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Effect of Anacardium occidentale Fruit Juice Extract on Haematological Parameters and Spleen of Paracetamol Induced Injury in Albino Rats

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Abstract

Introduction/Aim:The cashew tree (*Anacardium occidentale*) is a tropical evergreen tree that produces the cashew seed and the cashew apple accessory fruit. The fruit of cashew or cashew apple may be consumed fresh, but contains high quantities of tannins. The aim of this study is to determine spleen protective effect of *Anacardium occidentale* on paracetamol induced injury in albino rats.

Method: A total of thirty rats where used for this work. Group one served as the positive control receiving normal saline. Group two received normal saline, while group 3, 4 and 5 received 10, 20 and 40 ml/kg of the extract respectively. On the eighth (8th) day, animals in groups 2 - 5 received paracetamol 2000mg/kg bw orally. Twenty hours after the last administration, that is on the 9th day, all animals were weighed again and sacrificed under light diethylether vapour.

Result: *Anacardium occidentale* fruit juice extract caused significant increase (P<0.05) in the level of RBC, WBC, PCV, platelet and eosinophils when compared to the organotoxic group. The extract did not improve the level of haemoglobin caused by paracetamol administration. There was significant increase in body weight ratio of spleen of rats

Conclusion: *Anacardium occidentale* can be useful tissue/organ protective agents. The fruit may also be useful in improving hematological property in affected cases.

Introduction

The cashew tree (*Anacardium occidentale*) is a tropical evergreen tree that produces the cashew seed and the cashew apple accessory fruit^(1,2). The tree can grow as high as 14 m (46 ft), but the dwarf cultivars, growing up to 6 m (20 ft), prove more profitable, with earlier maturity and greater yields. The cashew seed is commonly considered a snack nut (cashew nut) eaten on its own, used in recipes, or processed into cashew cheese or cashew butter⁽³⁾. Like the tree, the nut is often simply called cashew. Cashew allergies are triggered by the proteins found in tree nuts, and cooking often does not remove or change these proteins. The true fruit of the cashew tree is a kidney– or boxing-glove–shaped drupe that grows at the end of the cashew apple⁽⁴⁾. The drupe develops first on the tree, and then the pedicel expands to become the cashew apple⁽⁵⁾. The true fruit contains a single seed, which is often considered a nut in the culinary sense⁽⁵⁾. The seed is surrounded by a double shell that contains an allergenic phenolic resin, anacardic acid—which is

a potent skin irritant chemically related to the better-known and also toxic allergenic oil urushiol, which is found in the related poison ivy and lacquer tree⁽⁶⁾.

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects⁽⁷⁾. The cashew (A. occidentale) is a tree in the family of the flowering plant Anacardiaceae. The family contains 73 genera and about 600 species. Anacardium contains 8 species, native to tropical America, of which the cashew is by far the most important economically. It is a multipurpose tree of the amazon that grows up to 15m high⁽⁸⁾. It has a thick and tortuous trunk with branches so winding that they frequently reach the ground The cashew tree produces many resources and products⁽⁹⁾. The bark and leaves are used medicinally. The cashew nut has international appeal and market value as food. Even the shell oil around the nut is used medicinally and has industrial applications in plastics and resin industries for its phenol content⁽¹⁰⁾. The pseudo-fruit, a large pulpy and juicy part, have a fine sweet flavor and is commonly referred to as the 'cashew fruit' or the 'cashew apple''. Cashew leaf and bark tea is used in Brazil and Peruvian herbal medicine, Tikinia in northwest Amazona, Wayapi tribe of Guyana as a douche for vaginal discharge and common diarrhea remedy. In Brazil, it is also used to treat diabetes, weakness, muscular debility, urinary disorders, asthma, eczema, psoriasis, scrofula, dyspepsia, genital problems, bronchitis, cough, intestinal colic, leishmaniasis, venereal disease, as well as impotence, and syphilis-related skin disorders⁽¹¹⁾. In the sixteenth-century, Brazil cashew fruits and their juice were taken by Europeans to treat fever, sweeten breath, and 'conserve the stomach''. It is taken for syphilis and as a diuretic, stimulant and aphrodisiac⁽¹²⁾. In addition to being delicious, cashew fruit is a rich source of vitamins, minerals and other essential nutrients. It has up to five times vitamin C than oranges and contains a high amount of mineral salts⁽¹³⁾. Because of its high amount of amount of vitamin C and mineral salts, cashew fruit is used as a catalyst in the treatment of premature ageing of the skin. Several clinical studies have shown that *anacardiac* acid is a component of cashew, with highest concentration in the nutshells curb the darkening effect of ageing by inhibiting tyrosinase activity, and that they are toxic to certain cancer cells reveal a unique function of *anacardic* acid in that, for dietary conditions enhancing body fat deposition that is consumption of a diet high in carbohydrates, dietary anacardic acid has the potential to decrease body fat deposition. In the last 20 years, the interest in medicinal plants has increased together with the number of investigations into their biological effects on human beings and animals^(14,15). Although, poisonous plants are ubiquitous, herbal medicine is used by up to 80% of the population in the developing $countries^{(16)}$.

The leaves and/or the bark is also used in Brazil for eczema, Psoriasis, Scrofula, Dyspepsia, genital problems, and venereal diseases, as well as for impotence, bronchitis, cough, intestinal colic, leishmaniasis and syphilis-related skin disorders⁽⁵⁾. A strong antioxidant capacity was also observed against hepatocarcinogenesis induced by aflatoxin B1 in Winstar rat.⁸ Preclinical studies reveals that metabolites isolated from the bark of cashew tree demonstrated antipyretic action for *anacardic* acid⁹ realized mutagenic tests with vegetable oil derived from cashew nut, which presented mutagenicity with or without the activation of the S9 fraction in *Salmonella thyphimurium*. *H*.⁽¹⁶⁾ demonstrated that tannic acid, a compound present in the cashew, presented an antimutagenic effect on the *Salmonella thyphimurium* TA98 lineage. The bark and leaves of cashew are a rich source of tannins, a group of plant chemicals with documented biological activity⁽¹²⁾.

Several clinical studies have shown that tannins has therapeutic property of curbing the darkening effect of aging by inhibiting tyrosinase activity, and that they also serve as therapeutic effect to certain cancer cells^(12,14,18). The aim of this study is to determine the effect of *A*. *occidentale* fruit juice on paracetamol induced injury on albino rats.

Materials and Method

Plants collection

of *Anacardium occidentale* were collected from its natural habitat from nearby Oye village, Ekiti State, Nigeria. The plant was authenticated from Department of Botany, Federal University, Ekiti State Nigeria.

Extraction

The plant material cashew apple, orange reddish, was harvested from the Oye Local government area of Ekiti State, Nigeria. Cashew apple fruits were cut into small pieces and crushed them under laboratory conditions, the resulting mash was pressed using a press to extract the juice

Animals

Adult albino rats of both sexes were obtained from Bingham university animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, Federal University, Oye Ekiti, Ekiti State, Nigeria..

Animal treatment

A total of thirty rats where used for this work. Group one served as the positive control receiving normal saline. Group two received normal saline, while group 3, 4 and 5 received 10, 20 and 40 ml/kg of the extract respectively. On the eighth (8^{th}) day, animals in groups 2 – 5 received paracetamol 2000mg/kg bw orally. Twenty hours after the last administration, that is on the 9^{th} day, all animals were weighed again and sacrificed under light diethylether vapour.

Hematological study

Blood samples were collected from each rat by cardiac puncture immediately after the animals were sacrificed under diethylether anesthesia, using 21-gauge (21 G) needles mounted on a 5ml syringe into ethylene diamine tetra-acetic acid (EDTA) – coated sample bottles for analyzed. Hematological parameters such as full blood count (FBC), hemoglobin, (Hb), packed cell volume (PCV), platelet concentration (PLC) and Total and differential white blood cell count (WBC). These parameters were analyzed using automatic hematological system.

Histopathological examinations

The spleen were processed and stained with haematotoxylin and eosin (H&E). Prepared slides of the organs were mounted on high-defination microscope. The result was interpreted by a Pathologist in the Department of Chemical Pathology, Ekiti State University, Ekiti State. Morphological changes in the excised organs of the sacrificed animals were observed and recorded. Histologic micrographs were taken.

Results

Effect of *A. occidentale* fruit juice on haematological parameters of paracetamol induced toxicity

Anacardium occidentale fruit juice extract caused significant increase (P<0.05) in the level of RBC, WBC, PCV, platelet and eosinophils when compared to the organotoxic group. Also, there was no significant difference in level of haemoglobin caused by paracetamol (Table 1).

Effect of A. occidentale on platelet count on paracetamol induced toxicity

Paracetamol caused decrease in platelet count in paracetamol administered group, while there was significant (P<0.05) increase in groups administered *Anacardium occidentale* fruit juice when compared to group 2 (Table 2).

Effect of *Anacardium occidentale* on body weight ratio of spleen of rats in paracetamol induced injury

A. occidentale cause significant (P<0.05) improvement in spleen of rats that receive paracetamol compared to the group that received paracetamol only (Table 3).

Effect of Anacardium occidentale of histopathology of rat spleen

The spleen showed lymphocyte degeneration in paracetamol group. Fruit juice caused histological improvement at all doses administered (Figure 3).

Effect of *A. occidentale* fruit juice on haematological parameters of paracetamol induced toxicity

Group	RBC (X	PCV (%)	Hb (g/dl)	WBC (X	Monoc
	1012/1)			109/1)	ytes (%)
Normal	7.4±1.11*	44.00±1.87*	13.46±0.87*	6.4±1.43*	4.13±1.81*
saline)			
РСМ	2.80±0.98	17.92±2.17	10.24±0.34	2.52 ± 1.00	2.9±1.62
2000					
mg/Kg					
PCM +	4.11±0.76*	34.67±1.28*	10.73±0.34	5.29±1.43*	4.01±1.71*
AO 10					
ml/kg					
PCM+	5.36±0.76*	37.7±2.12*	10.8±0.34	5.24±1.33*	5.6±1.25*
AO 20					
ml/kg					
PCM+	6.05±0.43*	4.22±1.8*	10.23±0.34	6.52±1.40*	5.7±1.19*
AO 40					
ml/kg					

Group	Platelet
	Count
Normal saline	350±3.42*
PCM 2000 mg/Kg	148±4.89
PCM + AO 10 ml/kg	296±3.92*
PCM+ AO 20ml/kg	309±3.15*
PCM+ AO 40 ml/kg	403±3.81*

Table 2: Effect of A. occidentale on platelet count on paracetamol induced toxicity

 Table 3: Effect of Anacardium occidentale on body weight ratio of spleen of rats in paracetamol induced injury

Group	SPLEEN BW RATIO
Normal saline	0.47±0.76*
PCM + 2000 mg/kg	0.19±0.15
A.O 1ML+PCM	0.41±0.11*
A.O 2ML+PCM	0.50±0.41*
A.O 4ML+PCM	0.49±0.16*

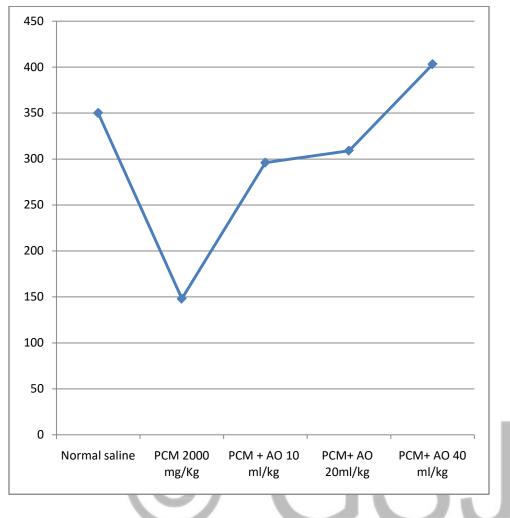


Figure 1: effect of Anacardium occidentale platelet count of paracetamol induced injury in rat.

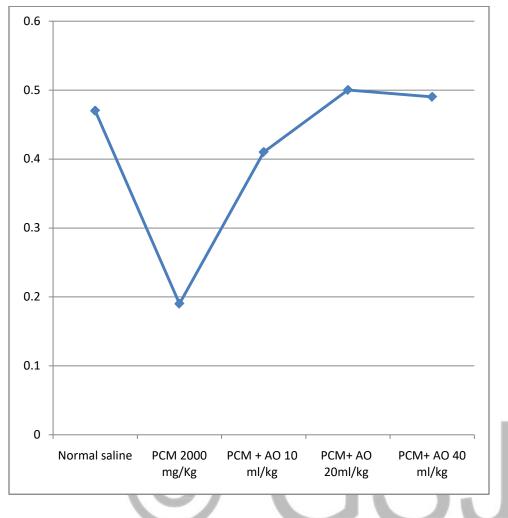


Figure 2: Figure 1: effect of *Anacardium occidentale* body weight ratio of spleen in paracetamol induced injury in rat.

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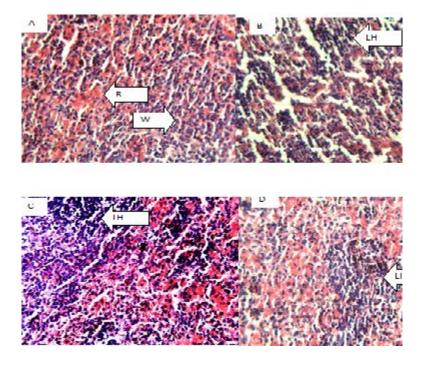


Figure 3: Histological section of the Spleen (H and E $\times 100$). (a) control group (b) PCM 2000 mg/kg (c) PCM 2000 mg/kg and A.O 10ml/kg (d) PCM 2000mg/kg and A.O 20 ml/kg,

Discussion

Cashew apple is rich in vitamin C, organic acids, antioxidants, minerals, and carbohydrates.^{1,4,} Its phytochemical profile reveals a complex source of natural antioxidants as phenolic compounds and carotenoids what makes this pseudofruit an excellent source of antioxidants that can scavenge free radical or reactive oxygen species (ROS), inhibit free radical formation, and prevent damage of cellular components, as well as cellular death^{19,2021)}. More recently, the interest and research on the nutritional and medicinal properties of cashew apple and its juice have grown⁽²²⁾, but little is known about it tissue protective ability against known and unknown toxicants.

Anacardium occidentale caused significant increase in the value of RBC, PCV, WBC and monocytes while there was no changes observed in the of hemoglobin value. Hematological parameters are useful indices that can be employed to assess the toxic potentials of plant extracts in living

systems^(21,22). Red blood cell and factors relating to it are major indices for evaluating circulatory erythrocytes and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce RBC as in mammals^(223,24).

significant increase in RBC and PCV following administration The of A. occidentalis may be an indication of erythropoiesis stimulation by the juice extract. It must have increase or prevented decrease in the rate of erythropoietin release in the kidney, which is the humoral regulator of RBC production. A platelet count may be used to screen for or diagnose various diseases and conditions that can cause problems with blood clot formation⁽²⁵⁾. It may be used as part of the workup of a bleeding disorder, bone marrow disease, or excessive clotting disorder⁽²⁶⁾. A high platelet count can happen when something causes the bone marrow to make too many platelets⁽²⁷⁾. When the reason is unknown, it is called primary, or essential, thrombocytosis. When excess platelets are due to an infection or other condition, it is called secondary thrombocytosis⁽²⁸⁾. A low platelet count can make it difficult for the blood to clot, putting a person at risk of excessive bleeding. The cause may be due to an inherited tendency to not produce enough platelets, but the cause may also be unknown. In other cases, it is due to an underlying medical condition⁽²⁹⁾. In this study thrombocytopenia induced by paracetamol was effectively reversed or prevented in the groups that received doses of the extract. This indicates that A. occidentalis may posses the ability to prevent thrombocytopenia and/or increase the production of platelet cells.

Hemoglobin is a main part of red blood cells and binds oxygen. If the level of RBC too few or there is abnormal red blood cells, or hemoglobin is abnormal or low, the cells in the body will not get enough oxygen⁽³⁰⁾. *A. occidentalis* fruit does not improve oxygen carrying capacity of the rat after been exposed to a toxin like paracetamol.

The spleen plays multiple supporting roles in the body. It acts as a filter for blood as part of the immune system^(31,32). Old red blood cells are recycled in the spleen, and platelets and white blood cells are stored there. The spleen also helps fight certain kinds of bacteria that cause pneumonia and meningitis. Spleen is a centre of activity of the Mononuclear Phagocyte System and can be considered analogous to a large lymph node, as its absence causes a predisposition to certain infections^(33,34,35). The spleen is an immunologic filter of the blood and it is made up of B cells, T cells, macrophages, dendritic cells, natural killer cells and red blood cells. In this study, aqueous fruit juice extract of *A. occidentalis* stimulated lymphoid follicular activation and sinus histiocytosis in the spleen which is capable of stimulating the production of more lymphocytes and histiocytes and eventually produced more antibodies, consequently improving the body immune/defence system. There were no evidence of such activation in the control group of the experimental animals . Increase to near normal in the values of WBC and

monocytes is also reflected in improvement of the body weight ratio of spleen of rats that received *A. occidentalis*.

Conclusion

Anacardium occidentale is a tropical evergreen tree that produces the cashew seed and the cashew apple accessory fruit. Study from this work suggests that cashew apple juice may be useful in protecting hematological disorder that may be caused by poisons or blood toxicant. It also reveals that the plant may be useful in improving the immune system and preventing thrombocytopenia and other possible blood disease.

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