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# EFFECT OF METHANOLIC EXTRACT OF *Annona muricata* SEED ON LIVER FUNCTION ENZYMES IN ALLOXAN-INDUCED DIABETIC MALE MICE

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**Abstract:** This study investigated the methanolic extract of *Annona muricata* seed on some liver enzymes: serum alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase in alloxan-induced diabetic rats as well as the liver histology. Twenty male mice were used in this study. Sixteen mice were alloxanized (200 mg/kg of alloxan intraperitoneal (i.p.). The four control (group A) mice were administered normal saline (i.p). The mice were confirmed diabetic and randomly divided into four experimental groups (B, C, D, and E). Group A (control) received normal rat chow, Group B (diabetic control) received normal rat chow, Group C received 200 mg/kg of *Annona muricata* seed extract, Group D received 400 mg/kg of *Annona muricata* seed extract, and Group E received 600 mg/kg of *Annona muricata* seed extract. At the end of 30 days experiment, result showed statistically significant difference in blood glucose level between Group A (CONT) (92.33 ± 8.81mg/ml) compared to Group B (485.67 ± 16.19 mg/ml) Group C (137.33 ± 11.61 mg/ml), and Group D (130.00 ± 9.54 mg/ml) at $P < 0.05$**.** There was no statistically significant difference in blood glucose level of Group A (CONT) (92.33 ± 8.81 mg/ml) compared with treated diabetic Group E (106.00 ± 7.23 mg/ml) at $P > 0.05$. There was a statistically significant decrease in body weight at $P < 0.05$ between the final body weight and initial body weight of Group C (32.73 ± 1.30 vs. 45.00 ± 1.50), Group D (33.47 ± 1.56 vs. 45.07 ± 1.65), Group E (32.33 ± 2.96 vs. 44.33 ± 1.45) but no statistically significant difference at $P > 0.05$ between the final body weight and initial body weight of Group A (CONT) (37.33 ± 3.71 vs. 37.00 ±3.51), and Group B (38.07 ± 1.55 vs. 44.20 ± 0.61). In serum ALP level, result showed a significant difference ($P < 0.05$) in Group A (CONT) (10.91 ± 3.34 μ/L) compared to Group B (29.50 ± 0.44 μ/L), statistically significant difference ($P < 0.05$) between Group B (29.50 ± 0.44 μ/L) compared to Group C (15.64 ± 7.78 μ/L) and Group E (13.64 ± 7.21 μ/L) and no statistically significant between Group A (CONT) (10.91 ± 3.34 μ/L) compared with Group C (15.64 ± 7.78 μ/L) and Group E (13.64 ± 7.21 μ/L) at $P > 0.05$. In serum AST level, result showed a significant difference ($P < 0.05$) in Group A (CONT) (56.25 ± 4.35 μ/L) compared to Group B (82.73 ± 1.16 μ/L), statistically significant difference ($P < 0.05$) between Group A (CONT) (56.25 ± 4.35 μ/L) compared to Group E (37.33 ± 25.96 μ/L) but no statistically significant between Group A (CONT) (56.25 ± 4.35 μ/L) compared with Group D (57.33 ± 25.45 μ/L) at $P > 0.05$. In serum ALT level, result showed a significant difference ($P < 0.05$) in Group A (CONT) (13.50 ± 3.66 μ/L) compared to Group C (5.33 ± 1.33 μ/L), statistically significant difference ($P < 0.05$) between Group A (CONT) (13.50 ± 3.66 μ/L) compared to Group B (32.00 ± 0.78 μ/L. However, there was no statistically significant between Group A (CONT) (13.50 ± 3.66 μ/L) compared with Group D (14.00 ± 5.13 μ/L) and Group E (13.67 ± 1.67 μ/L) at $P > 0.05$. Group E photomicrograph showed normalization of cells and reduced sinusoids compared to Group B photomicrograph which showed a marked focal dilation with congestion of the central vein. Therefore, data suggest that increased dose concentration of methanolic extract of *Annona muricata* seed restored significantly decreased serum ALP, AST and ALT, and restored cytoarchitecture of liver hepatocytes.

**Keywords:** Diabetes mellitus**,** *Annona muricata Linn***.** Alkaline phosphatase**,** Aspartate aminotransferase**,** Alanine aminotransferase**.**

## INTRODUCTION

*Annona muricata* (*Linn*), family of *annonaceae* commonly called "Soursop" is a small, upright evergreen tree growing 5 to 6 m in height. The flowers are borne singly, may emerge anywhere on the trunk, branches or twigs. They are short stalked, 4 - 5 cm long, plump and triangular-

conical; the 3 fleshy, slightly spreading, outer petals yellow-green, with 3 close-set inner petals pale yellow (Vasquez, 1990). In tropical Africa, including Nigeria, the plant is generally used as antiparasitic, antispasmodic, astringent, anticancer, sedative, hypotensive, insecticide, piscicide, vermifuge, and for coughs, fevers, pain and skin diseases (Watt and Breyer-Brandwijk, 1962) Several studies by different researchers demonstrated that the leaf, bark, roots, stem and seed extracts are antibacterial in vitro against numerous pathogens (Misas, 1979; Heinrich, 1992; Sundarrao, 1993). Research on *Annona muricata* has been on a novel set of phytochemicals (*Annonaceous acetogenins*) that are found in the leaves, seeds and stems which are cytotoxic against various cancer cells (Chang, 2001; Liaw, 2002; Chang, 2003).

One of the most potent methods to induce experimental diabetes mellitus is chemical induction by alloxan (Etuk, 2010). It is a well-known diabetogenic agent that is used to induce Type-I diabetes in experimental animals (Viana *et al.,* (2004). The liver is the main effector organ for maintaining plasma glucose levels within narrow limits. Herrman *et al.,* (1999) reported that streptozotocin (STZ) progressively decreased the volume of hepatocytes and their nuclei, as a result of cytoplasmic changes, and that a basal insulin level is also necessary to maintain the state of aggregation of the endoplasmic reticulum-bound polysomes for secretory protein synthesis. At the same time, hyperglycemia can generate a redox imbalance inside the cells, especially in the liver (Gallou *et al.,* 1993). Enzyme activities in the tissues are often used as "marker" to ascertain early toxic effects of administered foreign compounds to experimental animals (Akanji and Ngaha, 1989; Adesokan and Akanji, 2004). Alkaline phosphatase is a membrane bound enzyme while, alanine aminotransferase and aspartate aminotransferase are cytosolic enzymes. These enzymes are highly concentrated in the liver and kidney and are only found in the serum in significant quantities when the cell membrane becomes leaky and even completely ruptured (Ngaha, 1981; Cotran *et al.,* 1989) as a result of redox imbalance. In this view, this present study was aimed to evaluate the effect of methanolic extract of *Annona muricata* seed on liver function enzymes in male alloxanized mice.

## MATERIALS AND METHODS

Twenty mice weighing between 30 - 45 g were used in the study. The animals were obtained from the animal house of the Department of Veterinary Medicine, University of Nigeria, Nsukka. They were randomly assigned into groups and housed in a wire mesh cage (under temperature of $25^{0}$C - $30^{0}$C, 14 hours light and 10 hours dark cycle). They were acclimatized for two weeks. The mice were fed with vital feed rat chow and tap water *ad libitum*. Animal care and treatment were conducted with the institutional guidelines that are in compliance with international laws and policies.

### Experimental Grouping of Animals

The mice were fasted for 12 hours and fasting glucose level and body weight were recorded (basal level) before induction of diabetes. Sixteen mice were alloxanized (200 mg/kg of alloxan intraperitoneal (i.p.). The control mice were administered normal saline (i.p). After three days of induction, blood was collected from the tail vein, and glucose level was determined using one-touch glucometer, the mice exhibited plasma glucose level above 200mg/dl. The diabetic mice were divided into four groups and experiment lasted for 30 days after induction of diabetes mellitus

During the experiment the animals were weighed and randomly selected into four experimental groups (n = 5). Control group: (Group A) and was given distilled water and vital feed rat chow. Experimental diabetic group B mice were given distilled water and vital feed rat chow. Diabetic group C mice received oral administration of 200 mg/kg of *Annona muricata* seed extract. Diabetic group D received oral administration of 400 mg/kg of *Annona muricata* seed extract. Diabetic group E received oral administration of 600 mg/kg of *Annona muricata* seed extract.

**Extract Preparation**

*Annona muricata* fruits were bought from Elele market in Rivers State, Nigeria. The seeds were removed and sun dried for about two weeks. The dried seeds were then grounded into coarse form powder using a manual blender (Corona). About 200g of the grounded form was soaked in 600 ml of aqueous solution and placed in a mechanical shaker for 48 hours before filtering with a white handkerchief into a clean bottle. The filtrate was then concentrated to dryness at 50$^{o}$C in an electric oven (gallenkamp). A powdery form of the extract was then sieved to obtain very fine particles of the dried extract. The aqueous extract was then prepared by dissolving a given stock of 100 mg/ml in 10 ml of distilled water.

**Sample Collection**

At the end of 30 days experiment, the animals were anaesthetized in a chloroform chamber and 5ml of blood was obtained via cardiac puncture. Blood sample from each animal was put in a labeled EDTA anticoagulant bottle for enzyme assay. Biochemical analysis of serum enzymes for serum alkaline phosphatase levels were determined by the method of Bessey *et al.,* (1946), serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) were performed based on Randox diagnostic kit on the principles of Reitman and Frankel (1975). AST and ALT in serum were measured by monitoring the concentration of pyruvate hydrazone respectively formed with 2,4-dinitophenylhydrazine. The liver was routinely processed and stained with haematoxylin and eosin

**Statistical Analysis**

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS Version 15.0). Data was analyzed using one way analysis of variance (ANOVA). Turkey's multiple comparison was used to test for statistical significance. Results were presented as mean ± standard error of mean. Results were considered significant at $P < 0.05$.

## RESULT

At the end of thirty days of study, result no statistically significant difference in blood glucose level of Group A (CONT) (92.33 ± 8.81 mg/ml) compared with treated diabetic Group E (106.00 ± 7.23 mg/ml) at $P > 0.05$. However, there was statistically significant difference in blood glucose level between Group A (CONT) (92.33 ± 8.81mg/ml) compared to Group B (485.67 ± 16.19 mg/ml) Group C (137.33 ± 11.61 mg/ml), and Group D (130.00 ± 9.54 mg/ml) at $P < 0.05$.

Results showed a statistically significant decrease in body weight at $P < 0.05$ between the final body weight and initial body weight of Group C (32.73 ± 1.30 vs. 45.00 ± 1.50), Group D (33.47 ± 1.56 vs. 45.07 ± 1.65), Group E (32.33 ± 2.96 vs. 44.33 ± 1.45). There was no statistically significant difference at $P > 0.05$ between the final body weight and initial body

weight of Group A (CONT) (37.33 ± 3.71 vs. 37.00 ±3.51), and Group B (38.07 ± 1.55 vs. 44.20 ± 0.61).

In serum ALP level, result showed a significant difference ($P < 0.05$) in Group A (CONT) (10.91 ± 3.34 μ/L) compared to Group B (29.50 ± 0.44 μ/L). There was also statistically significant difference ($P < 0.05$) between Group B (29.50 ± 0.44 μ/L) compared to Group C (15.64 ± 7.78 μ/L) and Group E (13.64 ± 7.21 μ/L). However, there was no statistically significant between Group A (CONT) (10.91 ± 3.34 μ/L) compared with Group C (15.64 ± 7.78 μ/L) and Group E (13.64 ± 7.21 μ/L) at $P > 0.05$.

In serum AST level, result showed a significant difference ($P < 0.05$) in Group A (CONT) (56.25 ± 4.35 μ/L) compared to Group B (82.73 ± 1.16 μ/L). There was also statistically significant difference ($P < 0.05$) between Group A (CONT) (56.25 ± 4.35 μ/L) compared to Group E (37.33 ± 25.96 μ/L). However, there was no statistically significant between Group A (CONT) (56.25 ± 4.35 μ/L) compared with Group D (57.33 ± 25.45 μ/L) at $P > 0.05$.

In serum ALT level, result showed a significant difference ($P < 0.05$) in Group A (CONT) (13.50 ± 3.66 μ/L) compared to Group C (5.33 ± 1.33 μ/L). There was also statistically significant difference ($P < 0.05$) between Group A (CONT) (13.50 ± 3.66 μ/L) compared to Group B (32.00 ± 0.78 μ/L. However, there was no statistically significant between Group A (CONT) (13.50 ± 3.66 μ/L) compared with Group D (14.00 ± 5.13 μ/L) and Group E (13.67 ± 1.67 μ/L) at $P > 0.05$.

In the control group, the normal histology of the liver is observed. The photomicrograph showed the normal architecture exhibiting normal cells having moderate vacoulation of the cytoplasm. Group B photomicrograph showed a marked focal dilation with congestion of the central vein. Group C photomicrograph showed severe necrosis with hepatocytes disappearance, marked dilation of the central vein, some which are congested, increased intra-portal fibrosis and inflammation and also increased sinusoidal spaces. Group D photomicrograph showed moderate dilation of the central veins with reduced sinusoidal dilation, exhibited mild degree of necrosis and normalization of cells. Group E photomicrograph showed normalization of cells and reduced sinusoids.

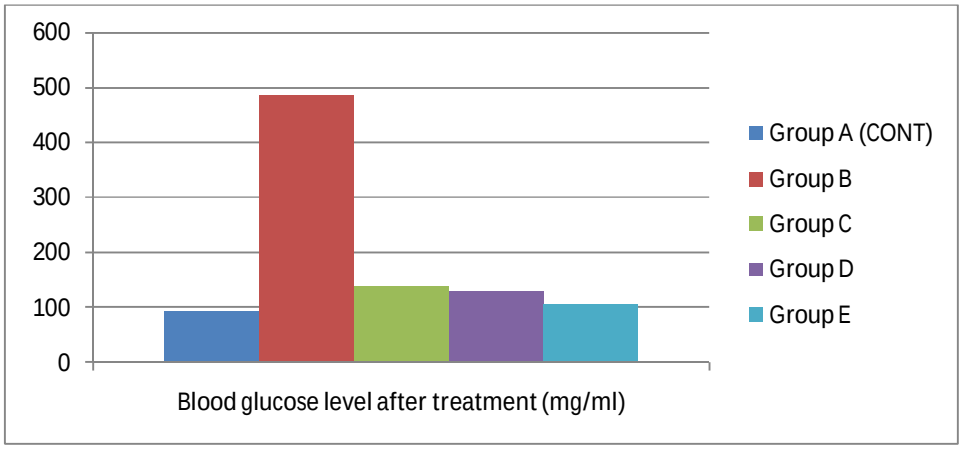


**Figure 1: Effect of Methanolic Extract of *Annona muricata* on Blood Glucose Level in Alloxan-induced Diabetic Mice**

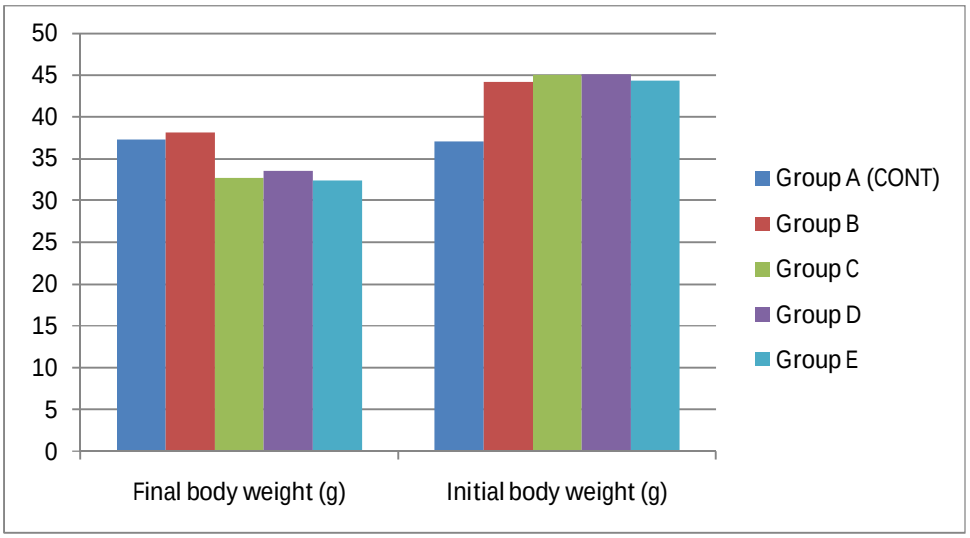


**Figure 2: Effect of Methanolic Extract of *Annona muricata* on Body Weight in Alloxan-induced Diabetic Mice**

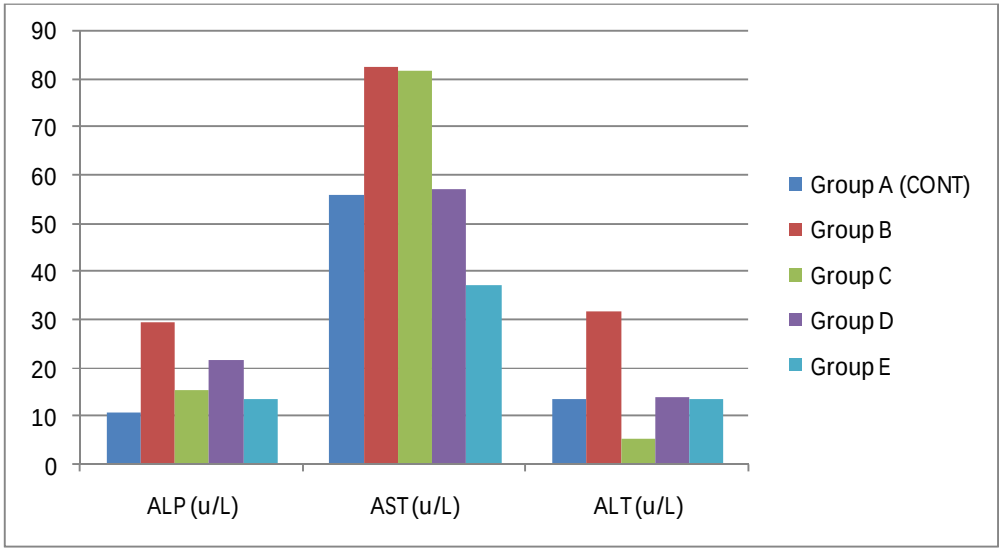


**Figure 3: Effect of Methanolic Extract of *Annona muricata* on Liver Function Enzymes in Alloxan-induced Diabetic Mice**

**Figure 4: Photomicrograph of Group A (CONT) Mice Showing Normal Architecture of the Liver**

**Figure 5: Photomicrograph of Group B Mice Showing Focal Marked Dilation with Congestion of Central Vein**

**Figure 6: Photomicrograph of Group C Mice Showing Severe Necrosis with Hepatocytes Disappearance, Marked Dilation of Central Vein, Some of Which are Congested, Increase Intraportal Fibrosis an Inflammation and with Increase Sinusoidal Spaces of the Liver**

**Figure 7: Photomicrograph of Group D Mice Showing Moderated Dilation of Central Vein, with Reduced Sinusoidal Spaces, Mild Necrosis of the Liver**

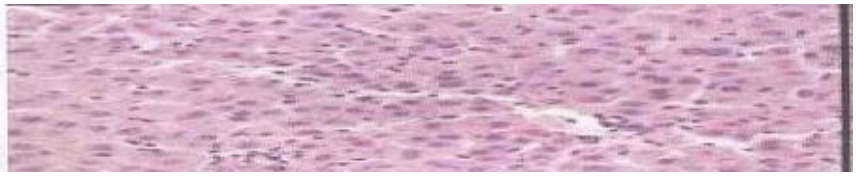

**Figure 8: Photomicrograph of Group E Mice Showing Normal Cells with Reduced Sinusoidal Spaces, and Scanty Portal Inflammation of the Liver**

## DISCUSSION

Results in figure 1 revealed that high dose concentration of methanolic extract of *Annona muricata* seed significantly reduced blood glucose level. Several studies have shown that *annona muricata* possesses antihyperglycemic activities (Adeyemi *et al.,* 2008; Rout *et al.,* 2013). Adewole and Ojewole (2009) have reported that treatment of diabetic rats with *Annona muricata* extract caused a marked amelioration of hyperglycemia with pronounced increase in serum insulin levels. These reports corroborate with our finding in figure 1 showing that *Annona muricata* seed extract lowers blood glucose levels.

Results in figure 2 showed that *Annona muricata* L. seed extract decreased the body weight in the treated diabetic rats. Adeyemi *et al.,* (2009) have reported that annona muricata possesses antihyperlipidemic activities. A strong relationship exists between relative increase in body weight and increase in serum lipid levels (Rifkind and Begg, 1966; Sanlier *et al.,* 2007), therefore, it could be established that extract reduced the body weight.

In this present study, the liver enzymes were determined to evaluate the effect of the extract on serum ALP, AST and ALT after alloxan induced diabetes mellitus. Results in figure 3 showed that serum ALP, AST and ALT were significantly increased in untreated diabetic mice (Group B). This increase could possibly result from the cell membrane becoming leaky and even completely ruptured (Ngaha, 1981; Cotran *et al.,* 1989). However, the extract reduced the liver enzymes in a dose-dependent fashion. 600 mg/kg of *Annona muricata* seed extract significantly reduced serum ALP, AST and ALT as well as 200 mg//kg of extract that significantly reduced only ALP and ALT respectively. Report has revealed that *Annona muricata* is hepatoprotective (Adewole and Ojewole, 2009), and causes pronounced increase in serum insulin. Our previous work have shown that insulin significantly reduced kidney enzymes AST and ALT in streptozotocin-induced diabetic rats (Agbai *et al.,* 2013), therefore, it is reasonable to suggest that this reduction of liver enzymes depends solely on the action of insulin. Several works have shown that tannins and other polyphenolic compounds (e.g., coumarins), flavonoids,

triterpenoid saponins, and a host of other plant secondary metabolites possess hypoglycaemic, hypolipidaemic, hypotensive, anti-inflammatory, and other pharmacological and biochemical properties in various experimental animal models (Ojewole, 2005). *Annona muricata* is known to contain ellagic acid, tannis, flavonoids, polyphenolic compounds, triterpenoids, β-sistosterol, and so on (Watt and Breyer-Brandwijk, 1962; TDRG, 2002; Chang, 2001). Histopathology revealed varying degrees of liver injury in the diabetic and treated mice at low dose of extract. Report has shown that hepatic damage is evident from increased liver enzymes (Kumar *et al.,* 2013). Plants with antioxidant defence system prevent atherosclerosis (Ebong *et al.,* 2011), and *Annona muricata* seeds significantly increased endogenous antioxidant enzyme activities (Adewole and Caxton-Martins, 2006). We have previously shown that insulin restored the cytoarchitecture of the glomerulus in streptozotocin diabetic rats (Agbai *et al.,* 2013), thus restoration of the cytoarchitecture of the liver may solely depend on the effect of *Annona muricata* antioxidant property and insulin effect in this present study.

Therefore, it can be concluded that increased dose concentration of methanolic extract of *Annona muricata* seed restored liver function by significant decrease in serum ALP, AST and ALT, and restoring cytoarchitecture of the hepatocytes in alloxanized mice.

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