Toxicity Studies of Moringa oleifera, Ocimum gratissimum and Vernonia amygdalina in albino rats

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Abstract

Ocimum gratissimum, Vernonia amygdalina and *Moringa oleifera* are leaves consumed by many families over varying periods of time. This study investigated the toxicity of aqueous extracts of *Ocimum gratissimum, Vernonia amygdalina, Moringa oleifera* leaves in albino rats. Forty-five albino rats were used for study. The animals were divided into nine groups A-I. Groups A-H were the experimental groups which received doses of the plant extracts at 150, 300, 500, 750 and 1200mg/kg singly and in combination while I served as control. The body weight of the animals was determined in the of course the experiment and effects of the extract were evaluated on the liver and blood parameters. The extracts significantly increased ($p<0.05$) the body weight of the test animals at varying doses than the control. The packed cell volume, haemoglobin and red blood cells significantly increased ($p<0.05$) in all the test groups except in 300mg/kg of the combined extract. Also, the levels of alanine aminotransferase (ALT) and aspartate aminotransaminase (AST) were higher in all the test groups when compared to the control group. The increase in packed cell volume, haemoglobin and red blood cells following the administration of leaves extract suggested that the plants had haemopoietic effect with *Ocimum gratissimum* having the highest. The liver enzyme assays also corroborated the safety of the plants as ALT and AST were within the standard values expected in healthy rats.

Keywords: *Ocimum gratissimum, Vernonia amygdalina, Moringa oleifera,* toxicity

INTRODUCTION

Herbal medicine has received much attention these days and these plants contain a variety of chemical substances with varied physiological effects (Lewis and Elvin-lewis, 1995). Acute toxicity as the adverse effect occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24 hrs, and subacute toxicity as the advance effects occurring as result of the repeated daily oral dosing of a chemical to experimental animal for part not exceeding 10% of the life span (OECD,2000).

The use of medicinal plants in most developing nations has attracted more concerns among health workers. Plants have taken the place of synthetic drugs as 80% of world's populations

rely on herbal remedies as primary source of medicinal agents for the treatment of diseases (Getie *et al.,* 2010).

Moringa oleifera, commonly known as "Horse radish or Drum stick tree" belongs to the family Moringaceae. It is an aboriginal of India subcontinent and has become naturalized in the tropical and subtropical areas of the world (Anwar *et al*.,2005). Various properties attributed to Moringa include; anti-spasmodic, diuretic, expectorant and abortifacent (Nadkarni, 2009). In Nigeria, this plant is seen as a curative agent to virtually all ailments among populace (Peltzer *et al*., 2008). *Moringa oleifera* leaves ether, ethanol and aqeous extracts contain profile of important phytochemicals (Kasolo *et al*., 2010, Kasolo *et al.,* 2011) and ingestion of nutrients and phytochemicals in large amounts over a period of time may cause animal toxicity (Kasolo *et al;* 2012).

Ocimum gratissimum, commonly known as African basil, sweet basil or scent leaf is a plant belonging to the Lamiceae family. It is found in Asia and West Africa Nigeria. *Ocimum gratissimum* is regularly used as food spices and condiment in most part of the world (Ezekwesilli *et al.,* 2004). The herb has been recommended for the treatment of various ailment and diseases (Chiu *et al*., 2012). Traditionally, it is used in the treatment of headache, diarrhea, wart, worms and kidney fraction (Simon and James, 2007). Aqueous extract of *Ocimum gratissimum* posses anti-hyperglycemic effect and therapeutic potentials on hepatic disorders (Lee *et al.*, 2011).

Vernonia amygdalina, a member of Asteracea is small shrub that grows in the tropical Africa. It is commonly known as bitter leaf Commonly known as bitter leaf with a characteristics odour produces a wide variety of flavonoids and bitter sesquiterpere lactones which contributes to the bioactivities of the plant (Favi *et al*., 2008). *Vernonia amygdalina* is used for a wide range of ailments such as ethnotherapy of asthma, malaria, fever, constipation, worm remover, measles, diarrhea and tuberculosis (Chukwuma, 2012). *Moringa oleifera, Ocmium gratissimum* and *Vernonia amygdalina* have been used traditionally by many africans for some ailments without giving serious consideration to their toxic potential or adverse effect. Hence, this study investigates the toxicity of these plants to determine tolerable dose for usage.

2. MATERIALS ANS METHODS

Plant materials

Fresh leaves of *Moringa oleifera, Ocimum gratissimum, Vernonia amygdalina* were collected separately at Irewon, Ijebu-ode, Ogun State and authenticated by a botanist in the Department of Biological Sciences, Tai Solarin University of Education, Ogun State.

Preparation of the plant materials

Fresh leaves of *Moringa oleifera, Ocimum gratissimum, Vernonia amygdalina* were washed with tap water and dried under shade and ground using electric blender. The dried leaves were milled to powdery form. 200g of each of the resulting powder was macerated in 2litres of distilled water for 48hours with intermittent stirring. The plant materials were filtered using clean muslin cloth and the filtrates were further filtered using Whatmann filter paper no 1. The filtrates were concentrated at 50^{o}c. desired yield of extracts was subsequently reconstituted in distilled water to form the stock solutions for the experiments.

Experimental animals

Forty-five adult albino male rats were obtained from animal facility of Faculty of Veterinary Medicine, University of Ibadan. They were kept and acclimatized in the laboratory of the Department of Biological Sciences, Tai Solarin University of Education, Ogun State before commencement of the experiment. The animals were divided into groups consisting of three animals each.

Experimental design

Forty-five albino rats were divided into nine groups which were further divided into sub-groups.

Group A- divided into A_1, A_2 and A_3. *Moringa oleifera* was administered at 150mg/kg, 300mg/kg and 500mg/kg respectively.

Group B- divided into B_1, B_2 and B_3. *Vernonia amygdalina* was administered at 150mg/kg, 300mg/kg and 500mg/kg respectively.

Group C- divided into C_1, C_2 and C_3. *Ocimum gratissimum* was administered at 150mg/kg, 300mg/kg and 500mg/kg respectively.

Group D- administered combined extracts *Moringa oleifera* and *Vernonia amydalina* at 300mg/kg

Group E- administered combined extracts *Moringa oleifera* and *Ocimum gratissmum* at 300mg/kg

Group F- administered combined extracts *Vernonia amygdalina* and *Ocimum gratissmum* at 300mg/kg

Group G- administered combined extracts *Moringa oleifera Ocimum gratissmum* and *Vernonia amygdalina* at 750mg/kg.

Group H- administered combined extracts *Moringa oleifera Ocimum gratissmum* and *Vernonia amygdalina* at 1200mg/kg respectively.

Group I- administered distilled water only.

Haematology

Blood samples were collected from the animals through cardiac puncture and analyzed using standard methods for Packed Cell Volume (PCV), Haemoglobin (Hb), White Blood Cell count (WBC). Total white blood cell counts were determined by the haemocytometer methods. The packed cell volumes were determined by the micro haematocrit method while hemoglobin (Hb) concentrations were determined by the cyanomethaemoglobin method. (Thrall and Weiser, (2002).

Enzyme assay

The activities of alkaline phosphatase (ALP) was determined, using the method of Bergemeyer and Brent (1974). The spectrophotometric methods described by Kings (1960) and Pavia *et al.,* (2003) were used for the determination of ALT and AST.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) software version 16.0 was used for data analysis. The results were expressed as Mean $\pm$ SEM. The statistical difference between groups was performed using one way analysis of variance (ANOVA) followed by Duncan multiple range test. Values of $p<0.05$ were considered significant.

RESULTS

Table 1 shows weight of experimental animals at 150mg/kg of the extract. At 150mg/kg, there was significant increase in the weight of animals administered single extract of the two plants except in *V. amygdalina.*.

Table 1: Weight of experimental animals at 150mg/kg of the extract

Groups	Day 1(PA)	Day 2(PA)	Day 3(PA)
O. gratissimum	160.00 ±1.00^{b}	164.00± 1.00^{b}	163.50 ± 4.50 ^{b}
M. oleifera	102.00 ± 2.57 ^{a}	108.33 ±3.17^{a}	109.33 ± 3.84^{a}
V. amygdalina	194.33 ± 2.33^{c}	193.67 ± 1.85^{c}	191.00 ± 4.00^{c}
Control	92.33 ± 7.17^{a}	104.00 ± 3.05^{a}	112.33 ± 3.38^{a}

Values are expressed as mean ± standard error of three replicate. Values in the same column followed by the same superscript are not significantly different ($p<0.05$).

Table 2 shows the weight of experimental animals at 300mg/kg. The weight of the animals increased significantly after the administration of single extract of *Moringa oleifera, Vernonia amygdalina and Ocimum gratissimum* when compared to control. However, the weight of administered combined extract of *Moringa oleifera* and *Vernonia amygdalina* reduced on day three.

Table2: Weight of the experimental animals after extract administration at 300mg/kg

Groups	Day 1(PA)	Day 2(PA)	Day 3(PA)
M. oleifera	147.33 ± 3.71^{c}	150.00 ± 4.04^{c}	150.00 ± 2.84^{b}
O. gratissimum	178.00± 4.61^{d}	181.67± 4.40^{d}	183.00 ± 5.29^{c}
V. amygdalina	111.67 ± 1.66^{b}	118.00 ± 1.52^{b}	119.33 ± 5.50^{a}
M. oleifera + V. amygdalina	202. 66 ± 1.76^{e}	203.00 ± 1.15^{e}	177.62 ± 6.35^{c}
V.amygdalina + O.gratissimum	163.00 ± 2.00^{d}	162.67 ± 3.66^{d}	169.00 ± 1.52^{c}
M.oleifera +O.gratissimum	145.00 ± 5.00 ^{c}	145.00 ± 3.84^{c}	146.00 ± 5.50^{b}
Control	92.33 ± 7.17^{a}	104.00 ± 3.05^{a}	112.33 ± 3.38^{a}

Values are expressed as mean ± standard error of three replicate. Values in the same column followed by the same superscript are not significantly different ($p<0.05$) ***PA-Post extract administration.**

Table 3 shows weight of experimental animals at 500mg/kg of the extract. At 500mg/kg, there was significant increase in the weight of animals administered single extract of the three plants when compared to control.

Table3: Weight of the experimental animals after extract administration at 500mg/kg

Groups	Day 1(PA)	Day 2(PA)	Day 3(PA)
O. gratissimum	147.33 ± 6.76[c]	152.33 ± 7.88[c]	152.00 ± 7.50[b]
M. oleifera	154.33 ± 0.33[c]	162.20 ± 6.32[c]	167.33 ± 9.93[c]
V. amygdalina	125.33 ± 8.74[b]	129.67 ± 5.23[b]	133.67 ± 4.09[b]
Control	92.33 ± 7.17[a]	104.00 ± 3.05[a]	112.33 ± 3.38[a]

Values are expressed as mean ± standard error of three replicate. Values in the same column followed by the same superscript are not significantly different ($p<0.05$). ***PA-Post extracts administration.**

Table 4 shows weight of the experimental animals at 750mg/ kg and 1200mg/ kg. The result showed there was significant increase in the body weight of rats at both 750mg/kg and 1200mg/kg throughout the experiment.

Table 4: Weight of the animals after administration of extract 750 mg/kg and 1200mg/kg

Groups	Day 1(PA)	Day 2(PA)	Day 3(PA)
M.oleifera+ *V.amygdalina* + *O.gratissimum* at 750mg/kg	154.33 ± 0.88[b]	157.33± 2.66[b]	158.67 ± 2.33[b]
M.oleifera+ *V.amygdalina*+ *O.gratissimum* at 1200mg/kg	145.00 ± 2.88[b]	152.67± 3.17[b]	196.67 ± 3.84[c]
Control	92.33 ± 7.17[a]	104.00± 3.05[a]	112.33 ± 3.38[a]

Values are expressed as mean ± standard error of three replicate. Values in the same column followed by the same superscript are not significantly different ($p<0.05$). PCV -Packed Cell Volume, RBC - Red Blood Cell Count, WBC - White Blood Cell Count, Hb-Haemoglobin

***PA-Post extract administration.**

Table 5 shows the haematology of experimental animals at 150 mg/kg. At 150mg/kg there was significant increase in packed cell volume, haemoglobin, and red blood cell count of the single extracts of *Moringa oleifera, Vernonia amygdalina* and *Ocimum gratissimum* when compared to control. At 150mg/kg, the highest packed cell volume was observed in *Vernonia amygdalina*. The white blood cell count of the single extract also increased in *Vernonia amygdalina* and *Moringa oleifera* except in *Ocimum gratissimum* when compared to control. Also, the highest platelet was recorded in *Moringa oleifera.*

Table 5: Haematology of the experimental animals at 150mg/kg of the extract

Groups	PCV (%)	Hb g/dl	RBC $x10^6$mm	WBC $x10^3$mm	PLATELET $x10^3$µl
O. gratissimum	40.00 ± 0.57 ^{bc}	13.16 ± 0.52 ^{bc}	6.37 ± 0.22 ^{ab}	6533.33 ± 130.17 ^{a}	127166 ± 440.95 ^{ab}
M. oleifera	38.00 ± 2.88 ^{ab}	12.53 ± 0.98^{ab}	6.46 ± 1.02 ^{ab}	11583.3 ± 440.95 ^{ab}	242500 ± 3752.77 ^{c}
V. amygdalina	43.00 ± 2.64 ^{bc}	13.76 ± 0.89 ^{bc}	6.57 ± 1.04 ^{ab}	16000 ± 6088.30 ^{b}	74500.0 ± 12990.38 ^{a}
Control	36.00 ± 0.57 ^{a}	11.8 ± 0.25 ^{a}	5.73 ± 0.51 ^{a}	6916.66 ± 1044. 16^{a}	137333 ± 16696.63 ^{b}

Values are expressed as mean ± standard error of three replicate. Values in the same column followed by the same superscript are not significantly different ($p<0.05$). PCV -Packed Cell Volume, RBC - Red Blood Cell Count, WBC - White Blood Cell Count, Hb-Haemoglobin

Table 6 shows the hematology of experimental animals at 300 mg/kg in various experimental groups. There was significant increase in packed cell volume, haemoglobin and red blood cell count of single extract of *Ocimum gratissimum.* However, the packed cell volume, haemoglobin and red blood cell count decreased in combined extract of *V. amygdalina +O. gratissimum*

There was significant reduction in White blood cell count of combined extract of *Moringa oleifera* and *Vernonia amygdalina*, *Vernonia amygdalina* and *Ocimum gratissimum*, and single extract of *Vernonia amygdalina* and *Moringa oleifera.*

Table 6: Haematology of the experimental animals at 300mg/kg of the extract

GROUPS	PCV (%)	HB g/dl	RBC $x10^6$mm	WBC $x10^3$mm	PLATELET $x10^3$µl
V. amygdalina	43.00±3.21 [c]	14.40±1.03 [c]	7.37±0.53 [c]	4733.30±88.19 [a]	128000±30022.21 [ab]
M. oleifera	43.00±0.57 [c]	14.46±0.32 [c]	7.55±0.34 [c]	5473.30±846.29 [a]	107500±22227.98 [ab]
O. gratissimum	52.00±1.15 [d]	17.70±0.66 [d]	9.47±0.38 [d]	7233.30±611.91 [ab]	116333±1452.96 [ab]
M. oleifera + *V. amygdalina*	33.67 ±1.76 [ab]	5.64 ±0.32 [ab]	5.64 ±0.32 [ab]	4750.00 ±476.96 [a]	70000 ±16822.60 [a]
V. amygdalina +*O. gratissimum*	29.66±5.04 [a]	4.98±0.84 [a]	4.98±0.84 [a]	5950.00 ±1247.33[a]	126000±3.575.30 [ab]
M. oleifera + *O. gratissimum*	40.33±0.33 [bc]	6.77±0.10 [bc]	6.77±0.10 [bc]	8600.00±1039.23 [b]	161000±19052.55 [b]
Control	36.00±0.57 [abc]	11.80±0.25 [abc]	5.73±0.29 [ab]	6916.70±1044.16 [ab]	137333±16696.63 [ab]

Values are expressed as mean ± standard error of three replicate. Values in the same column followed by the same superscript are not significantly different ($p<0.05$). PCV -Packed Cell Volume, RBC - Red Blood Cell Count, WBC - White Blood Cell Count, Hb-Heamoglobin

Table 7 shows the haematology of experimental animals at 500 mg/kg. At 500mg/kg there was significant reduction in packed cell volume, haemoglobin, and red blood cell of *Moringa*

oleifera compared to control. However, packed cell volume, haemoglobin, and red blood cell increased in single extract of *Ocimum gratissimum*. Also there was reduction in platelet count at 500mg/kg when compared to the control.

Table 7: Haematology of the experimental animals at 500mg/kg

Groups	PCV (%)	HB g/dl	RBC $x10^6$mm	WBC $x10^3$mm	PLATELET $x10^3$µl
O. gratissimum	44.33 ± 2.60 [c]	15.00 ± 0.79 [c]	7.52 ± 0.81[c]	5033.33 ± 1268.31 [a]	102000 ± 25403.41 [ab]
V. amygdalina	38.33 ± 0.33 [abc]	12.7 ± 0.36 [abc]	6.47 ± 0.27 [ab]	4333.33 ± 1084.87 [a]	88000 ± 20784.60 [a]
M. oleifera	32.00 ± 3.46 [a]	10.46 ± 1.18 [a]	5.33 ± 1.03 [a]	5803.33 ± 621.29 [a]	91599 ± 17031.83 [ab]
Control	36.00 ± 0.57 [ab]	11.8 ± 0.25 [ab]	5.73 ± 0.51 [a]	6916.66 ± 1044. 16[a]	137333 ± 16696.63 [b]

Values are expressed as mean ± standard error of three replicate. Values in the same column followed by the same superscript are not significantly different ($p<0.05$). PCV -Packed Cell Volume, RBC - Red Blood Cell Count, WBC - White Blood Cell Count, Hb-Heamoglobin

Table 8 shows haematology of experimental animals at 750mg/kg and 1200mg/kg of the extract. There was increase though not statistically significant in packed cell volume, haemoglobin, and red blood cell count of combined extracts of *Vernonia amygdalina, Ocimum gratissimum* and *Moringa oleifera* when compared to control. There was reduction in the white blood cell and platelet.

Table 8: Haematology of experimental animals at 750mg/kg and 1200mg/kg of the extract.

GROUPS	PCV (%)	HB g/dl	RBC $x10^6$mm	WBC $x10^3$mm	PLATELET $x10^3$µl
M.oleifera+V.amygdalina+ O.gratissimum 750	46.33±	14.56±	7.56±	3533.33±	94000.0±
	2.07 [b]	1.01 [a]	0.66 [a]	883.80 [a]	7135.46 [a]
M.oleifera+ V.amygdalina+ O.gratissimum 1200	36.00±	12.00±	6.65±	15500.00±	330000±
	2.08 [a]	0.57 [a]	0.65 [a]	77.35 [c]	5773.50 [b]
Control	36.00±	11.80±	5.73±	6916.66±	137333±
	0.57 [a]	0.25 [a]	0.29 [a]	1044.16 [b]	16696.63 [a]

Values are expressed as mean ± standard error of three replicate. Values in the same column followed by the same superscript are not significantly different ($p<0.05$). PCV -Packed Cell Volume, RBC - Red Blood Cell Count, WBC - White Blood Cell Count, Hb-Haemoglobin

Table 9 shows biochemistry of experimental animals at 150mg/kg and 500 mg/ kg of the extract. At 150mg/kg there was significant increase in aspartate aminotransaminase level (AST) and alanine aminotransferase level (ALT) of *Moringa oleifera, Vernonia amygdalina, Ocimum gratissimum* but there was decrease in alkaline phosphatase (ALP) levels. At 500mg/kg there was significant increase in all extracts of AST level and ALT level when compared to control.

Table 9: Biochemistry of the experimental animals at 150mg/kg and 500mg/kg of extract

Groups	AST	ALT	ALP
O. gratissimum at *150mg/kg*	36.00 ± 0.57^{ab}	26.67 ± 0.88^{a}	90.35 ± 1.45^{a}
M. oleifera at *150mg/kg*	37.00 ± 0.57^{ab}	27.00 ± 1.15^{a}	106.67 ± 1.45^{bcd}
V.amygdalina at*150mg/kg*	39.00 ± 0.57^{b}	27.67 ± 0.88^{ab}	109.67 ± 2.60^{cd}
O. gratissimum at *500mg/kg*	39.00 ± 0.57^{b}	27.66 ± 1.45^{ab}	96.00 ± 6.57^{ab}
V.amygdalina at 500mg/kg	36.67 ± 2.62^{a}	30.33 ± 6.88^{b}	92.33 ± 5.36^{a}
M.oleifera at 500mg/kg	38.33 ± 0.88^{ab}	26.00 ± 6.57^{a}	101.33 ± 0.66^{abc}
Control	35.00 ± 0.00^{a}	26.00 ± 6.00^{a}	115.00 ± 0.00^{b}

Values are expressed as mean ± standard error of three replicate. Values in the same column followed by the same superscript are not significantly different ($p<0.05$). ALP-alkaline phosphatase, AST- aspartate aminotransferase, ALT- alanine aminotransferase level

Table 10 shows biochemistry of experimental animals at 300mg/kg in various experimental groups. There was significant increase in the AST and ALT levels of all single and combined extracts while ALP level reduced significantly when compared to control

Table 10: Biochemistry of the experimental animals at 300mg/kg of the extract

GROUPS	AST	ALT	ALP
M. oleifera + *V. amygdalina*	37.33 ± 1.45^{ab}	28.66 ± 0.66^{b}	115.00 ± 7.23^{a}
V.amygdalina + *O. gratissimum*	37.66 ± 1.66^{ab}	26.66 ± 1.20^{ab}	95.66 ± 13.09^{a}
M.oleifera + *O.gratissimum*	40.33 ± 0.33^{b}	29.00 ± 0.57^{b}	
V. amygdalina	39.00 ± 2.08^{b}	28.66 ± 0.88^{ab}	
M. oleifera	38.33 ± 0.33^{ab}	28.33 ± 0.88 ^{ab}	98.33 ± 8.68^{a}
O. gratissimum	38.66 ± 0.33^{ab}	28. 00 ± 0.57^{ab}	106.33 ± 2.02^{a}
Control	35.00 ± 0.00 ^{a}	26.00 ± 0.00^{a}	94.66 ± 10.83^{a}
			118.00 ± 0.57^{a}
			115.00 ± 0.00^{a}

Values are expressed as mean ± standard error of three replicate. Values in the same column followed by the same superscript are not significantly different ($p<0.05$). ALP-alkaline phosphatase, AST- aspartate aminotransaferase, ALT- alanine aminotransferase level

Table 11 shows biochemistry of experimental animals at 750mg/kg and 1200mg/kg of the extract. At 750mg/kg and 1200mg/kg, there was significant increase in AST and ALT level when compared to control.

Table 11: Biochemistry of the experimental animals at 75 and 1200mg/kg

GROUPS	AST	ALT	ALP
M.oleifera+ V.amygdalina+ O.gratissimum at 750mg/kg	37.33 ± 0.57^{b}	27.00 ± 0.57^{ab}	107.00 ± 0.57^{a}
M.oleifera+ V.amygdalina+ O.gratissimum at 1200mg/kg	39.00 ± 1.00^{c}	28.00 ± 0.57^{b}	106.00 ± 0.57^{a}
Control	35.00 ± 0.00^{a}	26.00 ± 0.00^{a}	115.00 ± 0.00^{b}

Values are expressed as mean ± standard error of three replicate. Values in the same column followed by the same superscript are not significantly different ($p<0.05$). ALP-alkaline phosphatase, AST- aspartate aminotransferase, ALT- alanine aminotransferase level

DISCUSSION

The increase in the body weight recorded in the experimental rats after administration of single aqueous extract of *Moringa oleifera* and *Ocimum gratissimum* might be due to the fact that these plants are rich in amino acids, vitamins, and minerals especially iron (Faye 2011). The reduction in the body weight of rats administered V. *amygdalina* at 150mg/kg is in cosonance with the findings of Egedigwe (2016) where weight loss was observed when 100mg/kg of the extract was administered to obese animals. However, body weight increase in combined extracts *Moringa oleifera*, *Vernonia amygdalina* and *Ocimum gratissimum* agrees with the findings of Ekam *et al*., (2013) who reported that rats using different fractions of extracts of *Moringa oleifera, Ocimum gratissimum* and *Vernonia amygdalina* resulted in percentage growth and weight gained of the animals. This is also in consonance with the findings of Efiong, (2012) who reported that combined administration of these plants produced weight gain.

Heamatological parameters have been commonly used in the diagnosis of various diseases and pathological condition of foreign compounds including plant extracts, drugs, dyes and other blood constituents of animals (Oyedemi *et al.*, 2010). The increase in white blood cell of single extract of *M. oleifera* and *Vernonia amygdalina* at 150 mg/kg suggested the presence of some phytochemicals in them which induces the animal to respond as if there is infection (Aregheore *et al.,* 1998). The increase in packed cell volume, haemoglobin and red

blood cell count of single administration of *O. gratissimum* at all doses agrees with the findings of Ofem (2012) who observed same. The increase in WBC of single extract of *O. gratissimum* and when combined with *M. oleifera at 300mg/kg* shows that the principal function of phagocytes, which is to defend against invading microorganisms by ingesting and destroying them, which may account for its antibacterial activity.(Caceres, 1991; Fahey, 2005).

The increase in erythrocytes count in single and combined extract with *O. gratissimum* indicates a local tissue anoxia which leads to formulation of a glycoprotein called erythropoietin. It is likely that *O. gratissimum* leaves extract contain erythropoietin-like agents which are responsible for the increased production of erythrocytes.

The reduction in packed cell volume, haemoglobin and red blood cell of single extracts of *Moringa oleifera* at 500mg/kg agrees with Adedapo *et al.,(*2009) who reported that the plant could make the animals to be anaemic if exposed to this plant for a long period of time. The reduction observed in packed cell volume, heamoglobin level and red blood cell of combined extracts of *Vernonia amygdalina* and *Ocimum gratissimum*, *Moringa oleifera* and *Vernonia amygdalina* at 300mg/kg concurs with the findings of Obianime *et al.,* (2011) who observed same when he used *Ocimum gratissimum*. This suggests that when these plants are combined, they suppress haemopoietic effect and *Moringa oleifera* may be due to effect of compounds present in the extracts.

Aspartate aminotransferase(AST), alanine aminotransferase(ALT) and alkaline phosphate(ALP) have been associated with the liver enzyme and are diagnostic significance in clinical evaluation (Singah *et al*., 2000). It is important to analyze the liver parameter to ensure that livers are not damaged, because the damage can increase plasma level. (Edwards *et al*.,2005).

The increase observed in AST and ALT in single extracts of *Moringa oleifera, Vernonia amygdalina* and *Ocimum gratissimum* at all the doses agrees with (Adedapo *et al*., 2009) who reported that a rise in AST, ALT indicate damage to the liver cells including the biliary system. However, the reduction in ALP is in consonance with the findings Irvine, (2002) who reported that the reduction may have occurred due to lysis of blood cells and probably suppression of blood cell synthesis by saponin found in the leave extracts. Also, Ramanathan *et al.,* (2008) who reported that these extracts are found to contain flavonoids which are reported to exhibit antioxidant activity. Although there was increase in aspartate

aminotransferase and alanine aminotransferase levels the values still fall within the reference value for biochemistry i.e. AST 45.7-80.8U/L and ALT 17.5-30.2U/L (Johnson-Delaney, 1996).

In conclusion, the extracts are relatively safe at the doses investigated in this study, however higher dose should be investigated to determine the dose it will be toxic.

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