

Original Research Article

Pharmacological evaluation of the hypoglycemic and anti-Alzheimer's activities of aerial parts of *Breynia distachia* (Phyllanthaceae)

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Abstract

Purpose: To determine the cytotoxic, bronchorelaxant, spasmolytic, antidiabetic, α -glucosidase, acetylcholinesterase, butyrylcholinesterase, α -chymotrypsin and lipoxigenase inhibitory attributes of methanol and dichloromethane extracts of the aerial parts of *Breynia distachia*.

Methods: The dichloromethane and methanol extracts of the aerial parts of the plant were prepared by maceration. Various *ex vivo* assays were employed, such as the brine shrimp lethality assay, lipoxigenase inhibitory activity assay, α -glucosidase inhibitory assay and α -chymotrypsin assay, as well as assays to assess the spasmolytic and bronchorelaxant activity. Meanwhile, the hypoglycaemic effect were analysed using an alloxan-induced diabetic model in Wistar albino rats.

Results: The methanol extract (aerial) showed significant ($p \leq 0.05$) cytotoxicity towards brine shrimp larvae at concentrations of 10, 100 and 1,000 $\mu\text{g/mL}$, respectively, whereas the dichloromethane extract (aerial) of the plant showed non-significant ($p \geq 0.05$) results. The methanol extract (aerial parts) also demonstrated significant ($p \leq 0.05$) α -glucosidase inhibitory activity and lipoxigenase inhibitory activity, with IC_{50} (half-maximal inhibitory concentration) values of $40.37 \pm 5.29 \mu\text{g/mL}$ and $132.9 \pm 0.33 \mu\text{g/mL}$, respectively, while the dichloromethane extract exhibited significant ($p \leq 0.05$) α -glucosidase inhibitory activity, with an IC_{50} value of $135.43 \pm 8.29 \mu\text{g/mL}$. An *in vivo* antidiabetic model showed that the administration of 150 and 300 mg/kg methanol extract of the aerial parts significantly ($p \leq 0.05$) lowered the blood glucose level in alloxan-induced diabetic rats compared to control (treated with water).

Conclusion: Data from different *in vitro* and *in vivo* models suggest that the methanol extract (aerial parts) of *B. distachia* shows significant cytotoxic, bronchorelaxant, spasmolytic, antidiabetic and anti-Alzheimer's activity. Hence, these findings validate the folkloric use of *B. distachia* and highlight the need to further explore its medicinal potential and the phytoconstituents responsible for its pharmacological actions.

Keywords: α -Glucosidase; Acetylcholinesterase, Lipoxigenase, Cytotoxic, Antidiabetic, Alzheimer's disease

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INTRODUCTION

Breynia distachia is a plant of the genus *Breynia*, which belongs to the family Phyllanthaceae and consists of about 35 species, distributed from India to Australia. Different constituents isolated from the genus include friedelan-3, beta-ol, friedelin, aviculin (+)-isolariciresinol-9-rhamnopyranoside, arborinone, isoarborinol, flavone, 5-hydroxy-7,8,4-trimethoxy and 2,4-dihydroxy-6-methoxy-3-methyl acetophenone [1]. *B. distachia* is a shrub with purple, green and white leaves and has been reported to have antioxidant activity. A spiroketal glycoside, breynin I, containing sulphur atoms, and breyniaionoside E, a terpenic glycoside, have been isolated from the aerial parts of *Breynia* [2].

The present work focuses on the evaluation of multiple pharmacological activities, such as the hypoglycaemic and anti-Alzheimer's activity, of *B. distachia* (commonly named "snowbush"), using a series of *in vitro* and *in vivo* assays. The cytotoxic activity of plant extracts can be estimated by the brine shrimp lethality assay, which is a simple, rapid, inexpensive and reliable *in vivo* method to assess the bioactive potential of plant extracts, providing a preliminary overview of the cytotoxic, pesticidal and weedicide activities of plant extracts [3]. It is also a pre-screen study for antitumor activity, fungal and cyanobacteria toxins, pediculicidal effects and cytotoxicity of nanoparticles. A rapid and simple brine shrimp bioassay can be readily adopted by natural product researchers in order to detect and purify the bioactive components of plants that have diverse pharmacological actions.

Diabetes mellitus is a metabolic disorder marked with an elevated sugar level in the blood, as well as impaired insulin secretion or action. Different studies have shown that diabetes is the 5th major cause of the increasing morbidity rate, and around 2.8% of people are suffering from this disease [4]. According to folk history, *B. distachia* possesses antidiabetic potential; thus, it was assessed for antidiabetic activity.

Two more important hydrolysable enzymes, α -glucosidase and α -amylase have a pivotal role in the pathology of diabetes. These enzymes participate in the digestion or absorption of carbohydrates or sugars. Complex carbohydrates enter into the blood circulation by being first converted into disaccharides and then monosaccharides. This conversion is catalysed by different enzymes, such as pancreatic α -amylase and α -glucosidase, which are anchored to the border or surface of the intestinal cells [5]. The inhibition of α -glucosidase activity in

noninsulin-dependent diabetes mellitus (NIDDM) patients by the use of α -glucosidase inhibitors, e.g., acarbose, miglitol and voglibose, hinders these enzymes and proves very effective in controlling hyperglycaemia [6].

Alzheimer's disease is the most prevalent neurological disease; it is a progressive and degenerative disorder that affects brain neurons and manifests as memory loss, loss of thinking, language loss and abnormal behaviour. Neurons that slow down this disease use the chemical or neurotransmitter acetylcholine to contact the remaining neurons. Changes in enzymes such as acetylcholinesterase and butyrylcholinesterase can be detected in Alzheimer's disease, but acetylcholine deficiency is the most common and most significant sign [7]. Acetylcholinesterase enzyme is also involved in the apoptosis of β -cells in insulin dependent diabetic (IDDM) patients, so the acetylcholinesterase inhibitory activity was assessed in order to establish the effect of the plant on apoptosis as well as Alzheimer's disease [8].

Due to the α -glucosidase inhibitory activity and acetylcholinesterase inhibition of *B. distachia* extract, we selected this plant for antidiabetic and anti-Alzheimer's assessment.

EXPERIMENTAL

Plant collection

Plants growing in grain and rice fields from the surroundings of district Khanewal and district Layyah, Punjab (Pakistan) were collected in May 2017 and authenticated as *B. distachia* (*Phyllanthaceae*) by a plant taxonomist, Prof Dasti, Chairman of the Institute of Biology, Bahauddin Zakariya University, Multan, and a voucher specimen (no. Stewart 223) was deposited in the herbarium of the Bahauddin Zakariya University, Multan.

Preparation of extracts

The aerial parts of the plants were separated and shade-dried for 15 days at room-temperature. The dried plant was ground to a fine powder. For the extraction, 500 g powder was macerated at room temperature. The powder was extracted with dichloromethane by occasionally shaking, and the solution was filtered after 24 hours. The procedure was repeated three times with dichloromethane (Sigma Aldrich, USA). Residue was extracted with methanol by the same procedure. The extracts were concentrated using a rotary evaporator (Buchi-rotavapor R-200)

under reduced pressure at 35°C, and the concentrated material was collected in separate bottles, coded as BDAD (*B. distachia* aerial parts dichloromethane extract) or BDAM (*B. distachia* aerial parts methanol extract).

Brine shrimp lethality assay

The artificial sea water medium used to hatch the brine shrimp eggs was composed of sea salt dissolved in distilled water at a concentration of 4 g sea salt/1000 mL distilled water and was contained in rectangular tanks. Plastic sheets with holes were fixed into the tanks to divide the tanks into two compartments. The shrimp eggs were spread in the darker compartment, and the nauplii (shrimp larvae) migrated to the illuminated compartment after two days of hatching at 22–30°C. Then, 20 mg of each extract (methanol and dichloromethane) of the aerial plant parts was dissolved in 2 mL of methanol and the test vials initially tested at 1000, 100 and 10 µg/mL [9]. Each concentration was prepared in triplicate. Insoluble polar material was dissolved in dimethyl sulfoxide. About 50 µL/5 mL of artificial sea water was added after two days. Etoposide (7.4625 µg/mL) was used as standard. Finally, ten shrimps were added to each vial containing the 5 mL artificial sea water and all were illuminated. The survivors were counted after 24 hours and recorded. The Finney probit analysis method (Finney computer program) was used for data analysis [10].

Lipoxygenase inhibitory activity assay

Reactive oxygen species and free radicals cause tissue damage that contributes to stroke, artery disease, heart attack, neurodegenerative diseases, diabetes, inflammation and cancer. Antioxidants plays significant role for age-relevant diseases, being substances that can prevent or prolong the process of oxidation through an oxidizable-substrate. The Baylac and Racine method was used for the measurement of lipoxygenase activity [11]. Initially, a stock solution was prepared of plant extract (50 mg/mL). Then, phosphate buffer 970 µL having pH 9 and linoleic acid 17 µL was mixed with 5 µL of extract. The mixture was shaken and 4 µL of aliquot enzyme and phosphate buffer (4°C) were pipetted out to initiate the enzyme reaction. The measurement for absorbance was carried at 234 nm wavelength for 10 minutes using a spectrophotometer.

The inhibition was as in Eq 1.

$$H (\%) = \{Ac - At\}/Ac \times 100 \dots\dots\dots (1)$$

Ac = absorbance of control; At is absorbance of plant extracts, Control means the enzyme activity in total without inhibitor activity.

α-Glucosidase inhibitory assay

Acarbose and baker's yeast (α-glucosidase, p-nitrophenyl-α-D-glucopyranoside) were obtained from Sigma-Aldrich, USA. The yeast α-glucosidase (0.07 units) was premixed with phosphate buffer (100 mM) having pH 6.8, and p-nitrophenyl-α-D-glucopyranoside (3 mM) was used as substrate. Plant extracts (BDAD and BDAM) were tested at different concentrations, varying from 20-100 µg/mL. Various concentrations of 300 µL plant extracts were mixed with 100 mM phosphate-buffer (pH 6.8) and the mixture was incubated at 37°C for 30 min. Then, 3 mL of 50 mM NaOH was added to the mixture and the α-glucosidase activity evaluated in terms of the p-nitrophenyl release from p-nitrophenyl-α-D-glucopyranoside by measuring the absorbance at 410 nm. Acarbose (8 mg/mL) was employed as the reference inhibitor of α-glucosidase [12]. The control was prepared without plant extract. The inhibition was measured using the equation 1, described in the lipoxygenase activity assay.

Acetylcholinesterase and butyrylcholinesterase inhibitory assays

The acetylcholinesterase inhibitory activity was assessed using a well-known procedure, with minor alterations [13]. A total mixture volume of 100 µL was prepared by initially mixing 60 µL disodium phosphate buffer (50 mM, pH 7.7) and 10 µL test compound, 10 µL of the enzymes were then added. As the reaction started, about 10 µL of 0.5 mM substrate (butyrylthiocholine chloride) was added into each well, followed by 10 µL of 0.5 mM DTNB (5,5'-dithiobis (2-nitrobenzoic acid). When all the ingredients had been added, the incubation of mixture was done at 37°C for 10 min. The measurement of absorbance was done at 405 nm using a 96-well-plate reader. The procedure was carried out in triplicate, and as control 0.5 mM/well Eserine was used.

α-Chymotrypsin assay

This assay was performed using a well-known procedure, with minor alterations [14]. A 100 µL mixture was prepared by initially mixing 60 µL Tris-HCl buffer (50 mM, pH 7.6 (giving a final concentration of 0.5 mM/well)) and 15 µL of test compound Then, 15 µL (0.005 unit/well) enzyme was added and the ingredients pre-incubated at 37°C for 10 min. As the reaction started, 10 µL

N-succinyl phenylalanine-*p*-nitroanilide (substrate) was added, followed by 10 μ L (0.5 mM/well) DTNB. After incubation at 37°C for 30–60 minutes, the absorbance was measured at 410 nm using a 96-well plate reader. The experiment was conducted in triplicate, and a positive control in the form of Eserine 0.5 mM/well was applied.

Evaluation of spasmolytic activity

Rabbit jejunum was isolated and prepared for the evaluation of possible spasmolytic activity of the methanol extract of *B. distachia*. The animals were humanely sacrificed and dissected to excise the jejunum, which was cautiously freed of adherent mesenteries with sharp scissors. The jejunum was then cut into segments of about 2 cm in length and placed in 10 mL isolated Tyrode's solution in a tissue organ bath, which was bubbled with carbogen (95% O₂ + 5% CO₂) at 37°C. The isolated rabbit jejunum was equilibrated for 30 mins while being coupled to an isotonic transducer, and a tension of 0.50 mg was applied. The Power Lab Data Acquisition System was used to record the isotonic contractile and relaxation activities of the mounted isolated rabbit jejunum preparations [15].

The standard drug verapamil (1 mg/kg) was used to create a relaxant effect upon the spontaneous contractile activity of the isolated rabbit jejunum preparation. Different concentrations of the methanol extract (0.01–3 mg/mL), along with the standard drug, were exerted to the preparation of rabbit jejunum in various tissue bath concentration levels. The responses were measured to determine the EC₅₀ value and responses vs concentrations by software (Graph pad prism).

Determination of bronchorelaxant activity

Isolated rabbit tracheal preparations were used for the determination of potential bronchorelaxant activity. The trachea was removed by dissecting the rabbit body, and was then cut into rings of about 2–4 mm wide, each having two cartilages. A longitudinal incision on the ventral side, opposing the smooth muscle layer, was used to open the rings. The preparations were placed at 37°C in solution of Krebs and carbogen is used for aeration. The pressure about 1 g was exerted to each tracheal strip, and they were equilibrated about an hour before recording their isometric contractions. Bronchorelaxation of crude extract of methanol of the aerial parts of *B. distachia* (0.01–3 mg/mL) was tested on isolated rabbit tracheal preparations with carbachol (1 μ M), as well as K⁺ (80 mM), when applied in a cumulative

manner. To reduce animal to animal variance in the findings, the experiment was repeated three times on each preparation from five separate animals [12].

Ethical approval

The *in vivo* experiment was done according to Institution Review Board (IRB) guidelines and Bahauddin Zakariya University Multan Ethical Committee, after approval with authorization no. BZU/ERC/2524, Study No. 19486 and IRB no. 866. The studies also followed international guidelines for animal studies.

Screening for hypoglycaemic activity

The hypoglycaemic effect was examined using an *in vivo* alloxan-induced diabetes rat model. The rats, Sprague-Dawley (σ ♀; 200–250 g) were housed in cages of 47 x 34 x 18 cm³ dimension, with 6 rats per cage, at room temperature (25 \pm 2°C) with exposure to 12:12 h light and dark cycles. Commercially available feed and tap water was provided *ad libitum*.

Induction of hyperglycaemia

Alloxan monohydrate was injected to the Group III, Group IV and Group V animals, as described by [16]. Before administration, the alloxan was dissolved with fresh chilled citrate buffer (pH 4.5). Dextrose solution 20% was administered orally, 4 h after alloxan administration, to avoid hypoglycaemia, and subsequently massive release of insulin from necrotic pancreas. Glucose 5% was also provided to the rats for the next 24 h, in their drinking water.

The animals were selected for experimental work after screening for a fasting blood glucose level \geq 200 mg/dL. The occurrence of hyperglycaemia was examined at 72 h using a glucometer (Roche, Germany) that could measure glucose levels of 10–600 mg/dL. A drop of blood was obtained by pricking the animal's tail and placed on a test-strip. The glucose level was read from the display on the glucometer. An oxio-reductase reaction occurs when the sensitive area of the test strip comes into contact with the blood, and a colour change occurs as a result of this chemical reaction; the meter measures this change and converts it into a quantitative blood glucose level.

Administration of plants extracts

Diabetes mellitus was induced on the 5th day after alloxan administration. The animals were stabilized after one week.

Table 1: Basic profile of *Breynia distachia* extract

Plant name	Part used	Solvent	Weight of extract (g)	Abbreviation for the extracts
<i>Breynia distachia</i>	Aerial parts (1000 g)	Dichloromethane	39.85	BDAD
		Methanol	7.95	BDAM

Table 2: Brine-shrimp lethality of *Breynia distachia*

Extract	Dose ($\mu\text{g/mL}$)	No. of shrimps	No. of Survivors	LD ₅₀ ($\mu\text{g/mL}$)
BDAM	1000	30	0	45.643
	100	30	0	
	10	30	02	
	1000	30	20	
BDAD	100	30	22	430.675
	10	30	24	
	Standard	Etoposide		

Animals were grouped into 5 groups and the fasting blood glucose levels were measured on the 7th day. Treatment commenced on the same day, which was considered to be the first day of the experimental work [17]. The treatment was undertaken for 15 days, with the groups divided as follows: Group I: served as the normal control group and were administered citrate-buffer 4 mL/kg, i.p.; Group II: served as the positive control group and were administered alloxan 150 mg/kg and normal-saline 4 mL/kg, i.p.; Group III: served as the standard control group and were administered glibenclamide 10 mg/kg and alloxan 150 mg/kg, i.p.; Group IV (Test Group – A): administered alloxan 150 mg/kg + *B. distachia* methanol extract (aerial parts) 150 mg/kg, i.p.; and Group V (Test Group – B): administered alloxan 75 mg/kg + *B. distachia* methanol extract (aerial parts) 300 mg/kg, i.p.

Statistical analysis

Analysis was conducted using the post-hoc test in Graphpad-prism version #5 for inter group comparison. The analysis of the results was conducted by one-way or two-way analysis of variance (ANOVA) by Bonferroni post-test. The data are presented as mean \pm SEM, and $p < 0.05$ was considered statistically significant.

RESULTS

Basic profile of *Breynia distachia* extract

Table 1 shows the basic features of the plant extract.

Cytotoxic effect of *Breynia distachia*

Data in Table 2 indicates that the methanol extract of aerial parts (BDAM) of plant showed significant ($p \leq 0.05$) cytotoxic activity against

brine shrimp larvae, as compared to the dichloromethane extracts (BDAD). The methanol extract of the *B. distachia* aerial parts (BDAM) showed the highest cytotoxicity among all the tested extracts, with an LD₅₀ of 45.643 $\mu\text{g/mL}$, killing all the shrimp larvae (0% survival) at 1000 and 100 $\mu\text{g/mL}$ and leaving only 6% larvae alive at 10 $\mu\text{g/mL}$. The cytotoxic potential of the different extracts was in the order BDAM > BDAD. The dichloromethane extract of the aerial parts showed 66, 73 and 80% survival of larvae at concentrations of 1000, 100 and 10 $\mu\text{g/mL}$, respectively.

Table 3: Lipoxygenase inhibitory activity of *Breynia distachia*

Extract code	Lipoxygenase (% inhibition) at 0.5 mg/ml	Lipoxygenase (IC ₅₀) $\mu\text{g/ml}$
BDAD	27.82 \pm 0.74	Nil
BDAM	33.33 \pm 0.85	Nil
Control	Baicalein	22.41

BDAD: *Breynia distachia* aerial dichloromethane extract; BDAM: *Breynia distachia* aerial methanol extract

Acetylcholinesterase inhibition of *B. distachia*

Data in Table 4 highlights that the methanol extract of *B. distachia* aerial parts has significant ($p \leq 0.05$) acetylcholinesterase inhibition activity, having percentage inhibition values of 87.48 \pm 0.09. This table also shows that methanol extract of *B. distachia* aerial parts has significant ($p \leq 0.05$) butyrylcholinesterase inhibitory activity, with percentage inhibitory value 89.06 \pm 0.89 and IC₅₀ value 52.31 \pm 0.11. For the determination of acetylcholinesterase inhibition and butyrylcholinesterase inhibition the Eserine was used as standard drug.

Table 4: Acetylcholinesterase

Extract/standard drug	Inhibition (%) of 0.5 mg/mL acetylcholinesterase	IC ₅₀ value (µg/mL)	Inhibition (%) of 0.5mg/mL butyrylcholinesterase	IC ₅₀ value (µg/mL)
BDAD	11.73±0.75	-	88.52±0.39	55.25±0.07
BDAM	87.48±0.09	71.21±0.11	89.06±0.89	52.31±0.11
Standard drug (Eserine)	82.81±1.09 (0.5 mM)	0.04±0.001 (µM)	82.81±1.09 (0.5 mM)	0.84±0.001 (µM)
BDD (<i>Breynia distachia</i> aerial dichloromethane extract)				
BDM (<i>Breynia distachia</i> aerial methanol extract)				

Butyryl-cholinesterase inhibitory activity of *Breynia distachia*

Table 5: α- Glucosidase inhibitory activity of *Breynia distachia*

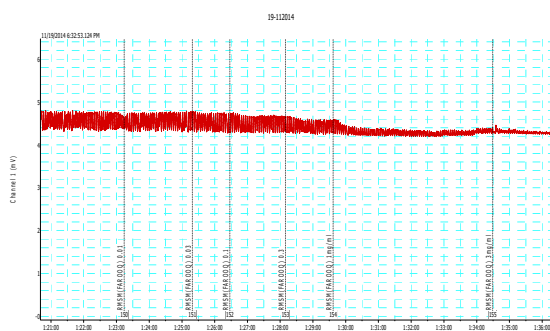
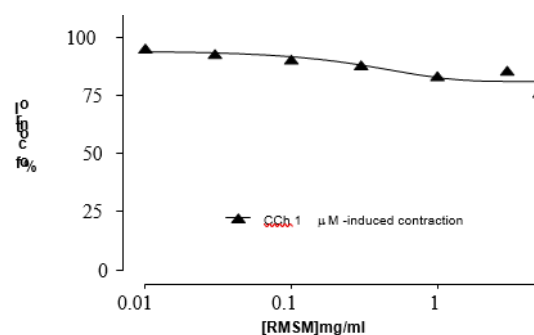
Concentration (µg/mL)	Inhibition (%) BDAM	IC ₅₀ (µg/mL) BDAM	Inhibition (%) BDAD	IC ₅₀ (µg/mL) BDAD
20	16.41		9.85	
40	29.80		28.20	
60	47.40	39.45	31.65	129.25
80	57.90	±6.15	39.42	±7.32
100	82.25		41.55	

Spasmolytic and bronchodilator activities of *B. distachia*

The aqueous methanol extract (30:70) of *B. distachia* (aerial parts) relaxed the spontaneous contractions induced by both K⁺ and carbachol (1 µM) in the rabbit jejunum preparations in a dose dependent manner, at a concentration of 0.01–3 mg/mL. The responses were found to be mediated through either muscarinic and/or blockage of voltage dependent Ca²⁺ channels.

Alpha-glucosidase inhibitory activity of *B. distachia*

Data in Table 5 shows that the methanol extracts of the aerial parts of *B. distachia* exhibited significant ($p \leq 0.05$) inhibitory activity at 100 µg/mL against yeast α-glucosidase, with maximum inhibition of 82.25 %, and IC₅₀ value 39.45 ± 6.15, respectively. The dichloromethane extracts of the aerial parts showed a maximum enzyme inhibition of 41.55 %, with IC₅₀ value of 129.25 ± 7.32 respectively.

**Figure 1:** Antispasmodic potential tracing of *B. distachia* aerial parts**Figure 2:** Isolated tracheal contraction of *B. distachia* aerial parts on carbachol (1µM)

In vivo antidiabetic effect of *B. distachia* extract

Table 6 shows that the glucose level of the animals were decreased significantly ($p < 0.05$) on treatment with the methanol extract of the aerial parts of *B. distachia* (150 mg/kg and 300 mg/kg) compared with the animals of the control group. On day 0, 1 and 4, the blood sugar level in the control group rats, who received citrate buffer (4 mL/kg), was 105.21±1.7, 104.25±1.8, and 105.20±2.1 mg/dL, respectively. The rats in the positive control group, who were given alloxan followed by normal saline (4 mL/kg), had blood sugar levels of 480.54±4.6, 478.56±4.2 and 469.55±2.2 mg/dL on day 0, 1 and 4, respectively.

Animals in the standard control group were administered glibenclamide (10 mg/kg) following alloxan treatment, and their blood glucose levels were 482.12 ± 5.3, 89.65 ± 5.2 and 105.43 ± 3.7 mg/dL, respectively, which indicated a significant ($p < 0.05$) reduction in blood glucose level on the 1st and 4th day of treatment.

Table 6: Antidiabetic attributes of *Breynia distachia* aerial parts in alloxan-induced diabetic rats

Group	Glucose level (mg/dL)				
	0 day	1st day	2 nd day	3 rd day	4th day
Normal control (4 ml/kg Citrate buffer)	105.21±1.7	104.25±1.8	108.25±1.2	106.12±2.25	105.20±2.1
Positive control (4 ml/kg Normal Saline)	480.54±4.6	478.56±4.2	476.85±3.9	472.42±2.9	469.55±2.2
Standard control (10 mg/kg Glibenclamide)	482.12±5.3	89.65±5.2	95.54±5.1	100.26±4.2	105.43±3.7
Test group-A (150 mg/kg) <i>Breynia distachia</i>	483.27±5.0	180.34±5.8	182.36±4.7	184.15±4.1	190.1±2.9
Test group-B (300 mg/kg) <i>Breynia distachia</i>	480.75±4.4	121.72±2.1	119.45±2.4	113.95±1.8	106.25±2.0

The animals in the test groups, A and B, were administered the methanol extract of the aerial parts of *B. distachia*. The test group-A who were administered 150 mg/kg of extract, their blood sugar levels on day 0, 1 and 4 were 483.27 ± 5.0, 180.34±5.8 and 190.1±2.9 mg/dL, respectively. The animals of test group B, who were administered the methanol extract of the aerial parts of *B. distachia* (300 mg/kg) revealed a significant ($p < 0.05$) decrease in blood sugar levels 480.7 ± 4.4, 121.7 ± 2.1 and 106.3 ± 2.0 mg/dL on day 0, 1 and 4.

DISCUSSION

In our present study, the cytotoxic activity of the plant was assessed using the brine shrimp lethality bioassay. This cytotoxicity bioassay is a marker of general toxicity and is predictive of the potential bioactive components of plants that could account for their pesticidal, cytotoxic and antitumor activities. This cytotoxicity assay was conducted on the basis of traditional significance and previous cytotoxic analysis of the genus *Breynia*. Species of this genus are well reputed for their significant cytotoxic effects [18]. An estimation of cytotoxicity against brine shrimps can provide a way to identify and purify novel bioactive compounds from these plant sources. The methanol extract of the aerial parts of the plant showed maximum cytotoxic activity and had an LD₅₀ value of 45.643 ± 0.05 µg/mL.

Enzyme inhibitors have now become a subject of great interest and attention, due to their excellent role in striking various pharmacological targets [19]. Due to the development of resistance against synthetic anti-hyperglycaemic agents, it is now crucial to build a large group of natural inhibitors with high activity and low toxicity [19]. Antidiabetic drugs are ideal for both their hypoglycaemic and antioxidant effects, with minimum or no side effects. Flavonoids of plants have been used since prehistoric times to treat diseases such as diabetes, heart disease and cancer [20]. Acetylcholinesterase enzyme

causes hydrolysis of acetylcholine to choline and acetyl-CoA. We evaluated in our study that level of acetylcholinesterase and butyrylcholinesterase enzymes was significantly decreased, resulting in the neuroprotection and down regulation of the Alzheimer's disease [22].

At present, plant polyphenols are accepted as a major class of natural antioxidants and a wide range of useful biological effects are attributed to these compounds, including antioxidant activity and the inhibition of carbohydrate-metabolizing enzymes, due to their ability to scavenge free radicals and bind with proteins. Phenolic compounds in green tea, berries and sweet potato have been shown to inhibit the carbohydrate-metabolizing enzymes sucrase, α -amylase and α -glucosidase [21].

The dichloromethane and methanol extracts of *B. distachia* possess lipoxygenase activity, which is significant ($p \leq 0.05$) and aerial parts of *B. distachia* showed 81% inhibition and IC₅₀ of 155.7 ± 0.55 and 132.9 ± 0.33 µg/mL, respectively. It is reported that lipoxygenase enzyme has been used in a variety of diseases, including asthma, and inflammation [22].

CONCLUSION

This study demonstrates that *B. distachia* possesses antidiabetic and anti-Alzheimer's activity and has the potential to inhibit α -glucosidase and lipoxygenase, as well as acetylcholinesterase and butyrylcholinesterase enzymes. Thus, these findings support the folkloric uses of this medicinally important plant.

DECLARATIONS

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Pakistan for providing facilities and support to perform this research work.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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