Haematopoietic and Safety Study of Methanolic Extract of the Bark of *Trema orientalis* (L.) Blume Fam. Ulmaceae

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ABSTRACT

Various plant parts are known and have been found to be of great benefit to humans either as condiments or spices in human diet or as drugs. Different parts of *Trema orientalis* are used as condiment or spices in human diet and as medicine. This study therefore focused on the haematopoietic and safety study of the stem bark of this plant. The stem bark of *T. orientalis* tree was chopped from the plant, dried, powdered and subjected to extraction using a soxhlet apparatus with methanol as solvent. Acute toxicity study of this methanol extract was carried out with twenty-four mice divided into six groups. Each group received a dose of 500mg/kg, 1500mg/kg, 2200mg/kg, 5000mg/kg and 7500mg/kg/body weight (bwt) orally as a single dose respectively while the control group received distilled water. The mice were observed over a period of 24-hours for any acute intoxication .Also Twenty-five albino rats were used for haematopoietic effect and they were divided into five groups, each group received 0.0625g/kg, 0.125g/kg, 0.25g/kg and 0.5g/kg bwt of the methanol extract daily respectively for 28 days while the control group received distilled water only. After 28 days of administration, the animals were sacrificed and the blood samples were collected through cardiac puncture and subjected to heamapoietic parameters evaluation using standard procedures. Twenty five per cent (25%) response was observed at a limit dose of 7500mg/kg for the acute toxicity test while the result of the haematopoietic effect showed a significant increase in the PCV, Hb, RBC and lymphocyte count ($P<0.05$) when compared with the control and there was a significant decrease in the WBC, Eosinophils, Neutrophils and Platelet counts ($P<0.05$) . The LD50 value was greater than 2000mg/kg. This study showed this plant had an appreciable hematopoietic effect and it was non-toxic. Therefore it confirms the ethnobotanical use of this plant as a blood booster.

Keywords: *Trema orientalis*, safety study, hematopoietic activity.

INTRODUCTION

Plants are the richest resource of ingredients for traditional systems of medicine, modern medicines, folk medicines, pharmaceutical and food industries and for provision of chemical entities for synthetic drugs (Hammer et al., 1999). The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants are a source of great economic value all over the world. Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. According to world health organization (WHO), more than 80% of the world's population rely on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemical substances that produce a definite physiologic therapeutic action on the human body. Green plants synthesize and preserve a variety of biochemical products known as secondary metabolites which are commercially important. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, glycosides and phenolic compounds. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan et al., 2006). Medicinal plants are defined as any plant which contains substances that can be used for therapeutic purpose or serve as precursors for the synthesis of useful drugs (Andrew, 2002). Medicinal plants are also plants whose roots, leaves, seeds, bark, or other constituent possess therapeutic, tonic, purgative, or other pharmacologic activity when administered to higher animals. The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing diseases has been documented in history of all civilizations. The onset of research in medicine revealed that plants

contain active principles which are responsible for curative activity.

Trema orientalis has been found to be useful in the treatment of yellow fever where the leaves is pounded and boiled with the leaves of other plants like *Combretum colinum* and *Erythrina abyssiniaca* (Mainen et al,2012). Also, it has been found to be useful in the treatment of viral infections (Abiodun et al, 2011). In recent pharmacological studies, an aqueous extract from the bark has been shown to reduce blood glucose level and thereby confirming its anti diabetic use (Dimo et al 2006). Extracts from *T. orientalis* showed anti-inflammatory, anti-arthritic and analgesic activity in rodents (Barbera et al, 1992). It is also used as an insect repellent and in a study to assess the antimicrobial activity of *T. orientalis* on six selected bacteria strains; it was observed that the selected bacteria strains were highly susceptible to the test materials indicating that *T. orientalis* is potentially a good source of antibacterial agent. Its antitrypanosomial activity against *T. brucei* rhodesiense activity has been confirmed by Abiodun et al, (2012).

In a study performed to investigate the safety of some medicinal plants in respect to genetoxicity and the evaluation of the bacterial reverse mutation of plants of which *T. orientalis* is one using *Salmonella typhimurium* and *Escherichia coli*, it was observed that *T. orientalis* showed negative results in the bacterial reverse mutation test, suggesting that it is potentially safe for the plant to be used in medicinal plant supplement at high doses (Honge and Lyn, 2011). Other research works carried out on *T. orientalis* showed also that the plant has antiplasmodial activity (Abiodun et al, 2011) and hence useful in the treatment of malaria. It has also anti-sickling activity and inhibitory effect on the aggregation of human deoxyhemoglobin (Mpiana et al, 2011).

A chemical investigation conducted on the ethyl acetate-soluble fraction of the stem bark of *T. orientalis* led to the isolation of seven methyl ester of fatty acids, hexadecanoic, (z,z)-9,12-octadecanoic, (z)-9-octadecenoic, octadecanoic, eicosanoic, docosanoic and tetracosanoic, and two triterpenoids identified as β-sitosterol and 3 β-acetoxyurs-12-en-28-oic acid (Abd Maleek et al, 2005). Some plants are found to be practically safe for human consumption whereas some despite their significant activity have some degree of toxicity. *T. orientalis* bark is used traditionally as heamopoietic agent. However available literature shows that this folkloric use of the plant has not been reported. This study was therefore carried out to verify the safety and heamopoietic activity of the bark of *T. orientalis*.

MATERIALS AND METHODS

Plant Collection: The stem bark of *T. orientalis* tree was chopped from the plant, at a bush in Luba area of Ijebu-Ode, Ogun state Nigeria in September, 2012 and identified with a herbarium specimen at the herbarium Department of the Forestry Research Institute of Nigeria (FRIN), Ibadan with voucher FHI109754. It was then chopped into smaller pieces to facilitate easy drying and was air-dried for two weeks after which it was milled into a powdery form.

Extraction Procedure: The powdered sample 530.00g was subjected to extraction using a soxhlet apparatus with methanol as solvent. The extract was concentrated using a rotary evaporator (Rotavapor- R, India) and brought to a mucilage form on a heater with the temperature at 40oC to prevent denaturising the content of the extract and preserved in the refrigerator until it was ready for use.

Phytochemical Screening: The Phytochemical test was carried out on the powdered stem of *T. orientalis* using standard procedure (Trease and Evans, 2002).

Experimental Rats: The rats and mice were purchased from the Animal House, University of Ibadan, Nigeria on 20th November, 2012 and acclimatized for four (4) weeks at the Animal House, Faculty of Pharmacy, Olabisi Onabanjo University Ogun state, Nigeria, and fed with growers mash and water ad libitum in accordance with the principle of laboratory animal care (NIH publication No. 85-23, revised 1985) .

Experimental mice: Twenty-four (24) mice were used for the experiment and were grouped into six (6) based on their body weight with four (4) mice per group. All experiments were examined and approved by the appropriate ethics committee of the faculty of Pharmacy, Olabisi Onabanjo University".

Administration of the Extract to the Mice: The method used was in relation to that described by Mailer and Tainter, (1944). The extract was administered to the mice via oral route. Group 1 to 5 of the mice received the extract while the sixth group served as control and received distilled water. Different concentrations of the extract were used in order to establish the LD50 of the extract: Groups 1, 2, 3, 4 and 5 received 500, 1500, 2200, 5000 and 7500mg/kg dose of the extract, respectively. Group 6(Control) received 0.9mls of water. The mice received this dose as a single daily dose. The control group received distilled water equivalent to the volume of extract. After

administration of the extract to the mice, they were closely observed at intervals for twenty-four hours for signs and symptoms of acute toxicity.

Administration of the extract to the rats: This was carried out according to the method of Friday et al (2010). Twenty five rats weighing between 120 and 280g were used. They were grouped into five (5) groups based on body weight and each group received a different concentration of the extract except the control group which received distilled water in place of the extract. The extract was administered to the rats via oral route. Groups 1 to 4 of the rats were given the extract. The concentration of the extract administered to each group was 0.0625g, 0.125g, 0.25g and 0.5g/kgbwt respectively. The control group was given distilled water which is equivalent to the volume of extract received by group 4. The extract was administered to the rats as a daily dose for 28 days after administration for 28 days within 24 hours the animals were sacrificed and the blood sample of each rat was collected by cardiac puncture into EDTA bottles for haematological parameters.

Determination of PCV: The Packed Cell Volume (PCV) was determined according to micro method using capillary tubes. Blood samples were collected by cardiac puncture into EDTA treated sample bottles. This was carried out by using capillary tubes of 75ml in length and internal diameter of about 1mm. The blood was allowed to enter the tube by capillarity, leaving at least 15mm unfilled. The tube was then sealed by plastic seal. After centrifuging for 5 minutes, the PCV was then measured using a reading device (Dacie and Lewis, 1991).

Determination of Haemoglobin Concentration: This was done by using the cyanomethaemoglobin method. (Dacie and Lewis, 1991). 20µl of blood was added to 4ml of diluents. The tube containing the solution was stoppered and inverted severally; it was then allowed to stand at room temperature for about 3-5mins to ensure complete reaction. The solution of HCN was then compared with the standard and a reagent blank in a spectrophotometer at 540nm.

Determination of Red Blood Cells: This was determined by the visual method. 20µl of blood was taken into a micro pipette into 4ml of diluting fluid contained in a glass tube. The diluted blood was then mixed for at least 2 minutes by tilting the tube. A clean Neubauer counting chamber was filled with solution with aid of stout glass capillary and covered with a cover glass. The cells were counted using a 4mm dry objective and x6 eye piece determined using the visual method as described by Dacie and Lewis (1991).

Determination of White Blood Cells: A1 in 20 dilution was made by adding 20µl of blood to 0.38ml of diluting fluid in a 75 x 10mm plastic tube. The tube was tightly corked and mixed for at least 1 minute then the Neubauer counting chamber was filled. The preparation was viewed using a 4mm objective and x6 eye piece determined using the visual method as described by Dacie and Lewis (1991).

Determination of Platelet: A 1 in 20 dilution of the blood sample was made. The suspension was then mixed on a mechanical mixer for 10-15 minutes. Neubauer counting chamber was filled with the suspension with the aid of a stout glass capillary. The counting chamber was closed in a moist petri-dish and left untouched for at least 20 minutes to give time for the platelets to settle. The preparation was examined with the 4mm objective and x6 eye piece determined using the visual method as described by Dacie and Lewis (1991).

Determination of Differentials (Neutrophils, Lymphocytes and Eosinophils): A thin blood film was prepared on a clean grease-free glass slide and was allowed to air dry, it was stained with Leishman stain containing methylene blue dyes and eosin for 2mins and double-diluted for 8 mins.It was rinsed with buffer solution of pH 6.8. Examination of the slides was carried out under microscope using x100 objective lens.

RESULTS AND DISCUSSION

The final yield of the extract was 26.8gram. The table 1 below shows the result of phytochemical tests of the extract.

Table 1: Result of Phytochemical Tests of *T. orientalis* bark

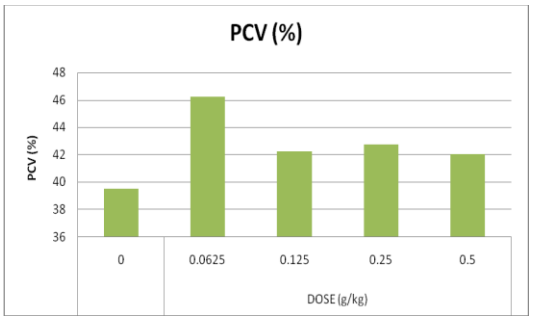
Test	Result
Combined Anthraquinone	Negative
Free Anthraquinone	Negative
Saponins	Positive
Alkaloids	Positive
Cardiac glycosides	Negative
Flavonoids	Positive
Tannins	Positive

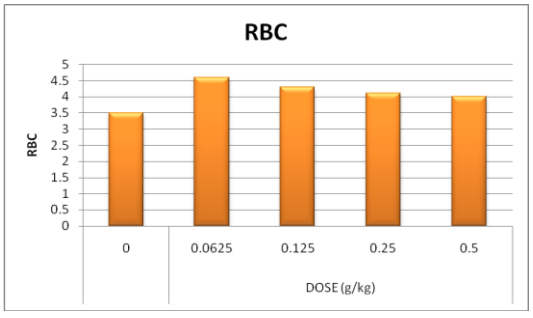
Result of Acute toxicity and Estimation of LD50 from Probit Analysis: The result of the toxicity effect of methanol extract of *T. orientalis* is as shown in table 2. From the result, there were no signs and symptoms of toxicity; the maximum death was one (1) death per group given a

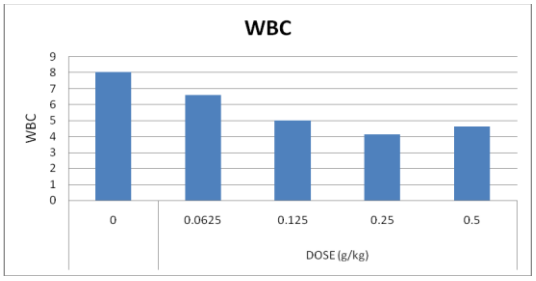
percentage response/death of 25% and hence from probit analysis LD50 was greater 7500mg/kg dose of the extract. The results of the methanol extract of *T. orientalis* bark on haematological parameters are as shown in the table 3. These results are graphically represented in Figures 1-8.

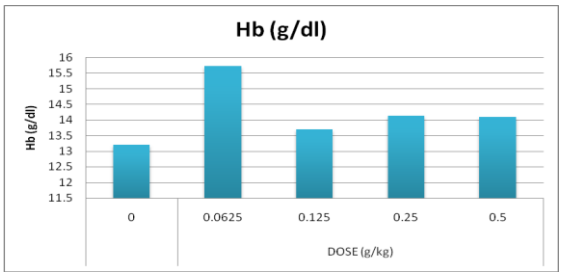
Table 2: Percentage (%) Response

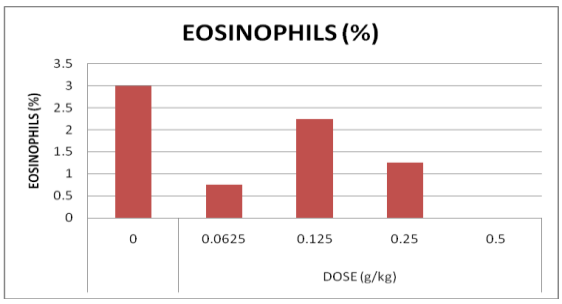
Group	Dose	Mortality rate and signs of toxicity	% Response/Death
1	500mg/kg	0, No sign	0%
2	1500mg/kg	0, No sign	0%
3	2200mg/kg	1, No sign	25%
4	5000mg/kg	1, No sign	25%
5	500mg/kg	1, No sign	25%
6(control)	0.9mls of water.	0, No sign	0%

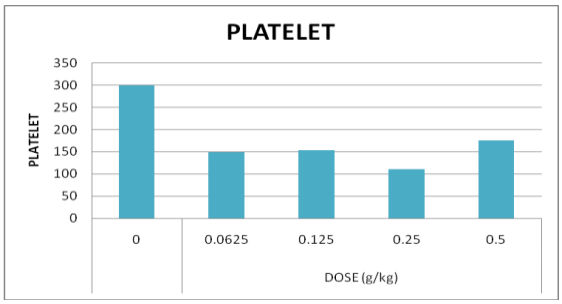


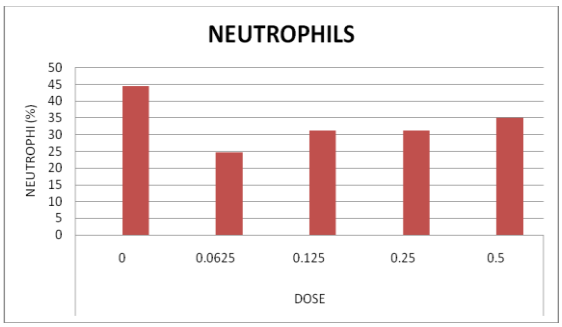


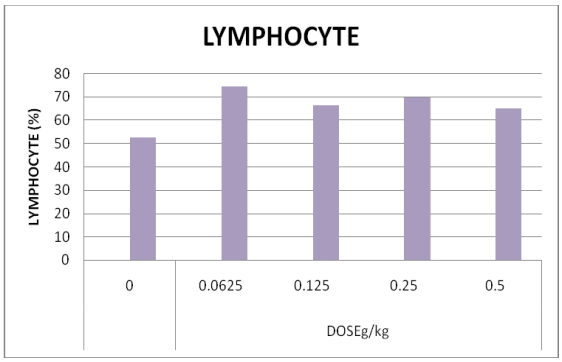












Figures 1-8: Graphical presentation of effects of *T. orientalis* bark on haematological parameters. 1: PCV, 2: RBC,.3:WBC, 4:Hb, 5:Eosinophils, 6:Platelets, 7: Neutrophils, 8: Lymphocyte

Table 3: Effects of methanol extract of *T. orientalis* bark on haematological parameters

Haematoogical parameters	Group 1 (0.0625g/kg) Mean ± SEM	Group 2 (0.125g/kg) Mean± SEM	Group 3 (0.250g/kg) Mean± SEM	Group 4 (0.500g/kg) Mean± SEM	Control rats Mean ± SEM	Reference Range
PC V (%)	46.25 ± 1.5*	42.25 ± 0.9	42.75 ± 1.5	42.00 ± 1.0	39.50 ± 1.7	35.00 - 51.00
Hb (g/dl)	15.73 ± 1.0*	13.70 ± 0.1	14.13 ± 0.5	14.10 ± 0.1	13.20 ± 0.1	10.80 - 17.50
RBC (X 10^9)	4.6 ± 0.2*	4.3 ± 0.0	4.1 ± 0.2	4.0 ± 0.1	3.50 ± 0.0	7.40 – 9.60
WBC (X 10^9/l)	6.60 ± 0.7	5.03 ± 0.7*	4.13 ± 0.2*	4.65 ± 0.3*	8.05 ± 0.9	6.40 - 26.20
Neutrophils (%)	24.75 ± 3.4*	31.25 ± 4.3*	31.25± 2.8*	35.00 ± 5.0	44.5 ± 5.10	12.00 – 46.00
Lymphocytes (%)	74.50 ±3.8*	66.50 ± 4.3*	70.00±2.8*	65.00 ± 5.0	52.5 ± 5.0	53.00 – 83.00
Eosinophils (%)	0.75 ± 0.5	2.25 ± 1.0	1.25 ± 0.5	0.00*	3.00 ± 1.0	0.00 - 3.40
Platelets (X 10^9/l)	149.75±24.2*	154.00 ± 33.7*	111.00±8.1*	175.00± 15.0*	300.00 ± 37.4	150 – 400

DISCUSSION

The phytochemical screening of the stem bark of *T. orientalis* as shown in table 1 revealed that it contained saponin, tannins, flavonoids and alkaloids as was reported by Abd maleek et.al (2005). The result of the estimation of LD50 of methanol extract of *T. orientalis* as shown in table 2, revealed that the administration of the extract at doses of 500mg/kg and 1500mg/kg, showed no death with no signs and symptoms of toxicity but from concentration of 2200mg/kg to 7500mg/kg, each group gave 25% response of a single death from the groups but there were no observable signs and symptoms of toxicity. The death observed in group 3 (2200mg/kg) occurred at 20 minutes after administration of the extract while the ones observed in groups 4 and 5 at concentrations of 5000 and 7500mg/kg respectively occurred immediately after administration of the extract. The death observed in group 4 and 5 could be said to be due to trauma and poor handling of the animals as no other death was recorded after 6 hours and 24 hours of administration and no signs and symptoms of acute toxicity observed in the rest of the animals (OECD, 2001). However it was observed that the mice became less active immediately after administration for about ten (10) minutes after which they became very active again. There was no change in their appearance and eating ability. Also from the result, the maximum response was 25% at the highest administered dose (7500mg/kg) and according to OECD guidelines for determination of acute toxicity, the upper limit dose to be administered should be 5000mg/kg body weight (OECD, 2001) and even at a higher dose of 7500mg/kg, only 25% response was observed. Hodge and Sterner's classification of toxicity states that if LD50 of a chemical falls within a concentration range of 5000-15000mg/kg, such chemical can be said to be "practically non-toxic" (OECD, 2001). Since from the result, the maximum administered dose was 7500mg/kg which gave a response of 25%, the LD50 of the plant was greater than 7500mg/kg and hence the plant could be said to be practically non-toxic and this was in agreement with a study performed by Hong and Lyn 2011 who investigated the safety of some medicinal plants with respect to genotoxicity, and *T. orientalis* was implicated to be non toxic genetically and potentially safe to be used as a medicinal plant in high doses.

From the data obtained, it can therefore be concluded that the methanol extract of the bark of *T. orientalis* could be classified to be in the Fifth class of toxicity (stated to be practically non-toxic) according to Hodge and Sterner Scale of toxicity classification; hence it is safe for acute administration.

The result of the Haematological effect of methanol extract of *T. orientalis* is as shown in table 3 and in fig. 1 the mean packed cell volume (PCV) of group of rats that received the methanol extract increased significantly than the control rats ($P<0.05$). It was also observed that the group with the lowest dose of extract have the highest PCV value. The mean values for RBC and Hb count figures 2 and 4 were higher in administered rats as compared to the control. The RBC count for the group with the dose of 0.0625 g/kg gave the highest count which is considerably significant ($P<0.05$) when compared with control and higher than the mean value for other groups (including the group with the highest administered doses).

The Hb count gave a significant increase with the administered rat especially with the group that

received the lowest dose when compared with the control but the increase was only significant with the lowest dose ($P<0.05$), and insignificant with other doses and this lent credence to recent pharmacological work done on *T. oreintalis* that revealed its ant-sickling effect (Mpiana et al, 2011). There was a decrease in WBC count in administered rats which was significant ($P<0.05$) especially in groups that received doses of 0.125, 0.25 and 0.5g/kg in comparison with the control. There was also a significant lymphocyte count with administered rats especially with 0.0625, 0.125 and 0.25g/kg doses. For eosinophils figure 7, a decrease in eosinophil count was found with the administered groups as compared to the control. The decrease is only found to be significant with group of 0.5g/kg and insignificant with others. The neutrophils count figure 5 showed a significant decrease ($P<0.05$) in all the groups except for group with 0.5g/kg dose. Figure 8 shows result for platelet count with a significant decrease in the platelet count of the administered rats ($P<0.05$). The reduction in WBC, neutrophil, eosinophil and platelet level in this present study might have occurred due to lyses of blood cells and or probably through suppression of blood cells synthesis by saponins found in the bark extract of *T. orientalis* because saponins are known to cause blood lyses (Watt et al, 1962).

It is obvious that the methanol extract of the plant *T. orientalis* has an effect on all the haematological parameters on PCV, Hb, RBC and Lymphocyte, an increase in the levels of these parameters was observed while for neutrophils, Eosinophils, WBC and platelets, a considerable decrease was observed. In general, 0.0625g/kg of the extract seem to exert the highest effect than other administered dose.

CONCLUSION

From the data obtained, it can therefore be concluded that the methanolic extract of the bark of T. orientalis felt into the Fifth class of toxicity (stated to be practically non-toxic) according to Hodge and Sterner Scale of toxicity classification, hence it is safe for acute administration. Also, the methanol extract of the plant T. orientalis has haematopoietic effect on treated rats which can be said to be beneficial and hence establish the folkloric use of the plant as a blood booster.

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