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TOXICOLOGICAL EVALUATION OF BERBERIS BALUCHISTANICA AHRENDT CRUDE METHANOLIC EXTRACT IN RABBIT

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ABSTRACT

Berberis baluchistanica (Berberidaceae) is an indigenous medicinal plant of the Balochistan. It is used in traditional medicine of Balochistan, Pakistan, for the cure of cough, Jaundice and removal Kidney stone. Current research work was carried out to determine toxicological effects. *B. baluchistanica* crude methanolic extract was administered at 300mg/kg oral dose for 90 days. Chronic toxicity test was carried out by determining Kidney function test, blood glucose, Cardiac Enzymes, Total Protein test, serum calcium and urea, Liver function test, Lipid profile and hematological parameters. *B. baluchistanica* crude methanolic extract results in non significant decrease in urea and creatinine level, a significant decrease in blood glucose level was observed. On cardiac enzymes, total protein test, serum calcium and urea, liver function test, lipid profile and hematological administration of *B. baluchistanica* crude methanolic extract showed non-

significant change in the level of these parameters. Findings of the current study suggest that *B. baluchistanica* have non-toxic profile on biochemical and hematological parameters of rabbit. Current studies are first study on toxicological profile of *B.baluchistanica.*

Keywords: Balochistan, *Berberis baluchistanica*, Toxicology, Kalat, Biochemical parameters

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Many indigenous plants have been in the use of man since time immemorial for curing various ailments without the actual knowledge of their toxic potential. Medicinal plants are now widely being used in many parts of the world for the remedy of many disease. One of the problems of using plants as medicines is that in many cases no definite doses are prescribed, often resulting in overdose [1].

Regarding safetythe herbs can be classified into three classes. First type are those herbs that contain poisonous constituents in a concentration which cannot be given internally by unqualified practioner, e.g. Atropa belladonna, Arnica spp, Aconitum sppand Digitalis spp. Second type are those herbs which have powerful actions, under suitable conditions these drugs are safe. Third type is known ascharacteristic grouping of plants, which have been suspected to show specific type of poisonousness. Most common example is the Comfrey plant, which contain pyrrolizidine alkaloids that produce hepatotoxicity. Some other examples are Viscum, Dryopteris and Corynanthe [2].

Berberis baluchistanica Ahrendt, Vern. is a shrub which belongs to the family Berberidaceae. This plant is endemic to Balochistan, locally it is known as Zralga in Pashto [3]and zarchin brahivi. It is found in Kalat, hananurak [4].

Root decoction *B.baluchistanica* is employed as remedy for the treatment of cough, Jaundice internal injury and infection (human being and livestock), and ophthalmic disorders, alongside it is also employed for kidney stone removal, and this action is may be attributed berberine, which is identified to remain beneficial for numeros diseases [4].

Phytochemical studies of plant extract (and fractions) exhibited presence of Flavonoids, Alkaloids, Phenols, Diterpenes and Saponins. Its antibacterial [3], antileishmanial, cytotoxic, antioxidant activities [5] and anti-diabetic activity [6] have been reported. Literature reveals that no toxicological

studies were available, as this plant is in use since prehistoric times.

MATERIAL AND METHODS

Chronic oral toxicity testing

Oral chronic toxicity tests were carried out on rabbits. *B. baluchistanica* was administered for 90 days, and observation was carried out periodically. Chronic toxicitytest provides information about safety of the drug substance to the humans. The report on oral chronic toxicity is vital for innovative drug substance. Here should be slight individual difference between the test animals, and the permissible variation inweightis ±20%. A cable group may be comprised in study protocol. This group comprised of both, control group and high-dose group. During the study period, the rabbits were observed for its standard physiological functions, changes in the biochemical parameters and behavioral differences [7] references about long duration effect of test drug in animals may be included [8].

Animals

Rabbits weighing about 1000 -1400g were used. 2 groups of the rabbits were made (each group consist of 5 animals). Control group (Group A) administered orally distilled water at 1ml/Kg dose and, *B. baluchistanica* CME treated group (Group B)administered orally at the dose of 300 mg/ kg, given the drug for 90 days.

Mortality and clinical signs

During the 90 daysdosing period, all rabbits were daily observed for any clinical signs and death, just the once before dosing, instantly after the dosing and up to four hours after dosing.

Drug administration

The drug was administered per body weight, were calculated for each rabbit on body weight basis 300 mg/kg

Dosage Period

B. baluchistanica crude extract was administered 300mg/kg orally as singly dose daily for 90 days.

Termination of dose

On day 91, administration of *B. baluchistanica* crude extract was terminated.

Blood collection

On day 91 of the experiment blood of the animal was drawn (by cardiac puncture) using sterile syringe [9] into anticoagulant containing tubes, thatallowed to stand for one hour to clot.Than it was centrifuged for ten minutes (at 3000 rpm)by using Eppendorf 5810R centrifuge machine. The serum was transferred in tubes, and kept in refrigerator at -20^{o}C till supplementary use [10,11].

Biochemical tests

Method described by Baig *et al*., (2014) was used for biochemical tests. Tests were carried out to determine the Kidney function test (urea and Creatinine), Cardiac enzymes (LDH, CKMB and SGOT) Total proteins (Total proteins, Albumin, Globulin, Protein A/G ratio), Serum calcium and uric acid, Liver function test (Total Bilirubin, Direct Bilirubin, alkaline Phospatase, amd SGOT) Electrolytes, Bicarbonate, Potassium, Sodium and Chloride, Lipid Profile (Cholesterol, and Triglycerides, by using Roche Diagnostics (Germany) kits [11].

Hematology

After completion of dosing period (90 days), blood was drawn in to an anticoagulant (EDTA) containing test tube (to stop clotting). Blood sample was investigated in automatic Hemaological analyzer (Beckman Coulter, HMX analyzer, USA). Study parameters were, Hb(g/dl), RBC Count (million/ul), Hematocrit (HCT/PCV) %, MCV (fl), MCH (pg), MCHC (g/l),Total WBC Count (×10^9/L), Platelet Count (×10^9/L)[12].

Statistical analysis

The data were articulated as mean ± SEM. Whilephysiological and biochemical parameters were statistically analyzed by using ANOVA (one way) followed by Dunnets t test, compared with control group and extract treated group, $P< 0.05$ was considered as significant, while $P < 0.01$ considered as highly significant [13].

RESULTS

Kidney function test

Level of Urea In Control group was 68.96±3.438 (mg/dL), and in *B. baluchistanica* crude extract treated value was 67.70±4.25 (mg/dL), level of Creatinine In Control group was 1.106±0.132 (mg/dL), and in *B. baluchistanica* crude extract treated value was 1.06±0.136 (mg/dL),

Blood Glucose

Level of Blood Glucose In Control group was 94.7±9.06 and in *B. baluchistanica* crude extract treated value was 88.72±5.20

Cardiac Enzymes

Level of LDH in Control group was 720.34±4.31 (U/L) and in *B. baluchistanica* crude extract treated value was 619.45+6.20 (U/L). Level of CK-MB in Control group was 23.6±2.62(U/L), and in *B. baluchistanica* crude extract treated value was 24.83±3.77 (U/L). Level of SGOT in Control group was 92.8±2.13 (U/L), and in *B. baluchistanica* crude extract treated value was 90.62±3.51 (U/L).

Total Protein test

Level of Total proteins in Control group was 7.30±0.07 (g/dL), and in *B. baluchistanica* ncrude extract treated value was 7.38+0.06

(g/dL). Level of Albumin in Control group was 3.63±0.12 (g/dL), and in *B. baluchistanica* crude extract treated value was 3.50±0.14 (g/dL). Level of Globulin in Control group was 3.31±0.07 (g/dL), and in *B. baluchistanica* crude extract treated value was 3.39±0.06 (g/dL). A/G ratio in Control group was 1.39±0.14, and in *B. baluchistanica* crude extract treated value was 1.37±0.12.

Serum Calcium and Uric acid

Level of Serum Calcium in Control group was 13.04±0.570 (mg/dL), and in *B. baluchistanica* crude extract treated group value was 12.88±0.63 (mg/dL). Level of uric acid in Control group was 0.142±0.0497 (mg/dL), and in *B. baluchistanica* crude extract treated group value was 0.12±0.03(mg/dL).

Liver function test

Level of Total Bilirubin in Control group was 0.30±0.01 (mg/dL), and in *B. baluchistanica* crude extract treated group value was 0.29±0.04 (mg/dL). Level of Direct Bilirubin in Control group was 0.11±0.03 (mg/dL), and in *B. baluchistanica* crude extract treated group value was 0.13±0.04 (mg/dL). Level of Total Alkaline Phospatase in Control group was 60.78±1.41 (U/L), and in *B. baluchistanica* crude extract treated group value was 62.24±1.88(U/L).

Level of SGOT in Control group was 92.8±2.13 (U/L), and in *B. baluchistanica* crude extract treated group value was 0.29±0.04 (U/L).

Lipid profile

Level of Cholesterol in Control group was 35.04±1.195 (mg/dL) and in *B. baluchistanica* crude extract treated group value was 35.61±1.20 (mg/dL). Level of Triglycerides in Control group was 57.84±2.749 (mg/dL), and in *B. baluchistanica* crude extract treated group value was 58.67±2.67 (mg/dL).

Hematological parameters

Level of Hb in Control group was 13.26±0.081 (g/dl), and in *B. baluchistanica* crude extract treated group value was 12.88±0.63 (mg/dL). Level of RBC in Control group was 6.28±0.06 (million/ul) and in *B. baluchistanica* crude extract treated group value was 6.07±0.24 (million/ul). Hematocrit (HCT/PCV) % in Control group was 41.58±0.19 and in *B. baluchistanica* crude extract treated group value was 41.06±0.63.MCV in Control group was 63.44±0.57 (fl) and in *B. baluchistanica* crude extract treated group value was 62.89±1.00 (fl).

MCH in Control group was 21.52±0.59 (pg) and in *B. baluchistanica* crude extract treated group value was 21.00±0.85 (pg).MCHC in

Control group was 32.47±1.28 (g/l) and in *B. baluchistanica* crude extract treated group value was 31.29±1.94 (g/l).Total WBC Count (×10^9/L) in Control group was 9.21±0.88 and in *B. baluchistanica* crude extract treated group value was 8.61±0.92. Platelet Count (×10^9/L) in Control group was 343.06±8.22 and in *B. baluchistanica* crude extract treated group value was 332.72±4.30.

Table 1: Effect of *B. baluchistanica* crude methanolic extract on Kidney function test on rabbit

S NO.	Test	Control (Mean± SEM)	Drug treated (Mean±SEM)
1	Urea (mg/dL)	68.96+3.438	67.70+4.25
2	Creatinine	1.106+0.132	1.06+0.136

All values are mean ± SEM; n=5; * = Significant (*P*<0.05), ** = highly significant (*P*<0.01)

Table 2: Effect of *B. baluchistanica* crude methanolic extract on Blood Glucose (random) of rabbit

S NO.	Test	Control (Mean± SEM)	Drug treated (Mean±SEM)
1	Blood Glucose Random	94.7+9.06	88.72+5.20

All values are mean ± SEM; n=5; * = Significant (*P*<0.05), ** = highly significant (*P*<0.01)

Table 3: Effect of *B. baluchistanica* crude methanolic extract on Cardiac Enzymes of rabbit

S NO.	Test	Control (Mean± SEM)	Drug treated (Mean±SEM)
1	LDH (U/L)	720.34+4.31	619.45+6.20
3	CK-MB(U/L)	23.6+2.62	24.83+3.77
4	SGOT(U/L)	92.8+2.13	90.62+3.51

All values are mean ± SEM; n=5; * = Significant (*P*<0.05), ** = highly significant (*P*<0.01)

Table 4: Effect of *B. baluchistanica* crude methanolic extract on Total Protein test of rabbit

S NO.	Test	Control (Mean± SEM)	Drug treated (Mean±SEM)
1	Total proteins (g/dL)	7.30+0.07	7.38+0.06
2	Albumin (g/dL)	3.63+0.12	3.50+0.14
3	Globulin (g/dL)	3.31+0.07	3.39+0.06
4	A/G ratio	1.39+0.14	1.37+0.12

All values are mean ± SEM; n=5; * = Significant (*P*<0.05), ** = highly significant (*P*<0.01)

Table 5: Effect of *B. baluchistanica* crude methanolic extract on Serum Calcium and Uric acid of rabbit.

S NO.	Test	Control (Mean± SEM)	Drug treated (Mean±SEM)
1	Calcium-Serum (mg/dL)	13.04+0.570	12.88+0.63
3	Uric acid (mg/dL)	0.142+0.0497	0.12+0.03

All values are mean ± SEM; n=5; * = Significant (*P*<0.05), ** = highly significant (*P*<0.01).

Table 6: Effect of . *B. baluchistanica* crude methanolic extract on Liver function test of rabbit.

S NO.	Test	Control (Mean± SEM)	Drug treated (Mean±SEM)
1	Total Bilirubin (mg/dL)	0.30+0.01	0.29+0.04
2	Direct Bilirubin (mg/dL)	0.11+0.03	0.13+0.04
4	Alkaline Phospatase(U/L)	60.78+1.41	62.24+1.88
6	SGOT(U/L)	92.8+2.13	90.62+3.51

All values are mean ± SEM; n=5; * = Significant (*P*<0.05), ** = highly significant (*P*<0.01)

Table 7: Effect of *B. baluchistanica* crude methanolic extract on Lipid profile of rabbit

S NO.	Test	Control (Mean± SEM)	Drug treated (Mean±SEM)
2	Cholesterol (mg/dL)	35.04+1.195	35.61+1.20

3	Triglycerides (mg/dL)	57.84+2.749	58.67+2.67

All values are mean ± SEM; n=5; * = Significant (*P*<0.05), ** = highly significant (*P*<0.01).

Table 8: Effect of *B. baluchistanica* crude methanolic extract on Blood of rabbit

S NO.	Test	Control (Mean± SEM)	Drug treated (Mean±SEM)
1	Hb (g/dl)	13.26+0.081	12.88+0.40
2	RBC Count (million/ul	6.28+0.06	6.07+0.24
3	Hematocrit (HCT/PCV) %	41.58+0.19	41.06+0.63
4	MCV (fl)	63.44+0.57	62.89+1.00
5	MCH (pg)	21.52+0.59	21.00+0.85
6	MCHC (g/l)	32.47+1.28	31.29+1.94
7	Total WBC Count (×10^9/L)	9.21+0.88	8.61+0.92
8	Platelet Count (×10^9/L)	343.06+8.22	332.72+4.30

All values are mean ± SEM; n=5; * = Significant (*P*<0.05), ** = highly significant (*P*<0.01).

DISSCUSION

For the determination of the safety of herbaldrugs for human use, toxicological studies are conceded in numerous experimental animals to calculate toxicity. The maximum whole concordances of the toxicity in the animals with that of humans are with gastrointestinal, haematological, and cardiovascular adversarial effects, with definite adverse effects in humans, particularly idiosyncratic reactions and hypersentivity, and are poorly connected with the toxicity experiential in animals. Moreover, it is relatively difficult to determine adversative effects in the animals such as abdominal pain, headache, dizziness and visual disturbances. In addition, the differences between interspecies in pharmacokinetic parameters make it problematic to translate some adversative effects from animals to humans [14]. In Kideny function test *B. baluchistanica* crude extract treated group there was no significant change in the serum creatinine (Cr) and urea level. Results shows that *B. baluchistanica* crude extract have no toxic effects on rabbit kidneys serum creatinine (Cr) and urea could indicate the effect of xenobiotics on kidney structure and function. The findings of normal activity of Cr and indicated normal renal function [15].

B. baluchistanica crude extract treated group showed significant decrease in blood glucose level. Previous studies show that hypoglycemic effect in rabbit may be due to the saponin and glycosidic components present in the plants [16]. *B. baluchistanica* is reported to contain saponins [3] so decrease in blood glucose is may be due to saponins.

Findings of the current study shows that administration of *B. baluchistanica* crude extract at 300mg/kg dose produced non-significant change in Cardiac Enzymes, Total Protein test, serum calcium and urea Liver function test, Lipd profile test.

The study showed that there was a non-significant change in the hematological parameters after administration of *B. baluchistanica*. Plants containing toxic constituents do not show a direct effect on WBCs, such as lymphocytes, neutrophils, monocytes and eosinophils. However, in divergence, extradigestion of inclusive variety of plant products has been establish to cause non-regenerative or hypoproliferative anemia. This is disease of stem cell, which is characterized by decrease production of allcomponents of blood, in the absence of a primary disease process suppressing haemopoiesis or infiltrating the bone marrow (Adeyemo-Salami et al., 2015). Results of the hematological parameters indicates that *B. baluchistanica* crude extract have non toxic profile on hematologoical parameters of the rabbit.

CONCLUSION

The findings of the present study are congruent with the above mentioned and go further to support the fact that most of the phytochemical constituents of *B. baluchistanica* are non toxic at 300mg/kg dose. Current study was carried out limited number of animals. However further studies are required on large number of animals.

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