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EVALUATION OF ANTI-DIABETIC, ANTI-EPILEPTIC AND CARDIOPROTECTIVE ACTIVITIES *BERCHEMIA PAKISTANICA*

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Abstract

Berchemia *pakistanica* is a medicinal plant of Balochistan, used for treatment of various diseases in traditional medicine. In current study, preliminary phytochemical tests, FT-IR studies, determination of the heavy metal concentrations, acute toxicity, anti-diabetic, anti-epileptic and cardioprotective activities were evaluated. In preliminary phytochemical tests, alkaloids, saponins, flavonoids, glycosides, Terpenoids and tannins were present. In FT-IR studies presence of various functional groups were confirmed. The concentration of Se, Cr, Co, Sb and Mn were within the permitted limit. Up to dose of 5000 mg/kg *B. pakistanica* did not showed any sign of toxicity. *B. pakistanica* ethanol extract showed significant ($p<0.05$) anti-diabetic effects and alloxan induced diabetes. *B. pakistanica* ethanol extract also showed significant ($p<0.05$) anti-epileptic effects in strychnine and picrotoxin induced anti-convulsant activity. In Cardioprotective activity *B. pakistanica* ethanol extract produced significant ($p<0.05$) protective effects. It is concluded that *B. pakistanica* showed significant anti-diabetic, anti-epileptic and cardioprotective activities and can be used as an alternative drug for management of Epilepsy, diabetes and as an cardioprotective agent.

Key words: Anti-diabetic, Anti-epileptic, Cardioprotective, Berchemia *pakistanica*

Introduction

Traditional medicine originates back thousands of years and is the culmination of all knowledge, abilities and irrespective of how they can be explained, are derived from culturally specific theories, experiences and beliefs. It is employed to preserve health and to stop, determine, manage, or treat diseases of the body and mind [1].

There are approximately 6000 plant species in Pakistan, which are divided into different climatic zones. Of these, 400–600 have been studied for potential medical benefits [2].

People from all over the world have studied the northern Balochistan biodiversity, which is well known for providing traditional medicines for millions of years. It is one of the main hubs for the diversity of cultures and traditional practices, herbal remedies, and an abundance of rare and endemic plants. Balochistan rural communities rely heavily on biological resources for their livelihood. Therapeutic herbs appear remain the best option for various health related problems in these rural communities [3].

Ten species in the genus Berchemia (Rhamnaceae) are found in the tropical regions of Asia, Africa, and America. *B. pakistanica, B. floribunda* and *B. edgeworthii* are the species present in Pakistan [4]. In traditional Chinese medicine, the stem and roots of these plants are utilized as diuretic, antipyretic and a remedy for lumbago and rheumatism. They are also used to treat gallstones, liver disorders, neuralgia, and stomach cramps [5]. *B. pakistanica* is grows in Waziristan, Ziarat and Quetta areas of the country [4]. Phytochemical investigation of *B. pakistanica* shows the presence of berchemin B, berchemin A, dimeric lignan glycosides and, *β*-sitosterol 3-*O*-*β*-D-glucoside, daphnetin 8-*β*-D-glucoside, , *β*-sitosterol and vavain. Berchemins B and A were reveals inhibit *α*-glucosidase and lipoxygenase inhibition and anti-oxidant activity is also reported [4].

Material and Methods

Preparation of Plant Extracts

B. pakistanica, entire plant was collected from ziarat district of Balochistan, in the month of June 2019. Plant was authenticated and specimen number AJ-06-23 was placed in Department of Pharmacognosy, University of Balochistan, Quetta. Collected plant was washed and shade dried, powdered and soaked in ethanol for 15 days, ethanol was removed by using rotary evaporator (Buchi R-200) under reduced pressure. The dark green semi solid residue was obtained.

Animals used in experiments

Wistar Albino rats of both sex having weight about 200-220 g, albino mice weighing about 25-28g and rabbits around 1200-1500 g weight, were utilized in the study. The animals were kept at the animal laboratory at Faculty of Pharmacy University of Baluchistan Quetta. The temperature was 22 ± 2 °C, and the light/dark cycle was 12/12 hours. The study protocol was permitted by the Ethical Committee (NO. FOPHS/Res/PCOG-05-21) of Pharmacy Faculty, University of Balochistan Quetta. All experiments of the study were performed according to the established public health protocols in the Laboratory Animal Use and Care Guide [6].

Phytochemical Screening

The presence of various phytoconstituents in extract of *B. pakistanica* was evaluated by implementing standard procedures. Tests were accomplished for presence of flavonoids alkaloids, saponins, steroids, terpenoids, tannins and glycosides [7].

Fourier-Transform Infrared Spectroscopy (FTIR)

Thin pellet of KBr salt and ethanolic extract *B. pakistanica* were mixed. FTIR scan range was between 4000 and 500 cm^{-1} **[8].**

Heavy metal detection

Freshly prepared samples were air-dried at room temperature. For digestion, 500 mg of the plant extract was weighed, transferred to digestion vessel, HCl (37%) 0.5 ml, HNO3 (69%) 9.0 ml and H_2O_2

(30%) 1 ml were added, and the combination of ingredients was prepared in a microwave with microwave assistance., samples were filtered. The metal concentration in the plant sample was measured by utilizing atomic absorption spectrophotometer. Heavy metal concentrations were represented as the dry weight ± SD of three subsamples and their mean value (mg/kg−1). [9].

Acute Oral toxicity study

A study on acute oral toxicity was conducted by using a plant extract of *B. pakistanica* in accordance with OECD guideline 425. Male Wistar albino rats were used in this experiment. Seven random groups of five rats each were created from the rats. A starting dose of 125 mg/kg was given to each animal, and they were all observed for 48 hours. The animal was monitored for signs of toxicity, such as bodyweight variation, continuously for the first half hour after administration, then once a day for the next four hours. Before the subsequent doses of 100, 250, 500, 750, 1000, 1500, 2000, and 5000 mg/kg were given, symptoms included changes in the eyes, skin, fur, eyes, mucous membranes, diarrhea, salivation, shaking, convulsions, and death. The animals were also observed every day for a further twelve days in order to look for any delayed indicators of toxicity and death [10].

Assessment of Anti-Diabetic Activity

Diabetes Induction

Alloxan monohydrate was diluted with normal saline (5% w/v) and injected intravenously (via the marginal ear vein) once at a body weight of 120 mg/kg. After five days, blood samples were taken, and glucose levels were observed. The experiment was conducted on rabbits that were diagnosed as diabetics based on levels of blood glucose exceeding 250 mg/dL.

Effects of Plant Extracts Acutely on Rabbits with Alloxan-Induced Diabetes

Five groups of 30 rabbits had been established. There were six rabbits in each group. Group I consisted of healthy rabbits and served as the control group. The only tween 80 was given to them orally in 10% (v/v) 10 milliliters per kilogram of distilled water. There were diabetic rabbits in the other groups (groups II through V). Group II was diabetic control. Plant extract was given to animals in groups III and IV, suspended (10 mL/kg) within a vehicle. Group V was given a reference anti-diabetic drug (Glibenclamide) suspended in a vehicle (10 mL/kg) at a dosage of 5 mg/kg, P.O. To measure the glucose levels in fasting animals, blood was drawn from one of the exposed marginal ear veins prior to, during, and six hours following dosage [11].

Analytical Method

Determination of blood glucose levels

Using a commercially available glucose kit, the blood glucose level was measured in milligrams/dL [11].

In Vivo Anti-Convulsive Activity

Strychnine induced convulsions

Strychnine induced convulsion method was used to measure the anticonvulsant potential of plant extract. There were four groups (n = 6) established from the mice. Group I, the standard control that was given vehicle; The animals in groups II and III were given doses of 250 and 500 mg/kg body weight, respectively, of the plant extract. Group IV, the standard group, was given a s dose of 1 mg/kg of diazepam. We measured the onset and duration convulsions in the mice exhibiting convulsive activity. The onset and duration of the convulsions were expressed in minutes [12].

Picrotoxin-Induced Convulsions.

For picrotoxin-induced seizures 04 groups (n=6) of rats were selected. In each group, 5 mg kg^{-1} of picrotoxin was injected 30 minutes after the corresponding treatment. All groups' tonic-clonic convulsion duration, as well as the start of mild jerks, were recorded and compared to those in the vehicle-treated control group [13].

Acute Myocardial Infarction Study

In this test five groups (n=06) of rabbits were used. Group 1 rabbits were given normal saline for two days in a row, while Group 2 rabbits were given adrenalin (ADR) 2 mg/kg b.w. Sc every 24 hours. For a period of 14 days, the rabbits were administered doses of 250 and 500 mg/kg of plant extract were daily to groups 3 and 4. The rabbits were given ADR at a dose of 2 mg/kg on day 14 and 15. The sixteenth day the collection of blood samples from the rabbits' ear veins on the margin were drawn to measure the levels of biochemical markers including AST, ALT, LDH, and CK in the blood[11].

Analytical Statistics

Each and every result is given as the standard error of the mean (SEM), or mean ±. One-way analysis of variance (ANOVA) was used for statistical analysis, and post hoc analysis was done using SPSS (version 16.0.) to identify significant variations [10].

RESULTS

Phytochemical tests

Alkaloids, saponins, flavonoids, glycosides, terpenoids, and tannins were all identified in phytochemical tests (Table 1).

FTIR Studies

The peak position at 3320.19 cm^{-1} showed the presence of an -OH (hydroxyl) functional group (alcohols, carboxylic acids, phenols). The peak position at 2924.85 cm^{-1} corresponds the stretching vibration of the C-H (carbon-hydrogen) bond. This peak is typically associated with alkanes. The peak position at 1700.89 cm^{-1} indicates the presence of a carbonyl functional group. This peak corresponds to the stretching vibration of the C=O (carbon-oxygen) bond in compounds such as aldehydes, ketones, carboxylic acids, esters, amides, and other carbonyl-containing compounds. The peak position at 1634.70 cm^{-1} suggests the presence of a conjugated carbonyl functional group (C=C bond in compounds such as conjugated alkenes, conjugated ketones, or aromatic compounds with a carbonyl group). The peak position at 1516.30 cm^{-1} suggests the presence of an aromatic ring or a conjugated system. This peak corresponds to the aromatic C=C stretching vibration in compounds such as benzene or other aromatic compounds. The peak position at 1437.45 cm^{-1} suggests the presence of a methyl (-CH3) group. This peak corresponds to the symmetric deformation vibration of the methyl group, it indicates the presence of methyl groups in a molecule, which can be found in various compounds such as alkanes, alkyl halides, ethers, and many other organic molecules. The peak position at 1375.07 cm^{-1} suggests the presence of a nitro (-NO2) functional group (nitroalkanes and nitroaromatics). The peak position at 1253.27 cm^{-1} suggests the presence of an amide (C=O-N) functional group. This peak corresponds to the amide C-N stretching vibration in compounds such as primary, secondary, and tertiary amides (peptides). The peak position at 1158.62 cm^{-1} suggests the presence of a C-N (carbon-nitrogen) stretching vibration, indicating the presence of an amine (R-NH2) functional group. This peak is commonly observed in infrared (IR) spectroscopy for compounds containing primary, secondary, or tertiary amines. The peak position at 1035.47 cm^{-1} suggests the presence of a C-O (carbon-oxygen) stretching vibration, indicating the presence of an ether (R-O-R') functional group (table 02). This peak is commonly observed in infrared (IR) spectroscopy for compounds containing ether linkages.

Heavy metal detection

In the current study the concentration (ppm) for Se was 56.4040, Cr was 0.3960, Co was 1.4806, Ni was 45.445, Sb was 27.082 and Mn was 1.5152 (table 03).

Study on Acute Oral Toxicity

The investigation of the acute oral toxicity of whole plant extracts of *B. pakistanica* was conducted at the dose of 100, 250, 500, 750,1000, 1500, 2000mg/kg and 5000mg/kg (table 04). Results shows that Up to a dosage of 5000 mg/kg of *B. pakistanica*, there was no mortality in the acute toxicity test

(table 2).

Anti-diabetic activity

The blood glucose levels of each animal were observed on the first, third, fifth, and eighth day after the alloxan injection. The results clearly indicate that the diabetic control rabbits' blood glucose levels were consistently higher than the untreated control rabbits during the experiment.

For eight days in a row, the alloxan-induced rabbits were given crude ethanolic extract of B. pakistanica in order to test its subacute antidiabetic effects. Compared with the diabetic control group group, the ethanol extract of *B. pakistanica* markedly lowered the blood glucose level on day one. When compared to the blood glucose levels of the diabetic control rabbits, all of the tested *B. pakistanica* plant extracts showed a significant decrease on the third, fifth, and eighth days; however, the diabetic rabbits treated with the methanolic plant extract during this period showed a more significant ($p<0.05$) effect (table 05). The glibeenclamide led to a highly significant decrease in blood glucose levels in a comparable manner.

Anti-epileptic activity

Strychnine induced anti-epileptic activity

For control group mean onset of convulsion was 2.26+0.02 and duration of convulsion was 3.15$\pm$0.01. For *B. pakistanica* 250mg onset of convulsion was 3.048$\pm$0.20 and duration of convulsion was 1.378$\pm$0.07. For *B. pakistanica* 500mg/kg treated group was onset of convulsion 3.944$\pm$0.23 and duration of convulsion was 2.174$\pm$0.01. For standard drug treated group onset of convulsion was 4.54$\pm$0.05 and duration of convulsion was 0.58$\pm$0.05 (table 06).

Picrotoxin induced anti-epileptic activity

For control group mean onset of convulsion was 2.97$\pm$0.16 and duration of convulsion was 4.33$\pm$0.10. For *B. pakistanica* 250mg/kg onset of convulsion was 3.38$\pm$0.19 and duration of convulsion was 2.99$\pm$0.07. For *B. pakistanica* 500mg/kg treated group was onset of convulsion 3.49$\pm$0.08 and duration of convulsion was 2.53$\pm$0.14. For standard drug treated group onset of convulsion was 5.27$\pm$0.05 and duration of convulsion was 1.25$\pm$0.04 (table 07).

Evaluation Cardioprotective Activity

When compared to control, ADR significantly raises cardio-hepatic biomarkers (ALT and AST) and cardiac markers (CK LDH) ($p \leq 0.05$). The rabbits (Group 2) that were given ADR-induced MI showed noticeably elevated cardiac markers. However, *B. pakistanica* treated groups at 250 and 500 mg/kg b.w. showed dose-dependent resistance to ADR-induced cardiac injury and showed significantly ($p< 0.05$) decreased average AST, ALT, CK, and LDH levels (Table 08).

Discussion

Currently herbal remedies are of great importance in the field of medicine despite tremendous advancements in the realm of chemical synthesis [14]. Numerous biological attributes, such as anticancer, antioxidant, antibacterial, and anti-inflammatory effects, have been demonstrated by natural products [15]. Thus, the goal of this work was to evaluate the acute toxicity, anti-diabetic, anti-epileptic and cardioprotective activities of *B. pakistanica* ethanolic extract. To the best of our knowledge, this research represents the first report on *B. pakistanica* anti-diabetic, anti-epileptic, and cardioprotective properties.

The *B. pakistanica* extract was tested for acute oral toxicity at doses of 100, 250, 500, 750, 1000, 1500, 2000, and 5000 mg/kg respectively. The findings indicate that throughout the acute toxicity test, the GIT, mucous membranes, eyes, skin and hair, somatic motor systems, autonomic nerve systems, did not significantly changed. According to the findings of the acute toxicity test, indicating that the LD50 of the *B. pakistanica* extract in rats is significantly higher than 5000 mg/kg [16].

Previous research has highlighted various health issues associated with uncontrolled diabetes. It was suggested that a novel therapeutic approach would prevent and completely remove the harmful effects of diabetes [17]. An unstable chemical substance called alloxan is frequently utilized in research on experimental diabetes. In current study alloxan induced diabetic rabbit model was used. Alloxan has a well-known history of destroying pancreatic beta cells, resulting in hyperglycemia in mice, and specifically causing necrosis in pancreatic β-cells of the islets of Langerhans [18,19, 20]. Numerous animal models have been used to study the cellular regeneration associated with diabetes. The balance between these cells' renewal and loss is reflected in the total mass of cells. Additionally, it was proposed that the main reason rabbits treated with alloxan recovered from the drug's effects could be the regeneration of islet ß cells after they were destroyed by alloxan. The current study findings, *B. pakistanica* extract significantly ($P<0.05$) lowered the glucose levels in alloxan-induced model of diabetic rabbits, making it an antihyperglycemic or antidiabetic effect. Thus, this finding supports the *B. pakistanica* extract antihyperglycemic effects. The antihyperglycemic action has been accompanying to secondary metabolites found in plants, such as saponins, alkaloids, terpenoids and flavonoids [21].

The development of new drugs to treat epilepsy-related cognitive impairment has slowed down significantly during the past few decades. On the other hand, in addition to reducing seizures, roughly one-third of epileptic patients experience cognitive alterations as a result of the currently available AEDs. Epilepsy is a complex neurological condition with a variety of recognized and unidentified causes as well as poorly understood processes. Memory and learning impairment are common in epileptic patients. Furthermore, standard anti-epileptic drugs impair memory in epileptic patients. In this sense, the research presented here provides insights towards the creation of a novel medication derived from natural sources to treat seizures and associated cognitive impairment [22].

Strychnine's convulsive effect results from interfering utilizing postsynaptic suppression, in which glycine acts as a mediator, a crucial spinal cord, interneurons and motor neurons inhibitory transmitter. Glycine receptors are selectively competitively antagonistic by strychnine. The animals' mean time to convulsion commencement was decreased in *B. pakistanica* extract treated group, compared to the untreated group, despite the fact that the plant extract had no discernible effect on the length or start of convulsions brought on by strychnine at the tested doses. Additionally, a small amount of death was prevented, suggesting a modest anticonvulsant effect against convulsions brought on by strychnine. [23]

PTZ is frequently used to cause seizures in rodents and other animals. Convulsant drug PTZ is utilized in anticonvulsant drug screening. By obstructing the GABA (gamma aminobutyric acid) pathway at GABAA receptors, it causes seizures, one of the main neurotransmitters implicated in the inhibitory pathway of epilepsy [24], the PTZ induced epileptic animal model was used to produce seizures, the B. pakistanica extract showed dose-dependent protection, as a result, the *B. pakistanica* extract may be useful in treating myoclonic seizures. The phytochemical analysis confirmed the presence of terpenoids, saponins, tannins, flavonoids, and alkaloids, previous studies shows that steroids, terpenoids, and flavonoids have been shown to have anticonvulsant properties [25], therefore it is hypothesized that anticonvulsant activity of *B. pakistanica* may be attributed due to presence of flavonoids, terpenoids and steroids.

Numerous studies have focused on the prevention or treatment of cardiovascular disease using natural products and herbal treatments [26]. The capacity of medicinal plants to lessen damage to endothelial cells, vascular smooth muscle cells, cardiomyocytes, and monocytes has been shown to have cardioprotective qualities [27]). *B. pakistanica* ethanolic extract produced significant cardioprotective effects and the cardiac markers AST, ALT, CK, and LDH levels were significantly ($p< 0.05$) decreased.The presence of alkaloids, saponin, flavonoids, glycosides and terpenoids, was confirmed by phytochemical tests of the said plant. The presence of cardioactive phytochemicals in

the plant extract, including as flavonoids, tannins, may be responsible for the cardioprotective effects [28]. Flavonoid consumption has demonstrated an inverse relationship with measures associated to cardiovascular disease in a number of epidemiological and experimental studies. The majority of herbal plants include flavonoids that contribute to cardio protection by inducing anti-inflammatory effects [29, 30, 26] therefore it is hypothesized that *B. pakistanica* ethanolic extract cardioprotective effect is attributed due to presence of flavonoids and tannins.

Table 1. *B. pakistanica* crude ethanolic extract phytochemical tests

S No.	Phytoconstituents	Result
1	Alkaloids	Positive
2	Saponins	Positive
3	Flavonoids	Positive
4	Steroids	Negative
5	Terpenoids	Positive
6	Glycosides	Positive
7	Tannins	Positive

Table 2: FT-IR Peak Positions of *B. pakistanica* crude ethanolic extract

S No	Peak positions	Functional Group
1	3320.19	-OH (hydroxyl)
2	2924.85	C-H (carbon-hydrogen)
3	1700.89	C-H (carbon-hydrogen)
4	1634.70	C=C (conjugated carbonyl functional group)
5	1516.30	C=C (aromatic ring or a conjugated system)
6	1437.45	-CH3 (methyl group)
7	1375.07	-NO2 (nitro functional group)
8	1253.27	C=O-N amide functional group.
9	1158.62	C-N (carbon-nitrogen, indicating the presence of an amine (R-NH2) functional group)
10	1035.47	C-O (carbon-oxygen, indicating the presence of an ether (R-O-R') functional group.

Table 3: Heavy metal determination of *B. pakistanica* crude ethanolic extract

Sno:	Metals	Conc (ppm)	Permitted limit
1	Se	56.4040C	25
2	Cr	0.3960	2
3	Co	1.4806	5
4	Ni (Ni)	45.4457	1.5
5	Sb	27.082	29
6	Mn	1.5152	2

Table 4: Aute toxicity to crude ethanolic extract of *B. pakistanica*

Sno	Treatment	No of deaths
1	Control	Nil
2	*B. pakistanica* 100mg/kg	Nil
3	*B. pakistanica* 250mg/kg	Nil
4	*B. pakistanica* 500mg/kg	Nil
5	*B. pakistanica* 750mg/kg	Nil
6	*B. pakistanica* 1000mg/kg	Nil
7	*B. pakistanica* 1500mg/kg	Nil
8	*B. pakistanica* 2000mg/kg	Nil
9	*B. pakistanica* 5000mg/kg	Nil

Table 05: Anti-diabetic activity of *B. pakistanica* crude ethanolic extract

S NO	Treatments	Day 1	Day 03	Day 5	Day8
1	Control	100.27+ 0.72	103.31+0.84	98.69+0.50	102.22+0.49
2	Diabetic control	297.97+0.33	306.29+0.46	314.06+0.54	311.32+0.95
3	*B. pakistanica* 250mg/kg	293.47+ 0.71*	283.32+0.85*	267.86+0.47*	246.08+0.30*
4	*B. pakistanica* 500 mg/kg	292.48± 0.81*	272.81±0.31*	254.57±0.65*	222.28±0.75*
5	Standard drug Glibenclamide 5mg/kg	278.20+ 0.46**	266.58+0.57**	246.91+0.39**	211.59+0.54**

Every value is presented as the mean ± SEM. (*=p<0.05, ** =p<0.001)

Table 06: Antiepileptic activity of *B. pakistanica* crude ethanolic extract

S No.	Treatments	Onset of Convulsion (Mean+SEM)	Duration of Convulsion (Mean+SEM)
1	Control	2.26+0.02	3.15+0.01
2	*B.pakistanica* 250mg/kg	3.04+0.20*	1.37+0.07*
3	*B. pakistanica* 500mg/kg	3.94+0.23*	2.17+0.01*
4	Standard drug (Diazepam 1mg/kg)	4.54+0.05**	0.58+0.05**

Every value is presented as the mean ± SEM. (*=p<0.05, ** =p<0.001)

Table 07: Picrotoxin induced antiepileptic activity of *B. pakistanica* crude ethanolic extract

S No.	Treatments	Onset of Convulsion (Mean+SEM)	Duration of Convulsion
1	Control	2.97+0.16	4.33+0.10
2	*B. pakistanica* 250mg	3.38+0.19*	2.99+0.07*
3	*B. pakistanica* 500mg	3.49+0.08*	2.53+0.14*
4	Standard drug (Diazepam 1mg/kg)	5.27±0.05**	1.25±0.04**

Every value is presented as the mean ± SEM. (*=p<0.05, ** =p<0.001)

Table 08: Effect of *B. pakistanica* ethanolic extract on cardiac parameters

Group	Treatment and Dose	AST	ALT	LDH	CK
Group I	Control 5ml/kg	49.34+0.11	59.09+0.34	1009.41+2.23	309.31+ 0.56
Group II	Adrenalin 2mg/kg (Sc)	98.23+0.78**	121.39+0.12**	1876.07+0.43**	650.21+0.73**
Group III	*B. pakistanica* 250mg/kg	69.19+0.43*	58.61+0.39*	1145.89+2.32*	350.25+0.19*
Group IV	*B. pakistanica* 500mg/kg	47.12+0.43*	41.67+0.23*	1094.82+1.77*	335.43+0.65*

Every value is presented as the mean ± SEM. (*=p<0.05, ** =p<0.001)

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