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A Study of Acute Toxicity and Cytotoxic Activity of *Prunus avium* Extracts against *Artemia salina* Larva

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Authors' contributions

This work was carried out in collaboration between the two authors. Author SOB designed the study, wrote the protocol and managed the literature search. Author AMO managed the laboratory work, performed the statistical analyses and wrote the drafts. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: This investigation was aimed at determining the cytotoxic activity of *Prunus avium* leaf and stem bark extracts as well as their acute toxicity in rats.
Methodology: The plant materials were air dried and grinded mechanically followed by solvent (ethanol) percolation for 48 h. Freshly hatched brine shrimp nauplii were exposed to the extracts in artificial sea water in the cytotoxic assay. Also, twelve adult male albino rats were placed in three groups of four (4) rats each, group I received distilled water, II and III were given orally, 300 and 200 mg/Kg body weight of the leaf and stem bark extract respectively for a period of 7 days. Haematological indices such as WBC, RBC, PCV, Hb and platelets were assayed while the internal organs including heart, liver and kidneys were weighed. The levels of serum ALT, ALP and AST were also determined.
Results: In the cytotoxic assay, *A. salina* showed high mortality against the plant crude extracts. The LC_{50} value of 8.89 ppm and 3.07 ppm were obtained for the leaf and stem bark extracts respectively. In the acute toxicity assay, an oral median lethal dose LD_{50} of the plant leaf and stem bark extracts were found to be 2738.61 mg/kg bw and 1870.83 mg/kg bw respectively. There were

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significant (p=0.05) reduction in the levels of RBC, PCV and Hb in the treated rats while there were increase in WBC and platelets compared with the control. Moreover, the plant extract had a significant ($p<0.05$) increase on the weight of liver of the treated groups II (5.323), III (6.055) compared with the control (4.881g). Also, there was a significant increase in the heart weight in group III (0.738g) while that of control (0.577g) and group II (0.609g) were not significantly different. Further, there was a significant increase in ALP and AST in treated groups (II and III) while ALT increased in group III whereas it was comparable in group II and control.

Conclusion: *P. avium* extracts may possess antitumor activity as shown with the effect on brine shrimps. Also, the plant leaf and stem bark appears to possess adverse effect on heart and liver of rats at high doses. Therefore it should be used in moderation as it is capable of causing suppression of haematopoietic system as well as multiple organ failure at high doses.

Keywords: Prunus avium; leaf; stem bark; cytotoxic; acute toxicity; haematology.

1. INTRODUCTION

Traditional medicine involves the use of whole plant or their parts for medicinal purposes. This form of health care provision system is becoming very popular these days throughout the world with more and more evidences from several clinical trials confirming the potency of these plants in the management of several human diseases [1]. Different people from different parts of the world have used plant as their primary source of remedy for the treatment of several human diseases from time immemorial. With the increase in resistance of pathogens to antimicrobial agents, there have been renewed interests in the development of potent antimicrobial agents of plant origin since they have been reported to be very effective in the management of diseases, both communicable and non-communicable [2].

Several plants have been studied for their medicinal potentials; some of the plants have been found nourishing while others were harmful due to toxic nature of the constituents of such plant. *Prunus avium* is such a plant whose fruits and other parts have been studied for medicinal properties [3]. It is a deciduous tree in the rose family (*Rosaceae*) growing to a height of 50 feet and having a broad round crown. The smooth bark is gray-brown with long horizontal lenticels and often peels. The leaves are simple, alternate, oval to obovate, 2 – 5 inches long, toothed on the margins and have 2 small glands at the base of the blade. Flowers are white and appear in clusters of 3 - 5 in early spring [4].

The fleshy fruit is dark red to black and edible. As with native cherries the wilted leaves, stems, and seeds of *P. avium* are toxic. Symptoms of gasping, weakness, excitement, pupil dilation, spasms, convulsions, coma, and respiratory failure can occur if ingested [5]. This study was therefore designed to determine the toxicity of *P. avium* leaf and stem bark extracts on brine shrimps and its acute toxicity in albino rats.

2. MATERIALS AND METHODS

2.1 Collection, Identification and Extraction of the Extracts

Fresh leaves and stem bark of *P. avium* were harvested from matured trees in a farmland in Owo, Ondo State, Nigeria in June, 2017. The plant materials were then authenticated at the Herbarium section of the Department of Forest Resources Technology and voucher specimens (X-PA7124L and X-PA7124B) of leaf and stem bark respectively were deposited in the same department, Rufus Giwa polytechnic, Owo. The authenticated plant materials were washed and cleaned thoroughly with tap water and then air-dried. Thereafter, dried material samples were then pulverized into powder with the aid of an electric grinder and were stored in clean air- tight containers, and kept in a cool, dry place until required for use.

One hundred gram (100 g) of the powdered sample was soaked in 200 ml of ethanol for 48hr with intermittent stirring using sterile spatula. The plant extracts were then filtered through muslin cloth into bijou bottles and then dried using rotary evaporator at a temperature of 50°C to yield crude extracts [6].

2.2 Laboratory Animals

Six (6) weeks old male albino rats of wistar strain (85-100 g) were sourced from Department of Animal Production and Health, Federal University of Technology, Akure. They were handled according to standard protocol and fed with rat

crumbs (Top feeds, Sapele, Nigeria) and water *ad libitum*.

2.3 Determination of Cytotoxic Effect of Plant Extracts

The brine shrimp (*Artemia salina*) lethality bioassay was carried out according to the method described by Haq et al. [7]. Brine shrimp eggs were hatched in artificial sea water prepared by dissolving 38 g of salt in 1 liter of distilled water, filtered and put in shallow rectangular dish. A plastic divider with several holes of 2 mm size was clamped in the dish to make two equal compartments. Brine shrimp eggs were placed in one side of the compartment while the other compartment was illuminated. After 48 h of illumination, phototrophic nauplii (Brine shrimp larvae) were collected by using pipette from the lightened side. Samples were then prepared by dissolving 20 mg each of the extracts in 2 mls of DMSO from where further diluted concentrations of 1000, 100, 10 and 1 ppm were prepared. A 4ml portion of the artificial sea water was added into each test tube and 20 shrimps were transferred into it. This was followed by the addition of 1 ml of each of the test extracts and of previously prepared concentrations and maintained under illumination at room temperature. Survivors were counted with the aid of magnifying glass after 24 h. The percentage mortality was calculated using Abbot's formula and the LC_{50} was also determined [8].

2.4 Acute Toxicity Study

The animals were divided into 15 groups of four animals per group; control group and 14 treated groups. They were maintained on standard rat feed (Top feed, Warri, Nigeria) and water and allowed to acclimatize for seven days to the laboratory environment before the experiment. After an overnight fast, the control group received 0.3 ml sterile distilled water while each treated group received 10 mg/kg, 100 mg/kg, 1000 mg/kg, 1,500 mg/kg, 2,250 mg/kg, 3,500 mg/kg and 5,000 mg/kg body weight administered orally with the aid of a feeding needle connected to syringe at stated doses in appropriate volume of sterile distilled water according to the method of Ubon et al. [9]. The animals were observed for signs of toxicity including writhing, paw-licking, stretching, respiratory distress, diarrhoea and mortality for the first critical 4 hours and thereafter at 3 hours interval for 24 hours.

The oral median lethal dose (LD_{50}) was calculated as the geometric mean of dose that caused 0% (a) and 100% (b) mortality respectively [10] using the formula; $LD_{50}=\sqrt{ab}$.

Twelve (12) mature male *albino* rats were used for this part. Animals in group I (control) received an equivalent volume (0.34 ml) of distilled water. The test groups II and III received orally, 300 and 200 mg/kg body weight of leaf and stem bark extract respectively representing 1/10 of each extracts' LD_{50} for a period of seven days. The extract was dissolved in distilled water as vehicle and delivered in 0.45 and 0.30 ml to group II and III respectively. After which they were fasted overnight before being sacrificed 24 hr after the last administration using standard protocols.

2.5 Determination of Haematological Parameters and Internal Organ Weight

At the end of the treatment period, the animals were anaesthetized in chloroform vapour and the blood collected via cardiac puncture into a plane tube. Heparinized test tubes were used to collect blood samples for haematological indices assay. White blood cells (WBCs), Red blood cells (RBCs), packed cell volume (PCV), platelets and Haemoglobin (Hb) were assayed by automated techniques using the Sysmex (Sysmex K21, Tokyo, Japan) automated machine respectively. After the blood collection, major internal organs including heart, liver and kidneys were weighed in triplicates.

2.6 Determination of Blood Biochemical Parameters

Test kits for estimation of serum alanine amino transferase (ALT), alkaline phosphatase (ALP), aspartate amino transferase (AST), alkaline phosphatase (ALP) were obtained from Teco Diagnostics, USA.

2.7 Data Analysis

Data were presented as mean±standard error (SE). Significance difference between different groups was tested using two-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range Test using SSPS window 7 version17.0 software. The significance was determined at the level of p=0.05.

3. RESULTS AND DISCUSSION

In the cytotoxicity assay of the plant's leaf ad stem bark extracts, brine shrimp lethality test was used which was based on the ability of tested samples to kill laboratory cultured larva of brine shrimp (*Artemia salina*). This assay is an important tool for probing toxicity level of substances since the brine shrimp is highly sensitive to a variety of chemical substances and it is a rapid and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-cancer properties [11].

Percentage mortality of brine shrimp larvae at four different concentrations (1, 10, 100, 1000 ppm/ml) of the plant's leaf and stem bark ethanol extracts revealed a concentration dependent activity as higher mortality percentage was observed with the increase in concentrations of the extracts. The LC_{50} value significantly (p0.05) less than 100 ppm/ml were recorded for both extracts with 8.89 and 3.07 ppm respectively for leaf and stem bark extracts (Table 1). LC_{50} values higher than 1000 ppm are not significant while those within the range of 0-100ppm/ml are considered to be very toxic [12]. The lethality concentration (LC_{50}) of the plant extracts were found to be within the range considered to be very toxic and earlier reports of brine shrimps assay already available [13] suggests that plant extracts with LC_{50} values of 20 mg/ml have a likelihood of yielding anticancer compounds. This indicates that *P. avium* leaf and stem bark extracts may contain active substances that may have potential antitumour properties.

The results of the acute toxicity of the ethanol extracts of both leaf and stem bark of *P. avium* are presented in Table 2. The Table showed an oral LD_{50} of 2738.61 mg/kg bw and 1870.83 mg/kg bw respectively for leaf and stem bark extract including the percentage mortality of the rats at various concentrations in the acute toxicity study.

At the doses above 2250 mg/kg bw and 1500 mg/kg bw of leaf and stem bark extracts of the plant, there were marked symptoms of toxicity such as salivation (stem bark extract), drowsiness, wobbling gait, weakness, pupil dilation, tremors, convulsion and death among the rats. These observations are in consonance with the ethnobotanical information on the plant [14].

The results suggests that *P. avium* leaf and stem bark extracts are not toxic in low concentrations

Table 1. Percentage mortality of brine shrimps at different concentrations of *P. avium* extracts

Dosage (ppm)	Leaf				Stem bark		
	Initial larvae	No. of survivors	No. of deaths	% mortality	No. of survivors	No. of deaths	% mortality
1000	20	0	20.00±0.10^b	100	0	20.00±0.00^b	100
100	20	4	16.00±1.05^b	80	0	20.00±0.01^b	100
10	20	8.67	11.33±1.00^b	56.65	3	17.00±0.05^b	85
1	20	12	8.00±0.05^b	40	10.3	9.70±0.10^b	48.5
LC_{50}				8.89			3.07

Values followed by different superscripts across each row are significantly different at p=0.05

Table 2. Acute toxicity test (LD_{50}) of ethanol extracts of *P. avium* leaf and stem bark

Sn.	Dosage (mg/kg bw)	Mortality ratio	Percentage mortality	Mortality ratio	Percentage mortality
		Leaf		Stem bark	
1	Control	0/4	0	0/4	0
2	10	0/4	0	0/4	0
3	100	0/4	0	0/4	0
4	1000	0/4	0	0/4	0
5	1500	0/4	0	1/4	25
6	2250	1/4	25	2/4	50
7	3500	2/4	50	4/4	100
8	5000	4/4	100	4/4	100
LD_{50}			2738.61 mg/kg bw		1870.83 mg/kg bw

Table 3. Effect of *P. avium* extracts on haematological indices of male albino rats

Group	RBC ($x10^6 \mu L^{-1}$)	HB (g/dl)	PCV (%)	WBC ($x10^3 \mu L^{-1}$)	PLAT ($x10^5 \mu L^{-1}$)
I	7.26	12.67	41.76	11.15	12.39
II	6.41	11.32	38.14	13.06	14.57
III	6.03	9.89	37.04	15.01	15.11

but may be very toxic at a higher doses and this supports the position of Clarke and Clarke [15], who reported that any compound or drug with oral LD_{50} estimates greater than 1000 mg/kg body weight could be considered to be of low toxicity and safe. Therefore, *P. avium* leaf and stem bark can be considered to be relatively safe and may be used for medicinal purposes.

The effect of the plant materials on the haematological parameters of the rats presented in Table 3 revealed a decrease in RBC, HB and PCV while there was an increase in WBC and platelets in the treated groups compared with the control group. The significant decrease in the RBC, Hb and PCV in the treated rats is an indication that ethanol extract of *P. avium* leaf and stem bark at the dosage used may have suppressed haematopoiesis which may be due to the reported presence of saponin, which has been reported to reduce haematological parameters probably due to lysis of blood cells or suppression of blood cell synthesis [16]. Meanwhile, the increase in WBC may be attributed to possible presence of antigens in the extract which elucidate this kind of immune reactions. Also, the increase in platelets in the treated group may be due to the production and secretion of thrombopoetin by the extracts. Thrombopoetin is known for regulating platelet production, therefore, *P. avium* extracts may possess haemostatic property [17].

The results of the effect of the plant materials on internal organs of the rats are presented in Fig. 1 which revealed that there was no significant changes in the weight of the kidneys of the treated groups and the control. However, the weight of liver of the treated groups II (5.323) and III (6.055) were higher than the control (4.881 g). Also, there was a significant increase in the heart weight in group III (0.738 g) while that of control (0.577 g) and group II (0.609 g) were not significantly different.

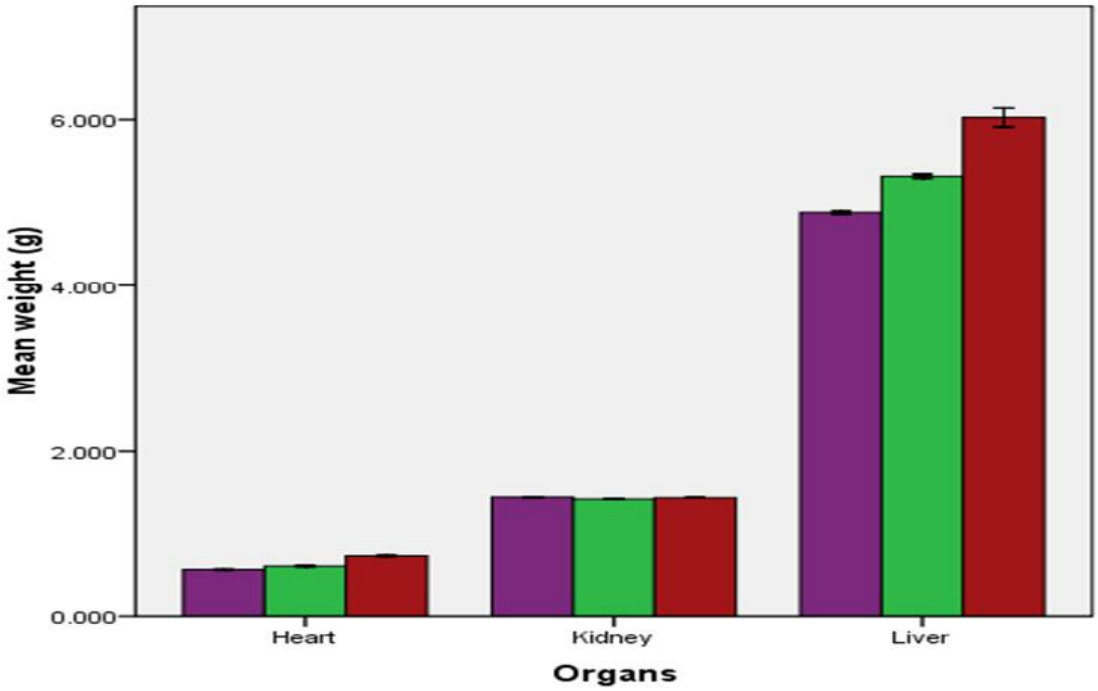

Fig. 1. Effect of *P. avium* extracts on weight of internal organ in male albino rats

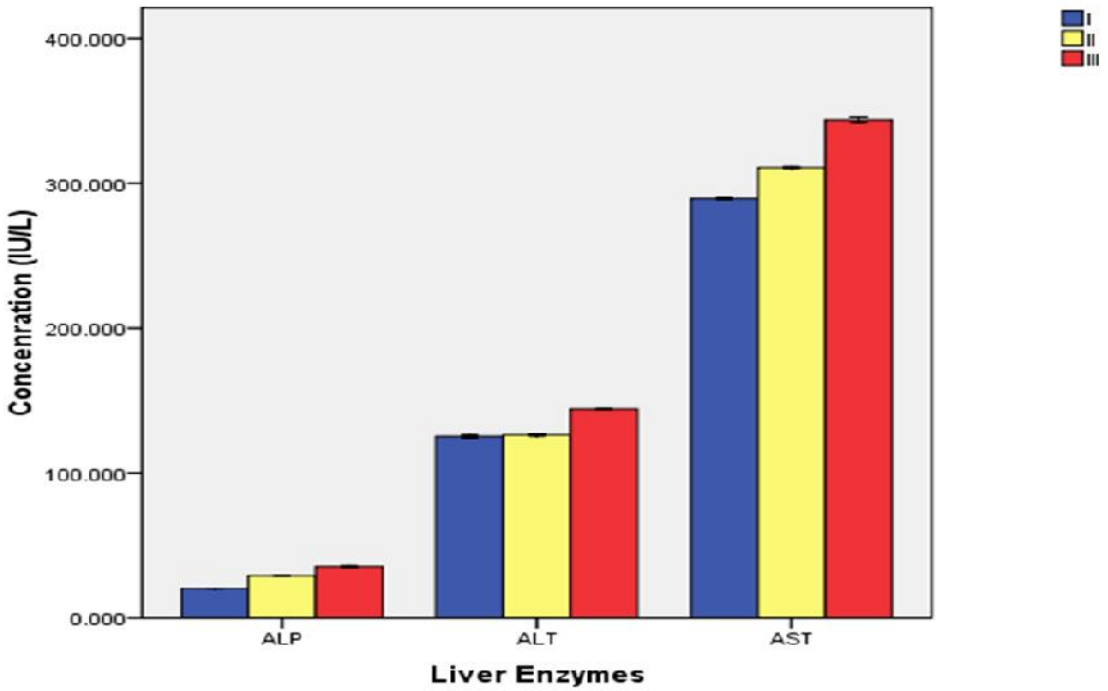

Fig. 2. Effect of *P. avium* extracts on liver enzymes in male albino rats

It has been reported that a change in organ weight is used as a veritable indicator of toxicity of a compound in animals [18]. In this research, there are indications of selective toxicity of the plant materials to different internal organs [19]. The increase in the liver weight (group II, III) and heart (III) signifies potential toxicity of these extracts on the organs nevertheless, the absence of changes in the kidney weight in treated and control group suggests a potential for kidney protection.

The result of the effect of *P. avium* leaf and stem bark extracts of on liver enzymes of rats is presented in Fig. 2. The figure showed a marked increase in alkaline phosphatase (ALP) and Aspartate amino transaminase (AST) in treated groups (II and III) while Alanine amino transaminase (ALT) increased in group III whereas it was comparable in group II and control. These enzymes are biochemical parameters linked with health indices and are of diagnostic significance in routine clinical evaluation of the state of health [20].

ALT and AST are generally employed in the assessment of liver damage by chemical substances [21]. The liver and heart release ALT and AST when under severe stress and an elevation in their plasma concentrations are indicators of liver and heart damage [22]. ALT on the other hand is a better parameter for detecting liver damage since it is more specific to the liver [23]. Therefore, the significant increase in all these enzymes in group III is an indication of potential hepatotoxicity, cardiotoxicity and kidney toxicity [24]. The increase in ALP in animals has been attributed to congestion or obstruction of biliary tract, which may occur within the liver [25].

4. CONCLUSION

In conclusion, *P. avium* extract may possess antitumor activity as shown with the effect on brine shrimps. Also, the plant leaf and stem bark appears to possess adverse effect on heart and liver of rats at high doses. Therefore it should be used in moderation as it is capable of causing suppression of haematopoietic system as well as multiple organ failure at high doses. Further studies are presently ongoing to determine the major active substances in this plant parts as well as their pharmacological activities.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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