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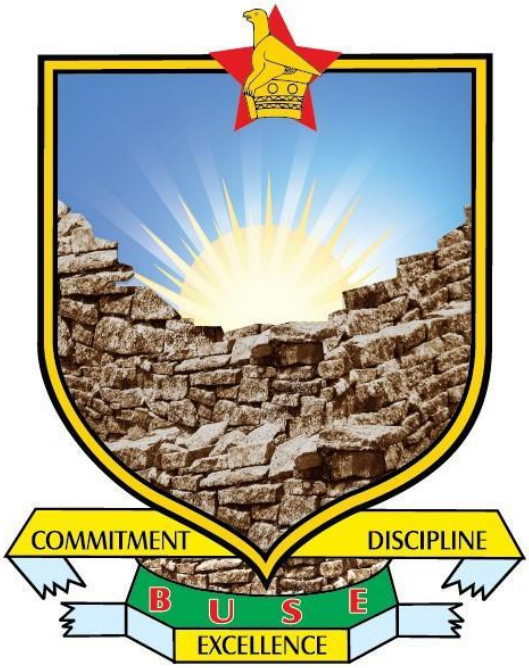

"Assessing Acute Toxicity of *Opuntia monacantha* (Mudhorofiya) as a Potential Nutraceutical for the Management of Chronic Diseases, Including Diabetes"

By

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A research project submitted in partial fulfillment of the requirements for the Bachelor of Science Honours Degree in Biological Sciences

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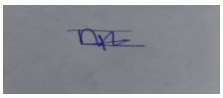
Approval form

The undersigned certify that they have read the dissertation titled 'Assessing acute toxicity of *Opuntia monacantha* as a potential nutraceutical for various chronic diseases including diabetes' and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.

Signature of student:  Date: 16 June 2025

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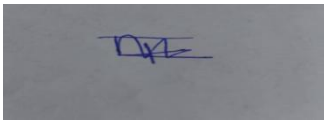
Signature:  Date: 16 June 2025

Supervisor

I, Dr N Mgocheki, declare that I have supervised this thesis and I am satisfied that it can be submitted to the Biological Sciences Department, Faculty of Science and Engineering, at Bindura University of Science Education.

Signature: NMgocheki Date: 20/06/2025

Dedication

I dedicate this project to my parents, Mr and Mrs Muurawa, for their unwavering presence and support.

Acknowledgements

I want to sincerely thank my supervisor, Dr. Mgocheki and my co-supervisor, Mr Hove for their unwavering support, tolerance and direction during the entire endeavor. I also want to express my gratitude to my family for their financial and moral assistance. I seek to acknowledge the help I got from my friends. Lastly; I would like to acknowledge the lab technicians Mr Gadaga and Mr Mutenure who helped me every step of the way. Above all, I want to express my gratitude to the Lord God Almighty for giving me the ability to finish this project.

List of abbreviations

ANOVA- Analysis of Variance

ECHA – European Chemicals Agency

FDA – Food and Drug Administration

HbAlc – Hemoglobin Alc

JREC - Joint Research Ethics Committee

LD 50 - Lethal dose 50

OECD - Organization for economic Co-operation Development

SADC – Southern African Development Community

WHO - World Health Organization

Abstract

Opuntia monacantha, a cactus found in Africa, particularly Zimbabwe, has long been used to treat chronic conditions like diabetes. However, nothing is known about its safety profile, which prevents it from becoming a reliable nutraceutical. This study looked at the acute oral toxicity and basic phytochemical composition of *O. monacantha* leaf extract in order to evaluate its potential as a diabetes-supportive nutraceutical. After utilizing 70% methanol for solvent extraction, 12.2 g of crude extract (6.10% yields) was obtained from 200 g of powdered *O. monacantha* leaves. A number of bioactive substances, including alkaloids, glycosides, terpenoids, flavonoids, tannins, sterols, and phenols, were detected by phytochemical screening; however, steroids and sapponins were not found. Bulb C mice were used to assess acute toxicity in accordance with OECD recommendations. A control group (distilled water), a diabetes model group (alloxan-induced), and treatment groups that received 500, 2000, and 5000 mg/kg of the extract were the five groups of mice. There was no mortality in either the 500 mg/kg or control groups. Death rates rose as dosage increased, reaching 1.33 and 3.33 fatalities at 2000 mg/kg and 5000 mg/kg, respectively. The mortality difference between groups was statistically significant, as proven by a one-way ANOVA ($F = 42.98$, $p < 0.05$). Intake of water and feed also decreased dramatically at increasing dosages; the group receiving 5000 mg/kg had the lowest intake (2.67 g feed, 6 ml water). ANOVA studies verified that these changes were statistically significant for both water ($F = 79.28$, $p = 0.000000156$) and feed ($F = 25.72$, $p = 0.00003$). The LD_{50} was calculated using probit analysis to be 2300 mg/kg, which suggests modest acute toxicity.

Conclusion: Although *O. monacantha* contains phytochemicals that are helpful, at high quantities it shows harmful effects that are dependent on dose. These results lend credence to its possible application as a diabetes nutraceutical; however, dose needs to be closely monitored to guarantee safety.

Contents

CHAPTER 1

1.1 Introduction

Opuntia monacantha, commonly known as prickly pear cactus, is a widely distributed plant in Africa, including Zimbabwe. It has been traditionally used to manage various health conditions, including diabetes, due to its reported hypoglycemic properties (Surine et al., 2020). However, its acute toxicity profile remains unknown, hindering its development as a safe and effective nutraceutical for diabetes management. Despite the potential health benefits of *O. monacantha*, there is a lack of comprehensive toxicity studies to support its safe use as a nutraceutical.

1.2 Background

Diabetes mellitus is a metabolic disorder affecting over 463 million people worldwide, with projections indicating a rise to 578 million by 2030 and 640 million by 2045 (International Diabetes Federation, 2021). The global nutraceutical market is growing rapidly, driven by increasing demand for natural health products, including those for diabetes management (Grand View Research, 2020). Africa bears a significant burden of diabetes, with an estimated 34 million people living with the condition (World Health Organization, 2021). Traditional medicine, including plant-based remedies, plays a vital role in African healthcare (World Health Organization, 2021). The African region has a rich diversity of medicinal plants, including *O. monacantha*, which has been used traditionally to manage various health conditions.

The Southern African Development Community (SADC) region faces significant challenges in diabetes management, including limited access to healthcare services and affordable treatments (SADC, 2017). The region has a high prevalence of diabetes, with countries such as South Africa, Mozambique, and Zimbabwe reporting significant numbers of diabetes-related deaths, highlighting the urgent need for improved prevention, diagnosis, and management strategies (World Health Organization, 2016). Zimbabwe has a high diabetes prevalence (8.6%) and limited access to healthcare services, particularly in rural areas (Ministry of Health and Child Care, 2019). Traditional medicine is widely used in Zimbabwe, with plants like *O. monacantha* being used to manage various health conditions, including diabetes.

Diabetes mellitus is a chronic disease characterized by high blood sugar levels. (Ministry of Health and Child Care, 2019). While conventional treatments exist, there is growing interest in natural alternatives, such as nutraceutical. *O. monacantha* has been traditionally used for various ailments, including diabetes. However, its safety when used as a potential nutraceutical for diabetes remains unclear. Understanding the different types of diabetes is essential for effective management and prevention strategies. (World Health Organization, 2016), have demonstrated the potential anti-diabetic effects of *O. monacantha*. These studies have shown that compounds extracted from the cactus can lower blood sugar levels, improve glucose tolerance, and enhance insulin sensitivity. However, before *O. monacantha* can be considered for clinical use as a nutraceutical for diabetes, it is essential to assess its safety profile. Acute toxicity studies are a fundamental step in evaluating the safety of any new substance. (Hepnarova et al., 2019) involve administering a single dose or multiple doses of the substance to animals and monitoring for any adverse effects. By identifying potential toxicities at early stages of development, researchers can minimize risks associated with human use. Effective diabetes management involves a multidisciplinary approach that includes healthcare professionals, education, lifestyle modifications, regular monitoring, and appropriate medical interventions. However, people now prefer to use herbs for they are nutraceutical, cost effective, less side effects and edible (Aronson, 2016).

1.3 Problem Statement

Despite the potential health benefits of *O. monacantha* in managing diabetes, its acute toxicity profile remains unknown. This knowledge gap hinders the development of safe and effective nutraceutical from this plant. Globally, the demand for natural products in diabetes management is increasing (Grand View Research, 2020). *O. monacantha* has shown promise in reducing blood glucose levels (Mostafa et al., 2014). Africa has a high burden of diabetes, with limited access to conventional treatments (World Health Organization, 2016). Traditional medicine, including plant-based remedies, is widely used (World Health Organization, 2013). SADC countries face significant challenges in diabetes management, including limited resources and accessibility (SADC, 2017). Regional research on indigenous plants like *O. monacantha* can inform local solutions. Zimbabwe has high diabetes prevalence (8.6%), is relatively high and with an estimated 10.3% of adults aged 18 and over living with diabetes and limited access to healthcare (Ministry

of Health and Child Care, 2019). Local research on *O. monacantha* can contribute to the development of affordable, effective diabetes management options. In comparison to rural areas, urban areas have a higher prevalence of diabetes (Bakris et al., 2018). A combination of factors, such as poor diet, physical inactivity and an increase in the number of overweight or obese people, are thought to be responsible for the high prevalence of diabetes. Adherence to diabetes management may be compromised in some individuals due to reluctance or refusal to initiate insulin therapy or oral hypoglycemic agents (Brazg et al., 2019). This plant-based intervention is primarily aimed at addressing type 2 diabetes mellitus, a condition often associated with insulin resistance and lifestyle-related factors. Many individuals with type 2 diabetes may struggle to manage their condition due to a variety of reasons, including fear of needles, concerns about the side effects of insulin or oral hypoglycemic agents, or a personal preference for natural, plant-based alternatives. Therefore, noncompliance with standard treatment can lead to insufficient glycemic control, raising the risk of major side effects like cardiovascular disease, neuropathy, retinopathy, nephropathy and poor wound healing. In addition to physical health challenges, individuals with diabetes frequently face emotional burdens, including anxiety, stress, depression, and a perceived loss of autonomy over their health and daily life (Vieira et al., 2019).

Both traditional knowledge systems and the scant scientific literature in Zimbabwe acknowledge the use of *O. monacantha*, in the treatment of a number of illnesses, such as diabetes, digestive issues, and skin diseases. The plant is used extensively in traditional medicine and is being used more and more in regional herbal medicines. Despite *O. monacantha's* increasing use and cultural importance, empirical toxicological data are scarce. No thorough research has been done to evaluate its possible acute or chronic toxicity, especially in relation to dosage, preparation method, or long-term health implications. This lack of scientific proof raises possible public health issues because uncontrolled plant usage could endanger consumers, particularly vulnerable groups including children, expectant mothers, and people with underlying medical disorders. To confirm *O. monacantha's* safe use in both conventional and contemporary medicinal applications, a thorough toxicological analysis of its safety profile is urgently required.

1.4 Research Objectives

1. To determine the LD_{50} (lethal dose 50) of *O. monacantha* extract when administered orally in Balb\c mice.
2. To identify the basic phytochemical profile of *O. monacantha* leaf extract.
3. To evaluate how *O. monacantha* affects general health metrics on the food and water intake

1.5 Research Question

1. What is the acute toxicity of *O. monacantha* extract when administered orally to mice?
2. What are the phytochemicals present in *O. monacantha?*
3. Are there any changes in water and feed intake associated with acute *O. monacantha* extract exposure?

1.6 Significance of the study

It offers a different kind of care for those who want not to take medicine or use insulin injections. New treatments with fewer side effects, additional advantages and greater efficacy than current ones that result from it. It also helps down the cost of treating chronic diseases which include diabetes because plants are frequently less expensive than prescription medications.

1.7 Assumptions of the study

This study used an animal model to assess the acute oral toxicity of *O. monacantha* extract in order to ascertain its safety profile or condition. This study's validity and interpretation are supported by a number of fundamental presumptions. Based on their genetic homogeneity and well-established physiological reactions in toxicological investigations, it is believed that the Balb/C mice chosen for the experiment are a suitable and trustworthy model for assessing the acute toxicity of *O. monacantha* extract. Additionally, it is expected that the plant extract used is bioavailable, chemically stable and processed consistently to guarantee consistency throughout dosage groups.

A quantitative and consistent dose-response relationship between the concentration of the *O. monacantha* extract and its biological action is another crucial premise.

This assumption underpins the ability to determine the median lethal dose 50 (LD_{50}), a key indicator of acute toxicity (Esquivel et al., 2012). The study's internal validity is further supported by the assumption that no major internal or external confounding variables, such as variations in handling practices, environmental fluctuations, or variations in animal health, had an impact on the results.

Throughout the trial, it was also believed that ethical standards would be maintained. All ethical and animal welfare rules should have been strictly adhered to in order to minimize needless suffering and increase the research's scientific usefulness. Finally, it is assumed that the statistical methods employed are suitable for accurately detecting toxicological effects at different dosage levels, contributing to a scientifically sound interpretation of the data (Díaz, 2017).

1.8 Limitations of the Study

This study's interpretability and generalizability may be impacted by a number of limitations. First off, although animal models like Balb/c mice are frequently employed in toxicological studies, they are not a perfect representation of human physiological reactions and metabolic processes. This restricts the ability to directly extrapolate results to populations of people. Furthermore, it may be challenging to predict reactions across many biological systems due to interspecies variations in sensitivity to harmful chemicals. Another difficulty is figuring out the right dose range for acute toxicity investigations. The procedure of choosing doses may not always accurately represent actual human exposures, but it must strike a balance between the requirements to monitor harmful consequences without inflicting needless harm. Moreover, the route of administration oral can significantly alter the absorption, distribution, metabolism, and excretion of a substance, thus influencing its toxicity profile.

Short-term exposure to the test chemical is usually required for acute toxicity investigations. As such, they might not fully account for the range of possible chronic or long-term consequences linked to long-term *O. monacantha* use. Finally, ethical and welfare considerations often limit the number and types of animals used in toxicity testing, which may restrict the statistical power and diversity of biological responses observed (Fokunang et al., 2018).

1.9 Delimitations of the study

This study was limited to assessing *O. monacantha's* immediate impacts on general health indicators, with a particular focus on food and water intake and identifying the phytochemicals found in the plant extract. This study did not address long-term toxicity, including possible mutagenic or carcinogenic consequences. Additionally, the study did not investigate detailed molecular mechanisms of toxicity (Rai et al., 2014). This research also did not specifically address the potential effects or toxicity of *O. monacantha* in vulnerable populations such as children, pregnant women or individuals with pre-existing medical conditions. Furthermore, possible interactions between *O. monacantha* and commonly prescribed antidiabetic medications were not explored (Wood, 2017). These delimitations were necessary to maintain a focused and manageable research scope.

1.10 Definitions of terms

- **Acute toxicity** is the term used to describe the negative consequences that follow one or more exposures to a substance within a brief time frame (typically less than 24 hours).
- ***Opuntia monacantha*** is a species of cactus that belongs to the Cactaceace family. It is also referred to as the Barbary fig, cochineal prickly pear, or drooping prickly pear.
- **A nutraceutical** is a food or food ingredient that offers health advantages above and beyond basic nourishment.
- **Diabetes** is a long-term, chronic illness that alters how your body uses food as fuel.
- **Lethal Dose 50 (LD_{50})** is a measurement of its acute toxicity and the quantity of the substance that kills 50% of test subjects.
- Flavonoids, steroids and other naturally occurring chemical substances are examples of **phytochemicals**.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This review of literature explored the major concerns centered on the effect of *Opuntia monacantha* fruit extract on potential nutraceutical for chronic diseases which include diabetes (Bulich et al., 1981). The review of literature focused on objectives one and two, which is to determine the acute toxicity of *O. monacantha* fruit extracts' when used concomitantly on mices and to identify the basic phytochemical profile of the fruit extract (Saleen et al., 2015).

The effect of *Opuntia* ficus-indica fruit on blood glucose levels in streptozocin-induced diabetic rats was evaluated in this study (Kianbakht et al., 2008). The results indicated that the fruit had no appreciable impact on the mice body weights or fasting blood glucose levels when compared to controls. But after two weeks of treatment, glibenclamide, a common antidiabetic medication, dramatically reduced blood glucose levels. While some studies revealed lower blood glucose levels and insulin resistance, the data was mixed, according to Gouws et al.'s (2019) systematic review of studies on the ingestion of prickly pear cacti and their products. The study emphasized that in order to validate these outcomes; larger, carefully planned clinical trials are required.
The soluble fiber found in the cacti cladodes (pads) may increase insulin sensitivity and decrease the absorption of glucose. Even though prickly pear cactus is usually seen as safe to eat, people with diabetes should speak with their doctors before including it in their diet, particularly if they are taking medication, to prevent any possible interactions. Supplement safety and suggested dosage are not well-established. There is currently some evidence in the literature that prickly pear cactus may have antidiabetic properties, especially in animal models. Clinical trials on humans, however, have produced conflicting findings and its acute toxicity and safety in the treatment of diabetes need to be confirmed by additional research. To ensure safe and informed use, those thinking about using prickly pear cactus as a supplemental diabetes treatment strategy should speak with medical professionals.

2.2 *Opuntia monacantha*

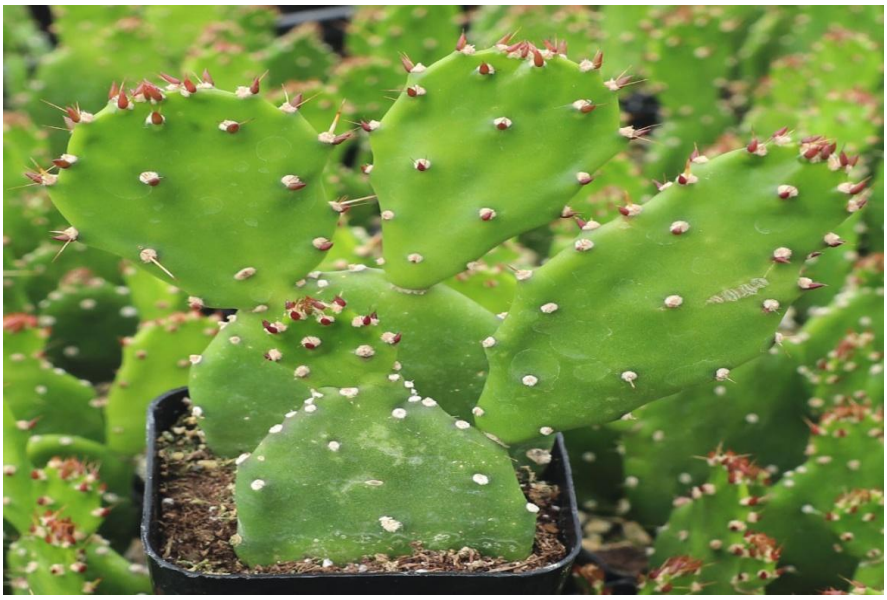


Figure 2.2: *Opuntia monacantha* cladodes. Image was taken from Bari, (2012**).**

Opuntia monacantha, sometimes referred to as prickly pear pads, (Binggeli, 2003) is edible parts of the cactus plant. A prickly pear is a succulent shrub that resembles a tree and can grow up to five meters tall. It has many branches and a large, enlarged crown on each branch. The elongated to oval shoots are shiny green and thin at the base. They range in length from four to ten inches and are fairly thin. *O. monacantha* is typically 2–5 meters tall. Cladodes are elongated, flattened, and have one or two prominent spines (Contreras-Padill et al., 2016). They shine green. Fruits are greenish with a reddish-purple and flowers are yellow. The plant thrives in containers, so don't over water it and keep it dry over the winter. *O. monacantha* is advertised as a remedy for hangovers, diabetes, high cholesterol and obesity. It is also praised for having anti-inflammatory and antiviral qualities. The plant's pads and fruits are both edible, making it an extremely versatile food source that also offers wildlife protection and sustenance. In Mexican civilizations, its fruits and stems are frequently consumed and utilized as medicine. Vitamins, antioxidants and plant

pigments like betalains and carotenoids are all present in prickly pears. It is a tropical plant that thrives in the Mediterranean region, Africa, Latin America and Mexico.

On the other hand, *O. monacantha* supports the immune system, increases moisture and unlocks the key to beautiful skin (Centre for Agriculture and Bioscience International, 2020). *Opuntia* cladodes are used extensively; however some people are worried about their toxicity. The existence of specific compounds and their possible interactions with other substances are the main causes of these worries. Oxalates, which are organic acids found in *Opuntia* cladodes, have the ability to bind to calcium and magnesium in the body (Galati et al., 2015). Kidney stones and other health issues may result from this. Cooking can lessen the absorption of oxalate, which is typically thought to be low in *Opuntia* cladodes. The existence of spines and glochids, which are microscopic barbed hairs, on the cladodes' surface is the main cause for concern. If they pierce the skin, these can result in infections, pain and skin irritation. It is essential to handle and prepare them properly, which includes removing the glochids and spines before eating (Martirosyan et al., 2018**)**. Researchers have looked into the possible health advantages of *Opuntia* cladodes. Antioxidants found in *Opuntia* cladodes may aid in preventing cell damage (Reda et al., 2020).

Furthermore, some research has suggested that *Opuntia* cladodes may aid in blood sugar regulation and that some of its constituents may have anti-inflammatory properties (Mazri, 2018), *Opuntia* cladodes' low calorie density and high fiber content may help in weight management. Cactus thrives in areas characterized by low rainfall and high temperatures, which have ideal conditions. However, it can also be found in other parts of Zimbabwe, including the Mashonaland East and Central provinces. Prickly pear cacti, also referred to as *Opuntia* species, have been used traditionally to treat a number of illnesses, including diabetes (Sabtain et al., 2021). The objective of this review is to examine the scientific data pertaining to *O. monacantha's* possible antidiabetic effects and to pinpoint the main mechanisms at play (Karym et al., 2014). They were a favorite garden because of their distinctive look and hardiness; the fruits, called tunas, were eaten for nutrition, the pads were used as animal feed and the massive root systems of the *Opuntia* helped to keep the soil from eroding. Many regions of Southern Africa have seen the naturalization of *Opuntia* species throughout time. Their vast distribution is a result of both their effective reproduction tactics and their capacity to flourish in arid conditions. Although they have been advantageous, they have also had several drawbacks: *Opuntia* species are becoming invasive in

some places, outcompeting native plants and upsetting ecosystems. Crop yields may be decreased if they infest agricultural fields. Both people and animals may sustain injuries from the spines and glochids of *Opuntia* plants (Patel, 2014). *Opuntia* species are nevertheless prized in Southern Africa for their diverse use in spite of these obstacles: Traditional medicine has utilized *Opuntia* species to treat a range of ailments, and the oil that is extracted from *Opuntia* seeds has the potential to be used as a source of biofuel (Sabtain et al., 2021). Tunas can be eaten fresh, processed into jams, or used in traditional dishes. The pads are also a valuable source of nutrition for animals, particularly during dry periods.

2.3 Taxonomy

Table 2.3: Taxonomic classifications of *O. monacantha* (Anderson, 2001).

Kingdom	*Plantae*
Division	*Angiosperms*
Class	*Eudicots*
Order	*Caryophyllales*
Family	*Cactaceae*
Genus	*Opuntia*
Species	*Opuntia Monocantha*

2.4 Diabetes

Diabetes mellitus is a long-term condition marked by elevated blood sugar levels. Despite the availability of conventional treatments, natural alternatives like nutraceutical are gaining popularity (WHO, 2019). In type 1 diabetes, the immune system unintentionally targets and kills the pancreatic cells that produce insulin. This causes insulin production to be insufficient or nonexistent, which raises blood sugar levels. People who have type 1 diabetes need to take insulin for the rest of their lives in order to control their blood sugar levels. Type 1 diabetes usually manifests in childhood or early adulthood (Scueren et al., 2021).

Conversely, insulin resistance, which occurs when the body's cells lose their sensitivity to the effects of insulin, is a hallmark of type 2 diabetes. After initially compensating by producing more insulin, the pancreas eventually loses its ability to keep blood sugar levels within normal ranges (Hou et al., 2022). The main lifestyle factors linked to type 2 diabetes are obesity, poor diet, and physical inactivity. Although type 2 diabetes is more common in adults, the rising rates of childhood obesity are contributing to an increase in its prevalence in children and adolescents. Insulin therapy, lifestyle changes, and oral medications to increase insulin sensitivity are all possible treatments for type 2 diabetes.

Women who have never had diabetes before are susceptible to gestational diabetes, which develops during pregnancy (Wang et al., 2022). If the pancreas is unable to produce enough insulin to compensate for insulin resistance brought on by hormonal changes during pregnancy, gestational diabetes results (Wang et al., 2022). Although it usually goes away after giving birth, women who have gestational diabetes are more likely to get type 2 diabetes in the future. Dietary changes, exercise, and occasionally insulin therapy are all part of managing gestational diabetes.

In order to avoid complications, blood sugar levels must be continuously monitored and managed for all forms of diabetes (Van der Scueren et al., 2021). Numerous health issues, such as kidney damage, nerve damage, cardiovascular disease, and vision impairment, can result from uncontrolled diabetes. As a result, a multidisciplinary strategy including medical professionals, education, lifestyle changes, routine monitoring, and the right medications is needed for effective diabetes management. Nonetheless, because herbs are edible, nutripharmacetical, affordable, and have fewer negative effects, people now favor using those (Abubakar et al., 2016).

2.5 Current Standard treatment\ Management

Diabetes can be treated in a number of ways, including regular exercise, a balanced diet, maintaining a healthy weight, While oral medications like metformin are frequently the first-line treatment for type 2 diabetes and sulfonylureas, which increase the pancreatic production of insulin, are required for type 2 diabetes, insulin therapy is required for type 1 diabetes (Kianbakht et al., 2008). Regularly checking blood sugar levels is essential to diabetes management. This helps people better understand how their diet, exercise, and medications affect their blood sugar levels. For effective diabetic care, blood sugar levels must be checked frequently (Wang et al., 2022). This aids people in understanding how their blood sugar levels are impacted by their diet, exercise, and medications. Effective diabetes management requires routine blood sugar monitoring. This aids people in understanding how their blood sugar levels are impacted by their diet, exercise and medications (Bakris et al., 2018).

2.6 Limitations of Current Standard treatment

Treatment for diabetes has improved thanks to contemporary medical procedures like insulin therapy and oral drugs, yet there are still drawbacks. Some patients are investigating complementary or traditional medicine as an alternative or supplement to traditional treatments as a result of these restrictions. Research has indicated that a considerable number of diabetic patients, including those who have the disease, use complementary, alternative and traditional medicine in addition to or instead of contemporary therapies. The influence on quality of life, resistance development and adverse effects are among the drawbacks of the diabetic treatments available today. To overcome these constraints and enhance general health, patients frequently turn to complementary and alternative medicine, including herbal medicines and mind-body therapies. Additionally, the use of traditional medicine in diabetes care is associated with higher quality of life and patient satisfaction (Heinemann et al., 2020). Research is ongoing to explore the potential benefits of integrating traditional medicine with modern treatments for diabetes. Studies have demonstrated the cytotoxic effects of certain compounds derived from herbal medicine, suggesting a potential role in enhancing the efficacy of current treatments (Brazg et al., 2021). Furthermore,

the use of psychological interventions combined with traditional Chinese and Western medicine has shown promise in improving patient outcomes and reducing diabetes.

2.7 Traditional Herbal Remedies

Since the people in underdeveloped nations, particularly those in Africa, find it difficult to pay for Western medications, medicinal plants have had a significant impact on their everyday lives. Traditional medicine is defined by the World Health Organization (WHO) as indigenous health practices, methods, knowledge and beliefs that can be used alone or in combination to diagnose, treat and preserve health. In certain medicinal systems, traditional herbal remedies are made from naturally occurring plant and animal materials that have undergone little to no industrial processing. In order to detect, stop and cure illness, traditional medicine is thought to be a synthesis of theory and practice.

This may be based on experience and observations passed down orally, typically in the form of stories, spiritually by ancestors or in modern times as written work **(**Sundarrajan et al., 2023). Since the start of time, people have utilized natural remedies derived from plants and trees to treat and prevent disease. Due to their affordability, traditional medicines are now being used by the majority. Africans' religious and societal practices include the use of traditional medicines.

Traditional doctors are among those who provide traditional medicine as they have extensive understanding of the various traditional practitioners specialize in certain conditions, such as those used in diabetes management. According to the type and stage of the disease, the plant part used differs from species to species and practitioner to practitioner (Rushton., 2008). Herbal medicines or supplements are natural substances made from plant parts like leaves, bark, roots, seeds, or flowers.

They are within the umbrella of medical practices known as complementary and alternative medicine and are natural botanical products derived from plants that individuals may use to cure and prevent illnesses. Although there is little formal research on these usages, some people use herbal supplements to relieve particular ailments. For those who take drugs or have certain medical conditions, taking supplements may not be safe (Elfahmi et al., 2014).

Reports indicate that twenty-five percent (25%) of the world's biodiversity is found in Africa. Up to 45,000 plant species with possible medical use are thought to exist in Africa. Much work has been done worldwide in the subject of traditional diabetes management and therapy over the years. However, the breadth of diabetes research has been restricted. This leads to the use of medicinal plants that contain nutraceutical ingredients as an alternative to traditional diabetes treatment methods, which are very costly and out of reach for diabetics, especially those from developing countries and frequently have adverse side effects (Majolo et al., 2019).

2.8 Medicinal Uses

Opuntia monacantha, a plant species native to Africa has been traditionally utilized for its medicinal properties. The stem bark, roots and leaves of *O. monacantha* are employed in treating diabetes management; wound healing digestive issues, anti-inflammatory properties, cardiovascular health (Akhtar et al., 2019). Furthermore *O. monacantha* has also been adopted in the Mediterranean region, where it's cultivated for its fruit and used in traditional remedies and as a traditional medicine systems in India also incorporate prickly pear for various therapeutic purposes. In addition to its medicinal uses, *O. monacantha* holds cultural significance, being considered sacred in certain regions like Mexico and Latin America (Muhammad Nadeem., 2012).

2.9 Phytochemicals

Phytochemicals are naturally occurring chemical substances that are physiologically active and present in plants. As nutrients and therapeutic agents, they are essential for promoting human health. They are in charge of shielding plants from harm and disease and they also add to the color, flavor and perfume of the plant. Based on their protective properties as well as their physical and chemical attributes, more than 4,500 phytochemicals have been identified. *O. monacantha* has garnered attention for its potential as a nutraceutical, particularly in management of diabetes. This plant has many bioactive substances, such as sterols, glycosides, alkaloids, flavonoids and tannins, which enhance its therapeutic potential. However, prior to its extensive use in the treatment of diabetes, it is important to assess the acute toxicity of *O. monacantha* and understanding classes of phytochemicals (Muhammad Nadeem et al., 2019).

2.10 Acute Oral toxicity

The impact of *Opuntia* on diabetics' capacity to regulate their blood sugar levels has been evaluated in numerous clinical investigations. Significant decreases in hemoglobin A1C (HbA1c), postprandial glucose and fasting blood sugar levels have been shown in some studies, despite mixed results. To demonstrate *Opuntia's* efficacy in diabetic treatment, more comprehensive randomized controlled trials are needed. Acute toxicity testing, a crucial component of toxicology research, is meant to evaluate the duration. These tests not only provide important data for risk assessment and regulatory decision-making, but they also help to identify the acute dangers associated with chemical exposure. The part of acute toxicity testing that determines the relationship between a substance's dose and its observed adverse effects is called dose-response evaluation. Finding the point at which adverse effects occur is useful. The lethal dosage 50 or LD_{50} is a medication that kills 50% of test animals (Kakkar, 2014). This statistic is frequently used to compare the levels of hazard associated with different substances. Medical signs and symptoms: Monitoring animals for signs of toxicity, such as behavioral abnormalities, weight loss, organ dysfunction, or death, is known as acute toxicity testing. In the event of an accidental exposure to a hazardous material, acute toxicity data may be used to influence emergency response plans. Acute toxicity testing helps to prevent damage from exposure to toxic substances by assessing potential dangers.

Although acute toxicity testing is a helpful method, it has limitations. For example, it might not effectively communicate the long-term or the relevance. To ascertain the possible risks connected to chemical exposure, acute toxicity data are essential. This information is used to design appropriate risk management strategies and safe exposure limits. Acute toxicity statistics are also used by regulatory bodies like the European Chemicals Agency (ECHA) and the Food and Drug Administration (FDA) to assess the safety of goods and chemicals. When creating new products, such as chemicals, medications and cosmetics, acute toxicity testing is a crucial stage.

It helps ensure that products have long-term effects of exposure, even though it might not be representative of all potential populations. To give a thorough grasp of a substance's safety profile, it is frequently coupled with other forms of toxicity testing, such as sub chronic and chronic toxicity testing (Atsamo et al., 2011).

To sum up, acute toxicity testing is necessary to ensure the safety of products and chemicals. By offering crucial details about the possible dangers of exposure, it supports public health protection and assists in guiding regulatory decision-making. Acute toxicity testing aims to determine the biologic activity of a chemical and provide crucial information about how it works. Risk management and hazard identification in the chemical's manufacture, handling and usage are aided by the data produced by the test. The statistically determined dose that results in 50% of treated animals dying in an acute toxicity test is known as the lethal dose 50 (LD_{50}) value. LD_{50} is the basis of toxicological classification currently. Mice are the laboratory animals normally used for the tests. The limit test and the main test are the two components of acute oral toxicity testing (Guidelines et al., 2011).

2.11 Limit test

A maximum of five mice are used in the step-by-step Limit test. 2000 mg/kg may be used as the test dose, or in extreme circumstances, 5000 mg/kg. The limit test is typically employed when the investigator has information suggesting that the test material is most likely nontoxic, meaning that its toxicity is below regulatory limit doses. A dose of 2000 mg/mg body weight is given to one animal. The primary test is performed to ascertain the LD_{50} if the animal die; however, if the animal lives, four additional mice should be dosed, for a total of five mice. If three or more animals survive, the LD_{50} exceeds 2000 mg/ml. Use the primary test to ascertain LD_{50} if fewer than three survive (Esquivel et al., 2012).

2.12 Main test

A single ordered dose progression made up the main test. One dose is given to each animal at a minimum of 48-hour intervals. A dose one step below the best estimate of the LD_{50} is given to the first animal. The dose for the subsequent animal is raised by 3.2 times the initial dose if the animal survives and lowered by a comparable dose progression if it dies (Rush et al., 2012).

Before determining whether and how much to dose the following mice, each one should be closely monitored for a maximum of 48 hours. This decision is based on the 48-hour survival pattern of every animal up until that point, at which point a confidence interval and an estimate of the LD_{50}

are computed for the test based on the condition of every animal at termination. Probit analysis was used to determine the LD_{50} (Rush et al., 2012).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

A range of laboratory apparatuses, including a freeze dryer, rotary evaporator and incubator, in addition to common tools like thermometers, pH meters and precision weighing scales, were used to evaluate the acute toxicity of *O. monacantha*. Laboratory Mill (*Thomas Wiley Model 4; serial number 870407*), Buchner funnel, centrifuge and Whitman filter paper are used for sample preparation and processing. A particular strain of mice called Bulb C mice are utilized in the animal tests; they are housed on wood shavings and fed regular rat chow. To guarantee precise and regulated administration of the *O. monacantha* extract, the doses are given straight into each mouse's stomach using oral gavage tubes and syringes. Throughout the experiment, meticulous records of procedures, observations and the results were maintained using manila folders and paper, ensuring organized documentation. The study also utilized normal saline, methanol and diethyl ether, handle samples with gloves and employ mutton cloth for specific procedures. Standard volumetric glassware, burettes and pipettes were used for accurate measurements (Campo et al., 2018).

3.2 Methods

Data collection procedure was done in the laboratory in the presence of laboratory assistants. The data was stored only for the purposes of this project.

3.3 Study design and Setting

The study was designed at Bindura University Of Science And Education and conducted an experimental investigation.

3.4 Plant Identification, Collection and Authentication

The *O. monacantha* leaves were collected from the *National Botanical Gardens and Herbarium* in Harare coordinates (17°47’56.4” S 31°03’08.6” E) in December 2023. At the *National Herbarium and Botanical Gardens in Harare*, a certified botanist identified the species. For future use, a voucher specimen was placed at the Herbarium.

3.5 Preparation and Extraction

The *O. monacantha* cladodes were washed under running water to eliminate any contaminants or impurities that might have been present on the surface. This step was crucial to ensure the purity of the extract**.** After washing, the plant was dried in a greenhouse, taking into consideration the degree of dryness required. This drying process typically took about two weeks. The dried cladodes were ground into fine powder using a grinding mill provided by the Department of Soil Science called the *Thomas- Wiley laboratory mill, model 4, Thomas scientific USA, serial number 87040, Catalogue number 33756 E20.* The sample of powdered leaves was weighed and kept in a dry, cool location. Over the course of three days, the extraction process was conducted using 70% methanol and a plant-to-solvent ratio of 1:5 (w/v). The choice of 70% methanol was based on its effectiveness in extracting a broad range of polar and moderately polar phytochemicals, including flavonoids and alkaloids. However, this solvent system may not effectively extract non-polar constituents such as certain lipids and terpenes, which would require the use of non-polar solvents like hexane or chloroform for comprehensive phytochemical profiling.

Figure 3.1: Extraction process (source taken by Tadiwa Muurawa)

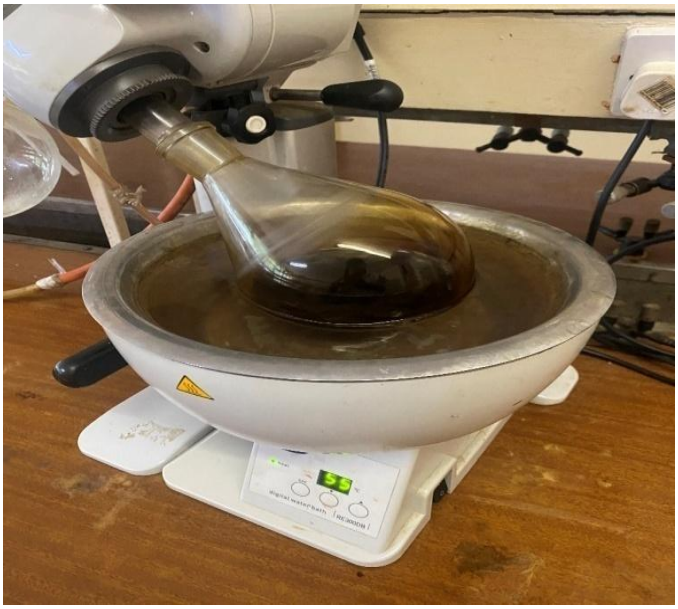


Figure 3.2: A Rotary evaporator (source taken by Tadiwa Muurawa)

A mutton cloth was used to filter both extracts in order to get rid of large, macroscopic particles. The resulting filtrate was once more filtered, this time with a vacuum pump, and then concentrated at 55°C under pressure using a rotary evaporator. The Department of Veterinary Science's freeze drier was used to freeze dry the filtrate that was produced following rotary evaporation. Before being used, the desiccator held the dried crude extracts. Weighing the dried extracts allowed us to establish the yield percentage: Equation 1

$$Extraction\ Yield\ (\%) = \frac{Weight\ of\ dried\ plant\ extract\ (g)}{weight\ of\ dried\ plant\ material\ (g)} \times 100 \qquad \textbf{[1]}$$

Figure 3.3: Maceration process of extraction (source taken by Tadiwa Muurawa)

3.6 Phytochemical Screening

Using standard protocols, a preliminary analysis of the extracts was conducted to determine the presence of different phytochemicals.

3.6.1 Test for Alkaloids

Two drops of Mayer's reagent were added to two milliliters of plant sample extract in a test tube to perform the Mayer's test. The presence of alkaloids was then confirmed by looking for the development of a white, creamy precipitate in the solution (Sintayehu et al., 2012).

3.6.2 Identification of Tannis

One milliliter of ethanol extract was combined with two milliliters of distilled water in a test tube. Two or three drops of ferric chloride are added after that. The test sample was examined for the development of a blue-black color, which denotes the presence of gallic tannins, and a green-blue color, which indicates the presence of catechic tannins (Bako et al., 2005).

3.6.3 Identification of Sapponins

After diluting 3 ml of extract with 20 ml of distilled water, the mixture was shaken for 15 minutes in a graduated cylinder. The test sample was examined for the development of a 1 cm layer of foam, a sign that sapponins are present (Ondeko et al., 2020).

3.6.4 Identification of glycosides

One drop of 5% iron chloride (FeCl3) and concentrated sulfuric acid were added to two milliliters of plant extract and glacial acetic acid. Glycosides are present if the top layer appears blue-green and a reddish-brown color appears where the two liquid layers converge (Ali et al., 2022).

3.6.5 Identification of terpenoids and Steroids

A solution of 0.5 ml acetic acid and 0.5 ml acetic anhydride was added to 4 mg of extract. Slowly, concentrated sulfuric acid was then added. According to Lawal et al. (2019), the sample was examined for the development of a blue-green color, which indicates the presence of terpenoids, and a reddish-brown color, which indicates the presence of steroids.

3.6.6 Identification of flavonoids

One milliliter of each extract was mixed with three drops of diluted sodium hydroxide. After a few drops of diluted acid were added to the test sample, it was noted that an intense yellow coloration of the colorless solution developed, indicating the presence of flavonoids (Solanki et al., 2019).

3.6.7 Identification of Sterols

One milliliter of an extract was treated with drops of acetic anhydride, chloroform, and sulfuric acid concentration. The formation of a dark pink or red color was monitored (Yanxiao et al., 2024).

3.7 Dose Preparation and Administration

Twenty-five healthy male and female Balb/c mice, ranging in age from four to seven weeks, were used for acute toxicity tests. Five mice were housed in each cage in three replicates and they were given free access to a standard diet that included 10 g of food and 20 ml of water. The mice were kept in a room with a 12-hour light/dark cycle, a temperature control of $22 \pm 2°C$ and a relative humidity of $55 \pm 10\%$. The animals were given a week to acclimate to the laboratory setting prior to the start of the experiments (Ellella et al., 2014).

The acute oral toxicity of *O. monacantha* was evaluated in mice using OECD Guideline No. 423. The extract was dissolved in the appropriate solvent to create new doses each day. The extract of *O. monacantha* was dissolved in ordinary saline. Alloxan and distilled water were administered to mice in the control groups, and the dosage was precisely administered by oral gavage. Each mouse was fasted for the whole night prior to dosing and for three hours following treatment. The initial dosage of *O. monacantha* was administered orally to three mice at a rate of 5 mg/kg BW.

These Bulb\c mice were monitored for clinical symptoms and mortality at regular intervals during the first 24 hours (0.5, 1, 2, 3, 4, and 24 hours). Once no mortality or overt toxicity symptoms were observed within 24 hours of treatment, the procedures were repeated three times using 500 mg/kg of *O. monacantha* extract. Following the survival of each animal in the first dose treatment group, the same protocol was used to test a higher dose group (2000 mg/kg BW). We also looked at a maximum dosage group of 5000 mg/kg. After receiving weekly feeding and

water intake, the animals underwent three repetitions of the procedure, with observations conducted once daily for 14 days (Awodele et al., 2012).

3.8 Data Analysis

Determining the extract's lethal dosage 50 (LD_{50}), or the quantity that kills 50% of test animals, is the primary goal. The well-liked statistical method known as probit analysis was used to estimate the LD_{50}. A regression analysis is carried out against the log-transformed doses after the fatality percentages have been transformed into probit values. The Mortality Rate was used to calculate the percentage of animals that died at each dose level. Clinical symptoms are noted and categorized based on feed and water intake. Mean +_ standard deviation (SD) was applied to quantitative data. Its statistical significance was investigated using ANOVA; a p-value of less than 0.05 was considered significant.

CHAPTER 4

RESULTS

4.1 Extraction

Yield of *O. monacantha* crude extract: 400g of leaf powder was obtained after grinding, 200g of the leaf powder was subjected to solvent extraction using methanol and 12.2g were obtained from freeze drying. The percentage yield was calculated as shown below:

% yield of *O. monacantha* = 12.2\200 x 100 = 6.10%

4.2 Phytochemical Screening

Phytochemical test were carried out using *O. monacantha* extract. The test confirmed the presences of Alkaloids, Glycosides, Terpenoids, Flavonoids, Tannis, Sapponins Phenols and Sterols.

Table 4.2: Phytochemicals present in *O. monacantha* extract:

Phytochemicals	*Results on O. monacantha* extracts	Detection
Alkaloids	Positive (+ +)	Reddish brown
Flavonoids	Positive (+++)	Yellow
Steroids	Negative -	Red
Sapponins	Negative	Foam
Terpenoids	Positive (++)	Reddish brown
Sterols	Positive (+++)	Dark pink or Red
Glycosides	Positive (++)	Brown Ring
Tannins	Positive (+++)	Green

Key: Positive(+++) mean Present , Negative(-) mean Absent and Positive (++ or +) mean Present in significant amount

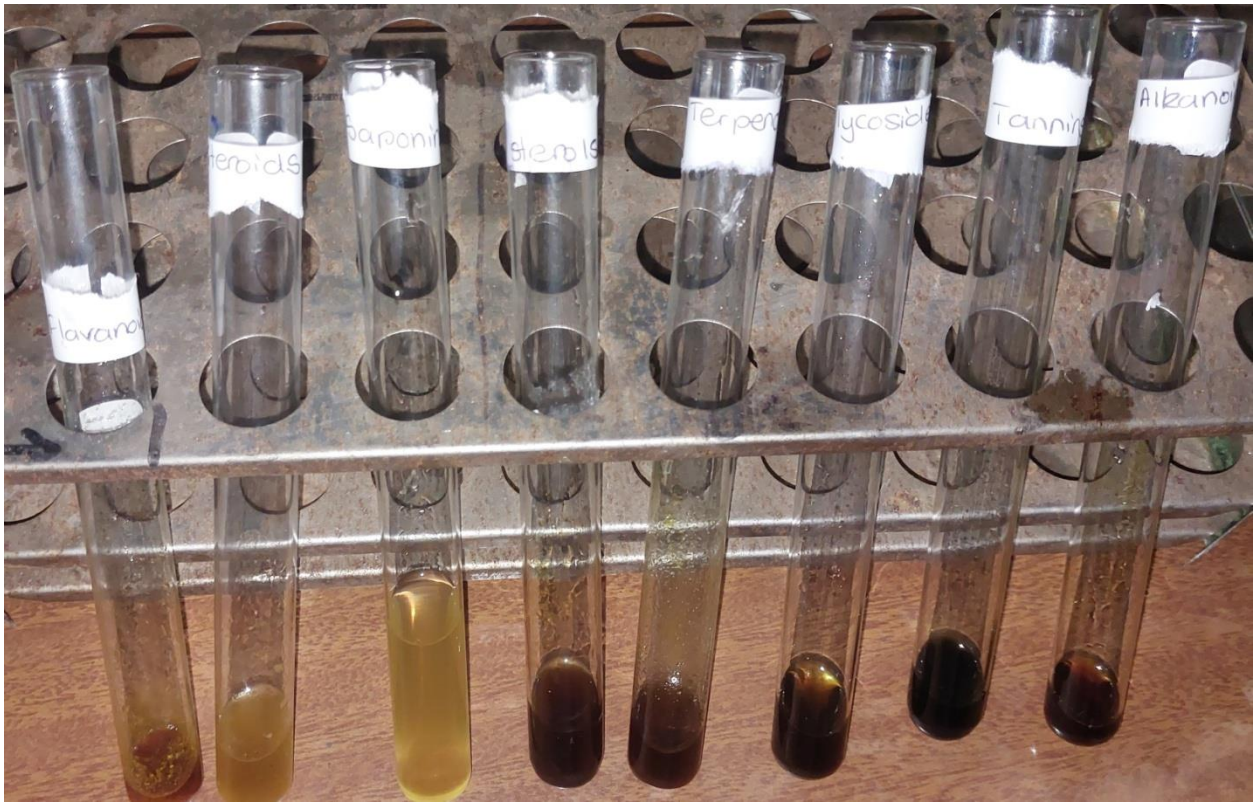


Figure 4.2: Results obtained after phytochemical testing.

The phytochemical testing found several bioactive components in the extract of *O. monacantha*. Color changes that were yellow, dark pink/red and green, which indicated high levels of flavonoids, sterols and tannins (+++). Reddish-brown and brown ring results were produced by the moderate levels of terpenoids and glycosides (++). Reddish-brown alkaloids (++) were detected in significant amounts. However, the lack of foam formation or color change indicated that steroids and sapponins were not present. These findings suggest that *O. monacantha* contains a range of compounds with anti-inflammatory, antioxidant and therapeutic properties.

4.3: Mortality rate in Balb\c mice

Table4.3.1: Results demonstrate a dose-dependent relationship between the administered doses and the observed mortality rates:

Treatments	Mortality			Mean
	Replication 1	Replication 2	Replicatio n 3	
T_0 (-ve) distilled water	0	0	0	0
T_1 (+ve) Alloxan	0	0	0	0
T_2 (500mg\kg *O. monacantha* extract)	0	0	0	0
T_3 (2000mg\kg *O. monacantha* extract)	1	1	2	1.33
T_4 (5000mg\kg *O. monacantha* extract)	3	4	3	3.33

The lower doses (T0, T1 and T2) resulted in no mortality, while higher doses (T3 and T4) caused increased mortality, with T4 having the most significant effect however the p-value =0.000016, since the $p<0.05$ the result is statistically significant. These results imply that the lethal effects of the treatment are more pronounced at higher doses, and the mortality rates provide a useful indication of the toxic potential of different dosages.

Table 4.3.2: One-Way ANOVA results showing significant difference in mortality:

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-Statistic
Between Groups	22.9149	4	5.7287	42.98
Within Groups	1.3334	10	0.1333	
Total	24.2483	14		

The exceptionally high 42.98 F-statistic suggests that the groups differ significantly from one another. Group differences might be the primary cause of the variation, as evidenced by the significantly greater Between Groups variation (SS = 22.9149, df = 4) than the Within Groups variation (SS = 1.3334, df = 10). There is at least one group mean that differs significantly from the others, as indicated by the F-value being significantly greater than 1.

4.4: Feeding and water intake in Balb\c in mice

Table 4.4.1: Results obtained after varying doses of *O. monacantha* extract on feed and water intake in mice.

Treatments	Replication 1		Replication 2		Replication 3		Mean	
	Feed	Water	Feed	Water	Feed	Water	Feed	Water
T_0 (-ve) distilled water	8	18	9	17	7	18	8	17.67
T_1 (+ve) Alloxan	7	18	7	18	8	16	7.33	17.33
T_2 (500mg\kg) *O. monacantha* extract	8	17	7	18	8	17	7.67	17.33
T_3 (2000mg\kg)*O. monacantha* extract	6	12	5	10	4	9	5	10.33
T_4 (5000mg\kg)*O. monacantha* extract	3	**7**	3	6	2	5	2.67	6

The dosage of *O. monacantha* extract caused a dose-dependent decrease in the amount of feed and water that mice consumed. Both the control group (T0) and the low-dose group (T2, 500 mg/kg) received comparatively high feed and water intakes. At larger doses (T3: 2000 mg/kg

and T4: 5000 mg/kg), however, both metrics experienced a significant reduction, with T4 showing the lowest intake levels. This suggests that mice that receive high dosages of the extract can show signs of dehydration and appetite suppression.

Table 4.4.2: One way ANOVA for feed and water intake, showing the effects of different treatments of *Opuntia monacantha* extract on mice:

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-ratio	p-value	Variable
Between Groups	61.73	4	15.43	25.72	0.00003031	Feed
Within Groups	6.00	10	0.60			
Total	67.73	14				
Between Groups	338.27	4	84.57	79.28	0.000000156	Water
Within Groups	10.67	10	1.07			
Total	348.93	14				

Since the p-value for feeding is less than 0.05, we reject the null hypothesis. This suggests that there is a statistically significant difference in feed intake between at least two treatment groups. The null hypothesis is disproved because p for water is less than 0.05. This suggests that there were statistically significant differences in the water intakes of the treatment groups. The water intake means of each group are equal, according to the null hypothesis (H_0). An Alternative Theory

(H_1): At least one group mean differs. These findings support the notion that higher dosages of *O. monacantha* extract cause quantifiable and noteworthy alterations in feeding and hydration patterns, which are in line with documented toxicity or stress reactions.

4.5 Probit Analysis Graph

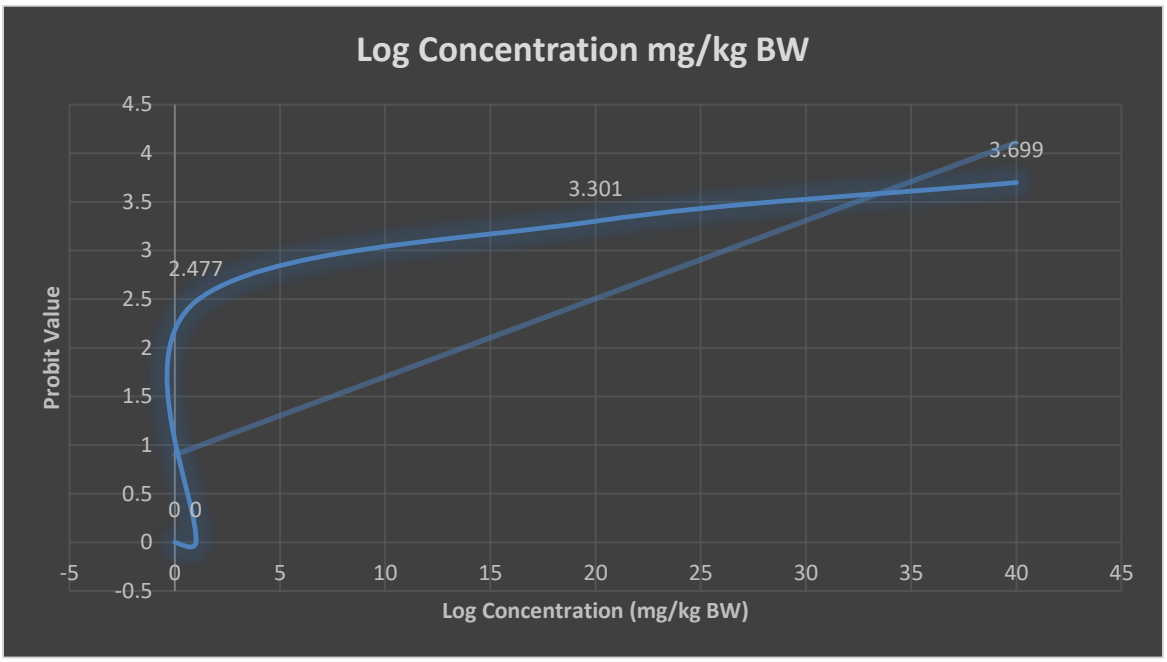


Figure 4.5: The LD_{50} using probit analysis

Interestingly, the probit value is roughly 3.3 at a log concentration of 3.301, which corresponds to 50% mortality, the threshold for LD_{50} and the estimated LD_{50} of roughly 2300 mg/kg body weight.

4.5.2: LD_{50} Calculations

The probit analysis confirms a dose-dependent response to the extract, with clear increases in probit values at higher log concentrations.

Linear Interpolation Formula:

$$LD_{50} = D_1 + (50 - M_1 / M_2 - M_1) \times (D_2 - D_1)$$

Where:

- D_1 = 2000 mg/k g (lower dose)

- D_2 = 5000 mg/kg (higher dose)

- M_1 = 44.4%

- M_2 = 100%

LD_{50} = 2000 + (50 - 44.4/100 - 44.4) × (5000 - 2000)

LD_{50}= 2000 + (5.6 /55.6) × 3000

LD_{50} = 2000 + (0.1007 × 3000) = 2000 + 302.1 = 2302 mg/kg BW (approx.)

Final Result:

Estimated LD_{50} = 2300 mg/kg BW

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CHAPTER 5

DISCUSSION

5.1 Introduction

The phytochemical composition was examined in this study, toxicity and physiological effects of *O. monacantha* extract on common house mice, with a focus on how the extract affects feed and water intake and mortality at varying doses. The findings contribute to our understanding of the plant extract's biological activity and safety profile.

5.2 Collection and Extraction

The identification and authentication of plant parts is a critical quality assurance step in herbal medicine research. In order to trace starting materials and distinguish them from related species or adulterants, this procedure makes sure that the right plant species and plant parts are used. In this study, *O. monacantha* cladodes were shade-dried for approximately two weeks. Shade drying is important for preserving vital plant constituents, particularly in the extraction of essential oils. To increase the surface area available for extraction and decrease particle size, the plant material was subsequently ground into a powder (Maldonado et al., 2010). The choice of extraction method is crucial, as it can significantly influence the extract's activity. Maceration with 70% methanol was selected because methanol's availability and cost-effectiveness, but also its superior ability to penetrate plant tissues and extract a wider range of compounds, including polar ones like flavonoids and alkaloids, compared to ethanol (Koubaa et al., 2015).

5.3 Phytochemical Composition

Phytochemical analysis of the *O. monacantha* extract identified various bioactive components, such as alkaloids, flavonoids, glycosides, terpenoids, sterols, phenols and tannins.
These secondary metabolites are known to have a wide range of pharmacological effects, including antioxidant, anti-inflammatory, antidiabetic and antibacterial characteristics.
The extract's capacity to regulate oxidative stress and metabolic processes is suggested by the abundance of flavonoids and tannins in particular (Chougui et al., 2013).

5.4 Extraction Yield

Maceration and rotary evaporation were used to successfully extract *O. monacantha's* methanol, producing 6.10% crude extract. This yield demonstrates that the leaf material contains extractable bioactive compounds and validates that methanol is a suitable solvent. This yield also provides a benchmark for future scale-up and standardization efforts (Priya et al., 2013). The selection of methanol was supported by its effectiveness in extracting polar compounds like flavonoids and alkaloids, as demonstrated in other research (Chaalal et al., 2013). The phytochemical analysis of the extract corroborated findings from prior research that identified chemicals with potential anti-inflammatory and anti-proliferative properties, indicating a prospective function in diabetes management and insulin generation (Mena et al., 2018).

5.5 Dose-Dependent Toxicity and Mortality

Mortality data indicated a dose-dependent increase in lethal effects, with higher doses of the extract leading to greater mortality. This finding underscores the toxic potential of the extract at elevated concentrations. The LD_{50}, estimated through probit analysis, suggests that the *O. monacantha* extract exhibits moderate toxicity at higher doses (Hachlafi et al., 2021).

5.6 Impact on Feed and Water Intake

The study demonstrated that the extract from *O. monacantha* significantly influenced feeding and hydration behavior. Increased extract concentrations may interfere with normal physiological processes linked to appetite and fluid balance, as indicated by the alterations in feed and water intakes. The extract's effects on the central nervous system, metabolic stress or gastrointestinal discomfort could all be responsible for these side effects. While lower doses may be safe or even have therapeutic potential, higher doses may compromise animal well-being and normal physiological functioning (Alamer et al., 2014).

5.7 Interpretation of Probit Analysis

Probit analysis validated the dose-dependent toxicity of the extract. The sigmoidal dose-response curve, with increasing probit values corresponding to increasing log concentrations, is characteristic of typical toxicological profiles. The probit analysis indicates a clear relationship between the administered extract doses and the observed mortality rates.

5.8 Implications and Future Directions

The possible applications of *O. monacantha* in toxicology and pharmacology are significantly impacted by the study's conclusions. Given its abundance of phytochemicals, the extract may have therapeutic value, especially when taken in little amounts. Nonetheless, the harmful consequences noted at increased dosages emphasize the necessity of vigilance and meticulous dosage control.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study used bulb\c mice to conduct in vivo experiments in order to examine the acute toxicity profile of *O. monacantha* methanolic extract. Both the lethal dose and any discernible sub-lethal effects of the extract on feed and water intake, as well as mortality patterns, were to be assessed in this study. The test mice were given a range of doses (500, 2000 and 5000 mg/kg) orally using a scientifically rigorous approach in compliance with OECD guidelines. Additionally, a thorough analysis of the phytochemical composition of the plant was carried out to shed light on the possible bioactive compounds that may be responsible for the effects that were observed.

Alkaloids, flavonoids, terpenoids, glycosides, sterols and tannins were among the secondary metabolites that the phytochemical screening verified were present. The various pharmacological characteristics of these bioactive substances, such as their cytotoxic, antibacterial and antioxidant actions, are well known. Remarkably, the extract showed no traces of steroids or sapponins, suggesting a phytochemical profile specific to *O. monacantha*. Depending on their quantity and how they interact with physiological systems, different classes of phytochemicals can show varying degrees of toxicity, which may help to partially explain the toxicological profile shown in the experimental mice.

The mortality results showed a clear dose-response relationship. No deaths were recorded at the lower dose levels (500 mg/kg) and minimal mortality was observed at 2000 mg/kg. However, significant lethality occurred at the highest dose of 5000 mg/kg, where an average mortality of 3.33 was recorded. These outcomes point to a dose-dependent toxicity pattern, with substantial physiological disruptions occurring at high doses. The calculated LD_{50}, derived from probit analysis, is a critical metric that places *O. monacantha* extract within a moderately toxic range. The one-way ANOVA further confirmed that these variations in mortality across the treatment groups were statistically significant, thereby strengthening the reliability of the dose-toxicity relationship.

In addition to mortality, other physiological consequences were noted as dosage increased, including decreases in feed and water intake. These toxicity indicators were most noticeable at 2000 mg/kg and 5000 mg/kg, with the latter group showing the most reduction in food and water intake. Systemic stress and possible organ malfunction are suggested by this decline, which is most likely the result of toxic chemical buildup or interference with regular metabolic or gastrointestinal functions. Both the observed mortality and decreased intake suggest that the animals' physiological equilibrium may be jeopardized by high quantities of *O. monacantha* extract. According to the data, *O. monacantha* extract has detectable harmful effects at high doses even if it also contains beneficial phytochemicals. This discovery has significant ramifications for the application of this plant in pharmacology and traditional medicine. It highlights how crucial it is to carefully control dosage, particularly if the extract is going to be utilized therapeutically on either humans or animals. To identify organ-specific effects, long-term toxicity, and the precise biochemical pathways impacted by the extract, more investigation would be necessary.

In summary, this work effectively determined the acute toxicity profile of *O. monacantha* methanolic extract in mice, pointing out the possible hazards connected with higher dosages and establishing a safe threshold for its administration. The findings show that even though *O. monacantha* has a variety of advantageous phytochemicals, using it should be done carefully. The results lay the groundwork for further pharmacological and toxicological studies and highlight the significance of evidence-based validation of traditionally used therapeutic herbs. The results of the probit analysis shed important light on the *O. monacantha* extract's toxicity profile. Future investigations and the development of the extract as a possible medicinal agent might be guided by the estimated LD_{50} value and the dose-response relationship noted in the study. These results also show that the *O. monacantha* is not toxic and proves that people may continue using the plant.

6.2.0 Recommendations

Opuntia monacantha medications should be taken in small doses. Without first undergoing a toxicological study, extracts especially in uncontrolled traditional formulations should not be ingested in excessive quantities. Standardization of Extracts: To lessen the possibility of toxicity from batch variability, herbal medicines containing *O. monacantha* should be standardized to guarantee constant quantities of bioactive components. Public awareness: initiatives should be

started to educate users and practitioners of traditional medicine about the possible toxicity linked to inappropriate or overuse of this strategy. Regulatory oversight: *O. monacantha* should be added to lists of medicinal plants that need pharmacological and toxicological proof before being approved for use in commercial therapy. Integration into guidelines: national or regional guidelines for the safe use of medicinal herbs should incorporate the findings of studies like this one.

6.2.1 Future Research

Sub-chronic and Chronic Toxicity Studies: Although acute toxicity was the main focus of this investigation, it is essential to look at the long-term consequences of repeated exposure to *O. monacantha* extract in order to evaluate cumulative toxicity concerns. Histopathological Analysis: A thorough organ-level analysis is necessary to determine which tissues are most and to what degree the extract affects them. Evaluations of liver and kidney function indicators as well as total blood counts would offer a more profound understanding of systemic toxicity through biochemical and hematological profiling. Mechanistic studies: to determine how toxicity is induced, researchers should investigate the molecular and cellular pathways that the phytochemicals in *O. monacantha* impact. Generalizability will be validated through testing in other animal models and subsequently human clinical trials, as the effects identified may be species-specific. Formulation development: attempts should be made to separate the extract's healthier components and transform them into less hazardous formulations.

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APPENDICES

APPENDIX 1: Approval letter from National Herbarium and Botanical Gardens

MINISTRY OF LANDS, AGRICULTURE, FISHERIES, WATER AND RURAL DEVELOPMENT
DEPARTMENT OF RESEARCH & SPECIALIST SERVICES (DR&SS)

All Communications to be addressed to
"THE HEAD"

Telephone: 263-4-744 170, 745230
E-mail: nhbg@drss.gov.zw
Fax:

NATIONAL HERBARIUM & BOTANIC GARDEN
Box A889
Avondale, Harare, Zimbabwe

ZIMBABWE

04 December 2023

Muurawa Tadiwa
UZ

Dear Sir/ Madam

RE: Plant Identification

The plant specimen you brought in were identified as *Opuntia ficus-indica.* It belongs to the family Cactaceae. Common names Idolofiya (N), Mudhorofiya (S), Prickly pear (E).

Thank you.

HEAD
Nat. Herbarium & Botanic Gardens
D.R. & S.S. Research Serv. Division
04 DEC 2023
P.O. BOX A 889, AVONDALE
HARARE, ZIMBABWE

Mazarire T. C. (Mrs.)
Research Officer
National Herbarium and Botanic Garden

Appendix 2: Approval letter from department of Pharmacy and Pharmaceutical Science Laboratories

DEPARTMENT OF PHARMACY AND PHARMACEUTICAL SCIENCES

P.O. Box MP 167
Mount Pleasant
Harare, Zimbabwe

Telephone: 263-4-303211 Ext. 1349
Telex: 26580 UNIVZ ZW
Telegrams: UNIVERSITY
Telefax: 263-4-307148
Email: pharmacy@medic.uz.ac.zw
Email:louisgada@gmail.com

Chairman: Mr L. Gadaga
BPharm (Hons) (*Univ Zim*), MPhil (*Univ Zim*)

FACULTY OF MEDICINE AND HEALTH SCIENCES UNIVERSITY OF ZIMBABWE

04 October 2024

To Whom It May Concern

This letter serves to confirm that Tadiwa Muurana

Student registration number B212194B

Has been granted permission to use the Department of Pharmacy and Pharmaceutical Sciences Laboratories to carry out his/her project entitled Assessing acute toxicity of Opuntia Monacantha as a potential nutraceutical for diabetes

and is being supervised by Dr Mugochekei, Mr P Hove and Mr L. Gadaga

Thank you

J. Mutenure
A/Chief Technician

UNIVERSITY OF ZIMBABWE
Department of Pharmacy and Pharmaceutical Sciences
OFFICIAL

Appendix 3: Approval letter for the use of experimental animal models

UNIVERSITY OF ZIMBABWE

FACULTY OF MEDICINE & HEALTH SCIENCES

ANIMAL HOUSE UNIT

DATE 10/10/2024

TO: J.R.E.C

FROM: A/Chief Technician

RE: CONFIRMATIONOF APROVAL FOR THE USE OF EXPERIMENTAL ANIMAL MODELS

This letter serve to inform you that Tadiwa Muurawa(B212794B) is a Bachelor Biologiacal Sciences student under the supervision of Mr Hove,Mr .L.Gadaga and Dr Mugocheki has been granted the permission to use laboratory animals in her research project

This approval has been granted on the condition that the student will carry out the research under the supervision of Dr.L.Gadaga who is licensed to use animals for research.

Supervisor's signature.....

A/Chief Technician .....

UNIVERSITY OF ZIMBABWE
CHIEF TECHNICIAN
2024 -10- 10
ANIMAL HOUSE
Faculty of Medicine & Health Sciences

Appendix 4: JREC Approval Letter

UNIVERSITY OF ZIMBABWE
Faculty of Medicine & Health Sciences

Joint Research Ethics Committee
For The University of Zimbabwe,
Faculty of Medicine and Health Sciences(FMHS) &
Parirenyatwa Group of Hospitals(PGH)

JREC Office No.4, 5th Floor, Faculty of Medicine and Health Sciences Building
Telephone: +263 242 708140/791631 Extns 2241/2242
Email: jrec.office@gmail.com - website: www.jrec.uz.ac.zw

Parirenyatwa Group of Hospitals

APPROVAL LETTER

Date: 16 January 2025

JREC Ref: 49/2025

Names of Researcher **Tadiwa Muurawa.**

Address: **UZ – Department of Pharmacy and Pharmaceutical Sciences.**

RE: **ASSESSING ACUTE TOXICITY OF PRICKLY PEAR CACTUS (MUDHOROFIYA) AS A POTENTIAL NEUTRACETICAL FOR DIABETES.**

Thank you for your application for ethical review of the above-mentioned research to the Joint Research Ethics Committee. Please be advised that the Joint Research Ethics Committee has reviewed and approved your application to conduct the above-named study. You are still required to obtain MRCZ and RCZ approval before you commence the study if required by the nature of your study.

- **APPROVAL NUMBER:** **JREC/49/2025**
- **APPROVAL DATE:** **16/01/2025**
- **EXPIRY DATE:** **15/01/2026**

This approval is based on the review and approval of the following documents that were submitted to the Joint Ethics Committee:

a) Completed Application Form
b) Full Study Protocol
c) Informed Consent in English and/or appropriate local language

After this date the study may only continue upon renewal. For purposes of renewal please submit a completed renewal form (obtainable from the JREC office) and the following documents before the expiry date:

a. Progress Report
b. A Summary of Adverse Events
c. A DSMB Report

- **MODIFICATIONS:**

  Prior approval is required before implementing any changes in the protocol including changes in the informed consent.

- **TERMINATION OF STUDY:**

  On termination of the study you are required to submit a completed request for termination form and a summary of the research findings / results.

Yours sincerely,

Dr Fiona Makoni
JREC Chairperson
FM/uh