Asian Journal of Research in Biochemistry

3(1): 1-8, 2018; Article no.AJRB.44499

Biochemical Alterations in the Liver and Kidney of Rats Following Sub-acute Administration of Aqueous Extract of Stem-bark of *Anacardium occidentale* (Cashew Tree)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ACF and FS designed the study, authors FS, AMF and ME carried out the study. Authors ME and PIO performed the statistical analysis. Authors ACF, PIO and MCO wrote the protocol and wrote the first draft of the manuscript. Authors ME, MCO and PIO managed the analyses of the study. Authors ACF, FS and AMF managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRB/2018/v3i129815
Editor(s):
(1) Dr. Mohamed Atiega Elbagermi, Associate Professor, Department of Chemistry, Misurata University, Libya.
Reviewers:
(1) Idorenyin Umoh, University of Uyo, Nigeria.
(2) Adeyomoye Olorunsola Israel, University of Medical Sciences, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/26783

Original Research Article

Received 19 July 2018
Accepted 10 October 2018
Published 23 October 2018

ABSTRACT

Background: The use of herbal medicines for treating ailments is rampant in recent years, and the toxicity implications of various plant preparations are sparingly reported. We investigated the potential effect of daily administration of aqueous extract of stem-bark of cashew tree on the liver and kidney status of rats.

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Methods: Rats were divided into 4 groups as follows: control rats received 1 mL of distilled water, G1 received 100 mg/kg, G2 received 200 mg/kg, while G3 received 400 mg/kg body weight of the extract for 28 consecutive days. The tissue homogenate supernatants were analysed for liver enzymes-alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) and kidney function indices- urea and creatinine.
Results: In comparison to control, total protein increased significantly (P< 0.05) at 400 mg/kg extract, whereas albumin level significantly decreased (P<0.05) in rats treated with extract. .Activities of AST, ALP and GGT increased markedly (P< 0.05) at 400 mg/kg, whereas a significant decrease was observed in bilirubin level when compared with the control. Levels of urea and creatinine in kidney tissue were significantly higher in extract-treated rats compared to control.
Conclusion: The findings suggest that the extract dose at 400 mg/kg may cause alterations with toxic implications in the liver and kidney of rats.

Keywords: Cashew; Anacardium occidentale; liver; kidney; urea; toxicity; herbal medicines.

1. INTRODUCTION

In recent years, there has been an exponential increase in the use of alternative and complimentary medicines for treatment of ailments in developed and developing countries [1]. The WHO estimate shows that about 80% of populations in some Asian and African countries still depend on herbal medicines for primary health care [2]. The thrust for the current trend may be associated with anecdotal reports that indigenous remedies are sources of new and natural substances with potential therapeutic effects against metabolic derangements and chronic degenerative disorders with little or no side effects. Furthermore, adverse effects and a high cost of conventional health care, affordability and accessibility to herbal medicines are factors underlying the increasing popularity and use of herbal therapy [3]. However, these indigenous drugs/medicines may be devastating and detrimental to the consumers and results in public health problems [4]. Herbal medications are claimed and widely believed to be beneficial; however, there are reports of nephropathy, hepatotoxicity, neurologic and heavy metal poisoning associated with their use [4,5]. Currently, public concerns trail the popular use of traditional remedies regarding the safety, efficacy and responsibility of practitioners using traditional remedies [1,5]. Systematic investigations of medicinal plants thus become a key to the discovery of drugs and also to help assess toxicity risks associated with the use of herbal preparations and consumption [6].

Cashew tree (*Anacardium occidentale L.)* belongs to a family of *Anacardiaceae,* and known to have numerous ethnopharmacological activities. Acute administration of aqueous or methanol extract of leaves and stem-bark of cashew plant are reported to show antidiabetic, antibacterial, anti-inflammatory and antiulcer-ogenic properties [7,8]. The leaves and the stem-bark are used in Nigeria and Brazil in the treatment of infections, gastrointestinal tract abnormalities, and skin problems [9]. Previous reports suggest that the plant improves antioxidant capacity against hepatocarcino-genesis induced by aflatoxin B_1 in Wistar mice [10]. In the existing literature, studies targeted at assessing the safety of various doses and potential toxic effect of the extract of cashew plant are sparsely available. However, a study has reported that cashew leaves extract may be toxic to the liver and kidney at a very high dose [11], whereas in a separate study, the leaves demonstrated no toxic effect on the brain and kidney of rats [12]. There is a dearth of literature on stem-bark extract of cashew tree in relation to safety and toxicity. This study was designed to evaluate and provide further scientific data on the safety and toxicity risk potential of aqueous extract of stem-bark of cashew tree.

2. MATERIALS AND METHODS

2.1 Chemicals

Diethyl ether from the British Drug House, United Kingdom was used. The assay kits for liver and kidney parameters were supplied by Randox Laboratories Limited, United Kingdom. All other reagents were of analytical grade and were obtained from British Drug House, United Kingdom.

2.2 Plant Identification, Authentication and Extraction

Fresh stem-bark of cashew tree (*Anacardium occidentale L.*) was obtained from the School farm of Ladoke Akintola University of Technology (LAUTECH), Ogbomosho, Oyo State, Nigeria, and was identified and authenticated at the Department of Pure and Applied Biology, LAUTECH, with the specimen voucher number LHO: 347. The stem-bark was gently washed and rinsed in distilled water thoroughly and air-dried at room temperature for 5 weeks and pulverised into a coarse powder using a mechanical grinder. The powdered stem bark (120 g) was first cold macerated in a water bath for 72 hours in 1 L of distilled water with frequent stirring, after which it was filtered using a muslin cloth to remove the cellulose and the filtrate was re-filtered with Whatmann filter paper No 1 to obtain the filtrate. The filtrate (extract) was dried in an oven (Genlab Widnes oven, England) at a temperature of 40°C to obtain concentrated extract. The dry extract obtained was weighed and stored in an air- and water-proof container kept in a refrigerator at 4ºC. The extract was reconstituted with distilled water to obtain the required concentrations of 100, 200 and 400 mg/kg body weight used for this study.

2.3 Animals

Twenty (20) Wistar rats weighing 150–180 g (8 to 10 weeks old) were used. The animals were obtained from the Animal Holding Unit, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria. Before the experiment, animals were housed for 2 weeks in polyethylene home cages with sawdust-covered floors cleaned every two days interval for acclimatisation. They were maintained in a room at 22 ± 2°C under conditions of constant humidity (55 ± 5%) and a 12 h light/dark cycle, with free access to water and standard commercial pellet chow. All experimental procedures were in agreement with the ethical rules for Care and Use of Laboratory Animals at LAUTECH.

2.4 Experimental Design

After the acclimatisation period, the animals were randomised into 4 groups of 5 animals each. Normal rats in control group received distilled water orally (1 mL daily) throughout the duration of the experiment. Rats in G1 G2, and G3 are normal rat that received different doses of 100, 200 and 400 mg/kg body weight, respectively, of the extract for 28 consecutive days by single oral gavage daily. At the end of the experimental period, the animals were sacrificed under diethyl ether anesthesia after an overnight fast.

2.5 Homogenate Preparation

The liver and kidney were excised, cleansed with cold saline water (0.9% NaCl w/v) and blotted dry with clean tissue paper. A clean sterile blade was used to cut each tissue and minced before being homogenised in ice cold phosphate buffered saline (1:5 w/v) using Teflon homogenizer. The homogenates were centrifuged at 3000 rpm for 20 minutes. The clear supernatant obtained was used to measure the liver and kidney parameters.

2.6 Biochemical Assays

The liver parameters, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total protein, total bilirubin and albumin were assayed with the homogenate supernatant using diagnostic kits of Randox Laboratory Limited. Urea and creatinine were analysed using Randox Laboratory Limited following standard methods.

2.7 Statistical Analysis

The data were expressed as mean ± SEM (n=5). Differences between group means were estimated using a one-way ANOVA followed by Tukey test, using SPSS version 20.0 for Windows (SPSS Inc., Chicago IL, USA). Results were considered statistically significant at $p< 0.05$.

3. RESULTS

3.1 Liver Parameters

In the present study, the administration of aqueous extract of stem-bark of cashew tree demonstrated alterations in homogenate biochemical parameters of liver functions as shown in Table 1. Activities of hepatic enzymes, ALP, ALT, AST, and GGT increased in hepatocytes dose-dependently. It was observed that the highest dose (400 mg/kg) of extract administered significantly increased ($p < 0.05$) ALP, AST, and GGT in comparison to control.

Figs. 1, 2, and 3 show that the extract had effects on the total protein, albumin, and bilirubin levels. The total protein levels in treatment groups increased dose-dependently with significant effect ($P< 0.05$) demonstrated at 400 mg/kg body weight of rats. However, when compared with the control group, albumin levels decreased significantly ($P< 0.05$) at every dose of aqueous extract of stem-back of cashew tree. Similarly, the three doses of extract significantly decreased total bilirubin in hepatocytes in comparison to control group.

3.2 Kidney Parameters

Table 2 presents the effects of the aqueous extract on urea and creatinine levels in kidney tissue. The treatments markedly increased the levels of kidney markers. Creatinine significantly increased ($P< 0.05$) in group G1, G2 compared to control group. Urea significantly increased ($P < 0.05$) in group G1, G2 and G3 compared to the control group.

4. DISCUSSION

A common misconception is that the use of natural substances in a variety of herbal preparations, concentrations and supplements cannot present toxic events [5]. However, although there is a paucity of published data on toxicity and safety profile of medicinal plant remedies, a few reports suggest their adverse effects on some organs, including liver and kidney [4,5,13-16]. In previous work, the nuts, kernels and leaf extract of cashew tree have demonstrated nutritional and biochemical health benefits [17-19]. In addition, extracts from stem-bark of cashew have also been reported with favourable effects in animal models [7-9]. However, the evaluation of its safety and toxic potential is important and this is currently sparsely available.

We have reported here, the perturbation that may be associated with the sub-acute administration of the extract of stem-back of cashew tree at different doses. In the current study, at the various doses of extract administration, the activities of hepatic enzymes increased dose-dependently (Table 1). Although ALT was comparable to control, the marked elevations of AST, ALP and GGT at 400 mg/kg may be important in relation to liver health status. The liver enzymes play a significant role in diagnosis, treatment and plant extract assessment for safety profile [6]. ALT and AST are hepatic enzymes although ALT is abundant in the cytosol, whereas AST is mitochondrial (80% of total activity) [20]. ALP and GGT are associated with hepatobiliary tract. Alterations in the enzyme activity may suggest tissue damage which may culminates into concomitant increase in their blood activities, as frequently observed in both acute and chronic exposures of liver to toxic agents [21-23]. The current elevations of these enzymes as compared to control might be caused by the interference of extract phytochemicals with protein synthesis or by the binding of the phytochemicals to some proteins involved in detoxification processes [23]. Possibly, the metabolic stress imposed on the liver by the extract at 400 mg/kg stimulates the enzyme activities to offset the stress [24,25]. And it has been suggested that there may be increased activities of various enzymes in some tissues under conditions of stress [26]. However, previous studies on stem-bark of cashew in the direction of the current study are limited. Furthermore, the significantly decreased concentration of albumin in the context of significant increase in total protein may suggest toxic alterations in protein and free amino acid metabolism and compromised protein synthetic capacity in the liver [23,27]. Our report corroborated the observations of Okonkwo et al [28] that reported significant increases in the activities of ALT and AST in serum although at higher doses of sub-acute administration of extract of stem-back of cashew. In this study, the hepatobiliary function was uncompromised as evidenced by the significant decrease in bilirubin levels in the extract-treated groups and the decreased levels of albumin support this observation. Hepatic damage usually results in increased serum level of bilirubin [6]. High bilirubin concentration causes a complementary increase in serum total protein, particularly albumin [28]. While the bilirubin level signaled no adverse effect, the albumin level in this study indicates possible unfavourable effect of the extract on oncotic pressure of the blood known to be sustained by albumin. Therefore, our findings for albumin and bilirubin levels in hepatic tissue may not sufficiently adversely affect serum levels of albumin and bilirubin. It may thus suggest that the extract elicited moderate toxic alterations in the liver tissue.

Urea is an important excretory product of amino acid metabolism. It is filtered from the blood by the glomerulus in the kidney. Creatinine is a nitrogenous product produced from creatine metabolism in skeletal muscles [29]. At the end

of 28 days, there were significant increases in the levels of urea and creatinine retained in the kidney tissue, and this may suggest alteration in renal function in the extract-treated rats compared to the control group. Urea and creatinine concentrations in serum are basic indicators of renal function [30], and the elevation of these parameters in the present study denotes inability of the kidney to excrete these products. In particular, creatinine level not only assesses kidney function status but also serves as a clinical indicator of renal toxicity associated with herbal extract or other compounds in experimental models [29]. Because many herbs contain pharmacologically bioactive compounds, they may exert toxic effect on various organs. Although they have historic traditional application in prevention, treatment and management of disorders, the scientific basis for such applications are not completely established [31]. The phytochemical screening of methanol extract of stem-bark of cashew tree revealed the presence of abundant amount of tannins and moderate amount of saponins to suggest evidence of tannins toxicity in the sub-chronic to chronic use of *Anacardium occidentale* stem-bark in complementary and alternative medicine [28]. It has been reported in the earlier studies that tannins toxicity may affect membranes, alter the excretion of cations, increases the excretion of proteins and essential amino acids in man and other monogastric animals [32,33]. However, the constellation of these toxic effects of tannins may be contributory to our observation of an increase in the levels of urea and creatinine in kidney tissue.

Table 1. Effects of daily administration of aqueous extract of stem-bark of cashew tree on liver enzymes in rats

Parameter	Control	G1	G2	G3
ALP (IU/L)	98.11 ± 6.50	98.18 ± 8.83	149.6 ± 13.67	164.4 ± 41.25*
ALT (IU/L)	67.33 ± 0.88	66.0 ± 0.58	68.0 ± 7.10	70.33 ± 1.45
AST (IU/L)	36.10 ± 3.22	36.70 ± 6.03	41.20 ± 4.36	62.10 ± 10.97*
GGT (IU/L)	3.35 ± 0.25	3.73 ± 0.46	4.12 ± 0.13	5.40 ± 0.22*

*Values are mean ± SEM (n=5). Control rats treated daily with 1 mL of distilled water; G1 rats treated with 100 mg/kg bw of extract; G2 rats treated with 200 mg/kg bw of extract; G3 rats treated with 400 mg/kg bw of extract. *Significantly different (p < 0.05) from control*

Table 2. Effects of daily administration of aqueous extract of stem-bark of cashew tree on urea and creatinine in rat kidney

Parameter	Control	G1	G2	G3
Urea (mg/dl)	13.42 ± 0.21	28.85 ± 0.95*	29.88 ± 1.03*	36.26 ± 1.84*
Creatinine (mg/dl)	0.59 ± 0.01	1.37 ± 0.10*	1.19 ± 0.20*	0.70 ± 0.01

*Values are mean ± SEM (n=5). Control rats treated daily with 1 mL of distilled water; G1 rats treated with 100 mg/kg bw of extract; G2 rats treated with 200 mg/kg bw of extract; G3 rats treated with 400 mg/kg bw of extract. *Significantly different (p < 0.05) from control*

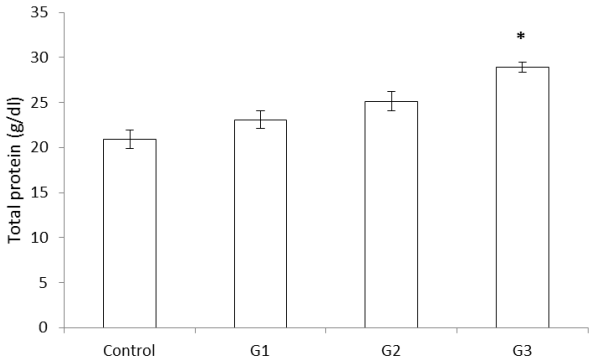

Fig. 1. Effect of daily administration of aqueous extract of stem-bark of cashew tree on total protein in rat liver

*Each bar represents the mean ± SEM (n=5); *Significantly different (p< 0.05) from control*

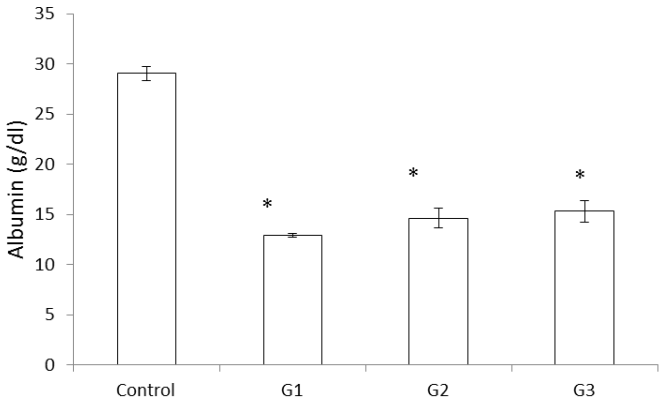

Fig. 2. Effect of daily administration of aqueous extract of stem-bark of cashew tree on albumin in rat liver

*Each bar represents the mean ± SEM (n=5); *Significantly different (p< 0.05) from control*

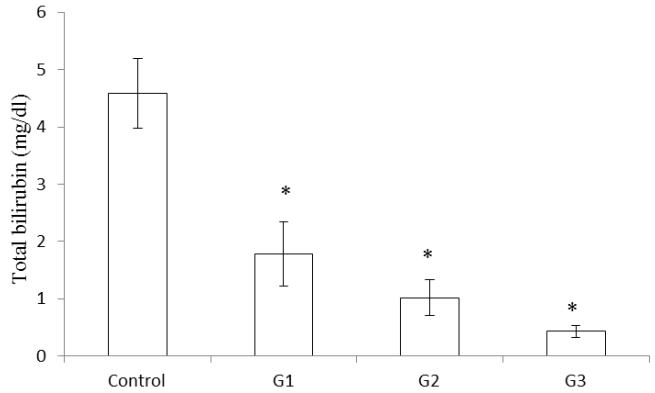

Fig. 3. Effect of daily administration of aqueous extract of stem-bark of cashew tree on total bilirubin in rat liver

*Each bar represents the mean ± SEM (n=5); *Significantly different (p < 0.05) from control*

5. CONCLUSION

This study indicates that daily administration of aqueous extract of stem back of cashew tree may alter liver and kidney status to exhibit toxic effects, particularly at 400 mg/kg body weight of rat. Given the growing use of herbs in a number of ailments, our findings suggest that traditional remedies from stem-bark of cashew warrants further investigations.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experimental procedures were in agreement with the ethical rules for Care and Use of Laboratory Animals at LAUTECH.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/26783