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Safety assessment of antidiabetic extract of *Berberis lycium* in rats

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ABSTRACT

Berberis lycium is an ethnomedicinally important plant. It is being used for the treatment of various ailments like jaundice, diabetes, internal wounds, diarrhea, etc. The plant is also being used as in ingredient in many Unani formulations. The plant has several experimentally proven pharmacological activities and we have also reported its antidiabetic potential in diabetic rats. However there has been no systematic study done to assess its safety. The present study was aimed to check the toxic effects of the methanol extract *of the plant in rats. The subchronic study was carried out at the dose level of 1000mg /kg of body weight in both sexes. The rats were observed carefully for any behavioral change till the completion of experimentation. The physiological parameters like water consumption, feed consumption and body weight were recorded on weekly basis. The rats were sacrificed on 91st day after overnight fasting. Blood was collected for hematological and biochemical parameter analysis. Organs were collected, observed and samples were taken for histological studies. No mortality/morbidity was reported in any of the treated rats. There were no significant changes found in any biochemical, hematological and histopathological parameters that could indicate any signs of toxicity, thus suggesting the extract is devoid of any adverse effect in rats.*

Keywords: ***Antidiabetic*** **Extract,** ***Berberis Lycium, Histopathology, Safety.***

I INTRODUCTION

Herbs and herbal products have been in use since times immemorial. Traditional healers often used the herbs for the treatment of the various diseases and illnesses. With the passage of time the larger masses consumed herbs and herbal products for their well being [1,2]. However a perception has evolved with the use of herbs that they are not

associated with the adverse effects, which reportedly has not been true [3]. With the marked increase in use and supposed safety of herbs, adverse effects have become a major safety issue for herbal products [4, 5]. There have been reports where herbs and herbal products have shown adverse effects, like some are hepatoxic [6,7], nephrotoxic [8-10], neurotoxic [11,12], cardiotoxic [13-14]. In order to increase the confidence and trust among the consumers, the toxicity assessment of herbal products is very much necessary and is also an important step in the development of pharmaceuticals [15,16]. *Berberis lycium* is a medicinal plant belonging to the family Berberidaceae. The plant is native to Himalayas found in Pkaistan, India, China Butan etc. It is called as Indian berberry in English, Kashmal or Kasmal in Hindi, Ishkeen in Urdu and Kwaray or Ziar Largay in Pashtu [17]. The plant is known as *Kaw Dach* in Kashmiri and the plant is also abundantly found in Kashmir. The plant has been traditionally used against various diseases including jaundice, diabetes, eye infections, fractured bones, internal wounds, diarrhea, rheumatism, stomachache, and it is also used as a general body tonic [18]. The plant has been reported to have antidiabetic activity [19, 20]**.** The plant has also been reported to have other pharmacological activates like antimicrobial [21, 22], hepatoprotective [23, 24], wound healing [25], pesticidal [26], antioxidant [27], anticoccidial [28]. However there has been no systematic study carried out on toxicity profiling of the plant. Thus, in order to obtain scientific information on its safety and potential toxicity, the present study was performed to assess the possible toxic effects of the *methanol* extract *of Berberis lycium* after repeated oral administration in rats following Organization of Economic Co-operation and Development (OECD) guidelines). The dose 1000 mg/kg was selected as it is the limit dose. Methanolic extract was selected for the study as it is the most bioactive extract as per reports.

II METHODS & MATERIALS

2.1. Collection of the plant material

The plant material of the *Berberis lycium* was collected from the hilly areas of Kashmir, India in the month of July. The identification of the plant material was authenticated from the University of Kashmir. Sample specimen (voucher specimen No: *709-KASH*) was deposited in the herbarium of Centre of Taxonomy, University of Kashmir. The root part was taken for the study and the root material was shade dried, cut into smaller pieces and coarsely ground.

2.2. Preparation of extracts

The powdered material was loaded into a soxhlet extractor and methanol was used as solvent for hot extraction. The solvent was repeatedly heated for a period of 96 hours in the flask to ensure the complete extraction. The extract was filtered and the solvent was recovered using a rotary evaporator. The extract thus obtained was kept in a desiccator to remove the extra bound solvent.

2.3. Animals and maintenance

The study was conducted in healthy Albino Wistar rats of both sexes. The rats were obtained from the animal colony of the Indian Institute of Integrative Medicine, Jammu. The rats were housed in polypropylene cages and acclimatized for a period of 15 days prior to the start of the experiment. They were maintained under standard laboratory conditions of regular 12h light/12h dark cycle and temperature (24 ±5 ºC) throughout the experimental period. They were fed clean tap water (Reverse Osmosis cleaned) and commercial pelleted rat feed *ad libitum* during the experimental period. The experimental protocol was approved by the Institutional Animal Ethics Committee of the Regional Research Institute of Unani Medicine, Srinagar, J&K, India.

2.4. Experimental Design

The study was conducted following the guidelines set by Organisation of Economic Cooperation and Development (OECD) guideline Number 408 [29]. The study was conducted in four groups of rats (*n* = 6 males and 6 females/group) namely Male Control group (MC), Female Control (FC) group, Male Treated group (MT) and Female Treated group (FT). The groups MC and FC were orally given water in comparable volumes. The groups MT and FT were orally administered daily methanol extract for 90 days. All the animals were closely examined for any adverse toxic signs, behavioural changes etc. The body weight of the rats was recorded weekly and at the end of the experiment the percentage of body weight gain was calculated. Feed and water consumption / rat / 24 hours were recorded weekly. On the 91st day, after over-night fasting, all the animals were sacrificed by exsanguination by withdrawing blood in a syringe from the dorsal vena cava after opening the abdomen under Isoflurane anaesthesia. Blood was collected for the haematological and biochemical parameter analysis. After euthanasia, the organs were removed for necropsy, organ weight measurement, and histopathological examination.

2.5. Biochemistry Parameters

Biochemical parameters were estimated in serum obtained after centrifugation of blood at 2000 RPM for 15 minutes on the day of the rat sacrifice. Biochemical parameters were determined on fully automatic biochemistry analyzer (XL-640 Transasia) using ERBA diagnostic kits. Liver function tests- aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphotase (ALP), total bilirubin (T Bil), total protein (TP) and albumin and kidney function tests- urea, uric acid and creatinine besides other biochemical substances such as glucose, cholesterol and triglycerides were estimated.

2.6.Haematological Parameters

Haematological parameters were analyzed in freshly collected blood in blue top vacutainer containing EDTA anticoagulant. The blood was gently mixed with the EDTA anticoagulant coated on the tube walls. Haematological parameters were determined on fully automatic haematological analyzer (Sysmex XT2000i Sysmex Corporation Japan). Haematological parameters such as haemoglobin conc. WBC count, RBC count, haematocrit value, mean

corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin, platelate count, differential leukocyte count – neutrophil %, lymphocyte %, monocyte %, eosinophil % and basophil %, and reticulocyte count were studied.

2.7. Necropsy and organ weight

All groups of rats were subjected to gross necropsy, which included the examination of the thoracic organs, external surface, and all of the internal organs. Vital organs were carefully examined macroscopically for any type of abnormalities. Thereafter, various organs, including the heart, liver, kidneys, lung, spleen, testes, and ovaries were surgically removed, cleaned with ice-cold saline solution, placed on absorbent papers, and then weighed (absolute organ weight in grams). The relative organ weight (ROW) of each animal was then calculated as follows: ROW= [Absolute organ weight (g) ÷ Bodyweight of rat on sacrifice day (g)]×100.

2.8. Histopathology

Tissue samples were collected from the organs of control as well as treated male / female rats of the subchronic study. The tissue collected from the organs such as liver, lung, kidney, pancreas, spleen, brain, ovary/testes and heart were numbered for identification and then transferred to tissue cassettes (SS) to enable fixation in 10 % formalin for 36-48 hours followed by the tissue processing which was carried on Automatic tissue processor Model No. TP-1020 (LEICA make Germany). The tissue processing included dehydration in graded isopropyl alcohol, clearing in xylene I & II, impregnation in paraffin wax and finally tissue blocks was prepared on paraffin block making Model No1150H+C (LEICA make-Germany). Section cutting of tissue blocks was done using microtome (YORCO) to the thickness of 5 – 8 microns. The tissue sections were fixed on the slide by heat technique following by the staining by Haematoxylin and Eosin stain. The staining was carried on Automatic slide stainer (Thermo Make-Germany) using haematoxylin and eosin staining. After staining the tissue sections were mounted with DPX to prevent any damage to the stained tissue. The stained tissue sections were examined under microscope 40X objective to check the adverse effects of drug on cell morphology as well as on the cell organelles.

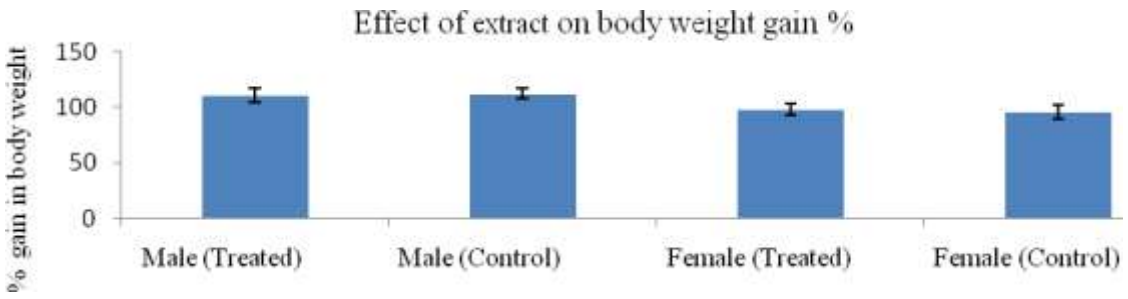
2.9. Statistical analysis

All the values of body weight, biochemical estimations, haematological parameters, feed intake values and water intake values were expressed as X ± SD and analyzed for one way ANOVA using SPSS 16.0 statistical software. Differences between groups were considered significant at $p < 0.05$ levels.

III RESULTS

3.1. Clinical observations and body weight of rats

None of the rats showed any observable signs of adversity and no report of any morbidity or mortality was recorded among the treated group of rats. The treated groups of rats were found to grow in a normal fashion as there was no statistically significant change observed in the percentage of body weight gain among the groups as shown in Fig 1.

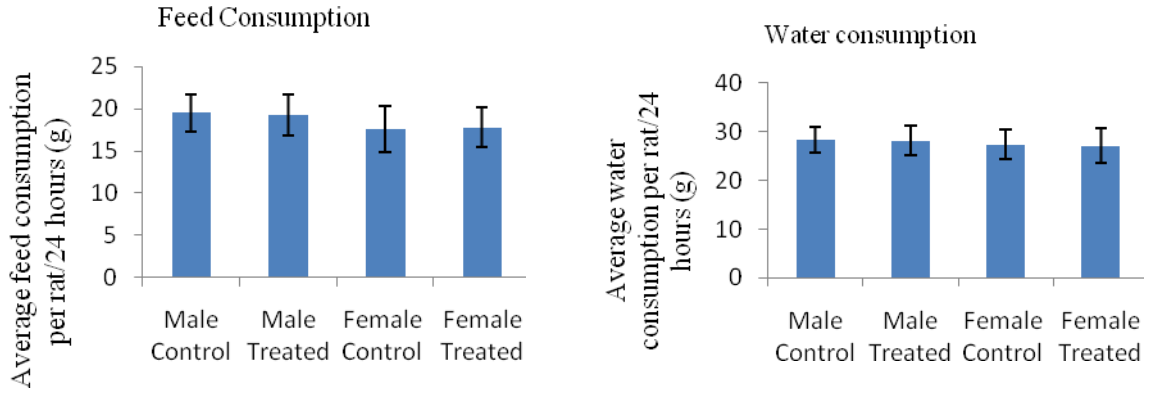


The values are expressed as mean ± SD. n=6 in each group.
**p<0.05 as compared with the controls at the same time (one-way ANOVA)*

Figure 1. Effect of extract on body weight gain % of rats. There is no adverse effect of extract on the body weight gain.

3.2. Effect of extract on feed and water consumption of rats.

The feeding habit of the treated group of rats was not found to be affected by the administration of the extracts. The extracts were also found to have no significant effect on the average water consumption of the treated rats as shown in Fig.2.

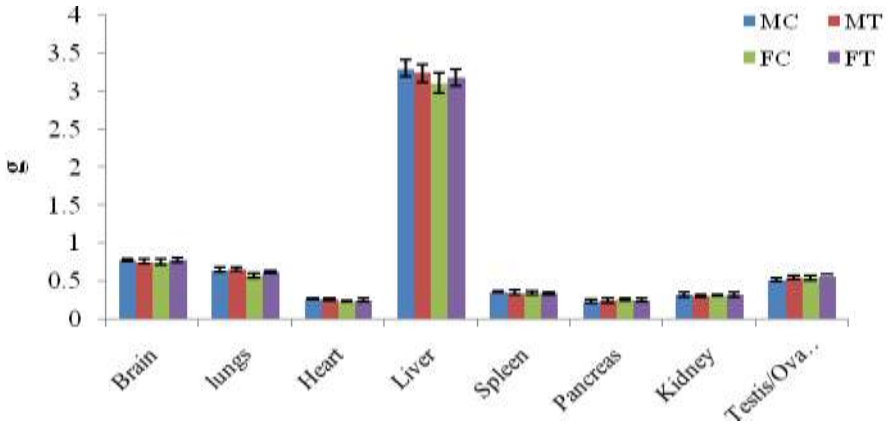


The values are expressed as mean ± SD. n=6 in each group.
**p<0.05 as compared with the controls at the same time (one-way ANOVA)*

Figure 2. Effect of extract on feed and water consumption of rats. The figure shows extract does not change the feeding habit along with the water consumption of treated groups of rats.

3.3. Effect of extract on the relative organ/ tissue weights of rats

The collected organs were observed for any morphological changes and the organs appeared to be normal in size, shape and texture when compared with the normal controls. There were no statistically significant difference in the relative weight of tissue/organs of extract treated rats and control rats as shown in Fig.3.



The values are expressed as mean ± SD. n=6 in each group.
**p<0.05 as compared with the controls at the same time (one-way ANOVA)*

Fig. 3. Relative Organ/tissue weight of treated and controls rats.

3.4. Effect of extract on clinical biochemistry parameters

The oral administration of the extract was found to have no statistically significant change in the biochemical parameters when compared with the controls as shown in the Table 1. The organ function tests like liver function test (LFT) and kidney function test (KFT) were found to be normal in treated male and female groups. The lipid profiles of the drug treated male and female rats was found to be unaffected by the drug administration when compared with respective controls as shown in Table 1.

Table 1. Biochemical parameters of treated and controls.

Parameter	Male		Female	
	Control	Treated	Control	Treated
Liver function tests				
ALT (IU/L)	91.01±6.5	87.36±7.8	84.3±9.2	87.4±8.7
AST (IU/L)	124.2±10.3	119 .5±13.2	129.2±8.9	123.2±9.4
ALP (IU/L)	171.5 ±14.1	173.8±11.87	163.4 ±13.3	169.2±10.7
GGT(IU/L)	6.3±0.23	6.8±0.33	5.7±0.28	6.1±0.22
Total bilirubin(mg/dl)	0.12±0.03	0.13±0.04	0.13±0.04	0.14±0.05
Total protein (g/dl)	7.23±0.22	7.42± 0.25	7.31±0.26	7.38±0.21
Albumin (g/dl)	4.12±0.01	4.18± 0.17	4.23±0.22	4.34±0.24
Kidney function tests				
Urea (mg/dl)	51.3±4.3	49.8±3.9	52.3± 3.1	53.3± 3.4
Uric acid (mg/dl)	2.44±0.52	2.41±0.51	2.38 ±0.51	2.5± 0.5

Creatinine(mg/dl)	0.73±0.05	0.72±0.06	0.74±0.04	0.75±0.06
Metabolic function tests				
Glucose (mg/dl)	84±9.7	87±8.3	88.3 ±8.5	90.3 ±9.9
Cholesterol (mg/dl)	65.2±8.5	68.3±8.1	63.2±7.7	59.7 ±8.3
Triglyceride (mg/dl)	82.8±8.6	87.3±9.1	92.4±10.7	92.3 ±9.7
LDH((IU/L))	209.0±14.4	203.5±15.4	205.2±14.1	199.3±15.7

The values are expressed as mean ± SD. n=6 in each group.
**p<0.05 as compared with the controls at the same time (one-way ANOVA)*

3.5. Effect of the extract on various hematological parameters of rats.

The hematological parameters of male and female rats are presented in Table 2. The extract was found to have no significant effect on changing the hematological parameters such as WBC counts, total leukocyte count, hemoglobin, hematocrit, total erythrocyte count, erythrocyte indices (MCV, MCH, and MCHC) and platelets count of both male and female groups as shown in Table 2.

Table 2. Hematological parameters of treated and control rats.

Parameter	Male		Female	
	Control (Mean ±SD)	Treated (Mean ±SD)	Control (Mean ±SD)	Treated (Mean ±SD)
WBC (10^3/µl)	11.17 ± 1.26	10.16 ± 0.91	11.54 ± 0.61	10.31 ± 1.04
RBC (10^3/µl)	7.53 ± 0.32	7.58 ± 0.26	7.69 ± 0.24	7.57 ± 0.23
Hb (grams %)	15.24 ± 0.45	16.15 ± 0.50	15.88 ± 0.33	14.98 ± 0.47
HCT (%)	46.54 ± 1.87	45.27 ± 1.09	45.48 ± 1.00	42.20 ± 1.54
MCV (Femtolitre)	58.44 ± 1.33	57.77 ± 0.69	61.04 ± 1.04	58.13 ± 0.83
MCH (pico grams)	19.84 ± 0.47	20.40 ± 0.17	21.40 ± 0.44	19.82 ± 0.27
MCHC (grams%)	33.96 ± 0.43	34.12 ± 0.42	34.20 ± 0.54	34.96 ± 0.34
Reticulocytes (%)	3.11±0.07	2.98±0.87	3.17 ± 0.56	3.45 ± 0.78
Platelet count (10^3/µl)	1140.2 ± 64.54	1191 ± 63.3	1005 ± 77.93	1018 ± 62.90
Differential Leucocyte Count				
Neutrophils %	16.03±0.54	17.17±0.75	15.44 ± 1.78	14.08 ± 0.93
Lymphocytes %	75.86±1.28	74.90±1.03	80.50 ± 1.02	79.14 ± 1.61
Monocytes %	5.03±0.65	4.50±0.46	6.22 ± 1.44	6.18 ± 0.87
Eosinophils %	3.56±0.16	4.03±0.99	2.62 ± 0.62	2.46 ± 0.94
Basophils %	0.28 ± 0.07	0.21 ± 0.03	0.22 ± 0.06	0.17 ± 0.02

The values are expressed as mean ± SD. n=6 in each group.
**p<0.05 as compared with the controls at the same time (one-way ANOVA)*

3.6. Effect of extract on histopathology of various organs/tissue.

The histopathological results show that the extract did not induce any pathological change in the organs. Microscopic examination of all the organs obtained from control groups and treated groups exhibited normal cytoarchitecture. Fig.4 to Fig. 11. shows different slides of drug treated and control tissues, as observable there is no major difference in treated and control slides, the photomicrographs were taken at 40X.

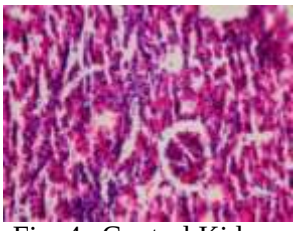


Fig. 4a.Control Kidney

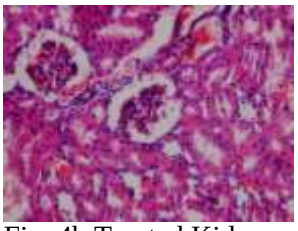


Fig. 4b.Treated Kidney

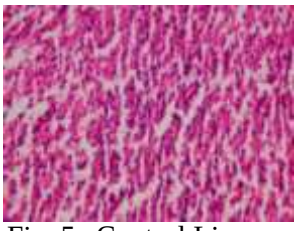


Fig. 5a.Control Liver

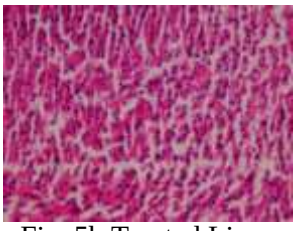


Fig. 5b.Treated Liver

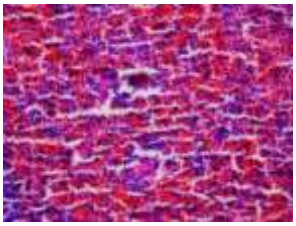


Fig. 6a. Control Spleen

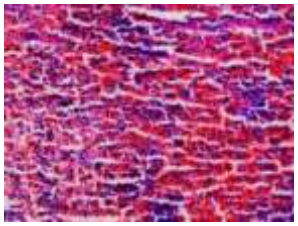


Fig. 6b.Treated Spleen

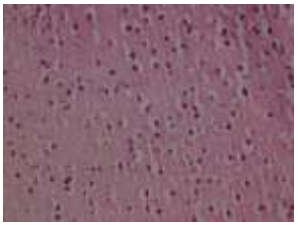


Fig. 7a. Control Brain

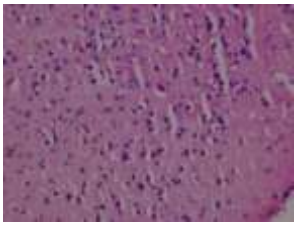


Fig.7b. Treated Brain

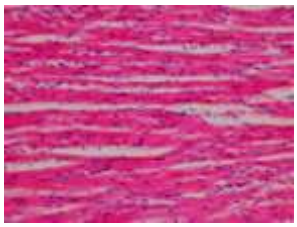


Fig. 8a. Control Heart

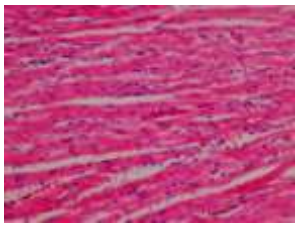


Fig. 8b. Treated Heart

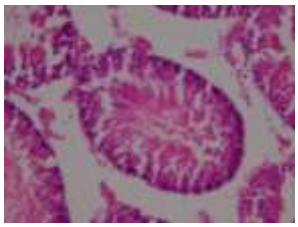


Fig. 9a. Control Testes

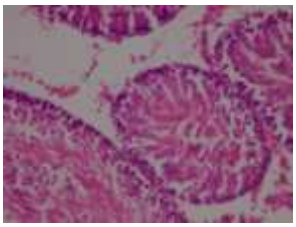


Fig. 9b. Treated Testes

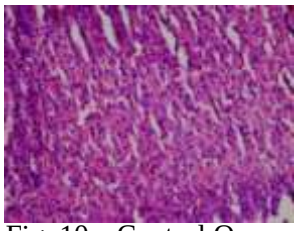


Fig. 10a. Control Ovary

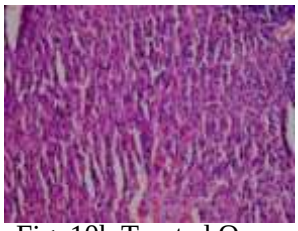


Fig. 10b.Treated Ovary

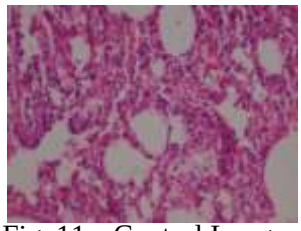


Fig. 11a. Control Lung

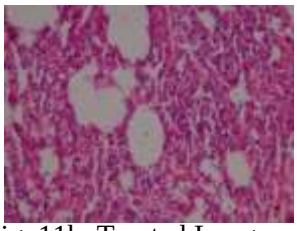


Fig. 11b. Treated Lung

IV DISCUSSION

All over the world and particularly in developing countries there has been a rise in the consumption of the medicinal plants and their products owing to their efficacy, low cost and easy availability. Nevertheless the safety data

regarding the toxicity and adverse effects of plants and plant products are not available at large [30]. Consequently, to ensure the safety of these plants and plant products among the consumers and traditional practitioners the systematic and experimental studies are need of the hour, so that any adverse or severe side effect may be projected among the humans and besides such studies will also help in choosing safe dosages among the humans [31].From the literature survey it was found that there has been no systematic study carried out on the safety assessment of the extract of the *Berberis lycium*. Hence, we carried out the present study to assess its toxicological profile by performing subchronic oral toxicity in rats. The aim of long duration repeated dosing toxicity studies is to provide data regarding the adverse effects of a test substance and the information about the potential health hazards expected to be a consequence of repeated exposure over a relatively limited period of time including information about target organs, the possibilities of cumulative effects, and an estimate of the dose at which there is no observed adverse effect. The repeated oral dosing of extract for a period of 90 days did not produce any deaths or clinical signs of toxicity.

The first and the foremost observable signs of toxicity are the changes in body weight and general behavior [32]. It was found in this subchronic toxicity study the extract administration did not induce any mortality and as such no abnormal behaviour was observed in any of the treated rats. The treated rats gained weight with age, and there was no significant change ($P > 0.05$) in their mean body weight gain compared to the control groups throughout the duration of the study.

The 90 day repeated oral administration of 1000mg/kg extract did not show significant change in any studied biochemical parameters. The biochemical parameters related to lipid metabolism (plasma triglycerides, cholesterol), kidney and muscle function viz. plasma LDH, protein, creatinine, urea and uric acid levels were in comparison to control animals. This suggested that *Berberis lycium* extract administration had no effect on cell membrane permeability, muscle metabolism and kidney function.

The liver being the central metabolic organ and its normal function is assessed by various serum biomarker parameters viz plasma bilirubin, ALAT, ASAT, ALP, GGT and albumin [33, 34]. Since the extract administration in rats did not produce significant changes ($P > 0.05$) in serum levels of ALT, AST, and ALP, in extract-treated rats when compared with the respective controls, it is suggested that extract has no apparent hepatotoxicity effect in rats.

Hematopoietic system is one of the most sensitive targets for toxic compounds [35]. Hematopoietic system gives an important index for physiological and pathological status in humans and animals in general. The chief medium of transport for many nutrients and foreign bodies in the body is blood. As such the constituents of the blood such as red blood cells, white blood counts, platelets and hemoglobin are first exposed to significant concentrations of toxic compounds. Damage to the blood cells has an adverse effect on the normal functioning of the body [36]**.** The hematological parameter analysis revealed a non significant change in the extract treated rats when compared to the respective controls. Thus suggests that extract did not induce any adverse effect in the hematopoietic system.

Any significant change in the relative weight of organs by the administration of any substance is a reliable sign that can be used in toxicological investigations to assess toxicity caused by substances [37]. In this study, no significant changes were observed in the relative weights of the collected vital organs in both male and female rats treated with extract at the dose of 1000 mg/kg. Therefore, these findings may suggest that the extract has no toxic effect on vital organs in rats.

The histopathological study of collected organs (brain, heart, lung, kidney, liver, adrenal spleen, testes, and ovary) of treated male and female showed that the drug is safe as there was no abnormality in the cytoarchitecture of the tissues/organs collected.

V CONCLUSION

In conclusion, the present study suggested that the root extract of *Berberis lycium* did not induce any toxic effects upon consumption in rats when administered orally at a dose of 1000 mg/kg body weight/day for 90 days.

VI ACKNOWLEDGEMENTS

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