ACUTE TOXICITY OF ETHANOL ROOT EXTRACT OF *Moringa oleifera* LAM. IN SWISS MICE

By

Osatohanmwen OPUTE

SR/2041/RPR/22/128

UNIVERSITY OF BENIN

BENIN CITY

AUGUST, 2023.

ACUTE TOXICITY OF ETHANOL ROOT EXTRACT OF *Moringa oleifera* LAM. IN SWISS MICE

By

Osatohanmwen OPUTE

A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (BSC.) IN PLANT BIOLOGY AND BIOTECHNOLOGY.

AUGUST, 2023.

CERTIFICATION

We certify that this research wok was carried out by Opute Osatohanmwen of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

Dr. Odaro Timothy _____

(Project Supervisor) Signature and

Date

Prof. E.D Vwioko _____

(Head of Department) Signature and

Date

DEDICATION

This work is dedicated to God almighty and to my invaluable parents, Mr and Mrs. Opute and to my wonderful supervisor Dr. Odaro Timothy.

ACKNOWLEDGEMENT

My utmost gratitude goes to God almighty, for His enabling grace all through the period of this project.

Also, I would love to sincerely appreciate the following persons; my kind-hearted, ever-warming and always responsive supervisor, Dr. Odaro Timothy for his support and immense contributions to the success of this work, Dr. Benjamin for his guidance, Prof. D.E Vwioko and the members of staff of the Plant Biology and Biotechnology.

My friends; Hokage, Nosa Ebeuku, Ezama Alvin, Uchiha Marvelous and Dylan for their encouragement and support. Also my fellow project colleagues and coursemates for their co-operation.

This acknowledgement would not be complete without my deep appreciation to my parents Mr and Mrs. Opute, they have thoroughly supported me for everything I have done. I really am truly thankful for such parents.

Special thanks also goes to my siblings; Osazeme, Phil, Osariemen, Gwain. They have never failed in carrying out their responsibilities as siblings. It is a great privilege to have such a great family. You are all very dear to me.

TABLE OF CONTENT

LIST OF TABLES

LIST OF PLATES

ABSTRACT

Moringa oleifera lam is a tree species belonging to the family moringaceae. It is widely appreciated for its ornamental and medicinal attribute. This study was carried out to determine the acute toxicity of *M. oleifera* in Swiss mice to determine the LD_{50} and observe any physiological and behavioral changes. Fresh roots of the *M. oleifera* were prepared by soaking dried powdered roots of the plant species in absolute ethanol for 72 hours. After filtering and concentrating, various doses of the extract were prepared for administration. Twenty-eight adult male Swiss mice weighing between 30-35 g were used. In phase I, twelve mice were randomly assigned into four groups (I – IV) of three mice each. Groups I - III received single oral doses of 10, 100, 1000 mg/kg respectively of *M. oleifera* ethanol root extract, and the control (Group IV) received distilled water. After 24 hours observation, Phase II experiment was conducted with three mice per group receiving single doses of 1600, 2900, 5000 and 10000 mg/kg of the extract respectively, control was given distilled water. They were observed for 14 days after administration. There was no mortality in both phases and no observable alterations in the mice treated with the *M. oleifera* ethanol root extract. Even at a high dosage of 10000 mg/kg, no observable alterations such as Writhing, Pilo-erection, Jerking, Lacrimation, Salivation, Hemorrhage and Nausea were observed. The lethal dosage (LD_{50}) of the ethanol root extract was determined to be >10000 mg/kg, which implies a relatively high safety limit.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Over the years, medicinal plants have gained a drastic growth and popularity in the making of herbal medicines. It is known in most developing countries but it has gained popularity in developing and developed countries because of its natural factor and lesser side effects. Herbal drugs have a major share in some recognized health systems in some countries like India. Acute toxicity testing plays a crucial role in evaluating the potential harmful effects of substances on living organisms.

Moringa oleifera lam belongs to the family Moringaceae, also known as drumstick or horse radish tree, commonly referred to as Moringa. They are native to Himalayan region of southern Asia, ranging from northeastern Pakistan to northern West Bengal of India but the species are widely cultivated in Africa and Southern America (Ayerza, 2011).

1.2 BOTANICAL DESCRIPTION

M. oleifera can grow up to about 9m (30 feet), the leaves are bi- or tripinnately compound and have oval shaped leaflets with conspicuous swellings where the parts join. The fruits of *M. oleifera* sometimes grow to 45 cm long and are dehiscent. The plants bear scented clusters of white pea like flowers with five stamens (male parts) held to one side. It requires tropical and subtropical regions and grows at a temperature of about 25–35 °C typically grown in tropical and subtropical regions across the globe. They are often called Miracle tree because

of its medicinal, nutritional and pharmacological benefits that has led to its popularity. They have been widely used as a source of food and traditional medicine for the treatment of various illnesses, water purification and natural supplements (Anwar *et al*., 2007; Fahey, 2005).

Plate 1: *M. oleifera* Tree (Source: Opute, 2023).

Plate 2: *M. oleifera* roots (Source: Opute, 2023).

Plate 3: *M. oleifera* leaves (Source: Opute, 2023).

Various parts of Moringa have been found to be very medicinal (Farooq *et al*., 2007). Some studies have shown the therapeutic benefits of moringa as shown below.

Regarding the Moringa leaves, a study was carried out to evaluate the toxicity and safety in recognized animal models used for allopathic medicines. This study indicated that oral administration of *Moringa oleifera* dried leaf powder up to 2000mg/kg showed no changes in clinical signs or gross pathology and that the LD50 was greater than 2000mg/kg (Moodley, 2017).

Another study was carried out to investigate the nutritional and phytochemical composition and 28-day dose toxicity and genotoxicity of *M. oleifera* leaves infusion and powder. Behavior alterations were observed in the first 2hours after administration at 5000mg/kg. Infusion did not present toxicity when administered for 28 days. 500 and 1000mg/kg promoted liver and kidney damages. Genotoxicity and mutagenicity were not detected at 2000mg/kg (Matheus *et al*., 2022)..

A study was carried out to determine the safety of methanolic extract of *M. oleifera* bark by performing acute and sub-acute (28 days) oral toxicity studies in Swiss albino mice. In all these tests no significant difference was observed between treated (extract administered) and control group mice. Histopathological examination of organs from the mice treated with extract at 2000 mg/kg b.wt for 28 days did not show any toxic effects compared to control group. Current study determined the non-toxic nature of oral administration of *M. oleifera* bark extract (Reddy *et al*., 2013).

A study was also carried out to determine the acute toxicity of *M. oleifera* using Swiss albino mice. A single oral dosage was given and observed for 24hours. The *M. oleifera* roots were found to contain protective phytochemicals and were relatively non-toxic when given in a single dose (Kasolo *et al*., 2011).

1.3 ETHNOMEDICINAL PROPERTIES

Throughout civilization, *M. oleifera* has been used because of its therapeutic effects. Traditional medicines have been made in various parts of the world to cure various ailments making use of all the parts of the tree (i.e leaf, fruits, bark, gum, flower, seed, seed oil, and root) e.g making use of the leaves of *M. oleifera* to make vegetable soup in Nigeria, etc. Moringa has been observed to be antihypertensive, anti-anxiety, anti-diarrheal and as a diuretic. Moringa is also used to treat dysentery. Moringa leaves is a helps as a remedy for inflammatory conditions such as glandular inflammation, headache, and bronchitis. The pods treat hepatitis and relieve joint pain. The roots are used to treat kidney stones, liver diseases, inflammation, ulcers, and pain associated with the ear and tooth. The bark of the stem is used to treat wounds and skin infections. The gum extracted from this plant to treat fever. The seeds of the plant act as a laxative and are used in the treatment of tumors, prostate, and bladder problems. Preparations from the plant leaves benefit nursing mothers and malnourished infants leading improved general public health. The leaves have been useful for patients suffering from insomnia and treating wounds. Moringa has gained popularity in cosmetic industries, and in ancient Egyptian history, it was used for preparing dermal ointments.

1.4 PHARMOCOLOGICAL PROPERTIES

Research has shown that various parts of the part contribute to the improvement of human health. Some pharmacological properties of Moringa include:

Antioxidant activity:

Moringa is rich in bioactive compounds, including glycosylates, isothiocyanates, thiocarbamates, flavonoids, and certain other compounds. These antioxidants help neutralize free radicals, reducing oxidative stress and protecting cells from damage.

Anti-inflammatory effects:

Studies have shown that Moringa extracts have anti-inflammatory properties, which may help reduce inflammation in the body. The plant contains active compounds like tannins, phenols, alkaloids, flavonoids, carotenoids β-sitosterol, vanillin, and moringin, all of which possess anti-inflammatory properties. In a study using *M. oleifera* leaf extract on mice with atopic dermatitis, it effectively reduced the expression of mannose receptor mRNA, thymic stromal lymphopoietin, and retinoic acid-related orphan receptor γT in ear tissues.

Antimicrobial and antibacterial activity:

Moringa extracts have demonstrated antimicrobial activity against various bacterial, fungal, and viral pathogens. It contains various compounds with potent antimicrobial and antifungal effects against a wide range of microbes and fungi.

Antidiabetic potential:

Research indicates that Moringa extracts may have a role in managing diabetes by reducing blood glucose levels and improving insulin sensitivity. The plant's ability to regulate glucose metabolism makes it a subject of interest in diabetes research.

Anticancer properties:

Preliminary studies suggest that Moringa extracts may possess anticancer activity by inhibiting the growth of cancer cells. However, further research is needed to fully understand and validate its potential in cancer treatment.

Cardiovascular benefits:

Moringa has shown promise in significantly lowered cholesterol levels by showing a protective effect on hypertensive rats. This could be beneficial in preventing cardiovascular diseases and promoting heart health.

1.5 LIMITATIONS

This project focuses on acute testing which involves observations of immediate adverse effects of *M. oleifera* extract on the animals. Therefore, long-term effects and chronic toxicity were not observed and addressed.

1.6 AIM OF THE STUDY

This study focused on evaluating the level of acute toxicity of *M. oleifera* ethanol root extract in Swiss mice.

1.7 OBJECTIVES OF THE STUDY

The primary objectives of the study were to;

- determine the mean lethal dose (LD_{50}) of *M. oleifera* root extract in mice.
- To access the level of mortality due to administration varying doses of *M. oleifera* root extract in mice after 24-hours and two weeks.
- investigate the acute toxicity of the plant extract on physiological attributes within two weeks in mice .

CHAPTER TWO

MATERIALS AND METHODS

2.1 PLANT COLLECTION AND IDENTIFICATION

The *Moringa oleifera* roots were procured from Obe quarters, Sapele road, Benin City, Edo state. It was later identified in the Department of Plant Biology and Biotechnology.

2.2 PREPARATION OF SAMPLE

Fresh roots of the plant samples were rinsed in clean water, sliced into pieces and shade-dried for fourteen (14) days. It was further oven dried at 45 ^{0}C for 24 hours. The powder root samples were weighed separately using various ration to acquire polyherbal mixture and extracted with ethanol using maceration technique for 72 hours with intermittent stirring and shaking. It was filtered and the filtrate was concentrated using crucibles on water bath, to concentrate into semi-solid. Percentage yield was calculated using the formula below

$$\% \text{ yield of extract} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times \frac{100}{1}$$

2.3 EXPERIMENTAL ANIMALS

Twenty-eight (28) Swiss mice weighed 30-35g of male sex was obtained in the Department of Biochemistry animal house, and House in the Unit of Phytomedicine, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo state for the experimental study. They were randomly divided into 8 groups (n=3). The ani-

mals were acclimatized to laboratory condition for fourteen days (14) prior to the experiment and were allowed free access to grower pellet diet and water *ad libitum*. Animals were fasted overnight with free access to water prior to each experiment. The ethical committee for the use of animals' guild was adhered to during the course of this study.

2.4 ETHICAL CONSIDERATIONS

The study will be conducted following ethical guidelines and regulations concerning animal experimentation (AREC, 1985). All efforts will be made to minimize animal suffering and ensure their well-being.

2.5 ACUTE TOXICITY STUDY

Acute toxicity study:

Determination of acute toxicity using modified method by Lorke (1983) and class method in adherence to the association of Economic Cooperation with Development (OECD) Instruction used in Testing Chemical No. 423 OECD, (2001). This was performed in two phases each for *M. oleifera* methanol root extract. Phase 1, twelve (12) mice were randomly divided into four (4) groups of three (3) each. Extracts were administered in a single dose of 10, 100 and 1000 mg/kg orally. Observable toxicological signs were recorded. With absence toxicity, while second phase was experimented.

Depending on the acquired results, phase 1, Phase 2 is schedule with three (3) mice per group administered with single doses (1600, 2,900 and 5,000 mg/kg) of the extract orally. Thereafter, the animals in both phases were left under similar conditions observed for general behavioral changes continuously for 30 minutes, every hour during the first 24 hours and once per

day for 14 days subsequent to administration of the extract. Observations were focused on parameters such as pilo-erection, sensitivity to sound and touch, locomotion, aggressiveness, appearance of feces, salivation, urinating, convulsing, coma and death. The number of survivors was noted after 24 hours. Animals' weights were taken at 0, 7 and 14 days. At the end of the study, all surviving animals were sacrificed and some internal organs such as lungs, liver, kidneys, spleen, stomach, intestine, and ovaries were removed and weighed. A gross pathological examination of these organs was also performed. The LD_{50} was evaluated and *Cu*AE classified according to the Globally Harmonized System (GHS) for the classification of chemicals (OECD, 1998). The LD_{50} was calculated based on the final results in square root (of product) with lowest fatal dose and highest non-fatal dose (geometric mean at repeated doses where 0 and 100 % as the survival rates recorded). Extracts above 10000 mg/kg doses stimulate no possible observable toxicity signs or death; extracts were certified safe. The control was orally given 1 ml distilled water.

Rats used in acute toxicity study were pre-administered daily for possible toxicity signs over a period of two weeks.

The LD_{50} calculation using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose with no mortality,

D_{100} = Lowest dose with mortality.

CHAPTER THREE

RESULTS

3.1 ACUTE TOXICITY EFFECT – PHASE I

Table 1 shows that no mortality/adverse effect was observed across the treatments 10 – 1000 mg/kg of the root extract.

Table 1: Phase I of acute toxicity (24 hours) assessment of *Moringa oleifera* ethanol root extract in mice

Observations	Control	Dose of plant extract (mg/kg)		
		10	100	1000
No. of mortalities	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
% mortalities	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Adverse effects	Nil	Nil	Nil	Nil

Values are expressed as Mean ± SEM; n=3; Control= Distilled water.

3.2 ACUTE TOXICITY EFFECT – PHASE II

Table 2 shows that no mortality/adverse effect was observed across the treatments 1600 – 5000 mg/kg of the phase II root extract treatment.

Table 2: Phase II of acute toxicity assessment of *Moringa oleifera* ethanol root extract in mice.

Observations	Control	Dose of plant extract (mg/kg)		
		1600	2900	5000
Number of mortalities	0.00	0.00	0.00	0.00
% mortalities	0.00	0.00	0.00	0.00
Adverse effects	Nil	Nil	Nil	Nil

Control = Distilled water. n=1

3.3 HIGHER DOSAGE MORTALITY RATE FACTOR

Table 3 shows that no mortality/adverse effect was observed from the administration of *Moringa oleifera* root extract on the mice for higher dosage treatments 1000 – 10000 mg/kg.

Table 3: Evaluation of mortality rate factors in higher doses 1000 – 10000 mg/kg.

Treatment	Doses (mg/kg)	Mortality
M. oleifera	DW	0
M. oleifera	1000	0
M. oleifera	1600	0
M. oleifera	2900	0
M. oleifera	5000	0
M. oleifera	10000	0

DW----Distilled water. polyherbal aqueous extract

3.4 ADVERSE EFFECT OF ETHANOL ROOT EXTRACT ON MICE

Table 4 shows the physical features and behavioral pattern of the Swiss mice which were no different from the control.

Table 4: Effects of 14-days oral administration of ethanol root extract of *Moringa oleifera* on the physical features and behaviours of Swiss mice.

Physical sign	Control	Dose of plant extract (mg/kg)					
		100	1000	1600	2900	5000	10000
Writhing	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Pilo-erection	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Stooling blood	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Jerking	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Lacrimation	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Salivation	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Hemorrhage	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Nausea	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Motor-movement	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Dizziness	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Nil = no change/adverse effect

CHAPTER FOUR

DISCUSSION

This study was done to evaluate acute toxicity of the *Moringa oleifera* root which was administered orally to the animals. The acute toxicity of the mice showed no mortality even at the high dosage of 10000 mg/kg. The physiology and behavioral pattern did not change as the animals did not exhibit any on the onset of the administration and even throughout the observation period of 14-days. This outcome is in line with a previous study carried out to determine the acute toxicity of *M. oleifera* dried leaf powder. The study indicated that oral administration of *M. oleifera* dried leaf powder up to 2000 mg/kg showed no changes in clinical signs or gross pathology and that the LD_{50} was greater than 2000 mg/kg. Thus, stating that the plant is safe for consumption and medicinal purposes (Moodley, 2017). In a related study, aqueous extract of *M. oleifera* leaf was evaluated for its oral toxicity by the oral route and for the sub-acute toxicity on hematological, biochemical and histological parameters in rats. There was no change after it was administered except changes in the body weight, slight dullness at the onset of administration and no change was also noticed in all the organs examined in the course of the study. Thus, concluding that the plant was safe for both nutritional and medicinal uses (Adedapo *et al*., 2009). In a similar study, the acute and sub-acute (28-days) study was carried out to determine the safety of methanol extract of *M. oleifera* bark in Swiss albino mice. Also, no significant difference was observed between treated and control group mice in an acute toxicity study to determine the oral administration of *M. oleifera* bark extract in mice (Reddy *et al*., 2013).

Several other studies have evaluated the safety of *M. oleifera* extract which corroborates with this study. However, some of the studies made use of dosages that are lower than the one used in the study.

The ethanol extract of *M. oleifera* helped mice against pentylenetetrazol induced convulsion and it was determined that the lethal dosage was above 6400 mg/kg (Bakre *et al*., 2013). Roots of *M oleifera* showed anti-inflammatory properties and acute toxicity tests showed low toxicity in mice (Ezeamuzie *et al*., 2008). The acute toxicity of the aqueous-methanolic *M. oleifera* leaf extract on female Wistar albino rats showed mild changes in the liver and hepatic index of the rats. The LD_{50} was found to be >2000 mg/kg (Okumu *et al*., 2016). *M. oleifera* leaves were extracted in 70% ethanol to determine the acute toxicity in albino rats and rabbits, which the lethal dosage was found 6616.67 mg/kg for rats (Osman *et al*., 2015). The evaluation of the effects of *M. oleifera* root on hematological and hepatorenal determined a lethal dosage of 7 mg/kg which showed no adverse effects were observed on liver and kidney functions at lower dosages (Mazumder *et al*., 1999).

This study on the other hand, was carried out on a 14-day basis (acute study) and is in line with previous studies which were carried out under tested conditions showed the various parts of the *M. oleifera* safe for consumption and medicinal use. Further research need to be carried out in order to gain more knowledge about the *M. oleifera* lethal dosage and its safety profile.

CONCLUSION

Ethanol root extract of *Moringa oleifera* root extract demonstrated no toxicity in mice with a value of LD_{50} greater than 10000 mg/kg. Furthermore, the extract was well tolerated as there no signs of debility as there was no change in the physiologic attributes. Conclusively, the plant extract can be said to be safe.

REFERENCES

Adedapo, A. A., Mogbojuri, O. M. and Emikpe, B. O. (2009). Safety evaluations of the aqueous extract of the leaves of *Moringa oleifera* in rats. *Journal of Medicinal Plants Research*, **3(8):** 586 – 591.

Ambi, A. A., Abdurahman, E. M., Katsayal, U. A., Sule, M. I., Pateh, U. U. and Ibrahim, N. D. (2011). Toxicity evaluation of *Moringa oleifera* leaves. *International Journal of Pharmaceutical Research Innovation*, **4:** 22 – 24.

Anwar, F., Latif, S., Ashraf, M. and Gilani, A. H. (2007). *Moringa oleifera*: A food plant with multiple medicinal uses. *Phythotherapy Research*, **21:** 17 – 25.

Awodele, O., Asekunle, I. O., Odama, S., Teixeira, J. A. S. and Oluseye, V. O. (2012). Toxicological evaluation of the aqueous leaf extract of *Moringa oleifera* (Moringaceae). *Journal of Ethnopharmacology*, **139:** 300 – 306.

Ayerza, R. (2011). Seed yield components, oil content and fatty acid composition of two cultivars of moringa (*Moringa oleifera*) growing in the arid chaco of Argentina. *Journal of Industrial Crops and Products*, **33(2):** 389 – 394.

Bakre, A. G., Aderibigbe, A. O. and Ademowo, O. G. (2013). Studies on neuropharmacological profile of ethanol extract of *Moringa oleifera* leaves in mice. *Journal of Ethnopharmacology*, **149(3):** 783 – 789.

Birendra, K. P., Hemant, K. J. D. and Bina, G. (2017). Phytochemistry and pharmacology of *Moringa oleifera*. *Journal of Pharmacopuncture*, **20(3):** 194 – 200.

Bose, S., Datta, R. and Kirlin, W. G. (2021). Toxicity studies related to medicinal plants. **In:** Mandal, S. C., Chakraborty, R. and Sen, S. (eds.) *Evidence Based Validation of Traditional Medicines*. Springer, Singapore. pp. 621 – 647.

Cajuday, L. A. and Pocsidio, G. L. (2010). Effects of *Moringa oleifera* Lam. (Moringaceae) on the reproduction of male mice. *Journal of Medicinal Plants Research*, **4(12):** 1115 – 1121.

Carceres, A., Saravia, A., Rizzo, S., Zabala, L., Leon, E. and Nave, F. (1992). Pharmacological properties of *Moringa oleifera* 2: Screening for antispasmodic, anti-inflammatory and diuretic activity. *Journal of Ethnopharmacology*, **36:** 233 – 237.

Ezeamuzie, I. C., Ambakederemo, A. W., Shode, F. O. and Ekwebelem, S. C. (1996). Anti-inflammatory effects of *Moringa oleifera* root extract. *International Journal of Pharmacognosy*, **34(3):** 207 – 212.

Fahey, J. W. (2005). *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Trees for Life Journal*. **1:** 5 – 15.

Farooq, F., Rai, M., Tiwari, A., Khan, A. A. and Farooq, S. (2007). Medicinal properties of *Moringa oleifera*: An overview of promising healer. *Journal of Medicinal Plants Research*, **6(27):** 4368 – 4374.

Foidl, N., Makkar, H. P. S. and Becker, K. (2001). The potential of *Moringa oleifera* for agricultural and industrial uses. **In:** Fuglie, J. L. (ed.) *The Miracle Tree/The Multiple Attributes of Moringa*. CTA. USA. pp. 45 – 76.

Kasolo, J. N., Bimenya, G. S., Ojok, L. and Ogwal, O. J. (2011). Phytochemicals and acute toxicity of *Moringa oleifera* roots in mice. *Journal of Pharmacognosy and Phytotherapy*, **3(3):** 38 – 42.

Matheus, C. B., Barros, A. G. S., Cristiano, A. C., Barbosa, J. C. M., Ferreira, M. R. A., Lira, . A. S., Xavier, V. L.,Larissa, C. C. A., Oliveira, E. F. B., Teresinha, G. S., Raion, R. V. A., Cassandra, L. B. C., Alisson, M. O., Henrique, T. N. and Guedes, P. G. P. (2022). Evaluation of acute toxicity, 28-day repeated dose toxicity and genotoxicity of *Moringa oleifera* leaves infusion and powder. *Journal of Ethnopharmacology*, **296:** 415 – 426.

Mazumder, U. K., Gupta, M., Chakrabarti, S. and Dilipkumar, P. (1999). Evaluation of hematological and hepatorenal functions of *Moringa oleifera* root treated mice. *Indian Journal of Experimental Biology*, **37(6):** 612 – 614.

Moodley, I. (2017). Acute toxicity of *Moringa oleifera* leaf powder in rats. *Journal of Medicinal Plants Studies*, **5(5):** 180 – 185.

Okumu, M. O., Mbaria, J. M., Kanja, L. W., Gakuya, D. W., Kiama, S. G., Ochola, F. O. and Okumu, P. O. (2016). Acute toxicity of the aqueous-methanolic *Moringa oleifera* Lam. leaf extract on female Wistar albino rats. *International Journal of Basic and Clinical Pharmacology*, **5(5):** 1856 – 1861.

Osman, H. M., Shayoub, M. E., Babiker, E. M., Faiza, A. O., Munzir, M. E., Osman, B., Elhassan, A. M. and Taha, K. K. (2015). Assessment of acute toxicity and LD_{50} of *Moringa oleifera* ethanolic leave extract in albino rats and rabbits. *Journal of Medical and Biological Science Research*, **1(4):** 38 – 43.

Pareek, A., Pant, M., Gupta, M. M., Kashinia, P., Ratan, Y., Jain, V., Aaushi, P. and Chuturgoon, A. A. (2023). *Moringa oleifera*: An updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical and toxicological aspects. *International Journal of Molecular Science*, **24(3):** 2098 – 2134.

Reddy, Y. J., Lokanatha, O., Ratnam, K. S. V. P., Shanmuga, C. R., Raju, I. N. and Damodar, C. R. (2013). Acute and sub-acute toxicity of *Moringa oleifera* stem bark extract in Swiss albino mice. *International Journal of Science, Biotechnology and Pharmacology Research*, **2(4):** 74 – 82.

Stohs, S. J. and Hartman M. J. (2015). Review of the safety and efficacy of *Moringa oleifera*. *Phytotherapy Research*, **29(6):** 796 – 804.