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Original Research Article

PRECLIINICAL SUB-ACUTE ORAL TOXICITY STUDY FOR ANTI-INFLAMMATORY EVALUATION OF METHANOL EXTRACT OF CUCURBITA PEPO L. SEEDS

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Abstract: *Cucurbita pepo, a plant known for its traditional use in managing various ailments, contains valuable phytoconstituents and nutritional components. Aim: This study aimed to assess the sub-acute toxicity of the methanol extract of C. pepo (MECP) seeds according to OECD 407 guidelines. Rats were orally administered MECP at 250, 500, and 1000 mg/kg doses for twenty-eight days. Throughout the study period, the animals were closely monitored for any signs of toxicity, and at the end, blood samples were collected for biochemical and hematological analyses. Histopathological examination was performed on vital organs after euthanizing the rats. The results demonstrated that MECP administration significantly increased body and organ weight in the treated rats. However, food and water intake remained unchanged, and no mortality was observed. Biochemical and hematological analyses revealed no significant changes in the treated groups compared to the control group, except for a notable decrease in red blood cells, suggesting potential anemic effects of MECP. Histopathological examination showed no structural alterations in the liver, heart, and kidney. Based on these findings, it can be concluded that C. pepo is safe for use and holds the potential for therapeutic efficacy.*

Keywords: Sub-Acute Toxicity, Cucurbita Pepo L., Methanol Extract, Seed

Introduction

Pharmacological assessment of medicinal plants has recently developed an increasing interest among scientists (Gupta et al., 2008). Herbal medicines must be evaluated before being included in the study concerning their toxicity. However, numerous plants lack safety information and have been reported to possess toxic effects. Toxicological screening is carried out to determine whether they are safe or not for use and assess their pharmacological potentials and mechanism of action (Arome and Chinedu 2013). *Cucurbita pepo* L. (*C. pepo*), usually called pumpkin, vegetable marrow, or summer squash, is an annual plant belonging to the Cucurbitaceae family (Okada et al. 2002) and is mostly utilized in America, Asia, Europe, India, Mexico, Egypt, China, Mongolia and United States (Nkang et al. 2003). Superior parts used are seeds for treating nephritis, anemia, hyperplasia, bronchitis, prostate cancer, and hemorrhoid (Omotayo and Borokini 2012). Fruits are edible parts in Pakistan and are used as vegetables. Leaves have folklore use in the management of various ailments such as urinary tract infections, fever, hemorrhoids, whopping cough, hyperplasia, constipation, rheumatism, miscarriage, blindness, and prostate cancer (Rahman et al. 2008). Fruits are also important due to their therapeutic potential in sore throat, stomach, fever, whopping cough, urinary problems, hyperplasia, miscarriage, hemorrhoid, blindness, prostate cancer, and rheumatism (Rahman et al. 2013). Its leaves, fruits, seeds, pulp, and whole plant have been reported to have pharmacological activities. Seeds containing bioactive compounds, including caffeic acid, vanillic acid, terpenoids, saponins, resins, and cardiac glycosides (Sood et al. 2012), have biological activities like antioxidant, antiulcer, antibacterial and anti-inflammatory, antidiabetic, antitumor, antihyperlipidemic and immunosuppressive (Nawirska-Olszanska et al. 2013). Fruits have cucurbitacin K, cholesterol, calotropoleanly ester, 2-O-β-D-glucopyranoside and linoleic acid (Rahman et al. 2013), which possess anticancer, antibacterial and antiviral properties (Badr et al. 2011). Flowers containing quercetin, 3,4-dihydroxybenzoic acid, myricetine, 3,4-dihydroxy methyl benzoate, and 5,7dihydroxy, 3,6,3 trimethoxy-flavone have been reported for antibacterial activity (Mohamed et al. 2009). Despite extensive consumption of *C. pepo*in, and myriad ailments, very few studies have been available about its toxicity profile. Therefore, this study evaluated oral sub-acute toxicity after administrating MECP in rats. *Cucurbita pepo* has been used as a folk remedy for a variety of illnesses, according to prior investigations. Phytoconstituents that contribute to the medicinal effects of *Cucurbita pepo's* fruit and seeds have undergone extensive characterization with quantification. It is to assure the safety of using plants as a new medication. Pre-clinical toxicity studies on animals occur throughout phases 1 and 2 of developing novel drugs. Because

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Cucurbita pepo has not yet been evaluated for sub-acute toxicity, the current investigation is necessary to fill this information gap.

Methodology

Preparation of extract

C. pepo plant was collected from Ayub Agriculture Research Faisalabad and authenticated by Dr. Mansoor in Botany, University of Agriculture Faisalabad (UAF) department. Seeds were washed, dried, and ground to form powder. The cold maceration method was used to form the extract. Powder (1000 g) was soaked with methanol (5 L) for 5 days. After filtration, it was concentrated by a rotary evaporator to prepare a methanol extract of C. pepo (MECP).

Approval for animals

The study was conducted after getting approval from the animal ethics committee of GCUF. Healthy male, non-pregnant, and nulliparous female rats were purchased from the animal house of UAF. Rats (100-150 g and 10-12 weeks) were used for sub-acute or repeated dose toxicity study. They were housed in individual cages for seven days in standard conditions (22 ± 4 °C, 30-70% humidity, and 12 hours of light-dark cycle). They were fed laboratory food and water. After 1 week of acclimatization, the study was conducted according to OECD 407 guidelines (Co-operation and Development, 2008).

Experimental protocol

Forty albino rats were divided into four groups; each had 5 males and 5 females. Group I served as normal control, and distilled water was given orally. Group II-IV were treated with three doses of MECP (250, 500, and 1000 mg/kg) administered orally according to their body weights. Rats were fasted overnight before being administered the dose. After dose administration, food was not given for 3-5 hours. During the experiment, water and diet intake were noted.

Observation of signs of toxicity

Cage side observations included vomiting, convulsion, diarrhea, difficulty in respiration, and abnormal posture. Changes in skin colour, piloerection, and pupil diameter were monitored at least once daily throughout the study period. Mortality rate was recorded for evaluating toxicity.

Determination of body and organ weight

Body weights were measured every week for 28 days. On 29th day, rats fasted overnight, and blood was obtained through cardiac puncture using anesthesia. Vital organs (heart, liver, and kidney) were removed from sacrificed rats, cleaned with normal saline, and weighed individually.

Determination of biochemical parameters

Blood samples were collected in tubes without ethylenediamine tetraacetic acid (EDTA). Biochemical analysis was done on serum.

Determination of hematological parameters

Blood collected in EDTA-containing tubes was used for analyzing hematological parameters.

Histopathological evaluation

Organs were fixed in 10% formalin solution at room temperature, embedded in paraffin wax, prepared at 4 mm, and then stained with hematoxylin and eosin. Slides were observed under the microscope.

Statistical analysis

All findings were presented as mean ± SEM. The statistically significant difference between the control and treated groups was assessed via two-way analysis of variance (ANOVA) using Bonferroni post-tests in Graph pad Prism version 5.

Results

The present study revealed that the extract did not produce any mortality during 28 days, even when the dose was 1000 mg/kg. Adverse signs were not observed in any rat. Food and water consumption did not change significantly in male and female rats exposed to doses (250, 500, and 1000 mg/kg) of MECP.

Effect on body and organ weight: Body weights were measured on 1st, 7th, 14th, 21st and 28th day (Table 1). The body weight of male rats in the normal control group gradually rose from 123.67 ± 1.28 g to 176 ± 1.18 g during 28 days. The body weight of male rats treated with MECP (250 mg/kg and 500 mg/kg) increased significantly ($P < 0.001$) from 118.17 ± 1.35 g to 157.17 ± 0.95 g and 131.67 ± 0.67 g to 162.50 ± 0.76 g respectively than the control group. Weight variation in 1000 mg/kg treated male rats was not statistically significant compared to the control group. Normal control female rats exhibited an increase in weight from 135.33 ± 1.26 g to 183 ± 0.58. Weight of female rats (250-, 500- and 1000 mg/kg) increased significantly ($P < 0.001$) from 112.50 ± 0.76 g to 148.33 ± 0.88 g, 127.50 ± 0.76 g to 132.50 ± 0.67 g and 133.67 ± 0.49 to 140.50 ± 0.89 respectively when compared to the normal control group. Weight of organs is summarized in Table 1. Male rats (250 mg/kg) indicated a significant ($P < 0.001$) change in the weight of all organs compared to the normal group. However, rats treated with 500 mg/kg extract exhibited a significant ($P < 0.001$) increase in weight of the liver and heart. However, the kidney's weight changed non-significantly in 500- and 1000 mg/kg. Administration of 1000 mg/kg of MECP caused a significant liver and heart weight alteration as ($P < 0.01$) and ($P < 0.05$), respectively. The liver and kidney weight changed over the normal control group in female rats at a dose of 250 mg/kg. Rats (500 and 1000 mg/kg) exhibited a significant change in the weight of the liver and the heart.

Effect on biochemical parameters: The effect of MECP on liver and kidney function tests has been illustrated in Table 2. Administration of MECP at 250 mg/kg in male rats caused a significant change in levels of AST ($P < 0.01$), ALT, ALP, and BUN ($P < 0.001$) for the normal control group. Changes in AST, ALT, ALP, and BUN were observed significantly ($P < 0.001$) at 500 and 1000 mg/kg treated groups than the control group but remained within normal limits. In female rats at 250 mg/kg there was a significant change in AST ($P < 0.01$), ALP, and BUN ($P < 0.001$), while no significant change in ALT was found in the control group. Nevertheless, bilirubin, albumin, globulin, serum creatinine, and uric acid were not statistically different in all treated groups of either sex from normal control rats. Lipid profiles are shown in Table 3. A significant change in cholesterol and LDL ($P < 0.05$) and triglycerides ($P < 0.001$) was shown in male rats (250 mg/kg of MECP) when compared to normal control rats but remained within the reference range. The cholesterol level (500 mg/kg) changed significantly ($P < 0.05$). Cholesterol and LDL changed significantly ($P < 0.001$) at 1000 mg/kg. In female rats (250- 500 and 1000 mg/kg), significant change in cholesterol, HDL, LDL, triglycerides ($P < 0.001$),

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and VLDL ($P < 0.05$) was observed in the control group but had no clinical significance.

Table 1.Effect of methanol extract of *C. pepo* on body and organ weight of rats

		Control group		MECP treated groups					
		Vehicle		250 mg/kg		500 mg/kg		1000 mg/kg	
		M	F	M	F	M	F	M	F
1st day body weight		123.67 ± 1.28	135.33 ± 1.26	118.17 ± 1.35***	112.50 ± 0.76***	131.67 ± 0.67***	127.50 ± 0.76***	123.50 ± 1.12	133.67 ± 0.49
7th day body weight		131.33 ± 1.15	143.50 ± 1.26	127 ± 1.53**	121.83 ± 0.95***	141.33 ± 1.15***	142.83 ± 0.60	130.83 ± 0.60	127.83 ± 0.60***
14th day body weight		148.33 ± 1.05	157.67 ± 0.76	140.83 ± 0.83***	130.83 ± 0.83***	150 ± 0.58	132.83 ± 0.60***	137.50 ± 0.76***	123.50 ± 0.76***
21th day body weight		157 ± 0.97	164.33 ± 0.88	147.17 ± 1.47***	137.33*** ± 0.80	158.33 ± 0.67	128.17 ± 0.48***	146.33 ± 0.92***	131.83 ± 0.60***
28th day body weight		176 ± 1.18	183 ± 0.58	157.17 ± 0.95***	148.33 ± 0.88***	162.50 ± 0.76***	132.50 ± 0.67***	157.33 ± 0.62***	140.50 ± 0.89***
organ weight	Liver	2.88 ± 0.01	2.92 ± 0.01	3.32 ± 0.01***	3.15 ± 0.01***	3.04 ± 0.01***	3.08 ± 0.01***	2.92 ± 0.01**	2.97 ± 0.01***
	Kidney	0.52 ± 0.01	0.59 ± 0.01	0.67 ± 0.01***	0.64 ± 0.01***	0.53 ± 0.01	0.59 ± 0.01	0.54 ± 0.01	0.57 ± 0.01
	Heart	0.54 ± 0.01	0.60 ± 0.01	0.73 ± 0.01***	0.62 ± 0.01	0.68 ± 0.01***	0.57 ± 0.01***	0.51 ± 0.01*	0.53 ± 0.01***

MECP: methanol extract of C. pepo, M: male, F: female.
*Values are expressed as mean ± S.E.M ***p < 0.001, **p < 0.01, *p < 0.05 as compared to control group.*

Table 2. Effect of methanol extract of *C. pepo* on liver and kidney function tests of rats

Parameters	Units	Control group		MECP treated groups					
				250 mg/kg		500 mg/kg		1000 mg/kg	
		M	F	M	F	M	F	M	F
AST	µ/L	68.33 ± 2.73	78.33 ± 2.95	62 ± 2.54**	87 ± 3.76**	88.50 ± 2.46***	113.17 ± 2.97***	121.17 ± 5.01***	154.33 ± 7.91***
ALT	µ/L	24.67 ± 2.33	29.67 ± 2.17	39 ± 2.02***	26.50 ± 1.60	46.50 ± 1.73***	47.83 ± 1.96***	58.83 ± 2.30***	49.67 ± 145***
Bilirubin	mg/dL	0.24 ± 0.01	0.29 ± 0.02	0.36 ± 0.02	0.37 ± 0.01	0.21 ± 0.02	0.37 ± 0.02	0.43 ± 0.02	0.43 ± 0.01
Albumin	g/dL	3.96 ± 0.07	4.12 ± 0.11	4.11 ± 0.11	4.27 ± 0.08	3.58 ± 0.12	4.52 ± 0.13	4.48 ± 0.14	4.61 ± 0.07
Globulin	g/dL	2.68 ± 0.09	2.92 ± 0.13	3.70 ± 0.10	3.27 ± 0.07	2.78 ± 0.08	3.28 ± 0.08	3.26 ± 0.08	2.97 ± 0.06
ALP	µ/L	69 ± 1.37	75 ± 1.63	84.83 ± 2.23***	94.50 ± 1.26***	107.67 ± 1.84***	115.67 ± 1.65***	82.67 ± 2.42***	95 ± 1.53***
BUN	mg/dL	10.85 ± 1.01	13.33 ± 1.20	17.29 ± 0.82***	18.21 ± 0.69***	24.17 ± 1.08***	21.83 ± 1.08***	21.33 ± 1.15***	24.83 ± 0.95***
Serum creatinine	mg/dL	0.29 ± 0.02	0.37 ± 0.01	0.33 ± 0.02	0.42 ± 0.03	0.71 ± 0.03	0.54 ± 0.06	0.46 ± 0.02	0.68 ± 0.05
Uric acid	mg/dL	0.63 ± 0.05	0.77 ± 0.08	0.86 ± 0.03	0.81 ± 0.06	0.98 ± 0.05	0.79 ± 0.03	1.32 ± 0.08	0.86 ± 0.02

ALT: Alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphate BUN: blood urea nitrogen,
MECP: methanol extract of C. pepo, M: male, F: female.
*Values are expressed as mean ± S.E.M ***p < 0.001, **p < 0.01 as compared to the control group.*

Effect on hematological parameters

Hematological parameters are illustrated in Table 4. A significant ($P < 0.001$) decrease in the level of RBC was observed in male rats treated with 250 mg/kg of MECP compared to normal control rats. No statistically significant variations were found in Hb, WBC, HCT, MCV, MCHC, monocytes, neutrophils, eosinophils, MCH, and platelets in 250 mg/kg treated group. Rats (500 mg/kg) exhibited no significant change in all parameters except RBC, which was significantly decreased ($P < 0.001$) for the control group. Platelets increased significantly ($P < 0.001$) in 500 mg/kg treated rats but remained within the normal range. RBC was significantly reduced ($P < 0.001$) in the 1000 mg/kg treated group than in the control group. Monocytes, platelets, and neutrophils were increased significantly ($P < 0.001$) in 1000 mg/kg but remained within normal limits. A significant ($P < 0.001$) decline in RBC was observed in treated female rats

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compared to the normal control group. Eosinophils and monocytes changed significantly as ($P < 0.001$) and ($P < 0.01$), respectively, at 1000 mg/kg, but were not clinically important.

Table 3. Effect of methanol extract of *C. pepo* on hematology parameters of rats

Parameters	Units	Control group		MECP treated groups					
		Vehicle		250 mg/kg		500 mg/kg		1000 mg/kg	
		M	F	M	F	M	F	M	F
Hb	g/dL	13.18 ± 0.33	12.16 ± 0.29	13.70 ± 0.09	14.57 ± 0.12	18.78 ± 0.46	16.60 ± 0.10	15.68 ± 0.15	14.93 ± 0.23
WBC	$\times10^3$/L	10.15 ± 0.59	9.53 ± 0.58	11.72 ± 0.45	9.42 ± 0.18	15.02 ± 0.14	14.78 ± 0.08	11.61 ± 0.15	12.19 ± 0.33
RBC	$\times10^{12}$/L	8.29 ± 0.22	9.59 ± 0.14	6.75 ± 0.08***	8.30 ± 0.01***	6.31 ± 0.08***	7.71 ± 0.07***	5.76 ± 0.07***	7.25 ± 0.08***
HCT	%	52.50 ± 3.96	54 ± 1.88	50.17 ± 1.47	49.50 ± 1.41	53.50 ± 0.76	52.50 ± 0.76	61.67 ± 1.05	62.50 ± 0.76
MCV	FL	53 ± 3.31	55.83 ± 3.26	47.50 ± 0.76	47.50 ± 0.76	57 ± 0.97	62.83 ± 0.95	61.50 ± 0.76	67.50 ± 0.76***
MCH	Pg	16.28 ± 0.90	16.56 ± 0.51	15.17 ± 0.29	15.62 ± 0.14	15.58 ± 0.14	17 ± 0.28	18.64 ± 0.15	17.78 ± 0.07**
MCHC	%	38.33 ± 2.17	50.17 ± 3.84	42.33 ± 2.29	52.22 ± 3.01	49.50 ± 1.93	54.67 ± 0.88	65.50 ± 1.52***	63.50 ± 0.76
Platelets	$\times10^9$/L	460.50 ± 16.28	549.83 ± 24.30	475.83 ± 1.58	489.67 ± 1.59	534.50 ± 1.61***	539.67 ± 2.91	476.50 ± 17.15**	506.83 ± 1.78
Neutrophils	%	26.50 ± 4.29	30.67 ± 0.80	25.50 ± 1.61	27.50 ± 0.76	16.17 ± 1.52	22.50 ± 0.76	8.69 ± 0.34*	17.33 ± 0.80
Eosinophils	%	2.15 ± 0.51	2.97 ± 0.29	3.15 ± 0.29*	3.58 ± 0.14	2.65 ± 0.14	2.55 ± 0.14	1.37 ± 0.22	1.66 ± 0.15***
Lymphocytes	%	66.17 ± 1.52	67 ± 0.99	71.50 ± 1.18	75.33 ± 1.02	57.67 ± 0.84	59 ± 0.97	66.83 ± 1.08	52.50 ± 0.76
Monocytes	%	2.67 ± 0.44	2.55 ± 0.47	2.57 ± 0.14	2.83 ± 0.33	3.57 ± 0.16	2.53 ± 0.15	4.36 ± 0.15***	1.63 ± 0.15**

*Hb: Hemoglobin, HCT: hematocrit, RBC: red blood cell, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cell, MECP: methanol extract of C. pepo, M: male, F: female. Values are expressed as mean ± S.E.M ***p < 0.001, **p < 0.01, *p < 0.05as compared to control group.*

Table 4. Effect of methanol extract of *C. pepo* on lipid profile of rats

Parameters	Units	Control group		MECP treated groups					
		Vehicle		250 mg/kg		500 mg/kg		1000 mg/kg	
		M	F	M	F	M	F	M	F
Cholesterol	mg/dL	75.17 ± 1.08	90 ± 0.58	99.33 ± 1.54*	110.50 ± 0.89***	99.50 ± 1.26*	112.17 ± 2.81***	117.17 ± 0.95***	111 ± 1.71***
Triglycerides	mg/dL	90 ± 0.58	95.83 ± 1.64	136 ± 1.07***	113.17 ± 3.10***	97.50 ± 0.76	125.67 ± 1.65***	83.59 ± 0.71	108.33 ± 0.88***
HDL	mg/dL	36.33 ± 1.63	38.33 ± 0.88	35.83 ± 1.30	33.50 ± 0.76***	38.33 ± 0.67	30.50 ± 0.76***	25.83 ± 0.95	22.50 ± 0.76***
LDL	mg/dL	113.83 ± 1.17	123.50 ± 0.76	90.50 ± 1.48*	85.17 ± 1.35***	94.83 ± 0.95	76.50 ± 0.76***	78.33 ± 0.67***	83.50 ± 0.76***
VLDL	mg/dL	16.17 ± 0.60	24.67 ± 1.05	27.50 ± 0.76	19.50 ± 0.76*	28.17 ± 1.35	17.67 ± 0.88*	21.50 ± 0.76	32.50 ± 0.76

*HDL: High-density lipoprotein, LDL: low-density lipoprotein VLDL: very low-density lipoprotein, MECP: methanol extract of C. pepo, M: male, F: female. Values are expressed as mean ± S.E.M ***p < 0.001, *p < 0.05 as compared to control group.*

Effect on histopathology

Histological examinations of organs are shown in Figures 1 and 2, respectively. The liver of the control group showed a portal triad with branches of hepatic artery portal veins and interlobular bile duct. Treated rats showed normal hepatocytes and hepatic sinusoids lined with endothelial cells. Necrosis (karyolysis and eosinophilic cytoplasm), hepatic congestion, and regeneration in treated groups were not seen. In kidney sections of control, rats exhibited normal glomerulus, proximal convoluted tubules, distal convoluted tubules, Bowman 's capsule, and macula densa. Treated rats showed no vacuolar degeneration, apoptosis, or renal congestion compared with controls. In control and treated rats of either sex, heart tissues showed normal muscle fibers with an intercalating disk. No microscopic changes were examined at 40X.

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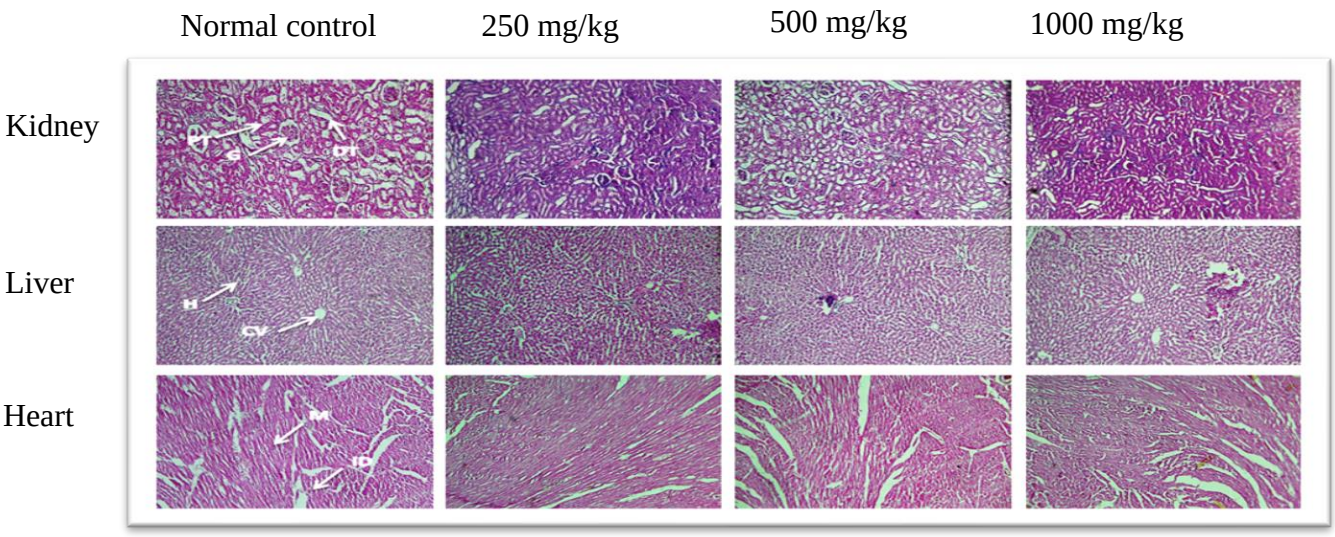

Figure 1: Histopathological examination of kidney, liver, and heart tissues of normal control and extract treated groups (250, 500, and 1000 mg/kg) of male rats. G: glomerulus, PT: proximal tubules, DT: distal tubules, CV: central vein, H: hepatocytes, M: myocardium, ID: intercalated disc (40XH & E stain)

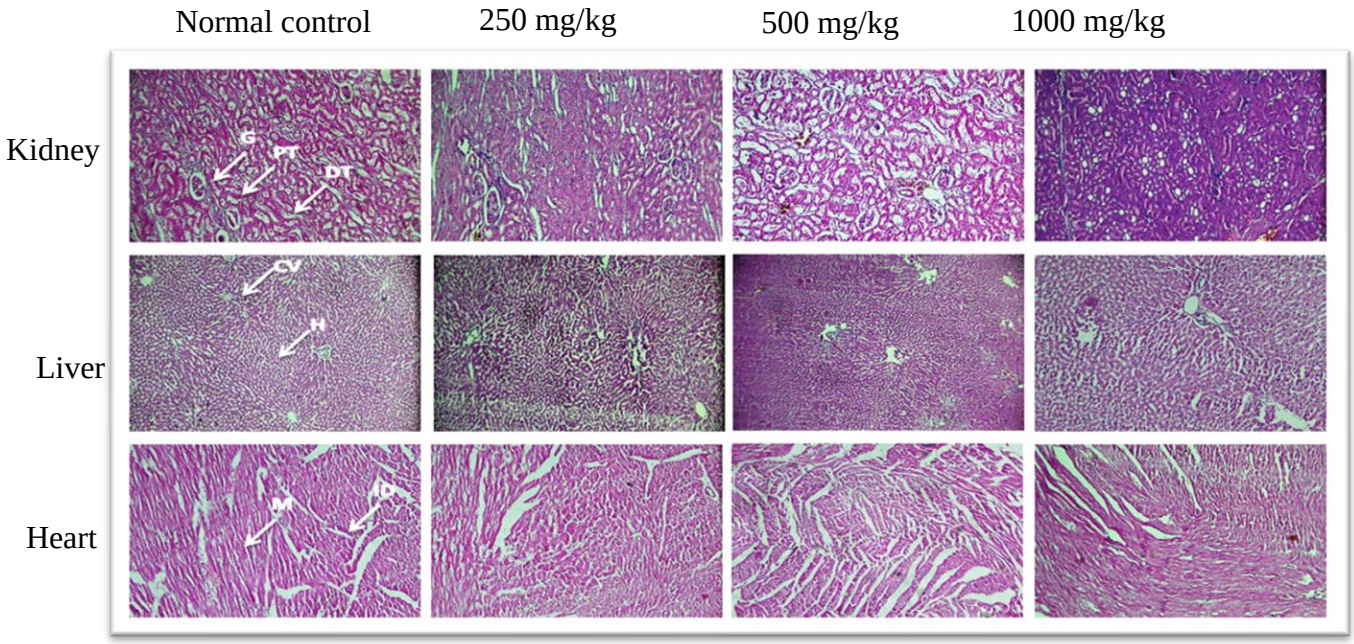

Figure 2: Histopathological examination of female rats' kidney, liver, and heart tissues of normal control and extract treated groups (250, 500, and 1000 mg/kg). G: glomerulus, PT: proximal tubules, DT: distal tubules, CV: central vein, H: hepatocytes, M: myocardium, ID: intercalated disc (40XH &E stain)

Discussion

Medicinal plants have attained more importance as an alternative to conventional treatment and are vital in expanding new drugs as clinically useful remedies. Uppsala Monitoring Committee of World Health Organization organizes data concerning the adverse effects of herbal preparations, while OECD sets procedures for executing different toxicological tests. An acute toxicity test of methanol extract of *C. pepo* seeds was performed in a previous study, and LD50 determined using Kerber's formula was 2975 mg/kg (OECD guideline 420). After sub-acute administration for 28 days, toxicological considerations deliver dose response suggestions on potential health hazards (Rivas *et al*. 2013). Body weight is an important indicator for evaluating the existence of toxic outcomes of tested material in animals (Teo *et al*. 2003). Both treated and control groups revealed a progressive increase in body weight, proven by better food and water consumption. Organ weight is also vital to animals' physiological and pathological status. The heart, liver, and kidney are the principal organs affected by toxicants

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(Amresh *et al*. 2008). There was no significant difference found in the organ weight of all groups. Biochemical and hematological profiles evaluate safety (Petterino and Argentino-Storino 2006). ALT, ALP, AST and bilirubin are sensitive indicators of hepato-cellular function (Traesel *et al*. 2016). After administration of MECP, mild change was observed in AST, ALT, and ALP, which was not clinically significant. Bilirubin is formed by hemoglobin degradation in spleen, bone marrow, and liver (Chitra *et al*. 2015). A decrease in albumin level may represent infection (Yakubu *et al*. 2003). Results showed that differences in albumin, globulin, and bilirubin were non-significant among groups. These findings recommend that MECP has a hepatoprotective effect, which a previous study has confirmed. The function of the kidney may be determined via evaluating variations in uric acid, blood urea nitrogen, and creatinine (Ezeja *et al.* 2014). BUN, serum creatinine, and uric acid did not change significantly among all groups. Current investigation indicated that MECP did not cause nephrotoxicity and corroborates with the literature. Furthermore gallic acid, caffeic acid, ferulic acid, and quercetin present in *C. pepo* were responsible for hepatoprotective and nephroprotective activity. Administration of MECP showed changes in triglycerides, cholesterol, and LDL, which were not clinically significant. Antihyperglycemic and antihyperlipidemic effects of plants support our results. Quercetin and caffeic acid can potentially decrease cholesterol through various metabolic pathways, including the efflux of cholesterol and controlling its metabolism in the liver by promoting its conversion into bile acid (Hsieh *et al.* 2016). The hematopoietic profile is important for determining pathological and physiological status since it is a sensitive indicator of toxicity in animals and humans (Diallo *et al.* 2010). MCV represents the volume of average red cells in the sample, while MCH presents the hemoglobin concentration in average red cells. MHC is an average level of hemoglobin in red blood cells. WBC and their sub-types, such as neutrophils, eosinophils, monocytes, and lymphocytes are part of the immune system (Odeghe *et al.* 2012). Platelets are the main indicators of thrombo-embolic ailments and play a role in blood coagulation. In the current study, treatment with MECP did not significantly change all parameters except RBC, which decreased significantly in the treated groups than the control group. Decreased levels of RBC have the potential to cause anemia.

Assessment of organ histopathological alterations is considered a basic test in the safety assessment of tested materials (Traesel *et al.* 2014). Histopathological analysis of kidneys of both control and treated groups exhibited a normal appearance of glomeruli, proximal and distal convoluted tubules without necrosis, vacuolar changes, and tubular degeneration. Liver sections displayed normal hepatocytes, portal triad, sinusoids, and central vein in treated groups similar to the control group. Heart tissues from treated groups showed normal morphology of cardiac muscle fibers having central nuclei without degeneration and fragmentation.

Conclusion

The current investigation demonstrated that MECP did not produce lethality and alteration in biochemical, hematological, and histopathological investigations after 28 days. Only significant change was observed in levels of RBC after oral administration of MECP at 250, 500, and 1000 mg/kg, suggesting its potential to cause anemia. On the other hand, to identify definite oral safe doses and analyze any unexpected variability of MECP, chronic toxicity, genotoxicity, and teratogenicity studies might be a prerequisite.

Declarations

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All data generated or analyzed during the study are included in the manuscript.
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Conflict of interest

The authors declared absence of conflict of interest.

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References

Arome D and Chinedu E 2020. The importance of toxicity testing. J Pharm BioSci. 4:146-148.

Badr SE, Shaaban M, Elkholy YM 2011. Chemical composition and biological activity of ripe pumpkin fruits (Cucurbita pepo L.) cultivated in Egyptian habitats. Nat Prod Res. 25:1524-1539.

Carrasco PC, Tan KN, Reyes FM 2016. The deleterious effect of cholesterol and protection by quercetin

on mitochondrial bioenergetics of pancreatic β-cells, glycemic control and inflammation: In vitro and in vivo studies. Redox Biology. 9:229-243.

Co-operation OfEconomic and Development 2008 Test No. 407 Repeated Dose 28-day Oral Toxicity Study in Rodents OECD Publishing.

Diallo A, Eklu GK, Agbonon A 2020. Acute and Subchronic 28 day Oral Toxicity Studies of Hydroalcoholic Extract of Lannea kerstingii Engl. and K. Krause(Anacardiaceae) Stem Bark. J Pharmacol Toxi. 5:343-349.

Ezeja MI, Anaga AO and Asuzu IU 2014. Acute and sub-chronic toxicity profile of methanol leaf extract of Gouania longipetala in rats. J Ethnopharmacol. 51:1155-1164.

GuptaD, Bleakley B and Gupta RK 2008. Dragon's blood: botany, chemistry and therapeutic uses. J Ethnopharmacol. 115(3):361-380.

Hsieh YL, Yeh YH, Lee YT and Huang CY 2016. Dietary potato peel extract reduces the toxicity of cholesterol oxidation products in rats. J Func Foods. 27:461-471.

Mohamed G, Ibrahim S and Sayed H 2009. Phenolic constituents of Cucurbita pepo L. cv 'Eskandrani'(Summer Squash) flowers. Bull Pharm Sci. 32:311-319.

Nawirska-Olszanska A, Kita A, Biesiada A 2013. Characteristics of antioxidant activity and composition of pumpkin seed oils in 12 cultivars. Food Chem. 139:155-161.

Nkang A, Omokaro D, Egbe A and Amanke G 2003. Variations in fatty acid proportions during desiccation of Telfairia occidentalis seeds harvested at physiological and agronomic maturity. Afric J Biotech. 2:33-39.

Odeghe O, Uwakwe A and Monago C 2012. Some biochemical and haematological studies on the methanolic extract of Anthocleista grandiflora stem bark. Int J Appl Sci Technol. 2:58-65.

OECD Guidelines for acute toxicity of chemicals. No. 420, 2001.

Okada M, Mitsuhashi H, Wada H 2002. Newly revised illustrated medicinal plants of world. Hokuryukan Co, Ltd: Tokyo, Japan:249.

Omotayo FO and Borokini TI 2012. Comparative phytochemical and ethnomedicinal survey of selected medicinal plants in Nigeria. Scient Res Essay. 7:989-999.

Rahman A, Anisuzzaman M, Ahmed F, Islam A and Naderuzzaman A 2008. Study of nutritive value and medicinal uses of cultivated cucurbits. J App Sci Res. 4:555-558.

Rahman A, Islam A and Rahman MM 2013. An anatomical investigation on Asteraceae family at Rajshahi Division, Bangladesh. Int J Biosci. 3:13-23.

Sood A, Kaur P and Gupta R 2012. Phytochemical screening and antimicrobial assay of various seeds extract of Cucurbitaceae family. Int J App Bio Pharmaceutical Tech. 3:401-409.

Traesel GK, de Souza JC, de Barros AL 2014. Acute and subacute (28 days) oral toxicity assessment of the oil extracted from Acrocomia aculeata pulp in rats. Food Chem Toxi. 74:320-325.

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