The Journal of Phytopharmacology 2021; 10(3):151-155
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2021; 10(3): 151-155
May- June
Received: 02-01-2021
Accepted: 01-03-2021

doi: 10.31254/phyto.2021.10301

Jamila Saleh
Department of Biology, Air Force Institute of Technology, Kaduna, Nigeria.

Funsho Olowoniyi
Department of Biochemistry, Kaduna State University, Kaduna, Nigeria.

Ekpa Emmanuel
Department of Biology, Air Force Institute of Technology, Kaduna, Nigeria.

Abdulrahman Abdullateef
Department of Biology, Air Force Institute of Technology, Kaduna, Nigeria.

Muhibat Komolafe Bolanle
Department of Biology, Air Force Institute of Technology, Kaduna, Nigeria.

Madinat Hassan
Department of Biology, Air Force Institute of Technology, Kaduna, Nigeria.

Correspondence:
Jamila Saleh
Department of Biology, Air Force Institute of Technology, Kaduna, Nigeria.
Email: j.saleh[at]afit.edu.ng

Acute Toxicity Assessment of the methanolic leaf extract of *Annona squamosa* Bark in Male Albino Rats

Jamila Saleh*, Funsho Olowoniyi, Ekpa Emmanuel, Abdulrahman Abdullateef, Muhibat Komolafe Bolanle, Madinat Hassan

ABSTRACT

Throughout the history of man, traditional and herbal method of treatment of diseases has been used without considering the dose effect. Therefore, this present study is an attempt on investigating the effect of different doses of *Annona squamosa* methanolic leaf extract on male wistar Rats especially the delicate organs. The work involves oral administration of different doses (10, 100, 1000, 1600, 2900, 5000 mg/kg body weight) of the extract to groups of rats according to Lorkes method. The animals were monitored for 30 days at every 24 hours interval in order to find the median lethal dose (LD_{50}) of the extract. Internal organ-body weight ratios (OBR) of animals in the test groups were determined and compared with those of the control group. LD_{50} was found to be greater than 5000mg/kg body weight without any significant decrease ($p>0.05$) in body weight. Biochemical analysis of Aspartate amino transferase (AST), Alanine amino transferase (ALT), Albumin and globulin of animals administered with extract showed no significant difference compared to the control groups ($p>0.05$) but concentration of Alkaline Phosphatase (ALP) indicated obvious changes in the treated groups compared to the control groups ($p<0.05$). Histopathology of the kidney revealed some inflammation at 1000, 1600, and 5000 mg/kg body weight. The implications of using this extract within safe doses in traditional medicine is hereby discussed.

Keywords: *Annona squamosa*, Acute toxicity, LD_{50}, Kidney congestion.

INTRODUCTION

Herbal and traditional medicine has been attracting attention in recent times. Literature on antidiarrheal, antimalarial and antitrypanosomal activities of plant-based products is on the increase as result of volume of work carried out by Scientists [1, 2, 3]. One major and overriding criterion in the selection of herbal medicines utilized in health care provision is safety [1]. Side effects should be reduced to the barest level if any herbal extract must be used as a drug. Plant's extract should not only be efficacious but safe for consumption. Therefore, screening of plants extract for disease management goes with the knowledge of their toxic level. *Annona squamosa* commonly known as custard Apple or Sweet Sop is a semi-evergreen shrub or small tree reaching 6-8m (20-26ft) tall. The plant is a native of tropical America and the West Indies, but its original home is uncertain [4]. Previous studies have shown that various chemical compounds, such as alkaloids, carbohydrates, tannins, phenolic compounds, isomeric hydroxyl ketones, cyclopeptides and acetogenins can be found in different parts of the A. squamosal plant [5, 6]. It has antibacterial activity against *Bacillus subtilis* and *staphylococcus aureus*. A number of antimalarial compounds have also been isolated from the plant which is traditionally used in diseases associated with malaria parasite [4]. Administration of the methanolic extract of *Annona squamosa* significantly prevent isoniazid-rifampicin-induced elevation in liver marker enzymes, together with increased total protein and reduced glutathione (GSH) levels [7]. Caryophyllene oxide was also the active agent isolated from an unsaponified petroleum ether extract of the bark of this plant [8]. Acute toxicity refers to the ability of a chemical to cause ill effect "relatively soon" after one oral administration or a 4 hour exposure of a chemical in the air. LD_{50} is an abbreviation for "lethal Dose 50%". The LD_{50} for a particular substance is essentially the amount that can be exposed to cause death in half of a group of some particular animals' species, when entering the animal's body by a particular route. There is a growing need to conduct toxicity study on herbal products to determine their safety for consumption [9]. Intake of medicinal plants without adequate guide on its safety and usage could lead to damaging of the organs and usually, liver and kidney are affected due to their involvement in metabolism and excretion of compounds. Renal toxicity is associated with the consumption of medicinal plants for treatment of diseases [10]. The aim of this current study was to evaluate the toxic potentials of the methanolic extract of this plant with a view to determining safe doses in traditional herbal application.

MATERIALS AND METHODS

Plant material (*Annona squamosa)*

Annona squamosa plant bark was purchased from Gardeners at Ungwan Rimi, close to Gamji Gate, Kaduna-Nigeria and authenticated at the Department of Biological Sciences, Ahmadu Bello University, Zaria. A sample was documented in the herbarium.

Preparation of Extract

The Fresh bark of *Annona squamosa* was air – dried under the shade and pounded to powder using mortar and pestle. 500g of the pounded sample was defatted with hexane using soxhlet apparatus. The Residue was then soaked in 95% methanol and divided into two portions and allowed to stand for 72 hours. This was subsequently filtered with Muslin cloth and the filtrate kept on a filter paper for further use. The filtrate was concentrated in a water bath and rotary evaporator with its temperature set at 40°C for 48 hours. The extract was finally concentrated by exposing to air to complete the drying process. The dried extract was stored in a refrigerator at 40°C until required.

Animal treatment

Thirty (30) male albino Wistar rats with body weights between 200 and 220 g were bought from the Nigeria Institute for Trypanosomiasis Research (NITR), Kaduna State, Nigeria. They were housed at the Kaduna State University (KASU) animal house to acclimatized for 14 days before the commencement of the experiment. The animals were maintained on a standard pellet diet throughout the acclimatization and administration period.

Acute Toxicity Test

The acute toxicity study was performed accordance to Lorke's method (Lorke, 1983). It was conducted in two phases using a total of sixteen rats. In the first phase, nine rats were divided into 3 groups of 3 rats each. Group 1, 2 and 3 animals were given 10,100 and 1000mg/kg body weight of the extracts respectively to possibly establish the range of doses producing any toxic effect. Each rat was given a single dose after at least 2 weeks of adaptation. In addition, a fourth group of 3 rats was set up as control group and animals in the group were not administered the extract. In the second phase, further specific doses (1600, 2900 and 5000mg /kg body weight) of the extract were given to three rats (one rat per dose) to further determine the correct LD_{50} value – the extract was usually dissolved in phosphate buffer saline (PBS) solution before given via oral route.

Biochemical Assay

Determination of ALP, AST and ALT activities in Plasma

Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), in plasma were determined using enzymatic kits (CYPRESS® Diagnostics, Langdorp, Belgium) following manufacturer's instructions.

Histopathology

The liver and kidney were carefully removed and weighed to determine relative organ weights and observed for gross lesions. All tissues were preserved in 10% formaldehyde for histopathological examination.

Relative organ weight

After 24 hours, animals were sedated in little quantity of chloroform while some organs like the kidney and liver were carefully dissected out and weighed in grams. The relative organ weight of each animal was then calculated as follows:

Relative organ weight = Absolute organ weight (g)/ Body weight of Rats (g) x100

Statistical Analysis

Statistical analysis was carried out using statistical package for social science (SPSS - computer package). Rat's body weights were expressed as mean ±S.D. Values in all groups were compared using the analysis of variance (ANOVA). For all analysis the level of statistically significant was fixed at $p< 0.05$

RESULTS

In the present study, the acute toxicity of the methanolic extract of *Annona squamosa* bark was evaluated by monitoring the levels of AST, ALT, ALP and total protein alongside histopathological changes. After the extraction; the percentage yield of the methanolic extract obtained from 500g of *Annona squamosa* bark was 3.654%. The acute lethal study of *Annona squamosa* on rats (Table 1) shows that no animal died within 24 hours after treatment with the extract and the LD_{50} was >5000mg/kg body weight. The biochemical analysis of AST, ALT, albumin and globulin shows no significant difference in any of the biochemical parameters examined in either the control or treated groups. But the analysis of ALP shows that there is significant difference in the treated groups compared to the control groups (table 2). The percentage organ-body weight ratio values (table 3) were not statistically significant ($p>0.05$). The photomicrograph shows the gross pathological features of some internal organs to capture the extent of damage to the tissues (figure 1). Kidney congestion was seen in all rats treated with the extract.

Table 1: Acute Lethal Effect of Methanolic Extract of *Annona squamosa* Bark Administered Orally to Wistar Albino Rats.

Experiment	Dose (mg/kg b.w.)	No of Dead rats at 24hrs	Treated rats at 24 hrs
Phase 1	10	0/3	0/3
	100	0/3	0/3
	1,000	0/3	0/3
Control	0	0/3	0/3
Phase 2	1,600	0/1	0/1
	2,900	0/1	0/1
	5,000	0/1	0/1
Control	0	0/1	0/1

Table 2: Changes in serum Biochemical Parameters due to the extract

Dose (mg/kg b.w.)								
Parameters	**10**	**100**	**1000**	**Control**	**1,600**	**2,900**	**5000**	**Control**
AST (IU/L)	218.00±34.04	249.00±25.24	189.67±27.61	187.00±28.93	112	112	220	72
ALT (IU/L)	60.33±10.12	42.67±7.77	43.00±6.00	42.33±6.11	22	33	62	31
ALP (IU/L)	172.00±7.94^{n}	131.33±19.86^{d}	174.67±10.97^{n}	173.30±13.87	99	109	143	140
ALBUMIN (mg/dl)	35.67±2.52	41.67±4.04	33.33±1.53	35.67±2.52	45	34	41	31
GLOBULIN (mg/dl)	13.33±3.79	20.33±5.13	15.33±7.57	21.67±3.51	20	16	22	14

Value are expressed as mean±S.D of three animals. Values with different superscript indicate significant different ($p < 0.5$). Parameters value in phase 2 ($n<3$) were not compared due to absence of measure of variability).

Table 3: Effect of Oral Treatment with Methanolic Extract of *Annona squamosa* on Percent Organ–body Weight Ratio of Rats.

(%) Organ – Body Weight							
Dose (mg / kg b.w.)							
Organ	**10**	**100**	**1000**	**1600***	**2900**	**5000**	**Control**
Kidney	0.49±0.05	0.49±0.09	0.49±0.06	0.32	0.18	0.88	0.54±0.07
Liver	3.37±0.64	4.26±0.38	3.72±0.36	1.83	2.24	2.01	3.51±1.07

*Test of significance was done in rows. Values (mean±S.D) with no significant difference ($p>0.05$).Those groups with single Rat per group ($n<3$) were not compared due to absence of measure of variability.

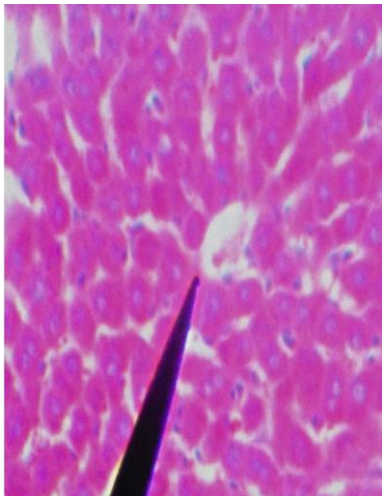

Plate 1: Photomicrograph of Liver section of control (H&E x 400)

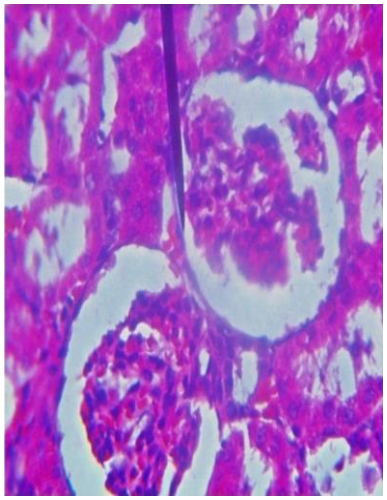

Plate 2: Photomicrograph of Kidney section of control (H&E x 400)

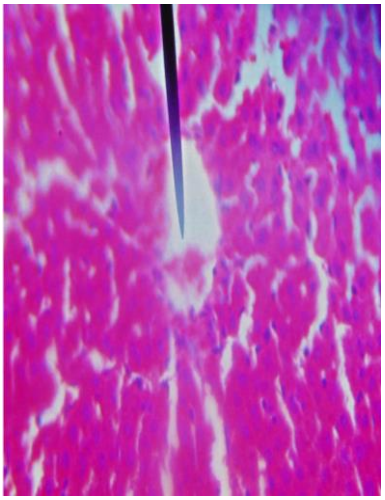

Plate 3: Photomicrograph of Liver section of animal treated with 1000mg/kg b.w. of extract (H&E x 400)

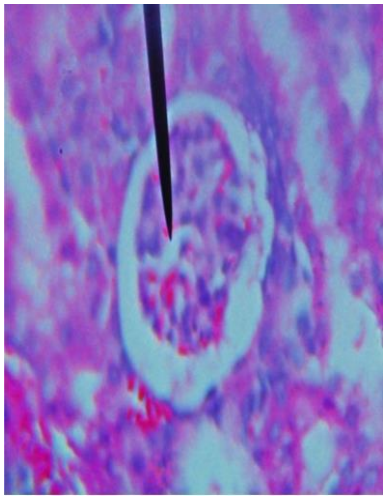

Plate 4: Photomicrograph of Kidney section of animal treated with 1000mg/kg b.w. of extract (H&E x 400)

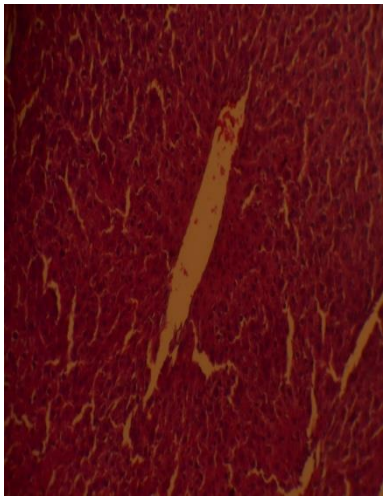

Plate 5: Photomicrograph of liver section of animal treated with 2900mg/kg b.w. of extract (H&E x 200)

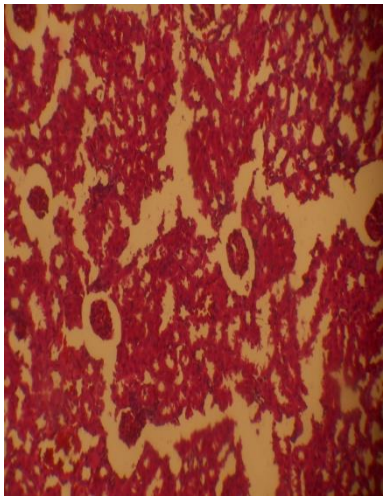

Plate 6: Photomicrograph of Kidney section of animal treated with 2900mg/kg b.w. of extract (H&E x 200)

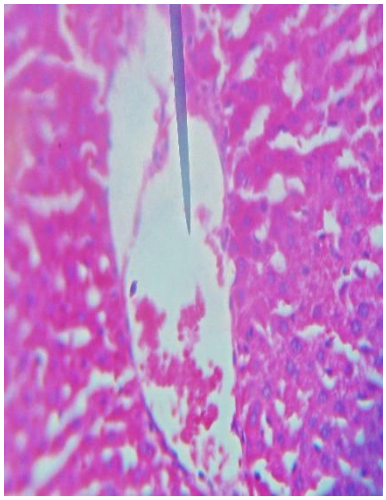

Plate 7: Photomicrograph of liver section of animal treated with 5000mg/kg b.w. of extract (H&E x 400)

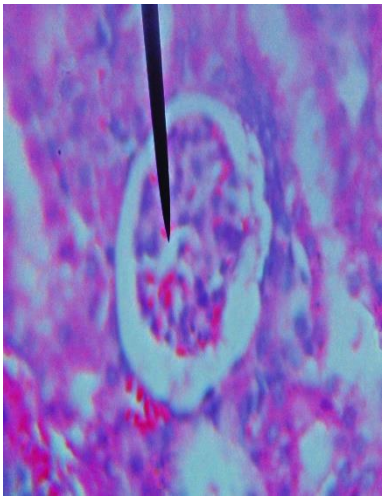

Plate 8: Photomicrograph of Kidney section of animal treated with 5000mg/kg b.w. of extract (H&E x 400)

Figure 1: Photomicrograph of Histopathological Studies of the organs due to different doses

DISCUSSION

The acute lethal effect of *Annona squamosa* bark extract on rats (table 1) showed that no animal died within 24 hours after treatment with the extract. The major signs of toxicity noticed within 24 hours included difficulty in breathing, loss of appetite and general weakness. These signs were not seen in 10 mg/kg, 100 mg/kg, and 1000mg/kg body weight, but progressed and became increasingly pronounced as the dose increased towards 5000mg/kg body weight. The LD_{50} being greater than 5000mg/kg body weight; is thought to be safe as suggested by Lorke (1983). Again, the absence of death among rats in all groups throughout the 24 hours of the experiment seems to support this claim. The biochemical analysis of AST, ALT, albumin and globulin shows no significant difference compared to the control groups ($p>0.05$). However, analysis of ALP shows that there is a distinction in the treated groups compared to the control groups ($p<0.05$) as presented in (table 2), this may be attributed to the obstruction of the biliary duct. Furthermore, the dose dependent weight loss observed, were not found to be statistically significant ($p>0.05$) when compared to the control groups (table 3). Kidney congestion appeared to be the major gross pathological damage accompanying treatment of rats with methanolic extract of *Annona squamosa* bark (figure 1). This observation is in harmony with the increase organ-body weight ratio values given in (table 3), though the values were not still significant ($p>0.05$). These were found to be toxic in acute toxicity studies at doses of 2000 and 3000mg/kg body weight. Extracts from *Annona squamosa* bark appear safer for usage in traditional medicine from the result of our investigation. It is possible that variations observed were due to the quantitative variation of saponin in this plant parts. Saponins are known to have deleterious haemolysing effect on circulating erythrocytes (5) and their presence in aqueous extract of *Annona senegalensis* accounted for its low therapeutic index found against *T. brucei brucei* infection in mice. Again, kidney congestion could be attributed, in part, to its role in biotransformation of xenobiotics. Survey by Darwin *et al.*, (2011) reported that *Annona squamosa* is a common plant with many folklore claims, which has medicinal properties like antifertility, antitumour and antimicrobial activities in experimental mice and rats. From the result of this study at this level, the methanolic extract of *Annona squamosa* bark at minimal dosage could be said to be relatively safe for usage in traditional medicine. Higher dosage should however be avoided and users should not rule out completely the possibility of chronic toxicity developing with continual usage of the plant as time progresses.

CONCLUSION

The findings of this study indicate that acute administration of *Annona squamosa* extract did not cause mortality in rats, but could lead to some physiological symptoms and Kidney congestion. The results obtained in respect to its toxicity study suggest that the leaf extract may lead to enlargement of organ-body weight. But relatively safe when administered in low dosage and lack of toxic effect, we recommend more clinical trials be conducted to ascertain the therapeutic dosage that will be suitable for pharmaceutical production.

Competing Interest

Authors have declared that no competing interests exist.

REFERENCES

1. Abdullahi AL, Agho MO, Amos S, Gamaliel KS, Wambebe C. Antidiarrhoel activity of the aqueous extract of*Terminaliaavicennoides* roots, *Phytotherapy Research*, 2001; 15:431-434.
2. Atawodi SE, Bulus T, Ibrahim S, Ameh DA, Nok AJ, Mamman M. In vitro trypanocidal effect of methanolic extract of some Savannah Plants, *African Journalof Biotechnology*, 2003; 2(9):317-321.
3. Nok AJ. Azanthraquinone inhibits respiration and in vitro Growth of long slender Blood stream forms of *Trypanosomacongolense, Cell Biochemistry and function*, 2002; 20:205-215.
4. Johns T, Windust A, Jurgens T, Mansor SM. Antimalarial Alkaloids isolated from *Annona squamosa, phytopharmacology* 2011; 1(3):49-53.
5. Pandey N, Barve D. Phytochemical and pharmacological review on Annona squamosa Linn. Int J Res Pharm Biomed Sci 2011; 2(4):1404-1412.
6. Singh Y, Bhatnagar P, Thakur N. A review on insight of immense nutra-ceutical and medicinal potential of custard apple (Annona squamosa Linn.). Int J Chem Stud 2019; 7(2):1237–1245.
7. Mohammad S, Thattakudia SU, Ramkath S, Azagusundharam M, Gnanaprakash K, Angala PS, *et al*. Protective effect of methanolic extract of *Annona squamosa Linn*. in Isoniazid –rifampicin induced hepatotoxicity in rats, *Pak. J. Pharm. Sci.*, 2011; 2:129-134.
8. Chavan MJ, Wakte PS, Shinde DB. Analgesic and anti-inflammatory activity of caryophyllene oxide from *Annona squamosaL*. Bark, *phytomedicine*, 2010; 17:149-151.

9. Saad B, Azaizeh H, Abu-Hijleh G, Said O. Safety of traditional Arab herbal medicine. Evid Based Complement Alternat Med. 2006; 3:433-9.
10. Mapanga RF, Musabayane CT. The renal effects of blood glucose-lowering plant-derived extracts in diabetes mellitus – an overview. Renal Failure. 2010; 32:132-138.

HOW TO CITE THIS ARTICLE
Saleh J, Olowoniyi F, Emmanuel E, Abdullateef A, Bolanle MK, Acute Toxicity Assessment of the methanolic leaf extract of *Annona squamosa* Bark in Male Albino Rats. J Phytopharmacol 2021; 10(3):151-155.