Liver Enzymes And Histopathology Assessments Of Alloxan-Induced Diabetic Wistar Rats Treated With Aqueous-Ethanolic Crude Extract Of Alysicarpus ovalifolius

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Abstract: The study was aimed to assess Liver enzymes level and histopathology of liver in diabetic wistar rats treated with crude extract of Alysicarpus ovalifolius. Rats were made diabetic by intraperitoneal injection of 150mg/kg body weight of Alloxan monohydrate, and divided into groups 1-5, with group 5 as a positive control (treated with insulin). Groups 2, 3 and 4 were treated with 50,100 and 200mg/kg body weight of the extracts respectively daily, and group 1 was used as a negative control which received normal saline. After 7 days of treatment. The animals were sacrificed, Serum liver enzyme levels were assessed, and found to be higher in all extract treated groups, with statistically significant (P≤0.05) higher value of AST (26.6±1.40) in extract 100mg/kg; ALT(50.6±5.20) and AST(28.8±2.63) in extract 200mg/kg treated groups, when compared to the control groups (ALT =34.6 ± 2.44 and AST =14.0 ± 1.30; in positive control) and (ALT=36.8 ± 3.71 and AST 18.8 ± 1.92; in negative control). Sinusoidal congestion, (lymphoid aggregation) and Slight hepatocytes necrosis on liver histology were observed in extract 200 and 100mg/kg per body weight treated groups. Higher levels of liver enzymes conform to liver histopathological derangement seen in extract 200 and 100mg/kg per body weight treated groups. High level of liver enzymes is a biomarker for liver injury, hence the extract might be toxic-potent. The results obtained, provide the scientific rationale for liver monitoring in certain medications, especially when using Alysicarpus ovalifolius plant in therapeutics.

Keywords: Liver enzymes, Alysicarpus ovalifolius, diabetes mellitus

Introduction

The human body identifies almost all drugs as foreign substances (i.e. xenobiotics) and subjects them to various chemical processes (metabolism) to make them suitable for elimination. This involves chemical transformations to; (a) Reduce fat solubility and; (b) To change biological activity. Although almost all tissues in the body have some ability to metabolize chemicals, Smooth Endoplasmic Reticulum in the liver is the principal "metabolic clearing house" for both endogenous chemicals (e.g., cholesterol, steroid hormones, fatty acids, proteins) and exogenous substances (e.g., drugs, alcohol) (Donald, et. al., 2006). Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic range, may injure the organ. Other chemical agents, such as those used in laboratories and industries, natural chemicals (e.g., microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins. More than 900 drugs, toxins, and herbs have been reported to cause liver injury, and drugs account for 20-40% of all instances of fulminant hepatic failure (Friedman, et. al., 2003, Many traditional plant treatments for diabetes mellitus are used throughout the world (Marles and Farnsworth, 1995). Challenges within the medical system is management of diabetes without any side effect. This had led to an increasing demand for natural products with antidiabeic activity and fewer side effects (Kameswara et al., 1999).

Many herbs and plant products have been shown to have hypoglycemic action. Alysicarpus ovalifolius provides a protein-rich fodder and a palatable feed for livestock grazing in ranchlands. In Niger, it is a valuable component of vegetation collected and traded as fodder. In Nigeria, it is reported as a wound medicine (Lamers, et. al., 1996). Alysicarpus ovalifolius (Schumach) plant, belongs to the family leguminosae papilionoideae and has been described as a multipurpose plant which is used extensively both for its nutritional and medicinal properties. The present study was to assess liver enzymes and histopathology of alloxan-induced diabetic wistar rats treated with Aqueous-ethanolic crude extract of Alysicarpus ovalifolius
Materials And Methods

Plant material

Fresh plant of *Alysicarpus ovalifolius* was collected from the Institute for Agricultural Research, Agronomy farm Samaru, Zaria. The plant was presented to the herbarium unit of Biological Sciences Department, Faculty of sciences, A.B.U. Zaria, and was authenticated by Malam Musa with a voucher specimen number of 354 as deposited.

Plant Extract Preparation

The leaves were dried under shade and ground into powder. The air-dried powdered plant (450g) material was extracted with 70% methanol and 30% aqueous using soxhlets apparatus; the solvent was removed in-vacuo and evaporated using rotatory evaporator to yield a residue of 13.6g of aqueous ethanolic extract.

Animal

A total of 25 adult Wistar rats weighing (150-250g) bred in the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences, A.B.U. Zaria were used for the study. The animals were kept in well aerated laboratory cages in Human Physiology Department Animal House and were fed with grower and starter mash from Vital Feeds Company and water ad libitum were provided.

Induction of Experimental Diabetes Mellitus

The animals were fasted for 16-18 hours with free access to water prior to the induction of diabetes. Induction was carried out by single intraperitoneal injection of Alloxan monohydrate (Sigma St Lous, M.O., USA) dissolved in 0.9% cold normal saline solution at a dose of 150mg/kg body weight (Katsumat et al., 1999). Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6hrs. The rats were then kept for the next 24hrs on 5% glucose solution bottles in their cages to prevent hypoglycemia (Dhandapani et al., 2002). Diabetes was assessed in alloxan- induced rats by determining the blood glucose concentration 72hours after injection of alloxan. The rats with blood glucose level above 200mg/dl were then selected for the study.

Experimental Design for Alloxan-induced Hyperglycemic Groups

Animals fasted overnight were randomly divided into 5 groups, N=5 rats:

- Group 1: As negative control, treated with normal saline (0.2ml) Ip.
- Group 2: received 50mg/kg body weight of *Alysicarpus ovalifolius* Ip.
- Group 3: received 100mg/kg body weight of *Alysicarpus ovalifolius* Ip.
- Group 4: received 200mg/kg body weight of *Alysicarpus ovalifolius* Ip.
- Group 5: As a positive control group were treated with insulin 6i.u/kg Ip.

![Graphical presentation of liver enzymes levels in controls, and alloxan induced hyperglycaemic Wistar rats treated with insulin (6 i.u), *Alysicarpus ovalifolius* extract at 200mg/kg, 100mg/kg, and 50mg/kg per body weights.](attachment:image)

Tests groups

**Fig.1:** Graphical presentation of liver enzymes levels in controls, and alloxan induced hyperglycaemic Wistar rats treated with insulin (6 i.u), *Alysicarpus ovalifolius* extract at 200mg/kg, 100mg/kg, and 50mg/kg per body weights.

Determination of Blood Glucose Level

Blood samples were collected by tail vein by bleeding of rats at tail tip using scissors on day; 0, 1,
3, 5, and 7 of the experiment. Determination of the blood glucose level was done by the glucose oxidase principle (Beach and Turner, 1958), with a digital glucometer using the one touch glucometer strips (Accu-Chek Advantage, Roche Diagnostic, Germany) the results were expressed in mg/dl (Rheney and Kirk, 2000).

**Termination of Experiment**

On completion of treatment, all animals from each group were exposed to overdose of chloroform vapour in an anesthetic box with the lid covered. From the unconscious animals; Blood sample were collected via cardiac puncture, which were centrifuge and from the sera liver enzymes were assayed. This include; Alkaline phosphatase, Alanine aminotransferase and Aspartate aminotransferrase using Reitman and Frankel method (1959).

Then animals were carefully dissected to remove liver tissue sample and dropped in label container each containing formalin solution, and later processed by method of Carleton (1967).

**Statistical Analysis**

All the data were expressed as mean ± S.E.M. Statistical comparisons were performed by one way analysis of variance (ANOVA) using the Duncan’s multiple range tests (Duncan, 1977). The value of p<0.05 was considered statistically significant. The data were analyzed using SPSS version 17.0.

**Results**

**Results Of Histopathology Studies**

*Plate I.* Photomicrograph section of the liver of normal wistar rat (positive control), Arrow pointing at central vein; N= Hepatocytes, H&E Stain, X250.

*Plate II.* Photomicrograph section of the liver of alloxan induced hyperglycemic wistar rat treated with normal saline, Arrow pointing at central vein; N= Hepatocytes; No observable microscopic cellular lesion. H&E stain, X250.
Plate III. Photomicrographic section of the liver of alloxan induced hyperglycemic wistar rat treated with insulin (6iu/kg) of body weight, Arrow pointing at central vein; N= Hepatocytes; No observable microscopic cellular lesion, H&E stain, X250.

Plate IV. Photomicrograph section of the liver in alloxan induced hyperglycemic wistar rat treated with 200mg/kg, Arrow pointing to central vein, H&E stain, X250. a= microscopic cellular lesion; B=sinusoidal congestion with lymphoid aggregation.

Plate V. Photomicrograph section of the liver in alloxan induced hyperglycemic wistar rat treated with 100mg/kg, Arrow pointed; central vein, N= Slight microscopic cellular lesion/necrosis with sinusoidal congestion, H&E stain X250
Plate VI: Photomicrographic section of the liver in alloxan induced hyperglycemic wistar rat treated with 50mg/kg of extract, Arrow pointing at central vein; There is no observable microscopic cellular lesion, N=Slight sinusoidal congestion, H&E stain X250.

Discussion

Alloxan induces diabetes by destroying the beta-cells of the islets of langerhans in the pancreas leading to reduction in synthesis and release of insulin (Szkudelski, 2001). This model has been used to study the anti-diabetic effect of several plant products (Abdel-Barry et al., 1997; Babu et al., 2002).

The hypoglycemic properties of numerous medicinal plants have been studied and reported (Vats et al., 2002; Maroo et al., 2003; Muhammed et al., 2007; Tanko et al., 2011). Throughout the world many traditional plant treatments for diabetes exist. However, few have received scientific or medical scrutiny and the World Health Organization (WHO) has recommended that traditional plant treatments for diabetes warrant further evaluation (WHO, 1980). Troglitazone (Rezulin) is a thiazolidinedione and was approved in 1997 as an antidiabetic agent. Over 3 years, more than 90 cases of hepatotoxicity were reported, which resulted in withdrawal of this drug. Many therapeutic drugs were withdrawn from the market primarily because of hepatotoxicity: This include; Troglitazone, Trovafloxacin, Ebrotidine, Nimesulide, Nefazodone, Ximelagatran and Pemoline (Shah, 1999; Andrade, et al., 2007), Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Ostapowicz, et al., 2002; McNally and Peter, 2006). In the present study Alanine aminotransferase (ALT) level was found to be statistically significantly (P≤ 0.05) higher in Alloxan induced hyperglycemic wistar rats treated with extract 200mg/kg per body weight than when compared to positive control group values with a statistical significant higher value difference, extract 100 and 50mg/kg per body weight treated groups also shows a high level of ALT, but with no statistical significant difference value when compared. Aspartate aminotransferase (AST) shows statistically significantly higher value difference in Alloxan induced hyperglycemic rats treated with extract 200, and 100mg/kg per body weights, While a higher level was seen in extract 50mg/kg per body weight treated group but with no statistical significant value difference. Alkaline phosphatase (ALP) levels were found to be high in all extract treated groups when compared with positive control group level but with no statistically significant value difference. The results obtained reveal a higher level of the assessed liver enzymes in extracts treated groups especially at 200mg/kg and 100mg/kg doses compared to levels in normal rats, This result conform with the histopathological effects observed in Photomicrographic section of the liver in alloxan induced hyperglycemic wistar rat treated with 200mg/kg, which shows microscopic cellular lesion and sinusoidal congestion. This high liver enzymes level is in line with the observed histopathological effect in photomicrographic section of study animals liver treated with extract 200mg/kg per body weight may therefore reveals acute related damages to hepatocytes or and linked to biliary tract damage, the liver enzymes are biomarkers for liver injury (Mengel, and Schwiebert, 2005), since this enzymes are release due to hepatocytes injury resulting in higher levels than normal in circulation (Patel, et al., 1998). The extract may be toxi-potent which may probably be due
to the presence of high concentration of tannins in the extract (Charles, 2008), since tannins are found in all parts of a plant (Kadam, et al., 1990), and the extract used is crude which comprises of the whole plant structures. This probably may lead to high concentration of tannins in the extract composition.

Conclusion

The results of the present study clearly indicates that the aqueous-ethanolic extract of Alysicarpus ovalifolius contained anthracene, flavonoids, glycosides, saponins, and tannins, significant high levels of ALT and AST were observed in alloxan-induced diabetic rats treated with extract 200 and 100mg/kg per body weight doses, and at these doses, deleterious histopathological signs were noticed, this reveal the extract is toxic-potent. Further studies are in fact currently on the way to isolate the active principle and elucidate the exact mechanisms of action of Alysicarpus ovalifolius.

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