VOL. 18 NO. 9
ISSN: 2278-1897
SEPTEMBER, 2020

African Scholar Publications & Research International

African Scholars Journal of pure and Applied Science (JPAS-9)

Acute Toxicity Study of Ethanol Extract of Neauclea *Latifolia* and *Moringa Oleifera* Plants on the Parameters of Haematology and Histopathology of Male Abino Rats.

Adeyemi, S. O.

Department of Biology, School of Science, EACOED, Oyo, Oyo State

Abstract

Neauclea latifolia and Moringa oleifera has been reported as a medicinal plant used traditionally in Nigeria for the treatment of many diseases. This study therefore investigated the acute toxicity of Neauclea latifolia and Moringa oleifera ethanol leaves extract. For the acute toxicity of N. latifolia and M. oleifer, each experiment used thirty (30) male albino rats weighing between 150-180g were randomly divided into six (6) groups of five (5) rats each. Mortality rate, weight change and behavioural response were observed during the experiments. More so, haematology and histopathology of liver were investigated in the sub-acute of each extracts. The findings were determined using analysis of Probits in SPSS 2019 and revealed that N. latifolia and M. oleifera has >1400mg/kg and 1500mg/kg> as respectively but the finding of this work has revealed that dose of >800 < 1600 mg/kg of both extracts are preeminent and safer doses as ED50 because of the less mortality recorded and its similar efficacy with higher doses on the histopathological study. Moreover, both the extracts had very low toxicity profile in all the tested animals, it is relatively safe for herbal oral medication and this research might become a highly effective guidance for the therapeutic agent in the treatment of diseases. Because of the first hand information provided on this study, the researcher recommended that efforts should be geared towards identification of specific fractions of the active components of the extracts which is the keys inhibitors against

diseases. More so, further study should be biogenic synthesis of silver nanoparticles exploiting M. oleifera lam., and N. latifolia leaves extract.

Keywords: *Nauclea latifolia, Moringa oleifera, Haematology and Histopathology.*

Introduction

Nauclea *latifolia* is a genus of flowering plants in the Rubiaceae family, the species are evergreen trees or shrubs that are native to the paleotropics and the terminal vegetative buds are usually strongly flattened [29]. It is commonly known as pin cushion tree, a straggling shrub or small native tree which grows up to an altitude of 200 m. it's a valuable medicinal plant, that is widespread in the humid tropical rainforest or in savannah woodland zone of West and Central Africa and Asia. Different parts of the plant possess remarkable therapeutic actions that can support the traditional usage of this plant in the treatment of several ailments [40]. The plant was described with the origin, distribution, and its local use. Infusions and decoctions of parts of N. *latifolia* are commonly prescribed traditionally as a remedy for diabetes mellitus and it has been reported that the aqueous extract of Nauclea *latifolia* had been found to exert hypoglycaemic activity in alloxan-induced diabetic rats [46]. The plant is also used as a chewing stick [9] and in the treatment of ailments like gastrointestinal tract disorders, tuberculosis, sleeping sickness, prolongs menstrual flow, hypertension and malaria [3, 4, 8, 11, 24, and 42]. More recently, it had been reported the presence of biologically active principles in the extract with anti-nociceptive, anti-inflammatory and anti-pyretic activities that justifies its use in malaria ethnopharmacy and subsequent development for clinical application [46]. Indeed, all plant parts of the Nauclea species are a rich source of monoterpene indol alkaloids namely indole alkaloid strictosamine [20, 36 and 45] but also terpenes [47] saponines and active polyphenols [3]. Moreover, the ethanolic extract of Nauclea *latifolia* had been found to posses anti-hepatotoxic and trypanocidal activities [3]. Despite the numerous studies on pharmacology activities of aqueous or ethanolic extract or biologically active principles of N. *latifolia*, very few studies were reported on its systemic

toxicity. The few data on the plant toxicity revealed that the was higher than 1600 mg/kg/bw after intraperitoneal (IP) injection of mice [10] and alkaloid rich extracts from the Nauclea *latifolia* can interact in vitro with DNA of bacteria and mammalian cells, leading to G2-M cell cycle arrest and heritable DNA-damage and cause single-strand breaks in liver, kidney and blood cells [35].

Moringa *oleifera* is a tree of the Moringaceae family, widely cultivated throughout the tropics and subtropics, and its leaves, seed pods, seeds, seed oil, roots, bark, flowers, and sap are commonly consumed as food and used in traditional medicines. Moringa *oleifera* is a multipurpose plant that fits properly into the above uses. It is economically useful as source of food, natural medicine, animal fodder, natural coagulants, forestry products,fertilizer, living fence, alley cropping and fueling [19, 38]. Moringa is considered rich with several medicinal properties as all the parts have been reportedly engaged singly or with other plants for treating diverse illnesses and diseases [19]. A review of medical evidence for the use of M. *oleifera* for nutrition, therapeutic and prophylactic properties indicated that the demand for it is on the increase in scientific research and in terms of global use [19]. Its use as an inexpensive component in foods, nutritional supplements, or medicines by patients with HIV/AIDS has been advocated by several African governments. It possesses anti-inflammatory, antioxidant, antimicrobial, antifertility, anticancer, antihepatotoxic and antiulcer activities. Reports have shown that the products of traditionally used plants such as Bryophyllum pinnatum and M. *oleifera* promoted healing in experimental animals. The M. *oleifera* roots and seeds are prescribed for the treatment of snake bites and scorpion stings. M. *oleifera* leaves are also reported to have hepatoprotective activity against carbon tetrachloride-induced hepatotoxicity in rats. All parts of M. *oleifera* are medicinally important. It mainly contains various glycosides of thiocarbamate and isocyanide class [1]. Pterygospermin, moringyne, Niaziridin, 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate, 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate, etc., are few of them which are isolated and therapeutically proved by scientific studies as antihypertensive, antiasthmatic, antidiuretic, anticancer, antibiotic, antiulcer, analgesic, CNS-depressant, antiepileptic, anti-inflammatory, anthelmintic, antiurolithiatic and many more [2, 5]. Recently, M. *oleifera* has been identified as an anti-venom herbal treatment. These plants contain a mixture of several hydrolytic enzymes, in which proteases are the key enzymes responsible for the observed

pharmacological actions [5]. Hence, the aim of the present study is to investigate the sub-acute toxicity study of N. *latifolia* and M. *oleifera* in male wistar albino rats. Therefore, this study will help to ascertain the lethal toxicity of the plants and veracity of the folkloric claims of the use of N. *latifolia* and M. *oleifera* in the treatment of diseases.

MATERIALS AND METHOD

Collection of the Plant material

Fresh leaves of *N. latifolia* and *M. oleifera* were collected from Oyo East Local Government, Oyo premises and the leave were identified and authenticated by trained Taxonomist with voucher no: UIH-22986 and UIH-22442 respectively in the herbarium section of the Department of Botany, University of Ibadan, Ibadan. The leaves were subsequently air-dried at room temperature between (25 to 27 ^{0}C) in the laboratory and grinded to powder form using a Qlink electric blender and sieved.

Extraction of the Ethanol Extract of the Plant Leaves

Cold maceration method was employed in each extraction of the plant material. Five hundred grammes (500 g) of each powdered leaves of *N. latifolia* and *M. oleifera* were soaked in 1liters of 100% ethanol for 72 hours separately to obtain the ethanol extract. The content of each extract was filtered through a Whatman filter paper lined funnel into a conical flask. The filtrates of each extract was concentrated using rotator evaporator at 40^{o}C before being gently evaporated to dryness at room temperature (25 to 27^{o}C) and then stored in separate clean dry bottles and kept in the refrigerator at 40^{o}C. Dilutions to desired concentrations were made for each extract when required.

Experimental Animals

Sixty adult male albino rats weighing 150-180 g were purchased from the Animal House of Department of Physiology, University of Ibadan, Nigeria. The rats were acclimatized for two weeks under standard conditions (12 h light and 12 h dark cycle), well ventilated, pathogen free cages at room temperature (27^{o}C) in the animal house of the Department of Biology, Emmanuel Alayande College of Education, Oyo, Oyo State. They were fed with standard mouse pellets and water was supplied *ad libitum.*

Lethal dose concentration () test of the Extracts

The method of Turner as reported by Adeyi, *et al.*, [5] was adopted for each extract to determine the percentage death of the animals 14 days after the daily oral dose. Thirty (30) adult male albino rats were divided randomly into six (6) groups of five (5) rats per cage for each extract. At the end of the two weeks of acclimatization, each extract was diluted as required and administered once orally to the rats at 10.00am daily for the period of the test (14 days) after the animals have been starved for 12 h before the onset of the treatment. The groups were constituted as follows for each extract:

For the *N. latifolia Leaves* Extracts:

Group 1 (Control group): They were given only normal saline.

Group 2: They were administered with 200 mg/kg of the dissolved ethanol leaves extract of *N. latifolia* in 1ml of normal saline.

Group 3: They were administered with 400 mg/kg of the dissolved ethanol leaves extract of *N. latifolia* in 1ml of normal saline.

Group 4: They were administered with 800 mg/kg of the dissolved ethanol leaves extract of *N. latifolia* in 1ml of normal saline.

Group 5: They were administered with 1600 mg/kg of the dissolved ethanol leaves extract of *N. latifolia* in 1ml of normal saline.

Group 6: They were administered with 3200 mg/kg of the dissolved ethanol leaves extract of *N. latifolia* in 1ml of normal saline.

For the M. *oleifera Leave* Extracts

Group 1 (Control group): They were given only normal saline.

Group 2: They were administered with 200mg/kg of the dissolved ethanol leaves extract of M. *oleifera* in 1ml of normal saline.

Group 3: They were administered with 400mg/kg of the dissolved ethanol leaves extract of M. *oleifera* in 1ml of normal saline.

Group 4: They were administered with 800mg/kg of the dissolved ethanol leaves extract of M. *oleifera* in 1ml of normal saline.

Group 5: They were administered with 1600 mg/kg of the dissolved ethanol leaves extract of M. *oleifera* in 1ml of normal saline.

Group 6: They were administered with 3200 mg/kg of the dissolved ethanol leaves extract of M. *oleifera* in 1ml of normal saline.

Behavioural Response, Weight Change and Mortality Study

Mortality, Weight change as well as the behavioural responses of the rats were recorded during the 14 days period to study of the lethal toxicity of the each dose and lethality of the each extracts of rats were determined using analysis of Probits in Excel 2019 and Graph pad 2019. After 14 days of treatment with the extracts, the blood samples were collected into heparinised tubes from each surviving rat of the study by tail vein puncture method and the sample bottles were gently rocked to allow a proper mixing of the blood with the anticoagulants in the sample bottles which was properly labeled for haematological study and the rats were sacrificed after 14 days according to guides of Rowett reported by Adeyemi, *et al.*, [2], and the liver of the rat samples were harvested from the various groups. These samples were bottled dry and fixed in 10% formaldehyde (pH 7.2 to 7.4) and sections of tissues were cut for histological analysis.

RESULT

Table 1: Weight Changes of the Rats during Assay of the Ethanol Extract of N. *latifolia*.

Mean Body Weight (g)

Groups	Initial	Final	Weight changes (%)
A	154.46±0.2	167.80±1.1	13.34±1.1††
B	159.30±2.4	161.20±1.4	1.9±0.8††
C	153.70±0.2	159.10±1.6	5.4±2.4††
D	156.70±1.0	158.90±1.4	2.2±0.7††
E	158.50±0.2	155.10±1.6	6.6±0.3††
F	157.40±1.0	162.90±1.4	7.5±0.1††

Weight gain =†† **Weight loss =** Nil†

Legend for Table 1.

Group A: –Control group

Group B: –administered with 200mg/kg of the ethanol extract of N. *latifolia* leaves.

Group C:– administered with 400mg/kg of the ethanol extract of N. *latifolia* leaves.

Group D:– administered with 800mg/kg of the ethanol extract of N. *latifolia* leaves.

Group E:– administered with 1600mg/kg of the ethanol extract of N. *latifolia* leaves.
Group F:– administered with 3200mg/kg of the ethanol extract of N. *latifolia* leaves.

Table 2: Weight Changes of the Rats during Assay of the Ethanol Extract of M. *oleifera*.

Mean Body Weight (g)

Groups	Initial	Final	Weight changes (%)
A	164.32 ±1.7	179.80 ±0.5	15.48±2.7††
B	169.31 ±2.2	176.20 ±1.0	10.89 ±1.7††
C	163.38 ±2.0	176.10 ±0.3	7.72 ±0.7†
D	176.70 ±0.7	183.90 ±1.0	7.2 ±1.2†
E	173.38 ±2.0	188.10 ±0.3	4.72 ±1.2†
F	178.70 ±0.7	179.90 ±1.0	3.2 ±1.3†

Weight gain = †† **Weight loss =**†

Legend for Table 2:

Group A: Control group
Group B:– administered with 200mg/kg of the dissolved ethanol M. *oleifera* extract.
Group C:– administered with 400mg/kg of the dissolved ethanol M. *oleifera* extract.
Group D:– administered with 800mg/kg of the dissolved ethanol M. *oleifera* extract.
Group E:– administered with 1600mg/kg of the dissolved ethanol M. *oleifera* extract.
Group F:– administered with 3200mg/kg of the dissolved ethanol M. *oleifera* extract.

Table 3: Determination of Assay of the Ethanol Leaf Extract of N. *latifolia*.

GROUP	LOG DOSE	TOTAL MORTALITY	% MORTALITY	PROBIT
A-**0**	0	0	0	0
B-**200**	2.30103	0	0	0
C-**400**	2.60205999	0	0	0
D-**800**	2.90308999	1	20	4.16

E-**1600**	3.20411998	1	20	4.16
F-**3200**	3.50514998	2	40	4.75
=10^3.44				
(2754.23mg/Kg)				

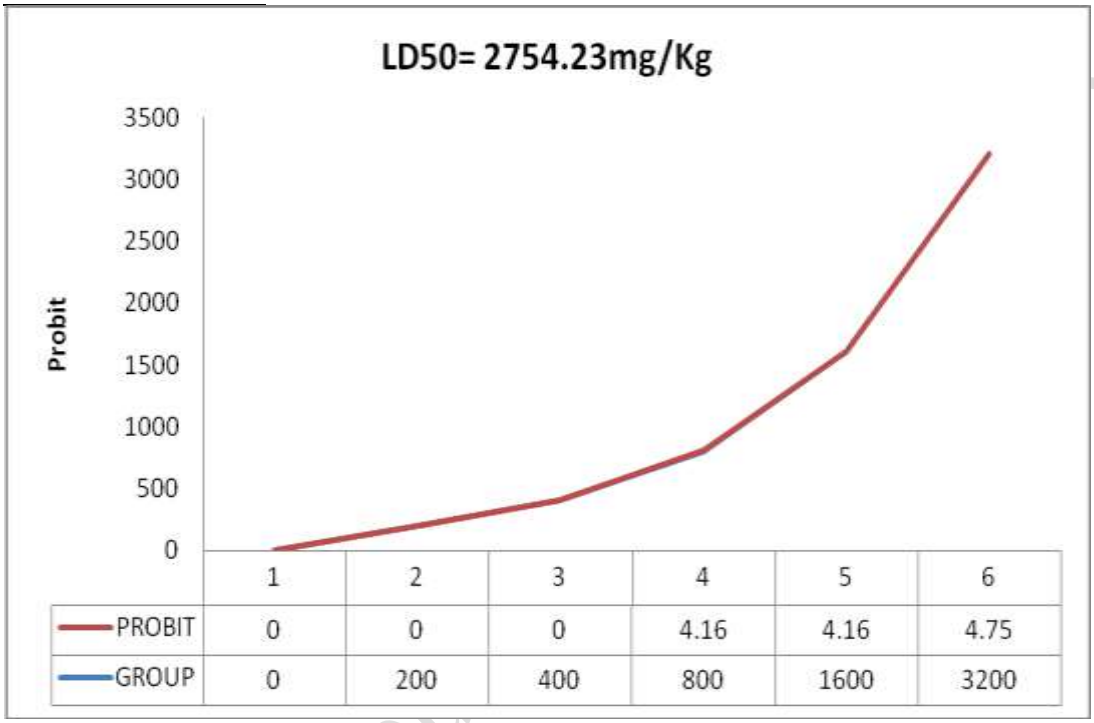


Figure 1. Graph of probit against Log doses of N. *latifolia* Ethanol Leaf Extract.

Legend for Table 3:

Group A: –Control group

Group B: –administered with 200mg/kg of the ethanol extract of N. *latifolia* leaves.

Group C:– administered with 400mg/kg of the ethanol extract of N. *latifolia* leaves.

Group D:– administered with 800mg/kg of the ethanol extract of N. *latifolia* leaves.

Group E:– administered with 1600mg/kg of the ethanol extract of N. *latifolia* leaves.

Group F:– administered with 3200mg/kg of the ethanol extract of N. *latifolia* leaves.

Table 4: Determination of Assay of the Ethanol Leaf Extract of M. *oleifera*.

GROUP	LOG DOSE	TOTAL MORTALITY	% MORTALITY	PROBIT
A-0	0	0	0	0
B-200	2.30103	0	0	0
C-400	2.60205999	1	20	4.16
D-800	2.90308999	1	20	4.16
E-1600	3.20411998	2	40	4.75
F-3200	3.50514998	3	60	5.25
=10^3.27				
(1862.1mg/Kg)				

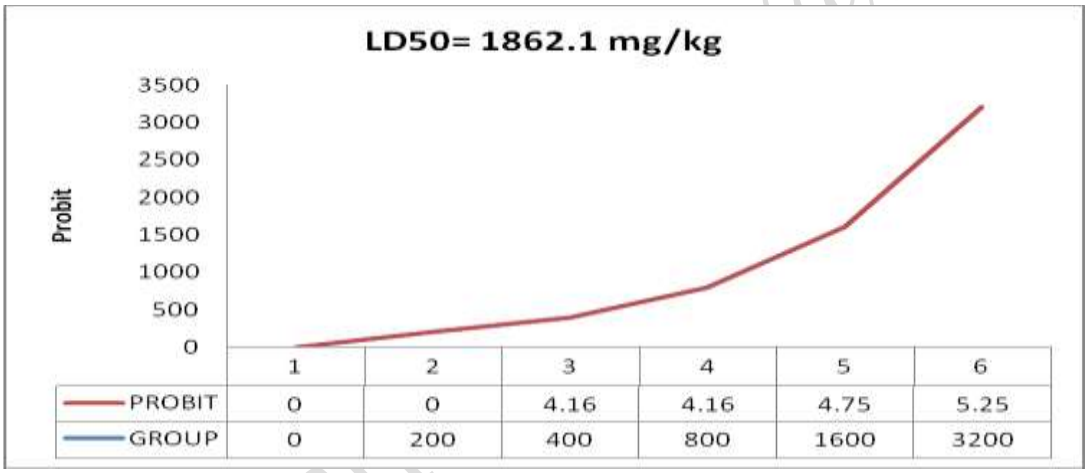


Figure 2. Graph of probit against Log doses of M. *oleifera* Ethanol Leaf Extract.

Legend for Table 4:

Group A: Control group

Group B:– administered with 200mg/kg of the dissolved ethanol M. *oleifera* extract.

Group C:– administered with 400mg/kg of the dissolved ethanol M. *oleifera* extract.

Group D:– administered with 800mg/kg of the dissolved ethanol M. *oleifera* extract.

Group E:– administered with 1600mg/kg of the dissolved ethanol M. *oleifera* extract.

Group F:– administered with 3200mg/kg of the dissolved ethanol M. *oleifera* extract.

Discussion

Table 1 and 2, showed the mean weight change of the experimental animals during the 14 days test of assay of ethanol leaves exact N. *latifolia*. In table 1, showed the mean body weight of the experimental animals during assay of the ethanol N. *latifolia* leaves extract and figure 1 showed the graph of the probit against log doses of the extract. The food and water consumption of control Group A (normal control) was consistence throughout the 14 days of the treatment compared to all administered groups. At the end of the 14th days of the experiment (day 14), no variation of the vivacity, sensitivity to noise and no painful stimulation was noted in both experimental and control groups and a total of 40% mortality were recorded in during the treatment of the assays. In addition, the macroscopic observation of each animal's tissues was not revealed any changes but, the water was rich in the faeces of experimental animal compared to control, animals treated with ethanol extract of *N. latifolia* at doses low and mid dose increased in body weights, in contrast to those treated with high dose of extract while the table 2, also showed no significant difference in term of behavioral observation of the experimental animals and mean body weight of the control Group A of ethanol leaves extract of M. *oleifera* but slightly differences in mean body weight of the treatment groups of ethanol leaves extract of M. *oleifera* compared to control group of this extract and that of N. *latifolia* ethanol extract and figure 2 showed the graph of the probit against log doses of the extract where total of 7 rats were recorded as mortality during 14 days assay and this is in line with work of Kasolo, *et al.,* (2012).

Furthermore, Table 3, showed the of the ethanol leaves extract of N. *latifolia* was established to be greater <450mg/kg of Basey, et al., [13, 12, 14, 15] but estimated to be 2754.23 mg/kg using Probit analysis while Table 4, showed the of the ethanol leaves extract of M. *oleifera* to be greater than that of Kasolo, *et al.,*[26, 27, 30] of 1600mg/kg but it was estimated to be 1862.1 mg/kg for the M. *oleifera* and this is in support of Okumu, *et al.,* [20, 21, 32] who also reported that the of the aqueous methanol leaves extract of M. *oleifera* leaves was found to be greater than 2000 mg/kg in female wistar albino rats.

Haematology of acute toxicity study of the extracts in rats.

Table 5 and 6, showed no significant differences between the two plant extracts of ethanol leaves of N. *latifolia* and M. *oleifera* in the haematological parameters of the experimental groups. Group A (normal control) showed a significantly ($P<0.05$) higher values in the Packed Cell Volume (PCV), Red

Blood Cell (RBC), Heamoglobin (Hb) compared to other groups and this showed that the dose dependent on decrease in the PCV, RBC, Hb across the treated groups. However, the group B showed a significantly (P<0.05) higher values in the PCV, RBC, Hb compared to group C, D, E and F. The white blood cell (WBC) of the group A (normal control) showed significantly (P<0.05) higher values compared to other groups. However, the (WBC) of group B showed no significantly (P<0.05) value compare to other treated groups. Also the platelet values were significantly (P<0.05) not different across all the experimental groups. Moreover, the result of Mean Corpuscular Volume (MCV) showed higher values in the all treated groups which were significantly (P<0.05) compared to group A (normal control). There were no significant (P<0.05) differences in the MCV (*fl*) values of Groups B compared to group C and Group D. And Group C, D, E and F showed no significant (P<0.05) differences in the MCV, MCHC and MCH values.

Table 5: Haematological parameters of ethanol leaf extract of N. *latifolia* in experimental rats.

Parameters	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	GROUP F
PCV (%)	26.00±2.00^{e}	22.01±0.58^{d}	21.33±0.58^{d}	20.34±0.58^{d}	22.33±0.58^{d}	23.14±0.18^{d}
Hb (g/dl)	15.23±0.82^{d}	14.88±0.06^{c}	14.43±0.06^{c}	13.44±0.06^{c}	14.66±0.06^{c}	15.44±0.16^{c}
RBC (cell/L)	7.92±0.35^{d}	7.99±0.08^{d}	7.77±0.08^{d}	7.78±0.08^{d}	7.77±0.08^{d}	8.78±0.22^{d}
WBC ($x10^3$ cell/L)	6.5±0.46^{b}	5.33±0.30^{ab}	5.33±0.20^{ab}	6.33±0.10^{ab}	6.43±0.20^{ab}	6.53±0.10^{ab}
PLATELET ($x10^5$ cell/L)	1.31±0.20^{a}	1.48±0.40^{a}	1.47±0.40^{a}	1.56±0.40^{a}	1.57±0.40^{a}	1.56±0.40^{a}
LYM (%)	44.0±3.0^{c}	47.0±1.0^{b}	46.0±1.0^{b}	45.2±1.0^{b}	46.0±2.0^{b}	46.2±3.0^{b}
NEUT (%)	32.3±3.5^{c}	20.0±1.0^{b}	21.0±1.0^{b}	23.2±1.0^{b}	22.0±2.0^{b}	23.2±3.0^{b}
MONO (%)	1.7±0.6^{bc}	1.7±0.1^{c}	1.5±0.3^{c}	1.5±0.0^{c}	1.6±0.3^{c}	1.7±0.0^{c}
EO (%)	2.0±1.0^{b}	1.6±0.3^{ab}	1.5±0.0^{ab}	1.6±0.0^{ab}	1.5±0.0^{ab}	1.6±1.0^{ab}
MCV (*fl*)	58.1±1.2^{c}	59.9±0.3^{a}	59.8±0.2^{a}	59.8±0.2^{a}	59.9±1.2^{a}	59.9±2.2^{a}
MCH(*pg*/cell)	19.2±0.2^{d}	19.8±0.2^{a}	19.7±0.3^{a}	19.6±0.1^{a}	19.7±0.3^{a}	19.7±1.1^{a}

Values are Means ± S.D, n ≤ 3

Mean with similar superscript on the same column are not significantly difference (P<0.05).

Legend for Table 5:

Group A: –Control group

Group B: –administered with 200mg/kg of the ethanol extract of N. *latifolia* leaves.

Group C:– administered with 400mg/kg of the ethanol extract of N. *latifolia* leaves.
Group D:– administered with 800mg/kg of the ethanol extract of N. *latifolia* leaves.
Group E:– administered with 1600mg/kg of the ethanol extract of N. *latifolia* leaves.
Group F:– administered with 3200mg/kg of the ethanol extract of N. *latifolia* leaves.

Table 6: Haematological parameters of ethanol leaf extract of M. *oleifera* in experimental rats.

Parameters	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	GROUP F
PCV (%)	24.00±2.00^{e}	12.56±3.62^{a}	13.66±3.60^{a}	12.56±3.62^{a}	12.56±3.62^{a}	12.56±3.62^{a}
Hb (g/dl)	14.23±0.82^{d}	7.24±0.95^{ab}	8.26±0.95^{ab}	9.24±0.95^{ab}	10.28±0.95^{ab}	11.29±0.95^{ab}
RBC (cell/L)	9.92±0.35^{d}	14.29±0.9^{b}	15.30±1.9^{b}	15.22±0.3^{b}	15.25±0.5^{b}	15.39±0.9^{b}
WBC ($x10^3$ cell/L)	35.5±0.46^{b}	37.95±2.17^{b}	38.95±2.17^{b}	39.55±2.17^{b}	39.65±2.17^{b}	39.95±2.11^{b}
PLATELET ($x10^5$ cell/L)	51.11±0.20^{a}	51.84±2.67^{a}	51.84±2.33^{a}	51.84±2.57^{a}	51.84±2.57^{a}	51.84±2.66^{a}
LYM (%)	19.0±3.0^{c}	19.23±1.31^{ab}	19.23±2.11^{ab}	19.23±5.11^{ab}	19.23±7.33^{ab}	19.23±8.51^{ab}
NEUT (%)	30.3±3.5^{c}	35.25±0.43^{a}	35.75±0.43^{a}	36.75±3.03^{b}	36.77±0.43^{b}	36.78±0.13^{b}
MONO (%)	41.6±0.6^{bc}	43.00±10.75^{b}	43.17±10.75^{b}	43.25±10.7^{b}	43.22±0.25^{b}	43.02±10.15^{b}
EO (%)	2.2±1.0^{b}	1.5±0.3^{ab}	1.5±0.4^{ab}	1.5±1.5^{ab}	1.5±1.3^{ab}	1.5±1.6^{ab}
MCV (*fl*)	51.1±1.2^{c}	49.9±0.3^{a}	49.9±1.3^{a}	49.9±1.6^{a}	49.8±0.6^{a}	49.9±4.3^{a}
MCH(*pg*/cell)	10.2±0.2^{b}	10.8±2.2^{a}	10.8±2.3^{a}	10.8±2.2^{a}	10.8±3.2^{d}	10.8±3.4^{d}

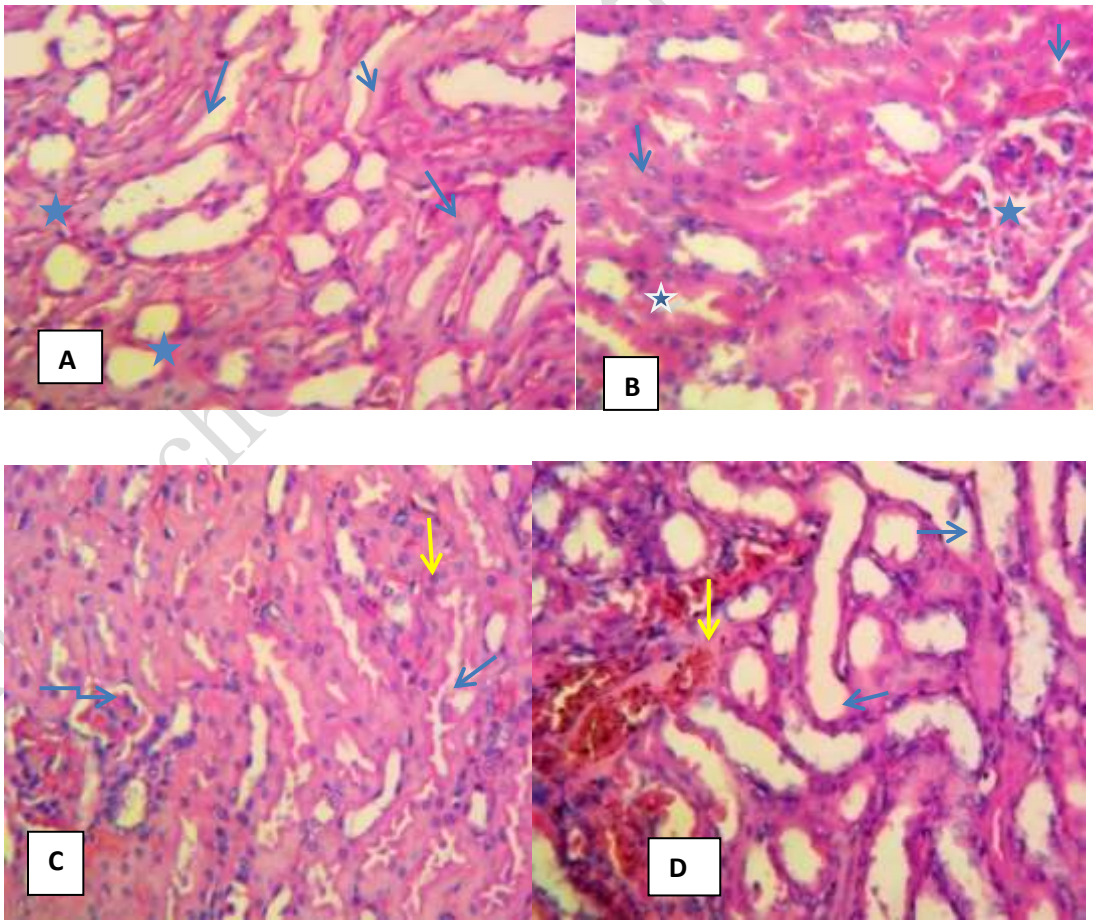
Values are Means ± S.D, n ≤ 3
Mean with similar superscript on the same column are not significantly difference (P<0.05).

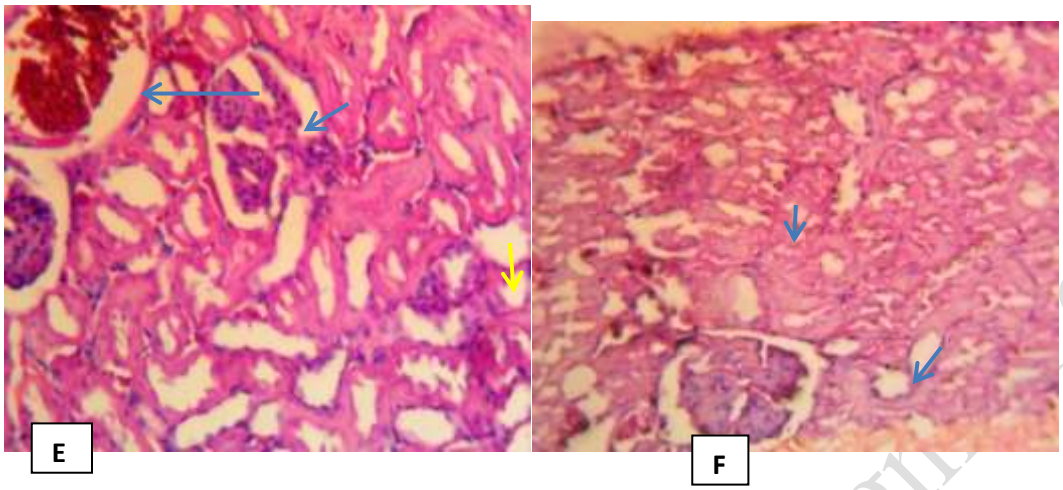
Legend for Table 6:
Group A: Control group
Group B:– administered with 200mg/kg of the dissolved ethanol M. *oleifera* extract.
Group C:– administered with 400mg/kg of the dissolved ethanol M. *oleifera* extract.
Group D:– administered with 800mg/kg of the dissolved ethanol M. *oleifera* extract.
Group E:– administered with 1600mg/kg of the dissolved ethanol M. *oleifera* extract.

Group F:– administered with 3200mg/kg of the dissolved ethanol M. *oleifera* extract.

Histopathology of Hearts of Envenomed Rats.

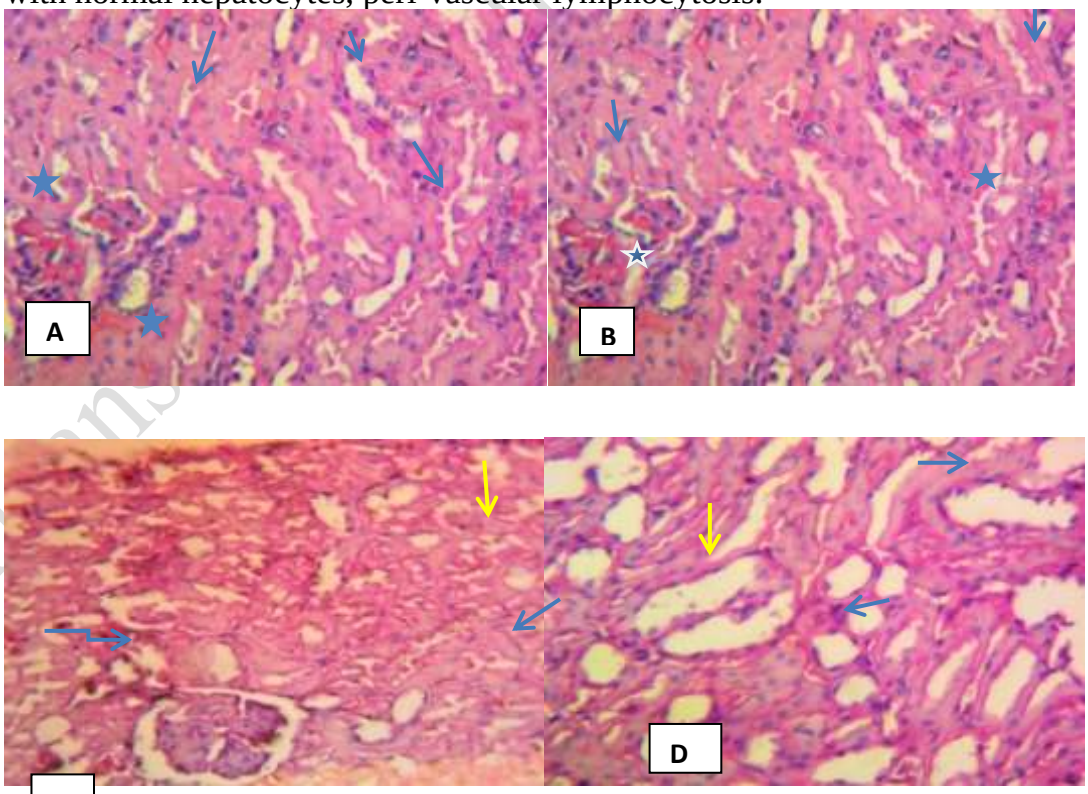
The results of the histopathological responses of the single daily dose of N. *latifolia* leaves ethanol extracts presented in Plate 1A-F, using light microscopy (x400 magnifications). There were minimal histopathological changes in the liver of the rats in group B and C doses and this is in support with the reports of the work of Adedapo, *et al.*, [1, 5, 26], who observed no abnormal features in the histopathology examination of the liver tissue and it could have been a result of the low doses used. The major histopathological changes occurred in the liver of animals that received the high doses which was observed in the group D - F doses such as congestion with scattered focal necrosis, scattered mononuclear cell infiltration, with normal hepatocytes, peri-vascular lymphocytosis were observed in group D – F and this is in line with the work of Adeyi, *et al.*, (2019).

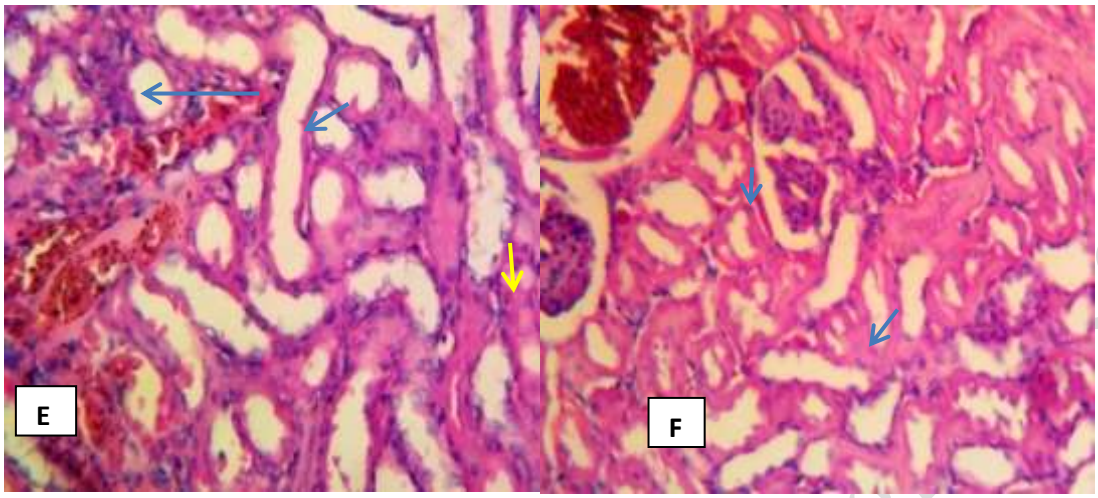




Light microscopy (x400 magnifications)

Plate 5 (A-F): Histological sections of the liver of animal treated with ethanol leaves extract of M. oleifera and each plate represent each group. Plate (A-C) Normal and no congestion of focal necrosis, compact mononuclear cell infiltration, with normal hepatocytes, vascular lymphocytosis (D) minimal congestion with scattered focal necrosis, small scattered mononuclear cell infiltration, with normal hepatocytes, peri-vascular lymphocytosis, (E- F) congestion with scattered focal necrosis, scattered mononuclear cell infiltration, with normal hepatocytes, peri-vascular lymphocytosis.





Light microscopy (x400 magnifications)

Plate 5 (A-F): Histological sections of the liver of animal treated with ethanol leaves extract of M. *oleifera* and each plate represent each group. Plate (A-C) Normal and no congestion of focal necrosis, compact mononuclear cell infiltration, with normal hepatocytes, vascular lymphocytosis (D) minimal congestion with scattered focal necrosis, small scattered mononuclear cell infiltration, with normal hepatocytes, peri-vascular lymphocytosis (E- F) congestion with scattered focal necrosis, scattered mononuclear cell infiltration, with normal hepatocytes, peri-vascular lymphocytosis.

CONCLUSION AND RECOMMENDATION

The obtained results of the values of the ethanol leaves extract of M. oleifera in this study was in line of the reports of the [26, 30, 35] reported that the values of the ethanolic and aqueous extracts of M. *oleifera* roots growing in Uganda were 17.8 g/kg and 15.9 g/kg, respectively and mortality and change in the behavioural patterns of the animals and Reddy, *et al.,* (2013) revealed that the of the acute oral toxicity study of the methanolic extract of M. *oleifera* bark was found to be >2000-5000 mg/kg bodyweight and mortality and change in the behavioural patterns of the animals was observed in all the studies and the present study didn't record any mortality but very severe changes in behavoural patterns were observed and this means the higher the dose, the increase the changes in the behavioural patterns and the mortality of the animals.. More so, the obtained result of the values of the ethanol leaves extract of N. *latifolia* in this study was supported with reports of Bassey, *et al.*,who reported the

photochemical composition and antidiabeticactivity of ethanol root extract of N. *latifolia*.

Moreover, the treatment of Wistar albino rats with ethanol leaves extract of N. *latifolia* and M. *oleifera* showed no significant difference on the biological alterations of the haematological, histopathological act ivies of the experimental animals in this study. Although, M. oleifera leaves ethanol extract in group E-F given to rats orally in a single for 14 days is associated with moderate liver necrosis which are features of mild organ toxicity but group D extract indicates safer outcomes but the rats with ethanol leaves extract of N. *latiofolia* only showed similar features on the group F of plate F. Therefore, an oral daily dose of M. oleifera leaves ethanol extract of group D could be used as effective dose (ED50).

RECOMMENDATION

This study has provided first hand information on the acute toxicity of ethanol leaf extract of N. *latifolia* and *M. oleifera* for the further study of treatment of ailment. However, with the growing knowledge on the biochemistry and pharmacology, efforts should be geared towards identification of specific fractions of the active components of the extracts which is the keys inhibitors against diseases. More so, the finding of this work has revealed that dose of >800 < 1600 mg/kg of both extracts are preeminent and safer doses because of the less mortality recorded and its similar efficacy with higher doses on the histopathological study. The dose of ethanol leave extract of M. *oleifera* above 800 mg/kg body weight showed some changes in the behavioural patterns of animals like a decrease in impairment in food intake, weight loss and an increase in the activity and this is in line with the previous studies of Manal, *et al.*, (2017) which revealed that the of the methanolic extract of M. *oleifera* was 3458.3 mg/kg in male wistar albino mice but mortality was recorded in doses above 1500mg/kg of the bodyweight with the changes in the behavioural patterns of animals like a decrease in impairment in food intake, weight loss and an increase in the activity.

REFERENCES

[1] Adedapo AA, Mogbijuri.OM, Emikpe BO (2009), Safety evaluations of the aqueous extract of the leaves of Moringa oleifera in rats. J.of Med. Plants Res., 3(8):586-591.

[2] Adeyemi, S.O, Larayetan Rotimi, Ajayi Abayomi, Yahaya Abdulrazaq, Ogunmola, O. Oluranti, (2020). Anti-haemorrhagic activity of ethanol extract of *Moringa oleifera* leaf on envenomed Albino rats; Scientific African 8 (2020) e00293.

[3] Adzu B, Mustapha KB, Masimirembwa C, Obodozie O, Kirim RA, Gamaniel KS. (2013) Simulation of metabolism-based herb-drug interaction: towards safe and efficacious use of NIPRD-AM1.Avicenna Journal of Phytomedicine. 3: (3), 201-204.

[4] Ajaiyeoba E, Ashidi J, Abiodun O, Okpako L, Ogbole O, Akinboye D, Falade C, Bolaji O, Gbotosho G, Falade M, Itiola O, Houghton P, Wright C, Oduola A. (2004) Antimalarial Ethnobotany: In Vitro Antiplasmodial Activity of Seven Plants Identified in the Nigerian Middle Belt. Pharmaceutical Biology. 42(8):588-591.

[5] Akindele Oluwatosin Adeyi, Siji Babafemi Ajisebiola, Esther Olubisi Adeyi, Chibuisi Gideon Alimba, Uchennaya Godwill Okorie, (2020). Antivenom activity of Moringa oleifera leave against pathophysiological alterations, somatic mutation and biological activities of Naja nigricollis venom; Scientific African 8 (2020) e00356

[6] Akinnibosun, F.I, Akinnibosun, H.A and Ogedegbe, D. (2009). Investigation on the antibacterial activity of Aqueous and ethanolic extracts of leaves of Boerhaviadiffusa. Science World Journal 4 (2): 15-18.

[7] Ali, G.H, El-Taweel, G.E. and Ali, M.A. (2004). The cytotoxicity and antimicrobial efficiency of Moringa oleifera seeds extracts. International Journal of Environmental Studies 61 (6): 699-708.

[8] Akubue PI, Mittal GC. (1982) Clinical evaluation of a traditional herbal practice in Nigeria: preliminary report. Journal of Ethnopharmacology. 6(3):355-359

[9] Anowi CF, Cardinal NC, Mbah CJ, Onyekaba TC. (2012a)Antimicrobial Properties of the Methanolic Extract of the Leaves of Nauclea latifolia. International Jouurnal Drug Res. Tech. 2 (1), 45-55.

[10] Arkhurst, FS, Arkhurst, JC. (2003) Method and Composition of Treating Malaria with Nauclea latifolia root. United States Patent Application Publication. p 1-2.

[11] Asase A, Oteng-Yeboah A A, Odamtten GT, Simmonds MSJ. (2005) Ethnobotanical study of some Ghanaian anti-malarial plants. Journal of Ethnopharmacology. 99: 273-279.

[12] Ayinde B, Omogbai E, Ameaechina F, (2007), Pharmacolgnosy and hypotensive evaluation of Ficus exasperata Vahl (Moraceae). leaf.Acta Pol. Pharm. 64(6):543-6.

[13] Bassey S. Antia, Jude E. Okokon, (2014). Phytochemical composition and antidiabetic activity of ethanol root extract of Nauclea latifolia; Journal of Phytopharmacology 2014; 3(1): 52-56

[14] Biomarkers Rausch W, Liu S, Gille G, Radad K (2006) Neuroprotective effects of ginsenosides. Acta Neurobiol. Exp. (Wars)., 66: 369-75.

[15] Broadhurst C, Leigh Polansky MM, Anderson R (2000), Insulin-like biological activity of culinary and medicinal plant aqueous extracts in vitro. J. Agric. Food Chem., 48: 894-52

[16] Builders MI, Isichie CO, Aguiyi JC. (2012) Toxicity Studies of the Extracts of Parkia biglobosa Stem bark in rats. British Journal of Pharmaceutical research. 2(1):1-16.

[17] Calderón-Montaño, J.M., Burgos-Morón, E., Pérez-Guerrero, C. and López-Lázaro, M. (2011). A review on the dietary flavonoid kaempferol. Mini Rev. Med. Chem., 11: 298–344

[18] Edduoks M, Jouad H, Maghrani M, Lemhadri A, Burcelin R. Inhibition of endogenous glucose production accounts for hypoglycemic effect of Spergularaia purpurea in streptozotocin mice. Phytomedicine 2003;10:594 – 599.
[19] Fahey, (2005). Moringa oleifera: A Review of the Medical Evidence for Its Nutritional, Therapeutic and Prophylactic Properties.
[19] Fils, J.M. (2000). The Production of Oils. In: Hamm, W. and R.J. Hamilton, (Eds.), Edible Oil Processing. Sheffield: Sheffield Academy Press, U.K.
[20] Goji ADT, Mohammed A, Tanko Y, Ezekiel I, Okpanchi AO, Dikko AUU. (2010) A Study of the Anti-Inflammatory and Analgesic Activities of Aqueous Extract of Nauclea latifolia leaves in Rodents. Asian Journal of Medical Sciences. 2(6): 244-247.
[21] Hu X, Sato J, Oshida Y, Xu M, Bajotto G, Sato Y. Effect of Goshajinki-Gan(Chinese herbal medicine:Niu-Che-Sen-Qi-Wan) on insulin resistance in streptozotocin induced diabetic rats. Diabetes Research and Clinical Practice. 2003;59:103 – 111.
[22] Idoko Sunday I., Andrew, Adamu and Iniobong Chukwuebuka I. U.(2017). Sub-acute Toxicity StudyofDefatted Moringa oleifera Seed Meal Administration on Wistar Rats; International Journal of Scientific & Engineering Research, Volume 8, Issue 2, February-2017 527 ISSN 2229-5518
[23] Jadhav, S.M., Bipinraj, N.K. and Milind, R.G. (2008). Moringa oleifera-Household Alternative Coagulant for Water Treatment, Paper for National Conference on Household Water Treatment Technology (July 2008) at Hindustan College of Sc. and Tech. Farah, Mathura.
[24] Kokwaro, JO. (1976) Medicinal plants of East Africa. East Africa Literature bureau, Nairobi; p35.
[25] Kapadia, G., Chung, E., Ghosh, B., Shukla, Y., Basak, S., Morton, J. and Pradhan, S. (1978). Carcinogenicity of some folk medicinal herbs in rats. Journal of National Cancer Institute, 60: 683-6.
[26] Kasolo, J. N., Bimenya, G. S., Ojok, L., and Ogwal-okeng, J. W., (2011). Phytochemicals and acute toxicity of Moringa oleifera roots in mice Journal of Pharmacognosy and Phytotherapy Vol. 3(3), pp. 38-42, April 2011.
[27] Li-Weber M (2009). New Therapeutic aspects of flavones: the anticancer properties of Scutellaria and its main active constituents Wogonin, Baicalein and Bacalin. Cancer Treat. Rev., 35:57-68.
[28] Lockelt CT, Calvert. C, Grivetti, L (2000) Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, Northeastern Nigeria. Int. J. Food Sci. Nutr., 51:195-208.
[29] Mabberley, D.J. (2008). Mabberley's Plant Book (3 ed.). Cambridge University Press. ISBN 978-0-521-82071-4.
[30] Marques, T.L., Alves, V.N., Coelho, L.M. and Coelho, N.M.M. (2012). Removal of Ni (II) from aqueous solution using Moringa oleifera seeds as a bioadsorbent. Water Science Technology, 65 (8); 1435-1440.

[31] Michael, L. (2010). Bioremediation of Turbid Surface Water Using Seed Extract from Moringa oleifera Lam. (Drumstick) Tree, Wiley Interscience, Current Protocols in Microbiology (February 2010), 1G.2.1-1G.2.

[32] Nandakuma V, Singh T, Katuiyar S, (2008). Mult-targeted prevention and therapy of cancer by proanthocyanidins. Cancer Lett., 269: 378-87

[33] Ochora J, Friis H, Aagaard-Hansen J (2005). Potential toxicity of some traditional leafy vegetables consumed in Nyang'oma division, Western Kenya. African J. Food Nutr. Sci., 5(1): 1-13

[34] Ogbunugafor, H.A., Eneh, F.U., Ozumba, A.N., Igwo-Ezikpe, M.N., Okpuzor, J., Igwilo, I.O, Adenekan, S.O. and Onyekwelu, O.A. (2011). Physico-chemical and anti-oxidant properties of Moringa oleifera seed oil. In Pakistan Journal of Nutrition, 10 (5): 409-414.

[35] Okei W, Ogunlesi M, Osibote EA, Binutu MK, Ademoye MA. (2011) Comparative Studies of the Antimicrobial Activities of components of Different Polarities from the leaves of Nauclea latifolia. Research Journal of Medicinal plant. 5 (3):321-329.

[36] Okwori AEJ, Okeke CI, Uzoechina A, Etukudoh NS, Amali MN, Adetunji JA. (2008). The antibacterial potentials of Nauclea latifolia; African Journal of Biotechnology; 7 (10):1394-1399.

[37] Okwu, D.E. and Josiah, C. (2006). Evaluation of chemical composition of two Nigeria Medicinal Plant Parts. Journal of Sustainable Agriculture and Environment, 6: 30-37. Orech FO, Akenga T,

[38] Pandey, R.V., Pushpendra, K.T., and Akhilesh, V.S. (2012). Moringa oleifera Lam. A Plant with a plethora of diverse therapeutic benefits: an updated retrospection; Medicinal and Aromatic Plants Journal;DOI:10.4172-2167-0412.10000101

[38] Paliwal, R., Sharma, V., Pracheta, Sharma, S., Yadav, S. and Sharma, S. (2011).Anti-nephrotoxic effect of administration of Moringa oleifera Lam in amelioration of DMBA-induced renal carcinogenesis in Swiss albino mice. Biology and Medicine, 3 (2) 27-35.

[39] Pari L, Amarnath S. Antidiabetic activity of Boerhavia diffusa L.: effect on hepatic key enzymes in experimental diabetes. J Ethnopharmacol 2004; 91: 109 -113.

[40] Quattrocchi, U. (2000). CRC World Dictionary of Plant Names. 3. Baton Rouge, New York, London, Washington DC: CRC ISBN:978-0-8493-2673-8.

[41] Raju J, Patlolla J, Swamy M, Rao C (2004). Diosgenin, a steroid of Trigonella foenum graecum (Fenugreek), inhibits azoxymethane Induced aberrant crypt foci formation in F344 rats and induces apoptosis in HT-29 human colon cancer cells. Cancer Epidemiol

[42] Tedong LP, Dzuefiet DD, Dimo J, Asongalem EA, Soken SN, Flejou JI, Callard P, Kamtchouring P. (2007) Acute and Subchronic Toxicity of Anacardium Occidentale Leaves Hexane Extract in Mice. Africa Journal of Traditional Alternative Medicine. 4:140-147.

[43] Traore-Keita F, Gasquet M, Di Giogio C, O'livier E, Dalmas F, Keita A, Diynbo O, Balansard G, Timon-David P. (2000) Antimalarial activity of four plants used in traditional medicine in Mali. Phytotherapy Research. 14 (1):45-47.

[44] Trease, G.E. and Evans, W.C. (1996). Phenols and phenolic glycosides in Trease and Evans pharmacognosy and Biliere Tindall London, 832.

[45] Udobre AS, Usifoh CO, Eseyin OA, Udoh AE, Awofisayo OA, Akpan AE. (2012). The wound healing activity of methanol extract of the stem bark of Nauclea latifolia International J PharmBiomed Sci. 3(3):136-139.

[46] World Health Organization. (2012) World Malaria Report. WHO, Geneva. pp 1-276.

[47] ZirihiGuédé N, N'guessan K, EtienDibié T, Grellier P. (2010) Ethnopharmacological study of plants used to treat malaria, in traditional medicine, by Bete Populations of Issia (Côte d'Ivoire). Journal of Pharaceutical Science and Research. Vol.2 (4), 216-227. XE "[1] Adedapo AA, Mogbijuri.OM, Emikpe BO (2009), Safety evaluations of the aqueous extract of the leaves of Moringa oleifera in rats. J.of Med. Plants Res., 3(8)\:586-591." \t "*See*" \i