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EFFECTS OF ETHANOL ROOTS EXTRACT OF *JATROPHA TANJORENSIS* ON HAEMATOLOGICAL INDICES OF MALE ALBINO WISTAR RATS

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ABSTRACT: *This study was carried out to determine the effects of ethanol roots extract of Jatropha tanjorensis on haematological indices of male albino wistar rats. Twenty (20) albino wistar rats weighing between 151-225g were used for the study. The rats were divided into four groups of five each based on body weight. Group I served as the control. Groups II, III and IV were administered varying concentrations (100, 250 and 500mg respectively) of ethanol roots extract of Jatropha tanjorensis. The administration was done daily for 14 days. The haematological indices analysed include Red blood cells (RBC), White blood cells (WBC), Haemoglobin (HGB), Haematocrit (HCT), Mean cell volume (MCV), Mean cell Haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC), Platelets count (PLT), Lymphocytes (LYM) and Neutrophil count. The study revealed a significant ($P< 0.05$) increase in White blood cell (WBC) count, Red blood cell (RBC) count and Haemoglobin (HGB) count in the groups administered the extract compared to the control group. There were also significant ($P< 0.05$) increase in the values of Haematocrit, Platelet count and Lymphocyte in the treated groups than the control. The values of MCV, MCH and MCHC in groups II and III were not significantly ($P< 0.05$) different from the control while that of group IV was significantly ($P< 0.05$) higher than the control group. For the values of NEUT, it was observed that there was no significant ($P< 0.05$) different between the treated groups and the control group. This study suggests that Jatropha tanjorensis roots possess the required ingredients capable of boosting the immune system and could prevent other opportunistic diseases associated with decreased immunity. The extract may be useful in the treatment and management of anaemia as well as improving the immune system in humans.*

KEYWORDS: Haemoglobin, *Jatropha tanjorensis* roots extract, Haematocrit, Red blood cells, white blood cells

INTRODUCTION

Plant based medications have been employed since the dawn of civilization for prolonging the life of man and for combating various ailments. For decades the screening of medicinal plant materials for their therapeutic values has continue to represent potential sources of new effective medicines (Omoregie and Osagie, 2012). Using plants for the treatment and cure of diseases is as old as human species itself, with popular knowledge making a great contribution to the dissemination of the therapeutic virtues of these plants. This knowledge has represented a therapeutic resource for many communities and ethnic groups (Maciel *et al.,* 2002).

Proteins, carbohydrates and fats as well as vitamins and minerals are made available to man and other animals through green plants (Iwalewa, *et al.,* 2005). Some plants apart from serving as food have also been known to possess medicinal properties (Shanti *et al*., 2010). Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases.

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Medicinal values of plants depend on these inherent substances that produce a definite physiological action on the human body. In developing countries, herbal medicine still serves as the mainstay of about 75-80% of the whole population (Parekh *et al.,* 2005). Herbal medicine has better cultural acceptability with the human body and fewer side effects (Goel and Sharma, 2014).

The plant kingdom offers a wide range of natural antioxidants and medicinal plants (Lewis and Manony, 2018). A number of studies have revealed the antioxidant activities of phytochemical constituents of medicinal plants (e.g. polyphenols, carotenoids, phenolics and vitamins C and E). These phytochemicals acting as antioxidants prevent damages to cell membrane and cellular oxidative processes that may give rise to diseases (Omoregie and Osagie, 2012). For instance, natural polyphenols from plant vegetables have been found to exert their beneficial effects by removing free radicals, chelating metal catalysts, and activating antioxidant enzymes (Ayoola *et al.,* 2006). In recent times, antioxidants from plant sources have received a lot of attention and are even preferred to synthetic ones especially due to their potential health benefits, availability, affordability and in many cases, reduced toxicity (Tarawneh *et al.,* 2010).

The continuous and perpetual people's interest in medicinal plants has brought about today's modern and sophisticated fashion of their processing and usage (Kelly *et al*., 2002). In all, the use of herbs to treat disease is almost universal among non-industrialized societies and is often more affordable than purchasing modern pharmaceuticals.

The name Jatropha is derived from the Greek words *jatrós* (doctor) and *trophé* (food) which implies medicinal use (Mousumi and Bisen, 2008). *Jatropha tanjorensis* is a member of the 'Euphorbiacea' family. It is popularly referred to as 'Hospital Too Far', 'Catholic Vegetable', Iyana-Ipaja or 'Lapalapa' by the local folks in different parts of Nigeria (Iwalewa *et al.,* 2005). The Igbo people of South Eastern Nigeria call it 'Ugu-Oyibo'. Other species of this plant are *Jatropha curcas, Jatropha gossypifolia, Jatropha padagrica, Jatropha glandulifolia, Jatropha multifida, Jatropha intergerrima* (Debnath and Bisen, 2008).

Jatropha tanjorensis is a bushy, gregarious shrub of about 1.8 metres in height. The leaves are 3-5 lobed palmate and contain glandular hairs. Jatropha species have a high adaptability for thriving under different climatic conditions (Debnath and Bisen, 2008). It is therefore suitable for all types of soils and barren land. Jatropha is a versatile plant owing to its excellent regeneration capability and long, productive life. *Jatropha tanjorensis* is a native of Central America and has become naturalized in many tropical and subtropical countries, including Africa, India and North America (Machlus *et al*., 2014). *Jatropha tanjorensis* is a common weed of field crops in rainforest zones of West Africa including Nigeria (Iwalewa *et al.,* 2005). Its primary use is for fencing while its secondary uses are a source of edible leafy vegetables and medicine. It is useful in herbal medicine, prepared locally in most parts of Southern Nigeria by collecting the leaves and squeezing out the juice (Manthey *et al*., 2013).

The plant has been investigated for hematopoietic activity, hypolipidaemia activity, hypoglycaemic and antidiabetic activity as well as antimicrobial effect (Madubuike *et al.,* 2015*). Jatropha tanjorensis* leaves are consumed in Nigeria as soups and as a tonic with the claim that it increases blood volume. The leaves are also employed traditionally in the treatment of anaemia, diabetes and cardiovascular diseases (Iwalewa *et al.,* 2005).

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Its healing potential is traceable to its phytochemical constituents. Phytochemicals screening of *Jatropha tanjorensis* leaf revealed that it contains bioactive principles such as alkaloids, flavonoids, tannins, cardiac glycosides, antraquinones and saponins (Ehimwenma and Osagie, 2007).

Olayiwola *et al.*, (2004) wrote that the leaf extract has hypoglycaemic properties and is taken as a remedy against diabetes. It is popular as a natural remedy against malaria infection and hypertension in Southern Nigeria where they drink the squeezed out juice. *Jatropha tanjorensis* leaves contain a high water and low protein content. The trace elements zinc, iron and selenium are in concentrations comparable to those in food regarded as good dietary source of these elements (Igbinaduwa *et al.*, 2011). The leaf extract also possesses antimicrobial properties and inhibit the growth of *S. aureus* and *E. coli* (Oboh and Masodje, 2009).

Jatropha tanjorensis leaf has also been documented to have antianaemic effect (blood replenishing potentials), in addition, the leaf was found to contain some important biogenic principles that are important for rapid hemopoiesis in the bone marrow (Omoregie and Osagie, 2012). According to Igbinaduwa *et al.*, (2011), *J. tanjorensis* leaf is also a potent anti-HIV agent (effective against HIV-1 vector).
Much work has been done on the leaf of *Jatropha tanjorensis* but little has been documented on the root. Due to the shortage of research work on the roots of *Jatropha tanjorensis*, this study was carried out with the aim to investigate effect of ethanol roots extract of *J. tanjorensis* on haematological indices of male albino wistar rats.

MATERIALS AND METHODS

Materials
Fresh roots of *Jatropha tanjorensis* were collected from an uncultivated farmland in Uyo, Nigeria.

Experimental Animals
Twenty (20) male albino Wistar rats weighing between 151-225g were used for this experiment. The rats were obtained from the animal house, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria. The animals were acclimatised for one month and maintained on water and animal feed ad libitum.

Reagents
Sysmex Automated Haematology Analyser kit Model KX-21N, produced by Sysmex Company, Japan was used for the biochemical analysis

Methods
Sample Preparation and Administration
The freshly collected roots sample was washed, air dried at room temperature for six weeks and pulverized to obtain a fine homogenate using a manual grinder. 500g of the homogenate was dissolved in 3.0 litres of 80% ethanol. The sample was macerated for 24hrs, after which the crude extract was filtered through a Whatman filter paper (125mm). The extract was placed in a water bath at 50^0C to remove the ethanol. The crude extract obtained was stored in a refrigerator at 4^0C prior to administration. 5g of the crude roots extract was dissolved in 20ml of distilled water and made up to 100ml mark, to make a concentration of 50mg/ml. This served as stock solution of the extract from where the required dosage of administration was prepared.

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Experimental Design and Treatment of Animals

A total of twenty (20) male albino Wistar rats were weighed and randomly divided into four groups of five animals each. The rats had free access to water and standard rat diet and exposed to 12h light/dark cycle in an optimum temperature. Group I served as control, groups II, III and IV were administered 100mg/kg body weight, 250mg/kg body weight and 500mg/kg body weight respectively of the roots extract daily for 14days. All treatments were done orally by gastric intubation.

Animal Sacrifice and Preparation of Plasma and Sera for Analysis

At the end of the administration (14 days), the animals were fasted overnight and anaesthetized by dropping each of them in a transparent jar with chloroform vapour. Incision was made on the abdomen and the blood samples collected using cardiac puncture into sterile plain tubes for sera preparation and anticoagulant (E.D.T.A.) bottles for plasma preparation which were used for whole blood analysis. Serum samples were obtained from clotted blood into sterile plain tubes after centrifugation at 2000rpm for 15mins using a bench-top centrifuge. The serum was stored in the refrigerator for analysis.

Haematological Studies

The samples were analysed for haematological indices within 24 hours using Sysmex Automated Haematology Analyser kit Model KX-21N, produced by Sysmex Company, Japan. The parameters analysed include Red blood cells (RBC), White blood cells (WBC), Haemoglobin (HGB), Haematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular Haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Platelets count (PLT), Lymphocytes (LYM) and Neutrophil count.

Statistical Analysis

Data obtained were subjected to statistical analysis using SPSS window version 9.0. Comparison was done using one way analysis of variance (ANOVA). P values < 0.05 were considered statistically significant. All values were expressed as mean ± SD.

RESULTS AND DISCUSSION

Results

The results of the effects of ethanol roots extract of *Jatropha tanjorensis* on haematological indices of male albino wistar rats are presented on table I below. Results obtained showed a significant ($P< 0.05$) increase in RBC count in groups treated with roots extract of *Jatropha tanjorensis* (II, III and IV) compared with control group I. The WBC count of the groups treated with the extract at 100 mg/kg body weight, 250 mg/kg body weight and 500 mg/kg body weight were significantly ($P< 0.05$) higher than the control. The values of HGB and HCT in the groups treated with the extract were also significantly ($P< 0.05$) higher than the control. Regarding MCV, MCH and MCHC values, only group IV was significantly ($P< 0.05$) higher than the control, while that of groups II and III were not significantly ($P< 0.05$) different. The values of PLT were significantly ($P< 0.05$) higher in groups II, III and IV than the control. There was also a significant ($P< 0.05$) increase in the values of LYM in groups treated with the extract (II, III and IV) compared with the control. For NEUT, groups II and III were not significantly ($P< 0.05$) different from the control but group IV was significantly ($P< 0.05$) higher when compared with the control group.

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Table I: Haematological parameters in rats administered with *Jatropha tanjorensis* roots extract

Parameter/Group	Group I Control	Group II (100mg/kg bw)	Group III (250mg/kg bw)	Group IV (500mg/kg bw)
RBC $x10^6/\mu L$	6.45 ± 0.12^a	9.65 ± 0.22^b	11.76 ± 0.47^b	15.29 ± 0.48^b
WBC $x10^6/\mu L$	8.88 ± 0.31^a	12.28 ± 1.32^b	14.79 ± 4.20^b	16.80 ± 1.20^b
HGB g/dl	12.58 ± 0.37^a	15.34 ± 0.54^b	16.01 ± 1.27^b	17.01 ± 1.49^b
HCT %	38.60 ± 1.34^a	49.38 ± 1.42^b	52.41 ± 1.22^b	54.82 ± 0.18^b
MCV fL	63.44 ± 0.52^a	64.01 ± 0.21^a	64.34 ± 1.58^a	69.01 ± 0.27^b
MCH pg	16.68 ± 1.22^a	16.79 ± 0.32^a	17.21 ± 0.57^a	19.54 ± 0.43^b
MCHC g/dL	26.40 ± 1.35^a	26.38 ± 1.28^a	28.89 ± 0.90^a	30.40 ± 0.25^b
PLT $\times10^3/\mu L$	588.18 ± 9.37^a	655.14 ± 7.61^b	658.70 ± 8.01^b	727.04 ± 9.25^b
LYM %	67.10 ± 2.34^a	73.44 ± 5.28^b	75.54 ± 3.20^b	78.08 ± 1.41^b
NEUT %	18.49 ± 4.02^a	18.38 ± 5.36^a	18.40 ± 2.28^a	19.86 ± 0.27^a

Values are mean of five rats in each group ±SD
Values with different alphabetical superscript in a row are significantly different at $P<0.05$

DISCUSSION

Assessment of haematological parameters can not only be used to determine the extent of deleterious effect of extracts on the blood of an animal, but can also be used to explain blood related functions of a plant extract or its product (Yakubu and Afolayan, 2009). The effects of *Jatropha tanjorensis* ethanol roots extract administration in adult male albino wistar rat were investigated to assess its benefits or possible risk involved. Literature is replete with the use of plant materials and its derivatives for the prevention and treatment of diseases. The beneficial therapeutic effects of these medicinal herbs are expressed in their scientific implications in health conditions of the users. Thus, medicinal herbs such as *Jatropha tanjorensis* have played a major role in the development of modern medicine and their traditional applications cannot be under estimated as some persons in the African societies do not have access to modern medications. Generally, there is still need to investigate the potential adverse effects associated with the use of some medicinal herbs and the possible way of ameliorating these toxic effects.

Haematological indices have a high diagnostic significance in routine clinical evaluation of health state. It is important in diagnosing toxicity in animals exposed to drugs or toxicant. The assay of haematological parameters reveals that *Jatropha tanjorensis roots extract* significantly increases haematological parameters in the blood of the animals investigated. There was a significant increase in RBC count in the test groups. This indicates that the roots extract may have induced an elevation of erythropoietic mechanism in the experimental animals compared to the control group. This finding is consistent with observations made previously by authors who worked on leaves of *Jatropha tanjorensis* (Iwalewa *et al.*, 2005 and Omoregie and Osagie, 2012). The present study also shows a significant difference in WBC count between the control and the test groups. This increase in WBC counts may indicate stimulatory effect of the extract on leucocytosis which perhaps explains the ability of the crude roots extract of *Jatropha tanjorensis* to improve immune related disease conditions.

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Haemoglobin is a bright red coloured pigment in the red blood cells. It contains iron which is a vehicle for transporting oxygen and carbon dioxide (Goodman, 2009). Haemoglobin can be used to assess total erythropoiesis and the amount of erythropoietic activity that is effective in producing circulating red cells. There was an increase in the Haemoglobin count in the test groups than in the control group.

Platelets play a critical role in the prevention of blood loss by adhering to exposed collagen to form platelet plug. Platelet count increased significantly ($P < 0.05$) in the test groups when compared with the control group. This indicates that the treatment induced a high platelet concentration in the test animals.

The values of lymphocytes in the test groups were observed to be significantly increased ($P < 0.05$) when compared with the control. Obviously, this extract may have induced lymphocytosis in the experimental animals, confirming the anti-infective potential of the extract. One of the major functions of lymphocytes is their response to antigen (foreign bodies) by forming antibodies that circulate in the red blood or in the development of cellular immunity.

The increases in the haematological indices observed following treatment with *Jatropha tanjorensis roots* extract might not be unconnected with the phytochemicals present in the roots of *J. tanjorensis*. The phytochemicals had been shown to include saponins, cardiac glycosides, flavonoids, terpenoids, tannins etc. Saponins have been reported to have beneficial effects on blood cholesterol levels. They bind with bile salt and cholesterol in the intestinal tract. This binding causes a reduction of blood cholesterol by preventing its re-absorption. The non-sugar part of saponins also has antioxidant activity which may help to reduce risk of heart diseases (Oakenfull and Sidhu, 1990). Some of the activities attributed to flavonoids include antioxidant, anti-allergic, anticancer, anti-inflammatory, antimicrobial, antidiarrheal activities as well as nervous and cardiovascular system benefits (Kozlowska and Szostak-Wegierek, 2014).

According to Oyewole and Akingbala (2011), the medicinal properties attributed to *Jatropha tanjorensis* as a useful herb in the treatment of heart diseases could be based on the antioxidant properties and positive modulatory effects of its phytochemicals on serum lipid. *Jatropha tanjorensis roots* have been proven to be rich in phenols which possess high antioxidant and blood boosting activities. According to Oboh, 2005, the high phenol contents could have contributed to the treatment and management of haemolytic anaemia. The additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for this potent blood boosting capacity. *Jatropha tanjorensis* is a rich source of phytochemicals that possess a protective potential against diseases such as anaemia and diabetes hence its use in folk medicine in Nigeria could be as a result of its ability to increase haematological parameters.

In summary, the result of this study shows that ethanol roots extract of *Jatropha tanjorensis* had significant impacts on various haematological parameters investigated.

CONCLUSION

Jatropha tanjorensis roots extract administered to albino Wistar rats at varying concentrations (100, 250 and 500 mg/kg body weight) significantly increase the haematological parameters like the red blood cell, white blood cell, haemoglobin concentration, lymphocyte and platelet count. Based on these findings, this work suggests that roots extract of *Jatropha tanjorensis* may be important in the management of anaemia as well as improving the immune system in humans.

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