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Research Article

Effect of ethanol leaves extract of *Justicia schimperi* on liver enzymes, serum proteins and bilirubin

Oboso E. Etim, Jessie I. Ndem, Utibe Evans Bassey*

Department of Biochemistry, University of Uyo, Nigeria

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***Correspondence:**
Utibe Evans Bassey,
E-mail: utibeevans@yahoo.com

ABSTRACT

Background: The effect of ethanolic leaves extract of *Justicia schimperi* on liver function was investigated. The liver enzymes studied include aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and bilirubin.
Methods: 30 adult albino Wistar rats with weight ranging from 150g to 224g were used. They were divided into five groups of 6 rats per group. Varying doses of 274mg/kg, 547mg/kg, 821mg/kg and 1095mg/kg per body weight of *Juisticia schimperi* leaves extract were orally administered daily to test groups II, III, IV and V for 14 days respectively. Group (I) however served as control that received normal rat pellets and water only.
Results: The results showed that there was a significant increase ($p<0.05$) in the activities of AST, GGT and ALT in all the test groups; ALP in Groups III and IV when compared to control. On the other hand, the results also showed no significant increase at ($p<0.05$) in the concentrations of total bilirubin and direct bilirubin of the test groups when compared to control. Also, albumin, globulins and total proteins showed significant increase ($p<0.05$) when compared to control.
Conclusions: Therefore, the observed changes in the parameters assessed, signifies the hepatotoxic impact of *Justicia schimperi* leaves which may be due to the doses administered and duration of administration or present of some bioactive substances.

Keywords: *Justicia schimperi*, Hepatotoxicity, Liver enzymes, Bilirubin

INTRODUCTION

Medicinal plants are the most important source of life saving drugs for the majority of the world's population. Herbalism is a traditional medicinal practice based on the use of plants and plant extracts.[1] Plants form the main ingredients of medicines in traditional systems of healing and have been the source of inspiration for several major pharmaceutical drugs. These drugs have been carefully standardized for their safety and efficacy.[2] Traditional use of any plant for medicinal purposes warrant the safety of such plant, particularly with regards to mutagenicity, nephrotoxicity, carcinogenicity and hepatotoxicity.[3] *Justicia schimperi* (family Acanthaceae) belongs to the class of plant used in traditional medicine for the management of certain illnesses.

The leaves of *Justicia schimperi* are used for the treatment of wounds and mixed with oil and salt, they are eaten to treat cardiac disorder. In Ghana, a leaf decoction is given to children for treatment of indigestion.[4] *Justicia shimperi* (mmeme) is outlined as one of the herbs used in paediatric care among the people of Akwa Ibom State, Nigeria. The leaves were used against respiratory tract infections such as cough where the leaves were squeezed and soaked in water and filtered. Some salt is added to

the fruit juice in a bottle and shaken before administration.[5] *Justicia schimperi* in concomitant administration with *Persea americana* is used in the treatment of high blood pressure among the people of Ilugun, Ilugun area of Ogun State, south-west Nigeria.[6] Medicinal properties of the plant mainly include; antispasmodic, fever reducer, anti-inflammatory, anti-bleeding, bronchodilator, anti-diabetic, disinfectant, anti-jaundice and oxytocic.[7]

It is antiperiodic, astringent, diuretic, purgative and is also used as an expectorant in addition to liquefy sputum.[8] The leaves, flowers and roots of this plant are used in herbal drugs against tubercular activities, cancer and possessed anti-helmintic properties.[9-11]

The leaf juice is stated to cure diarrhea, dysentery and glandular tumor.[11] The major phytochemicals commonly found in *Justicia schimperi* leaves are hydrocyanic acid, oxalate, phytic acid and tannins. Macro and micro elements also present in the leaf include; sodium, potassium, phosphorus, iron and magnesium. There is scarcity of information documented in literature on the effect of *Justicia schimperi* on liver enzymes and bilirubin in experimental models. Thus the present study is conducted to explore the toxicity or safety margin of *Justicia schimperi* on liver tissue of albino Wistar rats.

METHODS

Leaves of *Justicia schimperi* commonly called "mmeme" by the Ibibio people of Akwa Ibom State, Nigeria, were collected from Uyo-metropolis, Akwa Ibom State, Nigeria and identified by the Department of Botany and Ecological studies of the University of Uyo. A voucher specimen was kept at Department of Botany herbarium, University of Uyo, Nigeria. Fresh leaves of *Justicia schimperi* were properly washed with distilled water to remove accumulated dirt and were further air dried for two (2) weeks at room temperature. The sample was ground to powder using a grinder. Absolute ethanol (99.7%) was used for the extraction of the ground leaves sample. The filtrate was then placed in a water bath and was slowly evaporated to dryness at 50˚C for 72 hours. The dried extract obtained was stored in an air-tight covered container to prevent contamination and placed in a refrigerator at 4˚C.

Determination of LD50

Eighteen (18) albino mice purchased obtained from the College of Health Science Animal House, University of Uyo, were used to determine the LD50 of *Justicia schimperi*. They were divided into six groups with 3 animals per group. Different dosages of the plant extract was given thus; 1000mg/kg, 1500mg/kg, 2000mg/kg, 2500mg/kg, 3000mg/kg and 5000mg/kg.

After observation for 24 hours, mortality was observed at dosages of 3000mg/kg and 5000mg/kg. Using Lorke's method, the LD50 was calculated to be approximately 2737mg of *Justicia Schimperi* per kilogram body weight of mice. The dosages used in this study were 10%, 20%, 30% and 40% of the LD50.

Preparation of sample stock

3g of the extract was dissolved in 10ml of distilled water to make a concentration of 300mg/ml. the stock solution was prepared daily throughout the period of administration. The extract was administered to the experimental rats with their respective dosages base on their body weight

Experimental animals

The research involved the use of 30 adult albino rats with weigh ranging between 160g to 210g which were divided into 5 groups. The animals were obtained from the College of Health Science Animal House, University of Uyo. The animals were fed with rat pellets and clean drinking water *ad libitum*. The rats were housed in standard cages under laboratory conditions of humidity, temperature and 12 hours light/ darkness cycle. They were allowed to acclimatize for 14 days before commencement of administration. The study was designed thus:

Experimental design

Group I: Normal control group, received rat pellets and water only.

- Group II: Received doses equivalent to 274 mg/kg.
- Group III: Received doses equivalent to 547 mg/kg.
- Group IV: Received doses equivalent to 821 mg/kg.
- Group V: Received doses equivalent to 1095 mg/kg.

The rats were fasted twenty four hours after the last administration and then sacrificed under chloroform anaesthesia.

Blood samples were collected through cardiac puncture using sterile needles and syringes into labelled plain sample bottles and allowed to stand for two (2) hours and thereafter centrifuged at 2000g for ten minutes to obtain the serum. The serum obtained was preserved and used for biochemical assay.

Biochemical assay

The serum activity of AST was determined by kinetic method as reported by Young et al.[12] The serum ALT and GGT activities were assayed by colorimetric endpoint method as described by Young et al.[13]

The activity of ALP was determined by colorimetric endpoint method.[14] Total bilirubin concentration was estimated based on the use of van den Bergh reaction,

where diazotised sulfanilic acid (sulfanilic acid in HCl and sodium nitrite) reacts with bilirubin to form a purple-coloured complex.[15]

Statistical analysis

The results were expressed as mean ± standard error of mean (SEM). The statistical evaluation of data was performed using SPSS 7.5 for Windows. Student t-test and ANOVA were carried out on the data with value of P<0.05 accepted as significant.

RESULTS

The result in Table 1 shows the effect of *Justicia schimperi* leaves extract on the activities of serum liver enzymes, bilirubin and protein. A dose dependent significant increase is observed in the activities of serum GGT in the treated groups when compared to the control at p<0.05. The activities of serum AST and ALT were also observed to be significantly increased among the treatment groups when compared to the control group. Globulin, albumin and total protein increased significantly in the treatment groups.

Table 1: Effect of *Justicia Schimperi* leaves extract on the activities of serum liver enzymes, bilirubin and protein.

Groups	I	II	III	IV	V
GGT	0.50 ± 0.01	0.60 ± 0.01[a]	0.67 ± 0.02[a,b]	0.70 ± 0.02[a,b]	0.71± 0.01[a,b,c]
Direct bilirubin	0.04 ± 0.00	0.05 ± 0.00	0.06 ± 0.00[a]	0.05 ± 0.00	0.03 ± 0.00
Total bilirubin	0.05 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.08 ± 0.01[a,b]	0.06 ± 0.00
AST	72.00 ± 0.91	93.75 ± 0.85[a]	87.50 ± 1.44[a]	90.75 ± 0.48[a,c]	85.75 ± 0.63[a]
ALP	81.00 ± 5.31	68.50 ± 2.96	94.00± 3.24[a,b]	88.50 ± 2.10[a,b]	84.25 ± 0.85[b]
ALT	21.25 ± 0.85	26.00 ± 1.08[a]	26.00 ± 0.41[a]	30.00 ± 0.91[a,b,c]	24.50 ± 1.85[a]
Globulin	1.63 ± 0.09	2.45 ± 0.12[a]	2.18 ± 0.11[a]	2.05 ± 0.06[a]	1.53 ± 0.05
Albumin	3.28 ± 0.16	4.35 ± 0.12[a]	4.53 ± 0.14[a]	4.50 ± 0.18[a]	4.43 ± 0.13[a]
Total protein	5.48 ± 0.14	6.70 ± 0.18[a]	5.58 ± 0.12	6.03 ± 0.06[a,c]	6.65± 0.12[a,c,d]

Values represent mean ± SEM; a = significantly different at p<0.05 when compared to control (Group I); b = significantly different at p<0.05 when compared to Group 2; c = significantly different at p<0.05 when compared to Group 3; d = significantly different at p<0.05 when compared to Group 4.

DISCUSSION

Liver enzymes are well known biomarkers for the prediction of liver toxicity and as such, have been used in scientific reports.[16,17] Available evidence show that damage to liver cells results in elevations of these enzymes in the serum and the measurement of enzyme activities is of clinical and toxicological significance in determining liver damage by toxicants or in diseased conditions.[18,19] The level of these enzymes in the blood is directly related to the extent of the tissue damage.

The observed significant increase in ALT and AST activity in all the test groups compared to control may signify liver injury as seen in liver dysfunction, damage and liver diseases.[20] Increase in the activities of AST and ALT in the *Justicia schimperi* treated groups indicates that *Justicia schimperi* leaves have capacity to induce liver damage, while the observed dosage dependent alterations in enzyme activity imply that the toxic effect of *Justicia schimperi* increases with increase in dose. The findings from this study are in line with the reports by Singh et al and Navaro et al that the administration of plant extract increases the activities of ALT and AST. On the contrary however, Aquaisua et al reported that crude extracts of *Blighia unijugate* for example, have no toxic effects on both the kidney and liver of rats.[19-22] On the other hand, Abdulrahman et al and Ogunka-Nnoko et al reported that extracts of *Vitex doniana* and *Sorghum bicolor* have toxic effects on both the kidney and liver of rats.[23,24] Therefore, it is obvious that some plant extracts have hepatotoxic effect while some have hepatoprotective effect.

In this study, the comparison of means between control group and the test groups showed highly increasing levels in serum ALP. Hepatic alkaline phosphates are most densely represented near the canalicular membrane of the hepatocyte. Obstructive diseases, bile duct obstruction, primary biliary cirrhosis are some examples of diseases in which elevated alkaline phosphates levels are often predominant over transaminase elevation.[25]

Although elevated levels of alkaline phophatase (ALP) have been associated with bone diseases, it is also an indicator for obstructive jaundice and intra-hepatic cholestasis.[26] Hence, the observed higher activities of the enzymes in the entire test groups relative to control, suggests that *Justicia schimperi* can induce hepatic cell damage and/or other diseases like osteotoxicity. Increased gamma glutamyl transferase activity has been linked with hepatotoxicity, oxidative stress and

chromosomal aberrations in cells. The determination of serum GGT activity is well established, diagnostic test for hepatobiliary diseases, and is used as a sensitive marker of liver damage. Elevated serum GGT activity is associated with diseases of the liver, biliary system and pancrease. Significant increase in the GGT activity in all the test groups compared to control has shown a strong indicative that *Justicia schimperi* may induce hepatocellular damage.

Slight increments were recorded in the serum levels of Total and Direct bilirubin. Conjugated bilirubinaemia is one of the earliest signs of impaired hepatic excretion. The slight increase observed in the serum levels of total and conjugated bilirubin may be due to impaired excretory function of the liver.

CONCLUSION

Based on the findings of this study, therefore it can be concluded that excess consumption of leaf is toxic to the liver of albino Wistar rats. There is therefore need for further study on the toxicological profile of these plants and caution should be taken concerning its consumption.

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