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# DOSE RESPONSE OF AQUEOUS, METHANOLIC, AND LIME JUICE EXTRACTS OF COMBINED MORINGA OLEIFERA AND VERNONIA AMYGDALINA IN RABBITS.

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**ABSTRACT**
Over the years, medicinal plants have been used not only as a source of food but also in the treatment of many diseases in various parts of the world. In Nigeria, *Moringa oleifera* and *Vernonia amygdalina* are some of the common plants whose parts have contributed to the increased intake of essential nutrients and health-promoting phytochemicals. The lime, aqueous and ethanolic extracts of combined *Moringa oleifera* and *Vernonia amygdalina* was studies for their toxic effect and thus the LD50 was determined. It was seen that lime extract had the highest weight loss effect and also the highest mortality rate, however the LD50 values for the three extracts of 900mg/kg, showed that the extracts had an acceptable LD50 values and thus its use in trado-medicine is left uncontested.

**Keywords:** *Moringa oleifera, Vernonia amygdalina, LD50, extracts*

## INTRODUCTION

Over the years, medicinal plants have been used not only as a source of food but also in the treatment of many diseases and disorders in various parts of the world (Reference). In Nigeria, *Moringa oleifera* and *Vernonia amygdalina* are some of the common plants whose parts have contributed to the increased intake of essential nutrients and health-promoting phytochemicals (Bamisaiye *et al.,* 2011). These phytochemicals include tannins, pterygospermin, alkaloids, carbohydrates, terpenoids, flavonoids, phenols and saponins, which are commonly found in different parts of these plants using different extracting media like distilled water (aqueous extract), petroleum ether, methanol, ethanol and chloroform (Arun *et al.,* 2011; Audu *et al.,* 2012). *Moringa oleifera*, also called 'Drum stick' or 'Raddish tree' is known specifically to contain a rich and rare combination of zeatin, quercetin, beta-promsitosterol, cafteoyl, quinnic acid kaempherol and hydroxyl-anthraquinones from different parts of the plant at different stages of maturity (Kasolo *et al.,* 2011). Studies have shown that the leaves of *Moringa oleifera* have curative potentials in conditions of chronic hyperglycemia and hyperlipidemia which are symptoms of diabetes and cardiovascular diseases respectively (Asaolu *et al.,* 2010). Further reports, as documented by Gupta *et al.* (2010), have shown that extracts of *Moringa oleifera* is capable of modulating cellular immunity in animal models. Fruit and seed extracts of this very plant have been suggested to exhibit inhibitory virtues against HIV-1 reverse transcriptase (Momoh *et al.,* 2010). Arun *et al.* (2011) also reported the antibacterial properties of *Moringa oleifera*.

*Vernonia amygdalina* is a perennial shrub common in Nigeria and is known to contain carotenes, ascorbic acid, alpha-tocopherol, gluthathione peroxidase, superoxide dismutase and catalase, making it an outstanding antioxidant capable of mopping up free radicals that easily destroys tissues (Oyugi *et al.,* 2011). Previous studies have shown its effectiveness against dysentery, gastrointestinal disorders, microbial and parasitic infections, hepatotoxicity, cancer and hypertension (Momoh *et al.,* 2010). All of the studies conducted on these plants (*Moringa oleifera and Vernonia amygdalina*) were carried out on each plant separately; not much has been documented regarding the toxicological impact of the combination of these two plants. The study of the combination of these two plants is becoming of scientific interest because there is an increase intake of both plant leaves in food diets.

Citrus Aurantifolia, with generic name Lime, is the source from which we get Lime juice, a solvent for one of the extracts. It is consumed throughout the world in sorbets, beverages, refreshing drinks, etc. and the oil extracted from its peel is extensively used in soft drink concentrates, beauty products and toiletries (Ahmad *et. al.,* 2006). The health benefits of lime include weight loss, skin care, good digestion aid, etc. (Kawaii *et al.,* 2000). When lime is applied on human skin, its acids scrub out the dead cells, cures dandruff, rashes, bruises etc. Flavonoids found in lime help breakdown foods macro molecules, stimulate the digestive system and increase secretion of digestive juices (Ahmed et al., 2006), increase secretion of bile and acids and also stimulate the peristaltic motion. (Mohanapriya, 2013).

The acids present in lime helps clear the excretory system by washing and cleaning off the tracts, flavonoids inhibit microbial growth and potassium (Burt, S.A., 2004). Since lime helps to heal up ulcers and wounds in the digestive system and excretory system and gives relief from constipation too, it eradicates all the root causes of piles. The citric acid present in lime is an excellent fat burner (Mohanapriya, 2013). The high potassium content of limes is very effective in removal of the toxic substances and the precipitates deposited in kidneys, urinary bladder and its disinfectant properties help cure infections in the urinary system.

This research was aimed at determining the lethal dose (LD50) of *Moringa oleifera* and *Vernonia amygdalina* (here from refered to as Moringa and Vernonia) extracted in lime juice, water (aqueous and ethanol) for the treatment of diseases. The LD50 allows for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.

## MATERIALS AND METHODS

### Plant Material and Preparation

*M. oleifera* and *V. amygdolina* leaves were harvested at night from farm site in Anyigba Community, Kogi State, North Central Nigeria. Specimens were air-dried for three (3) weeks and then pulverized to powder. 90g of ground *V. amygdalina* leaves sample and 150g of ground *M. oleifera* leaves sample mixed together were soaked in 1000ml of ethanol, lime and distilled water (aqueous) for three (3) days respectively. The extracts of the various solutions were then concentrated in-vacuo to give the ethanol, lime and aqueous extracts respectively. The powder was further dissolved in 20ml of distilled water, packaged in various containers and kept in the refrigerator at 4°C.

**Animal Preparation**

Three groups containing ten (10) rabbits, weighing between 1.23 – 1.51kg in each group were used in the Oral Toxicity Study while three groups containing ten (10) rats, 200 – 250g were used in the Toxicity study. Each group received a dose of each of the extract of combined Moringa and Vernonia and administrations were done orally. Animals were weighed before the dose administration. All the animals were kept under continuous observation for 6 hours after the administration of the dose, for any change in behavior or physical activities. After 24 hours, all surviving mice were anesthetized with pentothal sodium (40 mg/kg) and autopsied. Conversely the time interval between the treatment groups was determined by the onset, duration, and severity of toxic signs. Observation of signs of appearance and disappearance of the toxicity were noted. The weights of the animals during the experiment were also determined and differences in weights were calculated. At the end of the experiment the surviving animals were weighed and humanely killed for histopathological analysis.

**Administration of Extracts**

The acute oral toxicity study was conducted using the up and down procedure according to OECD test guidelines on acute oral toxicity test 401 (OECD, 2001). Ten rabbits was used for the each of the extracts. The three extracts of combined Moringa and Vernonia, which are Ethanol, Lime Juice and Aqueous extracts, were administered to rabbits by varying the doses according to their body weights by gavage through oral intubation. A standard solution of 750g/L was prepared for all the extracts from which required volume corresponding to the required weight of extract per volume was taken. The weight of extract required was dependent on the weight of the animal to receive the extract. The rabbits were fasted for 24 hours but not deprived of water. The initial weights of animals were recorded prior to the administration of the extracts.

For the LD50, different doses of the three extracts were administered orally to the rats, each group receiving one extract but varying doses according to their body weight. Signs of toxicity and possible death of animals were monitored for 24 hours to calculate the median lethal dose (LD50). At the end of the study, all the animals in all the dose groups were sacrificed and the liver and kidney were processed for histological analysis.

## RESULTS AND DISCUSSION

Table 1: Oral Toxicity Result for the Lime Extract of Combined Moringa and Vernonia.

| Group | Initial Bwt (kg) | Administration (mg/ml) | Observation | Final Bwt (kg) | Bwt Difference (kg) |
|---|---|---|---|---|---|
| 1. | 1.34 | 15,000/20 | Weak, loss of appetite after 35 minutes and regained strength after 5 hours | 1.28 | 0.06 |
| 2. | 1.39 | 15,000/20 | Restless, staggered, coma and died after 40 minutes | 1.29 | 0.10 |
| 3. | 1.32 | 15,000/20 | Restless, loss of appetite and regained strength after an hour | 1.30 | 0.02 |
| 4. | 1.40 | 11,250/15 | Restless, loss of appetite and regained strength after an hour | 1.31 | 0.09 |
| 5. | 1.51 | 11,250/15 | Restless, loss of appetite and regained strength after an hour | 1.42 | 0.09 |
| 6. | 1.47 | 11,250/15 | Restless, loss of appetite and regained strength after an hour | 1.41 | 0.06 |
| 7. | 1.18 | 15,000/20 | Brief coma and died after 30 minutes | 1.05 | 0.13 |
| 8. | 1.30 | 15,000/20 | Survive | 1.10 | 0.20 |
| 9. | 1.29 | 11,250/15 | Survive | 1.26 | 0.03 |
| 10. | 1.23 | 11,250/15 | Survive | 1.20 | 0.03 |

Bwt/kg = body weight/kilogram; Mean of the difference in weight for Group A = 0.07 and Group B = 0.10. While Standard deviation for A = 0.03 and B = 0.08

Table 2: Oral Toxicity Result for the Aqueous Extract of Combine Extract of *Moringa and Venonia*

| Group | Initial Bwt (kg) | Administration (mg/ml) | Observation | Final Bwt (kg) | Bwt Difference (kg) |
|---|---|---|---|---|---|
| 1. | 1.38 | 30,00/40 | Weak & loss of appetite | 1.49 | 0.11 |
| 2. | 1.43 | 30,000/40 | Weak & loss of appetite | 1.46 | 0.03 |
| 3. | 1.25 | 30,000/40 | Weak & loss of appetite and occurrence of death after 6hrs | 1.25 | 0.00 |
| 4. | 1.52 | 30,000/40 | Loss of appetite | 1.56 | 0.04 |
| 5. | 1.01 | 30,000/40 | In coma for 48hrs and recovered | 1.04 | 0.03 |
| 6. | 1.52 | 30,000/40 | In coma for 4days and recovered | 1.56 | 0.04 |
| 7. | 1.02 | 30,000/40 | Loss of appetite, coma and death after 6hrs | 1.05 | 0.03 |
| 8. | 0.93 | 30,000/40 | Loss of appetite for 4days and gradually recovered | 0.99 | 0.06 |
| 9. | 1.15 | 30,000/40 | Recover after 24hours | 1.20 | 0.05 |
| 10. | 1.22 | 30,000/40 | Recover after 24hours | 1.27 | 0.05 |

BWT = Body Weight, BWTG = Body Weight Gained; Mean of the weight gained = 0.04 and standard deviation = 0.03

Table 3: Oral Toxicity Result for the Ethanol Extract of Combine Moringa and Venonia

| Group | Initial BWT (kg) | Administration (mg/ml) | Observation | Final BWT (kg) | BWTG Difference (kg) |
|---|---|---|---|---|---|
| 1. | 2.80 | 22,000/29 | Coma and regain in 7days | 2.91 | 0.11 |
| 2. | 2.15 | 22,000/29 | Coma and recovered in 5days | 2.17 | 0.02 |
| 3. | 2.13 | 22,000/29 | Coma and dead after 6hours | 2.15 | 0.02 |
| 4. | 2.14 | 22,000/29 | Coma and regained after 3hours | 2.13 | -0.01 |
| 5. | 2.70 | 22,000/29 | Coma and recovered after 4hours | 2.75 | 0.05 |
| 6. | 2.30 | 22,000/29 | Coma recovered after 4hours | 2.40 | 0.10 |
| 7. | 2.70 | 22,000/29 | Coma, weak and dead within 8hours | 2.73 | 0.03 |
| 8. | 2.40 | 22,000/29 | Coma and regained after 6hours | 2.41 | 0.01 |
| 9. | 2.30 | 22,000/29 | Coma and regained after 7hours | 2.31 | 0.01 |
| 10. | 3.12 | 22,000/29 | Coma and dead after 10hours | 3.19 | 0.07 |
| | | | | | |

BWT = Body Weight, BWTG = Body Weight Gained; Mean and Standard deviation are 0.05 and 0.04 respectively.

Table 4: Toxicity of lime extract of combined Moringa oleifera and Vernonia amygdalina

| Groups | Dose/day (mg/kg) | Mortality | Symptoms |
|---|---|---|---|
| 1 | 555.7 | nil | Weak, loss of appetite after 35min and regained strength after 5 hours |
| 2 | 539.5 | Yes | Restless, staggered, coma and died after 40 minutes |
| 3 | 568.1 | Nil | Restless, loss of appetite and regained strength after an hour |
| 4 | 535.7 | Nil | Restless, loss of appetite and regained strength after an hour |
| 5 | 496.7 | Nil | Restless, loss of appetite and regained strength after an hour |
| 6 | 510.2 | Nil | Restless, loss of appetite and regained strength after an hour |
| 7 | 635.6 | Yes | Brief coma and died after 30 minutes |
| 8 | 576.9 | Nil | Survive |
| 9 | 581.4 | Nil | Survive |
| 10 | 609.8 | Nil | Survive |

\Table 5: Toxicity of aqueous extract of combined Moringa oleifera and Vernonia amygdalina

| Groups | Dose/day (mg/kg) | Mortality | Symptoms |
|---|---|---|---|
| 1 | 543.5 | Nil | Weak & loss of appetite |
| 2 | 524.5 | Nil | Weak & loss of appetite |
| 3 | 600.0 | Yes | Weak & loss of appetite and occurrence of death after 6hrs |
| 4 | 493.4 | Nil | Loss of appetite |
| 5 | 742.6 | Nil | In coma for 48hrs and recovered |
| 6 | 493.4 | Nil | In coma for 4days and recovered |
| 7 | 735.3 | Yes | Loss of appetite, coma and death after 6hrs |
| 8 | 806.5 | Nil | Loss of appetite for 4days and gradually recovered |
| 9 | 652.2 | Nil | Recover after 24hours |
| 10 | 614.8 | Nil | Recover after 24hours |

Table 6: Toxicity of ethanol extract of combined Moringa oleifera and Vernonia amygdalina

| Groups | Dose/day (mg/kg) | Mortality | Symptoms |
|---|---|---|---|
| 1 | 270.9 | Nil | Coma and regain in 7days |
| 2 | 352.8 | Nil | Coma and recovered in 5days |
| 3 | 356.2 | Yes | Coma and dead after 6hours |
| 4 | 354.5 | Nil | Coma and regained after 3hours |
| 5 | 281.0 | Nil | Coma and recovered after 4hours |
| 6 | 329.8 | Nil | Coma recovered after 4hours |
| 7 | 281.0 | Yes | Coma, weak and dead within 8hours |
| 8 | 316.1 | Nil | Coma and regained after 6hours |
| 9 | 329.8 | Nil | Coma and regained after 7hours |
| 10 | 329.8 | Yes | Coma and dead after 10hours |

Table 7: Table Showing The Loss in Weights Caused by The Three Extracts from lowest to highest

| Extract | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Lime | 0.02 | 0.03 | 0.03 | 0.06 | 0.06 | 0.09 | 0.09 | 0.10 | 0.13 | 0.20 |
| Aqueous | 0.00 | 0.03 | 0.03 | 0.03 | 0.04 | 0.04 | 0.05 | 0.05 | 0.06 | 0.11 |
| Ethanol | -0.01 | 0.01 | 0.01 | 0.02 | 0.02 | 0.03 | 0.05 | 0.07 | 0.10 | 0.11 |

D = Difference; Differences arranged in ascending order for each extract

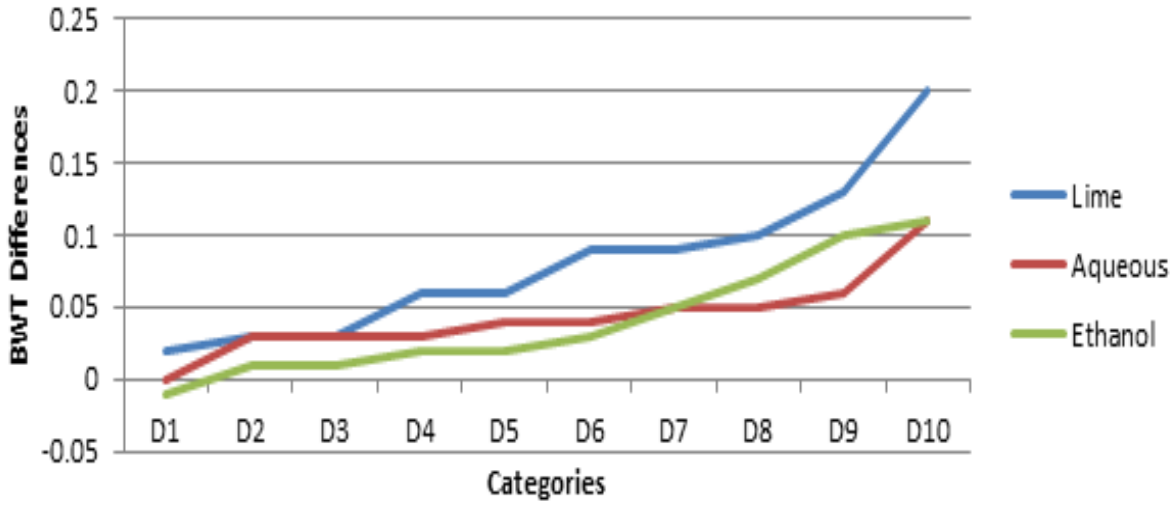
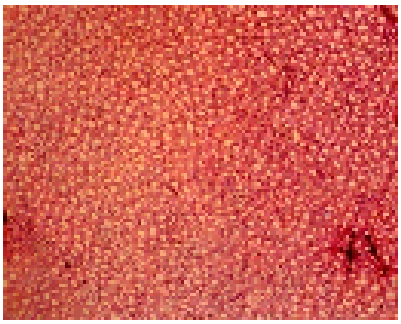
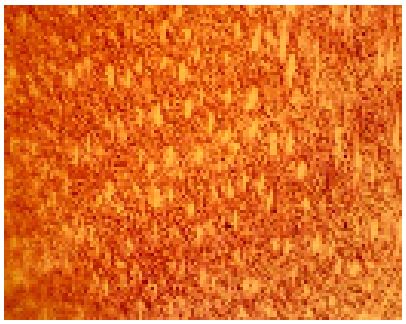
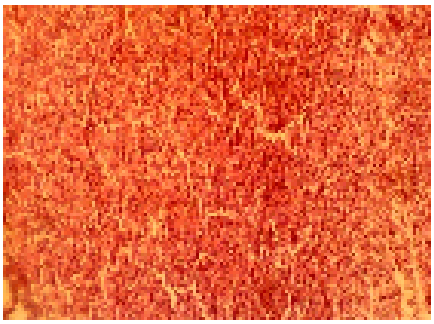
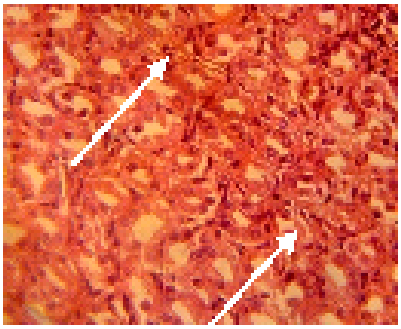
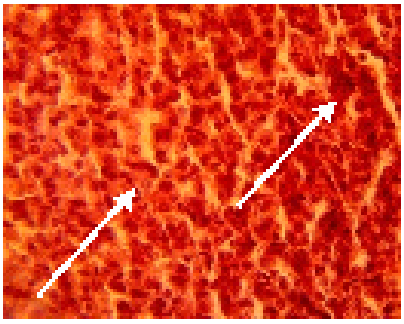
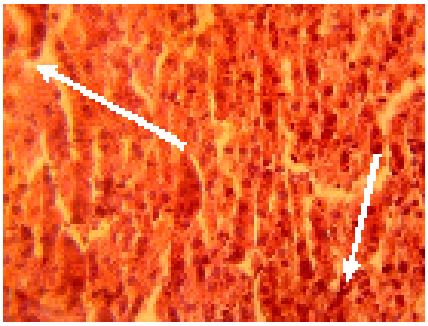


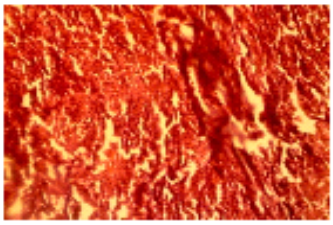
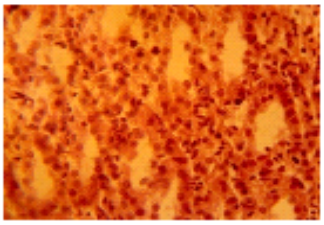
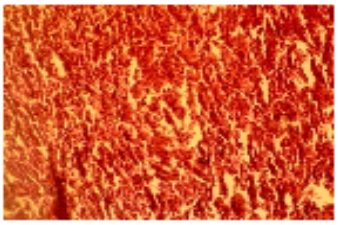
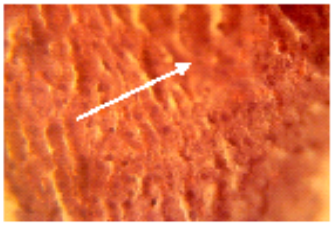
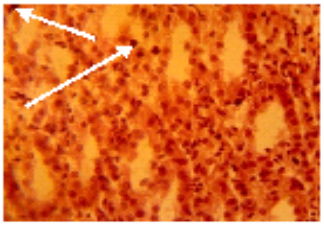
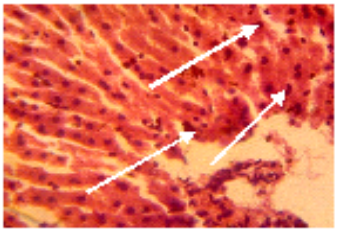

Figure 1: Graph Showing Comparison between Body Weight Losses Due to Each Extract

**The Photomicrographs Display the Histopathological Features of the Organs in the Three Solvents Used**

Kidney:

| Aqueous Extract | Lime Extract | Methanolic Extract |
|---|---|---|
|  |  |  |
| Kidney x10 | Kidney Mgx10 | Kidney x10 |
|  |  |  |
| *The liver section showing a mild edema, surface blebs and leaving the cells intact* | *Severe lost tubular arrangement and diffused necrosis (Arrowed)* | *The medulary region of the organ sectioned showed necrosis* |
| Kidney x40 | Kidney Mgx40 | Kidney Mgx40 |

Liver:

| Aqueous Extract | Lime Extract | Methanolic Extract |
|---|---|---|
|  |  |  |
| Liver Mg x10 | Liver x40 | Liver Mgx10 |
|  |  |  |
| *Liver section showing mild edema, and severe heamorrhage.* | *Liver section showing hydropic degeneration and severe hepatic necrosis.* | *Moderate hydropic degeneration and diffused necrosis(Arrowed)* |
| Liver Mg x40 | Liver Mg x40 | Liver Mgx40 |

The weight loss observed in each of the extracts administrations were arranged in ascending order and compared graphically. The graph above (Fig. 1) shows that the lime extract produced a rate of (or gradient in) weight loss. It is of interest to observe that the lime extract with the highest gradient on the graph also has the highest number of mortality of 3. This increased weight loss may be due to the weight loss effect of lime ingestion, thus the weight loss and the "normal" side effect of the lime extract resulted in the high mortality when compared to the other two extracts. The aqueous and ethanol extracts showed very similar differences and gradient. While the aqueous and ethanol have close range of 0.11 and 0.12 respectively that of lime was 0.18. The aqueous and ethanol extracts having close resemblance in their weight loss have same number of mortality, 2. The reasons for this closeness in the effect of these two extracts, aqueous and ethanol may probably be attributed to their closeness in polarity, miscibility with water, and the that they are a singular solvent with respect to composition. Though it is expected at lime juice should be very miscible with water, but probably the non-singularity of lime juice was responsible for its different effect on the animals compared to the other two extracts. Lime juice is much of a mixture compared to the water and ethanol.

The response during and after the administration of extracts are clearly showing in Tables 1, 2 and 3 in the above, which experimentally assessed the lethal doses of various solvents. In the lime extract, after the administration of 15,000mg/20ml, there was a difference in weight lost by the animal. Weakness, loss of appetite and restlessness were observed.

The histological section of the liver showed evidence of hydropic degeneration and severe hepatic necrosis as in the case of its kidney. However, in aqueous extract there was a great contrast in weight gain after the administration of 30,000mg/40ml/body weight. The observation of body weakness, loss of appetite and comatose was quite visible after some hours and similarly deaths recorded were after 6 hours interval. The majority recovered after 24hours to four (4) days. The histological review of the organ showed mild tubular edema and foci necrosis of the kidney. Similarly, the liver section showed mild edema, surface blebs of the cytoplasm with mild hemorrhage. Whereas the ethanolic extract was given at the dosage of 22,000mg/29ml leaving the animals with a slight gain in weight, restlessness, loss of appetite and regain strength after 6 hours to 24hours was observed, the death recorded was after 6hours of administration. Histological review showed moderate necrosis with little or no edema while the kidney showed necrosis at the medulary region. The death of the rabbits in the groups of ethanolic and aqueous extracts were caused by accumulation of excessive fluid and not as a result of toxicological effect of the plants. This evidence was proved by Thevenon *et al.,* (2013) as the fluid condition outside the cells becomes excessively low in amount of solutes such as sodium and other electrolytes in comparison to that inside the cells causing the fluid to shift through into the cells to balance its concentration. This eventually leads to swelling and perhaps rupturing of the cells. This is obvious in this experiment as the damaged tissue showed hemorrhage and edema.

However, in the lime juice extract, the review of the histology showed some damages such as necrosis tubular damage and hepatic death. Though these are found in the aqueous and methanol extract effects, but in a limitated state. Perhaps this is contrary to the recent study done by Asiedu-Gyeke *et al.* (2014) which investigated the oral toxicity of Moringa oleifera only and not in combination with Vernonia amygdalina to have no adverse effect on rabbit.

The LD50 of the aqueous extract of combined *Moringa oleifera* and *Vernonia amygdalina* was found to be 900mg/kg body weight upon oral administration in rabbits while that of combined methanolic extract of Moringa oleifera and Vernonia amygdalina was found to be 900mg/kg body weight. The LD50 of the lime juice extract of combined *Moringa oleifera* and *Vernonia amygdalina* was found to be 900mg/kg body weight. According to Hodge and Sterner toxicity scale, the obtained LD50 values classified the aqueous, methanolic and lime juice extracts of combined *Moringa oleifera* and *Vernonia amygdalina* as moderately toxic herbal medicine.

## CONCLUSION

Individual solvent that were used in this combined ratio of 5:3 of Moringa oleifera and Vernonia amygdalina when taking is less harmful provided this very established lethal doses (LD50) is not exceeded. However it is strongly believed, based on existing information that nearly half of the LD50 when consumed could be very efficacious in the management of some ailment. A very good example is the role of lime in the control of weight which has been practically demonstrated in this work. Lime as a solvent for the extraction of these leaves might contain active ingredients that could be of medicinal use. Therefore, based on the characteristic features demonstrated by these extracts, and the histological results, they can be used as agents of pharmaceutical formulations. It is thus recommended that other combined parts of these two plants can be studied for their degree of toxicity e.g. the seeds and stems.

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