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International Digital Organization for Scientific Research ISSN:2550-794X
IDOSR JOURNAL OF SCIENTIFIC RESEARCH 2(3) 51-58, 2017.

The implication of haematological indices in Wistar rats administered methanol-ethanol (1:1) leaf extracts of *Anacardium occidentale* and *Jatropha tanjorensis*

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ABSTRACT

Theeffect of administration of methanol-ethanol (1:1) leaf extract of *Anacardiumoccidentale* and *Jatropha tanjorensis* on haematological indices in Wistar rats was investigated in this study. The leaves were prepared and extraction done using standard methods. The low and high doses were established as 10% and 20% respectively of the determined LD_{50} was for methanol-ethanol extracts of *Jatrophatanjorensis*and corresponded to 381 mg/kg bwt and 762 mg/kg bwt respectively. Then 25 female rats weighing 100-150 g were divided according to body weight into 5 groups of 5 rats each. Group I was the normal control (NC) and animals were given normal feed and water *ad libitum.* Groups II amd III were given low (381mg/kg bwt) and high doses (762mg/kg bwt) respectively of *Anacardiumoccidentale,* while groups IV and were given low and high doses respectively of *Jatropha tanjorensis.* After 14 days of administration, serum was collected via cardiac puncture and used for evaluation of haematological indices.The results of the investigation showed significant (P<0.05) decrease in RBC, HGB, HCT, PLT and WBC in all the test groups. The absolute red blood indices were not clear-cut, however most of the test groups showed no significant (P≥0.05) difference compared with the control. The lymphocytes were significantly (P<0.05) decreased in group II but significantly(P<0.05) increased in group IV when compared with control.Conclusively, this study reveals that administration of methanol-ethanol (1:1) extracts of *Anarcardiumoccidentale* and *Jatropha tanjorensis* appears to signalanaemiafrom decreased RBC, HGB and HCT but there was very little or no alteration in their absolute red blood indices indicative of the fact that the anaemia may have resulted only in reduced number of RBC. While decrease in WBC may suggest immuno-suppression, but the lymphocyte and mixed cell blood increases at low and high doses respectively of *Jatropha tanjorensis*suggests that the extract also has the potential to boost the immune system. The extracts at the selected dose levels appeared to have exhibited the potential to induce thrombocytopenia.

Keywords:RBC count, WBC count, absolute RBC indices, throbocytopenia.

INTRODUCTION

Medicinal plants provide immense benefits in disease management. Plant medicineis relatively safe, more affordable and sometimes offer better therapeutic value than synthetic drugs.This is because human beings have co-evolved with plants over the past decades [1]. Medicinal plants arethe sources of many important scientific drugs of the modern world [2]. *Jatropha tanjorensis*(*J. tanjorensis*) belongs to the family, Euphorbiaceae

[2]. *J. tanjorensis*is a natural hybrid between *J. curcas*and*J. gossypifolia*[3]. Itis a native of Central America and has become naturalized in some tropical and subtropical countries, like India, Nigeria and Canada [3]. Its primary use is for fencing while its secondary uses are as a source of edible leafy vegetable and as medicine [4]. *J.tanjorensis*leaf is commonly consumed as vegetable in many parts of Southern Nigeria and is commonly called 'Hospital too far', 'Catholic vegetable'or 'Lapalapa' [5].
*J. tanjorensis*is popular as a natural remedy against diabetes in Southern Nigeria [6] .The leaf is used as heart tonic and remedy for hypertension in some parts of Nigeria [5]. The leaf extract has hypoglycemic activity and the aqueous leaf extract exhibit antibacterial activity against*Staphylococcus aureus* and *Escherichia coli* [7].
*Anarcadiumoccidentale*commonly called cashew is native to Brazil, and belongs to the family of *Anacardiaceae*.Vietnam, Nigeria, India and Cote d'Ivoire are major producers of Cashew [8].
The medicinal parts of cashew are the nuts, leaves, stem bark, root and gum. It exhibits antimicrobial andantifungal activities, with properties against worms and protozoa. In African herbal medicine the leaves are used to treat malaria[9].
The therapeutic values of most of these herbs are indisputable but their toxicities sometimes limit their clinical uses. Thus, the toxicity profile of these herbs must always be considered especially as the doses and dosing regimens of their preparations are not usually determined [10].The world is beset with problems which include - resistance to drugs, drug toxicity, protracted treatment protocol and limited repertoire of compounds [11]. The problem of drug toxicity and resistance is very pronounced in the management of many certain diseases, e.g. hypertension. Also, the problem of non-compliance where patients are usually unwilling to adhere to prescriptions, requiring the combination of several drugs which can be taken care of by the potential wholistic medicinal effects that may be elicited by these plants' extracts.Although, herbal medicines are widely preferred in the developing countries because of their availability and affordability, though, they are not without side effects, some of which are so severe that the plants may be considered as poisonous [12].Medicinal plants can also be a source of exposure to toxic elements depending on their origin and nature. The preparation and use can be harmful to human health, which has encouraged researchers to investigate medicinal plants [13].
The extracts of *A.occidentale*and *J.tanjorensis*leaves have been used in the management ofdifferent health disorders in Nigeria and the world at large.Theseand other reports have necessitated the assessmentofmethanol-ethanol (1:1) leaf extractsof *A.occidentale* and *J.tanjorensis*on renal function indices of Wistar rats at selected dose levels.

MATERALS AND METHODS

Equipment, Chemicals and Reagents Water bath (grant model:600303003, USA), Table top centrifuge (labofuge 300 Heraus, model D37520, USA), Thermocool freezer (model TH 170, China), Weighing balance (Denver, model:IR-30, USA), and Spectrophotometer (Jenway, model 6405, Japan).
The chemicals methanol, ethanol and diethyl ether were purchased from loba chemical Ltd., Nigeria. All chemicals and used in this research were of analytical grade,

Plant materials

The fresh leaves of *Anacardiumoccidentale* and *Jatropha tanjorensis* were obtained from Okuku village in Yala Local Government Area of Cross River State, Nigeria. The plants were identified and authenticated at the Department of Botany, University of Calabar.

Extract Preparation

The fresh leaves of *Anacardiumoccidentale*and *Jatropha tanjorensis* were collected and air-dried under room temperature at Medical Biochemistry Laboratory, Cross River University of Technology (CRUTECH) Okuku Cross River State. The dried samples were ground into fine, flour-like powder or constituency using a Q-link electric blender (model QBL-18L40, China) and stored in air-tight containers.
One hundred grams of *Anacardiumoccidentale* powder was weighed using an electric weighing balance and soaked in 1000mL of the mixture of methanol-ethanol at ratio 1:1.

The mixture was stirred for proper mixing of solvent with powder, then poured into air-tight container. The container with the mixture was kept under room temperature for 48h.*Jatropha tanjorensis*Were also subjected to the same treatment given to*Anacardiumoccidentale*.

Filtration and Concentration of plant extracts

The mixtures were first filtered using cheese material or cloth, followedby Whatman no.1 filter paper (24cm). The filtrates were then concentrated under reduced pressure, using a rotary evaporator (model RE52A, China) to 10% of its original volume at 37^0C. After which it was concentrated to complete dryness in the water bath, yielding 14.98g of *Anacardiumoccidentale* and 12.82g of *Jatropha tanjorensis*.The extracts were then stored in the refrigerator

Determination of Lethal Dose (LD_{50})

The assessment of the lethal dose (LD_{50}), (the dose that kills 50% of test animals population) has now been used as a major parameter in measuring acute toxicity and also as an initial procedure for general screening of chemical and pharmacological agents for toxicity. Apart from mortality, other biological effects and the time of onset, duration and degree of recovery on survived animals, are also important in acute toxicity evaluation. Acute toxicity study solely gives information about LD_{50}, therapeutic index and the degree of safety of a pharmacological agent [14].

Twelve (12) female Wistar rats weighing between 100 g-138g were obtained from animal house of the Department of Medical Biochemistry, CRUTECH, Okuku-Campus, Cross River State, Nigeria. The animals were kept in well-ventilated laboratory cages, the animals were fed with standard rat pelleted diet and water and also cleaning of beddings for 3 days, together with administration of *A. occidentale* and *J. tanjorensis* ethanol-methanol (1:1) leaf extracts.

In the determination of the $LD_{50,}$,Lorke's Method was employed. The animals were divided into three groups of three rats each and administered the graded doses of 1600, 2900 and 5000 mg/kg/bwt of test substance and then observed for 24 hours for behavior as well as mortality.

Group 1: 1600mg/kg per body weight of *Anacardiumoccidentale* and *Jatropha tanjorensis*extracts was administered to the Wistar rats.

Group 2: 2900mg/kg per body weight of *Anacardiumoccidentale* and *Jatropha tanjorensis* extracts respectively was administered to the Wistar rats.

Group 3: 5000mg/kg per body weight of *Anacardiumoccidentale* and *Jatropha tanjorensis* extracts was administered to the Wistar rats. The Wistar rat that was administered 5000mg/kg of *Jatropha tanjorensis* died which indicated that *Jatropha tanjorensis* is of high toxicity than *Anacardiumoccidentale.* It was calculated to be given 10% of the extracts to low doses and 20% of the extracts to high doses.

Then the LD_{50} was derived based on the formula:

$LD_{50} = \sqrt{D_0} \times D_{100}$

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

Thus,

10% for low dose and 20% for high dose of *J. tanjorensis* determined include:

LD_{50} = 381 mg/kg for low dose *J. tanjorensis.*

LD_{50} = 762 mg/kg for high dose *J. tanjorensis* respectively.

Animals

Twenty-five (25) female Wistar rats weighing between 100-150g were obtained from the animal house of the Department of Medical Biochemistry, CRUTECH,OkukuCampus, Cross River State, Nigeria. The animals were kept in a well-ventilated laboratory cages with 12-hours day/night cycles and fed with standard rat pelleted diet and water. Proper hygiene involving cleaning of beddingsthroughout the duration of the experiment was maintained.

Experimental Design

In the experiment, a total of 25 female Wistar rats were used. The rats were divided into five (5) groups of five (5) rats each.

Group I: Normal control (NC) animals was given normal fed and water only.

Group II:Low dose ofmethanol-ethanol extract of*Anacardiumoccidentale*administered to Wistar rats

Group III: High dose of methanol-ethanol extract of*Anacardiumoccidentale*was administered to Wistar rats.

Group IV: Low dose of methanol-ethanol extract of *Jatropha tanjorensis*was administered to Wistar rats.

Group V: High dose of methanol-ethanol extract of *Jatropha tanjorensis*was administered to Wistarrats.

Collection of blood sample

Blood samples were collected via cardiac puncture after anesthesia using syringes and needle and emptied into labelled EDTA bottles. The samples were briskly agitated to prevent clothing. The samples were used for the estimation of different haematological parameters within 12 h.

Estimation of haematologicalparameters

The haematological indices were determined using an Auto-hematology analyser, Model BC-3200, Shenzhen Mindary Biochemical Electrics Co. Ltd., Germany. The two independent measurement methods used in this analyser are the impedance method for determining the WBC, RBC and PLT data and the colorimetric method for determining the HGB. During each analysis cycle, the sample was aspirated, diluted and mixed before the determination for each parameter.

Statistical Analysis

Data obtained was analysed using Microsoft Office Excel 2007 and expressed as mean ± SEM. The statistical package SPSS version 17.0 using one-way ANOVA (Analysis of variance)was used to establish statistical significance at$P$ <0.05.

RESULTS

The result of haematological indices of Wistar rats administered methanol-ethanol (1:1) extracts of *Anacardiumoccidentale*and*Jatropha tanjorensis* are given in Table 1 and Table 2.

The results revealed a significant (P<0.05) decrease in WBC of all the test groups compared with the normal control (NC). Groups II and IV compared well with each other and so were groups III and V. There was a significant (P<0.05) decrease in lymphocytes (LYM) of group III only compared with the control, the rest were insignificant.

The mixed cell blood (MXD) levels showed significant (P<0.05) decrease and increase in groups III and Vrespectively compared with the control, while the granulocyte (GRA) and platelet (PLT) levelswere significantly (P<0.05) decreased in all the test groups compared with control.However, the mean platelet (MPV) level showed no significant (P≥0.05) difference in all the testgroups compared with the control. However, group II and III compared well with each other so were groups IV and V.The WBC count and its differentials may suggest that the immune system was not agitated.

The red blood cell (RBC) count, there was significant (P<0.05) decrease in the RBC count of all the test groups compared with the control.However, groups III and V compared well with each other. The haemoglobin (HGB) and haematocrit (HCT) levels showed a similar pattern as that of RBC.

The mean corpuscular volume (MCV) level revealed significant ($P<0.05$) increase in group IV only, compared with the control and other test groups, while themean corpuscular haemoglobin (MCH)level showed no significant ($P\geq0.05$) difference between all the test groups except group II that was significantly ($P<0.05$) decreased compared with the control. The mean corpuscular haemoglobin concentration (MCHC) level was significantly ($P<0.05$) decreased in group IV only,compared with control, however groups II and III compared well with each other.The absolute red blood cell indices put together, appeared to have given a picture of a well impacted action of the methanol-ethanol (1:1) leaf extracts of *Anacardiumoccidentale*and *Jatropha tanjorensis* on the blood of Wistar rats.

Table1. Administration of methanol-ethanol leaf extract of *Anacardiumoccidentale* and *Jatropha tanjorensis* on white blood cell count, platelet count, mean platelet volume and differential white blood cell indices of Wistar rats

PARAMETER / GROUP	WBC	LYM	MXD	GRA	PLT	MPV
I (NC)	5.32 ±0.16^{a}	2.89±0.10^{a,c,d}	1.08±0.10^{a}	1.46±0.13^{a}	899.40±0.04^{a}	7.24±0.35^{ab}
II (LAO)	4.75±0.63^{b}	2.56±0.09^{a,d}	0.86±0.09^{a,b}	0.73±0.79^{b}	822.60±9.95^{b}	6.68±0.07^{b}
III (HAO)	3.32±0.06^{c}	1.42±0.03^{b}	0.65±0.11^{b}	0.76±0.15^{b}	659.40±7.61^{c}	6.74±0.11^{ab}
IV (LJT)	4.62±0.13^{b}	3.18±0.02^{c}	0.78±0.12^{a}	0.50±0.06^{b}	783.80±6.88^{d}	7.4±0.06$^{a, c}$
V (HJT)	3.46±0.14^{c}	2.73±0.27^{a,d}	1.43±0.07^{c}	0.562±0.3^{b}	834.60±11.14^{b}	7.02±0.14$^{a, c}$

Values are mean ± SEM (n=5)

^{a,b,c,d}Values with different superscript are statistically significant at $P<0.05$

Note: NC= Normal control, LAO= Low dose *Anacardiumoccidentale*, HAO= High dose *Anacardiumoccidentale*, LJT= Low dose *Jatropha tanjorensis*, HJT= High dose *Jatrophatanjorensis.*

Table 2. Administration of methanol-ethanol leaf extract of *Anacardiumoccidentale* and *Jatropha tanjorensis* on red blood cell count, haemoglobin, haematrocrit and absolute red blood cell indices of Wistar rats

PARAMETR / GROUP	RBC	HGB	HCT	MCV	MCH	MCHC
I(NC)	8.94±0.22[a]	15.28±0.35[a]	42.44±1.01[a]	47.4 ±0.22[a]	17.08±0.21[a]	36.0±0.48[a]
II(LAO)	7.82±0.20[b]	12.76±0.32[b]	36.82±0.49[b]	47.0±0.49[a]	16.36±0.15[b]	34.7±0.10[a, b]
III(HAO)	8.26±0.04[b,d]	13.9±0.19[c]	40.01±0.47[c, d]	48.4±0.36[a, b]	16.84±0.17[a, b]	34.74±0.15[a,b]
IV (LJT)	7.34±0.06[c]	12.14±0.04[b]	37.76±0.75[b,c]	50.0±0.69[b]	17.02±0.17[a]	34.0±0.41[c]
V (HJT)	8.42±0.09[d]	14.5±0.32[c]	40.90±0.32[d]	48.4±0.54[a]	17.18±0.23[a]	35.38±0.64[a]

Values are presented as mean ± SEM (n=5).
[a,b,c,d]Values with different superscript are statistically significant at $P<0.05$
Note: NC= Normal control, LAO= Low dose *Anacardiumoccidentale*, HAO= High dose *Anacardiumoccidentale*, LJT= Low dose *Jatropha tanjorensis*, HJT= High dose *Jatrophatanjorensis.*

DISCUSSION

The effect of ethanol-methanol (1:1) leaf extract of *Anacardiumoccidentale* and *Jatropha tanjorensis* on haematological indices was evaluated. The assessment of haematological indices has been useful as a bio-maker for evaluating the haematotoxic potentials of extracts [15].

Therefore, thedecrease recorded for whole WBC count after administration of the methanol-ethanol leaf extract of *Anacardiumoccidentale* and *Jatropha tanjorensis* suggests that the extracts contain some agents which could causehaemolysis and thereby decrease in WBC count and other WBC differentials. White blood cell helps fight infection, therefore a low white blood cell count (leukopenia) leaves the body more open to infection and if any develops the body may be unable to fight it off [4]. There are also reports which suggest that the decrease in WBC count may be due to absence of foreign bodies on administration of the extracts hence providing a less agitated system which results in increased WBC count as observed in cigarette smoking, overall obesity etc which has a positive correlation with coronary heart disease [16]; [4] There was increase in some haemologicalindices such as lymphocyte(LYM) and(absolute mixed value (MXD)which equally suggests that the *Anacardiumoccidentale* and *Jatropha tanjorensis* extract may contain some bioactive constituents which could possiblyhave boosted the production of

lymphocytes, MXD in some of the groups. The decreased platelet count in all the groups is indicative of the fact that the extracts may have induced thrombocytopenia.

The RBC count, HGB and HCT reduction with administration of the-methanol-ethanol (1:1) extracts of*Anacardiumoccidentale* and *Jatropha tanjorensis*may also be as a result of haemolysis of red blood cells through interferencewith iron absorption [17], hence signaling anaemia.

The mean corpuscular volume was increased in almost all the groups administered with ethanol-methanol (1:1) leaf extract of *Anacardiumoccidentale* and *Jatropha tanjorensis*. Increased MCV is associated with microcytosis or large average RBC size but this occurred only in groups III and IV, the rest were normocytic.The absolute red blood cell indices put together, appeared to have given a picture of a well impacted action of the methanol-ethanol (1:1) leaf extracts of *Anacardiumoccidentale*and *Jatropha tanjorensis* on the red blood cells of Wistar rats.

CONCLUSION

This study reveals that administration of ethanol-methanol (1:1) extract of *Anarcardiumoccidentale* and *Jatropha tanjorensis* appears to signalanaemiafrom decreased RBC, HGB and HCT but there was very or no little alteration in their absolute red blood indices. While decreased in WBC may suggest immuno-suppression, the lymphocyte increase especially at low dose of *Jatropha tanjorensis*suggests that the extract also has the potential to boost the immune system. The extracts at the selected dose levels appeared to have exhibited the potential to induce thrombocytopenia.

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