IAA Journal of Biological Sciences 8(1):210-215,2022. ISSN: 2636-7254

Evaluation of the hepatotoxic effects of alloxan induced diabetic albino rat treated with ethanol extract of sweet potato (*Ipomoea batatas*)

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ABSTRACT

In most part of the world sweet potato (ipomoea batatas) is being heavily cultivated in some of these areas , local population is putting parts of the plant to a good medical. Therefore this study investigated the hepatotoxic effects of alloxan induced diabetic albino rat treated with ethanol extract of sweet potato (*Ipomoea batatas*). Twelve albino rats were used for acute toxicity test while forty eight were divided randomly into six groups of eight in each group. The animals were starved for 48hrs before the commencement of treatment. Group A: served as normal control, Group B: served as diabetic control, Group C:was treated with a standard drug (Glucophage) at 100mg/kg body weight, Group D:was treated extract 200mg/kg body weight, Group E was diabetic group treated with extract at 400mg/kg body weight and Group F 600mg/kg body weight by oral administration respectively and water was adlibitum. Diabetes was induced in albino rats by intraperitoneal injection of alloxan at a single dose of 120mg/kg body weight in group B, C, D,E and F. Group D, E,F were fed with ethanol extract of ipomoea batatas (except groupA, B,&C) for a period of 28days serum biochemical parameters were analyzed. The hepatotoxic effects of alloxan induced diabetic albino rat treated with ethanol extract of sweet potato (*Ipomoea batatas*) were done using standard methods. The results indicated that the diabetic group treated with 200 mg/kg body weight of extract recorded a significant decrease in ALT (5.60±0.49*IU/L*) as compared to the negative control group (7.6±0.49IU/L), while there were no significant differences in AST between the positive and negative control groups as well as between groups treated with 200 and 400mg/kg body weight respectively. In conclusion, the liver functions were improved in diabetics rats group, especially group E that were treated with 400 mg/kg body weight of the extract.

Keywords:Hepatotoxic effects, alloxan, diabetic rat, sweet potato and *Ipomoea batatas*.

INTRODUCTION

Diabetes mellitus (DM) is a critical general medical condition, considered one of the greatest difficulties in our century owing to the quantity of individuals suffering from DM has enormously increased over the most recent 20 years [1]. DM is a metabolic problem that is described by chronic high blood glucose level that prompts complications in the eyes, kidneys, heart, vessels and nerves [2]. Elevated diabetes level is a consequence of uncontrolled glucose and prompts hazardous injury to many of the body's systems [3]. Glucose happens either when the pancreas doesn't deliver adequate insulin (type I diabetes) or when the body can't utilize the insulin it produces (type II diabetes).Patients with glucose type II constitute about 90%-95% in around the world [3]. This chronic complex illness requiring permanent clinical consideration involving hazard reduction techniques is beyond glucose control [4]. More treatment drugs are financially accessible in the administration of glucose however they have results and extravagant, subsequently the requirement for natural items as a

substitution treatment [5]. Sweet potato is being intensely developed in many pieces of the world and in a portion of these spaces, local people use portions of the plants for medicinal purposes. In the Philippines for instance, the plant is professed to be valuable in the management of diabetes without logical proof [6]. The plant is plentiful in dietary fiber, minerals, vitamins and compound of substance with organic impact, for example, β-carotene, phenolic corrosive and anthocyanin which gives it the one of a kind tissue tones (cream, yellow,orange and purple) [7].The anthocyanin found in sweet potato could control the blood glucose level by inhibiting the alpha-glucosidase [8] and could likewise increase the phosphorylation of insulin receptor [9].

Aim of the Study

The aim of this study is to evaluate the hepatotoxic effects of alloxan induced diabetic albino rat treated with ethanol extract of sweet potato (*Ipomoea batatas*)

MATERIALS AND METHODS

MATERIALS

STUDY SUBJECTS

The study subjects were make wiser rats with varying size or weight ranged between 100g to 200g

Material

Fresh tubes of *Colocasia esulanta* was punrchased from nkwo market inmgbakwu town, Awka North Anambra State. It was sent to the department of Zoology. Nnamdi Azikiwe University, Awka for proper identification by Dr. Bibian Aziagba, a taxonomist Voucher specimens were number: NAUH: 147[A] and was deposited at the departmental Herbarium

Animals

sixty male wister albino rats weighing between 100 and 120 was purchased from Chris Research farms, Awka and used for the LD_{50} while Male rats weighing between 150g and 200g was used for the antidiabetic studies

Procurement, identification and preparation of plant

Fresh tubers of *I.batatas*were purchased from major market in Enugu south Local Government Area, Enugu State. The tubers were then taken to the Department of Botany, Nnamdi Azikwe University, Awka for identification by an expert. Dry cooked tubers of *I. batatas* were macerated and made into extract through sun-drying, crushing (in pestle and mortar) and dissolution in 4L of70% ethanol for 2 hours. The filtrate was concentrated using water bath (k420) at 50°C before use.

METHODS

Study design

A total of forty-eight male Wister albino rats of approximately the same age and an average body weight of between 150 and 200g were purchased from Chris Research farm, Ngbakwu Awka. They were housed in standard aluminum cages (4 per cage, such that the number per cage will not interfere with clear observation of eachrats), in a 12-hour light and dark cycle with temperature of 22±2°C. The rats were allowed two weeks' period of dietary accommodation to acclimatize before they were randomly grouped into six (group A, B, C, D, E, and F) as shown in Table 1 below:

Table 1: Protocol for treatment

Group	Treatment
A	Normal control (fed with rat growers feed+ H_20 adlibitum)
B	Diabetic untreated (negative control fed with rat growers feed + H_20 adlibitum)
C	Diabetic (positive control) – treated with standard drug e.g Glucophage + fed with rat growers feed+ H_20 adlibitum)
D	Diabetic treated with 200mg/kg of *ipomoea batatas* + growers feed
E	Diabetic treated with 400mg/kg of *ipomoea batatas* + growers feed
F	Diabetic treated with 600mg/kg of *ipomoea batatas* + growers feed

All the rats were given standard feed and water *ad libitum*. The rats in group A were not induced with diabetes; group B were induced but not treated; group C were induced but treated with standard drug (Glucophage); group D to F were induced but treated with extracts at 200, 400 and 600mg/kg body weight. The extracts were prepared with distilled water and given daily by oral route (cannula feeding).The standard drug dose were equivalents of human therapeutic dose of the drug and were prepared using standard methods.

Induction of Diabetes Mellitus

Following fourteen days of acclimatization, alloxan monohydrate was utilized to initiate type II diabetes in exploratory creatures. Intraperitoneal administration of 100mg/kg body weight of alloxan monohydrate was managed once. A gentle pressing factor was then applied at the spot of infusion to improve ingestion. Following three days of administration, creatures' fasting blood glucose levels were checked utilizing the glucose observing gadget (Acu Check).

Sample collection

At the end of experimental administrations, the wistar rats were anesthetized in a desiccator containing cotton wool soaked with chloroform. After they had attained deep anesthesia, they were brought out of the desiccator and a laparotomy was carried out (by making a V-shape incision in the abdominal region with the aid of a surgical scissors) and the visceral organ (liver) were then exposed and harvested for analysis.

Ethical clearance

Animal handling was performed with regard to Guide for the Care and Use of Laboratory Animals and the University's research ethics. Procedures were performed in strict accordance with the recommendations in the Guide of the Chukwuemeka Odumegwu Ojukwu University Animal Ethical Committee and the protocols were appropriately approved. Study was also conducted in accordance with the Current Animal Care Regulations and Standards approved by the institute for Laboratory Animal Research.

Liver function test

Determination of alanine aminotrnsferase (ALT)

Add 0.5ml of ALT substratetest tube, Test Blank, Standard, and Standard Blank. This was incubated for 5mins at 37^{o}C. Thereafter, 0.1ml of serum was added to Test, 0.1ml of pyruvate standard added to Standard and 0.1ml of Distilled water added to test blank and standard blank. They were mixed and incubated at 37^{o}C for 30minutes. After the incubation, 5.0mls of 2,4 dinitrophenylhydrazine was added to all the tubes, mixed and incubated for 20min at room temperature (25 ^{o}C). This was followed by the addition of 5.0mls of 0.4N NaOH to all the tubes and read at 505nmλ after zeroing the

spectrophotometer with the blank. The results were then read off using the calibration curve provided

Determination of aspartate aminotransferase (AST)

Add 0.5ml of AST substrate to test tube, Test Blank, Standard, and Standard Blank. This was incubated for 5mins at 37^{o}C. Thereafter, 0.1ml of serum was added to Test, 0.1ml of pyruvate standard added to Standard and 0.1ml of Distilled water added to test blank and standard blank. They were mixed and incubated at 37^{o}C for 30minutes. After the incubation, 5.0mls of 2,4 dinitrophenylhydrazine was added to all the tubes, mixed and incubated for 20min at room temperature (25^{o}C). This was followed by the addition of 5.0mls of 0.4N NaOH to all the tubes and read at 505nmλ after zeroing the spectrophotometer with the blank. The results were then read off using the calibration curve provided.

Determination of alkaline phosphatase (ALP)

To Test, blank and Standard, was added 1.0ml of Alkaline buffer and phenyl phosphate substrate. They were incubated for 3min at 37^{o}C. Thereafter 0.1ml of serum was added to test, 0.1ml of phenol standard was added to standard and 0.1ml of distilled water was added to blank they were equally incubated for another 15mins at 37^{o}C. following the incubation, 1.0ml of 0.5NNaOH, 1.0ml of 0.5NNaHCO_3, 0.1ml of 4-amino antipyrine, 0.1ml of potassium ferricyanide were each added to all the tubes, mixed and read immediately after zeroing the spectrophotometer with blank at 510nmλ wavelength

Determination of bilirubin

The concentrations of conjugated and unconjugated bilirubin were determined using the method of Jendrassik and Grof (1938) as outlined in Randox Kit.

Procedure for total bilirubin (T BIL)

The sample blank and sample tubes were set for the analysis. About 200 µl of sulphanilic acid was put into the sample blank and sample tubes. Then 50 µl of Sodium nitrite was added into the sample tube. Then 1000 µl of caffeine was added to both sample blank and sample tube. Also 200 µl of the sample was added to both sample blank and sample tubes. The tubes were mixed and allowed to stand for 10 minutes at 20 – 25^{o}C. Then 1000 µl of tartrate was added to both sample blank and sample tubes. The tubes were mixed and were incubated for 5- 30 minutes at 25^{o}C and then, the absorbance of the sample blank was read at wavelength of 578nm.

Procedure for direct bilirubin (D BIL)

The sample blank and sample tubes were set for the analysis. About 200 µl of sulphanilic acid was put into the sample blank and sample tubes. Then 50 µl of Sodium nitrite was added into the sample tube. Then 2000 µl of sodium chloride was added to both sample blank and sample tube. Also 200 µl of the sample was added to both sample blank and sample tubes. The tubes were mixed and allowed to stand for 10 minutes at 20 – 25^{o}C.The absorbance of the sample was read at wavelength of 546nm against the sample blank.

Statistical analysis

Data were presented as mean ± standard deviation (SD) following one-way analysis of variance (ANOVA) and Tukey–HSD test using Microsoft Excel 2016. Differences between $p< 0.05$ were considered significant.

RESULTS

Table 1 : Effects of *Ipomoea batatas* ethanolic extract onALT,ALP, and AST

Group	Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
A	Normal control	9.00±0.63	7.00±1.10	46.33±1.51
B	Diabetic (negative control)	7.60±0.49[a]	6.20±0.98	42.40±0.80[a]
C	Diabetic (positive control)	7.60±0.49[a]	6.20±0.98	44.60±0.80[b]
D	Diabetic + 2000mg	5.60±0.49[abc]	6.00±1.10	41.33±1.03[ac]
E	Diabetic + 4000mg	6.50±0.55[a]	5.60±0.49	44.20±0.98[ad]
F	Diabetic + 6000mg	7.17±0.98[ad]	5.00±0.00[a]	43.17±1.47[a]

Hints: values are Means±SD, n = 6. The superscript letters indicate a significant difference ($p<0.05$). The concentrations of ALT, AST and ALP was highest with group A.ALT and ALP was lowest with group D while AST waslowest with group F.

Table 2: Effects of *Ipomoea batatas* ethanolic extract on bilirubin (total and conjugated bilirubin)

Group	Treatment	Total bilirubin (µmol/L)	Conjugated bilirubin (µmol/L)
A	Normal control	16.17±1.33	2.4±0.03
B	Diabetic (negative control)	15.40±0.49	2.12±0.02[a]
C	Diabetic (positive control)	16.00±0.89	2.21±0.02[ab]
D	Diabetic + 2000mg	15.33±1.03	2.14±0.02[ac]
E	Diabetic + 4000mg	14.80±0.40	2.08±0.00[abcd]
F	Diabetic + 6000mg	15.00±0.63	2.12±0.03[ace]

Hints: values are Means±SD, n = 6. The superscript letters indicate a significant difference ($p<0.05$).Total bilirubin was highest with group A and lowest with group E.

DISCUSSION, CONCLUSION AND RECOMMENDATION

DISCUSSION

The *Convulvulaceae* is an important family in traditional medicine for the treatment of many ailments ,*I.batata s*tubers which is a member of the family as a vegetable has great economic importance. In the present study, the diabetic group treated with 200 mg/kg body weight of extract recorded a significant decrease in ALT (5.60±0.49*IU/L*) as compared to the negative control group (7.6±0.49*IU/L*), while there were no significant differences in AST between the positive and negative control groups as well as between groups treated with 200 and 400mg/kg body weight respectively. Also, there were no significant differences in AST and ALT between groups treated with 200 and 400 mg/kg body weight, but group treated with 600 mg/kg body weight indicates a significant difference when compared group treated with 200 mg/kg body weight of ethanol extract of *I. batatas*.AST and ALT activities act as an indicator of liver function, thus there conquest of these enzymes after the management of *I. batatas*, point out that the normal functioning of the liver was

restored(Udayakumar, *et al.*, 2009). In the same table, it was noticed that the positive control group recorded a significant increase in ALP (44.60±0.80IU/L) as compared to negative control group (42.40±0.80IU/L), and the result of rats' groups treated with 2000 mg/kg body weight were decreased to 41.33±1.03 *IU/L* when compared to positive control group (44.60±0.80 *IU/L*).The decrease in ALP activity following *I. batatas* treatment indicates that the cooked *I. batatas* could be effective in management of diabetes.

CONCLUSION AND RECOMMENDATION

In conclusion, the liver functions were improved in diabetics rats group, especially group E that were treated with 400 mg/kg body weight of the extract.

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