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COMPARATIVE EFFECT OF AQUEOUS AND ETHANOLIC EXTRACTS OF *TETRAPLEURA TETRAPTERA* (AIDAN) FRUIT ON SOME HAEMATOLOGICAL PARAMETERS OF FEMALE WISTAR RATS

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ABSTRACT

Extracts of *Tetrapleura tetraptera* have been employed in the management and treatment of various ailments. This study examined and compared the effects of aqueous and ethanolic extracts of *Tetrapleura tetraptera* fruit on some haematological parameters in female wistar rats. 15 female wistar rats (180-200g) fed with rat feed and water *ad libitum* were randomly assigned into three groups (n=5) thus: control, aqueous and ethanolic groups. The three groups were treated with daily oral administration of 0.2mL normal saline, aqueous (60mg/kg body weight) and ethanolic (40mg/kg body weight) extracts of *Tetrapleura tetraptera* respectively for 21 days. Blood samples were collected from each animal through cardiac puncture for haematological analysis. Red cell indices, platelets count and platelet large cell ratio were not significantly different among the three groups. Mean platelet volume and platelet distribution width were significantly decreased in aqueous ($p<0.05$) and ethanolic ($p<0.01$) groups compared with control. Total white blood cell and lymphocytes counts were significantly ($p<0.05$) increased in aqueous group compared with control. Neutrophils count was significantly ($p<0.05$) decreased in aqueous group compared with control. Total white blood cell count was significantly ($p<0.01$) decreased in ethanolic group compared with aqueous group. Both aqueous and ethanolic extracts of the fruit exhibited the same effect. Both had no significant effect on platelet count and red cell indices but decreased platelet distribution

width and mean platelet volume. Moderate use of both extracts is advised as excess use may be detrimental to individuals with bleeding and/or blood coagulation disorders.

KEYWORDS: *Extract; Haematological parameters; platelet; red blood cell; Tetrapleura tetraptera; white blood cell.*

INTRODUCTION

Several plants have been employed therapeutically for the management and treatment of several ailments. The plant, *Tetrapleura Tetraptera* (TTE) is an example of such plants. TTE is a medicinal and nutritional plant distributed in the lowland forest of tropical Africa. It is a deciduous tree belonging to the family "Minosaceae".[1] It is commonly called Aidan tree in English Language. In Nigeria, it is called Aridan or Aidan by the Yoruba tribe, Edeminang by the Efiks, Uyayak by the Ibibios, Oshosho by the Igbos, Dawo by the Hausas, and Ora ora, Ighimiakia, Ihokiriho and Imiminje by the people of Awka, Bini, Ngwa and Etsako respectively.[1, 2, 3] The fruit of TTE consists of a fleshy pulp with small, brownish-black seed and has a fragrant, characteristics pungent aromatic odour.[4] In addition to its medicinal property, TTE fruit is used popularly as a seasoning spice in Southern and Eastern Nigeria.[5, 6] The medicinal property of TTE is due to the presence of several phytochemicals and minerals in the plant. Phytochemical screening of ethanolic extract of TTE fruit revealed the presence of flavonoids, tannins alkaloids, sugar, saponins, cardiac glycosides, magnesium, phosphorus, potassium, calcium, sodium, zinc and vitamins A, C and E.[7, 8]

The medicinal property of different parts of the plant has been demonstrated in the treatment of malaria, diabetes mellitus, schistosomiasis, asthma, arthritis, hypertension and wound healing.[9, 10] Both aqueous and ethanolic extracts of TTE have exhibited several therapeutic effects. The fruits have been reported to have strong molluscicidal[4], antidiabetic[11], antioxidant[1,12,13], anticonvulsant[14], analgesic and anti-inflammatory[3, 14], antibacterial[15], anti-plasmodial[16], anti-insulin resistance and anti-obesity[17], wound healing[18], hypocholesterolemic[19] and hepatoprotective[20] effects.

It is worthy of note that both aqueous and ethanolic extracts of TTE fruit do not have the same potency. Ethanolic extract of the fruit has been reported to exhibit better antibacterial activity than the aqueous extract.[21] Also, both extracts of the fruit have been reported to exhibit antimalarial effect with ethanolic extract being more effective.[22] Ethanolic extract of TTE (50 and 100mg/kg b. w.) administered to rabbits caused significant decrease in red blood

cell (RBC) count and white blood cell (WBC) count and increase in mean corpuscular volume (MCV) with no significant effect on levels of haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).[7] The aqueous fruit extract (24.5, 49 and 73.5mg/kg) was reported to increase RBC, total WBC, platelet, lymphocyte and neutrophil counts and MCV, MCH and MCHC[23] in rats. From the foregoing, Reports on haematological effect of TTE are very scanty and the available reports[7, 23] are controversial. To date, no study has compared the effects of aqueous and ethanolic extracts of TTE fruit on haematological parameters in rats. The present study was therefore conducted to examine and compare the effects of aqueous and ethanolic fruit extracts of *Tetrapleura tetraptera* on blood parameters in female wistar rats.

MATERIALS AND METHODS

Preparation of Aqueous Extract

One hundred ripe fruits of *Tetrapleura tetraptera* were purchased from Ikono Local Government Area in Akwa Ibom, Nigeria. The fruits were botanically identified and authenticated at the herbarium of the Department of Botany, University of Calabar, Nigeria. They were washed with distilled water and dried using an oven (Astell Hearson, Germany) at 45-50°C. Fifty of the dried fruits were pulverized into coarse particles with mortar and pestle. The particles weighing 1200g were macerated in 2700mL of distilled water for 24 hours, filtered and the filtrate was concentrated to dryness at 45°C and then stored in a refrigerator until it was used. The yield of the dried extract was 96.50g.

Preparation of Ethanolic Extract

The remaining fifty dried fruits were also pulverized into coarse particles with mortar and pestle. The particles (1120g) were soxhlet extracted using 80% ethanol for 24 hours. The resulting extract was concentrated using a hot plate (Stuart, Great Britain) at 45°C and then stored in a refrigerator until it was used. Final yield of the extract was 120.90g

Experimental Animals

Fifteen (15) female wistar rats (180-200g) were used for the study. The rats were bought from Department of Agriculture, University of Calabar and handled according to Helsinki's 1964 laid down principles. They were kept in properly ventilated wooden cages in the animal house of Physiology Department, University of Calabar and given rat feed and water *ad*

libitum and exposed to 12/12 hours light/dark cycle. The rats were allowed for seven (7) days to acclimatize before treatment commenced.

Animal Grouping and Extract Administration

The 15 rats were assigned by randomization into 3 groups (n=5) thus: control, aqueous and ethanolic groups. The 3 groups received rat feed and water. In addition, control group received 0.2mL normal saline while aqueous group received 60mg/kg body weight of the aqueous extract of *Terapleura tetraptera* once daily and ethanolic group received 40mg/kg body weight of the ethanolic extract once daily. All treatments lasted for 21 days.

Collection of Blood Samples

At the end of 21 days of treatment, the rats were sacrificed under chloroform anaesthesia (3.8%). Collection of blood samples was done through cardiac puncture using 5mL syringes with 21G needles. The samples collected were put into pre-labelled ethylenediaminetetracetate (EDTA) vials and gently agitated to ensure uniform spread of EDTA after which the samples were immediately used for measurement of haematological parameters.

Measurement of Haematological Parameters

Haematological parameters were measured using automated cell counter (Coulter Electronics, Luton, Bedfordshine, UK) having standard calibrations in line with the instructions of the manufacturer. Parameters measured were: red blood cells (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV), red cell distribution wide standard deviation (RDW-SD), red cell distribution wide coefficient of variation (RDW-CV), platelet count, platelet distribution width (PDW), mean platelet volume (MPV), platelet large cell ratio (P-LCR), total white blood cells (TWBC) count, neutrophils count and lymphocytes count.

Statistical Analysis

Results are presented as mean ± standard error of mean (SEM). Data were analyzed using Statistical Package for Social Science (SPSS) (version 17). Statistics used was one way analysis of variance (ANOVA) followed by post hoc multiple comparison. $p<0.05$ was considered statistically significant.

RESULTS

Red Blood Cell Indices

Table 1 shows RBC count (x10^6 cell/µL), Hb concentration (g/dL), PCV (%), RDW-SD (fL) and RDW-CV (%) for control, aqueous and ethanolic groups. There was no significant difference in haematological parameters among the groups. However, Hb concentration was significantly ($p<0.01$) decreased in aqueous group compared with control.

Table 1: Comparison of red blood cell indices between the different groups.

Parameters	Control	Aqueous	Ethanoloic
RBC (x10^6 cell/µL)	7.04 ± 0.10	6.87 ± 0.37 ^{ns}	6.96 ± 0.27 $^{ns, nx}$
Hb (g/dL)	14.48 ± 0.27	14.70 ± 0.23^{ns}	14.24 ± 0.09 $^{ns, nx}$
PCV (%)	41.48 ± 0.51	42.68 ± 1.88 ^{ns}	43.50 ± 1.24 $^{ns, nx}$
RDW-SD (fL)	30.24 ± 0.27	33.68 ± 2.06 ^{ns}	34.14 ± 0.28 $^{ns, nx}$
RDW-CV (%)	12.56 ± 0.29	14.02 ± 1.15 ^{ns}	13.94 ± 0.77 $^{ns, nx}$

Values are expressed as mean ± SEM, n = 5.

ns = Not significant vs control

nx = Not significant vs Aqueous

Platelet Indices

Table 2 shows platelet count (x10^3 cell/µL), PDW (fL), MPV (fL) and P-LCR (%) for control, aqueous and ethanolic groups. There was no significant difference in platelet indices among the groups except PDW and MPV that were significantly decreased in aqueous ($p<0.05$) and ethanolic ($p<0.01$) groups compared with control.

Table 2: Comparison of platelet indices between the different groups.

Parameters	Control	Aqueous	Ethanolic
Platelet Count (x10^3 cell/µL)	907.00 ± 67.70	892.60 ± 60.43 ^{ns}	823.40 ± 70.15 $^{ns, nx}$
PDW (fL)	8.42 ± 0.20	7.48 ± 0.22 *	7.20 ± 0.28 $^{**, nx}$
MPV (fL)	6.96 ± 0.08	6.42 ± 0.12 *	6.32 ± 0.20 $^{**, nx}$
P-LCR (%)	6.74 ± 0.43	4.98 ± 0.39 ^{ns}	4.96 ± 1.05 $^{ns, nx}$

Values are expressed as mean ± SEM, n = 5.

*ns = Not significant, *p<0.05, **p<0.01, vs control;*

nx = Not significant vs Aqueous

Total White Blood Cell (TWBC), Neutrophil and Lymphocyte Counts

Table 3 shows TWBC (x10^3 cell/µL), neutrophil (%) and lymphocyte (%) counts for control, aqueous and ethanolic groups. TWBC and lymphocyte count was significantly ($p<0.05$) increased in aqueous group compared with control. There was a significant decrease in

TWBC and neutrophil counts in ethanolic ($p<0.01$) and aqueous ($p<0.05$) groups compared with aqueous and control groups respectively.

Table 3: Comparison of white blood cell indices between the different groups.

Parameters	Control	Aqueous	Ethanolic
TWBC ($x10^3$ cell/µL)	6.76 ± 1.16	12.60 ± 2.22 *	4.32 ± 0.43 ns, b
NEUT (%)	34.34 ± 3.72	20.88 ± 3.70 *	26.60 ± 1.85 ns, nx
LYM (%)	65.58 ± 3.75	79.12 ± 3.70 *	72.48 ± 1.73 ns, nx

Values are expressed as mean ± SEM, n = 5.

*ns = Not significant, *p<0.05 vs control;*

nx = Not significant, b = p<0.01 vs Aqueous

DISCUSSION

Tetrapleura tetraptera (TTE) is a plant that is distributed in the lowland forest of tropical Africa. In Nigeria, the fruit of the plant is used as a seasoning spice and for the management of various ailments. This study investigated and compared the effect of aqueous and ethanolic fruit extracts of *Tetrapleura tetraptera* on haematological parameters in female wistar rats.

RBC count, Hb concentration, PCV, RDW-SD and RDW-CV were not significantly different among all groups (Table 1). The results for Hb and PCV are in tandem with that of Odesanmi *et al.*[7] but contradict their result for RBC count which showed significant decrease. Jimmy and Ekpo[23] reported a significant increase in RBC count following administration of aqueous extract of TTE which is contradictory to the present result. Our results suggest that TTE at the administered doses did not affect erythropoiesis, heme biosynthesis, and oxygen-carrying capacity of blood and/or cause red blood cell lysing. Differentiation of precursor cells and maturation of red blood cells (RBCs) were also probably not affected by the extract because there was no variation in the sizes of circulating RBCs as indicated by the non-significant change in RDW-SD and RDW-CV. Aqueous and ethanolic fruit extracts of TTE therefore exhibited similar effect on red cell indices.

Platelet count and P-LCR were not significantly different among all groups but PDW and MPV were significantly decreased in the treatment groups compared with control (Table 2). This finding contradicts the report by Jimmy and Ekpo[23] where aqueous extract of TTE was reported to significantly increase platelet count. The non-significant change in platelet count and P-LCR from our results indicates that both aqueous and ethanolic fruit extracts of TTE

probably did not affect the role of platelets (i.e. aggregation and formation of platelet plug) in the blood clotting process. Platelet aggregation is not affected when P-LCR is not affected significantly. The decreased PDW indicates that both aqueous and ethanolic fruit extracts of TTE decreased platelet size. Both extracts exhibited the same effect on platelet indices.

TWBC count was significantly increased in aqueous group compared with control and significantly decreased in ethanolic group compared with aqueous group. Neutrophils and lymphocytes counts in aqueous group were significantly decreased and increased respectively compared with control (Table 3). The increase in TWBC and lymphocytes counts in aqueous group is in tandem with Jimmy and Ekpo[23] but the decrease in neutrophil count in this group contradicts their report where neutrophil count was significantly increased on the 14th and 28th day of administration of aqueous extract of TTE. Odesanmi *et al.*[7] previously reported that ethanolic extract of TTE significantly decreased WBC count. This finding contradicts ours- there was no significant difference in TWBC count between ethanolic and control groups in our study. The increase in TWBC and lymphocytes counts indicates the activation of the defence mechanism and immune system of the rats. Increase in lymphocytes count (lymphocytosis) occurs during infections. Certain cytotoxic substances (such as alkaloids and saponins) are present in the extract of *Tetrapleura tetraptera*.[7] The significant increase in TWBC and lymphocyte count in aqueous group compared with control which does not occur in the ethanolic group is probably due to difference in the doses administered (60mg/kg for aqueous and 40mg/kg for ethanolic) implying that more cytotoxic components were present in the aqueous extract than in the ethanolic extract used. Further work is needed for more clarification. It is possible that the increase in TWBC and lymphocyte counts is a consequence of the activation of the immune system in a bid to protect the body of the rats from attack by the cytotoxic components of the aqueous extract.

CONCLUSION

Both aqueous and ethanolic extracts of *Tetrapleura tetraptera* fruit exhibited similar effect. They did not affect red cell indices and platelet count but decreased PDW and MPV. Aqueous extract increased TWBC and lymphocytes counts and decreased neutrophil count whereas ethanolic extract did not exhibit any significant effect on TWBC, lymphocytes and neutrophils counts. We therefore advise that *Tetrapleura tetraptera* fruit should be consumed moderately as excess consumption may be detrimental to individuals with bleeding and/or blood coagulation disorders.

REFERENCES

1. Irondi EA, Oboh G, Agboola SO, Boligon AA, Athayde ML. (Phenolics extract of *Tetrapleura tetraptera* fruit inhibits xanthine oxidase and Fe2+-induced lipid peroxidation in the kidney, liver, and lungs tissues of rats *in vitro*). Food Science and Human Wellness, 2016; 5: 17-23.
2. Adesina SK, Iwalewa EO, Johnny II. (*Tetrapleura tetraptera* Taub ethnopharmacology, chemistry, medicinal and nutritional values- A review). British Journal of Pharmaceutical Research, 2016; 12: 1-22.
3. Ojewole JA, Adewunmi CO. (Anti-inflammatory and hypoglycaemic effects of *Tetrapleura tetraptera* (Taub) (Fabaceae) fruits aqueous extract in rats). J. Ethnopharmacol, 2004; 95: 177-82.
4. Aladesanmi JA. (*Tetrappleura tetraptera*: Molluscicidal activity and Chemical Constituents: A review). Afr. J. Trad. Complementary Alternative Med, 2007; 4: 23-36.
5. Okwu DE. (The potential of *Ocomum gratissimum, Pergularia extensa* and *Tetrapeura tetraptera* as spice and flavouring agents). Nig. Agric. J, 2003; 35: 143-8.
6. Essien EU, Izunwane BC, Aremu CY, Eka OU. (Significance for humans of the nutrients of the dry fruit of *Tetrapleura tetraptera*). Plant Food Hum. Nutr, 1994; 45: 47-51.
7. Odesanmi SO, Lawal, RA, Ojokuku SA. (Haematological effects of ethanolic fruit extract of *Tetrapleura tetraptera* in male Dutch white rabbits). Research Journal of Medicinal Plant, 2010; 4: 213-7.
8. Uyoh EA, Ita EE, Nwofia GE. (Evaluation of the chemical composition of *Tetrapleura tetraptera* [Schum and Thonn] Taub. Accessions from Cross River State, Nigeria). Int. J. Med. Arom. Plants, 2013; 3: 386-94.
9. Atawodi SE, Yakubu OE, Liman ML, Iliemene DU. (Effect of methanolic extract of *Tetrapleura tetraptera* [Schum and Thonn] Taub leaves on hyperglycemia and indices of diabetic complications in alloxan-induced diabetic rats). Asian Pac. J. Trop. Biomed, 2014; 4: 272-8.
10. Nwaichi E, Igbinobaro O. (Effects of some selected spices on some biochemical profile of Wister albino rats). Am. J. Environ. Eng, 2012; 2: 8-11.
11. Omonkhua AA, Adebayo EA, Saliu JA, Ogunwa TH, Adeyelu TT. (Liver function of streptozotocin- induced diabetic rats orally administered aqueous root-bark extracts of *Tetrapleura tetraptera* [Taub]). Nigerian Journal of Basic and Applied Science, 2014; 22: 99-106.

12. Irondi AE, Anokam KK, Chukwuma PC. (Phenological variation in the *in-vitro* antioxidant properties and alpha-amylase inhibitory activity of *Tetrapleura tetraptera* Pod). Int. J. Pharm. Sci. Drug Res, 2013; 5: 108-12.
13. Moukette BM, Pieme AC, Biapa PCN, Njimou JR, Stoller M, Bravi M, Yonkeu Ngogang J. (*In vitro* ion chelating, antioxidative mechanism of extracts from fruits and barks of *Tetrapleura tetraptera* and their protective effects against fenton mediated toxicity of metal ions on liver homogenates). Evid.-BasedComplement. Altern. Med, 2015:14. doi:10.1155/2015/423689.423689
14. Ojewole JAO. (Analgesic and anticonvulsant properties of *Tetrapleura tetraptera* Taub (Fabaceae) fruit aqueous extract in mice). Phytother. Res, 2005; 19: 1023-9.
15. Awofisayo SO, Udoh, IE, Mbagwu HOC. (Antibacterial effects of the aqueous and ethanolic effects of *Tetrapleura tetraptera* pods on the pathogens in nosocomial wound infections). IJPI's J. Pharmacognosy and Herbal Formulations, 2010; 1:18-23.
16. Okokon JE, Udokpoh AE, Antia BS. (Antimalaria activity of ethanolic extract of *Tetrapleura tetraptera* fruit). J. Ethnopharm, 2007; 111: 537-40.
17. Kuate D, Kengne APN, Biapa CPN, Azantsa BGK, Wan Muda WAMB. (*Tetrapleura tetraptera* spice attenuates high-carbohydrate, high-fat diet-induced obese and type 2 diabetic rats with metabolic syndrome features). Lipids in Health and Disease, 2015; 14:50. doi 10.1186/s12944-015-0051-0.
18. Effiong GS, Udoh IE, Essien GE, Ajibola DO, Archibong KO. (Effect of aqueous extract of *Tetrapleura tetraptera* on excision wounds in albino rats). Int. Res. J. Plant Sci, 2014; 5: 57-60.
19. Ajayi OB, Fajemilehin AS, Dada CA, Awolusi OM. (Effect of T*etrapleura tetraptera* fruit on plasma lipid profile and enzyme activities in some tissues of hypercholesterolemic rats). J. Nat. Prod. Plant Resour, 2011; 1: 47-55.
20. Okoli JTN, Agbo MO, Ukekwe IF. (Antioxidant and hepatoprotective activity of fruit extracts of *Tetrapleura tetraptera* [Schum & Thonn] Taubert). Jordan Journal of Biological Sciences, 2014; 7: 251-5.
21. Ekwenye UN, Okorie CF. (Antibacterial activity of *Tetrapleura tetraptera* Taub pod extracts). International J. Pharma and Bio Sciences, 2010; 1: 734-41.
22. Aina SA, Banjo AD, Lawal OA, Okoh HI, Aina OO, Dedeke GA. (The toxicity of extracts of *Tetrapleura tetraptera* [Aridan], *Delonix regia* [Flame of the Forest] and *Raphia vinifera* [Raffia Palm] on the larvae of anopheles gambiae). Academic Journal of Entomology, 2009; 2: 67-70.

23. Jimmy EO, Ekpo AJD. (Upgrading of lethal dose of *Tetrapleura tetraptera* extract enhances blood cell values). J Hematol ?rombo Dis, 2016; 4: 256. doi:10.4172/2329-8790.1000256.