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Full Length Research Article

Liver function of Streptozotocin- Induced Diabetic Rats Orally Administered Aqueous Root-Bark Extracts of *Tetrapleura tetraptera* (Taub).

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ABSTRACT: The aqueous root-bark extract of *Tetrapleura tetraptera* Taub. has been shown to ameliorate streptozotocin (STZ)-induced hyperglycaemia and dyslipidaemia. This study evaluated the liver status of STZ-induced diabetic rats treated with aqueous root-bark extract of *T. tetraptera* for 35 days. Twenty-four (24) rats in four groups (normal control, diabetic control, *T. tetraptera* treated STZ induced diabetic rats at 150 mg/kg b. w. and *T. tetraptera* treated STZ-diabetic rats at 300 mg/kg b. w.) were used. Serum and liver alanine and aspartate transaminases (ALT and AST), alkaline phosphatase (ALP), gamma glutamyl transferase (γGT) activities and protein concentration were assessed. Also, serum albumins, globulins, total and direct bilirubin concentrations were measured. STZ induced diabetes significantly ($P<0.05$) increased serum ALT, AST and ALP activities, while treatment of diabetic rats with *T. tetraptera* reduced the activities of these enzymes. *T. tetraptera* caused significant ($P<0.05$) increases in serum γGT, total and direct bilirubin levels, especially at the higher dose (300mg/kg body weight). While the treatment of STZ induced diabetic rats with the extract of *T. tetraptera* ameliorated hepatocellular damage, at the higher dose of 300mg/kg body weight, *T. tetraptera* treatment may cause bile duct obstruction. The use of this plant in the treatment of diabetes or other diseases should therefore be monitored with respect to hepato-biliary toxicity.
Keywords: *Tetrapleura tetraptera*, STZ diabetes, Liver function, Toxicity, Medicinal plants

INTRODUCTION

The increasing acceptability of the use of herbal remedies in the treatment of various ailments, including diabetes, must be closely accompanied by the assessment of the safety of these remedies. Despite the availability of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continue to be a major medical problem (Ji *et al.,* 2006). This has led to renewed interest in the use of medicinal plants in the treatment of diabetes as adjuncts to conventional treatments and as potential sources of hypoglycemic compounds (Tiwari and Rao, 2002).

Tetrapleura tetraptera (Schum. and Thonn) Taub., (locally known as *Aridan* in South Western Nigeria), has been used as a spice, a medicine and as a dietary supplement rich in vitamins (Uyoh *et al*., 2013a). The leaves, bark, root and kernel of *T. tetraptera* are used for medicinal purposes (Uyoh *et al.,* 2013b). Various methanolic extracts of the plant have displayed molluscidal activities (Aladesanmi, 2006). Crude extract of *T. tetraptera* has also shown anti-ulcer and anti-convulsant properties, confirming its ethnomedicinal use to treat these symptoms (Agomuo *et al*., 2011). In Nigeria and Africa, diverse extracts of *T. tetraptera* are popularly used because of the discovery that they contain crude protein, crude lipid, crude fiber, phytochemicals (especially saponins), carbohydrate and food energy (Ojewole and Adewunmi, 2004).

The role of the liver in metabolism, including detoxification, makes it particularly vulnerable to exogenous substances. In diabetes, liver damage may occur in the later stages of the disease due to disorders in lipid metabolism and increased gluconeogenesis and ketogenesis (Virdi *et al*., 2003). In addition, the oxidative stress imposed by both human and experimental diabetes may also result to multi-organ damage including liver damage (Kazeem *et al*., 2013). Our previous studies have shown that the aqueous root-bark extracts of *T. tetraptera* possess significant antidiabetic and antihyperlipidaemic effects in streptozotocin (STZ) diabetic rats (Omonkhua *et al*., 2014). This study was therefore designed to investigate the potential hepato-protective role of *T. tetraptera* aqueous root-bark extract in STZ induced diabetic rats.

MATERIALS AND METHODS

Chemical and Reagents

Streptozotocin and bovine serum albumin (Sigma, London). Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (γ-GT), albumin and bilirubin Randox kits were products of Randox Laboratory Ltd, United Kingdom. Other analytical grade chemicals were also used.

Experimental Animals

Twenty-four rats of the Wistar strain, weighing between 120-160g, were obtained from the Pharmacology Department of Obafemi Awolowo University, Ile-Ife, Osun State. The animals were given commercial feed (Ewu growers from the Bendel Feed and Flour Mill Ewu, Nigeria) and water *ad libitum*. The rats were allowed to acclimatize for three weeks before the commencement of the experiments. Treatment of the animals conformed to the guideline for the Care and Use of Laboratory Animals (The National Academy of Sciences, 2011).

The Medicinal Plant

T. tetraptera was obtained locally within Akungba-Akoko in Ondo State, Nigeria and identified in the Department of Microbiology and Botany, University of Ibadan, Nigeria. Herbarium specimen, with voucher number UIH22320 was deposited at the Herbarium of the University of Ibadan, Nigeria.

Preparation of Plant Extract

The aqueous root bark extract of *T. tetraptera* was prepared using a modification of the method described by Onoagbe *et al.* (1999). The shade dried root bark were pulverized and then soaked in distilled water for 72 hours in a plastic container and covered with cheesecloth. The content was stirred several times a day and at the end of the third day this was filtered through two layers of cheesecloth. The extract was quantified by drying 1 ml of the homogeneous filtrate (by controlled heating i.e. in an oven kept at 40^{o}C) in a pre-weighed watch glass; this was done in triplicates and the average determined. The extract was kept in the freezer until use, when it was allowed to thaw at room temperature.

Induction of Diabetes

Rats were injected (i.p.) with streptozotocin dissolved in acidified (pH 4.5) normal saline at a dose of 65 mg/kg body weight after a 12-hour fast. Seven (7) days later, diabetes was confirmed by measuring fasting blood sugar. Rats with FBS higher than 8.2 mmol/l and glucosuria were randomly distributed into groups 2, 3 and 4. After stable diabetes was established, treatment of rats commenced and lasted for 35 days.

Administration of the Plant Extract

The plant extract was orally administered to the rats at 150 and 300 mg/kg body weight daily for 35 days.
GROUP 1: Normal control
GROUP 2: Diabetic control
GROUP 3: Diabetic rats treated with 150 mg/kg body weight of *Tetrapleura tetraptera*
GROUP 4: Diabetic rats treated with 300 mg/kg body weight of *Tetrapleura tetraptera*
The rats were weighed weekly.

Blood Collection

At the end of *T. tetraptera* administration (i.e. after 35 days), the rats were sacrificed and blood was collected from the jugular vein. The thoracic and abdominal regions were opened and the liver was also collected.

Centrifugation of Sample

Blood samples were allowed to clot and centrifuge at 5,000 rpm for 5 minutes, the serum was then separated for analysis. The liver was homogenized in ice cold normal saline (1:4 w/v), centrifuged and the supernatant stored in the freezer until analysis.

Biochemical Analysis

Serum and liver alanine transaminase (ALT) and aspartate transaminase (AST) activities were assayed by Reitman and Frankel (1957) method. Serum and liver alkaline phosphatase (ALP) and gamma glutamyl transferase (γGT) activities were measured by an optimized standard method according to the recommendations of the Deutsche Gesellschaft fur Klinische Chemie (Rec. GSCC DGKC) (1972) and the Szasz (1969) methods respectively. Serum and liver protein concentrations were measured by Biuret method (Gornall *et al.*, 1949), serum albumins concentration was measured by Doumas and Biggs (1972) method while the amount of globulins was calculated as a difference between total serum proteins and serum albumins. Serum total and direct bilirubin levels were assayed by Jendrassik and Grof (1938) method.

Statistical Analysis

The data were expressed as means of 4 to 6 determinations ± S.E.M. The differences among groups were analyzed by the one-way analysis of variance (ANOVA). Inter-group comparisons was done by the Duncan's post hoc test. A value of $p<0.05$ was accepted as significant. The SPSS (15.0, SPSS[2] Inc., Chicago, Illinois, USA), was used for the analysis.

RESULTS AND DISCUSSION

The increasing global incidence of diabetes and its complications, despite the availability of well-known antidiabetic drugs, have led to a resurgence of interest in traditional methods of combating the disease. The abundance of literature on the efficacy of medicinal plants in the management of both human and experimental diabetes has contributed immensely to the growing acceptance of herbal remedies for the treatment of diabetes and other diseases. It is however of outmost necessity for scientists to evaluate the safety of these remedies. In addition, the effect of medicinal remedies on all aspects of the pathogenesis of diabetes should be assessed in order to gain knowledge on the full potential of these remedies to combat diabetes and its complications. Both human and experimental diabetes have been shown to exert some level of liver dysfunction (Virdi *et al*., 2003; Zafar *et al*., 2009; Najla *et al*., 2012). In this study, the effect of treatment with *T. tetraptera* aqueous root-bark extract on the hepatic status of STZ induced diabetic rats, was assessed. Untreated STZ diabetes caused increases in serum ALT and AST activities compared with normal control (Figures 1 and 2). This is consistent with several studies which show that STZ diabetes causes elevations in the amino transferases (Zafar *et al*., 2009; Najla *et al*., 2012; Soliman, 2013). Increase in oxidative stress imposed by STZ diabetes may be responsible for the hepatocellular damage that results in the leakage of ALT and AST from liver cells (Okechukwu *et al*., 2013). Treatment of diabetic rats with *T. tetraptera* reduced serum ALT activities (not statistically significant) compared to normal control. Whereas, compared to diabetic control, the reductions in ALT activities of the treated diabetic groups was significant. The reductions observed for serum AST was not as pronounced as ALT. ALT and AST are sensitive tests of hepatocyte injury (Ellis *et al*., 1978). This result implies that treatment of diabetic rats with *T. tetraptera* at both 150 and 300 mg/kg body weight doses, ameliorates the hepatocyte damage imposed by STZ diabetes. Liver ALT and AST activities for all test groups were not significantly different from normal control. It can therefore be suggested that some of the many phytochemicals present in *T. tetraptera,* including those that have antioxidant properties, have hepatocellular-protective effects. Again, several studies have shown that medicinal plant treatment can reduce the elevations of ALT and AST caused by STZ diabetes (Zafar *et al*., 2009; Najla *et al*., 2012; Soliman, 2013).

The untreated diabetic state produced significant ($p<0.05$) increase in serum ALP activities compared to normal control (Figure 3). This corroborates reports of the effect of STZ diabetes on serum ALP activities (Zafar *et al*., 2009**;** Okechukwu *et al*., 2013). This increase was again ameliorated by treatment of STZ diabetic rats with *T. tetraptera* aqueous extract as can be seen from the significantly ($p<0.05$) lower ALP levels of the treated diabetic rats compared to untreated diabetic control. Treatment of diabetic rats with several plant extracts has been reported to reverse the elevation of ALP imposed by STZ diabetes (Sabu *et al*., 2002; Ming *et al*., 2009). However, liver ALP activities were slightly reduced for both treated and untreated diabetic rats compared with normal control. Serum γGT activities of the untreated diabetic rats increased slightly compared to normal control (Figure 4). Much higher increases were seen in the treated diabetic rats compared to both normal and diabetic controls. Serum alkaline phosphatase (ALP) and γ-glutamyl transferase (γGT) activities usually rise to several times the normal value after several days of bile duct obstruction or intra-hepatic cholestasis. γGT is a more sensitive marker of bile duct obstruction or intra-hepatic cholestasis. The results from this study suggest that treatment of STZ diabetic rats with *T. tetraptera* aqueous root bark extract, especially at the higher dose of 300 mg/kg body weight, may have caused at least mild bile duct obstruction or intra-hepatic cholestasis. This conclusion is further supported by the significantly ($p<0.05$) higher total and direct bilirubin concentrations recorded for this group as compared to normal and diabetic controls (Figure 5).

Although there are reports of decreases in serum total proteins and albumins in STZ diabetic rats (Najla *et al*., 2012), in this study, the diabetic state or its treatment with *T. tetraptera* at both doses did not significantly alter serum and liver total protein concentrations (Figure 6) as well as serum albumins and globulins concentrations (Figure 7). Serum total protein, especially serum albumins concentration, reflects the synthetic capacity

of the liver (Braunwald *et al.*, 2001). Serum albumins concentration is not altered in mild liver injury but usually reduces in chronic liver disease such as liver necrosis (Rothschild *et al.*, 1988). It may therefore be concluded that under the conditions of this study, STZ diabetes or its treatment with *T. tetraptera* did not alter the liver's capacity to synthesize these proteins.

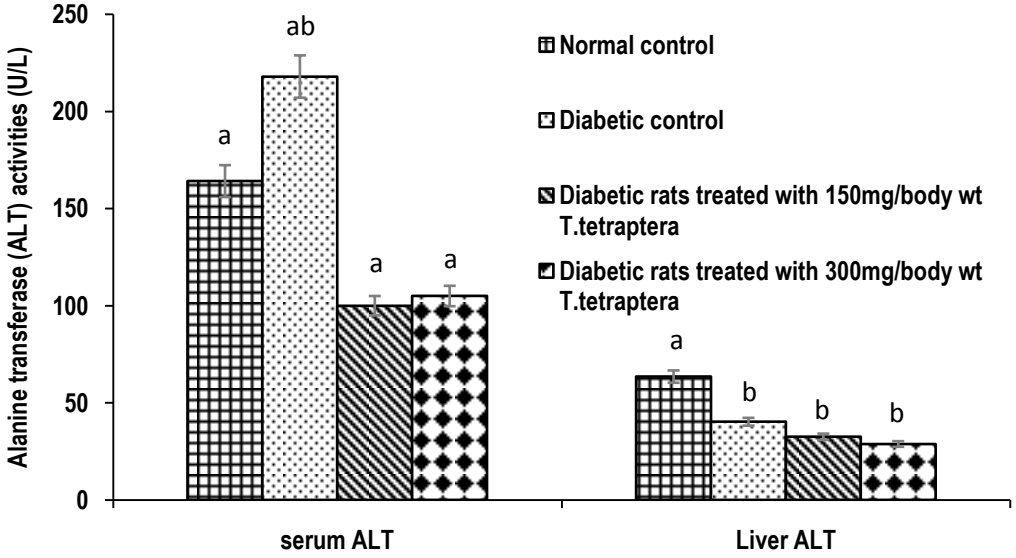

Figure 1: Effects of repeated daily oral administration of aqueous root-bark extract of *Tetrapleura tetraptera* for 35 days at 150mg/kg and 300mg/kg body weight on alanine transaminase activities of streptozotocin-induced diabetic rats. Values carrying different letters are statistically different at $p<0.05$.

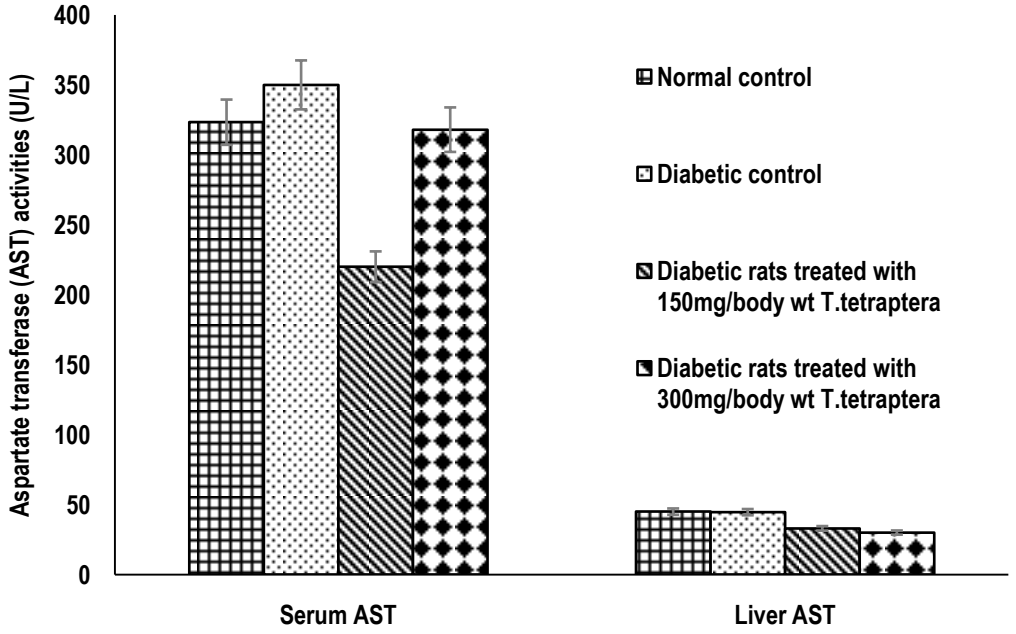

Figure 2: Effects of repeated daily oral administration of aqueous root-bark extract of *Tetrapleura tetraptera* for 35 days at 150mg/kg and 300mg/kg body weight on aspartate transaminase activities of streptozotocin-induced diabetic rats.

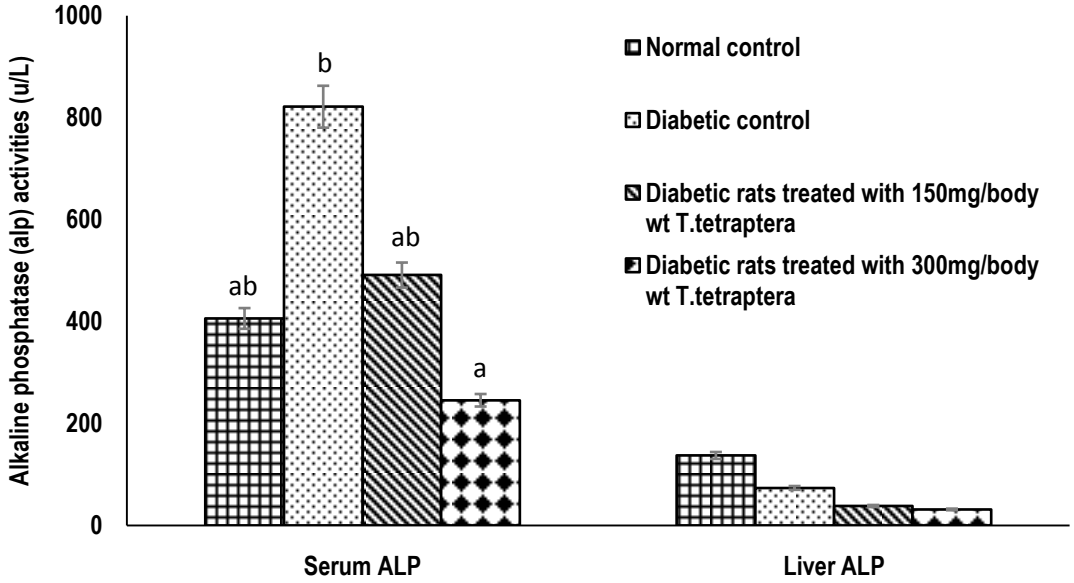

Figure 3: Effects of repeated daily oral administration of aqueous root-bark extract of *Tetrapleura tetraptera* for 35 days at 150mg/kg and 300mg/kg body weight on alkaline phosphatase activities of streptozotocin-induced diabetic rats. Values carrying different letters are statistically different at $p<0.05$.

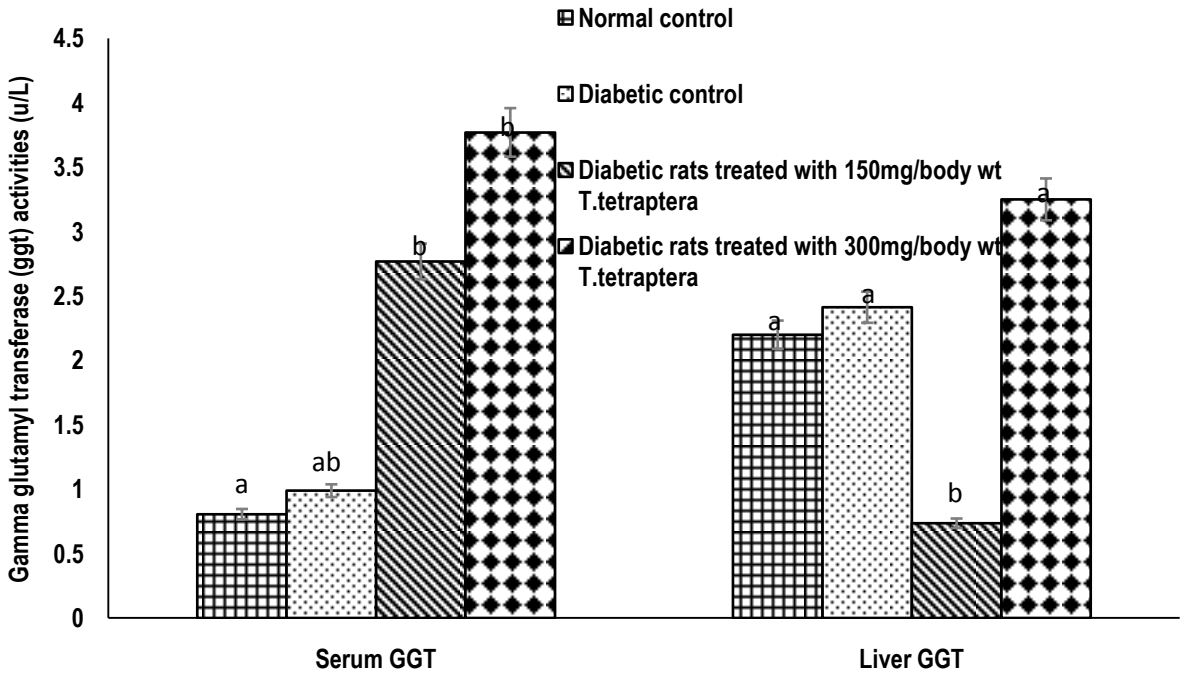

Figure 4: Effects of repeated daily oral administration of aqueous root-bark extract of *Tetrapleura tetraptera* for 35 days at 150mg/kg and 300mg/kg body weight on gamma glutamyl transferase activities of streptozotocin-induced diabetic rats. Values carrying different letters are statistically different at $p<0.05$.

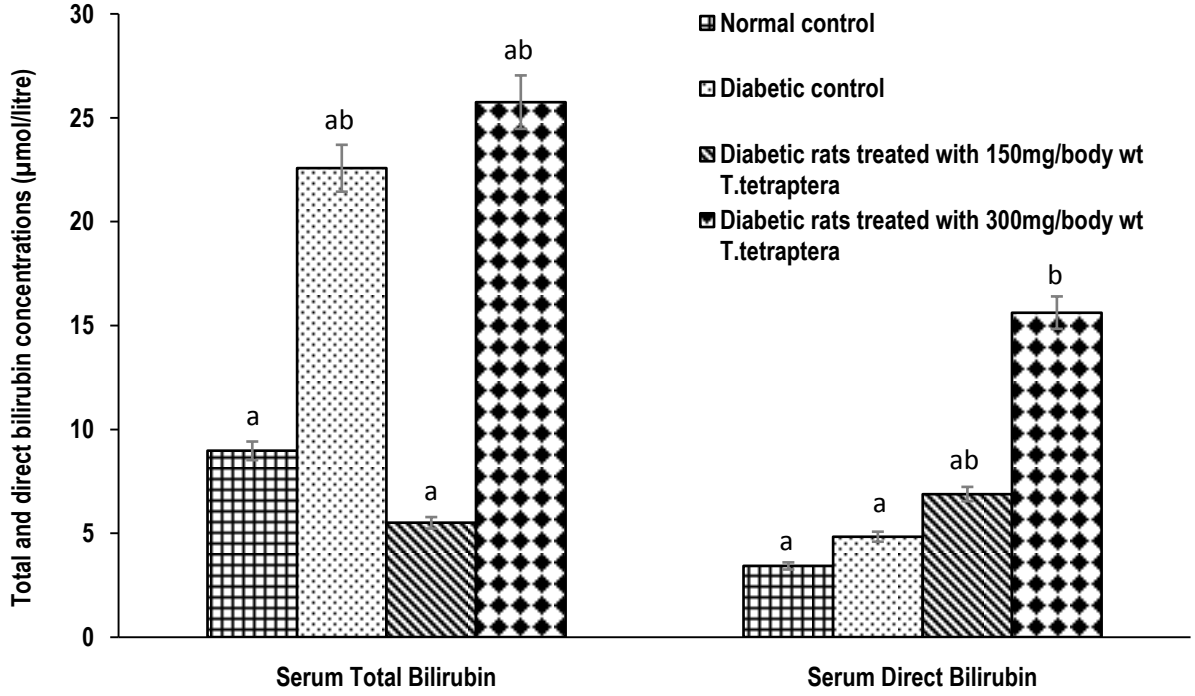

Figure 5: Effects of repeated daily oral administration of aqueous root-bark extract of *Tetrapleura tetraptera* for 35 days at 150mg/kg and 300mg/kg body weight on serum total and direct bilirubin concentrations of streptozotocin-induced diabetic rats. Values carrying different letters are statistically different at $p<0.05$.

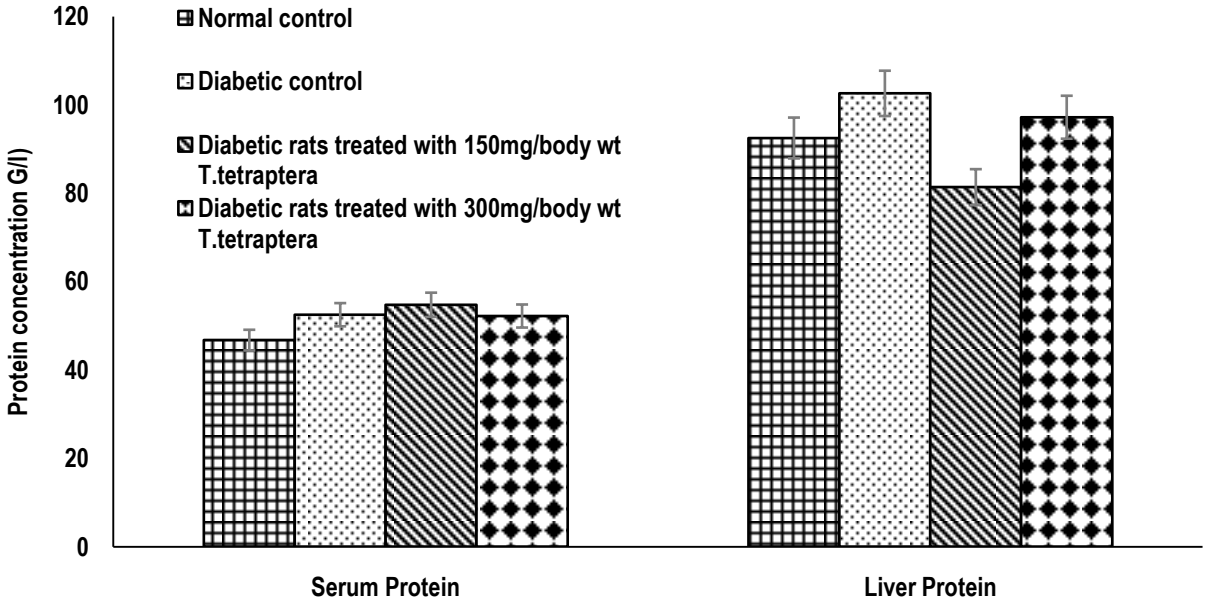

Figure 6: Effects of repeated daily oral administration of aqueous root-bark extract of *Tetrapleura tetraptera* for 35 days at 150mg/kg and 300mg/kg body weight on protein concentration of streptozotocin-induced diabetic rats.

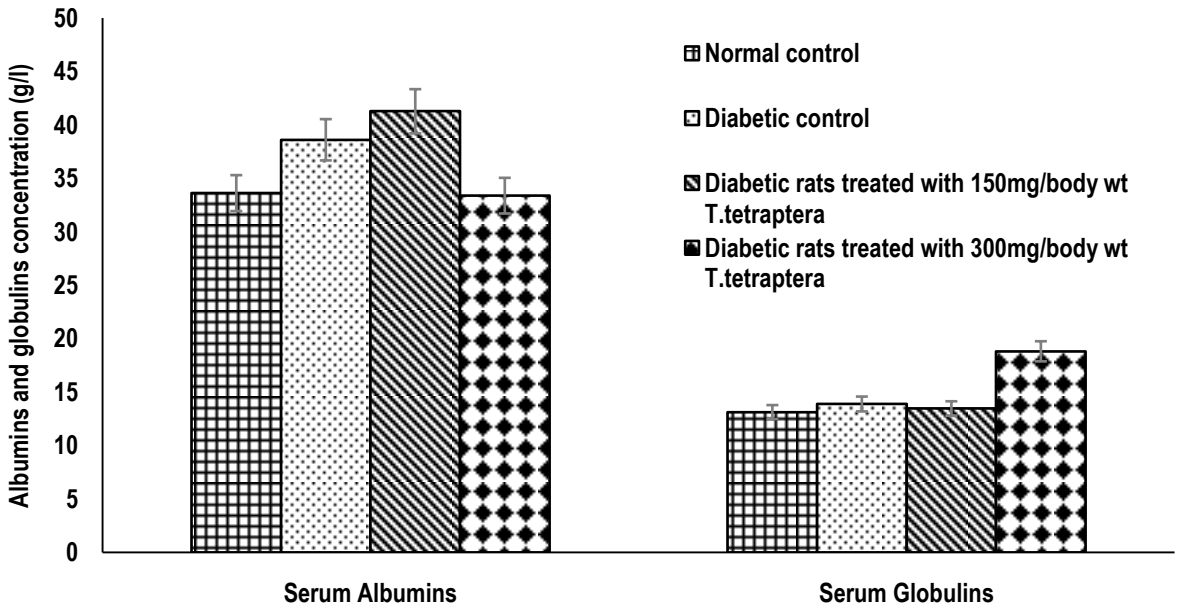

Figure 7: Effects of repeated daily oral administration of aqueous root-bark extract of *Tetrapleura tetraptera* for 35 days at 150mg/kg and 300mg/kg body weight on serum albumins and globulins concentrations of streptozotocin-induced diabetic rats.

CONCLUSION

This study showed that STZ induced diabetes caused hepatocellular damage in rats, which was ameliorated by treatment with aqueous root bark extracts of *T. tetraptera* at 150 and 300 mg/kg body weight. Also STZ diabetes and it treatment with *T. tetraptera* did not alter the liver's ability to synthesize proteins. However, treatment of diabetic rats with *T. tetraptera* at 300mg/kg body weight may have elicited some level of bile duct obstruction or intra-hepatic cholestasis. It is therefore important that long term users of this herbal remedy assess their hepatic function at regular intervals.

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