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Acute toxicity evaluation of *Opuntia stricta* Cladode grown in Zambia on *Wistar* albino rodents

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Abstract

Background: Traditionally, *Opuntia stricta* has been used to manage diabetes mellitus in many parts of the world, including Zambia. However, while a few studies elsewhere have assessed the safety of *Opuntia stricta*, no study to date has established its safety in Zambia. The study aimed to evaluate the acute toxicity of *Opuntia Stricta* cladode (OSC) on Wistar albino rodents.

Methods: At dosages of 0.5 g/kg, 1 g/kg, and 2 g/kg per os (p.o), an acute oral toxicity test was conducted in Wistar rats. Over a period of 14 days, all the groups of animals were watched for indications of pharmacotoxicity signs, mortality, and compared with the respective controls. Subsequently, tests for liver and kidney function, blood profile, relative organ weight, and histological examinations of the liver and kidney were conducted.

Results: The results showed that oral administration of methanolic extracts of Opuntia stricta cladodes (0.5 g/kg, 1 g/kg, and 2 g/kg) to Wistar rats for 14 days produced no observable behavioral changes (including restlessness, agitation, diarrhea, dullness, or convulsions), and no mortality occurred compared with the vehicle control group ($p > 0.05$). Furthermore, relative organ weights, biochemical and hematological parameters, as well as liver and kidney histology, did not differ significantly from the vehicle control group ($p > 0.05$). Therefore, the median lethal dose (LD_{50}) of the methanolic extract of Opuntia stricta cladodes was greater than 2000 mg/kg.

Conclusion: These results led to the conclusion that *Opuntia Stricta* extract for short-term use is non-toxic via the oral route

Keywords: Acute toxicity; cladodes; *Opuntia stricta; Wistar albino* rodents.

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Background

Opuntia stricta (OS) belongs to one of the most diverse and widely distributed genera of plants, found on every continent except Antarctica [1]. It is most diverse in Mexico, its likely place of origin, where various levels of domestication have been documented. From there, it has spread globally and is now established in temperate, subtropical, and tropical regions [2]. Medicinal plants are considered a mainstay of traditional medicine and their acceptance is continuously on the rise because 60% of the world population depends on herbal preparations against several ailments[3, 4]. Since ancient times, extracts from *Opuntia spp*. have been used to treat a variety of illnesses, including chronic and inflammatory disorders like diabetes, rheumatism, asthma, hypercholesterolemia, and hypertension [5]. Recent research indicates that *Opuntia* species demonstrate several pharmacological properties, including antioxidants, anti-inflammatory, antidiabetic, and hepatoprotective benefits [6-8]. Among these uses, the management of diabetes mellitus is well documented in traditional medicine. Diabetes mellitus is a chronic metabolic disorder characterized by impaired metabolism of carbohydrates, proteins, and lipids, leading to elevated blood glucose levels [9]. This condition arises from insufficient insulin secretion by the pancreatic β-cells or impaired responsiveness of peripheral cells to insulin. Insulin, a hypoglycemic hormone secreted by the β-cells of the islets of Langerhans, is crucial for regulating blood glucose by promoting its uptake and metabolism in peripheral tissues[9, 10]. Although many medicinal plants have demonstrated antidiabetic properties, they are not free from toxicity. For example, Bnouham et al. (2002) compiled a list of 94 antidiabetic plant species, 17 of which were found to be toxic, with toxicity often depending on the route of administration [11]. Traditional medicine continues to be widely practiced globally, particularly in developing countries, where medicinal plants are abundant, affordable, and readily available. While the therapeutic potential of these plants is well recognized and continues to be explored, concerns about their safety persist [12, 13]. In many cultures, medicinal herbs are often perceived as harmless or having low toxicity due to their long history of use [14, 15]. However, several studies have reported the toxicological and biological effects of juices and cladodes from *Opuntia* species [16, 17]. One such study reiterated that antidiabetic medicinal plants are not inherently safe and that toxicity is frequently related to the route of administration [18].However, there is dearth of data regarding acute toxicity of OSC. Like pharmaceutical drugs, plant-derived extracts can offer significant therapeutic benefits but are not inherently safe and may pose health risks if misused[19, 20]. Their use in health care should therefore be guided by regulation and scientific evaluation to minimize adverse effects, some of which can be severe or fatal [21, 22]. Establishing safety begins with assessing their overall biological effects, starting with toxicity studies [23]. Such studies form an essential part of preclinical evaluation and help determine whether a new plant extract presents immediate or long-term health hazards [23, 24]. General toxicity assessment typically involves acute, subacute, and chronic toxicity testing , conducted over varying durations and dosing regimens to provide a comprehensive safety profile [23, 25]. Currently, no published study in Zambia has assessed the acute toxicity of methanol extract of OSC. Therefore, the current study was carried out to evaluate the acute toxicity of methanol extract of OSC in Wistar albino rodents.

Methods

Plant materials and extraction

Opuntia stricta cladodes with an approximate length of 25–30 cm were manually harvested in chibombo district chief Mungule in October 2021. The collected specimens were taxonomically recognised by plant specialists at the University of Zambia, Department of Biological Sciences. Laboratory (Ref: OS-001). All the Cladodes were collected from their natural origin with no treatments applied on and maturity ranged from spring shoots to fully developed cladodes at 3 weeks. Cladodes were washed with distilled water and disinfected using commercial 10% (w/v) sodium hypochlorite solution. The spines were manually removed using a knife. The cladodes were cut into 2 x 2 cm^2, dried in the shade for 3 weeks at room temperature and then pulverized in an electric mill to get particles smaller than 4 mm. 100 g of each of the pulverized samples was weighed on the Toppan balance and was extracted separately with 330 mL methanol 48 hours. The extract was filtered using Whatman No. 1 filter paper and evaporated to dryness using a rotary evaporator under reduced pressure at various temperatures. The dried material was stored under refrigeration at 4-8°C until use.

Experimental animals

Wistar albino rats weighing 150 – 170 g of 8 - 10 weeks of either sex were acquired from the Department of physiology, School of medicine, University of Zambia. All the experiments were conducted in the Animal House of Department of physiology, School of medicine, University of Zambia (UNZA). Animals were kept in ventilated polypropylene cages for two weeks under regular laboratory conditions of temperature and humidity after randomization and before the experiment started. The rats were fed with a standard laboratory diet during this time, consisting of rat pellets bought from livestock feeds Limited in Lusaka, Zambia, and were provided unlimited access to water. Twelve-hour cycle of light and dark was also permitted in the entire period of the experiment [26].

Acute oral toxicity test (AOTT)

Acute oral toxicity study was executed as per the protocols of Organization for Economic Cooperation and Development (OECD) guidelines 425 with slightly modification [27]. 12 male and 12 nulliparous female animals were fasted overnight prior to dosing. They were randomly divided into four groups with each group containing six animals (three male and 3 female). The fasting body weight of each animal was recorded after weighing and the dose was determined accordingly. The crude methanol extracts 2000 mg/kg, 1000 mg/kg and 500 mg/kg of the cladode were administered in a single dose by gavage. The rats were then kept under strict observation for pharmacotoxicity signs such as motor activity, agitation, sedation, restlessness, gasping, touch and sound sensitivity for 24 h, with special attention during the first 4 h[28]. These observations continued for further 14 days period of administration for any signs of toxicity. On the 15th day, rats were weighed, humanly sacrificed by cervical dislocation. Thereafter, blood and organs were obtained and used as stated below.

Body weight and relative organ weight

In order to determine the relative organ weight , the liver and kidney were carefully removed and weighed using the analytical balance [29].

Determination of the hematological and biochemical parameters

Blood was drawn by cardiac puncture from three randomly selected rats per group and evaluated for hematological profile, liver function test (LFT) and kidney function test [30]. For the hematological profiling, a complete blood count (white blood cells; red blood cell count; Hemoglobin; hematocrit; mean corpuscular hemoglobin concentration; mean corpuscular hemoglobin; mean corpuscular volume and platelet count) was determined using the methods described in the previous study[28]. Regarding liver function test, Alkaline phosphatase (AP), Alanine aminotransferase (ALT), Gamma-glutamyl transferase (γ-GT) and total bilirubin were measured using the methods described in the previous studies[31-33].

Histological studies of the liver and kidneys

The liver and kidney samples obtained as previously indicated were fixed in neutral buffered formalin (10%) and then sectioned with paraffin. Sections (6 m) were stained with hematoxylin and eosin (H&E) and examined under a microscope using a Nikon Eclipse 90i and TE2000-E at a 20x magnification. Any morphological alterations in the hepatic and renal cells' anatomical architecture were noted [34]. The photos were taken with Nikon Imaging Software [NIS) element 3.0 AR)] and then underwent additional editing in Adobe Photoshop Creative Suite-2 CS2.

Statistical analysis

Independent experiments were performed thrice, and data were presented as mean ± standard deviation (SD). Shapiro-Wilk test was conducted to check for normality after which One-way analysis of variance (ANOVA) was performed using Graph Pad Prism Software Version 5.0 to determine differences among the results of the samples. Tukey's Honestly Significant Difference (HSD) test was performed as a post hoc to compare the results between the groups. The p value less than 0.05 was considered significant (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.005$).

Results

Acute toxicity in Wistar rats

Methanolic extract of *Opuntia stricta* cladodes, administered orally to Wistar rats at doses of 2000 mg/kg, 1000 mg/kg, and 500 mg/kg for 14 consecutive days, was well tolerated. No observable behavioral changes (including restlessness, agitation, tremors, diarrhea, dullness, or convulsions) were detected throughout the study period, and no mortality occurred. These findings did not differ significantly from those of the vehicle control group ($p > 0.05$).

Body weights and relative organ weights

Cladode methanol extracts of 2000 mg per kg, 1000 mg per kg, and 500 mg per kg were administered throughout the 14 days, and there was no effect ($p > 0.05$) on body weight (162.2 ± 6.0 g) as compared to vehicle control animals (162.7 ± 6.8 g) Figure 1. On the 15th day, rats were sacrificed, and relative organ weights (mg ± SD) from both sexes were weighed.

In the liver, the mean weight of the control male and female rats was 7.8 g ± 0.25 and 7.2 g ± 0.10, respectively. Following treatment with the extract at 500 mg/kg, liver weights were 7.8 g ± 0.38 in males and 7.0 g ± 0.06 in females. At the intermediate dose of 1000 mg/kg, liver weights were 7.7 g ± 0.15 in males and 7.1 g ± 0.11 in females. At the highest dose of 2000 mg/kg, the liver weights were 7.8 g ± 0.35 and 7.2 g ± 0.06 in males and females, respectively. No statistically significant differences ($p > 0.05$) were observed between the treated groups and their respective controls. The weight of the right and left kidneys in control male rats was 0.63 ± 0.02 g and 0.65 ± 0.01 g, and in female rats, it was 0.61 ± 0.02 g and 0.60 ± 0.00 g, respectively. After treatment with extract at 500 mg/kg, the weight of the right kidney in male and female rats was 0.63 ± 0.02 g and 0.61 ± 0.02 g, respectively. The weight of the left kidney in male and female rats was 0.64 ± 0.02 g and 0.61 ± 0.01 g, respectively. At an intermediate dose, the weight of the right kidney of male and female rats was 0.64 ± 0.02 g and 0.61 ± 0.01 g, respectively, and the weight of the left kidney at the same dose was 0.66 ± 0.01 g and 0.62 ± 0.01 g, respectively. At a high dose, the weight of the right kidney of male and female rats was 0.64 ± 0.01 g and 0.6 ± 0.01 g, respectively, and the weight of the left kidney at the same dose was 0.67 ± 0.03 g and 0.62 ± 0.01 g, respectively. We consistently noted a non-significant difference ($p > 0.05$) in the kidney weight between the control and treated groups (Table 1).

Determination of hematological and biochemical parameters

In rats under vehicle control, the levels (mean± SD) of hematological indicators like white blood cells (WBC: 7.1 ± 0.21 × 10^9/L), red blood cell count (RBC: 5.1 ± 0.25 × 10^6 /µL), hemoglobin (Hb: 13.1 ± 0.15 g/dL), mean corpuscular volume (MCV: 55.0 ± 1.1 fL), hematocrit (HCT: 46.2± 0.8%), mean corpuscular hemoglobin (MCH: 31.3 ± 1.0 pg), mean corpuscular haemoglobin concentration (MCHC: 32.7 ± 1.5 g/dL), and platelets count (796.7 ± 3.1 × 109/L) were obtained. When the group was given 500 mg/kg of methanol extract, their WBC (6.9 ± 0.21 × 109/L), RBC (5.1 ± 0.15 × 106 /µL), Hb (13.1 ± 0.26 g/dL), MCV (56.3 ± 1.5 fL), HCT (46.3 ± 1.5%), MCHC (32.7 ± 1.5 g/dL), MCH (30 ± 2.1 pg), and platelet count (798 ± 2.1 × 109/L) were similar ($p > 0.05$) to the vehicle controls. Concerning methanol extract (1000 mg/kg), the values of WBC (7.2 ± 0.06 × 109/L), RBC (5.3 ± 0.26× 106/µL), Hb (13.6± 0.14 g/dL), MCV (58.0 ± 1.0 fL), HCT (46.3 ± 1.3%), MCHC (32.9 ± 1.8 g/dL), MCH (31.7 ± 1.5 pg), and platelet count (799.9 ± 3.5 × 109/L) were equally comparable to vehicle control ($p > 0.05$). In relation to methanol extract (2000 mg/kg), WBC (7.1 ± 0.05 × 109/L), RBC (5.1 ± 0.06 × 106/µL), Hb (13.9 ± 0.15 g/dL), MCV (56.0 ± 2.0 fL), HCT (46.3 ± 1.3%), MCHC (33.3 ± 0.57 g/dL), MCH (31.1 ± 0.58 pg), and platelet count (801.1 ± 3.7 × 10^9/L) showed similar values to vehicle control ($p > 0.05$) in all respects.

The levels (mean ± SD) of neutrophils (39.7 ± 1.5%), lymphocytes (61.3 ± 1.5%), and monocytes (7.0 ± 1.0%) were found in rats that were given vehicle control. When the group was given methanol extract (500 mg/kg), neutrophils (40.9 ± 0.7%), lymphocytes (62.3 ± 1.2%), and monocytes (7.7 ± 0.6%) had values that were similar ($p > 0.05$) to those in the vehicle controls. Regarding methanol, extract (1000 mg/kg) increased the values of neutrophils (39.2 ± 0.7%), lymphocytes (62.7 ± 1.2%), and monocytes (7.3 ± 0.6%), and demonstrated comparable ($p > 0.05$) values to the vehicle controls. Neutrophils (39.3 ± 0.6%), lymphocytes (62.0 ± 2.0%), and monocytes (7.3 ± 1.2%) all had

similar values ($p > 0.05$) to those of the vehicle controls when methanol extract (2000 mg/kg) was used.

Regarding biochemistry, in-vehicle control rats, the blood levels of the products of kidney creatinine (25.0 ± 1.0 mmol/L) and urea (16.3 ± 0.6 mmol/L) were noted. The levels of liver enzymes alanine aminotransferase (ALT: 50.7 ± 2.1 U/L), aspartate aminotransferase (AST: 147.7 ± 2.5 U/L), and albumin (37.1 ± 0.25 g/dL) were noted.

The values of the treatment (500 mg/kg) group were ALT (51.7 ± 1.7 U/L), AST (148 ± 1.5 U/L), and albumin (37.5 ± 0.43). In addition, creatinine (26.0 ± 1.7 mmol/L) and urea (17.3 ± 1.6 mmol/L) were statistically non-significant ($p > 0.05$) in comparison to vehicle control. The levels of ALT (52 ± 1.2 U/L), AST (148.7 ± 1.5 U/L), albumin (39.8 ± 1.0 mmol/L), creatinine (26.9 ± 1.0 mmol/L), and urea (16.3 ± 0.6 mmol/L) in the 1000 mg/kg treatment group were all statistically not different from the vehicle control group ($p > 0.05$). Regarding the 2000 mg/kg concentration, the values of ALT (53.7 ± 1.28 U/L), AST (149.9 ± 2.5 U/L), albumin (37.6 ± 0.7 mmol/L), creatinine (27.3 ± 0.7 mmol/L), and urea (17.3 ± 1.0 mmol/L) were equally statistically non-significant ($p > 0.05$) when compared to vehicle control.

Histological studies of the liver and kidneys

Hematoxylin and eosin (H&E) staining of liver and kidney tissue from rats showed no evidence of necrosis, apoptosis, or other cellular damage or abnormalities. Furthermore, neither the vehicle control nor the treated groups displayed any abnormal alterations suggestive of any histological abnormalities. Figure 2 Kidney tissue composed of normal glomeruli (G) and normal interstitium with unremarkable tubules (T) (control group). Unremarkable glomeruli showing tuft-like vascular structure arranged in lobules of capillaries (C) which are surrounded by Bowman's Capsules (B) (1000mg/k) and Kidney tissue composed of normal glomeruli (G) and normal interstitium with unremarkable tubules (T) [2000 mg/kg]. Figure 3 (500 mg/kg, 1000 mg/kg and 2000mg/kg) shows congested central vein (CV) surrounded by liver parenchyma (P) containing normal hepatocytes. There is no evidence of congested Central Vein or swollen vacuolated hepatocytes across all the tree treatment groups.

Discussion

When conducting toxicological analyses on substances, including plant extracts, acute toxicity testing which entails LD50 estimation is essential[35]. In the current study, giving the animals acute oral doses of the methanol-cladoded extracts up to 2000 mg/kg did not result in mortality and obvious signs of toxicity. Consequently, the *Opuntia stricta* cladode extracts' LD50 was greater than 2000 mg/kg. This figure indicates that the *Opuntia stricta* cladode extracts are of relatively low acute toxicity, placing them into Globally Harmonized System (GHS) Category 5 [35, 36]. This finding is similar to what was reported in another study in which Siddiqui et al. (2021) assessed the acute toxicity of the *Opuntia dillenii* (Ker Gawl.) Haw. Cladode in adult rats, and found that during the 7-day study period, the cladode did not show any evidence of toxicity or death in animals given an oral dose of 1 g/kg or 5g /kg [28]. An extract or compound is generally regarded as harmless if its LD50 is higher than 5 g/kg [37]. Therefore, it can be inferred from this investigation that an oral dose of *Opuntia stricta* cladode methanol extract is not harmful.

In the current study, the mean body weight (162.2 ± 6.0 g) of the treatment groups did not differ significantly from that of the control (Figure 1). This suggests that throughout the course of the 14-day treatment and observation period, neither the rat's food intake nor their metabolic or physiological processes changed. Furthermore, there was no significant variation in the average relative weights of the rats' liver and kidney when compared to the control group (Table 1). The hematopoietic system is more vulnerable to harmful substances [35, 38]. Therefore, in order to determine how plant extracts affect an animal's blood system, hematological parameters must be evaluated [35, 39]. All the hematological parameters that were examined in this study did not differ significantly from the control group indicating that the hematopoietic system is not toxically impacted by *Opuntia Stricta* methanol extract (Table 2). The results of this investigation are consistent with those of the other investigations done on various Opuntia species which showed that all of the hematological profiles were normal when comparing the treated groups to the vehicle-treated group [13, 17, 28]. The kidney and liver are important organs for the excretion and metabolism respectively of foreign substances, such as metals, medicines, herbal remedies, and pesticides making the two organs susceptible to injuries [40]. Therefore, evaluation of biochemical markers is essential for determining how well an organ functions, particularly the kidney and liver. For both males and females, non-significant variations were observed in the majority of biochemical measures (urea, creatinine, albumin, AST and ALT) when compared to the control (Table 2). This finding in the current study is in contrast to what was reported in another study conducted on different species of *Opuntia* where the serum urea levels values were considerably higher in the male rats but not in the female rats when compared to male and female rats treated with vehicles implying that Serum urea levels were higher after renal damage or aberrant protein catabolism [13]. However, as revealed in the current study, there was no distinction between the creatinine levels of male and female rats compared to the control groups.

In the current study, no appreciable macroscopic alterations were observed in the liver or kidney histological results in rats treated with 2 g/kg of *Opuntia Stricta* methanol extract for 14 days. Furthermore, using hematoxylin and eosin stains to evaluate the architecture and morphological characteristics of the liver and kidney, the tissues maintained their similarity to the vehicle control tissues highlighting their non-toxic qualities even further. The current results are supported by what was reported in the study using 5 g/kg dose of Opuntia. *dillenii* methanol extract in mice [28]. On the contrary, when the *Opuntia. dillenii* methanol extract was administered intraperitoneally in another study at 5 g/kg, the mice's liver's morphology and structure showed utter disarray, suggesting that the intraperitoneal route of administration may be to blame for any toxicological symptom [41].

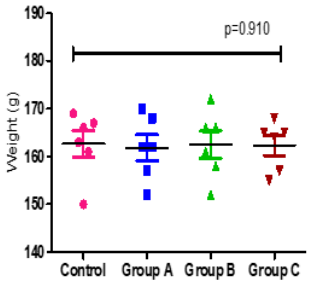

Figure 1. Effect of different doses of cladode methanol extract on the body weight of rats.

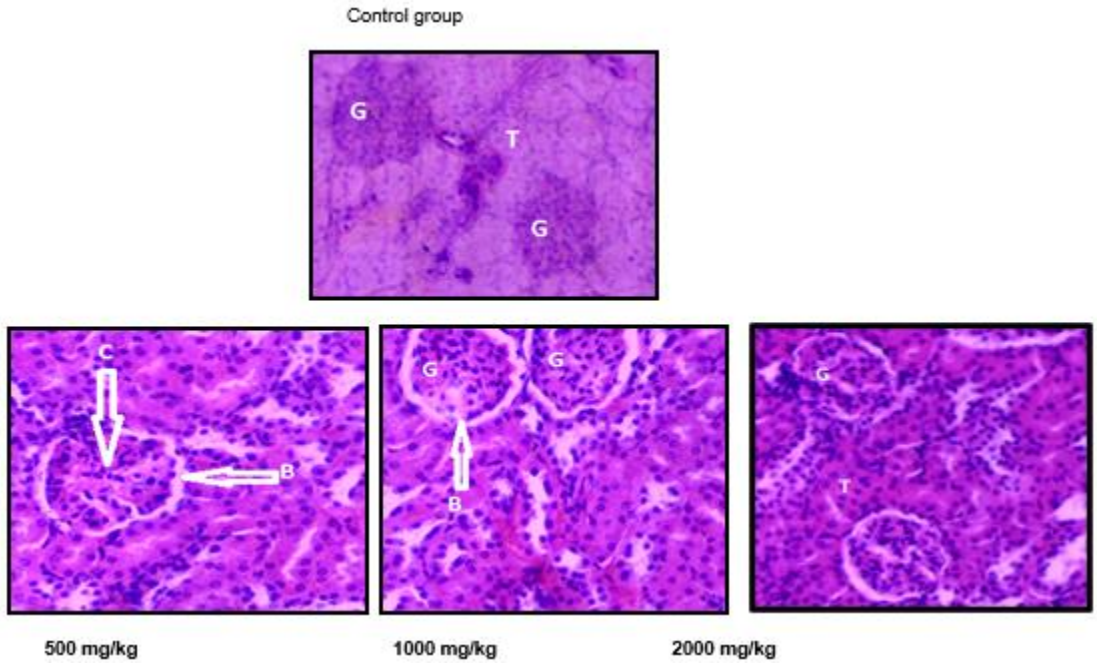

Figure 2. Kidneys from Rats (n = 3) were collected after 14 days of treatment with either saline (control), extract (500 mg/kg, 1000 mg/kg and 2000 mg/kg).

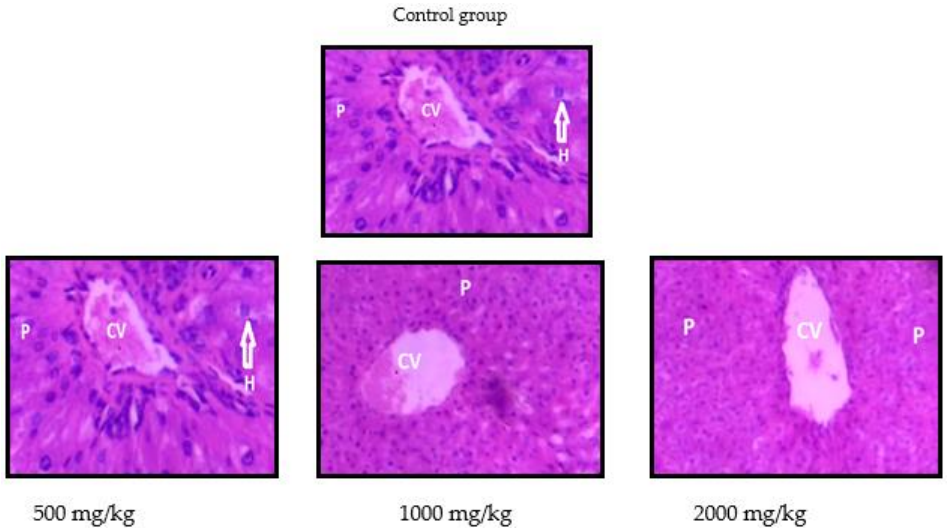

Figure 3. Liver from rats (n = 3) were collected after 14 days of treatment with either saline (control), extract (500 mg/kg, 1000 mg/kg and 2000 mg/kg)

Table 1. Relative organ weights (g) of the Wistar rats treated with methanol extract of *Opuntia Stricta* cladode

Organ	Control group	*Opuntia Stricta* cladode doses in mg/kg		
		500mg	1000 mg	2000mg
M-Liver	7.8 ± 0.25	7.8 ± 0.38	7.7 ± 0.15	7.8 ± 0.35
F-Liver	7.2 ± 0.1	7.0 ± 0.06	7.1 ± 0.11	7.2 ± 0.06
M-Right Kidney	0.63 ± 0.02	0.63 ± 0.02	0.64 ± 0.02	0.64 ± 0.01
M-Left Kidney	0.65 ± 0.01	0.61 ± 0.02	0.61 ± 0.01	0.61 ± 0.01
F-Right Kidney	0.61 ± 0.02	0.64 ± 0.02	0.66 ± 0.01	0.67 ± 0.03
F-Left Kidney	0.60 ± 0.00	0.61 ± 0.01	0.62 ± 0.01	0.62 ± 0.01

M: male, F: female

Table 2. Determination of hematological and biochemical parameters for combined male and female *Wistar* rat.

Organ	Control group	*Opuntia Stricta* cladode doses in mg/kg		
		500mg	1000 mg	2000 mg
WBC (10^9/L)	7.1 ± 0.21	6.9 ± 0.21	7.2 ± 0.06	7.1 ± 0.05
RBC (10^6/L)	5.1 ± 0.25	5.1 ± 0.25	5.3 ± 0.26	5.1 ± 0.06
Hb (g/dl)	13.1 ± 0.15	13.1 ± 0.15	13.6 ± 0.14	13.9 ± 0.15
MVC (FL)	55.0 ± 1.1	55.0 ± 1.1	58.0 ± 1.0	56.0 ± 2.0
HCT (%)	46.2± 0.8	46.2± 0.8	46.3 ± 1.3	46.3 ± 1.3
MCH (pg)	31.3 ± 1.0	30 ± 2.1	31.7 ± 1.5	32.9 ± 1.8
MCHC (g/dl)	32.7 ± 1.5	32.7 ± 1.5	32.9 ± 1.8	33.3 ± 0.57
Platelets (10^9/L	796.7 ± 3.1	799.9 ± 3.5	799.9 ± 3.5	801.1 ± 3.7
Neu (%)	39.7 ± 1.5	40.9 ± 0.7	40.9 ± 0.7	39.3 ± 0.6
Mon (%)	7.0 ± 1.0	7.7 ± 0.6	7.7 ± 0.6	7.3 ± 1.2
Lymph (%)	61.3 ± 1.5	62.3 ± 1.2	62.3 ± 1.2	62.0 ± 2.0
Creat (mmol/L)	25.0 ± 1.0	26.0 ± 1.7	26.9 ± 1.0	27.3 ± 0.7
Urea (mmol/L)	16.3 ± 0.6	17.3 ± 1.6	16.3 ± 0.6	17.3 ± 1.0
ALT (U/L)	50.7 ± 2.1	51.7 ± 1.7	52 ± 1.2	53.7 ± 1.8
AST (U/L)	147.7 ± 2.5	148 ± 1.5	148.7 ± 1.5	149.9 ± 2.5
Albumin g/dl)	37.1 ± 0.25	37.5 ± 0.43	39.8 ± 1.0	37.6 ± 0.76

Conclusion

Repeated administration of the methanol extract from the cladode of *Opuntia stricta* over 14 days did not lead to any deaths or severe signs of toxicity. There was no significant toxicity effects observed on body weight gain, organ weight, hematological and biochemical parameters. Additionally, no significant alteration in the histology of the kidney and the liver were observed. These results conclude *Opuntia stricta* extract for short term use is non-toxic via the oral route thereby supporting its safe traditional use against various ailments.

Abbreviations

AOTT Acute Oral Toxicity Test
OSC *Opuntia Stricta* clodode
UNZA University of Zambia
OECD Organization for Economic Cooperation and Development
LFT Liver function test
AP Alkaline phosphatase
γ-GT Gamma-glutamyl transferase
H&E Hematoxylin and eosin
NIS Nikon Imaging Software
CS2 Creative Suite-2
Hb Hemoglobin
MCHC Mean corpuscular hemoglobin concentration
WBC White blood cells
(HCT Hematocrit
MCV Mean corpuscular volume
GHS Globally Harmonized System

Authors' Contribution

The authors affirm that this work was conducted solely by those listed in this article, and all responsibilities and liabilities arising from claims related to the content herein shall rest with the authors. MK, CCE, and AGB conceptualized and designed the study. MK and KZ contributed to the execution of the research, while MK performed the data analysis. CCE and AGB provided overall supervision throughout the study process. All authors contributed to the writing, critically reviewed the manuscript, and approved the final version for publication.

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Conflict of interest

The authors declare no conflict of interest

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