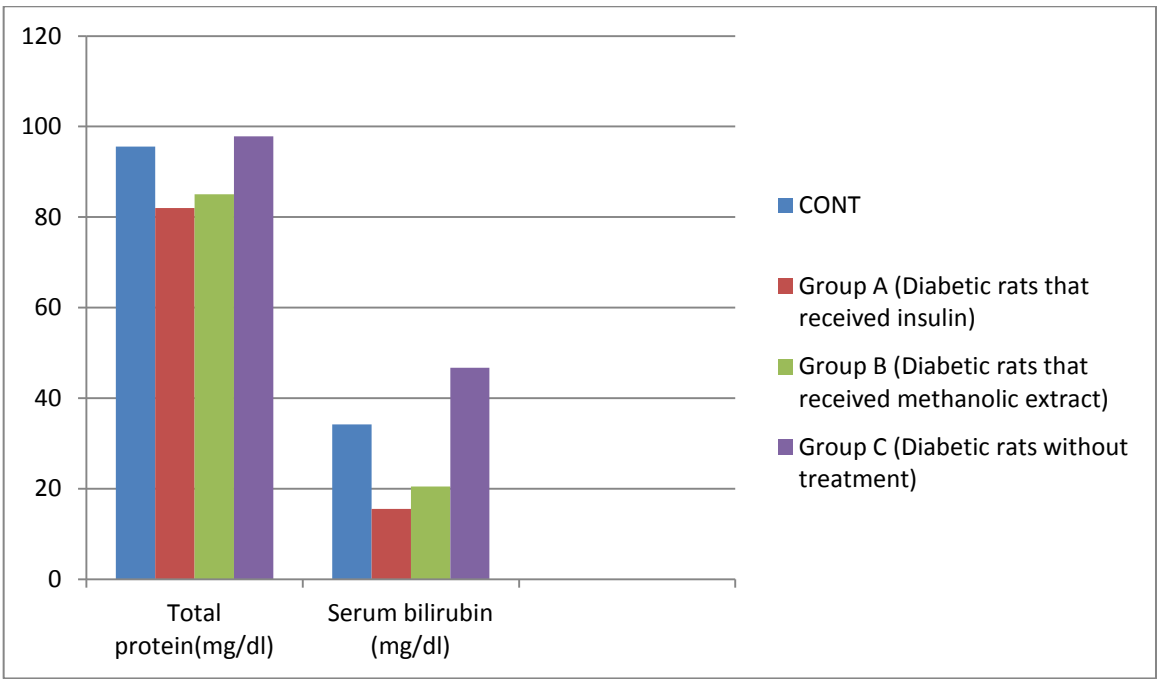

Figure 4. The serum level of total protein and bilirubin after two weeks of treatment

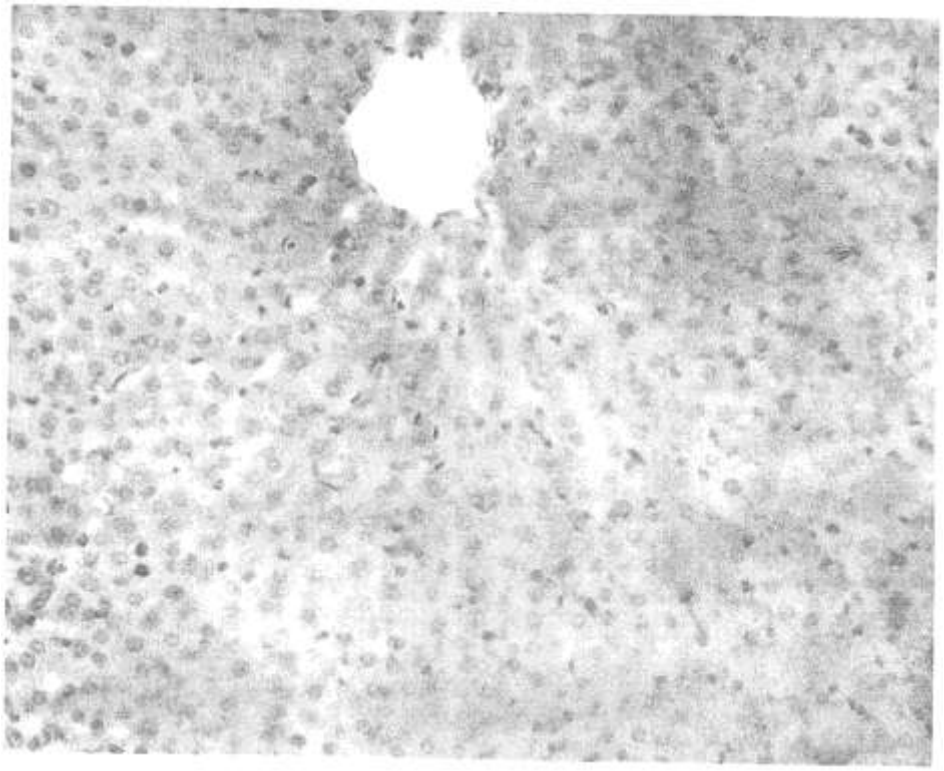

Figure 5. Photomicrograph of 3-5 mm thick H&E stained paraffin section from the liver of the normal control rats (CONT) showing normal lobular pattern with a pronounced central vein and radiating plates of hepatocytes with intervening sinusoids

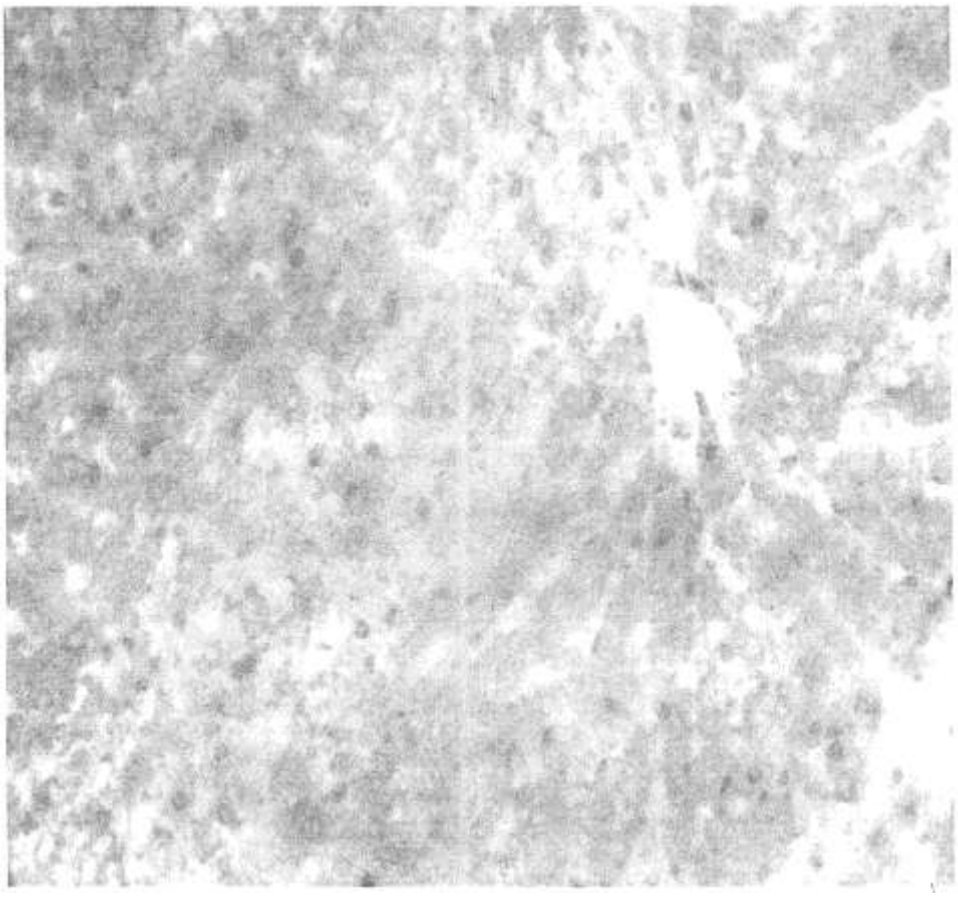

Figure 6. Photomicrograph of 3-5 thick H&E stained paraffin section from the liver in streptozotocin treated rats (group A) showing lymphatic infiltration and increased fibrous content in the portal tract

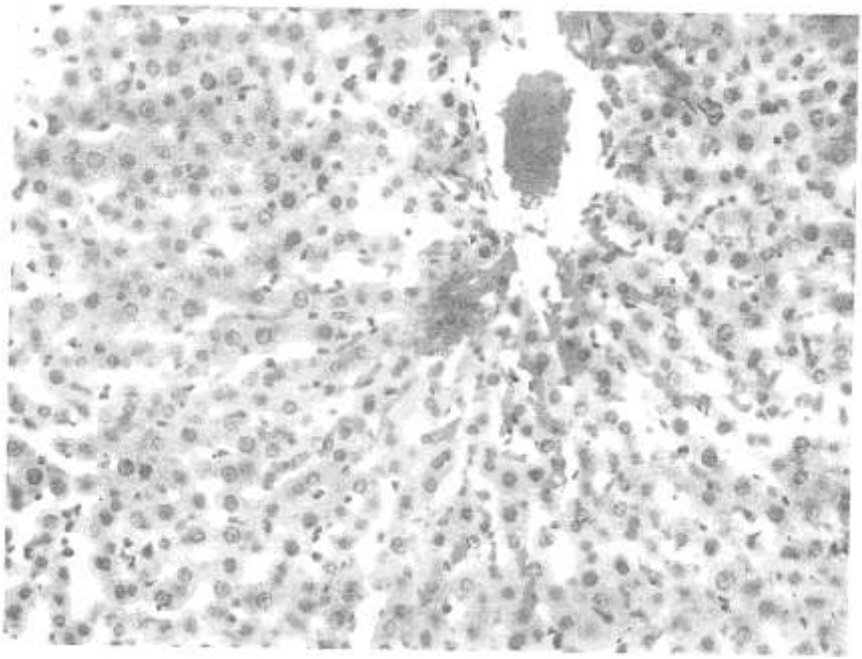

Figure 7. Photomicrograph of 3-5 mm thick H&E stained paraffin section of liver in streptozotocin treated rats with methanolic extract (Vernonia amygdalina and Ocimum gratissimum) showing lymphocytic infiltration

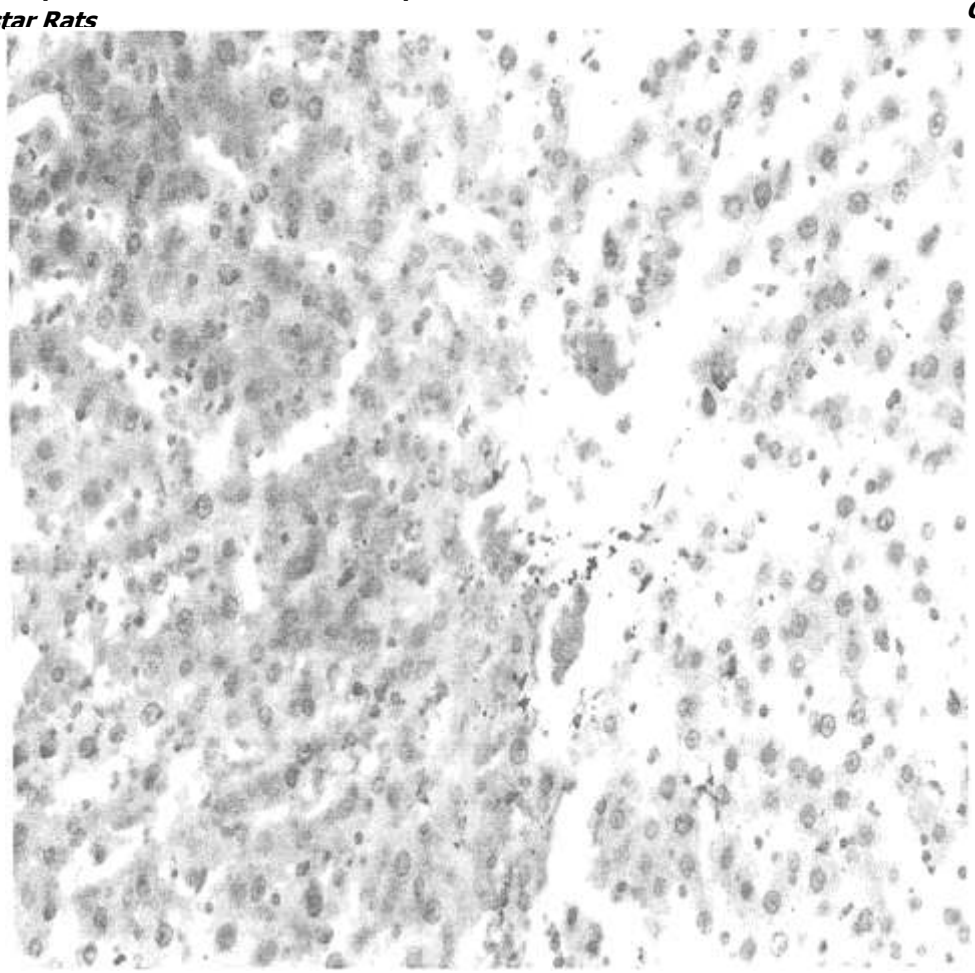

Figure 8. Photomicrograph of 3-5 mm thick H&E stained paraffin section of liver in streptozotocin rats without treatment showing pronounced lymphocytic infiltration and necrosis

DISCUSSION

Serum AST, ALT and bilirubin were significantly reduced, with increased levels of serum albumin in the extract treated rats and insulin treated rats. Total protein remained unchanged. Serum AST and ALT levels are used to evaluate liver function (Degirmenchi *et al,* 2002). Elevated activities of serum aminotransferases are a common sign of liver diseases and are observed more frequently among people with diabetes than in general population (Arkkila *et al*, 2001). This alteration in hepatic function may be because of increase activity and mRNA levels of arginase (Salimuddin *et al*, 2008). This present study showed that AST and ALT were significantly increased in group C rat (streptozotocin-treated rats). The increase in the serum AST and ALT indicated streptozotocin-induced diabetes produced alteration in hepatic function, and this may be due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro *et al*, 1997). On the other hand treatment of diabetic rats with extracts of these plants or insulin reduced the serum AST and ALT levels respectively. A possible explanation on the effect of extracts on the reduction of AST and ALT may be due to the antioxidant property of *Vernonia amygdalina* (Khallafalla *et al,* 2009) and *Ocimum gratissimum* (Akinmoladun *et al,* 2007) because free radicals have been implicated in the pathogenesis of diabetes hepatotoxicity. Oxidative stress may cause oxidative damage of cellular membranes and changes in their structural and functional integrity of subcellular organelles and may produce effects that

result in various complications in diabetic disease (Mecuri *et al*, 2000; West, 2000; Cam *et al*, 2003; Yavus *et al,* 2003). During streptozotocin metabolism, various toxic intermediates are produced, including methyl cations, methyl radicals, reactive oxygen species (ROS), and nitric oxide (Peschke *et al*, 2000; Gonzalez *et al*, 2002). The restoration of AST and ALT in the extract treated rats was similar to that observed in insulin treated and CONT rats and this may be due to direct effect of insulin on the streptozotocin-induced diabetic hepatocytes.

As observed the serum level of AST was lower compared with ALT. It is suggested that this may be due to the inactivation of cytosolic AST in the diabetic tissue by a glycation reaction, accompanied by impairment in glucose utilization in streptozotocin-induced diabetes (Okada *et al,* 1997) Histopathological studies of the liver in the extract treated group (group B) substantiate the cytoprotective action of the extract. This cytoprotective action may have been exerted through insulin-dependent mechanism as showed in the photomicrograph of the group A rats or its antioxidant property. Increased serum levels of AST and ALT are specific indicators of hepatocellular necrosis, therefore, the increased level of serum transferases as observed in diabetic rats without treatment corroborates with the necrosis seen in the photomicrograph in Figure 8.Albumin, produced only in the liver, is the major plasma protein that circulates in the blood stream. A low serum albumin indicates poor liver function. In this present study, streptozotocin-induced diabetic rats showed a significant decrease in the serum albumin concentration (35.6 ± 1.86). Similar depressant effect on albumin in streptozotocin diabetic rats has also been reported by some workers (Porte and Halter, 1981; Pepato *et al,* 1996; Sivajothi *et al,* 2007). Restoration of serum albumin concentration with methanolic extracts of *Vernonia amygdalina* and *Ocimum gratissimum* were similar to CONT (55.0 ± 3.80 vs 56.2 ± 6.04) mg/dl. Experimental study has reported that accelerated proteolysis of uncontrolled diabetes occurs as a result of deranged glucagons-mediated regulation of cyclic AMP formation in insulin deficiency (Dighe *et al*, 1984) thus a decrease in the plasma albumin. Therefore, the restoration of serum albumin concentration in the group B (extract treated) rats may be due to inhibition of proteolytic activity due to enhance insulin secretion and proper utilization of glucose.

However, the serum total protein did not show significant differences among groups; this may be dose- or duration-dependent. In this present study the serum bilirubin level was increased in the streptozotocin diabetic rats (group C, 46.74 ± 1.48) mg/dl compared with CONT (34.16 ± 1.22) mg/dl, doubled compared with methanolic extract (group B, 20.44 ± 1.58) mg/dl and tripled compared with insulin treated (group A,
15.54 ± 0.19) at $P < 0.05$. Elevation in serum bilirubin indicates liver damage. Rana *et al.* (1996) reported that in plasma bilirubin (hyperbilirubinimia) may be resulted from the decrease of liver uptake, conjugation or increase bilirubin production from haemolysis. Short-term streptozotocin-induced diabetes showed increased bilirubin production as well as enhanced hepatic conjugation and subsequent biliary excretion of pigment and these effects appear to be a direct consequence of diabetes (Tunun *et al,* 1991). The present results suggest that the methanolic extract of these plants may restore the serum AST, ALT, albumin, bilirubin and alleviated hepatic damage caused by streptozotocin induced diabetes via its antihyperglycemic and antioxidant property. These data indicate that

combined use of methanolic extract of *Vernonia amygdalina* and *Ocimum gratissimum* is beneficial in the treatment of diabetic-induced hepatic damage.

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