Original Research Article

Potential application of *Conyza canadensis* (L) Cronquist in the management of diabetes: *In vitro* and *in vivo* evaluation

Huma Aslam[1], Arif-ullah Khan[1]*, Humaira Naureen[1], Fawad Ali[2], Farhat Ullah[3], Abdul Sadiq[3]

[1]*Riphah Institute of Pharmaceutical Sciences, Riphah International University, Islamabad,* [2]*Department of Pharmacy, Kohat University of Science and Technology, Kohat,* [3]*Department of Pharmacy, University of Malakand, Chakdara 18000, Dir (L), KPK, Pakistan*

*For correspondence: **Email:** *arif.ullah@riphah.edu.pk;* **Tel:** *+92-51-2891835-38, +92-331-0054153*

Abstract

Purpose: *To investigate the antihyperglycemic activity of Conyza canadensis via α-glucosidase inhibition in alloxan-induced diabetic mice.*
Methods: *In vitro antidiabetic activity was investigated using α-glucosidase inhibition assay with acarbose (62.5, 125, 500 and 1000 µg/ml) as the standard drug. Conyza canadensis crude extract (Cc.Cr) in doses of 10, 30, 100 and 300 mg/kg were administered daily as a single dose to alloxan-induced (200 mg/kg) diabetic mice (Balb/c), and its effect on fasting blood glucose levels and body weight were evaluated for 15 consecutive days; oral glucose tolerance test was conducted. Metformin (500 mg/kg) was used as a standard antidiabetic drug for comparison. Acute toxicity of Cc.Cr was also evaluated at doses of 3 and 5 g/kg.*
Results: *Conyza canadensis crude extract (Cc.Cr) exhibited strong enzyme inhibition at concentrations (µg/ml) of 1000 (74.78 ± 0.92), 500 (65.11 ± 0.07), 250 (57.55 ± 0.41), 125 (51.55 ± 0.67) and 62.5 (44.00 ± 0.57), with a median inhibitory concentration (IC_{50}) of 107 µg/ml. Cc.Cr at all test doses (10 - 300 mg / kg) reduced fasting blood glucose levels in alloxan (200 mg/kg) - induced diabetic mice on days 5, 10 and 15 compared to the diabetic control group (p < 0. 001). These effects were similar to those caused by the standard antidiabetic drug, metformin. Cc.Cr at all test doses also increased body weight of treated animals. The extract (300 mg/kg) significantly improved tolerance of oral glucose overload in mice, like metformin. The extract did not cause any mortality up to the maximum dose of 5 g/kg.*
Conclusion: *The results reveal that Conyza canadensis possesses potent secondary metabolites which can cause inhibition of α-glucosidase. Moreover, the plant extract has the ability to reduce blood glucose level in diabetic animals and significantly improves oral glucose overload tolerance.*

Keywords: Conyza canadensis, α-Glucosidase, Blood glucose, Alloxan, Diabetes, Glucose tolerance

----

INTRODUCTION

Diabetes is one of the leading causes of death worldwide and according to World Health Organization (WHO), diabetes will be the 7th leading cause of death by 2030 [1]. It is a metabolic disease with major clinical manifestation of chronic hyperglycemia. The most prominent pathway in the management of diabetes is to reduce the postprandial hyperglycemia by reducing the absorption of glucose from the intestinal tract [2]. This reduction in glucose absorption is possible by inhibiting a key enzyme responsible for the process, i.e. α-glucosidase. The inhibitors of α-glucosidase will reduce the breakdown of complex carbohydrates to simple sugar units. The reduction in breakdown of complex carbohydrate will lead to the reduction in postprandial glucose level [3].

Conyza canadensis (L.) Cronquist (Asteraceae) is an annual herb, commonly known as Canadian horseweed, Canadian fleabane or Plaeet by the locals [4]. It is used in traditional medicine for the treatment of diarrhea, dysentery, wound healing, muscular pain, arthritis, cystitis, bronchitis and as a diuretic agent [5,6]. *Conyza canadensis* is reported to possess anti-inflammatory, antioxidant, antibacterial, antiplatelet, cytotoxic [7] , antiviral [8] and antimelanoma [9] activities.

The major constituent isolated from the aerial parts of *Conyza canadensis* is limonene [6], other constituents isolated and reported include 2Z, 8Z-matricaria ester, (*R*)-(+)-limonene, trans-α-bergamotene, erigeronol 1 (3-O-(hydroxy-acetyl)-23,28-dihydroxy-β-amyrin) [9], conyzapyranone A and B, 4 Z,8 Z-matricaria-γ-lactone, 4 E,8 Z-matricaria-γ-lactone, 9,12,13-trihydroxy 10(*E*) octadecenoic acid, epifriedelanol, friedeline, taraxerol, simiarenol, spinasterol and apigenin [10]. 3-β-erythrodiol was also isolated and found to be effective in inhibiting MKN-45 human gastric cells in mouse xenograft model [11]. The phytochemicals present in plants are responsible for their pharmacological activities. Various important phytocomponents present in *Conyza canadensis* have been previously reported as possessing antidiabetic activity in various animal models. D-Limonene, β-sitosterol and a sphingolipid have shown strong antidiabetic properties in streptozotocin-induced diabetic rats [12,13].

The present study is designed to investigate the effect of *Conyza canadensis* on the blood glucose level and oral glucose tolerence in alloxan induced diabetes in mice.

EXPERIMENTAL

Plant material and extraction

The whole plant of *Conyza canadensis* was collected from Islamabad, Pakistan in July 2015 and authenticated by Dr. Mushtaq Ahmad, a taxonomist at Department of Plant Sciences, Quaid-e-Azam University, Islamabad, Pakistan. Plant specimen was deposited at the Department herbarium (voucher no. 804).

The plant material was shade dried at room temperature and then grounded to a coarse powder. The powdered material was macerated in 70 % methanol for 10 days with regular shaking at intervals. The crude methanol extract was obtained by concentrating on rotary evaporator under reduced pressure at 40 - 50 ° C [14].

Chemicals

The details of chemicals used in the α-glucosidase inhibitory assay are; glucopyranoside (Sigma, product code 101547038), α-glucosidase (Sigma, product code 1001962968), acarbose (Alfa Aesar, CAS 56180-94-0) and sodium carbonate (Merck). Alloxan monohydrate was purchased from Sigma-Aldrich Co. LLC, USA and Metformin HCl from Caraway Pharmaceuticals, Islamabad, Pakistan. All the chemicals used were of analytical grade.

Animals

Balb-C mice (25 - 35 g) of either sex were used for this study and housed at the Animal House of Riphah Institute of Pharmaceutical Sciences, Islamabad, Pakistan, maintained at 23 - 25 °C and were given standard diet and tap water *ad libitum*. All procedures complied with the rules of Institute of Laboratory Animal Resources, Commission on Life Sciences University, National Research Council (National Research Council 1996) [15] and ethical approval was obtained from Ethical Committee of Riphah Institute of Pharmaceutical Sciences (ref no. REC/RIPS/2015/002).

Preliminary phytochemical screening

Preliminary phytochemical testing was performed for the detection of alkaloids, tannins, tri-terpenes, proteins, carbohydrates, flavonoids, saponins and steroids, using standard procedures [16]. Alkaloids were tested using Mayer's reagent and Dragendroff's reagent. Tannins were considered to be positive when

addition of lead acetate to the test material of Cc.Cr gave cream yellow color. Triterpenes were detected positive when addition of sulphuric acid produced a red colouration using Liebermann Burchard test.

Proteins were tested with ninhydrin, appearance of blue colour confirmed the presence of proteins. Benedict's test was used to detect carbohydrates, orange red precipitates were considered positive indication. If plant material treated with chloroform and sulfuric acid produced red colour, confirmed steroids presence [17]. Saponins were tested using Froth's test [18].

In-vitro α-glucosidase inhibition assay

The α-glucosidase inhibitory assay for Cc.Cr was performed according to standard procedure [19]. The enzyme solution was prepared by dissolving 0.5 unit / mL of α-glucosidase in 0.1M phosphate buffer (pH 6.9). The enzyme solution contains 20 µL of α-glucosidase (0.5 unit / ml) and 120 µl of 0.1 M phosphate buffer. Substrate solution of p-nitrophenyl-α-D-glucopyranoside (5 mM) was made in the buffer solution. Test samples were prepared in a concentration range of 62.5-1000 µg/ml, this was then mixed with the enzyme solution and incubated for 15 min at 37 $^{\circ}$C. Finally, substrate solution (20 µl) was added to the mixture and this was incubated for another 15 min at 37 $^{\circ}$C.

The reaction was completed by the addition of 80 µL of 0.2M sodium carbonate solution. The absorbance were measured at 405 nm using UV-spectrophotometer (Thermo Electron Corporation, USA). The system without α-glucosidase was used as blank and acarbose was used as positive control. The experiments were performed in triplicate and inhibitory activity (H) calculated as in Eq 1.

$$H (\%) = \{(Ac - As)/Ac\}100 \ldots\ldots\ldots\ldots (1)$$

where Ac and As are the absorbance of control and test samples, respectively.

Acute toxicity studies

The acute oral toxicity test was performed as reported by [19]. The mice (n = 6) in each group were fasted overnight and administered Cc.Cr orally at doses of 1, 3 and 5 g/kg. Animals were then kept under observation for 48 h and observed for any signs of diarrhea, discomfort, pain, behavioral, neurological changes, convulsions, coma or death.

Induction of diabetes, blood glucose level and body weight measurement

Animals were fasted for 12 h. The solution of alloxan (200 mg/kg) in 0.2 mL saline was injected intra-peritoneally for the induction of diabetes [20]. After 48 h, fasting glucose levels of test animals were obtained. Animals with blood glucose levels above 200 mg/dl along with signs of polyuria and polydipsia were considered diabetic and included in the study. The test animals were divided into 7 groups (n = 5). Group I (non-diabetic normal control, given saline), Group II (diabetic control treated with alloxan 200 mg/kg), Group III, IV, V and VI include alloxan treated diabetic animals which received 10, 30, 100 and 300 mg/kg doses of the methanolic extract respectively. The Group VII was diabetic animals, treated with metformin (500 mg/kg), as reference drug. Each group was administered the respective test drug daily for 15 consecutive days with monitoring of blood glucose levels, using EASYGLUCO Auto-coding Glucometer and body weight (g) at regular intervals.

Oral glucose tolerance test

The mice were fasted overnight (14-15 h) and divided into non-diabetic control group (treated with saline 0.2 mL), diabetic control group (administered alloxan 200 mg/kg for induction of diabetes), test group (treated with Cc.Cr 300 mg/kg) and standard drug (treated with metformin 500 mg/kg) group, 30 min prior to oral D-glucose (2 g/kg) challenge. Blood for glucose determination was measured by tail-prick method at different time points: 0 min (before glucose load), then at 30, 60, 90 and 120 min after glucose administration [21].

Statistical analysis

Data was expressed as mean ± standard error of mean (SEM) and were analyzed by one-way analysis of variance (ANOVA) followed by Tukey *post-hoc* test. $P < 0.05$ was taken as significantly different [22]. The plots were analyzed using the GraphPad software (GraphPad, San Diego, CA, USA).

RESULTS

Phytochemical profile

The methanol extract of *Conyza canadensis* showed positive results for alkaloids, tannins, tri-terpenes, proteins, carbohydrates, saponins, flavonoids and steroids.

α-Glucosidase inhibition

The α-glucosidase inhibition of the Cc.Cr was performed at different concentrations and compared with the standard drug acarbose at the same tested concentration (Table 1). Cc.Cr exhibited overwhelming enzyme inhibitory potential at various concentrations. At highest tested concentration (1000 μg/ml), Cc.Cr demonstrate 74.78 ± 0.92 % inhibition which was almost similar to that of the positive control at the same concentration (76.87 ± 0.06 % at 1000 μg/ml). Similarly, the observed inhibitions for Cc.Cr at concentrations of 500, 250, 125 and 62.5 μg/ml were 65.11 ± 0.07, 57.55 ± 0.41, 51.55 ± 0.67 and 44.00 ± 0.57 % respectively. The observed IC_{50}s for the Cc.Cr and acarbose were 107 and 23 μg/ml respectively.

Table 1: Alpha-glucosidase inhibition assay of crude extract of *Conyza Canadensis*

Sample	Concentration (μg/ml)	Inhibition (mean±SEM)	IC_{50} (μg/ml)
Crude extract	1000	74.78 ±0.92[ns]	
	500	65.11 ±0.07[**]	
	250	57.55 ±0.41[***]	107
	125	51.55 ±0.67[***]	
	62.5	44.00 ±0.57[***]	
Standard drug	1000	76.87 ±0.06	
	500	73.94 ±1.92	
	250	67.49 ±0.22	23
	125	61.53 ±0.89	
	62.5	55.50 ±1.00	

*All values are taken as mean ± SEM (n= 3). The P values less than 0.05 were considered as statistically significant. Values significantly different in comparison to standard drug i.e. ** = P < 0.01 and *** = P < 0.001*

Acute toxicity

The crude extract of *Conyza canadensis* did neither caused any mortality nor any signs of toxicity at the tested doses of 3 and 5 g/kg (n = 5).

Effect of Cc.Cr on blood glucose levels

The methanolic extract at doses of 10, 30, 100 and 300 mg/kg reduced the blood glucose of alloxan-induced diabetic mice (Figure 1), like that caused by metformin. The animals of non-diabetic control group at days 1, 5, 10 and 15 were having blood glucose levels of 110.0 ± 4.50, 113.0 ± 3.178, 115.4 ± 6.577 and 109.4 ± 4.106 mg/dl respectively. Blood glucose levels of alloxan (200 mg/kg) treated diabetic control group at days 1, 5, 10 and 15 were 256.2 ± 13.6, 259.0 ± 7.9, 269.8 ± 14.7 and 278.4 ± 10.8 mg/dl

respectively. The blood glucose levels of Cc.Cr (10 mg/kg) treated group at 1, 5, 10 and 15th days were 270.4 ± 13.8, 187.4 ± 4.190, 131.0 ± 3.8 and 115.8 ± 1.6 mg/dl ($^*p < 0.05$, $^{***}p < 0.001$ vs diabetic control group) respectively. The blood glucose levels of Cc.Cr (30 mg/kg) treated group at 1, 5, 10 and 15th days were 240.2 ± 10.47, 179.2 ± 7.4, 124.0 ± 2.6 and 113.6 ± 2.9 mg/dl ($^{**}p < 0.01$, $^{***}p < 0.001$ vs diabetic control group) respectively. The blood glucose levels of Cc.Cr (100 mg/kg) treated group at 1, 5, 10 and 15th days were 260.0 ± 14.2, 207.8 ± 14.1, 119.0 ± 5.1 and 102.8 ± 1.2 mg/dl respectively ($^{***}p < 0.001$ vs. diabetic control group). The blood glucose levels of Cc.Cr (300 mg/kg) at 1, 5, 10 and 15th days were 261.2 ± 25.1, 161.0 ± 20.6, 106.8 ± 1.9 and 79.8 ± 6.27 mg/dl ($^{***}p < 0.001$ vs diabetic control group) respectively. The blood glucose levels of metformin (500 mg/kg) treated group at 1, 5, 10 and 15th days were 212.0 ± 9.5, 157.0 ± 6.2, 109.2 ± 2.2 and 103.2 ± 3.9 mg/dl ($^{***}p < 0.001$ vs diabetic control group) respectively.
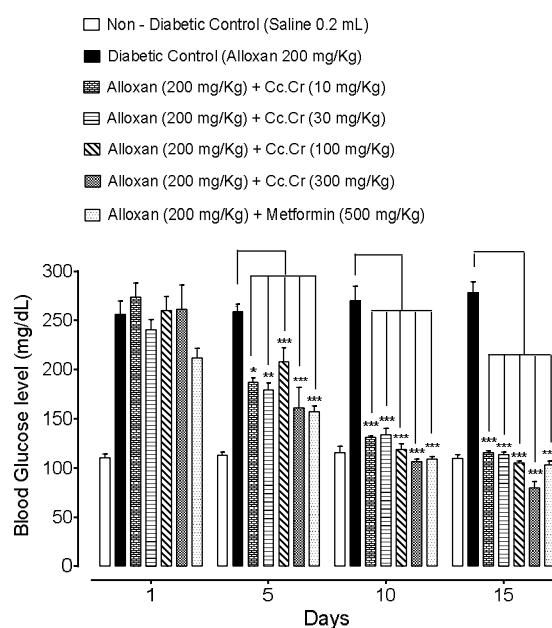

Figure 1: Blood glucose levels at different treatment days of non-diabetic control group (saline treated), diabetic control group animals (alloxan treated), dose-dependent (10 - 300 mg/kg) inhibitory effect of *Conyza canadensis* and metformin against alloxan-induced hyperglycemia in mice. Data are expressed as mean ± SEM (n = 5); $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, compared o diabetic control group

Effect of Cc.Cr on body weight

The methanolic extract at 30, 100 and 300 mg/kg doses increased body weight of the treated animals as shown in Table 2. The body weight of Cc.Cr (30 mg/kg) treated group at 1, 5, 10 and 15th days were 39.76 ± 2.6, 39.92 ± 2.6, 39.60 ±

2.4 and 40.44 ± 2.5 g respectively. The body weight of Cc.Cr (100 mg/kg) treated group at 1, 5, 10 and 15th days were 33.24 ± 2.3, 33.34 ± 2.5, 33.52 ± 2.3 and 34.56 ± 2.3 g respectively. The body weight of Cc.Cr (300 mg/kg) treated animals group at 1, 5, 10 and 15th days were 35.42 ± 2.4, 35.35 ± 2.2, 36.1 ± 2.3 and 36.9 ± 2.5 g respectively.

Effect of Cc.Cr on glucose tolerance

□ Non - Diabetic Control (Saline 0.2 mL) + D-Glucose (2 g/Kg)

■ Diabetic Control (Alloxan 200 mg/Kg) + D-Glucose (2 g/Kg)

▨ Cc.Cr (300 mg/Kg) + D-Glucose (2 g/Kg)

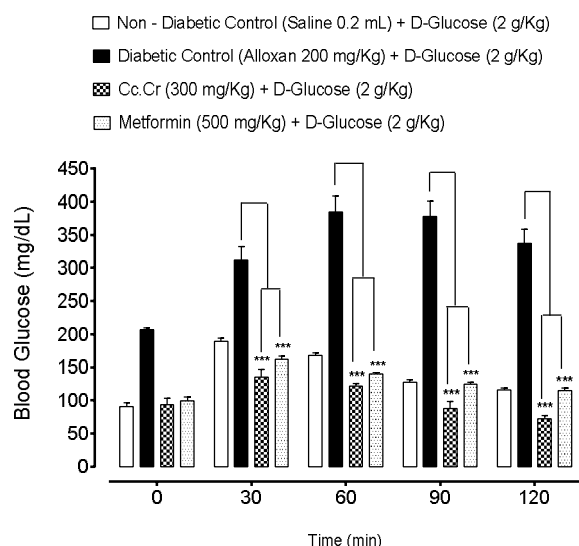▥ Metformin (500 mg/Kg) + D-Glucose (2 g/Kg)

Figure 2: Blood glucose levels of oral glucose loaded mice of non-diabetic control group diabetic control group , *Conyza canadensis* and metformin pre-treated groups. Data are expressed as mean ± SEM (n = 5); [***]*P* < 0. 001 compared to alloxan-induced diabetic control group

The crude extract (300 mg/kg) significantly (*p* < 0.001 vs diabetic control group) improved the tolerance of orally administered glucose, even better than that caused by metformin (500 mg/kg). After the glucose (2 g / kg) overload, blood glucose levels of non-diabetic normal control group at 0, 30, 60, 90 and 120 min were recorded as 91.9 ± 5.6, 179.3 ± 4.9, 160.2 ± 3.6, 137.4 ± 4.4 and 125.6 ± 3.2 mg/dl respectively. In the alloxan (200 mg/kg) treatment diabetic control group, blood glucose levels at 0, 30, 60, 90 and 120 min increased to 206.2 ± 2.9, 311.6 ± 20.2, 384.0 ± 24.4, 378.0 ± 23.05 and 336.8 ±

21.5 mg/dl, respectively. The blood glucose level of Cc.Cr (300 mg/kg) pretreated animals after glucose load (2 g/kg) at 0, 30, 60, 90 and 120 min were 93.40 ± 10.3, 135.2 ± 11.5, 122.0 ± 3.2, 87.80 ± 11.0 and 72.40 ± 4.9 mg/dl (*p* < 0.001 vs diabetic control group) respectively. The blood glucose levels of metformin (500 mg/kg) pretreated mice at 0, 30, 60, 90 and 120 min were 99.60 ± 6.1, 162 ± 4.8, 139.6 ± 2.1, 124.8 ± 2.6 and 114.6 ± 4.9 mg/dl (*p* < 0.001 vs diabetic control group) respectively (Figure 2).

DISCUSSION

Several agents have been used to induce diabetes in experimental animals, including alloxan. Alloxan, a pyrimidine derivative (chemically as 2,4,5,6 tetraoxypyrimidine; 2, 4, 5, 6- pyrimidinetetrone), gets transported into the beta cells through GLUT2 transporter where it induces diabetes by selectively inhibiting glucokinase and causing beta cells necrosis by formation of reactive oxygen species [23].

All animals treated with 200 mg/kg of alloxan showed marked increase in blood glucose levels up to 200 mg/dl and above after 72 h. The selected test animals were then treated with test drugs for 15 consecutive days. *Conyza canadensis* in a dose-dependent (10-300 mg/kg) and time-dependent (days 5-15th) fashion reduced blood glucose level in diabetic animals, similar to the effect of metformin, the standard antidiabetic drug. Metformin, acts by several pathways including impairing intestinal glucose absorption, enhancing insulin release from beta cells, inhibiting hepatic glucose production and increasing the uptake of glucose into the cells [24]. Weight loss is one of the complications of diabetes that occurs due to enhanced lipolysis. Reduction in body weight of diabetics is attributed to inadequacy of insulin and its function in fat metabolism [25]. Prevention of weight reduction in animals, treated with Cc.Cr indicates that *Conyza canadensis* has beneficial outcomes in preventing subsequent complication of weight loss, associated with hyperglycemia.

Table 2: Effect of *Conyza canadensis* crude extract on the body weight of alloxan-treated diabetic mice

Treatment (mg/kg)	Day 1	Day 5	Day 10	Day 15
Non diabetic control (saline 0.2 ml)	38.75 ± 1.2	38.91 ± 2.1	39.32 ± 1.9	39.86 ± 2.8
Diabetic control (Alloxan 200 mg / kg)	39.76 ± 2.6	39.44 ± 2.3	39.12 ± 1.8	38.92 ± 2.5
Alloxan + Cc.Cr 30 mg / kg	39.76 ± 2.6	39.92 ± 2.6	39.60 ± 2.4	40.44 ± 2.5
Alloxan + Cc.Cr 100 mg / kg	33.24 ± 2.3	33.34 ± 2.5	33.52 ± 2.3	34.56 ± 2.3
Alloxan + Cc.Cr 300 mg / kg	35.42 ± 2.4	35.35 ± 2.2	36.1 ± 2.3	36.9 ± 2.5
Alloxan + metformin 500 mg / kg				

Values shown are as mean ± SEM (n = 5); alloxan (200 mg/kg)

In the glucose-loaded hyperglycemia model, employed to evaluate oral glucose tolerance, Cc.Cr displayed significantly better tolerance of glucose overload at different time intervals (30-120 min), similar to metformin. The excessive amount of glucose in blood impels insulin secretion. This secreted insulin stimulates peripheral glucose utilization and controls processing of glucose through several mechanisms [26].

This property of *Conyza canadensis* in reducing blood glucose levels and simultaneously improving glucose tolerance, suggests its use as antidiabetic. However, further advanced molecular studies will be needed to elucidate the underlying pharmacodynamics involved.

The observed antihyperglycemic effect of the plant can be attributed to its diverse phyto-constituent profile. Limonene isolated from aerial parts of *Conyza canadensis* is reported to be effective as an antidiabetic agent by inhibiting protein glycation up to 85.61% and also effectively reduced the blood glucose levels in the *in*-vivo experiments [27]. Other compounds of the plant; β-sitosterol and stigmasterol are also known to possess antidiabetic and antioxidant potentials [28,29].

CONCLUSION

The study reveals that *Conyza canadensis* exhibits α-glucosidase inhibition and antihyperglycemic action in alloxan-induced diabetic mice and improves tolerance of oral glucose overload. This study thus provides scientific evidence for the use of *Conyza canadensis* in the treatment of diabetes mellitus in traditional medicine. Future studies will be carried out to identify the active principle(s) of the plant that are responsible for its antidiabetic effect.

DECLARATIONS

Acknowledgement

Conflict of interest

The authors have no conflict of interest with regard to 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. Huma Aslam and Arif-ullah Khan designed the project. Huma Aslam carried out literature search, experimental work, data analysis and drafted the manuscript. Arif-ullah Khan, Humaira Naureen and Fawad Ali supervised the study and reviewed the final manuscript. Abdul Sadiq also carried out the experimental work and assisted in drafting the manuscript. All authors reviewed and approved the final manuscript for publication.

REFERENCES

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. Plos Med 2006; 3e442.
2. Fonseca V. Clinical significance of targeting postprandial and fasting hyperglycemia in managing type 2 diabetes mellitus. Curr Med Res Opin 2003; 19: 635-631.
3. van de Laar FA, Lucassen PL, Akkermans RP, Van De Lisdonk EH, Rutten GE, Van Weel C. α-Glucosidase inhibitors for patients with type 2 diabetes. Diabetes care 2005; 28: 154-163.
4. Mahmood A, Mahmood A, Shaheen H, Qureshi RA, Sangi Y, Gilani SA. Ethno medicinal survey of plants from district Bhimber Azad Jammu and Kashmir, Pakistan. J Med Plants Res 2011; 5: 2348-2360.
5. Shinwari MI, Khan MA. Folk use of medicinal herbs of Margalla hills national park, Islamabad. J Ethnopharmacol 2000; 69: 45-56.
6. Veres K, Csupor-Löffler B, Lázár A, Hohmann J. Antifungal activity and composition of essential oils of Conyza canadensis herbs and roots. Sci World J 2012; 1-5.
7. Hayet E, Maha M, Samia A, Ali MM, Souhir B, Abderaouf K,Mighri Z, Mahjoub A. Antibacterial, antioxidant and cytotoxic activities of extracts of Conyza canadensis (L.) Cronquist growing in Tunisia. Med Chem Res 2009; 18: 447-454.
8. Edzir H, Laurent G, Mahjoub A, Mastouri M. Antiviral activity of Conyza canadensis (L.) Cronquist extracts grown in Tunisia. Afr J Biotechnol 2011; 10: 9097-9100.
9. Yan MM, Li TY, Zhao DQ, Shao S, Bi SN. A new derivative of triterpene with anti-melanoma B16 activity from Conyza canadensis. Chin Chem Lett 2010; 21: 834-837.
10. Csupor-Löffler B, Hajdú Z, Zupkó I, Molnár J, Forgo P, Vasas A, Kele Z, Hohmann J. Antiproliferative constituents of the roots of Conyza canadensis. Planta Med 2011;77: 1183-1188.
11. Liu K, Qin YH, Yu JY, Ma H, Song XL. 3-β-Erythrodiol isolated from Conyza canadensis inhibits MKN⬚45 human gastric cancer cell proliferation by inducing

apoptosis, cell cycle arrest, DNA fragmentation, ROS generation and reduces tumor weight and volume in mouse xenograft model. Oncol Rep 2016; 35: 2328-2338.

12. Murali R, Saravanan R. Antidiabetic effect of d-limonene, a monoterpene in streptozotocin-induced diabetic rats. Biomed Prev Nutr 2012; 2: 269-275.

13. Holland WL, Summers SA. Sphingolipids, insulin resistance, and metabolic disease: new insights from in vivo manipulation of sphingolipid metabolism. Endocr Rev 2008; 29: 381-402.

14. Ullah F, Ayaz M, Sadiq A, Hussain A, Ahmad S, Imran M, Zeb A. Phenolic, flavonoid contents, anticholinesterase and antioxidant evaluation of Iris germanica var; florentina. Nat Prod Res 2016; 30: 1440-1444.

15. National Research Council. Guide for the Care and Use of Laboratory Animals. Washington; National Academy Press 1996.

16. Khan SU, Khan AU, Shah SM, Hussain S, Ayaz M, Ayaz S. Heavy metals content, phytochemical composition, antimicrobial and insecticidal evaluation of Elaeagnus angustifolia. Toxicol Ind Health 2016; 32: 154-161.

17. Zeb A, Sadiq A, Ullah F, Ahmad S, Ayaz M. Phytochemical and toxicological investigations of crude methanolic extracts, subsequent fractions and crude saponins of Isodon rugosus. Biol res 2014; 47: 57-62.

18. Zeb A, Ullah F, Ayaz M, Ahmad S, Sadiq A. Demonstration of biological activities of extracts from Isodon rugosus Wall. Ex Benth: Separation and identification of bioactive phytoconstituents by GC-MS analysis in the ethyl acetate extract. BMC Complement Altern Med 2017; 17: 284-298.

19. Hullatti K, Telagari M. In-vitro α-amylase and α-glucosidase inhibitory activity of Adiantum caudatum Linn. and Celosia argentea Linn. extracts and fractions. Indian J. Pharmacol. 2015; 47(4): 425.

20. Zeb A, Ahmad S, Ullah F, Ayaz M, Sadiq A. Anti-nociceptive activity of ethnomedicinally important analgesic plant Isodon rugosus Wall. ex Benth: Mechanistic study and identifications of bioactive compounds. Front Pharmacol 2016; 7: 200.

21. Bano S, Khan AU, Asghar F, Usman M, Badshah A, Ali S. Computational and pharmacological evaluation of Ferrocene-based acyl ureas and homoleptic cadmium carboxylate derivatives for anti-diabetic potential. Front Pharmacol 2018; 8: 1001.

22. Khan AW, Khan AU, Ahmed T Anticonvulsant, anxiolytic, and sedative activities of Verbena officinalis. Front. Pharmacol. 2016: 7: 499.

23. Tripathi V, Verma J. Different models used to induce diabetes: a comprehensive review. Int J Pharm Sc 2014;i 6: 29-32.

24. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 2001; 50: 537-546.

25. Klip A, Leiter LA. Cellular mechanism of action of metformin. Diabetes care 1990; 13: 696-704.

26. Kumar AY, Nandakumar K, Handral M, Talwar S, Dhayabaran D. Hypoglycaemic and anti-diabetic activity of stem bark extracts Erythrina indica in normal and alloxan-induced diabetic rats. Saudi Pharm J 2011; 19: 35-42.

27. Murali R, Saravanan R. Antidiabetic effect of d-limonene, a monoterpene in streptozotocin-induced diabetic rats. Biomed Prev Nutr 2012; 2: 269-275.

28. Gupta R, Sharma AK, Dobhal M, Sharma M, Gupta R. Antidiabetic and antioxidant potential of β-sitosterol in streptozotocin-induced experimental hyperglycemia. J diabetes 2011; 3: 29-37.

29. Nualkaew S, Padee P, Talubmook C. (2015). Hypoglycemic activity in diabetic rats of stigmasterol and sitosterol-3-O--D-glucopyranoside isolated from Pseuderanthemum palatiferum (Nees) Radlk. leaf extract. J Med Plants Res 2015; 920: 629-663.