

BJP

Bangladesh Journal of Pharmacology

Research Article

Anti-diabetic activity of *Iphiona aucheri* leaf extract

Anti-diabetic activity of *Iphiona aucheri* leaf extract

Muhammad Abdur Rehman Shah, Rahmat Ali Khan and Mushtaq Ahmed

Department of Biotechnology University of Science and Technology Bannu 28100, Khyber Pakhtunkhwa, Pakistan.

Article Info

Received: 20 November 2019

Accepted: 1 August 2020

Available Online: 9 October 2020

DOI: 10.3329/bjp.v15i4.44170

Cite this article:

Shah MAR, Khan RA, Ahmed M. Anti-diabetic activity of *Iphiona aucheri* leaf extract. Bangladesh J Pharmacol. 2020; 15: 99-109.

Abstract

The methanolic extract of *Iphiona aucheri* leaves and its *n*-hexane, chloroform and aqueous fractions were screened for phytochemicals, cytotoxic effects, total phenolic contents, antioxidant and anti-diabetic properties. The methanolic extract was assessed for its *in vivo* anti-diabetic effects. The results indicated the presence of alkaloid, protein and amino acids, carbohydrates, glycosides, flavonoids, and saponins along with the highest brine shrimp lethality ($90.7 \pm 1.8\%$), α -amylase inhibition (60.2%) and free radical scavenging properties. The diabetic control indicated a significant ($p < 0.05$) decrease in weight and high-density lipoprotein and elevated level of blood glucose, bilirubin, creatinine, urea, triglyceride, low density lipoprotein, very low-density lipoprotein, transaminases, and alkaline phosphatase. All these changes were restored significantly ($p < 0.05$) by treatment of diabetic rats with methanolic extract of *I. aucheri*. The results show the potential of *I. aucheri* as a source of therapeutic compounds against diabetic, hyperlipidemia and free radical associated disorders.

Introduction

The common problems in diabetes mellitus include the occurrence of hypercholesterolemia, hypertriglyceridemia and low serum high-density lipoprotein cholesterol (HDL-C) in diabetics as compared to normal individuals (Schofield et al., 2016, Vijayaraghavan, 2010). Microvascular impediments resulting from chronic hyperglycemia affects nerves, kidneys, eyes, and increasing the risk for cardiovascular diseases (Skylar et al., 2017).

Diabetes mellitus and its complications are treated with synthetic anti-diabetic drugs such as glibornuride, glipizide, biguanides, pioglitazone, bromocriptine glitazones, rosiglitazone, pioglitazone, saroglitazar, bezafibrate and metformin (Holt et al., 2010, Jani et al., 2013, Kharbanda et al., 2014, Mehanna, 2013, Piero et al., 2009, Proks et al., 2005, Tschöpe et al., 2013, Varas-Lorenzo et al., 2014, Yousef et al., 2017) but they have side effects. To overcome this problem, the researchers

are continuously exploring natural products especially phytochemicals.

Anti-diabetic activities of several plants like *Eclipta alba* (Ananthi et al., 2003), *Artemisia vulgaris* (Daradka et al., 2014), *Glossocardia bosvallea* (Chethan et al., 2014), *Wedelia chinesis* (Senthilkumar et al., 2008), *Wedelia trilobata* (Gallé et al., 2014) *Crassocephalum crepidioides* (Bahar et al., 2017) and *Brachylaena elliptica* (Sagbo et al., 2018) have been documented. Hence, plant-derived bioactive compounds are accessible, less expensive, effective, safe, and is an important source for the treatment of diabetics.

Iphiona aucheri belongs to the family Asteraceae. The non-toxic pyrrolizidine alkaloid have been isolated from the aerial parts of *I. aucheri* (Stewart and Steenkamp, 2000). Antibacterial and antifungal activities of *I. aucheri* have been reported (Kakar et al., 2012, Shah et al., 2019). It has also showed hepatoprotective activity (Ahmed et al., 2019).



The intention of the current project was to find out the phytochemicals constituents, total phenolic contents, cytotoxicity, antioxidant and anti-diabetic activities of methanolic extract and its *n*-hexane, chloroform and aqueous fraction of *I. aucheri* leaves.

Materials and Methods

Plant material

The leaves of *I. aucheri* were collected in March 2017 from the District, Bannu, Pakistan. Its taxonomic recognition was carried out by Prof. Abdur Rehman, Govt. Post Graduate College Bannu, Khyber Pakhtunkhwa, Pakistan. A specimen voucher (AR-123) was submitted in the herbarium of University of Science and Technology Bannu, Khyber Pakhtunkhwa, Pakistan.

Preparation of crude extract

Fresh leaves of *I. aucheri* were shade dried and pulverized into a fine powder with the help of pestle and mortar. The powdered leaves (500 g) were put into 70% methanol (1.5 L) and kept at room temperature for 72 hours with frequent agitation and then filtered using Whatman No. 3 filter paper. The filtrate was dried under rotary vacuum evaporator (Strike202, Italy) at 40°C to obtain concentrated dry extract. The resulting gummy methanolic extract (29.6 g) was put into a falcon tube and stored for use.

Preparation of fractions

The stored gummy methanolic extract was subjected to fractionation. 20 g of the methanolic crude extract of *I. aucheri* was sequentially extracted each with 300 mL *n*-hexane, chloroform and water using separating funnel to avoid any sort of damages to the filtrate. Filtrate of organic solvents was dried under rotary vacuum evaporator at 40°C while aqueous fraction was obtained using lyophilizer. The resulting extracts of *n*-hexane (2.7 g), chloroform (5.2 g) and water (8.9 g) were stored for further designed assays.

Phytochemicals screening

The phytochemicals screening of the methanolic extract of leaves and its various fractions was conducted using standard methods to identify the presence of protein, amino acids, glycosides, saponins, flavonoids and carbohydrates (Trease and Evans, 1989; Rice-Evans et al., 1996).

Cytotoxic assay

The cytotoxic activity of methanolic extract and its various fractions of *I. aucheri* were carried out by brine shrimp lethality bioassay (Meyer et al., 1982). The artificial seawater (4%) was prepared and placed in a small tank comprising of two cabins with pores in the

central wall. A small amount of brine shrimp eggs (1 mg) was placed in one cabin, covered with aluminum foil to produce darkness inside it and the other illuminated with energy saver light for 24 hours. The working solutions having different concentrations of test samples in methanol i.e. 100, 250, 500, 1000 µg/mL were prepared and were put in test tubes arranged in duplicate such that the control was without test sample. The tubes were placed at room temperature for complete evaporation of methanol, added 10 brine shrimps to each test tube and were placed at room temperature for 24 hours. Then the shrimps were counted in each test tube, calculated % lethality by using Abbot's formula and compared the results (lethality) of control with experimental.

$$\% \text{ Death} = (\text{Sample-control}/\text{control}) \times 100$$

Total phenolic content

The total phenolic content contained in the plant extract and its various fractions were measured by using Folin-Ciocalteu reagents (Singleton and Rossi, 1965). 2.5 mL Folin-Ciocalteu reagents (10x diluted in distilled water) was mixed with 250 µL sample solution (1-5 mg/mL) and incubated for 5 min at room temperature. 2.5 mL saturated solution of sodium carbonate (60 mg/mL) was added to the reaction mixture and incubated again for 2 hours at room temperature. Gallic acid solution (1-5 mg/mL) was used as a standard. The absorbance was measured at 725 nm spectrophotometrically (UV-1602, Biotechnology Medical Services). The results were expressed as gallic acid equivalent.

ABTS radical cation assay

The 2, 2'-azino-bis (3-ethyl-benzothiazoline)-6-sulfonic acid disodium salt (ABTS⁺) free radical scavenging assay was carried out by following Roberta and Pellegrini protocol (Re et al., 1999). 7 mM ABTS solution was mixed with potassium persulfate (2.5 mM) solution and incubated in dark for eight hours due to its sensitivity to light. Followed by the incubation, the stock solution was diluted with the relevant solvent (50%) to adjust the absorbance of about 0.900 (± 0.02) at 745 nm at 30°C. 3 mL of the diluted ABTS solution was mixed with 300 µL (62.5-2,000 µg/mL in a respective solvent) of extract and measured the absorbance spectrophotometrically. The percentage scavenging effects of the samples were calculated by using the formula:

$$\text{Scavenging effect (\%)} = (\text{Control absorbance (ABTS)} - \text{Sample absorbance}) / (\text{Control absorbance}) \times 100$$

Hydrogen peroxide scavenging (H₂O₂) assay

The plant possesses natural hydrogen peroxide scavenger that can be estimated according to Wettasinghe and Shahidi (Amarowicz et al., 2000) hydrogen peroxide scavenging assay. A sample (125-2,000 µg/

Box 1: DPPH method**Principle**

The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay was done

Requirements

Ascorbic acid; DPPH (1, 1-diphenyl-2-picrylhydrazyl); Extract

Procedure

Step 1: DPPH (3 mg) was dissolved in 100 mL relevant solvent

Step 2: Incubate at 25°C for about 30 min in the dark because of its sensitivity to light

Step 3: Check its absorbance on the spectrophotometer at 517 nm.

Step 4: 200 µL from each sample solution i.e. 62.5, 125, 250, 500,

1000, 1500 and 2000 µg/mL were taken and mixed it with 1800 µL of DPPH solution.

Step 5: The same process was repeated with the ascorbic acid solution.

Calculation

By using the following equation the potential of the samples to scavenge the DPPH free radicals was calculated;

$$\% \text{DPPH free radicals scavenging effect} = (A_1 - A_2 / A_1) \times 100$$

Where, A_1 = the absorbance of DPPH (control) and A_2 = the absorbance in the presence of samples

Reference

Gyamfi et al., 1999

Reference (video)

Shylaja et al., 2018

mL; 300 µL) was added with 1.4 mL of 100 mM phosphate buffer (pH 7.4), followed by mixing of 300 µL of 43 mM hydrogen peroxide solution in the said buffer. The reaction mixture was incubated at room temperature for 40 min and measured its absorbance values at 230 nm. The percentage of scavenging properties of plant extract was calculated as:

$$\text{Scavenging effect (\%)} = [(\text{Control absorbance (H}_2\text{O}_2\text{)} - \text{Sample absorbance}) / (\text{Control absorbance})] \times 100$$

All tests were done in triplicate and the results were presented as means \pm SD.

Alpha-amylase inhibition

The Worthington enzyme manual (Kwon et al., 2007) was opted to determine the α -amylase inhibitory properties of the plant extract. Plant extract (300 µL) was mixed with 500 µL of 1% starch solution in sodium phosphate buffer (20 mM, pH 6.9 with 6 mM NaCl) followed by the addition of 500 µL of sodium phosphate buffer (20 mM, pH 6.9 with 6 mM NaCl) containing 0.5 mg/mL of α -amylase and pre-incubated the reaction mixture for 10 min at 25°C.

Dinitrosalicylic (DNS, 1.0 mL) acid, a color reagent was used to stop the reaction and incubated the test tubes in boiling water for 5 min and then cooled to room temperature. Distilled water (3 mL) was added to each test tube to dilute the reaction mixture. The absorbance of blank containing buffer instead of plant extract and experimental was measured at 540 nm spectrophotometrically. The percentage of α -amylase inhibition is calculated by the following formula:

$$\text{Amylase inhibition (\%)} = [(\text{Control absorbance (blank)} - \text{sample absorbance}) / (\text{Control absorbance})] \times 100$$

In vivo study on experimental animals

Male Wistar albino rats (200–250 g) were purchased from the animal house, National Institute of Health

Sciences, Islamabad, and were used during this study. Fresh tap water and pellet diet procured from the market in Bannu were provided to the experimental animals. The animals were kept in cages under standard husbandry environment (12 hours light/dark cycle; 25 \pm 0.5°C). The animals were acclimatized to the local laboratory situations for 1 week before launch the experiment. The animals described were deprived of food and water for 16 hours before experiments.

Toxicity study

Healthy male rats were arranged in one normal and two experimental groups each with 6 rats, deprived them all of food overnight and gave free access to water the day before the experiment. The possible toxicity of *I. aucheri* leaves extract was determined by oral administration (1 mL) of its different doses (1 and 2 g/kg) to experimental groups once. Behavioral modification as well as mortality of the experimental animals were monitored for 24 hours (Chinedu et al., 2013). The signs of acute toxicity and mortality in extract-fed rats during observation period were not found which indicate that LD₅₀ of *I. aucheri* leaves extract is >2,000 mg/kg. On the basis of acute toxicity test result, 150 and 300 mg/kg doses of *I. aucheri* extract were chosen for the determination of its anti-diabetic activities.

Induction of diabetic in experimental rats

The mentioned animals were deprived of water and food for 16 hours fasting. Thereafter, the Wistar albino rats (260–290 g body weight) were injected with single intra-peritoneal dose of freshly prepared alloxan (120 mg/kg) in normal saline in a volume of 1 mL/kg in order to induce experimental diabetes in the rats (Negreş et al., 2013; Etuk, 2010; Kulkarni, 2005; Mule et al., 2016). The fasting blood glucose levels of rats were examined after 72 hours (Borgohain et al., 2002) and animals with fasting blood glucose level of \geq 200 mg/dL were considered diabetic (normal range of blood

glucose in rat is 80–120 mg/dL) (Kulkarni, 2005; Borgohain et al., 2002; Annida and Prince, 2005) and chosen for further experimentation.

Experimental protocol

In the present experiment 25 albino Wistar rats were divided into five groups of five rats each. Group 1: Normal rats (control); Group 2: Untreated diabetic rats (negative control); Group 3: Diabetic rats treated with glibenclamide at 10 mg/kg body weight; Group 4: Diabetic rats treated with methanolic extract of *I. aucheri* leaves at 150 mg/kg body weight; Group 5: Diabetic rats treated with methanolic extract at 300 mg/kg body weight. Gastric intubation of 16 gauge was used to nourish the rats orally with plant extract and glibenclamide, every morning for 15 days as the people traditionally use ethnomedicines orally for the treatment of various ailments (Suleman and Alemu, 2012).

Measurement of blood glucose level and weight

The fasting blood glucose levels were estimated prior to the start of treatment (t=0) and on day 7, 14 and 21 of treatment by using *I. aucheri* leaves glucometer (Medisign, England). Blood samples from rats were acquired by puncturing the tail vein aseptically.

Collection of serum samples

After the day 21 of oral administration, the animals were fasted overnight and on the day 22 the animals were anesthetized with chloroform and then sacrificed. Collected the animal's blood by cardiac puncture and put into plain sample bottles. For recovery and analysis of serum, the blood was allowed to clot for 2 hours and centrifuged at 3,000 rpm for 10 min. The supernatant (serum) was collected and poured into test tubes for further analyses.

Determination of serum lipid profile

The concentration of total cholesterol, HDL and triglycerides in blood serum was evaluated on a chemistry analyzer (Selectra, Netherlands) using commercially available kits (Gesam productions, Italy) and following the instructions and methods of the manufacturer. The

Friedwald equation was used to find out the concentration of very low density lipoproteins (VLDL) and the concentration of low density lipoproteins (LDL).

Determination of some indicators of liver and kidney function

Alanine and aspartate aminotransferase activities (ALT and AST), total bilirubin and creatinine were determined using the chemistry analyzer (Selectra, Netherlands) using commercially available kits (Gesam productions, Italy) and following the instructions and methods of the manufacturer.

Statistical analysis

It was conducted by using GraphPad Prism software. All the experiments were performed in triplicate and recorded the results as mean \pm SD (standard deviation). Experimental results were further analyzed for Pearson correlation coefficient between total phenolic contents and different antioxidant and anti-diabetic assays. One way ANOVA and Dunnett's-test were used *in vivo* analysis. The $p < 0.05$ was considered to be statistically significant.

Results

Phytochemical screening

Protein and amino acids, carbohydrates, glycosides, flavonoids and saponins were present in the methanolic extract whereas they were absent in the *n*-hexane fraction except flavonoids. Protein and amino acids, carbohydrates and glycosides were found in both aqueous and chloroform fractions (Table I).

Cytotoxic assay

The crude methanolic extract, chloroform, aqueous and *n*-hexane fraction indicated brine shrimp lethality up to 90.7 ± 1.8 , 60.3 ± 1.4 , 80.2 ± 1.2 and $40.3 \pm 1.7\%$ respectively at the concentration of 1,000 $\mu\text{g}/\text{mL}$ during brine shrimp lethality bioassay.

Table I

Phytochemical screening of *I. aucheri* leaves methanolic extract and its different fractions

Phytochemical	Tests	Crude methanolic extract and its fractions			
		Aqueous	Methanol	Chloroform	<i>n</i> -Hexane
Saponins	Alkaline reagent test	-	+	-	-
Alkaloid	Wagner's reagent	+	+	+	-
Flavonoids	Ferric chlorides test	+	+	-	+
Glycosides	Fehling	+	+	+	-
Carbohydrates	Millon's test	+	+	+	-
Protein and amino acids	Biuret test	+	+	+	-

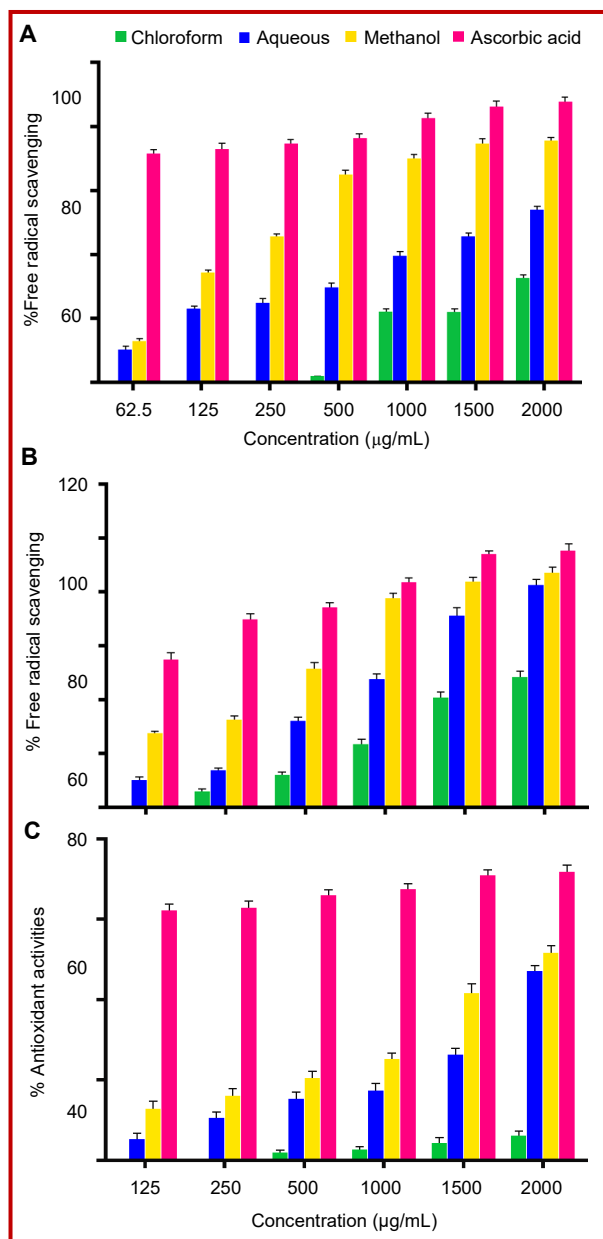


Figure 1: DPPH (A), ABTS (B) and H₂O₂ (C) free radical scavenging capability of *I. aucheri* leaves methanolic extract and its fractions

Total phenolic contents

The highest amount of phenolic contents was found in chloroform fraction (32.6 ± 2.0 mg GAE/g) while the lowest was observed in hexane fraction (5.0 ± 1.2 mg GAE/g).

Antioxidant assays

Antioxidant activities are measured indirectly. The antioxidant activities are based on the ability of compounds to interrupt free radicals by scavenging or trapping methods.

DPPH (1, 1-diphenyl -2- picrylhydrazyl) method

The crude methanolic extract exhibited maximum anti-oxidant activity (76.7%) followed by an aqueous fraction (51.4%) while chloroform fraction showed minimum activity (31.2%) at the concentrations of 2 mg/mL. At the same concentration ascorbic acid indicated 86% antioxidant activity. Figure 1A illustrates the antioxidant activities of applied standard and extracted samples.

The antioxidant activity of the *I. aucheri* methanolic extract and its various fractions were concentration dependent.

ABTS radical cation assay

In the current study, the maximum antioxidant activity was expressed by methanolic extract (97.3%) followed by an aqueous fraction (92.8%) and chloroform fraction (46.1%) (Figure 1B). The correlation between phenolic contents and antioxidant was well-known, however in the current study this correlation was different, the chloroform fraction containing higher phenolic contents (19.8 mg GAE/g) indicated lower antioxidant activity (46.1%), the methanolic extract had moderate phenolic contents (12.7 mg GAE/g), had the highest antioxidant activity (97.3%).

Hydrogen peroxide (H₂O₂) scavenging capacity

The applied extracts showed scavenging of hydrogen peroxide in a concentration-dependent manner (Figure 1C). The methanol extract (2,000 µg/mL) and its aqueous and chloroform fractions expressed 50.8, 49.0 and 4.7% free radical scavenging characteristics respectively whereas ascorbic acid revealed 71.0% at the same concentration.

Anti-diabetic: α -Amylase inhibition assay

Results of *in vitro* anti-diabetic activity of crude methanolic extract of *I. aucheri* and its different fractions by using alpha-amylase enzyme inhibition assay are presented in Figure 2A. The mentioned activities of glucophage (commercially available medicine used as a standard), methanolic extract and its aqueous fraction were found 49.3, 60.2 and 14.2% respectively.

Correlation among total phenolic contents, antioxidant and anti-diabetic activities

The correlation among total phenolic contents and percentage of antioxidant and anti-diabetic activities of the crude methanolic extract and its different fractions were represented by the Pearson correlation coefficient and found that it was non-significant in all cases. DPPH, ABTS and H₂O₂ methods expressed that the chloroform fraction with the higher phenolic content (32.6 mg GAE/g) indicated lower antioxidant and anti-diabetic properties.

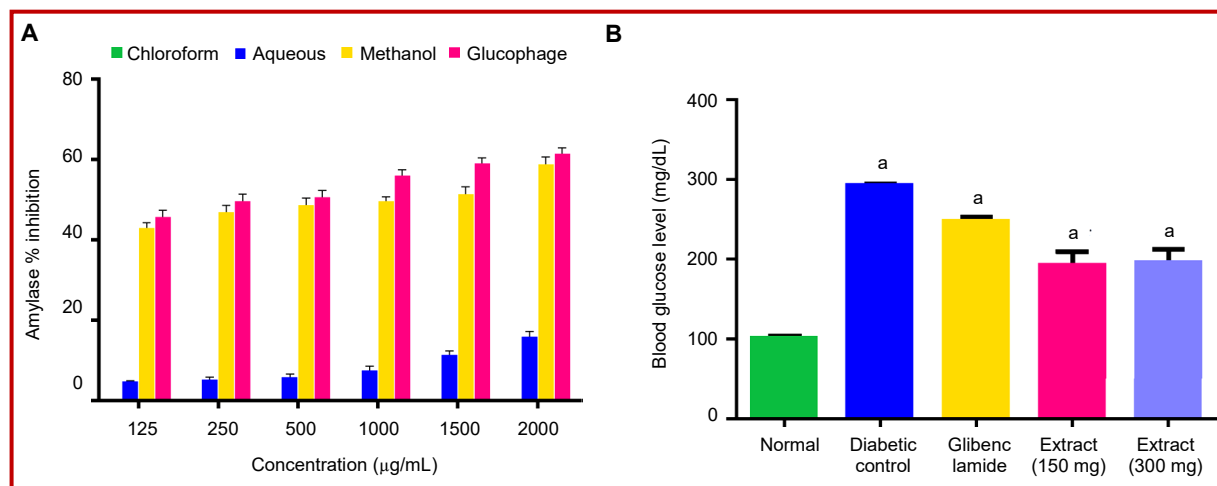


Figure 2: Anti-diabetic activity of *I. aucheri* leaves methanolic extract and its fractions as represented by %amylase inhibition (A). Chloroform extract had no effect; Effect of *I. aucheri* extract on blood glucose levels of diabetic rat (B). Data is represented as mean \pm SD; ^aSignificance at $p < 0.05$ (Dunnett's test); Normal was compared with the diabetic control, diabetic control were compared with the standard and extract-treated groups

Acute toxicity test

A single oral dose of *I. aucheri* methanolic extract (2 g/kg) did not reveal any symptom of acute toxicity or mortality in rats within 24 hours. The acute toxicity assessment expressed that the extract of *I. aucheri* was safe up to 2 g/kg and the approximate LD₅₀ value is more than 2 g/kg.

Effect of extract on blood glucose levels

The treatment of diabetic rats with the extract of *I. aucheri* (150 and 300 mg/kg) indicated considerable ($p < 0.01$) decrease in their blood glucose level (Figure 2B), hence expressing hypoglycemic nature of the applied extract. The diabetic control group showed a significant increase in the glucose level of blood during the study period with respect to normal control group.

Effect of extract on liver function tests

In experimental animals, the alloxan effects pancreas as well as it induces injuries in other organs like kidney and liver. To evaluate the hepatic injuries, the level of biochemical makers, serum transaminoferases, alkaline phosphatase and total bilirubin level, which were exceptionally susceptible to oxidative stress and toxic chemicals were used. The protective effects of methanolic extract of *I. aucheri* and glibenclamide against the alterations in the level of liver serum markers are indicated in Table II. The treatment of rats with alloxan considerably elevated the activity of liver serum marker enzymes which were lessened appreciably ($p < 0.05$) near to control group by oral administration of extract of *I. aucheri* in a dose-dependent manner. Likewise 10 mg/kg, of glibenclamide treatment considerably ($p < 0.05$) reversed the effects caused by alloxan intoxication.

Effect of extract on serum lipid levels

Increased level of lipid parameters like triglyceride, total cholesterol, LDL-cholesterol and VLDL-cholesterol were found in the serum of diabetic rats while HDL-cholesterol level has decreased (Table II). The parameters were considerably restored in dose-dependent manner in experimental rats treated with extract (150 and 300 mg/kg) of *I. aucheri*.

Effect extract on serum urea and creatinine levels

The defensive effect of *I. aucheri* extract at the concentration of 150 and 300 mg/kg had significantly ($p < 0.05$) recovered the raised serum level of kidney profile; serum creatinine and urea (Table III).

Discussion

The antioxidant and anti-diabetic activities of of *I. aucheri* leaf methanolic extract and its various fractions were found during *in vivo* and *in vitro* studies.

In the present study, the highest amount of total phenolic contents was found in chloroform fraction while the lowest was observed in hexane fraction. Another study of *Mimosa pudica* ethyl acetate fraction exhibited higher phenolic contents than chloroform fraction (Patro et al., 2016). The methanolic extract contains maximum quantity of total phenolic contents (Mahfuz et al., 2019). An indirect correlation between total phenolic content and antioxidant activity was found in this study. Several authors have found direct correlation between total content and antioxidant activity (Patro et al., 2016, Mahfuz et al., 2019) while others found inverse correlation (Ismail et al., 2004). The anti-diabetic activities of glucophage, methanolic extract and its aqueous fraction were found. The hexane fraction didn't show the mentioned activity

Table II					
Effect of <i>I. aucheri</i> methanolic extract on serum enzymes, total bilirubin and lipid profile levels					
Tests	Normal control	Diabetic control	Diabetic rat		
			Glibenclamide (10 mg)	Extract (150 mg)	Extract (300 mg)
Alanine transaminase ($\mu\text{mol/L}$)	145 \pm 1.9	236 \pm 3.4	158 \pm 2.9 ^b	154 \pm 25.6 ^b	143 \pm 5.9 ^b
Aspartate transaminase ($\mu\text{mol/L}$)	65 \pm 3.2	107 \pm 4.6	96.4 \pm 2.4 ^b	91.6 \pm 3.4 ^b	74 \pm 3.9 ^a
Alkaline phosphatase ($\mu\text{mol/L}$)	217 \pm 2.8	416 \pm 3.7	298 \pm 5.7 ^b	256 \pm 8.7 ^b	235 \pm 5.4 ^b
Total bilirubin ($\mu\text{mol/L}$)	1.1 \pm 0.3	1.96 \pm 0.4	1.2 \pm 0.9 ^b	1.3 \pm 0.2 ^a	1.1 \pm 0.1 ^b
Triglycerides (mg/dL)	96 \pm 1.9	175 \pm 11	112 \pm 4.2 ^b	122 \pm 7.8 ^b	114 \pm 7.2 ^b
Cholesterol (mg/dL)	88 \pm 1.9	97 \pm 3.2	93 \pm 2.9	95 \pm 4.5	91 \pm 2.2 ^a
LDL (mg/dL)	27 \pm 2.1	49 \pm 1.9	32 \pm 2.6 ^b	30 \pm 4.4 ^b	27 \pm 4.4 ^b
VLDL (mg/dL)	19 \pm 0.38	35 \pm 2.3	22 \pm 0.84 ^b	24 \pm 1.6 ^b	23 \pm 1.4 ^b
HDL (mg/dL)	45 \pm 5.8	28 \pm 1.7	36 \pm 2.4 ^a	41 \pm 5.7 ^b	45 \pm 5.6 ^b

Data are represented as mean \pm SD; Significance at ^ap<0.01 and ^bp<0.001 (Dunnett's-test), Normal was compared with the diabetic control, diabetic control were compared with the standard and extract treated groups

Table III		
Effect of <i>I. aucheri</i> leaves methanolic extract on serum level of serum creatinine and urea		
	Creatinine (mg/dL)	Urea (mg/dL)
Normal control	0.6 \pm 0.1	36.6 \pm 1.6
Diabetic control	1.6 \pm 0.2	53 \pm 4.2
Glibenclamide (10 mg)	1.0 \pm 0.2 ^a	42.4 \pm 2.4 ^a
Extract (150 mg)	0.9 \pm 0.3 ^a	40.2 \pm 3.7 ^a
Extract (300 mg)	0.7 \pm 0.1 ^a	37.4 \pm 2.8 ^a

Data is represented as Mean \pm SD, ^aIndicate significance at p<0.05 (Dunnett's-test), compared the normal with the diabetic control and in turn diabetic control was compared with the standard and extract treated groups

while in other studies hexane, ethyl acetate fractions and aqueous extract were found most active (Goel et al., 2012; Gurjar et al., 2016).

Identification of the mentioned phytochemicals provided the base for further study of plant extract. The results of the cytotoxic potential of *I. aucheri* methanolic extract and its various fractions were congruent with the cytotoxic activates *Chrysophthalmum montanum* (Aktiviteleri, 2017), *Arcangelisia flava*, *Coscinium blumeianum* and *Fibraurea tinctoria* (Keawpradub et al., 2005). It suggests that the applied extracts are possible potential sources of anticancer compounds. The phenolic compounds in the applied extract undergo a complex oxidation-reduction reaction with phosphotungstic and phosphomolybdic acids available in the Folin-Ciocalteu reagent. However this assay is not only specific for phenols but can be employed to any substance that could be oxidized by the said reagent and a number of researchers have mentioned the poor specificity of the assay (Singleton et al., 1999; Escarpa and González, 2001). Moreover, the

number of phenolic groups in phenolic compounds triggers variation in its response to the Folin-Ciocalteu reagent (Keawpradub et al., 2005). These results resemble with *Eclipta alba*, *Elephantopus scaber* and *Pluchea indica* that exhibited high total phenolic contents but lower antioxidant activities were observed (Indradi et al., 2017).

In the current study, we employed three different methods (DPPH, ABTS and H₂O₂ methods) to estimate antioxidant activities of plant methanolic extract and its aqueous, chloroform and hexane fractions because it is recommended to implement more than one method to achieve more confirmed results (Kazazic et al., 2016, Zengin et al., 2011). The observed differences in scavenging properties of applied extracts and its fractions against different systems may reveal that diverse antioxidant reactions mechanisms are adopted in the different assays. The higher free radical scavenging property of *Iphiona aucheri* leaves methanolic extract to scavenge more free radicals (ABTS⁺) might be due to higher molecular weight phenolics (tannins) and their usefulness depends on the number of aromatic rings, molecular weight and nature of hydroxyl group's substitution than the specific functional groups. Similar observations were reported in previous studies (Keawpradub et al., 2005, Hagerman et al., 1998). Different antioxidant activities are contributed by diverse antioxidant components, such as β -carotene, tocopherol, selenium, vitamin C or phenolic compounds (Ismail et al., 2004). Metabolic disorder of carbohydrate may lead to serious health problems including obesity and diabetic where deterioration in function or secretion of insulin causes reduction in the breakdown of disaccharides and polysaccharides and the ultimate onset of diabetes mellitus (Funke and Melzig, 2006).

In inhibition of alpha-amylase, carbohydrate digestive enzyme increases the time of carbohydrate digestion and decreases the glucose uptake by intestines (Cheng and Fantus, 2005). Thus, *in vitro* alpha-amylase inhibition assay was used to search out anti-diabetic properties of plant extract. The higher anti-diabetic properties of methanolic extract than glucophage provided a signal that the said extract is a potent source of phytochemicals having significant anti-diabetic characteristics. Congruent, *in vitro* results were shown by the extract of *P. guajava* leaves (Manikandan et al., 2016) and *Taraxacum officinale* leaves (Mir et al., 2015). Moreover, plants possess alpha-amylase inhibitors and are using for their protection from insects. The said inhibitors change the digestive action of alpha-amylases and proteinases in the gut of insects and reduce their usual feeding behavior (He, 1998). Owing to highest alpha amylase inhibition/anti-diabetic activities expressed by methanolic extract, it was subjected to *in vivo* studies and achieved good results. It has reported that phytochemical compounds have been effective antihyperglycemic, hypolipidemic, and antioxidant activity (Saxena and Vikram, 2004) The phytoconstituents can also restore enzymatic functions by healing pancreatic islets and repairing the kidneys and liver.

The methanolic extract of *I. aucheri* considerably restored the changes induced by alloxan in diabetic rats. Shiekuma and coworker, reported that the *Sorghum-tigernut Ibyer* extract due to its restorative effects on altered glucose level, enzyme and tissue damages, prevent the loss of weight in diabetic rats (Shiekuma et al., 2019). Similar results were obtained in previous anti-diabetic studies of *sorghum bicolor* grains (Olawole et al., 2018). As a rule, an increase in aminotransferases is a common sign of liver disease and is more common in patients with diabetes than in the general population. In addition, a number of diabetic complications, such as retinopathy, neuropathy, and limited joint mobility, are associated with liver enzyme activity, despite body mass index, alcohol consumption, and metabolic control of diabetes (Arkkila et al., 2001). A significant increase in the activity of certain enzymes, such as N-acetyl-beta-glucosaminidase, beta-glucuronidase, leucine aminopeptidase, and lysosomal acid phosphatase and cathepsin D, was noted after the introduction of alloxan in earlier studies (Witek et al., 2001).

In animals with diabetes, attenuation in serum enzymes is directly related to the metabolic changes in which these enzymes are involved. In the absence of insulin, the higher transaminase activity is due to the greater availability of amino acids in diabetes and is responsible for the enhanced ketogenesis and gluconeogenesis observed in diabetes. Therefore, a marked improvement in the level of these enzymes was the result of improved metabolism of carbohydrates,

fats and proteins. Recovery of bilirubin and ALT levels after treatment also indicated restoration of insulin secretion. Previous studies have also reported restorative characteristics of plant extracts of ALT, ALP, total bilirubin and AST in rats with alloxan diabetes (Khan et al., 2017, Rajaram, 2013). Diabetic hyperglycemia caused an increase in serum urea and creatinine, which were considered important markers of renal dysfunction (Saeed et al., 2008, Doi et al., 1991) and reflected a decrease in glomerular filtration rate. Congruent results were observed in an antidiabetic study of three varieties (Chal sorghum, Heuin sorghum and Hwangeumchal sorghum) from Korean sorghum (*Sorghum bicolor*) (Chung et al., 2011).

The occurrence of hypercholesterolemia with alloxan diabetes in rats has been reported and, therefore, of apparent hyperlipidemia which describes the state of diabetes can be considered as a result of inhibition of lipolytic hormones and, as a result, a decrease in the catalysis of fat deposits (Fernandes et al., 2007). The lipid-lowering effect may be associated with inhibition of fatty acid synthesis. As a rule, insulin activates lipoprotein lipase during metabolism which hydrolyzes triglycerides, while in a diabetic state insulin deficiency leads to inactivation of these enzymes and, therefore, causes hypertriglyceridemia (Khan, 2017). A significant decrease in serum lipid levels in diabetic rats after treatment with methanolic extract can be directly associated with an improvement in insulin levels. Similar results were obtained in an antidiabetic study of the aerial parts of *tricornutum* extracts (Khan et al., 2017). This finding suggests that the different chemical present in a plant did not separate according to the polarity of solvents applied in this study but may be accumulated and distributed non-directionally in terms of yield (Batoool et al., 2019). The changes in characteristics of extract might be ascribed to the joint influences of both growing conditions and genetic factors (Kaur and Arora, 2009, Ghahremanimajd et al., 2012).

Conclusion

I. aucheri leaves possess significant cytotoxic, anti-oxidant, hypolipidemic and anti-diabetic activities.

Financial Support

Self-funding

Ethical Issue

All experiments were performed according to the guidelines of Institutional Animals Ethics Committee (Ref. No. USTB/Biotech/Ethical/123).

Conflict of Interest

Authors declare no conflict of interest

Acknowledgements

We are thankful to all my friends, Dr. Mir Sadiq Shah and Sami Ullah Khan and my colleague, Manzoor Ullah Khan for their guidance and support.

References

- Ahmed J, Khan MR, Mughal ZU, Batool R, Naz I, Maryam S, Zahra Z. Methanol extract of *Iphiaea aucheri* ameliorates CCl₄-induced hepatic injuries by regulation of genes in rats. *Toxicol Res.* 2019; 8: 815-32.
- Aktiviteleri İ. Cytotoxic, Phytotoxic and Insecticidal Activities of *Chrysophthalmum montanum* (DC.) Boiss. *Turk J Pharm Sci.* 2017; 14: 290-93.
- Amarowicz R, Naczek M, Shahidi F. Antioxidant activity of various fractions of non-tannin phenolics of canola hulls. *J Agric Food Chem.* 2000; 48: 2755-59.
- Ananthi J, Prakasam A, Pugalendi KV. Antihyperglycemic activity of *Eclipta alba* leaf on alloxan-induced diabetic rats. *Yale J Biol Med.* 2003; 76: 97-102.
- Annida B, Prince PSM. Supplementation of fenugreek leaves reduces oxidative stress in streptozotocin-induced diabetic rats. *J Med food.* 2005; 8: 382-85.
- Arkkila PE, Koskinen PJ, Kantola IM, Rönnemaa T, Seppänen E, Viikari JS. Diabetic complications are associated with liver enzyme activities in people with type 1 diabetes. *Diabetes Clinl Pract.* 2001; 52: 113-18.
- Bahar E, Akter KM, Lee GH, Lee HY, Rashid HO, Choi MK, Bhattarai KR, Hossain MM, Ara J, Mazumder K, Raihan O, Chae HJ, Yoon H. β -Cell protection and antidiabetic activities of *Crassocephalum crepidioides* (Asteraceae) Benth. S. Moore extract against alloxan-induced oxidative stress via regulation of apoptosis and reactive oxygen species (ROS). *BMC Complement Altern Med.* 2017; 17: 179.
- Batool R, Khan MR, Sajid M, Ali S, Zahra Z. Estimation of phytochemical constituents and *in vitro* antioxidant potencies of *Brachychiton populneus* (Schott & Endl.) r. Br. *BMC Chem.* 2019; 13: 32.
- Borghain R, Lahon K, Das S, Gohain K. Evaluation of mechanism of anti-diabetic activity of *Terminalia chebula* on alloxan and adrenaline-induced diabetic albino rats. *Drugs* 2002; 19: 05.
- Cheng AY, Fantus IG. Oral antihyperglycemic therapy for type 2 diabetes mellitus. *Can Med Assoc J.* 2005; 172: 213-26.
- Chethan J, Kumar PM, Prakash HS. Anti-diabetic and antihypertensive potential of selected Asteraceae plant species. *Am J Adv Drug Deliv.* 2014; 2: 355-63.
- Chinedu E, Arome D, Ameh FS. A new method for determining acute toxicity in animal models. *Toxicol Int.* 2013; 20: 224.
- Chung IM, Kim EH, Yeo MA, Kim SJ, Seo MC, Moon HI. Antidiabetic effects of three Korean sorghum phenolic extracts in normal and streptozotocin-induced diabetic rats. *Food Res Int.* 2011; 44: 127-32.
- Daradka HM, Abas MM, Mohammad MAM, Jaffar MM. Antidiabetic effect of *Artemisia absinthium* extracts on alloxan-induced diabetic rats. *Comp Clin Pathol.* 2014; 23: 1733-42.
- Doi K, Kurabe S, Shimazu N, Inagaki M. Systemic histopathology of rats with CCl₄-induced hepatic cirrhosis. *Lab Anim.* 1991; 25: 21-25.
- Escarpa A, González M. Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic and spectrophotometric methods. *Anal Chim Acta.* 2001; 427: 119-27.
- Etuk E. Animals models for studying diabetes mellitus. *Agric Biol JN Am.* 2010; 1: 130-34.
- Fernandes NP, Lagishetty CV, Panda VS, Naik SR. An experimental evaluation of the antidiabetic and antilipidemic properties of a standardized *Momordica charantia* fruit extract. *BMC J Altern Complement Med.* 2007; 7: 29.
- Funke I, Melzig MF. Traditionally used plants in diabetes therapy: Phytotherapeutics as inhibitors of alpha-amylase activity. *Rev Bras Farmacogn.* 2006; 16: 1-5.
- Gallé JB, Groeber S, Ledoux A, Nicolas JP. Quelques plantes employées dans le Sud-Ouest de Madagascar. *Madagascar: Jardins du Monde.* 2014.
- Gahremani-majd H, Dashti F, Dastan D, Mumivand H, Hadian J, Esna-Ashari M. Antioxidant and antimicrobial activities of Iranian mooseer (*Allium hirtifolium* Boiss) populations. *Hortic Environ Biotechnol.* 2012; 53: 116-22.
- Goel R, Bhatia D, Gilani SJ, Katiyar D. Medicinal plants as anti-diabetics. A review. *Int Bull Drug Res.* 2012; 1: 100-07.
- Gurjar HP, Irchhaiya R, Verma A. Review on some medicinal plants with antidiabetic activity. *J. Drug Deliv. Ther.* 2016;6:45-51.
- Gyamfi MA, Yonamine M, Aniya Y. Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. *Gen Pharmacol-Vasc S.* 1999; 32: 661-67.
- Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel TL. High molecular weight plant polyphenolics (tannins) as biological antioxidants. *J Agric Food Chem.* 1998; 46: 1887-92.
- He L. Alpha-glucosidase inhibitors as agents in the treatment of diabetes. *Diabetes Rev.* 1998; 6: 132-45.
- Holt RI, Barnett AH, Bailey CJ. Bromocriptine: Old drug, new formulation and new indication. *Diabetes Obes Metab.* 2010; 12: 1048-57.
- Indradi RB, Fidrianny I, Wirasutisna KR. DPPH scavenging activities and phytochemical content of four Asteraceae plants. *Int J Pharmacogn Phytochem Res.* 2017; 9: 755-59.
- Ismail A, Marjan ZM, Foong CW. Total antioxidant activity and phenolic content in selected vegetables. *Food Chem.* 2004; 87: 581-86.
- Jani RH, Kansagra K, Jain MR, Patel H. Pharmacokinetics,

- safety, and tolerability of saroglitazar (ZYH1), a predominantly PPAR α agonist with moderate PPAR γ agonist activity in healthy human subjects. *Clin Drug Invest.* 2013; 33: 809-16.
- Kakar SA, Tareen RB, Kakar MA, Jabeen H, Kakar SU, Al-Kahrman YM, Shafee M. Screening of antibacterial activity of four medicinal plants of Balochistan-Pakistan. *Pakistan J Bot.* 2012; 44: 245-50.
- Kaur GJ, Arora DS. Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*. *BMC Complement Altern Med.* 2009; 9: 30.
- Kazazic M, Djapo M, Ademovic E. Antioxidant activity of water extracts of some medicinal plants from Herzegovina region. *Int J Pure App Biosci.* 2016; 4: 85-90.
- Keawpradub N, Dejaisai S, Yuenyongsawad S. Antioxidant and cytotoxic activities of Thai medicinal plants named Khaminkhruea: *Arcangelisia flava*, *Coscinium blumeianum* and *Fibraurea tinctoria*. *Songklanakar J Sci Technol.* 2005; 27: 455-67.
- Khan RA. Antidiabetic, antioxidant, and hypolipidemic potential of *Sonchus asper* Hill. *Altern Ther Health M.* 2017; 23: 34-40.
- Khan ST, Ahmed M, Khan RA, Mushtaq N, Khan N. Antidiabetic potential of aerial parts of *Galium tricornerutum* (Dandy) Rubiaceae. *Trop J Pharm Res.* 2017; 16: 1573-78.
- Kharbanda C, Alam M S, Hamid H, Javed K, Shafi S, Ali Y, Alam P, Pasha MAQ, Dhulap A, Bano S, Nazreen S, Haider S. Novel benzenesulfonylureas containing thiophenyl-pyrazoline moiety as potential anti-diabetic and anticancer agents. *Bioorg Med Chem Lett.* 2014; 24: 5298-303.
- Kulkarni S. Commonly used drugs, their doses and nature of action in laboratory animals. *Handb Exp Pharmacol.* 2005; 3: 190-95.
- Kwon YI, Apostolidis E, Shetty K. Evaluation of pepper (*Capsicum annuum*) for management of diabetes and hypertension. *J Food Biochem.* 2007; 31: 370-85.
- Mahfuz A, Salam FBA, Deepa KN, Hasan AHMN. Characterization of *in vitro* antioxidant, cytotoxic, thrombolytic and membrane stabilizing potential of different extracts of *Cheilanthes tenuifolia* and stigmasterol isolation from n-hexane extract. *Clin Phytosci.* 2019; 5: 39.
- Manikandan R, Anand AV, Kumar S. Phytochemical and *in vitro* anti-diabetic activity of *Psidium guajava* leaves. *Pharmacogn J.* 2016; 8: 392-94.
- Mehanna A. Anti-diabetic agents: Past, present and future. *Future Med Chem.* 2013; 5: 411-30.
- Meyer B, Ferrigni N, Putnam J, Jacobsen L, Nichols DJ, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Med.* 1982; 45: 31-34.
- Mir MA, Sawhney S, Jassal M. *In-vitro* anti-diabetic studies of various extracts of *Taraxacum officinale*. *Pharm Innov.* 2015; 4: 61.
- Mule V, Naikwade N, Magdum C, Jagtap V. Antidiabetic activity of extracts of *Pithecellobium dulce* Benth leaves in alloxan induced diabetic rats. *Int J Pharm Sci Drug Res.* 2016; 8: 275-80.
- Negres S, Chiriță C, Moroșan E, Arsene AL. Experimental pharmacological model of diabetes induction with alloxan in rat. *Farmacia* 2013; 61: 313-23.
- Olawole TD, Okundigie MI, Rotimi SO, Okwumabua O, Afolabi IS. Preadministration of fermented sorghum diet provides protection against hyperglycemia-induced oxidative stress and suppressed glucose utilization in alloxan-induced diabetic rats. *Front Nutr.* 2018; 5: 16.
- Patel D, Prasad S, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pac J Trop Biomed.* 2012; 2: 320-30.
- Patro G, Bhattamisra SK, Mohanty BK, Sahoo HB. *In vitro* and *in vivo* antioxidant evaluation and estimation of total phenolic, flavonoidal content of *Mimosa pudica* L. *Pharmacogn Res.* 2016; 8: 22.
- Piero M, Maria CB, Aldo B. NO-glibenclamide derivatives: Prototypes of a new class of nitric oxide-releasing anti-diabetic drugs. *Bioorg. Med Chem.* 2009; 17: 5426-5432.
- Proks P, Reimann E, Green N. Sulfonylurea stimulation of insulin secretion. *Diabetes* 2005; 51: S368-S376.
- Rajaram K. Antioxidant and anti-diabetic activity of *Tectona grandis* Linn. in alloxan-induced Albino rats. *Drugs* 2013; 4: 5.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Bio Med.* 1999; 26: 1231-37.
- Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med.* 1996; 20: 933-56.
- Saeed MK, Deng Y, Dai R. Attenuation of biochemical parameters in streptozotocin-induced diabetic rats by oral administration of extracts and fractions of *Cephalotaxus sinensis*. *J Clin Biochem Nutr.* 2008; 42: 21-28.
- Sagbo IJ, van de Venter M, Koekemoer T, Bradley G. *In vitro* anti-diabetic activity and mechanism of action of *Brachylaena elliptica* (Thunb.) DC. *Evid Based Complement Alternat Med.* 2018; 2018.
- Saxena A, Vikram NK. Role of selected Indian plants in management of type 2 diabetes: A review. *J Altern Complement Med.* 2004; 10: 369-78.
- Schofield JD, Liu Y, Rao-Balakrishna P, Malik RA, Soran H. Diabetes dyslipidemia. *Diabetes Ther.* 2016; 7: 203-19.
- Senthilkumar R, Ahmedjohn S, Archunan G, Manoharan N. Antioxidant activity of *Wedelia chinensis* in alloxan-induced diabetic rats. *Pharmacologyonline* 2008; 2: 640-51.
- Shah MAR, Khan RA, Ahmed M, Ullah M, Fazal D. *In vitro* evaluation of antibacterial and antifungal activities of *Iphionia aucheri* leaves extracts. *Int J Biosci.* 2019; 15: 175-82.
- Shiekuma S, Ukeyima M, Msendoo JA, Blessing I, Tughgba T. Effect of Sorghum-tigernut Ibyer (A Traditional Gruel) on the fasting blood glucose levels of alloxan-induced diabetic rats. *Eur J Nutr Food Saf.* 2019; 29: 260-68.
- Singleton V, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol*

- Vitic. 1965; 16: 144-58.
- Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Method Enzymol.* 1999; 299: 152-78.
- Shylaja G, Sasikumar K, Sathiavelu A. Antimycobacterial potential of resorcinol type lipid isolated from *Chaetomium cupreum*, an endophytic fungus from *Mussaenda luteola*. *Bangladesh J Pharmacol.* 2018; 13: 114-19.
- Skyler JS, Bakris GL, Bonifacio E, Darsow T, Eckel RH, Groop L, Groop P-H, Handelsman Y, Insel RA, Mathieu C. Differentiation of diabetes by pathophysiology, natural history, and prognosis. *Diabetes.* 2017;66:241-255.
- Stewart MJ, Steenkamp V. The biochemistry and toxicity of atracyloside: A review. *Ther Drug Monit.* 2000; 22: 641-49.
- Suleman S, Alemu T. A survey on utilization of ethnomedicinal plants in Nekemte town, East Wellega (Oromia), Ethiopia. *J Herbs Spices Med Plants.* 2012; 18: 34-57.
- Trease GE, Evans WC. *Phytochemical screening.* In: *Pharmacognosy.* Evans WC (ed). 11th ed. Canada, Macmillian Publishers, 1989.
- Tschöpe D, Hanefeld M, Meier JJ, Gitt AK, Halle M, Brämke P, Schumm-Draeger PM. The role of co-morbidity in the selection of antidiabetic pharmacotherapy in type-2 diabetes. *Cardiovasc Diabetol.* 2013; 12: 62.
- Varas-Lorenzo C, Margulis AV, Pladevall M, Riera-Guardia N, Calingaert B, Hazell L, Romio S, Perez-Guttham S. The risk of heart failure associated with the use of noninsulin blood glucose-lowering drugs: Systematic review and meta-analysis of published observational studies. *BMC Cardiovasc Disord.* 2014; 14: 129.
- Vijayaraghavan K. Treatment of dyslipidemia in patients with type 2 diabetes. *Lipids Health Dis.* 2010; 9: 144.
- Witek B, Krol T, Kolataj A, Ochwanowska E, Stanislawski I, Siewka A. The insulin, glucose and cholesterol level and activity of lysosomal enzymes in the course of the model alloxan diabetes. *Neuroendocrinol Lett.* 2001; 22: 238-42.
- Yousef F, Yousef N, Mansour O, Herbali J. Metformin: A unique herbal origin medication. *Glob J Med Res.* 2017; 17.
- Zengin G, Aktumsek A, Guler GO, Cakmak YS, Yildiztugay E. Antioxidant properties of methanolic extract and fatty acid composition of *Centaurea urvillei* DC. subsp. hayekiana Wagenitz. *Records Nat Prod.* 2011; 5: 123.

Author Info

Rahmat Ali Khan (Principal contact)

e-mail: Rahmatgul_81@yahoo.com