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Liver function status in Streptozotocin Induced Diabetic Rats Treated with Extracts of some Anti-diabetic Medicinal plants

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ABSTRACT: The anti-diabetic effects of the plants *Spondias mombin, Vernonia amgdalina, Annona murica* and *Nigella sativum* have been reported in streptozotocin induced diabetic rats. This study assessed the liver function status of diabetic rats treated with these plant extracts. 42 rats were randomly divided into seven groups. Groups 1-3 served as the normal control, diabetic control and positive control groups respectively. Groups 4-7 were the *Spondias mombin, Vernonia amgdalina, Annona murica and Nigella sativum* extracts treated diabetic rats respectively. The liver markers assessed includes serum AST, ALT, ALP, total protein, albumin, bilirubin concentrations. The result showed that treatment with all plant extract resulted in a significant decrease in AST, ALT and ALP concentrations. However, a higher percentage decrease in ALP and ALT levels were observed in the *Vernonia amgdalina* treated diabetic rats when compared to other extract treated rats while Annona muricata treated rats gave the highest percentage reduction in AST concentration. Also treatment with *V.amgdalina* and *Annona muricata* gave the highest percentage reduction in total and conjugated bilirubin. Non-significant change was observed in protein and albumin levels. Histological evaluation revealed that treatment with extracts of *Spondias mombin* leaves, *V. amgdalina* leaves, *Annona muricata* leaves reverted the damage to the liver caused by STZ induction. *V.amgdalina* and *Annona muricata* are two plants to consider as powerful hepatoprotective agents.

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Diabetes is a metabolic disorder whose long term effects leads to damage to most body organs such as the liver. Research has shown that the main cause of liver damage in diabetics is hyperglycemia-induced oxidative stress which eventually causes disturbances in carbohydrate, protein and lipid metabolisms (Mohamed *et al*., 2016). Fibrosis, abnormal fat and glycogen deposition, cirrhosis and increased hepatic enzyme activities are few of liver abnormalities linked with diabetes (Levinthal and Tavill, 1999). The liver is an important organ in the body as it is involved in the metabolism and detoxification of drugs and other substances hence damage or diseases to the liver is highly devastating. Liver diseases are among the leading cause of death worldwide. Herbal therapy plays a significant role in the treatment of liver disorders. Numerous amount of plant have been used to treat liver diseases in traditional medicine (Rao *et al*., 2006). Wills and Asha, (2006) reported that plants can serve as hepatoprotective agents. Streptozotocin is used to induce diabetes in animal model. It damages the beta cells of the pancreas leading to degranulation and inability of the pancreas in secreting the hormone insulin (Magee and Swann, 1969). Chronic hyperglycaemia of diabetes mellitus has been strongly associated with damage to several organs including the liver (Lyra *et al.*, 2006) hence, the need to assess the hepato-protective effects of plant extracts in STZ induced diabetic rats. *Spondias mombin* belongs to the family *Anacardiaceae*. It is native to the tropical America and found in abundance in parts of Africa, India and Indonesia and is among the medicinal plants in Southern Nigeria (Aiyeloja and Bello, 2006). *Vernonia amygdalina* is a shrub common in tropical Africa (Areghore *et al*., 1997). It belongs to the Asteraceae family and is a useful medicinal plant among the West Africans (Akah and Okafor, 1992; Amole *et al*., 2006). Soursop (Annona muricata L.) also referred to as graviola or guanabana is present in many parts of the world (Wélé, *et al.*, 2004). The

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antitumor, cytotoxic, antiparasitic, pesticidal, and antidiabetic properties have been investigated (Gleye *et al*., 1997; Gajalakshmi *et al*., 2012). *N. sativa* is a spice plant used as flavours and codiments that belongs to the Ranunculaceae family. Research on the seed has shown that nigella sativum possesses bronchodilatory, anti-bacterial, antioxidant, anti-tumoral, antidiabetic effects, anti-inflammatory (Al-Awadi *et al*., 1985; Bamosa *et al*., 1997; Burits and Bucar, 2000; El-Dakhakhny, 1965; Hajhashemi *et al*., 2004; Houghton *et al*., 1995; Meral *et al*., 2001; Worthen *et al*., 1998). These observed effects might have been due to the rich content of flavonoid, saponin, steroid/triterpenoid, quinone and alkaloid (Aisyah *et al*., 1995; Sharma *et al*., 2009). The lack of effective hepatoprotective orthodox drugs, has led to the use of medicinal plants as alternative therapy. This study was therefore designed to explore on more better the hepato-protective agents of plants origin that can be used to manage/ treat Streptozotocin induced liver damage in rats.

MATERIALS AND METHODS

Plant materials: Fresh leaves from the plants *Spondias mombin, Vernonia amgdalina, Annona muricata*, were gotten from gardens in the staff quarters of University of Benin Ugbowo campus while dried seeds of *Nigella sativum* were obtained from local markets, Benin City. Proper plant identification and authentication of the plants was done in the department of Plant Biology and Biotechnology, University Of Benin. Voucher specimens (UBH_S 345, UBH_V245, UBHa 0205, and UBH_N 506 and respectively) were deposited in the herbarium.

Extraction of plant material: Dried leaves of *Spondias mombin, Vernonia amgdalina, Annona muricata* and dried seeds of *Nigella sativum* were cleaned and then crushed to fine powder using a mechanical blender. 200g of pulverized plant materials were each macerated in ethanol (800ml) for 48hrs. Solvent was evaporated using a rotary evaporator to obtain ethanol extract of each plant.

Animals: A total of 42 male Wistar rats(of weight 200-250g) gotten from the animal house department of Anatomy, University of Benin were housed in clean galvanized cages with 12h-light and 12h dark cycle and were acclimatized for two weeks before the start of the experiment. The rats were allowed free access to food and water. The rats were randomly divided into seven groups of six rats each

Group 1- Normal untreated rats
Group 2- Diabetic control rats (diabetic untreated rats)
Group 3- Positive control (diabetic rats treated with 50mg/kg body weight of metformin)
Group 4- Diabetic rats treated with 200mg/kg body weight of *Spondias mombin*
Group 5- Diabetic rats treated with 200mg/kg body weight of *Vernonia amgdalina*
Group 6- Diabetic rats treated with 200mg/kg body weight of *Annona muricata*
Group 7- Diabetic rats treated with 2.5ml/kg body weight of *Nigella sativum* oil

The research guidelines for the handling of animals of the College of Medicine, University of Benin (CMR/REC/2014/57), was obtained, adopted and strictly adhered to. The respective extracts was administered to the rats for 28days. The animals were sacrificed on the 28th day by cervical dislocation. Blood and tissue (liver) samples were collected for liver function tests and histopathology respectively.

Induction of diabetes: After fasting the rats overnight, Streptozocin (STZ) was prepared fresh by dissolving in 0.1 M cold citrate buffer, PH 4.5 and administered intraperitoneally to the rats at a dose of 60mg/kg body weight. After 7days, fasting blood glucose was measured using Acchuchek one touch glucometer and only rats with blood glucose level $\geq$200mg/dl were considered diabetic and were used for this study.

Biochemical assays: Reitman and Frankel (1957) procedure was used to assay for Alanine transaminase (ALT) and aspartate transaminase (AST) activities. Gornall *et al.,* (1949) procedures for alkaline phosphatase (ALP), Biuret method for protein concentrations, Doumas and Biggs (1972) procedure for albumin concentration. Jendrassik and Grof (1938) method was used to assay for Serum total and conjugated bilirubin levels.

Histological evaluation: The liver collected were cleaned and fixed using hematoxylin and eosin and examined using the microscope.

Statistical Analysis: Data were expressed as mean ± SEM. Statistical analysis was done using one way analysis of variance and $p<0.05$ indicated statistical significant difference.

RESULTS AND DISCUSIION

Effects of extracts on serum liver enzyme levels: The activities of ALP, AST and ALT are shown in table 1. STZ induced significant elevation in ALP, AST and ALT levels when compared with the normal control. Treatment with all plant extracts showed significant decrease in the concentrations of these liver enzymes when compared with the diabetic control rats. A higher percentage decreases (45.9% and 71.86 % in ALP and ALT levels respectively) were observed in the *Vernonia amgdalina* treated diabetic rats when

compared to other extract treated rats. Treatment with *Annona muricata* resulted in a higher percentage decrease (65.84%) in levels of AST compared with the 61.46, 65.84 and 50.13% decrease observed for the *Spondias mombin, vernonia amgdalina, and Nigella sativum* treated diabetic rats.

Table 1: Serum liver enzyme activities in diabetic treated rats

Treatments	Liver function parameters		
	Alkanine phosphatase(ALP)(U/L)	Aspartate amino transferase(AST) (U/L)	Alanine amino transferase(ALT) (U/L)
Normal control	340±2.0	140±1.50	105.5±1.50
Diabetic control	578±3.50*	371±0.04*	330.5±1.05*
Positive control	286±2.54*	153±2.00*	219.5±2.00*
Spondias mombin treated diabetic rats	460.5±1.50*	143.5±1.54	105±2.00
Vernonia amgdalina treated diabetic rats	312.5±2.0	151±0.01*	93.0±1.55*
Annona muricata treated diabetic rats	359.5±2.55*	126.7±2.10*	132.5±1.20*
Nigella sativum treated diabetic rats	330.0±0.05	185±2.00*	161.0±1.40*

*Data are liver function parameters of rats treated with extracts for 28 days and are expressed as means ±SEM (n=6). *p ≤0.05 when compared with the normal control values*

Effects of extracts on serum total and conjugated bilirubin level in Streptozotocin induced diabetic rats: As shown in the table 2 below, significant increases ($p<0.05$) in total bilirubin and conjugated bilirubin were observed in the diabetic control rats. Treatment with extracts of *Vernonia amgdalina and Annona muricata* resulted in a profound decrease in total bilirubin and conjugated bilirubin when compared with the diabetic control. Whereas *Spondias mombin* and *Nigella sativum* treated rats produced non-significant reduction in total bilirubin and a significant reduction in conjugated bilirubin.

Table 2: Serum bilirubin levels in diabetic treated and non-diabetic rats

Treatments	Total bilirubin(mg/dL)	Conjugated bilirubin(mg/dL)
Normal control	0.17±0.01	0.08±0.02
Diabetic control	0.33±0.10*	0.19±0.01*
Positive control	0.23±0.01	0.17±0.01
Spondias mombin treated diabetic rats	0.31±0.05	0.10±0.05*
Vernonia amgdalina treated diabetic rats	0.27±0.01*	0.10±0.01*
Annona muricata treated diabetic rats	0.23±0.10*	0.13±0.02*
Nigella sativum treated diabetic rats	0.32±0.00	0.10±0.01*

*Data are total and conjugated bilirubin levels of rats treated with extracts for 28 days and are expressed as means ±SEM (n=6). *p ≤0.05 when compared with the normal control values*

Effects of extracts on total protein, and albumin levels in Streptozotocin treated diabetic rats: Table 3 shows the result of total protein and albumin levels. We recorded a non-significant change in total protein and albumin levels in the diabetic control rats, positive control and extracts treated diabetic rats when compared with the normal control. In this study, treatment of the diabetic rats with extracts of *Spondias mombin, vernonia amgdalina, Annona muricata and Nigella sativum* significantly improved the alterations in serum liver enzymes ALP, ALT and AST (Table 1). AST is a nonspecific marker for hepatic injury while ALT is a specific marker for hepatic parenchymal injury. They are both used in the evaluation of Liver disorders (Jus´kiewicz *et al.*, 2008; Sepodes *et al.* 2004; Bi *et al*., 2008). Alkaline phosphatase is a membrane bound glycoprotein enzyme. High amount of this enzyme is present in the sinusoids and in the endothelium of the central and periportal veins. An increase in these enzyme activities is indicative of liver damage (Elisa *et al*., 2009). Cell damage to the liver causes these cytosolic enzymes to spill into the sinusoids and finally into the blood stream. In this study we reported a significant increase in the levels of AST, ALT and ALP in the diabetic control (untreated) rats when compared with the normal control.

Table 3: Serum total protein and albumin concentration of diabetic treated and non-treated rats

Treatments	Total protein(g/dL)	Albuming/dL)
Normal control	9.1±1.20	4.2±1.05
Diabetic control	9.2±0.05	4.0±0.01
Positive control	9.13±1.00	4.3±1.05
Spondias mombin treated diabetic rats	9.45±1.10	4.15±0.02
Vernonia amgdalina treated diabetic rats	9.05±1.20	4.2±0.05
Annona muricata treated diabetic rats	9.27±0.20	4.3±1.00
Nigella sativum treated diabetic rats	9.0±0.05	4.2±0.01

Data are total protein and albumin levels of rats treated with extracts for 28 days and are expressed as means ±SEM (n=6).

Ohaeri (2001) reported that induction of diabetes with STZ resulted in necrosis of the liver of rats. Hence, the increases observed in the activities of AST and ALT may result from the leakage of these aminotransferase enzymes from the cytosol of the liver into the blood (Navarro *et al*., 1993), which therefore indicates the hepatotoxic impact of STZ. Our result is in agreement with the reports of other researchers who observed similar elevations in activities of liver enzymes following STZ induction (Zafar *et al*., 2009; Najla *et al*., 2012; Soliman, 2013; Omonkhua *et al*., 2014). Chronic and untreated diabetes tends to induce liver injury and damage, since this organ is the central processing unit for fuels whose metabolism have been drastically altered in diabetes Although, all plant extracts resulted in a significant decrease in ALP, AST and ALT activities, treatment with extracts of *Vernonia amgdalina* gave the highest percentage decrease in ALP and ALT activities. Whereas treatment with *Annona muricata* leaves extract gave the highest percentage decrease in serum AST activities. The high percentage reduction in these liver enzyme activities may not be far -fetched as several authors have reported on the hepato-protective effects by extracts of *V. amgdalina*. Studies by Atangwho *et al*., (2007); Buraimoh *et al*., (2010) and Ojiako & Nwanjo, (2006) have shown that *Vernonia amgdalina* has hepatoprotective effects. Igile *et al*., (1994) also reported that the protective effects of *V. amgdalina* may also be as a result of its antioxidant properties, and high flavonoid, sesquiterpene, lactones and saponins constituents. Some of these phytochemicals are important antioxidants which can help protect against oxidative stress induced organ damage resulting from STZ induction. Ajayi *et al*., (2021) has revealed the presence of important phtoconstituent such as hexadecanoic acid, methyl ester, 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester, cis-13-Octadecenoic acid, methyl ester, phytol and 9, 12-Octadecadienoic acid in *V.amgdalina* all of which may have acted synesgestically with other metabolites present in the plant to protect the liver against oxidative damage from STZ induction. The hepatoprotective properties of the other plant extracts have been demonstrated. Calderone *et al*., (2000) showed that the non-hepatotoxic effect of *Spondias mombin* may be due to its rich antioxidant properties. Al-Logmani and Zari, (2009) showed that *Nigella sativum* extract significantly reduced the levels of these liver enzymes in streptozotocin-induced diabetic rats. Bilirubin is a product of heme metabolism and it is lipid-soluble. The significant increase in total and conjugated bilirubin levels observed in the diabetic control groups in this study corroborates with the studies done by Omonkhua *et al*., (2014); Elkhateeb *et al*., (2015). The significant decrease in bilirubin levels in the extracts treated groups (table 2), therefore suggests that *Spondias mombin leaves, V. amgdalina leaves, Annona muricata leaves, and Nigella sativum* seeds possesses bilirubin lowering effects. Significant decrease in serum total proteins and albumins concentrations have been reported in other studies in Streptozotocin induced diabetic rats (Najla *et al.*, 2012), but we recorded a non-significant changes in total protein and albumin level in this study. In chronic liver disease such as liver necrosis, significant reduction in serum albumin occurs (Rothschild *et al.*, 1988). Since treatment with all plant extract did not significantly affect the albumin level we could therefore say that the ability of the liver to synthesize liver proteins were not affected by STZ induction or by treatment with the plant extracts.

Histology of the liver of rats treated with the various plant extracts: Histological examination of the liver revealed that induction of the rats with STZ resulted in a characteristic periportal infiltrates of inflammatory cells, portal vascular congestion and oedema as well as portal vascular ulceration of the liver cells. Treatment of the diabetic rats with extracts of *Spondias mombin* leaves, *V. amgdalina* leaves, *Annona muricata* leaves showed a characteristic normal hepatocytes as those recorded in the control groups. On the other hand, treatment with *Nigella sativum* seeds extract did not reverse the histological damage caused to the liver by STZ induction (Plates 1-7).

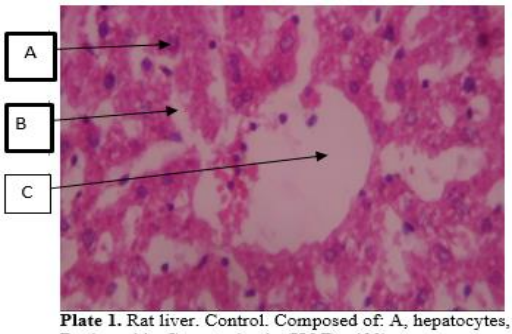

Plate 1. Rat liver. Control. Composed of: A, hepatocytes, B, sinusoids, C, central vein (H&E x 400)

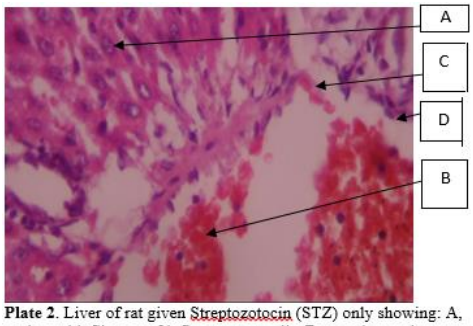

Plate 2. Liver of rat given Streptozotocin (STZ) only showing: A, periportal infiltrates of inflammatory cells, B, portal vascular congestion and C, Oedema as well as D, portal vascular ulceration (H&E x 400)

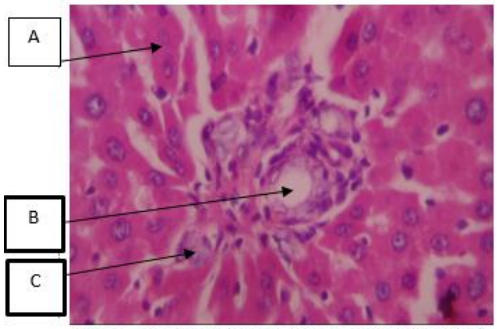

Plate 3. Liver of rat given Streptozotocin + Metformin showing: A, normal hepatocytes, B, normal biliary and vascular architecture and C, kuppfer cell activation (H&E x 400)

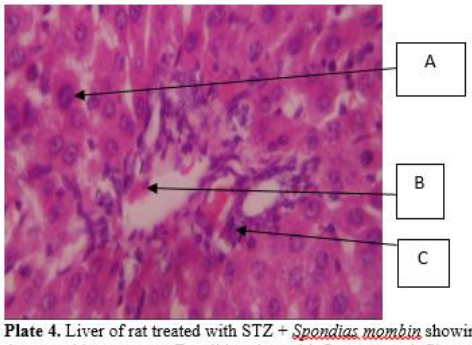

Plate 4. Liver of rat treated with STZ + *Spondias mombin* showing: A, normal hepatocytes, B, mild periportal inflammatory infiltrates and C, vascular ulceration (H&E x 400)

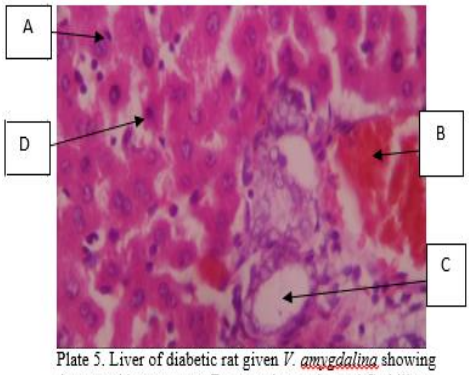

Plate 5. Liver of diabetic rat given *V. amygdalina* showing A, normal hepatocytes, B, normal vascular and C, biliary architecture and D, kupffer cell activation (H&E x 400)

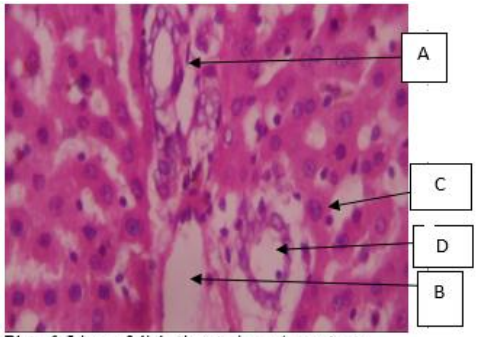

Plate 6. Liver of diabetic rat given *A. muricata* showing A, normal hepatocytes, B, normal vascular and C, biliary architecture as well as D, kupffer cell activation (H&E x 400)

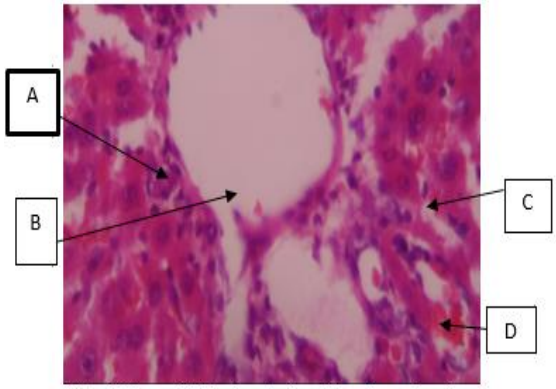

Plate 7. Liver of diabetic rat given *N. sativum* showing A, periportal infiltrates of inflammatory cells B, focal vascular ulceration C, tissue oedema and D, kupffer cell activation (H&E x 400)

Conclusion: The result obtained from this study has shown that of the four plant extracts investigated for their hepatoprotective effect, *V. amgdalina* gave the highest percentage reduction in liver enzymes activities in Streptozotocin induced diabetic rats. We also established that treatment with extracts of *Spondias mombin* leaves, *V. amgdalina* leaves, *Annona muricata* leaves reverted the damage to the liver caused by STZ induction.

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