AUCTORES
Globalize your Research

Clinical Research Notes
Mihir Y. Parmar *

Open Access

Research article

Toxicological Evaluation of Methanolic Extract of Vetiveria Zizanioides Roots in Male and Female

Mihir Y. Parmar [1*], Purvi A. Shah [2], Vaishali T. Thakkar [2], Tejal R. Gandhi [2]

[1]Sanjay College of Pharmacy, SKS group of institution, Dr. A. P. J. Abdul Kalam Technical University, Mathura-281406, UP, India.

[2]Anand Pharmacy College, Shree Ram Krishna Seva Mandal, Opp. Town Hall, Sardar Patel University, Anand-388001, Gujarat, India.

***Corresponding Author:** Mihir Y. Parmar, Sanjay College of Pharmacy, SKS group of institution, Dr. A. P. J. Abdul Kalam Technical University, Mathura-281406, UP, India.

Received date: October 30, 2021; Accepted date: December 20, 2021; published date: January 03, 2022

Citation: Mihir Y. Parmar, Purvi A. Shah, Vaishali T. Thakkar and Tejal R. Gandhi. (2022). Toxicological Evaluation of Methanolic extract of Vetiveria zizanioides roots in male and female rats: A Rare Research Report. Clinical Research Notes. 3(1). DOI: 10.31579/2690-8816/044

Abstract

Vetiveria zizanioides roots were used from tradition in the treatment of various types of liver disorder. The objective of present study was to evaluate the oral acute and sub-acute toxicity (28 days) of the methanolic extract of *Vetiveria zizanioides* roots (MEVZ) in Wistar albino rats of both sexes. In the acute toxicity study, Wistar rats were treated orally with a single dose of 5 g/kg of body weight, and were observed for 14 days. Animals were monitored continuously for the first 4 h, for every 6 h for the next 24 h, upto 14 days. In the sub-acute toxicity study, MEVZ was administered orally at doses of 175, 550 and 1750 mg/kg daily for 28 days to Wistar albino rats. Treatment with MEVZ did not produce any toxic signs or deaths. Treatment with MEVZ did not alter either the body weight gain or the food and water consumption. The hematological and biochemical parameters did not show any significant differences in any of the parameters examined in both gender groups. Organ weight, necropsy and histopathological examination, did not reveal any remarkable treatment related changes. A no-observed adverse-effect level for MEVZ is 5 g/kg for rats in our study. Consumption of MEVZ for various medicinal purposes is safe.

Keywords: acute toxicity; sub-acute; toxicity; *vetiveria zizanioides* roots

Introduction

Phytomedicine or herbal medicine is considered as the most common form of complementary alternative medicine (Ogbonnia *et al*., 2011). The use of herbal medicine can be traced back to 2100 BC in ancient China, and in India during the Vedic period (Schuppan *et al*., 1999). The basic concept is that the disease is a manifestation of a general imbalance of the dichotomous energies that govern human life in particular, and focus on medicine that can balance these energies and maintain good health. In Indian traditional system of medicine Ayurveda, the forces are said to be *Agni* (strength, health and innovation) and *Ama* (weakness, disease and intoxication) (Thyagarajan *et al*., 2002).

The World Health Organization (WHO) estimates that 80% of the world's population relies on these herbal medicines. Traditional medicine is majorly practiced in the prevention, diagnosis, and treatment of various diseases, as this type of medicine is easily accessible (Humber. 2002), especially in the developing and in the developed countries (Rickert *et al*., 1999; Kroll and Shaw, 2003; Ogbonnia *et al*., 2008). Plant-derived foods, especially vegetables and fruits, are generally considered to be highly important components of the human diet by providing wide range of nutrients, vitamins, etc which widen the therapeutic ordnance (Newman *et al*., 2003). The historic use of natural products in the treatment of various diseases has been very successful and useful in novel drug discovery development (Zhu *et al*., 2002).

The popularity and availability of the botanicals are enjoying widespread use of plants for treatment of several diseases by the rural communities but still little known about their toxicity and safety issue which are always a major concern (Chan, 1995). As such herbal medicines are considered safe and less damaging to the human body than synthetic drugs (Alam *et al*., 2011). However, the lack of standardization has been a major concern regarding use of herbal medicines (Angell and Kassierr 1998). Toxicity is a stage of being poisonous. The toxic effects may take place prior to the binding of the toxicants to the vital organs such as liver and kidneys (Asante-Duah *et al*., 2003). Some herbal medicines are known to be toxic at high doses and others may have potentially adverse effect after prolonged use. The general public is largely unaware that adverse effects can be associated with the use of herbal drugs resulting from overdosing, contaminated formulations to the inherent toxicity of the herbs of choice (Hazel *et al*., 1999). The safe use of herbal drugs requires knowledge of

their chemical properties and possible adverse effects (Santos *et al*., 2012). However, it should be a cardinal requirement to find the toxic effects of some of the major substances present in the plants when considering for public health protection because it can be hazardous and results to adverse effects on human being (Asante-Duah *et al*., 2003; Bellini *et al*., 2008).

Vetiveria zizanioides root (Commonly known as: Ushira, Family: Poaceae), is a perennial herb, which found throughout the plains and lower hills of India, particularly on the river banks and in rich marshy soil. The plant used as digestive, carminative, stomachic, constipating, haematinic, expectorant, antispasmodic, antiasthmatic, antigout (Sharma *et al*., 2002). It possesses various pharmacological activities such as anthelmintic (Gilbert *et al*., 1972), antimicrobial (Hammer *et al*., 1999), diuretic (Rao *et al*., 1994) and antioxidant activity (Kim *et al*., 2005). In our recent study methanolic extract of *Vetiveria zizanioides* roots has found hepatoprotective effects on ethanol (Parmar *et al*., 2008) [14], paracetamol and carbon tetrachloride-induced liver damage in rats (Parmar *et al*., 2013).

Despite knowledge of biological activities of *V. zizanioides* roots, toxicological studies are not reported so far. With the continuous increase in the use of herbal medicines worldwide, the safety and quality of herbal medicines have become a major concern (Santos *et al*., 2012). Hence, preclinical acute and sub-acute toxicological evaluations using Organisation for Economic Cooperation and Development (OECD) guidelines need to be undertaken to establish safety profiles of drugs of herbal origin (Jadeja *et al*., 2011; OECD. 2001; Joshi *et al*.2007). Plant materials are used throughout the world as home remedies or raw materials for the pharmaceutical industry and represent a substantial proportion of the global drug market (Santos *et al*., 2012). The present study therefore aims to investigate the acute and sub-acute oral toxicity of MEVZ in animal models.

Materials and methods:

Plant material and extraction

The plant *V. zizanioides* was collected from forest area of Jodhpur, Rajasthan, India in the month of July. The plant was identified and authenticated by Dr A. S. Reddy, Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India where a voucher specimen (No. MP-1) was kept for future reference. The dried roots were powdered mechanically and defattened using petroleum ether (60-80°C), and cold extracted with methanol. The ethanol crude extract (10.5 % yields) was obtained by evaporation using Rotavapour ® (BÜCHI, Switzerland) under reduced pressure. The dry methanol extract of *V. zizanioides* (MEVZ) was stored at -20°C until use and suspended in distilled water for the evaluation of toxicity studies.

Phytochemical Screening

To determine the chemical constituents, qualitative phytochemical screening of MEVZ was carried out using following standard procedures routinely (Khandelwal., 2000; Kokate *et al*., 2000) for alkaloids (Dragendoff's and Meyer test), tannins ($FeCl_3$ test), saponins (frothing test), lipids (Wattman paper test), flavonoids (Schinoda's test), glycosides (Borntrager's and Keller Killiani's test), phenols ($FeCl_3$), polyphenols ($K_3Fe(CN)_6$), terpenes and sterols (Salkowski's and Liberman Burchard's test), Reducing sugars (Fehling's test), resins and essential oils (Boiling test).

Acute Oral Toxicity Study

The acute oral toxicity study was conducted using the limit test procedure according to OECD test guidelines on acute oral toxicity test 425. The rats were divided into 4 groups of 12 animals (06 males and 06 females) where each groups received orally a single dose of MEVZ (5 g/kg) and control, received distilled water only. They were observed continuously for the first 4 h, for every 6-hour for the next 24 h and upto for 14 days to observe any changes in general behavior or other physiological activities with the purpose of recording any symptoms of ill-health or behavioural changes. The signs of toxic effects and/or mortality were observed carefully and the body weights were recorded for first and on day 14. LD50 value was calculated following the previous method (Litchfield and Wilcoxon., 1949).

Sub-acute Oral Toxicity Study

The method was performed according to the OECD test guidelines 407 (OECD. 2001). Eight-week-old Wistar rats were housed in the same conditions as described above. The eighty animals were randomly divided into four groups containing 20 rats each (06 females and 06 males) where each groups received orally a single dose of MEVZ (175, 550, 1750 mg/kg/d) and control, received distilled water only for 4 weeks. The animals were observed for signs of toxicity and mortality throughout the experimental period. The weight of each rat was recorded at weekly intervals throughout the course of the study. Food and water consumption were measured at weekly intervals. At the end of the 4-week experiment, the animals, fasted for 24 h, were sacrificed under light anesthesia.
Blood was collected into two set of tubes: tube 1 containing EDTA was processed immediately for hematological parameters; tube 2 without EDTA was centrifuged at 3000 g at 4°C for 10 min to obtain serum (stored at -20°C till further analysis). The all major organs (kidneys, liver, lungs, heart, testes and glands annexes, ovaries, spleen, and) were weighted. Organ samples were fixed in 10% formalin for histopathological examination.

Hematological and Biochemical Analysis

Hematological analysis was performed using an automatic hematological analyzer (Coulter STKS, Beckman). Parameters included red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets count, and mean platelet volume (MPV). For biochemical analysis, following parameters were determined: glucose, blood urea nitrogen (BUN), Creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (GGT), total, direct bilirubin level (TBL and DBL), triglyceride (TG) total cholesterol (TC), high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL). These levels were determined using an auto analyzer (Hitachi 7080, Japan).

Pathological Examination

All animals were subjected to necropsy at the end of the toxicity studies, or earlier in case of death. Necropsy was performed to analyze the macroscopic external features of the heart, liver, spleen, lungs, kidney, esophagus, stomach, small intestine (length of 6-7 cm), hypophysis, hypothalamus, brain, and reproductive organs (uterus and ovary or testicle, prostate, seminal vesicle, epididymis and vas deferens). These organs were carefully removed and weighed individually. Organ weights were expressed in absolute and relative terms (g and g/100 g of body weight, respectively).

Histopathological examination tissues

Tissues were fixed in 10 % neutral buffered formalin, embedded in paraffin wax and sectioned at 5 µm. Sections were then stained with Hematoxylin and Eosin (H & E) stain and placed in slides for light microscopic examination. Slides were evaluated by a histopathologist who was blinded to the treatment groups to avoid any kind of bias.

Statistical Analysis

Data are expressed as the mean ± standard deviation (S. D). Statistical analysis was performed with one-way analysis of variance (ANOVA). The Dunnett's multiple comparison test (DMCT) using Graph Pad prism 5.0 software were used to determine the source of significant differences where appropriate. A *P* value <0.05 was considered statistically significant.

Results

Phytochemical Screening of MEVZ:

The phytochemical screening of MEVZ revealed the presence of the following classes of chemical compounds: alkaloids, tannins, saponins, flavonoids, phenols, terpenes, and essential oils **Table 1.**

Sr. No	Chemical compounds	Test methods	Methanol Extract
1	Alkaloids	Dragendorff's and Meyer test	-
2	Tannins	Ferric chloride test	+
3	Saponins	Frothing test	+
4	Lipids	Wattman paper test	-
5	Flavonoid	Shinoda's test	+
6	Anthraquinones glycosides	Borntrager's test	-
7	Cardiac glycosides	Keller Killiani's test	-
8	Phenols	Ferric chloride test	+
9	Polyphenols	$K_3Fe(CN)_6$ test	-
10	Terpenes	Salkowski's test	+
8	Sterols	Liebermann's test	-
9	Reducing sugars	Fehling's test	-
10	Volatile oil	Boiling test	+
11	Resins	Boiling test	-

Table 1: *Phytochemical screening methanolic extract of V. zizanioides (MEVZ)*

Acute Toxicity:

The mean body weight of female rats increased from 205.7 ± 6.7 g to 238.6 ± 11.2 g during the 14 days, and that of males increased from 209.2 ± 6.4 g to 253.2 ± 11.3 g (data not given). No mortalities had occurred during the entire study period. Additionally clinical observations and measurements did not indicate evidences of substance related toxicity.

Table 2 indicates the behavioural parameters changes observed before and after the administration of the MEVZ. After sacrifice on the 14th day, macroscopic and gross pathology findings conducted at the necropsy examination revealed no visible changes in any rats. Thus, no evidence of acute toxicity of MEVZ in rats was found. The oral LD50 values for both sexes of animals must be greater than 5 g/kg.

No	Animal No	1	1	2	2	3	3	4	4	5	5	6	6
	Response	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	Alertness	N	N	N	N	N	N	N	N	N	N	N	N
2	Grooming	A	A	A	A	A	A	A	A	A	A	A	A
3	Touch and pain response	A	A	A	A	A	A	A	A	A	A	A	A
4	Torch response	N	N	N	N	N	N	N	N	N	N	N	N
5	Tremors	A	A	A	A	A	A	A	A	A	A	A	A
6	Convulsion	A	A	A	A	A	A	A	A	A	A	A	A
7	Righting reflux	P	P	P	P	P	P	P	P	P	P	P	P
8	Gripping strength	N	N	N	N	N	N	N	N	N	N	N	N
9	Pinna reflux	N	N	N	N	N	N	N	N	N	N	N	N
10	Corneal reflux	P	P	P	P	P	P	P	P	P	P	P	P
11	Writhing	A	A	A	A	A	A	A	A	A	A	A	A
12	Pupils	N	N	N	N	N	N	N	N	N	N	N	N
13	Urination and Salivation	N	N	N	N	N	N	N	N	N	N	N	N
14	Skin colour	N	N	N	N	N	N	N	N	N	N	N	N
15	Lacrimation	N	N	N	N	N	N	N	N	N	N	N	N

Table 2: *Effect of acute treatment of methanolic extract of V. zizanioides (MEVZ) on behaviour of rats*

Sub-acute Toxicity:

There were no signs of behavioural toxicity or deaths were recorded during the 4 weeks of MEVZ orally treatment at doses of 175, 550, or 1750 mg/kg. No significant differences were found between the initial and final body weight, food and water consumption of the rats treated with MEVZ and control rats (**Table 3**).

Week (W) of Treatment		Female MEVZ (mg/kg/day)				Male MEVZ (mg/kg/day)		
	Vehicle	175	550	1750	Vehicle	175	550	1750
Body weight (g)								
W1	222.50±12.14	224.67±10.68	225.83±10.68	227.50±12.14	233.33±9.30	233.33±14.37	240.00±7.07	242.50±5.24
W2	236.67±5.16	235.00±8.37	231.67±11.69	228.33±5.43	238.33±4.08	244.17±7.36	240.83±5.84	243.33±5.16
W3	249.17±12.82	250.83±9.17	246.67±16.0	248.33±17.51	252.50±6.08	252.50±6.12	251.50±5.16	252.17±10.59
W4	240.83±15.30	242.50±14.75	239.17±13.57	249.17±16.56	250.83±7.36	249.17±6.64	245.83±8.61	248.67±8.38
Food intake (g/ day/rat)								
W1	23.33±6.83	26.67±2.58	27.50±2.74	27.00±3.46	24.17±7.36	27.83±3.19	27.83±3.19	27.33±3.33
W2	21.67±7.53	23.38±6.83	24.17±5.84	24.67±4.97	23.33±6.83	24.50±5.61	25.33±4.46	25.67±4.59
W3	24.17±7.36	25.83 ±4.84	26.67 ±6.05	26.23 ±5.81	26.33 ±5.89	27.33 ±2.94	28.33 ±3.20	28.67±3.93
W4	20.83±7.36	22.50±6.89	23.33±6.08	23.00±6.32	24.00±6.03	24.50±4.97	25.17±4.12	26.83±2.93
Water intake ml/day/rat								
W1	22.83±6.73	23.17±6.91	23.67±7.58	24.00±7.69	23.67±5.82	23.83±5.98	25.33±5.95	25.67±5.35
W2	22.17±7.83	22.33±7.66	22.67±7.84	22.83±7.65	23.17±7.25	24.00±6.36	24.33±7.17	25.50±5.79
W3	25.33±7.47	25.83 ±7.55	26.35 ±7.81	26.86 ±7.00	26.33 ±6.65	26.50 ±6.86	28.00 ±5.69	28.5 ±5.50
W4	21.33±7.86	21.00±7.92	21.68±7.47	22.17±7.37	24.17±6.24	24.33±6.28	25.00±6.32	25.17±5.04
Data are expressed as mean ± S.D. Wistar rats (n = 6/group) were treated orally with MEVZ daily for 4 weeks.								

Table 3: *Effects of sub-acute treatment of the methanolic extract of V. zizanioides (MEVZ) on body weight, food and water intakes in rats.*

Hematological and Biochemical Parameters:

The hematological profile of the treated and control groups as shown in Table 4. MEVZ did not induce any significant change in the hematological parameters such as WBC counts, total leukocyte count, hemoglobin, hematocrit, total erythrocyte count, erythrocyte indices (MCV, MCH, and MCHC), platelets count and MPV (**Table 4**). The biochemical profile of the treated and control groups are presented in **Table 5**. The plasma levels of glucose, BUN, Creatinine, AST, ALT, ALP, LDH, GGT, TBL, DBL, TG, TC, HDL, LDL, VLDL of rats treated with MEVZ up to 1750 mg/kg were found to be comparable with those of the control group at end of the 28 days treatment (**Table 5**).

Parameters		Female MEVZ (mg/kg/day)				Male MEVZ (mg/kg/day)		
	Vehicle	175	550	1750	Vehicle	175	550	1750
WBC (mm^3)	5.55±0.28	5.76±0.43	5.46±0.18	5.63±0.14	6.50±0.32	6.80±0.29	5.67±0.58	6.03±0.54
Lymphocytes (10^3 μL^{-1})	6.72±0.40	6.68±0.41	6.73±0.42	6.73±0.42	6.88±0.19	6.85±0.23	6.90±0.21	6.90±0.21
Erythrocytes (mm^3)	4.19±0.06	4.40±0.11	4.41±0.11	4.52±0.19	5.11±0.18	5.10±0.60	5.35±0.53	5.4±0.38
Hemoglobin (g/dl)	16.05±0.29	16.16±0.26	16.74±0.49	17.64±0.13	16.59±0.18	16.34±0.39	16.63±0.22	16.76±0.35
Hematocrit (%)	45.78±5.50	45.45±5.33	44.97±5.08	45.07±5.61	46.61±3.65	46.28±3.50	45.95±4.21	45.78±3.80
MCV (fL)	50.75±6.58	50.44±6.14	50.76±6.57	50.77±6.56	51.61±6.00	51.63±6.10	51.65±6.01	51.66±5.69
MCH (pg)	25.71±6.82	25.54±6.99	25.38±6.62	25.04±5.88	26.36±6.94	26.19±7.12	26.02±6.77	25.69±6.09
MCHC (g/dl)	29.30±10.50	29.13±10.68	28.97±10.31	28.63±10.20	29.95±10.30	29.78±10.58	29.61±10.38	29.28±10.05
RDW (%)	11.86±0.81	11.87±0.82	11.89±1.01	12.02±0.98	12.36±1.17	12.37±1.53	12.38±1.89	12.53±1.22
Platelets (mm^3)	272.0±31.38	276.0±27.48	301.60±26.36	346.01±27.25	241.8±13.08	243.8±29.66	268.01±34.20	276.0±15.94
MPV (fL)	5.70±1.42	5.72±1.19	5.73±1.41	5.75±1.07	6.01±1.28	6.05±1.24	6.03±1.26	6.06±0.89
Data are expressed as mean ± S.D. Wistar rats (n = 6/group) were treated orally with MEVZ daily for 4 weeks.								

Table 4: *Effects of sub-acute treatment of the methanolic extract of V. zizanioides (MEVZ) on hematological parameters in rats.*

Parameters			Female MEVZ (mg/kg/day)				Male MEVZ (mg/kg/day)	
	Vehicle	175	550	1750	Vehicle	175	550	1750
Glucose (mg/dl)	88.17±13.21	89.0±11.93	89.33±11.99	90.17±10.92	89.83±10.89	90.67±10.15	91.00±10.58	91.83±9.76
BUN (mg/dl)	45.50±7.82	46.00±7.32	45.83±7.30	46.67±6.32	46.33±6.65	46.83±7.49	46.67±6.31	47.50±5.89
Creatinine (mg/dl)	0.556±0.06	0.558±0.07	0.565±0.06	0.567±0.08	0.560±0.06	0.561±0.05	0.568±0.06	0.570±0.07
AST (U/L)	51.28±5.64	51.45±5.15	51.11±6.64	51.61±6.50	51.61±5.37	51.78±5.16	51.45±5.49	51.95±6.04
ALT (U/L)	46.28±4.37	45.94±3.53	45.61±3.86	45.45±4.20	46.61±3.65	46.28±3.50	45.95±4.21	45.78±3.80
ALP (U/L)	27.95±6.12	27.78±5.22	27.61±4.20	27.28±4.37	28.28±6.19	28.11±5.86	27.95±5.53	27.61±4.92
LDH (U/L)	364.11±43.30	370.78±44.33	374.11±45.03	373.78±46.08	368.28±39.88	374.95±39.60	377.45±41.20	378.28±41.92
GGT (U/L)	26.45±7.06	26.28±7.27	26.11±6.38	25.78±5.72	26.61±6.27	26.45±7.06	26.28±6.71	25.95±6.04
TBL (mg/dl)	0.188±0.016	0.191±0.02	0.193±0.02	0.197±0.015	0.195±0.013	0.198±0.017	0.200±0.017	0.203±0.019
DBL (mg/dl)	0.072±0.01	0.071±0.01	0.073±0.01	0.073±0.01	0.070±0.01	0.080±0.01	0.080±0.01	0.080±0.01
Triglycerides (mg/dl)	48.10±12.04	51.19±8.38	49.52±6.87	50.19±6.46	48.93±11.01	52.02±7.77	50.35±6.38	51.02±5.82
Total cholesterol (mg/dl)	51.68±10.32	52.51±9.13	54.18±8.23	55.18±7.12	53.35±13.63	54.18±10.93	55.85±9.85	55.91±9.57
HDL (mg/dl)	24.62±7.10	24.45±6.82	24.37±7.42	24.29±7.54	25.45±8.65	25.29±8.33	25.20±8.95	25.12±9.05
LDL (mg/dl)	30.23±4.45	30.33±4.42	30.62±4.27	31.14±3.67	30.56±4.30	30.66±4.26	30.96±4.25	31.47±3.58
VLDL (mg/dl)	9.62±2.41	9.97±1.89	9.63±1.57	9.50±1.68	9.78±2.20	10.13±1.82	9.80±1.52	9.66±1.64

Data are expressed as mean ± S.D. Wistar rats (n = 6/group) were treated orally with MEVZ daily for 4 weeks.

Table 5: *Effects of sub-acute treatment of the methanolic extract of V. zizanioides (MEVZ) on biochemical parameters in rats.*

Morphological Parameters:

The absolute and relative tissue weights were not changed so much by MEVZ treatment (Tables 6, 7). No treatment-related macroscopic findings were observed in treated animals at necropsy. For the histological investigation, no pathological changes were observed in the livers (**Figure 1)**, lungs (**Figure 2)**, hearts (**Figure 3)** and kidneys (**Figure 4**) of animals in any of treated group. Other organs including spleen, adrenal gland, thymus, thyroid gland, brain, uterus, testis, prostate and bladder showed no sign of pathological changes compared with the corresponding organs of the controls.

Discussion

The results of present acute toxicity study indicated that oral treatment of MEVZ at dose of 5 g/kg did not produce any sign of toxicity or death in rats, suggesting a LD50 above 5 g/kg. Thus, referring to the Hodge and Stemer scale, the orally treated MEVZ could be considered practically nontoxic (CCOHS. 2005; Sasidharan *et al*., 2008). In this study of acute toxicity evaluation, rats are orally treated with MEVZ at single dose of 5 g/kg. The clinical symptom is one of the major important observations to indicate the toxicity effects on organs in the treated groups (Eaton and Klaassen., 1996). The animals were observed daily until day 14 for any toxic signs and mortality. All group showed no overt signs of distress, and there were no observable symptoms of either toxicity or deaths. All of the rats gained weight and displayed no significant changes in behavior. Apart from that, the physical appearance features such as skin, fur and eyes were found to be normal and whilst the body weight of the rats showed as increase, this indicates that the treatment of the crude extract has negligible level of toxicity on the growth of the animals.

Sub-acute toxicity studies are conducted to evaluate the adverse effects of a test substance after prolonged use. Also to collect information about the possible health effects arise from repeated dosage over a relatively limited period of time and about target organs, the possibilities of cumulative effects, and an estimate of the dose at which there is no observed adverse effect. The sub-acute treatment indicated that MEVZ in doses of 175, 550, and 1750 mg/kg per day during 28 consecutive days did not produce any deaths or clinical signs of toxicity.

Generally, the alterations of body weight and internal organ weights of animals would be an indicator of adverse effects after exposure to the toxic substances in the form of drugs and chemicals (Raza *et al*., 2002; Teo *et al*., 2002). There were no significant changes in animal behaviour, body weight, food and water consumptions in MEVZ-treated group at any dosage in both species.

Investigation of haematological and serum biochemical parameters alterations are of diagnostic significance in routine clinical evaluation of the state of health system in humans (Olson *et al*., 2000). No significant alterations of the haematological and biochemical parameters were found in both gender of animals treated with MEVZ.

Kidney is a sensitive organ, whose function is to be affected by drugs including phytochemicals of plant origin that ultimately lead to renal failure (Saidu *et al*., 2007). Results show no significant alteration in the serum urea and creatinine levels due to MEVZ treatment. This is suggestive of no kidney damage specifically by renal filteration mechanism (Crook. 2006) or probably indicates that MEVZ did not interfere with the renal capacity to excrete these metabolites. Therefore, it was evident that the drug at doses employed did not cause renal impairment or kidney damage. Moreover, there was no effect on the levels of AST and ALT, which is considered to be sensitive indicators of hepatocellular damage and within limits, can provide a quantitative evaluation of the degree of damage to the liver (Ramaiah, 2011). ALP activity on the other hand is related to the functioning of hepatocytes and an increase in its activity may be due to its increased synthesis in the presence of increased pressure (Manjunatha *et al*., 2005).

Results show no significant alteration in AST, ALT, ALP, TBL, DBL, LDH and GGT due to MEVZ treatment. This is suggestive of no liver damage. Thus, MEVZ did not induce any damage to the liver and kidneys. The significant decrease in levels of triglycerides, total cholesterol and other cholesterol observed in groups treated with MEVZ may be attributed to the presence of hypolipidemic agents in the polyherbal drug (Ogbonnia *et al*., 2011). The serum cholesterol levels also decreased which is considered as an indirect indicator of liver function (Hilaly *et al*.,

2004). This is further confirmed by the histological assessment of these organs.

Organ weight also is an important index of physiological and pathological status in animals. The relative organ weight is fundamental to diagnose whether the organ was exposed to the injury or not. The heart, liver, kidney, spleen and lungs are the primary organs affected by metabolic reaction caused by toxicant (Dybing *et al*., 2002). There is no changes were observed in gross observation or structure of systemic organs of both control and treated groups. In our study, the relative and absolute of organs weight in both control and treated groups was increased but not significantly which shows that the extract nurtures the organs (Table 6, 7). Treatment with MEVZ did not show any adverse effect on organs weight of all important organs. Hence, it can be suggested that, MEVZ is virtually nontoxic and did not induce prejudicial changes and morphological alterations in these organs.

Additionally, histological evaluation is the golden standard done for further confirmation about the treatment related pathological changes in tissues and organs. In our study, oral treatment with MEVZ for 28 days showed no adverse effect on the morphology of animal major organs. This agrees with the results of hematological, serum biochemical parameters also collaborated with the results of body weight and organ weight.

Since the oral dose of 1750 mg /kg per day of MEVZ administered for 28 consecutive days did not induce any biochemical, hematological, anatomical, and histopathological signs of toxicity, it can be defined as the no-observed adverse- effect level (NOAEL) for Wistar rats of both sexes under the experimental conditions used. However, it should be emphasized that this NOAEL was derived from a sub-acute study only. Since toxicity in humans cannot always be entirely extrapolated from animal studies. For a more reliable safety evaluation performed on the basis of the acceptable daily intake concept, data on the chronic toxicity, reproductive toxicity, genotoxicity, and carcinogenicity of MEVZ would also be required. Also clinical evaluation should be performed to precisely define the safe dosage to advice in humans.

Conclusion

The present results show that in acute and sub-acute toxicity studies in male and female rats we did not observe any mortality or signs of toxicity due to treatment with MEVZ and no significant weight loss was recorded thus establishing its safety in use.

Thus, the plant, at least its methanol extract, could be considered with a wide margin of safety for oral use. The haematological and serum biochemical parameters and histology examination revealed no changes in the architecture of the internal organs mice in both control and treated groups. Hence, *V. zizanioides* can be used as a medicinal agent in known dosages, especially in rural communities where conventional drugs are unaffordable because of their high cost. A detailed experimental analysis of its chronic toxicity is essential for further support of this drug. Since toxicity in humans cannot always be entirely extrapolated from animal studies, clinical evaluation should be performed to precisely define the safe dosage to advice in humans. However this study provides the basis for further study on the detailed toxic and pharmacological effects of the extracts of *V. zizanioides* and their active components.

Competing Interests

The authors declare that they have no competing interests.

Authors Contributions

MP and TG have performed experimental designed, literature search and animal treatment. MP and PS have carried out biochemical and statistical analysis as well as interpretation of the data. MP and VT participated in histopathological investigation. MP, PS and TG involve in writing of the manuscript, review and edited manuscript.

Acknowledgment

The authors express sincere thanks to Dr. A. S. Reddy, Professor, BRD School of biosciences, S. P. University, V. V. Nagar for the authentication of the plant. The authors also thank Dr. Prera Desai, Ayurvedic practitioner for her interest and encouragement.

References

1. Alam, m.b hossain, m.s.chowdhury, n.s. Mazumder, M.E.H. Haque, M.E. (2011). In vitro and in vivo antioxidant and toxicity evaluation of different fractions of Oxalis corniculata linn. J. Pharmacol. Toxicol. 6; 337-348.
2. Angell, m. Kassierr, j.p. (1998). Alternative medicine–the risk of untested and unregulated remedies. N. Engl. J. Med. 339; 839-834.
3. Asante-Duah, K. (2002). Public Health Risk Assessment for Human Exposure to Chemicals (illustrateded.); Kluwer Academic Publishers: Dordrecht, The Netherlands, 6.
4. Bellini MF, Cabrioti L.N, Terezan A.P, Jordao B.Q, Ribeiro L.R, Mantovani M.S. (2008). Cytotoxicity and genotoxicity of Agaricus blazei methanolic extract fractions assessed using gene and chromosomal mutation assays. Genet. Mol. Biol. 31; 122-127.
5. CCOHS. (2005). What is an LD50 and LC50, Canada's National Occupational Health and Safety Resource: Canadian Centre for Occupational Health and Safety.
6. Chan i. (1995). Progress in traditional Chinese medicine. Trends. Pharm. Sci. 16; 182-187.
7. Dybinge Doe J, Groten J, Kleiner J, O'brien J. (2002). Hazard characterization of chemicals in food and diet: dose response, mechanism and extrapolation issues. Food. Chem. Toxicol. 42; 237-242.
8. Crook, M. A. (2006). Clinical chemistry and metabolic medicine. 7th ed. London: Hodder Arnold.
9. Eaton d.l, klaassen c.d. Principles of toxicology. In Casarett and Doull's Toxicology: The Basic Science of Poisons, 5th edm, Klaassen, C.D Ed. McGraw-Hill: New York, NY, USA, 13.
10. El Hilaly J, Israili Z.H, Lyoussi B. (2004). Acute and chronic toxicological studies of Ajugaiva in experimental animals. J. Ethnopharmacol. 91; 43-50.
11. Gilbert b. (1972). Anthelmintic activity of essential oils and their chemical components. An. Acad. Bras. Cienc. 44; 423-428.
12. Hazel B.M, george w.l, kenneth d.F. (1999). Medicinal herbs in the United States: research Needs. Environ. Health. Perspect. 107; 773-778.
13. Hammer K.A, Carson C.F, Riley T.V. (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86; 985-990.
14. Humber, J.M. (2002). The role of complementary and alternative medicine: Accomodating pluralism. J. Am. Med. Assoc. 288; 1655-1656.
15. Jadeja R.N, Thounaojam C, Ansarullah, et al. (2011). Toxicological evaluation and hepatoprotective potential of Clerodendron glandulosum Coleb leaf extract. Human. Exp. Toxicol. 30; 63-70.
16. JOSHI C.S, Priya, E.S, Venkataraman S. (2007). Acute and sub-acute toxicity studies on the polyherbal antidiabetic formulation diakyur in experimental animal models. J. Health. Sci. 53; 245-249.
17. Khandelwal, K.R. Practical Pharmacognosy. 9th ed, Nirali Prakashan. (2000). Pune, India. 149-156.
18. Kim H.J, Chen F, Wang X, Chung H.Y, Jin Z. (2005). Evaluation of antioxidant activity of vetiver (Vetiveria zizanioides L.) oil and

identification of its antioxidant constituents. J. Agric. Food. Chem. 53; 7691-7695.
19. Kokate C.K, purohit A.P, goshala sub. (2000).Text Book of pharmacognosy. 38th edn. Nirali prakashan. Pune, India. 7135-7145.
20. Kroll, D.J.; shaw, H.S. (2003). Complementary and alternative medicine (CAM): relevance to laboratory medicine. Clin. Lab. Int. 27; 14-16.
21. litchfield J.T, wilcoxon, H.A. (1949). A simplified method of evaluating dose-effect experiments. The. J. Pharmacol. Exp. Therap. 96; 99-113.
22. Manjunatha B.K, Vidya S.M, Dhiman P, Pallavi R. (2005). Hepatoprotective activity of Leucas hirta against CCl4 induced hepatic damage in rats. Indian. J. Exp. Biol. 43; 722-727.
23. Newman D.J, cragg G.M, snader K.M. (2003). Natural Products as Sources of New Drugs. J. Nat. Prod. 1022-1103.
24. OECD. (2001). Guidelines for Acute Toxicity of Chemicals; Organization for Economic Co-operation and Development: Paris, France. 425.
25. OECD. Repeated Dose 28-day Oral Toxicity Study in Rodents; Organization for Economic Co-operation and Development: Paris, France. 407.
26. Ogbonnia S, Adekunle A.A, Bosa M.K, Enwuruv N. (2008). Evaluation of acute and subacute toxicity of Alstonia congensis Engler (Apocynaceae) bark and Xylopia aethiopica (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. Afr. J. Biotechnol. 7; 701- 705.
27. Ogbonnia, S.O, Mbaka G.O, anyika, E.N, osegbo O.M, igbokwe, N.H. (2010). Evaluation of acute toxicity of hydro-ethanolic extract of chromolaena odorata (L.) king and robinson (Fam. Asteracea) in rats. Agric. Biol. J. North. Am. 1; 859-65.
28. Ogbonnia S.O, Mbaka G.O, Anyika E.N, Mordi J.E, Nwakakwa N. (2001). An evaluation of acute and sub chronic toxicities of a Nigerian polyherbal tea remedy. Pak. J. Nutr. 10; 1022-1028.
29. Olson H, Betton G, Robinson D et al. (2000). Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul. Toxicol. Pharmacol. 32; 56-67.
30. parmar M.Y, shah P.A, thakkar V.T, gandhi T.R. (2008). Hepatoprotective activity of Vetiveria zizanioides Linn against ethanol-induced liver damage in rats. Pharmacog. Mag. 4; 216.
31. parmar M.Y, shah P.A, thakkar V.T, salim R, gandhi T.R. (2013). Hepatoprotective potential of methanolic extract of Vetiveria zizanioides roots against carbon tetrachloride induced acute liver damage in rats. Digest. J. Nanomat. Biostr. 8; 835-844.
32. Ramaiah S.K. (2011). Preclinical safety assessment: current gaps, challenges and approaches in identifying translatable biomarkers of drug induced liver. Clin. Lab. Med. 31; 161-172.
33. Raza M, Al-Shabanah O.A, El-Hadiyah T.M, Al-Majed A.A. (2002). Effect of prolonged vigabatrin treatment on haematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. Sci. Pharm. 70; 135-145.
34. Rao R.C, Serradeil-Le G.C, Granger I, Gleye J, Augereau J.M, Bessibes C. (1994). Khusimol, a non-peptide ligand for vasopressin VI a receptors. J. Nat. Prod. 57; 1329-1335.
35. Rickert K, Martinez R.R, Martinez T.T. (1999). Pharmacist knowledge of common herbal preparations. Proc. West. Pharmacol. Soc. 42; 1-2.
36. Sasidharan S, darah I, jain K. (2008). In vivo and in vitro toxicity study of Gracilaria changii. Pharm. Biol. 46; 413-417.
37. Saidu Y.L.S, Bilbis M.L, Isezuo S.A, Hassan S.W, Abbas, A.Y. (2007). Acute and sub-chronic toxicity studies of crude aqueous extract of Albizzia chevalieri harms (Leguminosae). Asian. J. Biochem. 2; 224-236.
38. Santos L.K.X, Cunha G.H, **Fechine** F.V, Pontes A.V, Oliveira J.C, Bezerra F.A.F, Moraes M.O, Moraes M.E.A. (2012). Toxicology and safety of the tincture of Operculina alata in patients with functional constipation. Braz. J. Pharm. Sci. 48; 469.
39. Schuppa ND, JIA J.D, Brinkhaus B, Hahn E.G. (1999). Herbal products for liver diseases: A therapeutic challenge for the new millennium. Hepatology. 30; 1099-1004.
40. Sharma P.C, Yelne M.B, Dennis T.J. (2002). Database on Medicinal Plants used in Ayurveda, Central council for research in ayurveda and siddha, New Delhi. 1; 454-461.
41. Teo MS.D, Stirling S, Thomas A, Kiorpes A, Vikram K. (2002). A 90-day oral gavage toxicity study of D-methylphenidate and D, L-methylphenidate in Sprague-dawley rats. Toxicol. 179; 183-196.
42. Thyagarajan S.P, Jayaram S, Gopalakrishnan V, Hari R, Jeyakumar P, Sripathi M.S. (2002). Herbal medicines for liver diseases in India. J Gastroenterol and Hepatol. 17; 370-76.
43. Zhu M, Lew K.T, Leung P. (2002). Protective effects of plant formula on ethanol-induced gastric lesions in rats. Phytother. Res. 16; 276-280.

DOI: 10.31579/2690-8816/044