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Pharmacological, Phytochemical and histopathological basis of *Conyza bonariensis* in the potential management of diabetes mellitus

Mohammad Saleem[1]*, Hira Shahid[2], Muhammad Furqan Akhtar[3*], Ammara Saleem[2*], Ali Sharif[4], Bushra Akhtar[5], Bilal Tufail[6], Irfan Hamid[7], Zeeshan Javaid[8] and Rabia Iqbal[7]

[1]Department of Pharmacology, University College of Pharmacy, University of the Punjab, Lahore, Pakistan
[2]Department of Pharmacology, Faculty of Pharmaceutical Sciences, GC University Faisalabad, Faisalabad,
[3]Riphah Institute of Pharmaceutical Sciences, Riphah International University, Lahore Campus, Pakistan,
[4]Department of Pharmacology, Faculty of Pharmaceutical and Allied health Sciences, Lahore College for Women University, Lahore, Pakistan
[5]Department of Pharmacy, University of Agriculture, Faisalabad, Pakistan
[6]Department of Pharmacology, Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan
[7]Cadson College of Pharmacy, University of the Punjab, Lahore, Pakistan
[8]Department of Pharmacy, Mirpur University of Science and Technology, Mirpur, AJK, Pakistan

Abstract: To evaluate the anti-diabetic potential of aqueous methanolic extract of *Conyza bonariensis* amongst the Wistar rats. Phytochemical and High Performance Liquid Chromatography (HPLC) analyses of phenols and flavonoids were examined. The plant extract (250 and 500mg/kg/day) was explored for its anti-hyperglycemic effect for 14 days in normoglycemic and alloxan-induced diabetic rats using the oral glucose tolerance test (OGTT). HPLC analyses demonstrated the composition of the plant extract as gallic acid, cinnamic acid, quercetin, p-coumaric acid and syringic acid. The blood glucose concentrations in experimental diabetic as well as non-diabetic rats significantly decreased with doses 250 and 500 mg/kg in OGTT. Moreover, the significant drop in fasting glucose level was observed following 14 days of therapy. It also ameliorated the serum cholesterol, total protein, low and high density lipoproteins, glycosylated hemoglobin A1C and serum amylase with respect to untreated rats suffering from diabetes. There appeared to be no significant alteration with regard to body weight amongst the treated rats. The plant extract revamped the pancreatic islets of Langerhans and abridged alloxan-induced degenerative changes in the liver. It can be concluded that *Conyza bonariensis* extract has a pronounced hypoglycemic effect on diabetes due to the presence of phytochemicals.

Keywords: *Conyza bonariensis*, anti-diabetic potential, high performance liquid chromatography, OGTT, glycosylated hemoglobin,

INTRODUCTION

Diabetes mellitus (DM), a multifaceted metabolic disease, is a crucial health burden. Every year about 318 million people suffer from impaired glucose tolerance worldwide and 21 million develop gestational DM (Fatima *et al*., 2019). The prevalence of DM is increasing due to large population, urbanization, low birth weight, poor diet, inactive living routine, obesity, stress and smoking. High blood sugar concentrations may be due to insulin resistance or beta cell dysfunction (Erukainure *et al*., 2012, Cerf, 2013). Estimation of fasting blood glucose (FBG), HbA1c and OGTT are used to diagnose or monitor the disorder. Untreated DM leads to complications (Seo *et al*., 2016, Mbaka *et al*., 2012). Major organs affected by uncontrolled DM include adipose tissues, skeleton, smooth and cardiac muscles, liver, kidney, and pancreas. Acute complications include ketoacidosis, whereas chronic complications include macro and micro-vascular abnormalities (Sabir *et al*., 2018). Medicines used for the treatment of type 2 DM are Sulfonylureas, Biguanides, Thiazolidinediones, Alpha-amylase, and Dipeptidyl peptidase-IV inhibitors (Gupta, 2018). Practice of this allopathy results in severe side effects, including hypoglycemia, excessive weight gain, gastrointestinal disturbances, breathing and kidney problems, headache, and nasopharyngitis (Seo *et al*., 2016, Ahmed, 2017).

Demands of traditional medicines are increasing significantly owing to lesser side effects and higher therapeutic efficacy than the allopathic drugs (Bibi *et al*., 2014). More than 21000 plants have been studied for their antidiabetic activities, antioxidant and alpha amylase inhibitory activities of *Fumaria officinalis* and its antidiabetic potential against alloxan induced diabetes (Fatima *et al*., 2019). Phytoconstituents present in medicinal herbs decrease the FBG levels through different mechanisms such as stimulation of insulin secretion, inhibition of alpha-amylase or glucosidase, and proliferation of islet beta cells (Beidokhti *et al*., 2020; Akhtar *et al*., 2021). *Conyza bonariensis,* also called Asthma weed, Fleabane or Sadaf, belongs to the family Asteraceae (Bibi *et al*., 2014). It is traditionally used in

**Corresponding author:* e-mail: saleem2978@hotmail.com; furqan.pharmacist@gmail.com; amarafurqan786@hotmail.com

the treatment of various ailments such as ulcers, myalgia, rheumatism, dysmenorrhea, cystitis, headache, nephritis, diarrhea, piles and hemorrhage (Bibi *et al*., 2014, Ganesan and Xu, 2017). *Conyza bonariensis* has shown anti-inflammatory, antipyretic, antimicrobial, cytotoxic, and cardiotonic activities (Chhetri *et al*., 2015). This experimental research was conducted to explore the phytoconsitutents in aqueous methanolic extract of *C. bonariensis* to look into its anti-diabetic potential among alloxan-inducted diabetic Wistar rats.

MATERIALS AND METHODS

Chemicals

Methanol, formalin, disodium hydrogen phosphate, and sodium dihydrogen phosphate were obtained from Medipak®, Pakistan. Alloxan monohydrate (Ark Pharma®, USA) as well as glibenclamide were procured from CCL laboratories®, Pakistan. Serum total cholesterol (TC), triglycerides (TG), low-density lipoproteins (LDL), high-density lipoproteins (HDL), total protein, as well as HbA1c (Crescent diagnostic kits®) were used in the study. Analytical standards of phenolic acids, flavonoids (Sigma-Aldrich®, USA) and HPLC grade solvents such as acetonitrile, methanol, and acetic acid (Merck®, Germany) were consumed in the study.

Procurement and Maceration of Whole Plant

The whole plant of *Conyza bonariensis,* gathered up from Faisalabad district, was identified and deposited at the botany department of the University of Agriculture, Faisalabad under voucher number (1008-1-2017). The desiccated aerial parts of the plant dried in air were extracted in aqueous methanol (30:70) solution by the cold maceration technique. The vaporization of the filtrate was carried out through a Rotary evaporator to collect crude aqueous methanolic extract that was kept at -20°C until further use (Akhtar *et al*., 2021).

Qualitative phyto-chemical analyses

The methanolic extract of *Conyza bonariensis* was assessed by qualitative methods for detecting primary and secondary metabolites according to standard procedures (Saleem *et al*., 2019c).

HPLC analysis

HPLC analyses were performed through the utilization of Shimadzu shim-pack CLC-ODS (C-18) column with (25cm × 4.6mm - 5µm) dimensions. Mobile phases in the proportion of, H_2O: Acetic acid-94:6 and acetonitryl-100% were run through flow rate of about 1mL/minute. The UV-visible detector was calibrated on 280nm to determine peaks and retention time (Saleem *et al*., 2019b).

Housing of Research Animals

Rats of either sex of Wistar species (150-250 grams) procured from the animal housing area of Department of Pharmacology University of Faisalabad were kept in the same with 12 h dark as well as light cycle and given rodent pellet dietary food along with water *ad libitum*. The animal study was approved by Animal Ethics Committee of GC University Faisalabad (Approval No. GCUF/ERC/1677). Moreover, this animal research was carried out and performed under the guidance of the National Institute of Health (NIH publication 85-23).

Acute toxicity

Aqueous methanol extract of *Conyza bonariensis* was assessed for acute toxicity according to OECD guidelines 423. Extract (5000mg/kg) was given orally to fasting rats to determine any signs of immediate toxicity in the first day. Rats were also observed daily for 14 days to demonstrate any delayed toxicity and behavioral changes (Saleem *et al*., 2019b).

Diabetes induction

An alloxan monohydrate (150 mg/kg) solution formulated in normal saline was injected to rats by intra-peritoneal route for inducing diabetes (Saleem *et al*., 2019c, Al-Qudah *et al*., 2016). The blood glucose level was measured after 48- 72h of alloxan administration. Rats having blood glucose greater than 200 mg/dL were employed in this research.

Oral glucose tolerance Testing

The oral glucose tolerance test (OGTT) was done on normoglycemic and diabetic wistar rats. Twenty-four rats had been subjected to uniform distribution into four groups. All rats fasted overnight before treatment. Group (I) and group (II) were made to serve as normal control and standard respectively. Group II was subjected to Glibenclamide (10mg per kg per day in distilled water). Group (III) and group (IV) were subjected to 250 and 500 mg/kg/day of the aqueous methanol extract respectively. Glucose (4g/kg) was administered to all the groups 30 min before any treatment. OGTT was also performed on diabetic rats according to the method described for normoglycemic rats. The blood glucose concentration in rats was measured on 0, 0.5, 1, 2, 3 and 6h post glucose administration (Zulfqar *et al*., 2020, Chan *et al*., 2015).

Anti-diabetic activity in alloxan-inducted diabetic rats

Forty-two rats had been subjected to distribution within seven uniform groups. Group (I) was kept as Normal control, while group (II) served as Diabetic control. Both groups received distilled water only. Group III was standard. Group IV and group V were orally given aqueous methanol extract at 250mg per kg per day as well as 500mg per kg per day, respectively. Blood samples had been withdrawn from the tail vein to quantify the blood glucose concentration prior to and after the administration of therapy at 1, 3, 6, 9, 12, and 14 days. Body weights were calculated before and after the study (Zulfqar *et al*., 2020, Zaheer *et al*., 2019).

Determination of biochemical parameters

Blood was collected in serum tubes from each rat by cardiac puncture under mild ether anesthesia. Serum total cholesterol (TC), serum triglyceride, serum (HDL) and serum (LDL) have been analyzed utilizing commercially available diagnostic kits. Serum amylase and total protein were also investigated. Glycated hemoglobin was estimated by the HbA1c assay kit (Akhtar *et al*., 2021, Suman *et al*., 2016).

Histopathological evaluation

Rats were sacrificed on the 14th day. Pancreas and liver were dissected, subsequently washed in saline, were maintained in 10 percent solution of formalin. Tissues were embedded within paraffin wax and then sections prepared with a microtome. Tissue sections were then stained by hematoxylin as well as eosin to perform histopathological evaluation (Saleem *et al*., 2019a, Hamishehkar *et al*., 2015, Olayode *et al*., 2020).

STATISTICAL ANALYSIS

Results obtained were subjected to expression as mean ± standard deviation (S.E) while being analyzed through SPSS-20. The effect of aqueous methanol extract on OGTT was subjected to analyses via Two-way (ANOVA), subsequently following Tukey's test. The parameters such as lipid profile, serum amylase, total protein, and HbA1c were subjected to analyses via one-way (ANOVA). Statistically, the values were estimated to be considered significant as $p<0.05$.

RESULTS

Qualitative phytochemical profile

Qualitative tests performed on the aqueous methanolic extract confirmed the composition of tannins, terpenoids, alkaloids, as well as saponins.

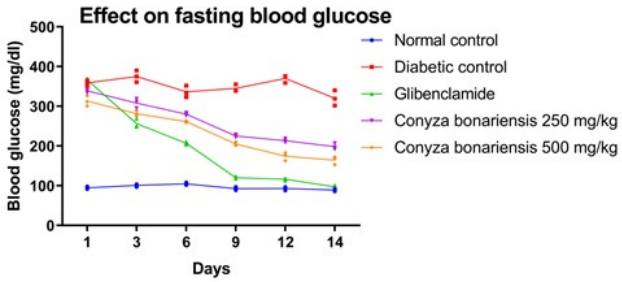


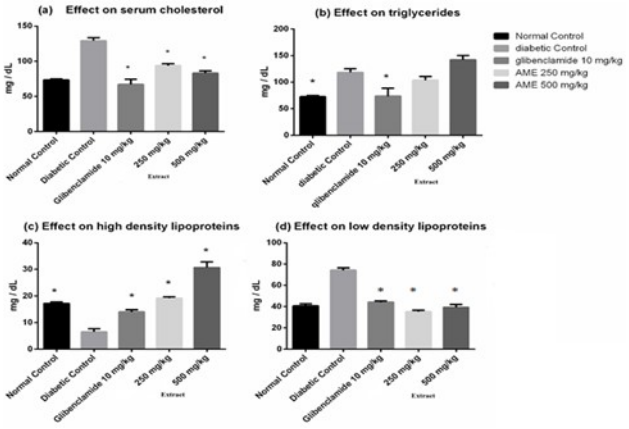
Fig. 1: Effect of the aqueous methanol extract of *Conyza bonariensis* on (FBG) of diabetic rats Values were expressed as mean ± S.E.M

HPLC analysis

Phenolic acids and flavonoids in the aqueous methanol extract were determined by HPLC. The HPLC analysis indicated that gallic acid was present in the highest concentration (118.72ppm), followed by quercetin, cinnamic acid, p-coumaric acid and syringic acid (table 1).

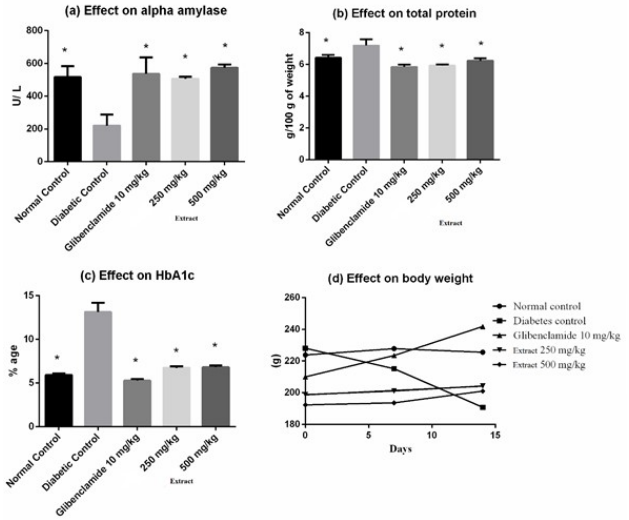
Acute toxicity of the aqueous methanol extract

A single oral administration of 5000mg per kg aqueous methanolic extract of *Conyza bonariensis* did not induce any abnormal behavior and toxicity sign in rats. There was no fatality seen among animals during the time duration of 14 days. Therefore, the extract was considered safe up to 5000mg/kg.



Values were expressed as mean ± S.E.M.* showed statistically different in comparison to diabetic animals ($p<0.05$)

Fig. 2: Effect of *Conyza bonariensis* extract on lipid profile in diabetic rats



Values were expressed as mean ± S.E.M.* showed statistically different in comparison to diabetic animals ($p<0.05$.

Fig. 3: Effect of aqueous methanolic extract of *Conyza bonariensis* on body weight, glycated hemoglobin, total protein and alpha amylase in diabetic rats

Effect of the aqueous methanolic extract on oral glucose tolerance

The plummeting of blood glucose concentrations in normoglycemic rats with 250 as well as 500 mg per kg dose of the extract had been statistically significant with '$p<0.05$' in relation to untreated normal control rats at 3 h post glucose administration (table 2).

Table 1: Phytochemicals determined by HPLC analysis in aqueous methanolic extract of *Conyza bonariensis*

Compound	Retention time	Area (mV.s)	Area (%)	Concentration of compound in extract (µg/gm)
Quercetin	3.367	246.587	2	13.13
Gallic acid	5	3298.157	27	118.72
Syringic acid	16.407	237.853	1.9	5.92
p-coumaric acid	17.3	620.995	5.1	8.17
Cinamic acid	24.947	272.627	2.2	9.52

Table 2: Blood glucose levels in OGTT of normoglycemic animals treated with the extract of *Conyza bonariensis*

Time (h)	Groups			
	Normal animals (mg/dL)	Glibenclamide 10 mg/kg (mg/dL)	250 mg/kg (mg/dL) extract	500 mg/kg (mg/dL) extract
0	73.83±3.50	76.67±5.17	73.83±3.05	72.33±1.86
0.5	118.50±2.42	120.83±2.34	105.67±1.33	110.67±0.99
1	129.00±1.03ab	133.00±1.00	114.17±3.35*	129.17±0.83
2	114.00±1.79	114.50±0.67	103.00±1.90	105.00±1.63
3	104.67±1.36	72.33±5.90*	85.50±1.93*	74.17±1.68*

Values were shown as mean ± S.E.M.* showed that the values were significantly different in comparison to normal animals ($p < 0.05$)

Table 3: Blood glucose levels in OGTT of diabetic animals treated with the extract of *Conyza bonariensis*

Time (h)	Diabetic control (mg/dL)	Glibenclamide 10 mg/kg (mg/dL)	250mg/kg (mg/dL) extract	500mg/kg (mg/dL) extract
0	233.83±3.66	272.67±3.94	258.83±10.19	271.50±1.82
0.5	381.50±1.48	348.67±8.86	315.83±24.48	349.83±10.60
1	393.33±1.17	366.50±6.26	343.67±26.72	384.33±7.73
2	394.00±1.83	322.50±5.44	309.83±31.03	348.00±7.30
3	392.17±1.89	281.67±12.80*	240.33±23.75*	287.83±7.43*

Values were expressed as mean ± S.E.M.* showed statistically different in comparison to diabetic animals ($p<0.05$)

Untreated diabetic rats showed higher FBG than the normoglycemic rats due to the induction of diabetes. FBG of diabetic rats reached a peak level at 2h post glucose administration and then decreased at 3h. The aqueous methanol extract and standard decreased the FBG in diabetic rats. The decrease FBG by aqueous methanol extract and glibenclamide treatment were significant statistically with '$p<0.05$' in relation to the untreated diabetic rats at 3 h post glucose administration (table 3).

Effect of the aqueous methanolic extract on fasting blood glucose

Estimation of FBG during the 14 days' study depicted that the orally administered aqueous methanolic extract and Glibenclamide had reduced the glucose level in blood. Aqueous methanol extract with doses 250 as well as 500mg/kg started lowering the FBG ($p<0.05$) at day 14 compared to untreated diabetic controls. Glib enclamide treated rats depicted a significant plummeting '$p<0.05$' of FBG as early as subsequent to 12 days of therapy as compared to the normal rats (fig. 1).

Effect of the aqueous methanolic extract on lipid profile and other parameters

The aqueous methanol extract markedly '$p<0.05$' plummeted the cholesterol concentration in serum as well as the LDL level in relation to the diabetic control group. The aqueous methanolic extract did not demonstrate a decrease in triglycerides. The plant extract also showed a sufficient increase '$p<0.05$' of HDL concentrations in relation to the diabetic control group. The effect of aqueous methanolic extract of *Conyza bonariensis* on lipid profile of diabetic rats is depicted in fig. 2.

The aqueous methanol extract significantly reduced the total protein and glycated hemoglobin in diabetic animals at 250 and 500mg per kg per day in relation with the diabetic control group. The plant-extract markedly increased ($p<0.05$) serum amylase activity (fig. 3).

Effect of aqueous methanolic extract on body weight

Induction of diabetes caused a decrease in weight of rats. Treating via plant extract caused slight moderate increase in body weight that was statistically insignificant in relation with the diabetic control group (fig. 3d).

Effect of the aqueous methanolic extract on diabetic pancreas and liver

Histological examination of the pancreas demonstrated shrinkage of islets in the pancreas of diabetic rats in relation to the normal control. Pancreatic islets of Langerhans were restored after treatment with the plant

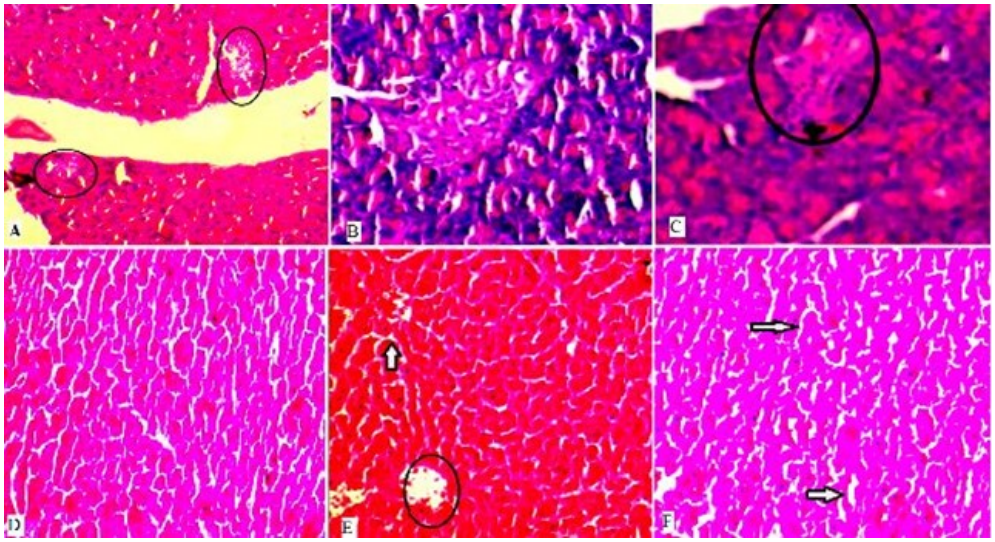


Fig. 4: Effect of aqueous methanolic extract of *Conyza bonariensis* on pancreas and the kidneys of diabetic rats at 40X. (a) Diabetic untreated rats showed degenerative changes in the islets of Langerhans, shrinkage of cells and densely basophilic nucleus. (b) Aqueous methanol (250mg/kg) treated rats showed some degeneration in the beta cells without any lymphoid infiltration; (c) Aqueous methanol (500mg/kg) treated rats showed less vacuolization and healthy beta cells; (d) Normal rats showed intact liver architecture (e) Diabetic untreated rats showed widespread vacuolization with the fading of nuclei associated with glycogen infiltration and (f) Aqueous methanolic (500 mg/kg) extract showed normal cell architecture with dilated sinusoids.

extract with doses 250 as well as 500 mg per kg per day. Histological examination of the diabetic liver also demonstrated a decrease in degenerative changes in hepatocytes, cell necrosis and congestion of diabetic liver owing to treatment via aqueous methanolic extract of *Conyza bonariensis* (fig. 4).

DISCUSSION

DM and its complications are affecting a huge population of developing countries (Sabir *et al*., 2018). In the last few years, the traditionalistic utilization of herbal medicines for the potential management of diabetes acclaimed significant appraisal due to the presumed lesser side effects associated with their phytochemical constituents (Gupta, 2018). *Conyza bonariensis* has historical medicinal importance in the treating different ailments. This experimental research was performed to demonstrate the anti-diabetic potential of *Conyza bonariensis* in alloxan-inducted diabetes rats. The glucose plummeting effects of the aqueous methanolic extract of *Conyza bonariensis* has been determined in non-diabetic as well as diabetic rats via OGTT method. Histopathological evaluation of the treated and untreated pancreas and livers was also carried out.

In our study, phyto-chemical analyses affirmed the composition of alkaloids, saponins, phenolic compounds, tannins, as well as flavonoids within the aqueous methanol extract of *Conyza bonariensis*. HPLC analyses revealed the compsition of gallic acid, quercetin, syringic acid, p-coumaric acid, and cinnamic acid within the aqueous methanol extract which had previously shown significant hypo-glycemic as well as hypo-lipidemic effects. Flavonoids assisted in regenerating of beta cells, while saponins prevented hyperglycemia through increasing secretion of insulin and decreasing gluconeogenesis and alpha-glucosidase activities (Fatima *et al*., 2019). Phenolic compounds are secondary metabolites which tend to decrease blood cholesterol and lipids and exhibit antioxidant property. Tannins also exhibit a hypoglycemic effect.

Acute toxicity depicted that the plant extract did not show any toxic effects while being given on a dose of 5000mg per kg. A similar study conducted on *Conyza dioscoridis* extract also did not show any mortality among animals up till a dose of 5000mg per kg in 14 days (Nassar *et al*., 2014).

It has been observed that diabetes was induced within 48 h of alloxan injection due to the formation of nitric oxide. The toxicological profile of alloxan on tubular cells of the kidney is also well- documented (Zulfqar *et al*., 2020, Akhtar *et al*., 2021). It can be reasoned out that the hypoglycemic effect of *Conyza bonariensis* may be due to 1) improvement and regeneration of the beta cells resulting in increased secretion of insulin, 2) protection and enhancement of insulin receptors to suppress insulin resistance and/or 3) inhibition of glycogenolysis. These explanations are consistent with several previous investigations (Beidokhti *et al*., 2020, Akhtar *et al*., 2021). An increase in triglycerides, serum cholesterol, total protein as well as the plummeting of the level of

HDL were the consequences of abnormal protein and lipid metabolism associated with diabetes. The dosing of aqueous methanolic extract of *Conyza bonariensis* depicted significantly an increased concentration of HDL and serum amylase as well as a plummeting of serum cholesterol with Low density lipoprotein (LDL) in diabetic rats in relation to untreated diabetic rats. Aqueous methanol extract did not show any decrease in serum triglycerides of diabetic rats at 500mg per kg when compared to untreated diabetic rats. Effect of the extract at (500mg per kg) may be due to the inhibition of the activity of HMG-CoA reductase (Seo *et al*., 2016). A significant increase in serum amylase level in extract-treated diabetic rats might be in due part to the polyphenolic compounds present in the aqueous methanol extract which inhibited the glycolytic activity of brush border enzymes (Beidokhti *et al*., 2020, Akhtar *et al*., 2021). HbA1c is an indicator of the control of diabetes in patients. This study showed a decrease in HbA1c in extract-treated diabetic rats following a two-week therapy (Zulfqar *et al*., 2020).

Histological interpretations demonstrated that the aqueous methanol extract reduced fatty acids and inflammatory changes up to a considerable extent in the liver as well as pancreas of diabetic rats. It can be assumed that the aqueous methanol extract of *Conyza bonariensis* may reduce the secondary diabetic changes in the liver (Beidokhti *et al*., 2020). Regeneration of pancreatic islet cells may be in due part to a plummeting of the oxidant status in diabetic rats owing to the extract (Seo *et al*., 2016).

CONCLUSION

The current study demonstrated the hypoglycemic and hypolipidemic effect of *Conyza bonariensis* against glibenclamide in alloxan-inducted diabetic rats. However, it can be proposed that the amelioration effect of *Conyza bonariensis* against diabetes might be in due part to the composition of phenolic compounds as well as the flavonoids contained in it, which needs further investigation. In conclusion, this experimental research rationalizes the potential utilization of *Conyza bonariensis* in diabetic patients and provides the pre-clinical basis for research on its use in humans.

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REFERENCES

Ahmed A (2017). Evaluation of Anti-hyperglycemic effects of methanolic extract *of Cinnamomum zeylanicum* in laboratory animal models. Doctoral dissertation, East West University, **9**(1): 25-35.

Akhtar M, Shagufta A, Saleem A, Baig M, Sharif A, Rasul A and Abdel-Daim M (2021). *Tylophora hirsuta* L. leaf extract attenuates alloxan-induced diabetes in mice by suppressing oxidative stress and α-amylase: *Asian. Pac. J. Trop. Biomed.,***11**(9): 394-401.

Al-Qudah M, Haddad M and EL-Qudah J.M (2016). The effects of aqueous ginger extract on pancreas histology and on blood glucose in normal and alloxan monohydrate-induced diabetic rats. *Biomed. Res*., **27**(2): 350-360.

Beidokhti M, Eid H, Villavicencio M, Jager A, Lobbens E, Rasoanaivo P, McNair L, Haddad S and Staerk D (2020). Evaluation of the antidiabetic potential of *Psidium guajava* L.(Myrtaceae) using assays for α-glucosidase, α-amylase, muscle glucose uptake, liver glucose production and triglyceride accumulation in adipocytes: *J. Ethnopharm*., **257**: 112877.

Bibi S, Sultana J, Sultana H and Malik R (2014). Ethnobotanical uses of medicinal plants in the highlands of Soan Valley, Salt Range, Pakistan. *J. ethnopharm*., **155**(1): 352-361.

Cerf M (2013). Beta cell dysfunction and insulin resistance. *Front. Endocrinol*., **4**(4): 37-43.

Chan L, Pyle L, Newnes L, Nadeau K, Zeitler P and Kelsey M (2015). Continuous glucose monitoring and its relationship to hemoglobin A1c and oral glucose tolerance testing in obese and prediabetic youth. *J. Clin. Endocrinol. Metab*., **100**(3): 902-910..

Chhetri B, Ali N and Setzer W (2015). A survey of chemical compositions and biological activities of Yemeni aromatic medicinal plants. *Medicines*, **2**(2): 67-92.

Erukainure O, Adeboyejo O, Elemo N and Ebuehi O (2012). Modulation of lipid peroxidation, hypolipidemic and antioxidant activities in brain tissues of diabetic rats by fibre-Enriched biscuits. *J. Acute. Dis*., **1**(1): 42-46.

Fatima S, Akhtar M, Ashraf, K, Sharif A, Saleem A, Akhtar B, Peerzada S, Shabbir M, Ali S and Ashraf W (2019). Antioxidant and alpha amylase inhibitory activities of *Fumaria officinalis* and its antidiabetic potential against alloxan induced diabetes. *Cell. Mol. Bio.,* **65**(2): 50-58.

Ganesan K and Xu B (2017). Ethnobotanical studies on folkloric medicinal plants in Nainamalai, Namakkal District, Tamil Nadu, India. *Trend. Phytochem. Res*., **1**(3): 153-168.

Gupta R (2018). Active phytoconstituents for diabetes management: A review. *J. Complement. Integ. Med*., **15**(3): 32-41

Hamishehkar H, Shokri J, Fallahi S, Jahangiri A, Ghanbarzadeh S and Kouhsoltani M (2015). Histopathological evaluation of caffeine-loaded solid lipid nanoparticles in efficient treatment of cellulite. *Drug. Dev. Ind. Pharm*., **41**(10): 1640-1646.

Mbaka G, Ogbonnia S, Oyeniran K and Awopetu P (2012). Effect of *Raphia hookeri* seed extract on blood

glucose, glycosylated haemoglobin and lipid profile of alloxan induced diabetic rats. *J. Adv. Med*., **12**(3): 621-635.

Nassar M, Elshamy A and El Gendy A (2014). Phenolics, essential oil and biological activity of *Conyza dioscoridis* growing in Egypt. *Planta Med*., **80**(16): 10-15.

Olayode OA, Daniyan MO and Olayiwola G (2020). Biochemical, hematological and histopathological evaluation of the toxicity potential of the leaf extract of Stachytarpheta cayennensis in rats. *J. Tradit. Complement. Med*., **10**(6): 544-554.

Sabir S, Saleem A, Akhtar F, Saleem M and Raza M (2018). Increasing beta cell mass to treat diabetes mellitus. *Adv. Clin. Exp. Med.,* **27**(9): 1309-1315.

Saleem A, Saleem M, Akhtar M, Sharif A, Javaid Z and Sohail K. (2019). *In vitro* and *in vivo* anti-arthritic evaluation of Polystichum braunii to validate its folkloric claim. *Pak. J. Pharm. Sci.*, **32**(7): 1167-1173.

Saleem M, Ali H, Akhtar M, Saleem U, Saleem A and Irshad I (2019). Chemical characterisation and hepatoprotective potential of *Cosmos sulphureus* Cav. and Cosmos bipinnatus Cav. *Nat. Prod. Res*., **33**(6): 897-900.

Saleem M, Tanvir M, Akhtar M, Iqbal M and Saleem A (2019). Antidiabetic potential of *Mangifera indica* L. cv. Anwar Ratol leaves: Medicinal application of food wastes. *Medicina*., **55**(7): 353-361.

Seo Y, Shon M, Kong R, Kang O, Zhou T, Kim D and Kwon D (2016). Black ginseng extract exerts anti-hyperglycemic effect via modulation of glucose metabolism in liver and muscle. *J. Ethnopharm*., **190**(2): 231-240.

Suman RK, Ray Mohanty I, Borde MK, Maheshwari U and Deshmukh YA (2016). Development of an experimental model of diabetes co-existing with metabolic syndrome in rats. *Adv. Pharmacol. Sci.*, **45**: 23-32

Zaheer J, Najam-Us-Saqib Q, Qamar M and Akram M (2019). *In vitro* (anti-alpha-glucosidase) activity and in vivo anti-diabetic activity of *Androsace foliosa* (common rock jasmine) in alloxan-induced diabetic BALB/c mice: *Eur. J. Inflamm*., **17**(3): 205-211.

Zulfqar F, Akhtar M, Saleem A, Akhtar B, Sharif A and Saleem U (2020). Chemical characterization, antioxidant evaluation and antidiabetic potential of *Pinus gerardiana* (Pine nuts) extracts. *J. Food Biochem*., **44**(6): 13199.