



Research Article

Phytochemical Constituents and Haematological Profile of Female Rats Following Oral Administration of Aqueous Extract of *Tetrapleura Tetraptera* Fruits

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Abstract

The phytochemical screening and the effect of administration of aqueous extract of *T. tetraptera* fruits at the doses of 50, 100 and 150 mg/kg body weight on daily basis for 14 days on haematological profile of female Albino rats was investigated. Female albino rats weighing 90g-125g were grouped into four (1-4) such that animals in group 1 received 0.5 ml of normal saline as a placebo while those in groups 2, 3, and 4 received same volume of the extract corresponding to 50, 100 and 150 mg/kg body weight respectively. The extract contained saponins (3.87%), phytosterols (9.89%), tannins (2.34%), phenols (7.08%), flavonoids (13.11%), cardiac glycosides (2.48%), terpenoids (8.92%) while alkaloids and anthraquinones were absent. All the doses of the extract significantly decreased ($p < 0.05$) the levels of haemoglobin (Hb) and Packed Cell Volume (PCV). In contrast, the levels of Total White Blood Cell (TWBC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) increased significantly ($P < 0.05$). While the levels of Red Blood Cell (RBC), Platelet (PLT) and Mean Corpuscular Volume (MCV) were not significantly altered ($p < 0.05$) by all the doses of the extract, there was dose specific effects on the neutrophils, lymphocytes, monocytes and eosinophils of the animals without any significant alteration in their levels. Findings from this study have revealed that the administration of the aqueous extract of *T. tetraptera* fruits at the doses of 50, 100 and 150 mg/kg body weight once daily for 14 days selectively and mildly altered the levels of haematological parameters in the experimental animals.

Keywords

Tetrapleura Tetraptera, Fabaceae, Phytochemicals, Haematopoiesis, Medicinal Plant

1. Introduction

The great medicinal properties of plants have been recognized and appreciated from time immemorial [1]. Thus, the use of plants as medicine has long been in existence and is widely documented in records kept in ancient China, India and Egypt [2]. Medicinal plants typically contain several different

pharmacologically active compounds that may act individually, additively or in synergy to improve health [3, 4]. Phytochemicals are chemical compounds that occur naturally in plants and are derived from them. Some phytochemicals may have evolved through defunct metabolic pathways in the

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plants. The availability of several medicinal products from these plants has been of interest to the pharmacological biochemist for decades. Plants produce these bioactive compounds as part of defense mechanisms against predators and to protect themselves [5, 6]. These numerous phytochemicals including the alkaloids, flavonoids, tannins, saponins, carotenoids and many others exhibit various physiological actions that make them effective in disease prevention and treatment [2].

Since many plants have great potential uses, especially as traditional and pharmacopoeia drugs, the modern-day use of traditional medicine is also rapidly increasing in many countries including Nigeria where the vast biodiversity is a potential source of new and more affordable treatments for numerous conditions [7]. Medicinal plants have also provided the modern medicine with numerous plant-derived therapeutic agents and lead compounds with pharmacological or biological activity likely to be therapeutically useful [8]. Plant-derived active compounds can also be screened using *in silico* experiments during rational drug design for their ability to inhibit or stimulate a receptor of interest [9].

Since the discovery by man through customary and ancestral practice that some of these plant chemicals have medicinal values, they are now used as medicines, flavourings, and recreational drugs. With the upsurge of interests in medicinal plants, there is the need therefore for scientific investigations of these plants for both efficacy and potential toxicity [10]. The phytochemical studies of the medicinal plants have also provided some biochemical basis for their ethnopharmacological uses, in the treatment, management, and prevention of various diseases [11, 12].

Uhiokrihi tree (*Tetrapleura tetraptera*) is a species of the pea family endemic to West Tropical Africa. It is a deciduous flowering plant of the Fabaceae-Mimosoideae's family, which grows luxuriantly in the rainforest regions of West and Central Africa. The height and girth can reach up to 20-25 metres and 1.2-3 metres respectively. The leaves are sessile (have no stalk), glabrous or minutely hairy. The stem-bark is greyish-brown, thin and partially smooth. It has a unique four wing fruit consisting of a woody shell, fleshy pulp, and small brownish-black seeds with a distinct flavor. The fruits appear green when still tender but the mature dark purplish-brown fruit ripens from September to December. The tree is commonly harvested from the wild for its edible fruit and medicinal applications [13].

T. tetraptera fruit is used in ethnomedicine for the management of a wide range of diseased conditions and is believed to be rich in antioxidants, vitamins, minerals, essential oils, sugars and phytochemicals [14]. In the Eastern part of Nigeria, nursing and weaning mothers are fed with foods prepared with this plant, and assumed to have therapeutic effects on gestational diabetes and associated ailments. It is also consumed to improve and stimulate uterine contractions, hence help to return the stomach walls to shape.

The fruit is reported to have anti-arthritic, anti-inflammatory and antidiabetic properties. It is used to treat cardiovascular disorders, hypertension, convulsions and epilepsy, gastrointestinal disorders, malaria and other fevers, diabetes, asthma and chest pain, low body immunity, flu and colds, reproductive disorders, cancer of the breast and uterus, skin disorders for newly born babies, wounds and burns, back pain and general body pains and weakness, and dental disorders [13].

Rigorous scientific studies are required in order to unfold the complete utilization of *T. tetraptera*. The fruit is washed with water before adding into the soup. Some consumers add the fruit 30 minutes before the end of the soup boiling or at the beginning: the sweet aromatic odour takes over the air surrounding the house when the soup starts boiling. The dry fruit is softened when boiling commences, thus dissolving and leaking the oily odoractive compounds, including the accounted and unaccounted bioactive substances. However, the release mechanism involved in this process is still ambiguous. Yakubu *et al* [4] have shown that *Garcinia kola* (Heckel) seeds extract used in ethnomedicine for the treatment of various diseases exhibited selective haematotoxicity on experimental animals. Their findings highlight the need for toxicological studies of the many medicinal plants used in folk medicine.

Despite the acclaimed myriad uses of *T. tetraptera* fruits in folklore medicine, the effects of the fruit extract on the haematological parameters (Hb, PCV, RBC, MCV, MCH, MCHC, TWBC, platelets, neutrophils, Lymphocytes, monocytes, and eosinophils) of female rats has not been reported in the open scientific literature. Haematological analysis is often used for the diagnosis and treatment of diseases.

Therefore, the present study is relevant to ethnopharmacology and was designed to evaluate the phytochemical constituents and the effects of the aqueous extract of *T. tetraptera* fruits on haematological parameters of female Albino rats as the quest to fully profile its medicinal utility continues.

2. Materials and Methods

2.1. Plant Materials and Authentication

Tetrapleura tetraptera (Benth) fruits purchased at a market (Eke Okigwe Market), Okigwe, Imo State Nigeria, was authenticated at the Department of Biosciences and Biotechnology, University of Medical Sciences, Ondo, Nigeria. A voucher specimen (UNIMED P. B. T. H. No: 0237) was deposited at the herbarium of the Department.

2.2. Experimental Animals

Twenty albino rats (*Rattus norvegicus*) weighing 90g-125g obtained from the Animal Holding Unit of the Department of Biochemistry, Abia State University, Uturu, Nigeria, were maintained under standard housing conditions (temperature: 23±4°;

photoperiod: 12h natural light and 12h dark; humidity: 50-54%). The animals were allowed free access to rat pellets (Bendel Feeds and Flour mill Ltd, Ewu Nigeria) and tap water *ad libitum* throughout the period of the experimental study.

2.3. Preparation of Aqueous Extract of T. Tetraptera Fruits

The fleshy pulp of the fruits was washed, chopped into pieces and oven-dried at 40°C for 24h to a constant weight using Uniscope Laboratory Oven, (SM9053 Surgifriend Medicals, England). The dried pieces were then blended into a pulp using Arthur Miller Machine, and preserved in cellophane bags until when used.

Blended sample 150g was extracted with 250ml of distilled water by warm maceration for 24 hours with occasional stirring. This was then filtered using Whatman No 1 filter paper [15]. The volume of the extract was noted to be 155 ml and percentage yield / concentration of the extract was determined to be 28mg/ml. An appropriate volume was taken each time to make a desired dose. The choice of the doses 50mg/kg, 100mg/kg and 150mg/kg body weights used in this study was as a result of the findings from ethnobotanical survey. Ethnobotanical survey was conducted between September, 2017 and March, 2018 in Isuikwuato Local Government Area of Abia State, Nigeria. The Local Council is inhabited primarily by the Igbo ethnic group, and agriculture is the major economy.

Ethnobotanical information was obtained from participants using a semi-structured questionnaire. The participants were purposively selected, comprising herb sellers, traditional health practitioners, farmers and individuals with indigenous knowledge. The questionnaire was prepared in English. However, to facilitate efficient communication, informal discussion with herb sellers and traditional health practitioners was done in Igbo, the local language in the Local Government Area. A total of 46 participants were interviewed individually to maintain confidentiality. Data collected include age, gender, occupation, educational background, residence. plant part used, method of preparation/extraction, dosage, and administration method. The study and data collection were done following the regulations made by the International Society of Ethnobiology (available at <https://www.ethnobiology.net>; accessed on 20 April, 2017).

2.4. Phytochemical Screening

Standard laboratory methods as described for total phenolic compounds [16], saponins, flavonoids, cardiac glycosides and tannins [17], alkaloids and anthraquinones [18], phytosterols and terpenoids [19], were used in detecting the presence of phytochemicals in the plant.

2.5. Animal Grouping

Twenty apparently healthy female Albino rats aged 8 weeks

and weighing 90g-125g were randomly grouped into four (1-4) of five rats each. Rats in group 1 (control) received 0.5 ml of normal saline by means of oropharyngeal cannula. Those in groups 2-4 were administered 50, 100 and 150 mg/kg body weights of the extract respectively. The treatments were done once daily for 14 days.

2.6. Collection of Blood and Analysis of Haematological Parameters

On the 14th day of the experiment, the animals were starved overnight after treatment, anaesthetized with chloroform and sacrificed. Their jugular veins were cut and blood samples collected into sample bottles containing EDTA was analyzed for haematological indices such as PCV, RBC, and TWBC [20], Hb and platelets [21]. The MCV, MCH and MCHC were computed using appropriate formula as described by Jain [22].

2.7. Ethical Approval

The experimental protocols were approved by the Ethical Committee for Animal care and use of the College of Medicine, Abia State University, Uturu. Nigeria.

2.8. Statistical Analysis

The results were pooled and expressed as mean $\pm$ S. D. Analysis of variance (ANOVA) was used to analyze the results and Duncan Multiple Range Test (DMRT) was used for the post hoc analyses to compare group means for significance at $p < 0.05$. DMRT was used to compare the treatment means for significant differences and to test specific number of comparisons besides comparing them to the controls. DMRT was also used because of the large pairs of means being compared on a table format.

3. Results

Phytochemical screening revealed the presence of saponins, phytosterols, tannins, phenols, flavonoids cardiac glycosides, and terpenoids (Table 1). Quantitatively, flavonoids showed the highest concentration followed by phytosterols, terpenoids, phenols and saponins, while cardiac glycosides and tannins were present in smaller quantities. However, alkaloids and anthraquinones were absent (Table 1).

All the doses of the extract significantly decreased ($p < 0.05$) the levels of haemoglobin (Hb) and Packed Cell Volume (PCV). In contrast, the levels of Total White Blood Cell (TWBC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) increased significantly ($P < 0.05$). While the levels of Red Blood Cell (RBC), Platelet (PLT) and Mean Corpuscular Volume (MCV) were not significantly altered ($p < 0.05$) by all the doses of the extract, there was dose specific effects on the neutrophils, lymphocytes, monocytes and eosinophils (EOs) of the animals

without any significant alteration in their levels (Table 2).

Table 1. Phytochemical Constituents of Aqueous Extract of *Tetrapleura Tetraptera* Fruits.

Phytochemicals	Concentrations (%)
Saponins	3.8±0.04
Phytosterols	9.89±0.06
Alkaloids	Absent
Tannins	2.34±0.02
Phenols	7.08±0.03
Flavonoids	13.11±0.05
Cardiac glycoside	2.48±0.03
Terpenoids	8.92±0.04
Anthraquinones	Absent

Data in triplicates ± S. D

Table 2. Effect of Aqueous Extract of *T. Tetraptera* Fruits on Haematological Parameters Parameters.

Treat-ment	RBC (X10 ⁶ /m ³)	PCV (%)	Hb (g/Dl)	TWBC (X10 ³ /m ³)	PLT (X10 ³ /m ³)	MCV (FL)	MCH(Pg)	MCHC (g/dL)	Neu-trophil (%)	Lym-phocyte (%)	Mono-cyte (%)	Eosin-ophil (%)
Normal Control	6.99±0.1 1c	38.53 ±1.62c	12.63±0. 68d	5.63±0.2 4a	606.29 ±51.35a	51.60± 0.40a	16.80± 0.39a	32.32±0. 38a	29.34± 0.84b	63.69±1. 86a	4.37±0 .48a	2.34±0 .43a
T. tetrapa 50mg/kg b.w.	6.93 ±0.15c	31.10± 0.35a	10.90±0. 38b	7.64±0.3 6b	631.25± 58.81a	52.05± 0.40a	17.23± 0.39b	38.10±0. 79d	27.85± 0.63ab	64.57±1. 38a	5.26±0 .77ab	2.37±0 .39a
T. tetrapa 100mg/kg b.w.	6.89 ±0.17bc	31.97± 2.35a	11.07±0. 72b	9.02±0.8 7c	624.57± 28.21a	51.94± 1.03a	17.44± 0.39b	37.52 ±0.59c	28.02± 1.56ab	62.45±1. 37b	5.33±0 , 76ab	2.33±0 .43a
T. te-trapta 150mg/kg b.w.	6.95 ±0.18c	34.87± 2.17b	10.92±0. 36b	8.97±0.7 7c	625.38± 40.49a	51.72± 0.79a	17.32± 0.29b	33.30±0. 80b	28.83± 0.48b	62.29±1. 38b	5.10±0 .75ab	2.24±0 .26a

Values represent Mean ±SD for n = 5. Superscripts of the same alphabet along the same column are not significantly different from each other at (p < 0.05). RBC, red blood cell; PCV, packed cell volume; Hb, hemoglobin; TWBC, total white blood cell count; PLT, Platelet count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin; concentration.

4. Discussion

Medicinal plants or synthetic drugs can alter a wide range of haematological parameters [23] and this may be due largely to differences in the biological functions of the active components in such plants or drugs. Haematological parameters are good indicators of the physiological status of animal and alterations in the indices could be used in haematology to assess

the response of animals to various physiological situations [24]. Changes in blood parameters can also be used to explain the blood-relating functions of the plant extract or its products [25]. In a healthy adult human, about ten billion (10¹⁰) new blood cells are produced per day, in order to maintain steady state levels in the peripheral circulation [26]. The non-significant reduction in RBC by the extract in the present study may be an indication the erythropoiesis was mildly affected, which is characteristic of many therapeutic agents. The extract might

have slightly interfered with the balance between the rate of production and destruction of the blood corpuscles [27]. The accompanying decrease in Hb and PCV is quite understandable since they are directly related to the RBC. Therefore, the release of erythropoietin in the kidney which is the humoral regulator of RBC production was mildly affected [28]. The phytocompound saponins have been reported to have haemolytic properties [29]. Therefore, the presence of saponins in the extract may be responsible for the mild reduction in RBC and consequently the decrease in PCV and Hb. The MCV, MCH and MCHC are related to individual red blood cells. However, these haematological parameters may not be affected when the individual red blood cells in terms of their average size (microcytes) and weight of haemoglobin per RBC were not affected [30]; although the total population slightly reduced as evidenced by the decrease in RBC, Hb and PCV (Table 2).

TWBC, lymphocytes, neutrophils, monocytes and eosinophils are immunologic machines of the body against infections. Alterations in these haematological parameters may lead to immunologic incapacitation. The increase in TWBC may suggest that the rate of entrance of the corpuscles into the blood circulation from the bone marrow was enhanced compared to its rate of removal from circulation. It is also possible that the haematopoietic regulatory elements of the stromal cells and macrophages in the bone marrow were enhanced by the extract. This is further corroborated by the stimulatory effect of the extract on thrombopoietin which is directly related to platelet production [31]. Thrombopoietin is a glycoprotein hormone produced by the liver and kidney which regulates the production of platelets. It stimulates the production and differentiation of megakaryocytes, the bone marrow cells that bud off large numbers of platelets [32]. Since the TWBC increase the immunological action of the body by fighting pathogenic antigens either by phagocytosis or cytotoxicity, the significant increase in TWBC following the administration of the extract at all the doses suggests a boost in the immune system of the experimental animals. The mild reduction in lymphocyte counts at the higher doses of 100 mg/kg body weight and 150 mg/kg body weight and lack of an effect at the lower dose of 50 mg/kg body weight of the extract may indicate parameter- and dose-specific effect of the extract. However, the small sample size (n=5) used in this study may not adequately represent the realities of the population being studied. Small sample sizes may produce wide confidence intervals that increases the vulnerability of results to random error and potential bias. Consequently, further studies with a larger sample size are advocated to improve the applicability and reliability of findings.

5. Conclusion

This study has revealed that the aqueous extract of the fruits of *T. tetraptera* at the doses of 50, 100 and 150 mg/kg body weight selectively and mildly altered the levels of the haematological parameters of the experimental animals. Therefore,

further biochemical and toxicological evaluation is necessary to fully characterize its therapeutic effects.

Abbreviations

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Conflicts of Interest

The authors declare no conflicts of interest.

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