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Research Article

HEPATIC EVALUATION OF ALLOXAN-INDUCED DIABETIC ALBINO RATS TREATED WITH ETHANOL EXTRACT OF THE LEAF OF *SENNA ALATA*

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ABSTRACT

Hepatic cells of alloxan-induced diabetic albino rats treated with oral administration of ethanol extract of leaf of *Senna alata* were evaluated. Forty rats were grouped into eight on weight bases. Diabetes was induced by a single intra-peritoneal injection (1650mg/kg) of alloxan monohydrate. After 120hours of alloxan injection, the rats from group 2-8 were confirmed diabetic with fasting blood sugar levels greater than 9.5mMol/L. Group 1 rats were not induced with alloxan and served as normal control. The extract of *S. alata* was administered twice daily for 21 days at a dose of 500mg/kg – 2500mg/kg in Groups 4-8 respectively, Group 2 was treated with a standard diabetic drug, 'metformin' while Group 3 (was untreated and represent negative control). Blood sample and liver tissue were collected for investigation of liver function markers: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Total Bilirubin, Total Protein (TP) and Albumin (AL) as well as photomicrographs of the liver. When compared to the negative control, ALT, AST and ALP showed significant ($p<0.05$) decrease for 500-2500mg/kg treated groups. Similarly, Total protein showed significant ($p<0.05$) increase in 500-2500mg/kg treated groups when compared with the negative control. Total Bilirubin showed significant ($p<0.05$) increase in 2500mg/kg treated group and a significant decrease in 500 and 1000mg//kg treated groups and albumin showed significant ($p<0.05$) increase in 1000-2500mg/kg treated groups when compared with normal control. Results from photomicrographs indicated abnormal liver architecture for the negative control. The present investigation suggests that the extract may have a protective effect on hepatocytes.

Keywords: *Senna alata,* alloxan-induced, diabetic, liver function markers, photomicrographs

INTRODUCTION

The use of plants in medicine predates written human history. Ethnobotany (the study of traditional uses of plants is recognized as an effective way to discover future medicine). Green plants synthesize and preserve a variety of biological products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolites of plants are commercially important and of use in a number of pharmaceutical companies. However, a sustained supply of the source material often becomes difficult due to factors such as environmental changes, labour cost, cultural practices and over exploitation by pharmaceutical industries. [1]Over 120 therapeutic agents of known structure have been identified in about 90 species of plants so far investigated.[2] This small fraction has yielded some useful plant drugs which include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digotoxigenin, codeine, aspirin, atropine, pilocapine, capsciscine, allicin, artemesinin ephedrine among others.[3] Of paramount importance is the isolation, identification of active principles and elucidation of the mechanism of action of the active compounds present in these plants. Where the active molecule cannot be synthesized economically, the product must be obtained from the cultivation of plant material hence, works in both mixture of traditional medicine and single active compound are very important. The scientific study of traditional medicines, derivation of drugs through bioprospecting and systematic conservation of the medicinal plants are thus of immense benefit.

Senna alata is a tropical perennial herb which belongs to the family, Fabaceae. It has a thick downy branched shrub and its leaflets is 8-12 pairs having the lower leaf and upper ones being oblong-elliptic and broadly obviate respectively.[4] *S. alata* is native to Southeast Asia, Fiji, Northern Australia, Africa and Latin America.[5] It is an ornamental shrub which grows well in forest areas of West Africa especially in aquatic environment like ponds, rivers, ditches, roadsides and drainage channels. It is commonly called Ringworm shrub and this may be likened for its use in the treatment of fungal related diseases (fungicidal and fungistatic properties) like ringworm. In Nigeria, it is used locally in the treatment of several infections, which include ringworm and parasitic skin diseases.[6] *S. alata* has been shown to possess antibacterial, antifungal, antioxidant activities and can be used as an abortifacient.[7] The leaves, roots and stem of *S. alata* have been reported to be useful in treating convulsion, gonorrhoea, heart failure, abdominal pains and oedema and are also used as purgative[6]. Leaf extract of *S. alata* is a good antioxidant and the compound obtained has been identified as a flavonoid and named as "Kaempferol".[8, 9] Pharmacological actions elicited by these medicinal plants is a function of the biological chemical compounds possessed by them called phytochemicals and they are found in the different parts of the plants, the roots, stems and leaves.[10] Phytochemicals, otherwise known as secondary plant metabolites have biological properties such as antioxidant activities, antimicrobial effects, modulation of detoxification enzymes, stimulation of the immune system, modulation of hormone metabolism and anti-cancer property. They equally help in ameliorating diseases.[11] Phytochemicals like phenols, tannins, saponins, alkaloids and flavonoids present in plants have been observed to give plants its antimicrobial

activity.[12] The toxicological evaluation and effects of this plant have not been properly elucidated. The aim of this study is to evaluate the hepatic cells of alloxan-induced diabetic albino rats treated with oral administration of ethanol leaf extract of *S. alata*.

MATERIALS AND METHODS

Plant Material and Preparation of Extract

The leaves *S. alata* were collected from UNIPORT botanical garden Abuja campus, University of Port Harcourt, Rivers State, Nigeria and authenticated by biotechnologist Dr. Ekeke Chimezie of Department of Plant science and Biotechnology, Faculty of Science, University of Port Harcourt, Rivers state, Nigeria. Its voucher specimen Number is UPH /V/1225.

Experimental Animals

Forty (40) albino rats weighing 160-280g of a particular sex (female), were obtained from Font Scientific Laboratory Animal Farm, physiology laboratory Ofrima complex, Abuja campus, University of Port Harcourt, Choba, Rivers State, Nigeria. The rats were allowed to acclimatize in the animal house unit for 10days, during which they were fed with standard rat chow and separated into eight (8) groups, (5 each). Water was given liberally, *ad libitum.*

Ethanol Extraction of *Senna alata*

The leaves of the plant were air dried and triturated in a mechanical mill and then wrapped in a filtered paper and put inside the soxhlet extraction thimble and heated under reflux until the reflux was clear. The filtrate was concentrated on a rotary evaporator at 45^0C and the extract was then kept in sterile bottle under refrigerated condition at 4^0C throughout the experiment.

Preparation of Plant Extract

The stock solution of the plant extract was prepared by weighing the required quantity according to dose to be administered (500-2500mg/kg) after weighing the rats to get their average body weight.

Preparation of the chemicals

All chemicals used in this study were of analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstand, Germany. Reagents used for the assays were products of Randox Commercial Kits. Normal saline: 0.9g of sodium chloride (NaCl) was weighed and dissolved in few ml of distilled water, mixed and the solution was made up to 100ml and stored in clean, dry, screw-cap bottle.

Alloxan (1.5g) was weighed and dissolved in few ml of normal saline, stirred and the solution was made-up to 100ml. The required quantity of alloxan for the treatment was calculated based on the average body weight of each group of the animals.

Grouping and Treatment of the Animals

The use of experimental animals was conducted in compliance with NIH guide for care and use of laboratory animals (pub No: 85-23Revised 1985). Animal ethical clearance number (DPGCAE: BCH 042) for this study was obtained from the department of Biochemistry, University of Port Harcourt. The animals were divided in to 8 groups of 5 rats each. Treatment of animal was carried out according to the dosage required for each group and once daily in full dose.

Group 1: (normal control), these were rats fed with ordinary rat feed throughout the course of the experiment.
Group 2: These were rats treated with known standard diabetic drug (Metformin) administered at a dose of 200mg/kg.
Group 3: (Negative control): Rats in this group were diabetics and did not receive any treatment
Group 4: These were diabetic rats treated with plant extract administered at a dose of 500mg/kg.
Group 5: These were diabetic rats treated with extract administration at dose of 1000mg/kg.
Group 6: These were diabetic rats treated also with extract at dose of 1500mg/kg.
Group 7: These were diabetic rats treated with extract at a dose of 2000mg/kg
Group 8: Rats in this group were diabetic and treated with plant extract administered at 2500mg/kg.
The treatment processes lasted for a period of three (3) consecutive weeks with fasting glucose level monitored seven (7) days interval.

Induction of Diabetes

The animals were fasted overnight, few hours prior to induction of alloxan, fasting blood glucose test were conducted to obtain a baseline result. The animals (albino rats) in all the groups were induced by a single intra-peritoneal administration of alloxan monohydrate except group 1 (normal control). This causes an insulin –dependent diabetes mellitus (called ‘‘Alloxan Diabetes’’ in these animals, with characteristics similar to type 1 Diabetes in humans. The dose of alloxan induced to the rats was 160mg/kg body weight (standard method). The diabetic states of the rat were confirmed 120hrs after induction.

Method of Blood collection

After 96hours of induction of alloxan, the vein puncture technique or method of collecting blood from the tail of the rats was used, using a sterile lancet. A drop of blood from the tail of the rat was placed on the glucometer test strip and monitored on the electronic glucometer.

Estimation of Blood Glucose level

The fasting blood glucose level of blood samples drawn from the tail vein puncture was determined using One-touch ultraeasy glucometer. The strip was dipped into animal blood and inserted into the glucometer which automatically displayed the level of glucose in the blood.

Determination of liver function markers

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) Bilirubin, Total protein and Albumin were analyzed by kinetic methods kits from Randox (United Kingdom) using a double-beam spectrophotometer.

Histology

The plastics sample jar was labelled, and the vial was filled to about 2/3 full with the fixative. The organs were removed from the animal after dissection, and placed into the vial containing the fixative. This was done to preserve the cellular structure of the tissue. Fixation was done by immersion using 10% formalin**.** The tissues were subjected to standard routine histological procedures.[13] The slides were viewed using the light microscope

and histopathological changes were observed and recorded at X400 magnification identifying both the normal and the degenerated cells.

RESULT

Table 1: Effect of Ethanol Leaf Extract of *Senna alata* on liver enzyme activity of Alloxan-Induced Diabetic Albino Rats

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
1	12.00 ± 0.00	19.67 ± 1.53	24.19 ± 2.67
2	9.00 ± 1.00	20.00 ± 1.00	26.69 ± 1.53
3	34.67* ± 8.51	109.67* ± 10.50	34.00* ± 4.00
4	9.00 ± 1.00	29.00* ± 2.00	28.28* ± 0.10
5	9.00 ± 1.00	33.33* ± 3.22	28.77* ± 2.48
6	13.00 ± 1.00	41.00* ± 0.00	27.36 ± 0.94
7	10.00 ± 2.00	18.67 ±0.58	26.87 ± 0.58
8	12.00 ± 0.00	29.67* ± 2.52	32.22* ± 2.07

Values are shown in mean ± standard deviation, n=3

Results obtained from Table 1 showed a significant ($p<0.05$) increase in alanine aminotransferase activity when Group3 was compared to normal control (Group 1) while Groups 2, 4, 5, 6, 7 and 8 showed no significant increase ($p< 0.05$) when compared to group 1, also when Group 3 was compared to positive control (Group 2) a significant ($p< 0.05$) increase in alanine aminotransferase activity was observed. While group 1, 4, 5, 6, 7 and 8 showed no significant ($p<0.05$) increase when compared to Group 2. However, when Groups 1, 2, 4, 5, 6, 7 and 8 were compared to negative control (Group 3) a significant ($p<0.05$) decrease was observed.

Similarly, a significant ($p<0.05$) increase in aspartate aminotransferase activity was observed when Groups 3, 4, 5, 6 and 8 were compared to normal control (group 1) while Groups 2 and 7 showed no significant increase ($p<0.05$) when compared to Group 1, also when Groups 3, 4, 5, 6 and 8 were compared to positive control (Group 2) a significant ($p<0.05$) increase in aspartate aminotransferase activity was observed while Groups 1 and 7 showed no significant ($p<0.05$) increase when compared to Group 2. But when Groups 1, 2, 4, 5, 6, 7 and 8 were compared to negative control (Group 3) a significant ($p<0.05$) decrease was observed.

Furthermore, a significant ($p<0.05$) increase in alkaline phosphatase activity was observed when Groups 3, 4, 5 and 8 were compared to normal control (Group 1) while Groups 2, 6, and 7 showed no significant increase ($p>0.05$) when compared to Group 1, also when Groups 3 and 8 were compared to positive control (Group 2) a significant ($p<0.05$) increase in alkaline phosphatase activity was observed while Groups 1, 4, 5, 6 and 7 showed no significant increase ($p<0.05$) when compared to Group 2. But when Groups 1, 2, 4, 5, 6 and 7 were compared to negative control (Group 3) a significant ($p<0.05$) decrease in alkaline phosphatase activity was observed while group 8 showed no significant decrease ($p<0.05$) when compared to Group 3.

Table 2: Effect of Ethanol Leaf Extract of *Senna alata* on Total Protein and Albumin of Alloxan- induced Diabetic Albino Rats

Group	Total protein	Albumin
1	46.79 ± 1.37	2.88 ± 0.29
2	51.29* ± 1.04	34.9 ± 0.22
3	10.39* ± 0.84	41.9* ± 0.07
4	51.83* ± 1.74	35.1 ± 0.26
5	58.10* ± 3.57	35.7* ± 0.06
6	55.39* ± 4.47	41.6* ± 0.03
7	58.34* ± 1.83	39.4* ± 0.70
8	66.72* ± 1.41	38.4* ± 0.67

Values are shown in mean ± standard deviation, n=3

Result from Table 2 showed a significant ($p<0.05$) increase in total protein levels when Group 2, 4, 5, 6, 7 and 8 were compared to normal control (Group 1) while Groups 3 showed a significant ($p<0.05$) decrease. However, when Groups 1 and 3 was compared to positive control (Group 2) a significant ($p<0.05$) decrease in alkaline phosphatase levels was observed. Group 5, 7, and 8 showed a significant increase while Group 4 and 6 showed no significant increase ($p<0.05$) when compared to Group 2. But when group 1, 2, 4, 5, 6, 7 and 8 were compared to negative control (Group 3) a significant ($p<0.05$) increase in total protein level was observed.

Result also showed a significant ($p<0.05$) increase in albumin levels when Groups 3, 5, 6, 7 and 8 were when compared to Group 1, also when Groups 3 and 6 were compared to positive control (Group 2) a significant ($p<0.05$) increase in the albumin level was observed, while Group 4, 5, 7 and 8 showed non-significant increase ($p<0.05$), Group 1 showed a non- significant decrease when compared to Group 2. But when Groups 1, 2 and 4 were compared to negative control (Group 3) a significant ($p<0.05$) decrease in the albumin level was observed, while when group 5, 6, 7 and 8 were compared to group 3 a non – significant ($p<0.05$) decrease in the albumin was observed.

Table 3: Effect of Ethanol Leaf Extract of *Senna alata* on Total Bilirubin level of Alloxan- induced Diabetic Albino Rats

Group	Total Bilirubin (umol/L)
1	9.18 ± 1.07
2	8.63 ± 0.68
3	16.00* ± 2.00
4	4.67* ± 0.46
5	6.23* ± 1.04
6	8.57 ± 0.18
7	7.75 ± 1.36
8	13.96* ± 1.28

Values are shown in mean ± standard deviation, n=3

Total bilirubin level (Table 3) was significantly ($p<0.05$) increased when Groups 3 and 8 were compared to normal control (Group 1) while Group 2 showed non- significant increase ($p<0.05$) when compared to Group 1, However, when Groups 4 and 5 were compared to normal control a significant ($p<0.05$) decrease was observed, while when Groups 6 and 7 were compared to normal control a non- significant ($p<0.05$) decrease was observed. Also when Groups 3 and 8 were compared to positive control (Group 2) a significant ($p<0.05$) increase in the total bilirubin level was observed, while Group 1 showed non-significant increase ($p<0.05$) when compared to Group 3. On the other hand, when Groups 6 and 7 were compared a non-significant decrease was observed. But when Groups 1, 2, 4,5, 6, 7 and 8 was compared to negative control (Group 3) a significant ($p<0.05$) decrease in the total bilirubin level was observed.

Histology

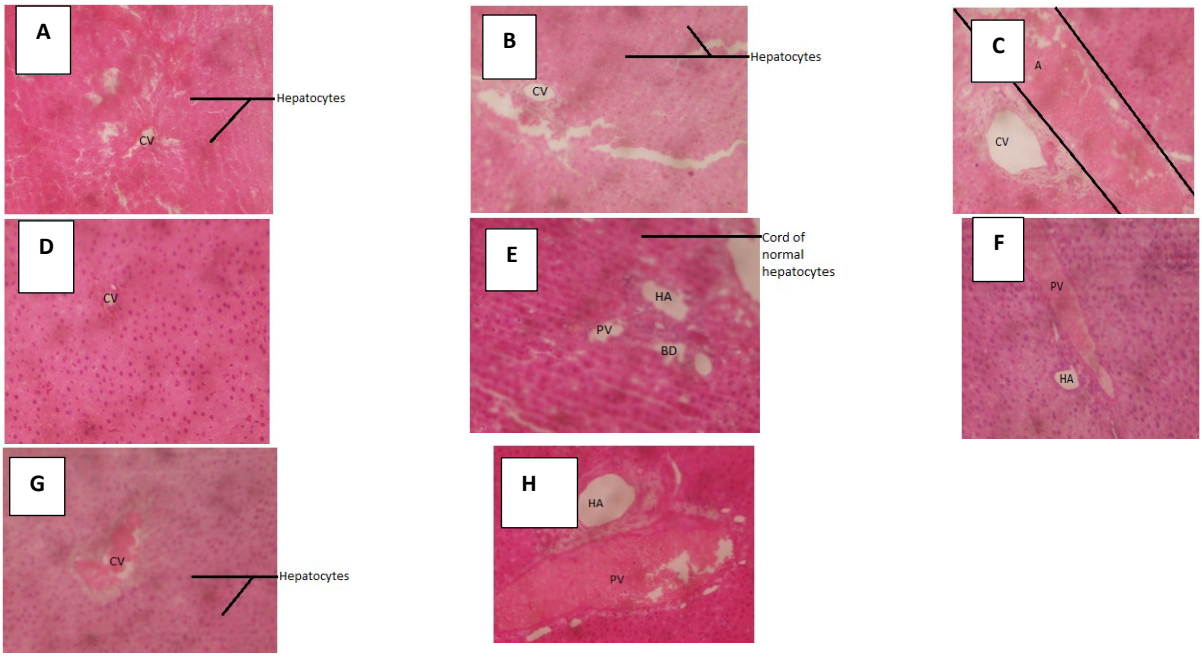

Plate 1: Photomicrograph of hepatocytes of rat.
A: Group 1 showing cords of normal hepatocytes, central vein (CV) is patent and normal.
B: Group 2 treated with metformin showing cords of normal hepatocytes, patent central vein (CV).
C: Group 3 (negative control) alloxan diabetic-induced rats showing partly cords of normal hepatocyte and congested dilated central vein with very large area of wiped out hepatocytes labelled A, bounded by two parallel lines patent central vein (CV).
D: Group 4 treated with 500mg/kg B.W extract of *S. alata* showing cords of normal hepatocytes and patent central vein (CV).
E: Group 5 treated with 1000mg/kg B.W extract of *S. alata* showing normal hepatic triad (hepatic artery, portal vein and bile duct) and cords of normal hepatocytes.
F: Group 6 treated with 1500mg/kg B.W extract of *S. alata* showing normal hepatocytes congested portal vein (PV).
G: Group 7 treated with 2000mg/kg B.W extract of *S. alata* showing normal hepatocytes and congested central vein (CV).
H: Group 8 treated with 2500mg/kg B.W extract of *S. alata* showing excessively dilated and congested portal vein (PV) normal hepatocytes.

DISCUSSION

Herbal medicines have received greater attention as alternative to clinical therapy in recent times leading to subsequent increase in their demand. The use of herbal medicines by the traditional practitioners for treatment of diseases remains the main stay of health care system and is gaining increasing popularity especially among the rural populace in the developing countries.[14] In rural communities, the exclusive use of herbal drugs, prepared and dispensed by herbalists without formal training, for the treatment of diseases is still very common. The effect of ethanol leaf extract of *S. alata* on hepatic cells of alloxan-induced diabetic albino rats was investigated. The liver enzymes along with bilirubin are the most commonly measured parameters in liver function test. These enzymes are hepatic in origin and they are leaked into the serum with the destruction of hepatic cells. The liver is an insulin-guided organ. Its behaviour changes depending on the level of the hormone insulin in the body. The destruction of insulin producing beta cells of the pancreas caused by alloxan leads to diabetes. Glucose level of the diabetic rats monitored were significantly reduced as treatment with *S. alata* progresses and before the end of the third week all the rats were diabetic- free except group three that were untreated. Diabetes raises the risk of non-alcoholic fatty liver disease, a condition in which excess fat builds up in the liver even if little or no alcohol is taken. Fatty liver disease raises the risk of developing liver inflammation or scarring (cirrhosis).[15] The result of negative control Group (alloxan-

induced without treatment) when compared to the normal control group showed a significant ($p< 0.05$) increase in ALT, AST, ALP, TB, AL and a significant ($p<0.05$) decrease in TP, Indicating that the liver was damaged and this agrees with the information from El-serag.[15] Metformin a standard drug for the management of diabetes prevents the liver from dumping more glucose into the blood and also causes no damage to liver. The result of group 4-8 (groups treated with various concentrations of *S. alata*) when compared to the metformin groups showed a non-significant ($p<0.05$) increase in ALT, ALP, Albumin while AST, T.P and T.B level of some groups showed significant increase. Following the fact that, ALT is a more specific markers of hepatic injury than other parameters, reason being that, other parameters example AST elevation, can also be seen in cardiac tissue injury, haemolysis and muscle tissue[16] would indicate that no damage was done to the hepatic cells of rats treated with *S. alata.*

Photomicrograph of liver of different groups of rats - normal, diabetic and treated groups - showed well-arranged cells and clear central vein for the normal groups. Rats treated orally with the extract of *S. alata* for 21 days, showed little abnormalities such as wiped-out hepatocyte, congested central vein and excessive dilated and congested portal vein. However, in the diabetic group, it showed the complete destruction of hepatocytes, degeneration of central vein, fatty degeneration, damaged hepatocytes and various size vacuoles.

CONCLUSION

This study presents strong evidence of the nontoxic effect of the leave extract of *S. alata.* It shows that the aqueous leaves extract of *S. alata* may have a hepatocyte regenerative potential thereby improving liver architecture of alloxan-induced diabetic albino rats. This could explain the extensive utilization of the plant in traditional medicine.

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