Original Articles
Toxicology International, *Sept-Dec 2015 / Vol-22 / Issue-3, 108-114*

ISSN (Print) : 0971-6580
ISSN (Online) : 0976-5131
DOI: 10.22506/ti/2015/v22/i3/137634

Hepatotoxic and Nephrotoxic Potentials of Aqueous Extract of Stem-Bark of Cashew Tree (*Anacardium occidentale L*) in Rats

Ademola C. Famurewa[1*], Funmilayo A. Showunmi[2], Abiola M. Folawiyo[3], Patience N. Ogbu[1], Michael A. Epete[4] and Emeka C. Igwe[4]

[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Federal University, Ndufu-Alike Ikwo, Ebonyi State, Nigeria; clementademola@yahoo.com
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria
[3]Department of Human Physiology, College of Medicine, Ebonyi State University, Abakaliki, Nigeria
[4]Department of Anatomy, College of Medicine, Ebonyi State University, Abakaliki, Nigeria

Abstract

The beneficial potential of the fruit, leaves and bark of cashew tree against health disorders have been reported; however, the effects of aqueous extract of stem-bark of cashew tree (*Anacardium occidentale L.*) on biochemical and haematological indices are sparse in the literature. Twenty Wistar rats were divided into 4 equal groups – one control group and three treatment groups. The control group was placed on normal rat feed, while group 1,2 and 3 received 100, 200 and 400 mg/kg bodyweight extract orally, respectively, for 28 consecutive days with distilled water *ad libitum*. The blood and serum obtained were used for haematological and biochemical analyses, while histopathological alterations were examined in the liver and kidney tissues. The treatment significantly increased serum ALP, AST, ALP, γ-GT, total bilirubin and urea at 400mg/kg ($P<0.05$) as compared to control. Although there was a significant increase in total protein, we observed significant decrease in serum albumin at 400mg/kg aqueous extract ($P<0.05$). Similarly, the low to high doses of the aqueous extract significantly decreased ($P<0.05$) red blood cell count. Our results suggest hepatotoxic and nephrotoxic potentials of aqueous extract of stem-bark of cashew tree at 400mg/kg, although lower oral dose may have beneficial effects.

Keywords: *Anacardium occidentale*, Cashew Tree, Creatinine, Urea, Stem-Bark

Received: 25-09-2015 **Revised:** 06-10-2015 **Accepted:** 15-12-2015

1. Introduction

In recent decades, there has been a renewal of interest in herbal medicine as an alternative source for prophylactic and therapeutic treatment against metabolic and malignant disorders in humans. In line with the increasing importance of traditional medicine in various climes of the world, (Ezuruike and Prieto, 2014) there is a surge in the number of publication of scientific data evaluating the beneficial efficacy, safety and toxicity of phytochemicals in medicinal plants. The need for chronic intake of a large number of drugs with their attendant side effects in addition to their high costs which is often borne by the patients is the identified reason for the paradigm shift in favour of herbal remedies (Ezuruike and Prieto, 2014). This form of natural remedies is accessible, affordable and acceptable among the indigenous communities in West Africa, Africa and worldwide, depending on the customs, tradition and religion (Okpashi *et al.*, 2014).

In Nigeria, herbal medicine has a long history of traditional use and its currently common compare to several decades past (Obi *et al.*, 2006). A large number of medicinal plants indigenous to Nigeria have been used as traditional remedies for various ailments with abundant reports from studies on their effects on lipid metabolism, free-radical scavenging potential and beneficial role in

* *Author for correspondence*

metabolic processes and disorders associated with major organs like the liver, kidney, pancreas, and cardiovascular system, although there is a paucity of data on their toxicological profile (Ezuruike and Prieto, 2014; Obi *et al.,* 2006; Chiu *et al.,* 2015; Adaramoye and Arisekola, 2013; Ngondi *et al.,* 2009).

The cashew tree (*Anacardium occidentale L*) is a medicinal plant of the Amazon, indigenous to Brazil (Ukwenya *et al.,* 2012) and known for variegated therapeutic potentials associated with its fruits, leaves, stem-bark and root. The fruits, stem-bark and leaves of cashew are used for the treatment of several diseases such as hypertension, asthma, bronchitis, and intestinal disturbances (Edet *et al.,* 2013). *Anacardium occidentale* leaves and stem-bark extracts are utilized widely for the treatment of diarrhea, dysentery, and colonic pain. It has also been reported to possess antidiabetic, antibacterial, anti-inflammatory, and antiulcerogenic properties (Okpashi *et al.,* 2014; Ojewole, 2004; Kamtchouing *et al.,* 1998). Phytochemical analyses of extracts from this plant report the presence of flavonoids, anthocyanins, tannins, alkaloids, quercetin-glycoside, phytate, micronutrients and nutritional elements (Belonwu *et al.,* 2014; Okonkwo *et al.,* 2010). Anacardic acids are phytochemical constituents of stem-bark extract of cashew tree which has been shownto inhibit tyrosinase activity in malanocytes and kill certain cancer cells (Okonkwo *et al.,* 2010; Mendes, 1990). Other chemicals demonstrated by a study include an acardol, hydroxybenzoic acid, kaempferol, salicylic acid and tannins (Mendes, 1990).

However, the toxic effects of cashew herbal preparations on organs of metabolic importance are not severally reported in scientific literature. The potential toxic substances in plants such as antinutritional factors, heavy metals and phytochemicals are emerging to be the subject of many investigations (Mendes, 1990). The broad use of cashew plant extract in metabolic disorders necessitates data on its toxicological profile. Although some studies have reported the hepatoprotective and nephroprotective effects of methanolic and ethanolic extracts of stem-bark of cashew, effect of aqueous extract which is a similitude of the traditional extract preparations, is not well reported. Thus, we set out to investigate the effect of aqueous extract of stem-bark of cashew tree on haematological and biochemical indices associated with hepatic and renal status in rats.

2. Materials and Methods

2.1 Chemicals

Formaldehyde was purchased from E-Merck, Mumbai, India, diethyl ether from BDH, UK, liquid paraffin from SD Fine Chemicals Limited, Mumbai, India. The assay kits for liver and kidney function parameters were supplied by Randox Laboratories Ltd. (Admore, Crumlin, Co-Antrim, UK). All other reagents were of analytical grade and were obtained from BDH (Poole, Dorset, UK).

2.2 Plant Material

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, by Dr. A.T.J Ogunkunle, an angiosperm Taxonomist with the specimen voucher number LHO: 347.

2.3 Plant Extract

The stem-bark was gently washed and rinsed in distilled water thoroughly and completely air-dried at room temperature for 5 weeks and pulverized 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 muslin cloth to remove the cellulose fiber and the filtrate was re-filtered with Whatmann filter paper No 1 to obtain the filtrate. The filtrate (extract) was dried in an oven (GenlabWidnes oven, England) at 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 4°C. 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.

2.4 Phytochemical Screening

A preliminary phytochemical screening of the aqueous extract of stem-bark of cashew tree for the presence of various phyto-constituents like alkaloids, saponins, tannins, phlobatannins, glycosides, phenols, anthraquinones and flavonoids was done using standard methods of analysis (Trease and Evans, 2002).

2.5 Animals

The study was carried out in 20 Wistar albino rats of either sex weighing 150-180 g (8 to10 weeks old). 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 acclimatization. They were maintained in a room at 22 ± 2°C under conditions of constant humidity (70 ± 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.

2.6 Animal Treatment

After the acclimatization period, the animals were randomized into 4 groups of 5 animals each. Rats in control group received distilled water orally throughout the duration of the experiment. Rats in experimental group 1, 2 and 3 received different doses of 100, 200 and 400 mg/kg body weight, respectively, of the extract for 28 consecutive days by oral gavage daily. At the end of the experimental period, the animals were sacrificed under diethyl ether anesthesia after an overnight fast. The blood samples were obtained by cardiac puncture into a plain and EDTA bottles to obtain serum and for haematological analysis. The blood samples in plain bottles were allowed to stand for 30 minutes. Serum samples were obtained by centrifuging blood samples at 3000 rpm for 15 min and stored at −4 °C in aliquots until analysis. The blood samples in EDTA bottles were stored in refrigerator at 4 °C until the time of analysis.

2.7 Biochemical Assays

The serum levels of Total Protein (TP), Albumin (Alb), Total Bilirubin (TB), urea and creatinine were estimated spectrophotometrically using the available diagnostic assay kits supplied by Randox Laboratories (Admore, Crumlin, Co-Antrim, UK) following standard methods. Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) were determined using the method of Reitman and Frankel (1957). γ-Glutamyltransferase (GGT) was assayed by the method of Szasz (1969), while serum Alkaline Phosphatase (ALP) was determined according to the method of Klen *et al*., (1960). All haematological parameters were determined by an automated hematological analyzer, SYSMEX KX-21, (SYSMEX Corporation, Japan).

2.8 Histopathological Analysis

Liver and kidney tissue samples from rats of different groups were removed, washed and immediately rinsed with saline and then fixed in10% buffered formalin, dehydrated in graded alcohol and embedded in paraffin. Fine sections obtained from microtome were mounted on glass slides and counter-stained with hematoxylin and eosin. These sections were then examined under a light microscope for histological changes and the photomicrographs taken by a Pathologist who was blinded to the treatment procedures.

2.9 Statistical Analysis

The data were expressed as mean ± SD (n = 5 in each group). 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 Phytochemical Screening

The aqueous extract of stem-bark of cashew tree was analysed for phytochemical constituents commonly associated with medicinal plants. The results showed that the most abundant phytochemicals were alkaloids, saponins, glycosides and phenols among others as shown in Table 1.

Table 1. Phytochemical constituents of aqueous extract of stem-bark of cashew tree

Phytochemical constituent	Qualitative level
Alkaloids	+++
Saponins	+++
Tannins	++
Phlobatannins	+
Glycosides	+++
Phenols	+++
Anthraquinones	+
Flavonoids	+

+++ abundant, ++ moderate, + trace

Table 2. Effect of administration of aqueous extract of stem-bark of cashew tree on serum total protein, albumin, total bilirubin and renal status markers in Wistar rats

Treatment	TP(g/L)	Alb (g/L)	TB (mg/dl)	Urea(mg/dl)	Creatinine(mg/d)
Control	33.20±0.99	3.43±0.44	1.75±0.88	26.16±1.11	3.48±0.99
Grp 1(100mg/kg)	36.58±1.01	2.61±0.71	1.66±0.49	29.55±1.40	0.33±0.10*
Grp 2(200mg/kg)	52.01±1.21*	2.37±0.62	1.87±0.29	32.74±1.19	0.31±0.12*
Grp 3(400mg/kg)	53.62±1.04*	1.40±0.81*	1.94±0.28*	33.29±1.25*	0.34±0.11*

Data represented as mean±SD (n=5). Significantly different from control in each column: *P <0.05.

Table 3. Effect of administration of aqueous extract of stem-bark of cashew tree on serum liver enzymes in Wistar rats

Treatment	ALT(U/L)	AST(U/L)	ALP(U/L)	GGT(U/L)
Control	56.01±1.73	8.67±1.53	11.96±1.13	2.66±0.71
Grp 1(100mg/kg)	63.33±5.13	11.67±1.53	13.77±1.04	3.71±0.86
Grp 2(200mg/kg)	64.33±2.52	25.33±1.53*	13.80±0.99	3.98±1.02*
Grp 3(400mg/kg)	73.11±4.58*	45.00±3.00*	20.24±1.09*	4.63±0.55*

Data represented as mean±SD (n=5). Significantly different from control in each column: *P <0.05.

3.2 Biochemical Parameters

The effects of administration of aqueous extract of stem-bark of cashew tree on serum total protein, albumin, total bilirubin, urea and creatinine are shown in Table 2. There were significant effects of the extract particularly at a dose of 400 mg/kg body weight of rats. However, the extract at different doses of 200 and 400 mg/kg significantly increased (P <0.05) total protein and urea, whereas significant increase was only observed for total bilirubin at 400 mg/kg, as compared to control. Serum creatinine decreased significantly (P <0.05) by the doses administered in the current study, as compared to control. Albumin levels decreased dose-dependently, but significantly (P <0.05) at 400 mg/kg of aqueous extract. In Table 3, the sub-acute treatment significantly increased the serum AST and GGT of rats administered 200 and 400 mg/kg aqueous extract of stem-bark of cashew tree, while increase in ALT and ALP activities were significant at 400 mg/kg of the aqueous extract.

3.3 Haematological Parameters

In Table 4, the administration of extract at different doses appeared not to have marked effect on haematological parameters considered in this study. However, we observed negative significant effect (P <0.05) of the extract at various doses on Red Blood Cell count (RBC). There were insignificant dose-dependent increases (P >0.05) in haemoglobin, Mean Cell Haemoglobin (MCH) and Mean Cell Volume (MCV). Packed Cell Volume (PCV) and Mean Cell Haemoglobin Concentration (MCHC) showed inconsistent changes (P >0.05).

3.4 Histopathological Changes

Light microscopy of the tissue sections of control group showed normal hepatic and renal structures. In extract-treated rats, there were mild to moderate alterations of hepatic structures ranging from vascular infiltration of lymphocytes, congested central vein to dilated sinusoids. The renal structures appeared normal but only with moderate tubular necrosis and collapsed tubules with moderate infiltration of inflammatory cells in interstitial space in group 4 administered 400 mg/kg aqueous extract.

4. Discussions

The use of medicinal plants in the treatment of ailments in both the developed and developing countries is on the increase. The therapeutic efficacy of most of these herbs is indisputable but their toxicities which sometimes lead to biochemical derangements may limit their possible clinical uses (Okonkwo *et al.,* 2010). Limited evidence demonstrates toxicity profile of these herbs in relation to potential health impact (Olatunji *et al.,* 2005; Tédong *et al.,* 2007). Thus, it is important that they are considered in this regard especially that the doses and dosing regimens of their preparations are not usually standardized (Okonkwo *et al.,* 2010).

In the present study, we investigated effect of aqueous extract of *A. occidentale* stem-bark on liver and kidney markers and haematological parameters. The findings suggest that the aqueous extract of *A. occidentale* stem-bark, after daily oral administration for 28 consecutive days, may be toxic to rat hepatic and renal status

particularly at 400 mg/kg. There was a significant increase in serum total protein. However, albumin level decreased markedly at 400 mg/kg contrary to expectation (Table 2). It is well established that albumin is the major serum protein that maintains colloidal oncotic pressure and binds a number of biological compounds for transport, including bilirubin, in the systemic circulation (Johnson *et al.,* 2001). It is known that low protein diet may be responsible for low albumin level. However, the animals in the present study were fed a diet with suitable protein content. It is conceivable to associate this discrepancy with liver damage which causes imbalance between the total protein and albumin. This may be responsible for accumulation of bilirubin as evident by significant increase at 400 mg/kg in this study, as it is well known that albumin plays a key role in bilirubin metabolism. Our result here suggests adverse alteration in biosynthetic capacity of hepatic parenchymal cells and increased production of γ-globulins. In previous studies however, total bilirubin and total protein levels in treated animals were reported to be insignificantly altered at higher doses, although with methanolicextract of *A. occidentale* stem-back (Okonkwo *et al.,* 2010). An earlier report indicated that serum total protein was significantly decreased by hexane extract of *A. occidentale* leaves at doses above 400 mg/kg (Tédong *et al.,* 2007). However, the current study reports possible hepatotoxicity of aqueous extract of *A. occidentale* stem-bark at lower dose of 400 mg/kg in comparison to previous reports (Tédong *et al.,* 2007). In support of this importantly, the activities of liver enzymes, ALT, AST, ALP and γ-GT, were found to be significantly elevated above control levels at a dose of 400 mg/kg (Table 3). ALT and AST are important diagnostic markers for evaluation of early liver damage especially by chemicals (Bukar *et al.,* 2005). ALT is more specific for liver injury, and thus a better index for hepatocellular leakage and loss of functional integrity. Alkaline phosphatase is a biochemical marker enzyme for plasma membrane architecture. These findings further suggest damage to plasma membrane of hepatocytes with consequent release of marker enzymes of hepatotoxicity. This was further confirmed by histological assessment of these organ tissues that showed liver infiltration, congestion and mild necrosis (Figure 1(d)). These results are in consonance with those observed in Okonkwo *et al.,* (2010) for significant increase in activities of ALT and AST after sub-chronic administration of extract of *A. occidentale* stem-bark. Contrary to our evidence, Tedong *et al.,* (2007) showed significant decrease in the serum activities of ALT, AST, while Fazil *et al.,* (2011) demonstrated marked reduction in GGT, ALP and aminotranferases by aqueous extract of *A. occidentale,* although this was found in a model of hypercholesterolemic rabbits.

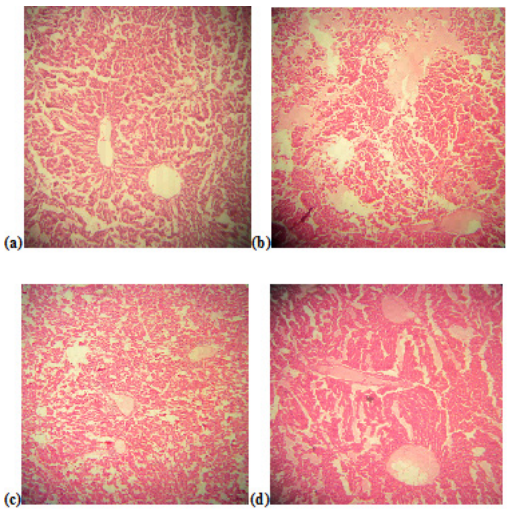


Figure 1. Photomicrographs of liver sections of rats from different treatment groups (H & E,×100): **(a)** control group with normal central vein, sinusoidal spaces and hepatic parenchyma without congestion; **(b)** group 1 (100mg/kg) showing moderately dilated sinusoids with fluid; **(c)** group 2 (200mg/kg) showing mild perivascular infiltration by lymphocytes; **(d)** group 3(400mg/kg) showing congested central veins and portal veins with mild infiltration of inflammatory cells.

Assessment of nephrotoxicity was determined by serum concentrations of urea and creatinine, one of the most commonly indicated endogenous markers of kidney injury (Sharifudin *et al.,* 2013). Urea is a nitrogenous waste product from protein metabolism. It is carried by plasma to kidney where it is filtered into urine by the glomerulus. Creatinine is a by-product of muscle metabolism and under normal physiological condition the amount excreted per day is constant and correlated with body mass (Adeyemi and Elebiyo, 2014). Serum urea and creatinine are considered the major nephrotoxicity markers, although serum urea concentration is often considered a more reliable renal function predictor than serum creatinine (Naggayi *et al.,* 2015). Elevation of urea and creatinine in plasma or serum is indicative of reduced renal function, defective glomerular filtration rate and biochemical endpoint for toxic effects of extracts/compound on the kidney in experimental models (Sharifudin *et al.,* 2013; Adeyemi and Elebiyo, 2014; Adeyemi and Akanji, 2012).

Table 4. Effect of administration of aqueous extract of stem-bark of cashew tree on haematological parameters in Wistar rats

Parameters	Control	Group 1(100mg/kg)	Group 2(200mg/kg)	Group 3(400mg/kg)
Hb (g/dl)	7.97±1.10	8.87±0.45	9.17±1.10	10.2±0.95
MCH (pg)	14.67±0.15	19.07±5.16	21.07±8.64	21.9±1.99
RBC($\times10^{12}$/L)	0.63±0.06	0.43±0.03*	0.45±0.06*	0.43±0.10*
PCV(%)	26.67±1.53	30.67±2.52	27.67±3.22	24.00±3.00
MCHC(g/dl)	33.27±0.31	33.23±0.35	33.10±0.26	33.20±0.44
MCV(fl)	43.93±0.45	59.00±7.41	63.67±26.52	57.30±14.8

Data represented as mean±SD (n=5).Significantly differentfrom control across each row: *P <0.05.

In the present study, serum levels of urea and creatinine were determined. The oral administration of the extract at 400 mg/kg for 28 days significantly elevated urea, whereas contrasting significant decreases were observed in creatinine levels. To our knowledge, it seems this is the first study to report significant elevation of serum urea at lower dose of 400 mg/kg in comparison to previous studies. Similar result was obtained by a study (Tédong *et al.,* 2007) for serum urea but at 6 g/kg hexane extract dosage. Our result is important because apparent toxicity of *A. occidentale* at 400 mg/kg in the present study is a pointer that the aqueous traditional remedies or regimen of the stem-bark may elicit hepatotoxicity and nephrotoxicity. Moreover, current study demonstrates that sub-acute oral administration of stem-back extract of *A. occidentale* may cause a significant increase in serum urea without adversely altering serum creatinine in normal rats. The presence of alkaloids and saponnins in the aqueous extract from our phytochemical screening corroborated the findings of others that stem-bark and fruits of *A. occidentale* contain alkaloids and tannins (Paris *et al.,* 1977), and this may be noteworthy in relation to its toxicity. For example, Igile *et al.,* (1995) reported in small animals like mice the toxicity of saponins extracted from *V. amygdalina*. We also report significant decrease in RBC count among haematological parameters evaluated in this study (Table 4). This would have undoubtedly contributed to the increased bilirubin level observed in this study. Although the fruits of *A. occidentale* are commonly eaten, leaves and barks have been shown to beneficially impact health outcomes in experimental models, the aqueous extract of stem-bark of this plant may exert important cytotoxic effects.

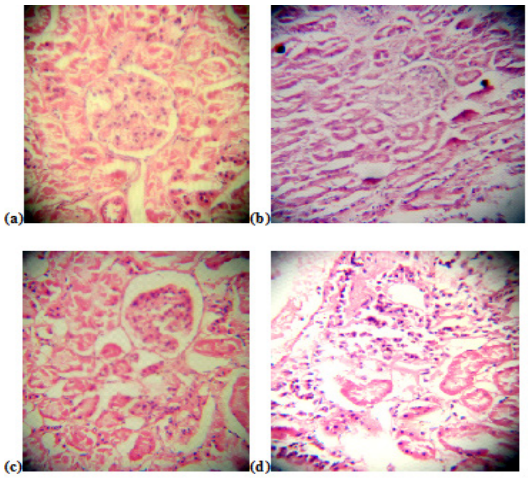


Figure 2. Photomicrographs of kidney sections of rats from different treatment groups (H & E, ×100): **(a)** control rats showing normal appearance of glomeruli, tubules and interstitium; **(b)** group 1 (100mg/kg) normal renal cortex with glomerulus containingmesengial cells and minimal capsular spaces, the renal tubules appear normal. The interstitial spaces show few infiltrating inflammatory cells; **(c)** group 2 (200mg/kg) renal cortex with normal glomerulus andcapsular spaces, thetubules appear normal without evidence of infiltrated interstitial spaces; **(d)** group 3 (400mg/kg) renal cortex with moderate tubular necrosis and collapsed tubules with moderate infiltration of inflammatory cells in interstitial space.

5. Conclusions

The present study has shown that aqueous extract of *A. occidentale* stem-bark may adversely alter biochemical parameters associated with the liver and kidney status particularly at 400 mg/kg to suggest hepatotoxicity and nephrotoxicity of the medicinal plant in experimental animals. In comparison to previous studies, current study found toxicity at a lower dose that may be comparable to

the levels traditionally consumed in remedies. Further studies are recommended to delineate on biochemical effects of aqueous extract of *A. occidentale*.

6. References

1. Ezuruike UF, Prieto JM. The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. J Ethnopharmacol. 2014; 155:857–924.
2. Okpashi VE, Bayim BP, Obi-Abang M. Comparative effects of some medicinal plants: *Anacardium occidentale*, *Eucalyptus globulus, Psidiumguajava, and Xylopiaaethiopica* extracts in Alloxan-induced diabetic male wistar albino rats. Biochem Res Int. 2014. Available from: doi.org/10.1155/2014/203051
3. Obi E, Akunyili DN, Ekpo B, Orisakwe OE. Heavy metal hazards of Nigerian herbal remedies. Sci Total Environ. 2006; 369:35–41.
4. Chiu C, Chen J, Chou F, Lin H. *Hibiscus sabdariffa* leaf extract inhibits human prostate cancer cell invasion via down-regulation of Akt/NF-κB/MMP-9 Pathway. Nutrients. 2015; 7:5065–87.
5. Adaramoye OA, Arisekola M. Kolaviron, a biflavonoid complex from *Garcinia kola* seeds, ameliorates ethanol-induced reproductive toxicity in male wistar rats. Niger J PhysiolSci. 2013; 28:009–15.
6. Ngondi JL, Etoundi BC, Nyangono CB, Mbofung CM, Oben JE. IGOB131, a novel seed extract of the West African plant *Irvingiagabonensis*, significantly reduces body weight and improves metabolic parameters in overweight humans in a randomized double-blind placebo controlled investigation. Lipids Health Dis. 2009; 8:7–13.
7. Ukwenya VO, Ashaolu JO, Adeyemi AO, Akinola OA, Caxton-Martins EA. Antihyperglycemic activities of methanolic leaf extract of *Anacardium occidentale (Linn.)* on the pancreas of streptozotocin-induced diabetic rats. J Cell Anim Biol. 2012; 6(11):169–74.
8. Edet EE, Adewuni D, Uti DE. Effect of ethanolic leaf extract of *Anacardium occidentale* on histoarchitecture of brain and kidney. J Pharm Biomed Science. 2013; 30(30):S31–5.
9. Ojewole JA. Potentiation of the anti-inflammatory effect of *Anacardium occidentale (Linn)* stem-bark extract by grapefruit juice. Exp J Clin Pharmacol. 2004; 26:183–8.
10. Kamtchouing P, Sokeng DS, Moundipa PF, Watcho P, Jatsa BJ, Lontsi D. Protective role of *A. occidentale* extract against streptozotocin-induced diabetes in rats. J Ethnopharmacol. 1998; 65:95–9.
11. Belonwu DC, Ibegbulem CO, Nwokocha MN, Chikezie PC. Some phytochemicals and hydrophilic vitamins of *Anacardium occidentale*. Res J Phytochem. 2014; 8(3):78–91.
12. Okonkwo TJ, Okorie O, Okonta JM, Okonkwo CJ. Sub-chronic hepatotoxicity of *Anacardium occidentale (Anacardiaceae)* inner stem bark extract in rats. Indian J Pharm Sci. 2010; 72(3):353–7.
13. Mendes NM. Molluscacide activity of a mixuture of 6-n-alkyl salicylic acid (anacardic acid). Rev Soc Bras Med Trop. 1990; 23:217–24.
14. Trease GE, Evans WC. Pharmacognosy. 15th ed. London: Saunders Publishers; 2002. p. 221-393.
15. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Amer J Clin Path. 1957; 289(1):56–8.
16. Szasz GA. Kinetic photometric method for serum glutamyltranspeptidase. ClinChem. 1969; 15:124.
17. Klen B, Read PA and Babson LA. Rapid method for the quantitative determination of serum alkaline phosphatase. ClinChem. 1960; 6:269–75.
18. Olatunji LA, Adebayo JO, Akinola OB, Olatunji VA, Adekoya A, Badaki OJ, Soladoye AO. Haematological effect of aqueous extract of *Hibiscus sabdariffa* petals in rats. The Tropical Journal of Health Sciences. 2005; 12(1):40–5.
19. Tédong L, Dzeufiet PD, Dimo T, Asongalem EA, Sokeng SN, Flejou J, Callard P, Kamtchouing P. Acute and subchronic toxicity of *Anacardium occidentalelinn* (*anacardiaceae*) leaves hexane extract in mice. Afr J Tradit Complimentary Altern Med. 2007; 4(2):140–7.
20. Johnson AM, Rohlfs EM, Silverman LM. Proteins. In: Burtis CA, Ashwood ER, editors. Tietz Fundamentals of Clinical Chemistry. 5th ed. India: Elsevier; 2001. p. 325–51.
21. Bukar BB, Uguru MO, Edache PJ, Wannang NN, Dayon BW. Sub-chronic hepato-toxicity evaluation of the aqueous extract of the leaves of *Xylopiaparviflora (Benth)* in rats. Nig J Pharm Res. 2005; 4:55–9.
22. Fazil F, Amom Z, Esa NM, Hasan MK, Kamilah K, Kadir A, Shah Z, Sulaiman S. Toxicity screening and hypocholesterolemic effect evaluation of aqueous extract of *Anacardium occidentale Linn* in hypercholesterolemic-induced rabbits. International Journal of Phytomedicine. 2011; 3:192–7.
23. Sharifudin SA, Fakurazi S, Hidayat MT, Hairuszah I, Moklas MA, Arulselvan P. Therapeutic potential of *Moringaoleifera* extracts against acetaminophen-induced hepatotoxicity in rats. Pharm Biol. 2013; 51(3):279–88.
24. Adeyemi OS, Elebiyo TC. *Moringaoleifera* supplemented diets prevented Nickel-induced nephrotoxicity in wistar rats. J Nutr Metab. 2014. Available from: doi.org/10.1155/2014/95821.
25. Naggayi M, Mukiibi N, Iliya E. The protective effects of aqueous extract of *Carica papaya* seeds in paracetamol-induced nephrotoxicity in male wistar rats. Afr Health Sci. 2015; 15(2):599–605.
26. Adeyemi OS, Akanji MA. *Psidiumguajava* leaf extract: Effects on rat serum homeostasis and tissue morphology. Comp Clin Path. 2012; 21(4):401–7.
27. Paris R, Phat M, Giono-Barber, Linhard J, Laureus J. Recherchechimique et pharmacologiquesur les feuillesd' Anacardium occidentale L. (Anacardiacées). Bull Soc Med Afr Noire. 1977; 22(3):275–81.
28. Igile GO, Oleszek W, Burda S, Jurzysta M. Nutritional assessment of *Vernoniaamygdalina* leaves in growing mice. J Agric Food Chem. 1995; 43:2162–6.