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Effects of aqueous leaf extracts of *Tithonia diversifolia* and *Moringa oleifera* on haematological, biochemical and histopathological parameters in albino rats

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Africa is a vast reservoir of medicinal plants which need substantial research to understand their therapeutic potential for safe use. *Tithonia diversifolia* (TD) and *Moringa oleifera* (MO) are two such plants commonly used in South-west Nigeria for their therapeutic properties. This study therefore evaluated the effects of single and combined aqueous leaf extracts of both plants on hematological, biochemical and histopathological indices of albino rats aimed at providing baseline information on their systemic health impact when orally administered. Thirty albino rats of both sexes were allotted to 6 groups of 5 rats each and given the extracts: Group I (control) distilled water only; Groups II to VI-200 mg/kg TD; 200 mg/kg MO; 200 mg TD + 200 mg MO; 400 mg TD + 400 mg MO and 800 mg of TD + 800 mg MO respectively. These were orally administered daily for 14 days at the end of which blood samples, liver and kidney tissues were collected. Body weights were measured weekly. Results were compared with rats administered distilled water only. No significant differences (p≥ 0.05) were observed in the body weights of the rats. Alterations in the values of hematological indices were observed within the treatment groups showing significant differences (p<0.05) when compared with control. Significant differences (p< 0.05) were observed in the values of serum biochemical indices when compared with the control. However, the combination of both extracts above 200 mg/kg resulted in injury to the liver and kidney tissue indicating toxicity at high doses. This study demonstrated that 200 mg/kg of the combined leaf extracts is safe and effective for use.

Key words: Albino rats, haematology, histopathology, serum biochemistry, *Moringa oleifera*, *Tithonia diversifolia.*

INTRODUCTION

Assessment of medicinal plants and their properties by systematic screening is essential for possible toxic effects when used in therapy. These plants can be screened singly or in combinations using animal models in order to obtain a robust evaluation. One method of achieving this is by exposing laboratory animals to a range of

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concentrations of the test substance over time. The assessments help to determine the potential hazards a test substance may likely produced and the characterization of its action. (Cunny, 2004). These studies usually precede clinical trials with human subjects. Using plants for medicinal purposes indiscriminately without consideration of appropriate dosage is potentially hazardous and can make them ineffective due to factors like herb/herb, food/herb, drug/herb interactions and overdose. The information obtained when medicinal plants are screened serves as the basis for determining their safety and the dose at which they can be used therapeutically (Cunny, 2004). The laboratory animals employed in such screenings undergo regular monitoring of physiological, biochemical indices and histopathological examination during and at the end of such studies (Klaassen, 2008).

The use of plants as medicines is becoming more popular, as the campaign for consumption of natural, rather than processed foods, continues to spread; herbal concoctions are made with water. Traditionally, the use of medicinal plants involves a combination of more than one plant and may also include other materials (Funmilayo et al., 2016). This combination therapy is widely used and also recommended because it is believed to produce rapid clinical and parasitological response and may circumvent or delay resistance (Bukirwa and Orton, 2005).

Tithonia diversifolia (Hemsl. A. Gray), commonly known as Mexican Sunflower, tree marigold, shrub sunflower or Japanese sunflower “sepeleba” (Yoruba), is an annual plant that grows throughout the year. It is 2-3 m (6.6-9.8 ft.) in height with ligneous stalks in the form of woody shrubs. It can be used as chicken feed, fuel wood, soil erosion control, and building materials (Olabode et al., 2007). Having a characteristic bitter taste, it is used to induce a fever to help fight poisoning, although not used for direct medicinal purposes then but recent research has gone far in finding its usefulness as a medicinal plant.

Moringa oleifera plants is the most widely cultivated species of a monogeneric family, the Moringaceae, It is a perennial softwood tree which for centuries, has been advocated for traditional, medicinal and industrial uses. It is considered as one of the World’s most useful trees, as almost every part of the Moringa tree can be used for food, medication and industrial purposes (Khalafalla et al., 2010). Some people use its leaves, flowers and fresh pods as vegetables, while others use it as livestock feed (Anjorin et al., 2010). It is a plant with exceptional medicinal properties which is used to resolve the health care needs in several situations. It has the potential to improve nutrition and boost food security (Hsu, 2006).

T. diversifolia and *M. oleifera* are medicinal plants used both singly and in combination with other plants to treat many diseases and infections in folk medicine. There is little or no significant information reported on the efficacy, potency and effects of both plants when used in combination therapy, hence the need to test for safety. This study therefore used haematological, biochemical and histopathological investigations in albino rats to assess the effects of these plants when used singly and in combination. The systemic health impact of these extracts (used singly and in combinations) were evaluated in order to make available, information on their safety when used in therapy and also add to the overall value of the medicinal and nutritional potential of these plants.

MATERIALS AND METHODS

Plant collection and extract preparation

Fresh leaves of *T. diversifolia* and *M. oleifera* harvested by hand-plucking were used for the study between August-September which falls within the rainy season in South-west Nigeria. The plants were collected within the vicinity of Institute of Agricultural Research and Training (IAR&T) and College of Agriculture, Moor Plantation Apata, Ibadan, and was identified and authenticated by a Botanist in Department of Botany, University of Ibadan, Nigeria. The leaves were cleaned of extraneous materials and air dried for 2 weeks at room temperature and humidity of 45%. They were kept under shade with no direct contact of sunlight in order to maintain the essential components of the leaves. The leaves were spread on newspapers placed on a clean concrete floor. They were always turned-up on 2 days interval to prevent any form of contamination from the ground and access to local insects. The dried leaves were examined to exclude any form of contamination after which they were ground to particle size of 3 mm (coarse texture) using an electrical grinder. The grounded leaves were immediately soaked in 5 L of distilled water at room temperature for 72 h. They were filtered with Muslin cloth and Whatman paper No.1 in order to separate the filtrate from the debris. The filtrate was immediately poured into a conical flask and taken to Pharmacognosis Laboratory of the University of Ibadan where it was concentrated and evaporated to dryness using rotary vacuum evaporator. The dried residue obtained as the crude extract was stored in a refrigerator at 4°C until it was used.

Experimental site

The experiment was conducted at the Animal HOUSE of Institute of Agricultural Research and Training, Moor Plantation, Ibadan. The institute is located in the humid zone of the Rainforest belt 0703.25 of Oyo State, South-west of Nigeria; it lies on latitude 07°23N and longitude 03°51E with an elevation of 650 m above sea level. It has mean annual rainfall of 1220 mm, mean temperature of 26°C and relative humidity of 74%.

Experimental design and management

Thirty healthy albino rats *(Rattus norvigicus),* 8-10 weeks old of both sexes and with weight range of 130-200 g purchased from the Animal House of University College Hospital Ibadan (UCH) were used for this study. Animals were kept inside animal house of the Institute of Agricultural Research and Training, Moor Plantation Ibadan where the experiment was carried out. The experiment was a completely randomized design. Animals were randomly allocated into six treatments and housed in groups of five rats of same sex

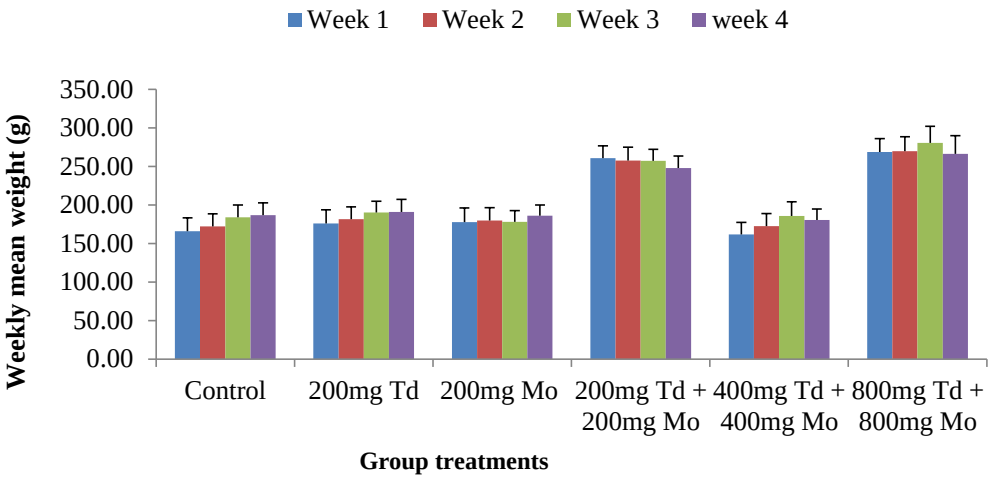


Figure 1. Observable differences in mean weekly body weight of the experimental animals among treatment groups.

per treatment based on body weights. Rats in group one was the control while those in groups 2, 3, 4, 5, and 6 were the treatments groups. They were kept in metal cages covered with wire gauze with one open side for easy access to the animal. The rats were maintained on 12 h light and dark cycle in the experimental house and were allowed to acclimatize for fourteen days (14 days) before commencement of the experiment. The rats were fed with pelletized commercial rat feed commercially obtained from Ladokun Feeds located at Mokola Market, Ibadan with clean drinking water provided ad libitum. Administration of extracts and distilled water to animals was oral (by trained personnel) for 2 weeks using oral cannula. The experiment lasted for four weeks. The rats groups with their treatments are as follows:

Group 1 (control) = Distilled water only (vehicle of the extracts)
Group 2 = 200 mg/kg of *T. diversifolia* extract only
Group 3= 200 mg/kg of *M. oleifera* extract only
Group 4 = 200 mg/kg of *T. diversifolia* + 200 mg/kg of *M. oleifera* extracts
Group 5 = 400 mg/kg of *T. diversifolia* + 400 mg/kg of *M. oleifera* extracts
Group6 = 800 mg/kg of *T. diversifolia* + 800 mg/kg of *M. Oleifera* extracts.

Extracts administration and clinical observations

0.2 ml of each concentration was administered orally to each rat once daily for 14 days; 1 ml per day of the prepared extracts of 200, 400 and 800 mg/kg was administered to each treatment group; while rats in Group 1 was administered distilled water only. The doses were calculated in their different concentrations from a stock solution of the extracts. Each dose calculated concentration level was dissolved in 14 ml of distilled water to get the supposed value needed for the 14 days administration. The animals were observed daily for physiological changes, mortality and change in behavior.

Weekly body weight

Body weight of each rat was measured using a sensitive balance scale. The measurements were taken before the commencement of extract administration, during and after the administration. Mean value of the body weight was obtained using the data collected from the weekly weight recorded. Weight gain was calculated using:

Weight gain (g) = Weight before commencement of extract administration – Weight after administration.

Statistical analysis

Data obtained were subjected to one-way ANOVA and analyzed using SPSS version 20. PostHoc was used as a multiple comparison test to identify possible differences between both haematological and biochemical values that were obtained. Significant means were separated using Duncan multiple range test of the same software. The level of significance was set at p value of less than 0.05 ($p \leq 0.05$) taken at 95% confidence interval and 5% level of probability.

RESULTS

Results from clinical observations

The animals were clinical observed daily in terms of their reactions with the extracts during and after the administration. No mortality was recorded during the experimental period. There was neither abnormal behavior nor physiological changes observed from the animals.

Results from mean weekly body weight

There was a slight observable increase of weight in all the groups between first to third weeks of extract administration while there was slight decrease a week after the end of extract administration within Groups 4, 5, and 6 given the combined therapy of the extracts (Figure 1). Body weight between the groups was however not significantly different ($p > 0.05$) (Table 1).

Table 1. Effect of the extracts on mean weekly weight of albino rats.

Variables	Group 1 (control)	Group 2 (200 mg TD)	Group 3 (200 mg MO)	Group 4 (200 mg TD & MO)	Group 5 (400 mg TD & MO)	Group 6 (800 mg TD & MO)
Week 1	165.80±17.40[a]	175.80±17.88[a]	177.80±18.12[a]	260.60±16.15[a]	161.60±15.55[a]	172.40±16.49[a]
Week 2	172.20±16.32[a]	181.40±15.95[a]	179.60±16.95[a]	257.60±17.21[a]	172.40±16.49[a]	269.60±18.76[a]
Week 3	183.80±16.25[a]	190.00±14.75[a]	179.00±14.76[a]	257.20±15.07[a]	185.80±1836[a]	280.40±21.59[a]
Week 4	186.80±15.95[a]	191.00±16.04[a]	186.00±14.02[a]	247.80±15.57[a]	180.40±14.41[a]	266.20±23.59[a]

TD-*Tithonia diversifolia;* MO-*Moringa oleifera.* Values are mean ± S.E. where S.E. stands for standard error. [a.] Means within the same column having the same superscript are not significantly different *(p >0.05).*

Table 2. Effect of orally administered *T. diversifolia* and *M. oleifera* on haematology of albino rats.

Parameter	Treatment groups					
	Group 1 (Control)	Group 2 (200 mg TD)	Group 3 (200 mg MO)	Group 4 (200 mg TD & MO)	Group 5 (400 mg TD & MO)	Group 6 (800 mg TD & MO)
PCV	42.33[a]	46.67[ab]	51.6 [bc]	54.00[c]	40.67[a]	44.33[a]
Hb	13.90[ab]	15.10 [ab]	17.07[b]	17.00[b]	13.13[a]	12.18[a]
RBC	6.95[ab]	7.80[abc]	8.73[c]	8.37[bc]	6.79[a]	7.48[abc]
WBC	2983.33[a]	4116.67[bc]	3275.00[a]	3600.00[ab]	3166.67[a]	4300.00[c]
Platelet	1257.67[a]	1347.67[a]	1275.00[a]	1425.00[a]	2620.00[b]	1553.33[a]
Lym.	67.33[a]	66.00[a]	67.67[a]	67.00[a]	67.33[a]	71.00[a]
Neu	29.33[a]	31.67[a]	30.00[a]	29.67[a]	29.61[a]	25.67[a]
Mono	1.67[a]	1.67[a]	1.67[a]	2.00[a]	2.03[a]	1.67[a]
Eos	1.67[a]	1.00[a]	1.00[a]	1.67[a]	1.03[a]	1.66[a]
MCV	61.10[a]	59.73[a]	59.23[a]	64.70[a]	60.07[a]	59.27[a]
MCH	20.07[a]	19.37[a]	19.57[a]	20.40[a]	19.40[a]	16.57[a]
MCHC	32.77[a]	32.40[a]	33.03[a]	31.47[a]	32.33[a]	27.93[a]

[a, ab, abc, bc, c.] Means along the same row with different superscript are significantly different (p< 0.05). TD-*Tithonia diversifolia;* MO-*Moringa oleifera.*

Effects of orally administered aqueous extracts of *T. diversifolia* and *M. oleifera* leave on haematological indices of albino rats

This study showed that there was no significant difference (p> 0.05) in the values of lymphocytes, neutrophil, monocytes, eosinophil, Mean cell volume (MCV), Mean cell haemoglobin (MCV) and mean cell haemoglobin concentration (MCHC) among the treatment groups when compared with control value; but significant differences (p<0.05) were observed in the pack cell volume (PCV) within Groups 2, 3, and 4 (±46.67, ±51.67, ±54.00), when compared with control group (±42.33), except in Groups 5 and 6 (±40.67 and ±44.33) where the PCV value showed no significant difference (p>0.05) . Significant differences were observed in red blood cell (RBC) values in groups 2, 3, 4, 5 and 6 (±7.80, ±8.73, ±8.37, ±6.79, ±7.48) when compared with control group (±6.95). Significant differences (p<0.05) were observed in haemoglobin (Hb) values within Groups 3, 4, 5 and 6 (±17.07, ±17.00, ±13.13, ±12.18) when compared with control group (±13.90) except in Group 2 (±15.10) where no significant difference (p>0.05) was observed. For platelets values, no significant difference (p>0.05) was observed among the groups except in Group 5, (±2620.00) when compared with the value of control group (±1257.67). Significant differences were observed in white blood cell (WBC) values within Groups 2, 4 and 6 (±4116.67, ±3600.00, ±4300.00) when compared with the control value (±2983.33) except in Groups 3 and 5 (±3275.00 and ±3166.67) where there was no significant difference compared to control group (Table 2).

Effects of orally administered aqueous extracts of *T. diversifolia* and *M. oleifera* leaves on serum biochemical indices of albino rats

The results of serum biochemical indices of albino rats obtained in this study are presented in Table 3. It showed

Table 3. Effect of orally administered *T. diversifolia* and *M. oleifera* on serum biochemical indices of albino rats.

Treatments	Parameter								
	Total protein	Alb	Glob	A-G ratio	AST	ALT	ALP	Creatinine	Glucose
Group 1(contro)	6.20^{ab}	2.63^{ab}	3.57^{a}	0.70^{a}	44.33^{a}	32.33^{a}	113.33^{a}	0.67^{a}	107.33^{ab}
Group 2(200 mg TD)	7.47^{c}	3.37^{c}	4.30^{b}	0.77^{a}	46.67^{a}	33.33^{a}	116.00^{ab}	0.73^{a}	106.00^{ab}
Group 3(200 mg MO)	5.77^{a}	2.37^{a}	3.40^{a}	0.70^{a}	43.00^{a}	31.00^{a}	126.00^{c}	0.57^{a}	108.67^{ab}
Group 4 (200 mg of TD & MO)	7.17^{bc}	3.00^{ab}	4.17^{b}	0.70^{a}	42.67^{a}	31.67^{a}	114.00^{ab}	0.70^{a}	89.67^{a}
Group 5 (400 mg of TD & MO)	7.03^{bc}	2.90^{ab}	4.13^{b}	0.67^{a}	44.67^{a}	32.67^{a}	104.67^{a}	0.63^{a}	112.33^{bc}
Group 6 (800 mg of TD & MO)	6.93^{bc}	3.00^{ab}	3.93^{ab}	0.73^{a}	43.33^{a}	31.33^{a}	109.00^{a}	0.63^{a}	126.00^{c}

$^{a, b, ab, bc, c.}$ Mean within the same column having different superscript are significantly different ($p< 0.05$).

(Histology of Liver)

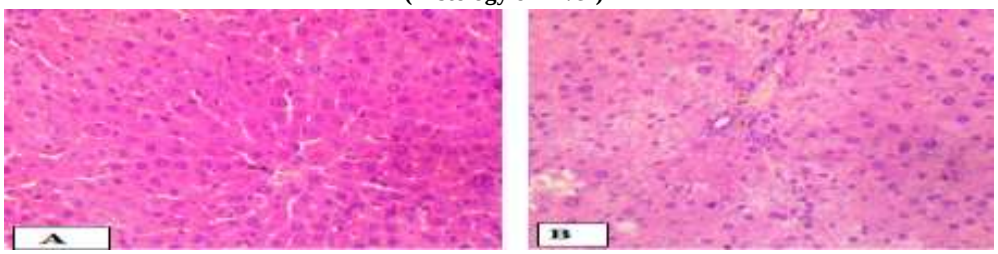
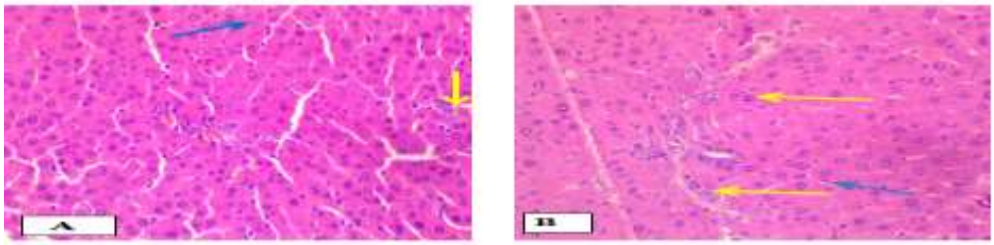


Plate 1. (A) Hepatic plates are closely-packed, (**B)** No visible lesion.



Plates 2. (A) Hepatic plates are closely-packed. There is mild bile ductular hyperplasia at portal tracts (yellow arrows).There is mild KCH (blue arrows), **(B)** Hepatic plates are closely-packed. There is moderate bile ductular hyperplasia (yellow arrow). There is mild Kupffer Cells (KCH) (blue arrows).

that there was no significant difference ($p>0.05$) in some serum biochemical indices like Aspartate amino-transferase (AST), Alanine Aminotransferase (ALT), Creatinine and Albumin-Globulin ratio (AG Ratio), but there were significant differences ($p<0.05$) in total protein value within Groups 2, 3, 4, 5 and 6 (±7.47, ±5.77, ±7.17, ±7.03, ±6.93) when compared with the control group (±6.20). In albumin value, significant differences were observed in the treatment Groups of 2 and 3 (±3.37, ±2.37), when compared with control group (±2.63).

Significant differences were observed in glucose level of groups 4, 5 and 6 (±89.67, ±112.33, ±126.00) when compared with the value of control group (±107.33). The values obtained in globulin showed significant differences in Groups 2, 4, 5 and 6 (±4.30, ±4.17, ±4.13, ±3.93), when compared with the value obtained in control group (±3.57). The mean result obtained in Alkaline phosphatase (ALP), showed significant differences in Groups 2, 3 and 4 (±116.00, ±126.00, ±114.00), when compared with the value obtained in control group (±113.33).

Effects of orally administered aqueous *T. diversifolia* and *M. oleifera* on histological sections of liver and kidney of albino rats

The histological profiles of the liver section from rats in different treatment groups are presented in Plates 1 to 6. From the results, the analyzed tissues especially from

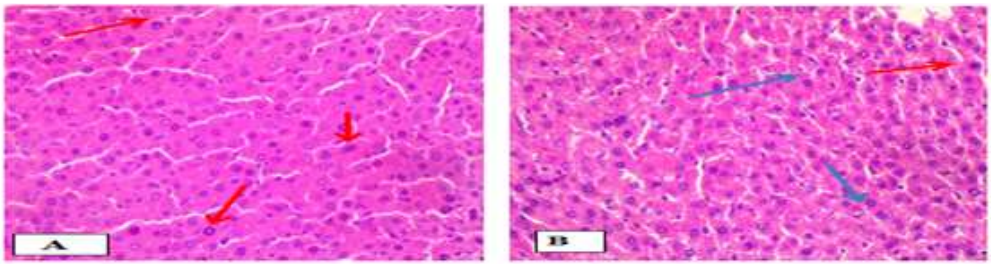


Plate 3. (A) Hepatic plates are closely-packed. There are a few megalocytes (red arrows). **(B)** There are a few megalocytes (red arrows). There is mild KCH (blue arrows).

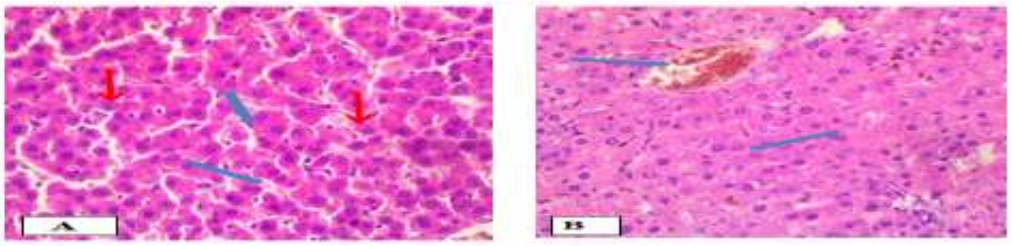


Plate 4. (A) There are random foci of single-cell hepatocellular necrosis (red arrows). There is moderate KCH (blue arrows), **(B)** Hepatic plates are closely-packed. There is moderate congestion of blood vessel (thick arrow) there is mild KCH (blue arrows).

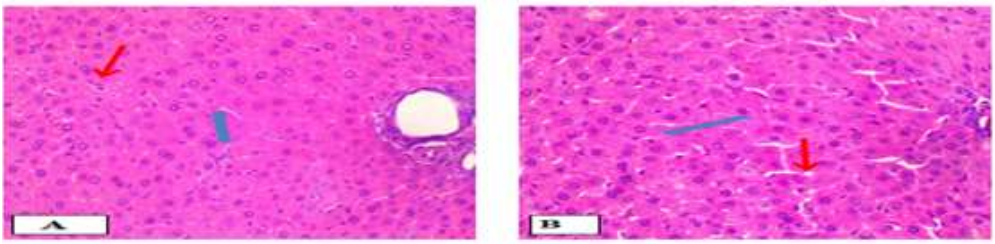


Plate 5. (A) Hepatic plates are closely-packed. There are a few foci of single-cell hepatocellular necrosis (red arrows).There is moderate KCH (blue arrow), **(B)** Hepatic plates are closely-packed. There are a few foci of single-cell hepatocellular necrosis (red arrows) and mild KCH (blue arrow).

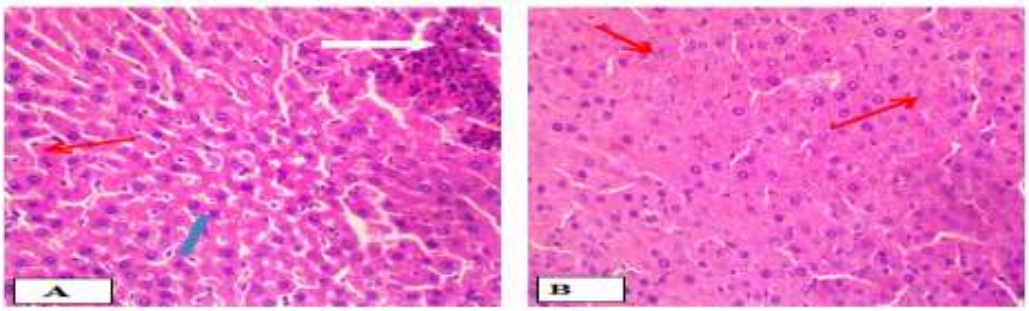


Plate 6. (A) There are a few foci of mild coagulation necrosis of hepatocytes (red arrow). There is mild KCH (blue arrows).There are a few aggregates of mononuclear inflammatory cells (thick white arrow), **(B)** Hepatic plates are closely-packed. There are scant foci of single-cell hepatocellular necrosis (red arrows).

(**Histology of Kidney)**

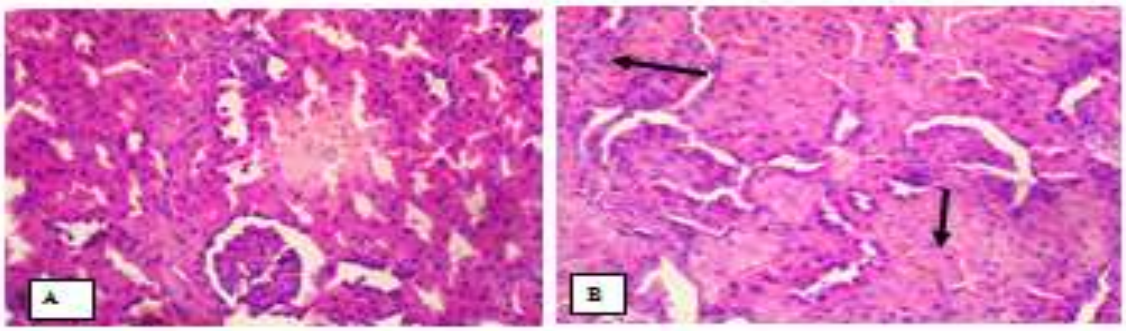


Plate 7. (A) The glomeruli and tubules appear normal, **(B)** The glomeruli and tubules appear normal. There is mild congestion of interstitial blood vessels (black arrows).

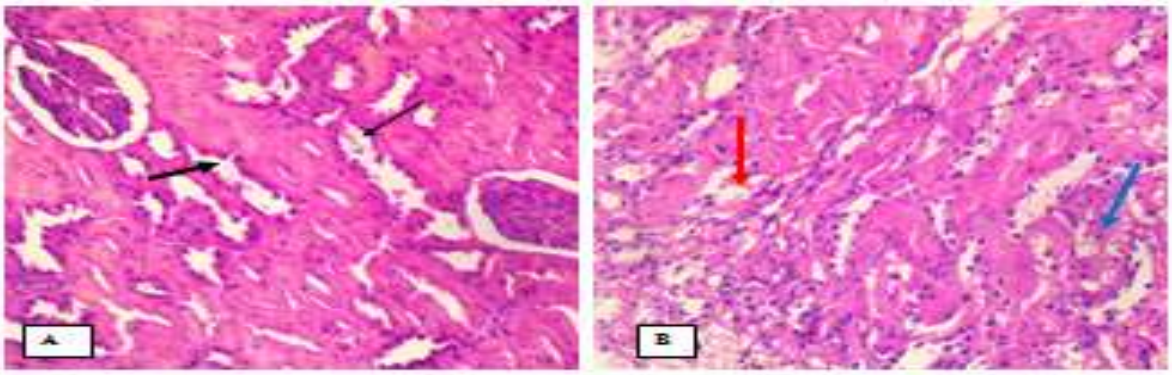


Plate 8. (A) There are multiple foci of marked flattening of tubular epithelium (arrows). The glomeruli and renal interstitium appear normal (**B**) There are locally extensive foci of moderate desquamation (red arrow) and vacuolar change (blue arrow) of tubular epithelial cells.

Groups 5 and 6 (Plates 5a -6b) showed hepatocellular necrosis of the liver while that of Group 4 administered 200 mg/kg combination of the extracts (Plates 4a-b) showed a little damage. Meanwhile rats administered 200 mg/kg alone of each extract respectively (Plates 2a-3b) when compared with that of the control group showed moderate effects of the extracts on the liver tissues.

Histological section of the kidney from selected animals in different treatment groups in this experiment is presented in Plates 7 to 12. From the kidney tissues analyzed especially from Groups 5 and 6, it is shown that the extracts had some adverse effects on the kidneys of treated animals when compared to that in control group; while that of Group 4 which was administered combined 200 mg/kg dose level of both extracts showed little adverse effect with evidence of regeneration of the tubules following acute tubular necrosis. Meanwhile that of the treatment groups administered 200 mg/kg alone of each extracts respectively show moderate effects on the kidney tissues analyzed.

DISCUSSION

The results in Table 1 suggest that exposure of the animals to aqueous leaf extracts of both plants did not change significantly ($p > 0.05$) their body weights as well as other physical and behavioral characteristics neither was there any mortality recorded. This implies that the extracts had no major adverse effects on the metabolic activities of the treated animals.

The values obtained for pack cell volume (PCV), haemoglobin (Hb), platelets, red blood cell (RBC) and white blood cell (WBC) were significantly different ($p < 0.05$) at 200 mg/kg dose level of both extracts administered solely and in combination therapy when compared with the control group. This shows that the extracts of both plants are effective having the potency that can alter the blood profile of experimental animals. However, the result is different for that of MO as obtained from Group 3 administered 200 mg/kg of extracts alone. The result shows an increase in the above mentioned

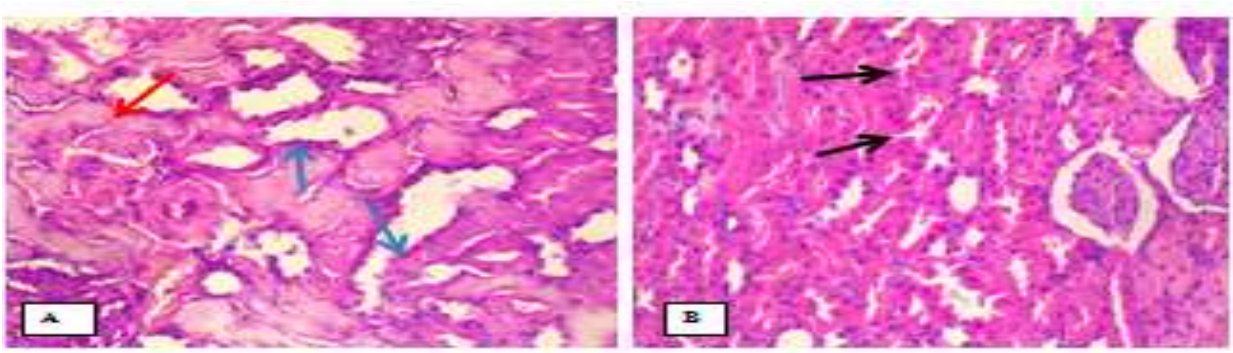


Plate 9. (A) There are multiple foci of sloughing off of tubular epithelial cells (red arrows) as well as flattening of epithelial cells (blue arrows) which may suggest some regeneration following acute tubular necrosis, (**B**) There are multiple foci of intraluminal pinkish tubular casts (arrows).

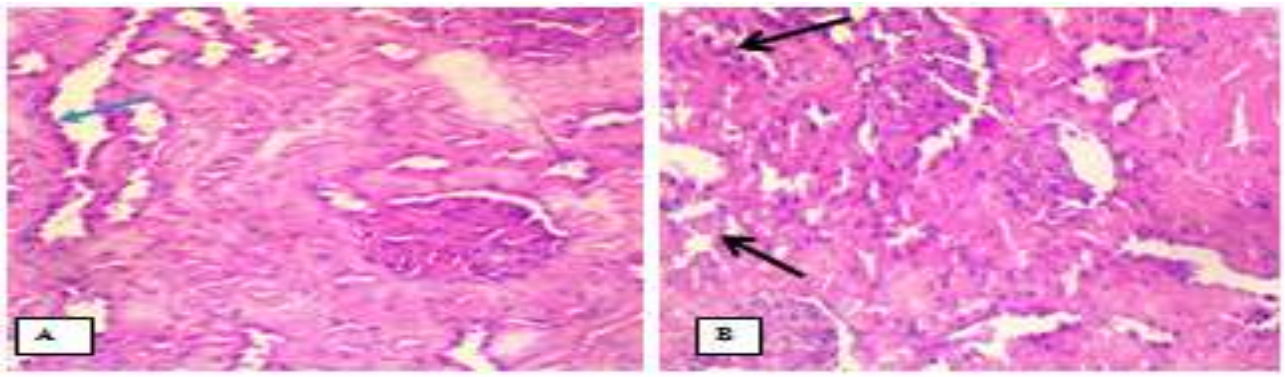


Plate 10. (A) There are a few foci of flattened tubular epithelial cells (blue arrows) which might be evidence of regenerating tubules following acute tubular necrosis, **(B**) There are a few foci of desquamation and vacuolar change of tubular epithelial cells (arrows).

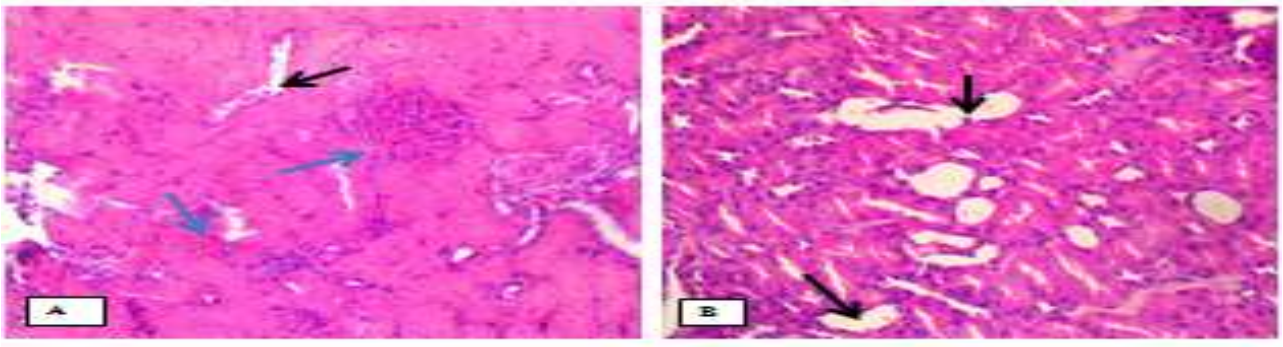


Plate 11. (A) There are a few foci of moderate sloughing off of tubular epithelial cells (black arrows). There is marked congestion of glomerular capillary tufts and interstitial blood vessels (blue arrows), (**B**) There are locally extensive foci of marked sloughing off of tubular epithelial cells. Affected tubules (black arrows) appear cystic and dilated.

haematological parameters when compared with control group. This can be attributed to the many potential nutrients contained in *Moringa* leaves as it has been found to be a good source of micronutrients and are concentrated with proteins (Busani et al., 2011). The leaves are an exceptional excellent source of β-carotene,

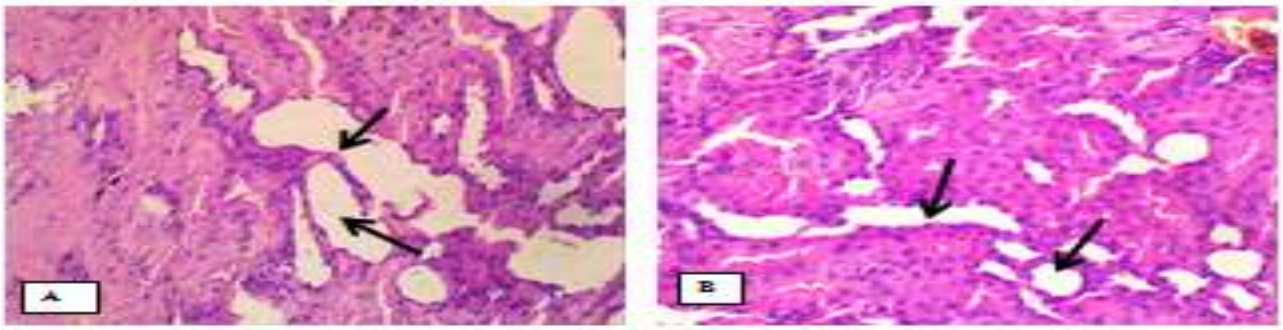


Plate 12. (A) There are locally extensive foci of severe sloughing off of tubular epithelium (blackarrows), (**B**) Affected tubules appear cystic and distended. All photomicrographs are at magnification of 400X.

vitamin C, calcium, iron, potassium, magnesium, selenium, zinc and a good balance of all the essential amino acids. Meanwhile, the combination therapy at 200 mg/kg dose level of both extracts appears to have better effect than the groups administered 400 and 800 mg/kg when compared with the control group. This shows that both extracts at higher dose than 200 mg/kg were no longer beneficial in relation to the blood profile of experimental animals.

Platelets are known to be involved in blood clotting. A significant higher value was observed ($p< 0.05$) in blood platelets count in group 5 which has the highest value when compared to control and other treatment groups. This suggests that there was no loss of blood in the case of injured tissues as a result of the plant extracts administered. This correlates with Nse-Abasi et al. (2014) who reported that low platelet concentration indicates that the process of clot formation (blood clotting) will be prolonged in the case of injury resulting in excessive loss of blood. The effects of the leaf extracts on heamatological parameters of albino rats showed a significant increase ($p <0.05$) in white blood cells (WBC) especially in treatment groups administered 200 mg/kg of *T.D* alone, 200 and 800mg/kg combination of both extracts. This shows that *T. diversifolia* contains active compounds or nutrient capable of fighting diseases and its combination with *M. oleifera* can help in balancing and maintaining the immune system of animals against diseases. This agrees with the report of Asomugha et al. (2015) who studied the impact of *M. oleifera* on blood parameters of wistar rats and found that *M. oleifera* raised the white blood cell count. White blood cells (WBC) are part of blood cells which stimulate cell mediated immunity and help B-cells to make antibodies that fight against antigens. This suggests that the plants could be good positive immunomodulators. There was no significant difference ($p >0.05$) in lymphocytes counts in all treated groups as the haematological alterations were characterised by decreased values of MCV, MCH, MCHC with non-significant differences ($p> 0.05$) in neutrophils, Eosinophils and monocytes. These effects may be related to the nutritional adequacy and immune potency attributed to both plants.

Packed cell volume (PCV) is an indicator of blood dilution and also an index of toxicity. Any reduction in its concentration in the blood usually suggests presence of toxic factors (haemaggutins) which have adverse effect on blood formation (Oyawoye and Ogunkunle, 1998). The PCV value obtained in this study from the rats administered both extracts indicates variations in the PCV when compared with the control group. This suggests that the combination of the extracts at dose level higher than 200 mg/kg is toxic to the body system of the animals.

Haemoglobin content is used to determine the oxygen carrying capacity of animal circulatory system thereby measures the ability of an animal to withstand some level of respiratory stress (Akintunde et al., 2017). Results from this study showed a confirmation of this. From the haemoglobin values obtained in this study, significant differences ($p < 0.05$) were observed in treatment groups administered 200 mg/kg of TD, 200 mg/kg of MO alone and 200 mg/kg combination of both extract respectively. The extracts of both plants are rich in essential nutrients/compounds such as iron, copper, vitamins, tagitinin C, chlorogenic acid derivatives (CAs) which are relatively known to be safe and present in some vegetables consumed in human diet. The good physiological state of the animals clinically observed during the experiment which contributed to the normal behavioral pattern of the rats used in this study, can therefore be attributed to the positive effects of the extracts.

Values of RBC obtained from animals in the treatment groups showed significant difference ($p < 0.05$) when compared to that obtained in control group. This shows that the animals administered the extracts at 200 mg/kg dose level may not be prone to anemia; meanwhile animals administered 200 mg/kg of *M.O* alone had the highest value of RBC. Good value of red blood cell indicates nutritional adequacy of a test substance and its safety which signifies erythrocyte production and oxygen

utilization (Akintunde et al., 2017); hence *M. oleifera* at dose level of 200 mg/kg is safe for the body system. Results from this study as obtained in Group 3 suggest that MO in combination with *TD* at the same dose level of 200 mg/kg has a positive impact in balancing and maintaining the body system since high concentration of RBC and haemoglobin (Hb) is said to show the absence of toxic factors such as haemoglutinin which normally have adverse effect on blood formation (Akinmutimi, 2004).

The effect of *M. oleifera* and *T. diversifolia* leaf extracts on selected enzymes (Table 3) shows an enhancement in the activities of alkaline phosphatase, albumin, globulin, glucose and total protein, indicating that the combination of both plants may have the capacity to enhance the proper functioning of the liver, kidney and hepatobiliary activity. These views are not at variance with the report of Ibeh (1992) with respect to the functions of plasma enzymes.

Creatinine is a measure of waste product from muscle phosphocreatin, an energy rich compound generated from muscle metabolism (Polat et al., 2011) produced at a fairly constant rate by the body depending on muscle mass (Aguihe et al., 2014). Values of creatinine obtained from the treatment groups when compared to that of control were not significantly different ($p > 0.05$) with the lowest value obtained in group 3 rats. This reveals inconsequential muscular wastage which indicates no obvious organ damage.

AST, ALT, A-G ratio values were not significantly ($p>0.05$) affected by the extracts when compared to the values of control group, indicating that the liver cell membrane was protected. This prevented the leakage of cellular enzymes into the blood circulation of experimental rats. Clinical surveys and animal model experiments (Adedara et al., 2014; Eidi et al., 2006) revealed that raised levels of ALT and AST activities are indicative of organ damage; specifically, in pathologic and toxicological events leading to cardiac and hepatic necrosis. In this study with administration of aqueous extracts of both TD and MO, no increase was observed in ALT and AST levels rather there was a decrease in values of AST and ALT. This signifies no organ damage but an injury as observed from the group of albino rats administered high doses of the extracts in combination therapy (Plates 5a-6b). The values obtained are in comparison with the reference ranges of serum biochemical indices by Sharp and La Regina (1998). The non-significant ($p>0.05$) variations of these values in all the treatment group could be due to the presence of essential minerals, vitamins and amino acids present in both extracts. The trend supports the reports of Pierre (2015) who opined that *M. oleifera* leaves contain proteins, vitamins, beta-carotene and amino acids, important phytochemicals, such as gallic tannins, steroids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars; they can only cause adverse effects in rats when taken in large quantities over varying periods of time, thereby having the possibility of causing organ toxicity.

Glucose level is related to the energy usage by experimental animals hence the increase and decrease in the glucose level of albino rats at high dose of both extracts 800 mg/kg (*TD/MO*) and 200 mg/kg of *M. oleifera* alone. This may be due to differences in energy expenditure because of certain factors which might be attributed to the sex of animals since rats in group 4 are females while that of group 6 are males. This agrees with the report of Mary et al. (2008) on clinical laboratory parameters of different glucose levels obtained from male and female Wistar rats.

ALP is one of the liver enzymes. Elevated ALP results from increased osteoblastic activity including traumatic, neoplastic and infectious disease (Kendel, 2006). The significant difference ($p < 0.05$) observed in Alkaline phosphatase value within the treatment groups when compared with the control value agrees with the research work of Adebayo et al. (2010) who tested an aqueous extract of *T.D* via oral administration in Wistar rats at 100 and 200 mg/kg for seven days. From the study, the extract caused no alterations at 100 mg/kg in the haematological and biochemical parameters analysed while at 200 mg/kg, the alkaline phosphatase levels increased in the liver and heart demonstrating toxicity of the aqueous extract of *T. diversifolia.* However from this study, combination of *T. diversifolia* with *M. oleifera* leaf extracts at 200 mg/kg showed a significant difference, suggesting that both plants can protect and maintain the health of vital organs such as the liver and bone by supplementing each other with their respective nutrients.

Results from Table 3 show a significant difference ($p<0.05$) in total protein and albumin values. There was a decrease of value in total protein and albumin of treated animals administered 200 mg/kg of *MO* alone while an increase in value was observed in treatment animals administered 200 mg/kg of *TD* alone when compared with the control. This indicates the availability of proteins for utilization and a non- disturbance in the osmotic balance of plasma and tissue fluid. However, the high level of albumin and total protein recorded in Group 2 showed increased protein levels and mild changes in the liver and kidney tissues. This Increased value resulted in the effects observed in the liver and kidney sections of Group 2 animals (Plates 2a-b) and (8a-b) showing multiple foci of marked flattening of tubular epithelium though glomeruli and renal interstitium appeared normal. There was also locally extensive foci of moderate desquamation and vacuolar change of tubular epithelial cells (Plate 8a-b); while mild bile ductular hyperplasia at portal tracts was observed with mild KCH in the liver; also hepatic plates were seen closely-packed with moderate bile ductular hyperplasia (Plate 2a-b). This agrees with the work of Fankule and Abatan (2007).

Globulin is a measure of immune resistance to

infections and high globulin levels are mostly due to production of antibodies to fight infections (Agboola et al., 2013). Significant differences ($p<0.05$) were observed in globulin values obtained from the treatment groups when compared to control group with highest value obtained in rats administered 200 mg/kg of *T. diversifolia* alone; while the lowest value was recorded in rats administered 200 mg/kg of *M. oleifera* alone. This indicates that TD has high potency in production of antibodies to fight infections. This agrees with the work of Agboola et al. (2013) on serum biochemistry and hematological indices of broiler chickens. Meanwhile the combination therapy of both extracts at increasing dose level showed significant difference ($p<0.05$) when compared with the control group. This suggests that both extracts at increasing dose level from 200 mg/kg possess a positive effect which might be attributed to the respective functions of both plant extracts in trying to balance the body system against invading diseases and infections.

Research studies of Elagib et al. (2012) and Martin (2011) reported that increase in serum Alkaline phosphatase (ALP) which is one of the liver enzymes used to detect bone and liver health indicates a metabolic change in the liver developed during administration of toxin; therefore, the alterations in liver and kidney tissues observed from the treated groups administered 400 and 800mg/kg combination therapy of both extracts (Plates 11a-12b) showed significant injury of kidney tissues analyzed, a sign of non-safety of both extracts at high dose.

T. diversifolia extract alone at dose level of 200 mg/kg was found to cause mild and moderate bile ductular hyperplasia in the liver (Plates 2a-2b) and moderate desquamation and vacuolar change of tubular epithelial cells of the kidney (8a-8b) but a recovery or regeneration of injured tissues occurred as a result of its combination with *M. oleifera* extracts (4a-b). This shows a positive impact of *Moringa* extracts on histopathology of the kidney and liver of rats, indicating their anti-inflammatory activities. Therefore the use of these plant extracts in combination therapy should be considered but thorough consideration should be given to their use at dose level not higher than 200 mg/kg. It was observed that the combination therapy of both extracts gave the mildest or highest adverse effect on the kidney and liver especially at dose level above 200 mg/kg' it shows that they are capable of causing damage or injury to the vital organs such as the liver and the kidney of mammalian species.

Conclusion

This research has shown that the combination of aqueous extracts of *T. diversifolia and M. oleifera* is safe at dose level not exceeding 100-200 mg/kg of body weight for the period of 14 days administration used in this study. They were effective in boosting the immune system of the experimental rats and there was no record of mortality or change in metabolic activities of the animals used. Therefore the plants could be positive immune-modulators, enhancers of glucose metabolism and can help in the proper functioning of the liver and kidney by improving hepatobiliary and anti-inflammatory activities at dose level not higher than 200 mg/kg of body weight. Therefore, aqueous leaf extracts *of* both plants when used in therapy should not exceed dose level of 100-200 mg/kg and only for a short period of time. However, more studies are needed to properly evaluate if the extracts in combination at dose level higher than 200 mg/kg will be toxic or safe using shorter term study protocol like 7 days administration.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Adedara IA, Abolaji AO, Odion BE, Okwudi IJ, Omoloja AA (2014). Impairment of hepatic and renal functions by 2,5-hexanedione is accompanied by oxidative stress in rats. Journal of Toxicology Volume 2014 |Article ID 239240, 9 p. https://doi.org/10.1155/2014/239240.

Adebayo AH, Abolaji AO, Opata TK, Adegbenro IK (2010). Effects of ethanolic leaf extract of Chrysophyllum albidum G. on biochemical and haematological parameters of albino Wistar rats. African Journal of Biotechnology 9:2145-2150.

Agboola AF, Ajayi HI, Ogunbode SM, Majolagbe OH, Adenekun OO, Oguntayo CT, Opaleye RO (2013). Serum biochemistry and haematological indices of broiler chickens fed graded levels of frog (*Rana esculata*) meal as replacement to fish meal. International Journal of Agriculture and Biosciences 2(5):260-265.

Aguihe PC, Kehinde AS, Fatokun BO, Omotugba SK, Ashifat AA (2014). Effect of enzyme supplementation on haematology and Serum biochemistry of broiler finishers fed cassava peel meal based diets. Tropical Animal Production Investment 17(1):47-51.

Akinmutimi AH (2004). Evaluation of sword bean (*Canavalia gladiata*) as an alternative feed resources for broiler chickens. Ph.D. Thesis, College of Animal Science, Micheal Okpara University of Agriculture, Umudike, Nigeria.

Akintunde AR, Omage JJ, Bawa GS, Onimisi PA, Samuel I (2017). Haematological parameters and serum biochemistry of Japanese quail chicks (*Coturnixcoturnix japonica*) fed raw and processed pigeon pea (*Cajanus cajan*) seed meal based diets. Nigerian Journal of Animal Production 44:89-96.

Anjorin TS, Ikokoh P, Okolo S (2010). Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria. International Journal of Agricultural Biology 12:431-434.

Asomugha AL, Ezejindu DN, Asomugha RN, Anyabolu AE, Ojukwu PC (2015). Evaluation of toxicity effect of graded doses of *Moringa oleifera* leaf extract on blood indices using 20 adult Wistar rats. International Journal of Biomedical and Advance Research 6(2):

98-102.
Bukirwa H, Orton L (2005). Artesunate plus mefloquine versus mefloquine for treating uncomplicated malaria. Cochrane Database of Systematic Reviews 4:CD004531.
Busani Moyo, Patrick JM, Arnold H, Voster M (2011). Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. African Journal of Biotechnology 10 (60):12925-12933.
Cunny HE (2004). Toxicity testing. In: Hodgson E, (ed). A textbook on modern toxicology. 3rd edition. A John Wiley & Sons. Inc. Publication pp. 353-384.
Eidi A, Eidi M, Esmaeili E (2006). Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats. Phytomedicine 13: 624-629.
Elagib HAA, Nabiela EM, Abbass SA, Ginawi TAN (2012). Effects of natural spices on plasma proteins in broiler chicks. Journal of Nutrition and Food Science 2:152,
Fakule JO, Abatan M (2007). The toxicological effects of aqueous leaf extract of *Tithonia diversifolia* Gray in Rats. Journal of Animal and Veterinary Advances 6(10):1223-1226.
Funmilayo IA, Afolayan OM, Adegbolagun BI, Lucky K, Jennifer O, Chiaka A (2016). Antimalarial actions of *Lawsonia inermis*, *Tithonia diversifolia* and *Chromolaena odorata* in combination. Journal of Ethnopharmacology 191:188-194.
Hsu R (2006). *Moringa oleifera* medicinal and Economic uses. International Course on Economic Botany, National Herbarium, Leiden, The Netherlands.
Ibeh IN (1992). The Response of the Mammalian Reproductive System to Dietary Exposure to Aflatoxin. University of Benin, Benin City, Nigeria.
Kendel EH (2006). Diagonistic value of biochemistry. Clinical Avian Medicine 11:611-630.
Khalafalla MM, Abdellatef E, Dafalla HM, Nassrallah AA, Aboul-Enein KM, Lightfoot DA, El-Deeb FE, El-Shemy HA (2010). Active principle from *Moringa oleifera* Lam Leaves effective against two leukemias and a hepatocarcinoma. African Journal of Biotechnology 9(49):8467-8471.
Klaassen CS (2008). Principle of toxicology and treatment of poisoning. In: Parker BK, Blumenthal D, Buxton L (eds.). Goodman & Gilman's; Manual of Pharmacology and Therapeutics, McGraw Hill, pp. 1115-1119.
Martin P (2011). Approach to the patient with liver disease. In Goldman L.S. Chafer A.I. Edns Cecil Medicine, 24th ed. Philadelphia, Pa: Saunders Elsevier Chapter 148.
Mary LA, Gikinis MLA, Charles B, Clifford DVM (2008). Clinical laboratory parameters for Crl: WI (Han) Charles River Laboratories.
Nse-Abasi NE, Williams ME, Akpabio U, Edem EAO (2014). Hematological parameters and factors affecting their values. Agricultural Science 2(1):37-47.
Olabode OS, Sola O, Akanbi WB, Adesina GO, Babajide PA (2007). Evaluation of *Tithonia diversifolia* (hemsl.) A gray for soil improvement. World Journal of Agricultural Sciences 3(4):503-507.
Oyawoye FO, Ogunkule M (1998). Physiological and biochemical effects of raw jack beans on broilers. In Proceedings of Animal Conference of Nigerian Society for Animal Production, Abeokuta, Nigeria. pp. 141-142.
Pierre L (2015). Is Moringa bad for malaria. Malaria World Journal 20:26. The World's Scientific and Social Network for Malaria Professionals. https://malariaworld.org
Polat U, Yesilbag D, Eren M (2011). Serum biochemical profile of broiler chicken fed diets containing Rosemary and Rosemary volatile oil. Journal of Biology and Environmental Science 5(13):23-30.
Sharp PE, La Regina MC (eds) (1998). The laboratory rats. CRC Press, New York.