http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2020.1357.1368

Research Article
Evaluation of Acute and Chronic Antidiabetic Activity of Ivy (*Hedera helix* L.) Aqueous Leaf Extract in Rat Model

[1]Saira Saeed Khan, [1]Anum Adil, [2]Sadaf Naeem, [3]Nazish Jaffar, [4]Humera Khatoon, [2]Humera Ansar and [2]Yousra Shafiq

[1]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
[2]Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan
[3]Sindh Medical College, Jinnah Sindh Medical University, Karachi, Pakistan
[4]Jinnah University for Women, Karachi, Pakistan

Abstract

Background and Objective: *Hedera helix* L. (Ivy) has been utilized as an alternative medicine for cough however, through extensive literature search; we found no reported activity of ivy on α-glucosidase inhibition, HbA1c levels and its protective effect on vital organs. Therefore, the present study aimed to evaluate the antidiabetic and protective effect of ivy in alloxan induced rat model. **Materials and Methods:** The hypoglycemic activity of ivy was examined in normoglycemic, glucose overloaded and alloxan-induced rats. The antidiabetic potential was also confirmed by estimation of HbA1c and α-glucosidase inhibitory activity. **Results:** Results of acute and chronic study revealed that ivy produced highly significant decline ($p<0.01$) in fasting and post-prandial blood sugar levels as compared to diabetic control and standard group respectively. Furthermore, highly significant decline ($p<0.01$) in HbA1c levels were seen after chronic administration of ivy indicating its therapeutic effect in lowering HbA1c levels during long term use. It was found that ivy produced stronger and highly significant ($p<0.05$) inhibition of α-glucosidase activity than the standard agent acarbose at 500 μg mL^{-1}. **Conclusion:** The histopathological studies of vital organs revealed protective effect of ivy via maintaining the normal architecture as compared to alloxan model. Hence, our findings support the potential use of ivy for diabetes management.

Key words: *Hedera helix* L. (ivy), antidiabetic activity, alloxan, glycated hemoglobin, α-glucosidase activity

Citation: Khan, S.S., A. Adil, S. Naeem, N. Jaffar, H. Khatoon, H. Ansar and Y. Shafiq, 2020. Evaluation of acute and chronic antidiabetic activity of ivy (*Hedera helix* L.) Aqueous leaf extract in rat model. Pak. J. Biol. Sci., 23: 1357-1368.

Corresponding Author: Sadaf Naeem, Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi Pakistan Tel: +923012925497

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hedera helix L. also known as English ivy or ivy is a perennial climbing plant of Araliaceae family, with lustrous, deep green, coriaceous leaves. Ivy is a famous herbal plant that has been utilized for the management of conditions associated with diabetes amongst the native populations of Asia, East Africa, India, South America and the Caribbean[1]. The active biological compounds accountable for the beneficial therapeutic use of *Hedera helix* L. are triterpene saponins (2.5-6%): the bidesmosidic glycosides of hederagenin: hederacoside C (1.7-4.8%), hederacoside D (0.4-0.8%), hederacoside B (0.1-0.2%) and monodesmoside α-hederin (0.1-0.3%)[2]. Several previous studies have demonstrated the medicinal value of this plant such as antibacterial[3], anthelmintic[4], antileishmanial[5,6], *in vitro* antispasmodic[7], hemolytic[8], anticarcinogenic/antitumor[9] and antifungal properties of *H. helix* L. extract[10]. Extract of *H. helix* L. also expressed anti-elastase, anti-hyaluronase, secretolytic, spasmolytic, antimicrobial and hepatoprotective activities[11].

Beside all these medicinal uses only few researchers explored its antidiabetic potential[12,13]. Although several parts of *Hedera helix* L. such as fruit, leaf and stem, have been utilized as alternative medicines for the management of diabetes. However, there is narrow pharmacological basis for its application as a therapeutic agent. Researcher did extensive literature search but could not find any reported α-glucosidase inhibitory effect of ivy leaf extract. Moreover, the effect of ivy leaf extract on HbA_1c levels and its protective effect on vital organs such as Liver and Kidney have not been reported. Therefore, the present study was carried out to evaluate the antidiabetic and protective effect of ivy leaf extract in rat model. In addition to the effects of ivy leaf extract on RBG, FBG, HbA_1c, *in vitro* α-glucosidase activity were also examined as one of the possible mechanisms underlying antidiabetic activity of the leaf extract.

MATERIALS AND METHODS

Study area: The study was carried out from the month of March to July 2019 in the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi Pakistan.

Chemicals and plant material: The standard hypoglycemic drug gliclazide was purchased from market manufactured by local pharmaceutical company and given in the dose of 10 mg kg^{-1}. Other reagents used in the study such as alloxan monohydrate, glucose and DMSO (3%) were purchased from Sigma Aldrich Pvt. Ltd, NaCl (0.9%) and distilled water were obtained from Otsuka Pakistan Ltd. Dry powdered leaf extract of *Hedera helix* L. (Filzenberg, Germany) was obtained from Medics Laboratories Pvt. Ltd, Karachi, Pakistan.

Test sample preparation: The aqueous extract was prepared by dissolving the powdered leaf extract of *Hedera helix* L. in distilled water, stirred well until dissolved. The standard hypoglycemic drug gliclazide was suspended in isotonic saline solution with DMSO (3%) and was given orally, after an overnight fast, maintaining the volume of the vehicle constant at 1 mL. The dose for each animal was calculated according to their body weights and extract was then administered orally by gavage technique.

Animals: Adult male Sprague-Dawley (SD) rats (200-250 g) were purchased from the animal house of the HEJ, University of Karachi. The design of the experiment was endorsed by the BASR Committee under the reference number BASR/No. /00368/Pharm. and the animals were handled as per the guidelines provided by National research council, 1996. Rats were accommodated in standard cages where rat chow and water were provided ad libitum.

Measurement of α-glucosidase activity: The α-glucosidase activity of the sample was measured by reference to the method of Kim *et al.*[14]. The enzyme α-glucosidase was dissolved in 100 mM sodium phosphate buffer (pH 7.0) at 0.2 unit mL^{-1} concentration. A total of 2 µL of the sample and 100 µL of the enzyme solution were mixed well. Mixed samples were transferred to 96-well plate and absorbance at 405 nm was measured. Then, 2 mM p-nitrophenyl α-D-glucopyranoside (100 µL) was rapidly added into 96-well plate and the absorbance at 405 nm was further measured. Acarbose (100 µg mL^{-1}) which was used to proven the α-glucosidase inhibitory activity was used as a positive control and the α-glucosidase activity was calculated according to the following formula[15]:

$$\text{Inhibitory activity (\%)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

Normoglycemic rats: Normoglycemic blood glucose test was conducted to evaluate the effect of *H. helix* L. leaf extract on normal blood glucose level. Forty non-diabetic male SD rats were separated into 4 groups (n = 10). Control Group only received distilled water (10 ml kg^{-1}). Standard Group was given gliclazide (10 mg kg^{-1}). Two groups were given aqueous

leaf extract of *Hedera helix* L. in the doses equivalent to 3 and 4 g kg^{-1} of dried leave powder[12]. The dose of extract was calculated from simple unitary method. The yield of extract was 1 kg per 6 kg of dried powder leave. Therefore the dose of extract was approximately 500 mg kg^{-1} for 3 and 650 mg kg^{-1} for 4 g kg^{-1} of dried powder respectively. All drugs and distilled water were given orally by using gavage technique[16].

The alteration in normal blood glucose level were determined at 0, 30, 60 and 120 min interval after administration of drugs using Accu Chek Glucometer, Roche, Switzerland.

Rats subjected to Oral Glucose Tolerance Test (OGTT): The oral glucose tolerance test was performed as modified by Du Vigneaud and Karr[17] to assess the effect of *Hedera helix* L. aqueous leaf extract on raised serum glucose levels stimulated by glucose challenge. The same SD rats used to assess normoglycemic effect were reused for OGTT after a washout period of fifteen days. Forty non-diabetic male Sprague Dawley rats were separated into four groups (n = 10). Control group received only distilled water (10 mL kg^{-1}), standard group received gliclazide (10 mg kg^{-1}), while remaining two groups received aqueous leaf extract of *Hedera helix* L. in the doses[12] of 500 and 650 mg kg^{-1}. All drugs and distilled water were given orally using gavage technique.

The baseline blood sugar levels (BGLs) were measured by Accu-Check Active glucometer Roche, Switzerland. After 30 min of administration of drugs, the rats in each group were orally administered with 200 mg kg^{-1} glucose in the form of solution. The blood glucose levels were determined by collecting blood sample from tail vein at the time interval of 0, 30, 60 and 120 min after drug administration.

Alloxan induced diabetic rats: Rats were made diabetic by intraperitoneal administration of alloxan monohydrate in sterile normal saline at a dose of 150 mg kg^{-1} body weight. Prior to administration of alloxan, the animals were kept fasted for 12 h with free access to water. The animals were kept under observation. After 48 h, the animals were tested for BGL using Accu-Chek strips Roche, Switzerland. The blood glucose level was checked before and 72 h after alloxan injection to ensure the development of diabetes. The diabetic animals were stabilized for one week and the experiment was commenced on the next day (day 0). Animals having blood glucose >250 mg dL^{-1} were only considered diabetic and used for the study[18].

Acute hypoglycemic effect of *Hedera helix* leaf extract

Fasting blood sugar level: After two weeks of acclimation, forty diabetic SD rats were randomly separated into four groups each group having 10 animals.

The animals in diabetic control group were given only distilled water (10 mL kg^{-1}), while animals in standard group, received gliclazide in the dose of 10 mg kg^{-1}. The animals in treatment group received *H. helix* L. aqueous leaf extract in two different doses[12] i.e. 500 and 650 mg kg^{-1}.

All the rats were fasted over a period of 12 hours, then drugs were administered and fasting blood glucose level were determined at 0, 30, 60 and 120 min time interval using Accu Check Glucometer, Roche, Switzerland.

Post-prandial blood sugar level: The same protocol and grouping pattern were followed for testing post-prandial blood sugar level. The rats were fed in the morning and then after 2 hours the drugs were administered and random blood glucose level were checked at 0, 30, 60 and 120 min time interval using Accu Check Glucometer, Roche, Switzerland.

Chronic hypoglycemic effect of *Hedera helix* leaf extract: After two weeks of acclimation, forty diabetic SD rats were randomly separated into four groups each group having 10 animals.

The animals in diabetic control group were given only vehicle, while animals in standard group, received gliclazide in the dose of 10 mg kg^{-1} and animals in the treatment groups received *Hedera helix* L. aqueous leaf extract in two different doses i.e. 500 and 650 mg kg^{-1} daily for sixty days[12].

After sixty days blood samples from all rats were collected and centrifuged to estimate HbA_1c in serum. However both fasting and post-prandial blood sugar level in all animals used in the study were determined at various time intervals i.e. at day 15, 30, 45 and 60. Fasting Blood Sugar (FBS) was determined after 12 h of fasting, the rats were then fed and drug was administered and then post-prandial blood sugar level was estimated 2 h after meal.

After estimation of HbA_1c, fasting and post prandial glucose level animals were randomly sacrificed. The liver and kidney were removed and were preserved in 10% formalin solution for histopathological examination. Sections of 5 µ thickness were cut and paraffin embedded blocks was prepared. Hematoxylin and eosin staining was performed and all slides were examined under light microscopy using scanner (4×), low power (10×) and high power (40×) lenses by a histopathologist.

Statistical analysis: All the values were shown as Mean±SEM (standard error of mean). Statistical analysis were performed by one way ANOVA (analysis of variance) followed by Post-Hoc Tukey's Multiple Comparison Test. Statistically significant differences were considered in relation to control and standard at the value of probability less than 5% ($p<0.05$). However the values less than 1% ($p< 0.01$) were considered as highly significant.

RESULTS

Determination of α-glucosidase inhibitory effect: Table 1 show that the higher the concentration *Hedera helix* L. leaf extract, the greater the inhibitory activity of α-glucosidase. The inhibitory activity of ivy leaf extract at 100 μg mL^{-1} concentration was insignificant as compared to the standard acarbose. However, at 250 μg mL^{-1} concentration ivy leaf extract produced a significant ($p<0.05$) inhibition of α-glucosidase enzyme than the standard group. At the concentration of 500 μg mL^{-1}, ivy leaf extract exhibited highly significant inhibition ($p<0.01$) of α-glucosidase enzyme than the standard Acarbose group. Ivy aqueous leaf extract showed better inhibition of α-glucosidase enzyme than acarbose which is commercially used antidiabetic drug to manage type 2 diabetes mellitus. Based on our results, ivy leaf extract may improve antidiabetic effects by upregulating α-glycosidase activity.

Effect in normoglycemic rats: The effects of two doses of *Hedera helix* L. aqueous leaf extract were assessed in normoglycemic animals on FBS at different time intervals.

Table 2 revealed the effect of 500 and 650 mg kg^{-1} of *Hedera helix* L. aqueous leaf extract in normoglycemic animals. The effects were almost similar to control after 60 and 120 min administration of *Hedera helix* L. leaf extract. The aqueous extract at both doses showed significant increase in FBS after 30 min as compared to control. However, 500 mg kg^{-1} dose showed significant increase at 30 min as compared to standard.

Effects after oral glucose tolerance test: Table 3 showed the levels of blood glucose in normal, standard and treated groups after oral glucose tolerance test (200 mg kg^{-1} b.wt). Standard drug gliclazide and *H. helix* L. aqueous leaf extract at 500 mg kg^{-1} dose showed significant increase in blood glucose level at 30 min. Whereas, at 120 min there was significant and highly significant decline in blood glucose at 500 and at 650 mg kg^{-1} dose of *Hedera helix* L. aqueous leaf extract as compared to normal control respectively. However at the same time interval, *Hedera helix* L. 500 mg kg^{-1} produced a comparable decline in blood glucose level as compared to the standard group. Overall in both the standard and experimental groups (500 and 650 mg kg^{-1}), there is a gradual decline in blood glucose level

Table 1: Effect of *Hedera helix* L. on α-glucosidase enzyme activity

Concentration (μg mL^{-1})	Standard acarbose	Ivy leaf extract
100	22.66±3.67	16.25±2.62
250	27.91±5.21	13.28±1.72$^{\#}$
500	35.71±2.79	18.59±3.22$^{\#\#}$

Values are in Mean±SEM, n = 5, $^{\#\#}p<0.01$ highly significant as compared to standard, $^{\#}p<0.05$ significant as compared to standard

Table 2: Effect of *Hedera helix* L. on normal blood glucose level

	Blood glucose (mg dL^{-1})			
Time (min)	Normal control	Standard	*H. helix* L. (500 mg kg^{-1})	*H. helix* L. (650 mg kg^{-1})
0	80.50±1.59	89.00±3.05	89.90±3.59	83.90±2.14
30	78.40±2.06	82.80±3.11	94.60±3.69*$^{\#}$	89.00±2.27*
60	87.78±1.79	76.8±3.37	85.00±3.46	83.11±2.25
120	87.20±1.49	69.10±2.82	79.00±3.34	73.00±2.67

Values are in Mean±SEM, n = 10, *$p<0.05$ significant as compared to normal control, $^{\#}p<0.05$ significant as compared to standard drug

Table 3: Effect of *Hedera helix* L. on glucose level after oral glucose tolerance test

	Blood glucose (mg dL^{-1})			
Time (min)	Normal control	Standard	*H. helix* L. (500 mg kg^{-1})	*H. helix* L. (650 mg kg^{-1})
0	79.60±1.57	85.60±3.91	89.20±3.67	89.60±4.67
30	117.50±2.26	131.50±3.76*	131.20±3.51*	128.40±4.29
60	118.30±1.98	111.00±3.86	106.20±3.29	111.20±4.47
120	126.90±1.59	97.70±3.59	98.69±2.75*	103.72±4.59**

Values are in Mean±SEM, n = 10, *$p<0.05$ significant as compared to normal control, **$p<0.01$ highly significant as compared to normal control

Table 4: Acute Hypoglycemic effect of *Hedera helix* L. on fasting blood sugar

	Blood glucose (mg dL^{-1})			
Time (min)	Diabetic control	Standard	*H. helix* L. (500 mg kg^{-1})	*H. helix* L. (650 mg kg^{-1})
0	449.10±17.28	388.20±18.55	356.40±23.66**	372.10±20.67*
30	455.10±17.52	292.00±18.76**	346.60±23.8**	363.70±20.43**
60	464.10±17.45	249.30±17.45**	329.90±23.93**	360.60±19.86**
120	473.90±16.97	224.30±18.75**	309.40±23.56**	325.90±22.93**

Values are in Mean±SEM, n = 10, *p<0.05 significant as compared to diabetic control, **p<0.01 highly significant as compared to diabetic control

Table 5: Acute hypoglycemic effect of *Hedera helix* L. on post-prandial blood glucose level

	Blood glucose (mg dL^{-1})			
Time (min)	Diabetic control	Standard	*H. helix* L. (500 mg kg^{-1})	*H. helix* L. (650 mg kg^{-1})
0	449.10±17.28	388.20±18.55	356.40±23.66**	372.10±20.67*
30	467.70±17.02	336.40±12.58**	310.60±21.03**	325.60±17.92**
60	489.40±18.16	280.00±12.41 **	269.40±21.16**$^{\#}$	280.10±17.67**
120	505.80±17.90	223.10±12.98**	223.50±17.16**	216.30±26.92**

Values are in Mean±SEM, n = 10, *p<0.05 significant as compared to normal control, **p<0.01 highly significant as compared to normal control, $^{\#}$p<0.05 significant as compared to standard drug

Table 6: Chronic hypoglycemic effect of *Hedera helix* L. on fasting blood sugar

	Blood glucose (mg dL^{-1})			
Time (min)	Diabetic control	Standard	*H. helix* L. (500 mg kg^{-1})	*H. helix* L. (650 mg kg^{-1})
15	395.40±3.24	296.50±13.42**	148.00±5.50**$^{\#\#}$	346.10±17.29**$^{\#}$
30	385.50±2.70	244.80±15.03**	134.00±5.44**$^{\#\#}$	313.40±16.79*
45	386.30±3.21	192.90±13.10**	119.60±5.50**$^{\#\#}$	278.70±15.92**
60	393.80±2.47	79.30±2.73**	78.50±3.87**	176.30±12.00**

Values are in Mean±SEM n = 10, *p<0.05 significant as compared to normal control, **p<0.01 highly significant as compared to normal control, $^{\#}$p<0.05 significant as compared to standard drug, $^{\#\#}$p<0.01 highly significant as compared to standard drug

with increasing time interval which is in contrast with the normal control group where the blood sugar level elevated with increased time interval.

Effects in alloxan induced diabetic rats

Acute effect on FBS: Table 4 shows the acute effect of *Hedera helix* L. aqueous leaf extract and animals received standard drug in alloxan induced diabetic rats on FBS.

Aqueous extract of *Hedera helix* L. at both doses (500 and 650 mg kg^{-1}) exhibited highly significant decline in blood glucose level at 30, 60 and 120 min as compared to diabetic control group, these effects were equivalent to standard drug gliclazide.

Acute effect on post-prandial glucose: Table 5 shows the acute effect of *Hedera helix* L. aqueous leaves extract in alloxan induced diabetic rats on the post-prandial blood glucose level.

Aqueous extract of *Hedera helix* L. at both doses (500 and 650 mg kg^{-1}) exhibited highly significant decline in post-prandial blood sugar level at 30, 60 and 120 min time interval as compared to diabetic control group. These effects were similar to standard drug gliclazide. However, the effect of *Hedera helix* L. at 500 mg kg^{-1} was significant than gliclazide at 60 min.

Chronic effect on FBS: Table 6 reveals the chronic effect of *H. helix* L. aqueous leaf extract and animals received standard drug in alloxan induced diabetic rats on FBS.

Following the administration for 15, 30, 45 and 60 days of *Hedera helix* aqueous leaf extract at the dose of 500 mg kg^{-1} exhibited highly significant decline in FBS as compared to both diabetic control and standard drug gliclazide however, *Hedera helix* L. at 650 mg kg^{-1} showed highly significant decline in FBS after 15, 45 and 60 days but the response after 30 days was only significant.

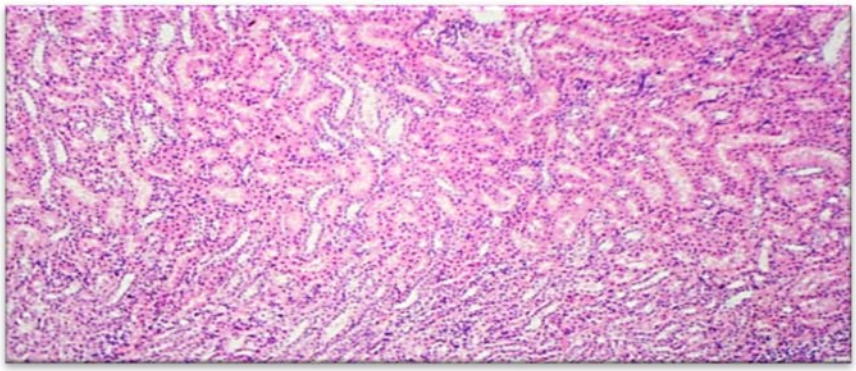

Fig. 1: Renal parenchyma with normal architecture (20×)

Table 7: Chronic hypoglycemic effect of *H. helix* L. on post-prandial blood sugar level

	Blood glucose (mg dL^{-1})			
Time (min)	Diabetic control	Standard	*H. helix* L. (500 mg kg^{-1})	*H. helix* L. (650 mg kg^{-1})
15	422.10±4.23	173.50±12.94**	308.70±15.99**	364.10±13.70**
30	441.40±5.01	159.90±11.07**	264.40±14.64**	339.5±12.00*
45	460.80±4.76	148.40±8.52**	222.80±12.50**	317.30±12.98 **
60	491.80±2.85	120.20±5.36**	148.90±3.86**	224.10±19.20**

Values are in Mean±SEM, n = 10, *p<0.05 significant as compared to normal control, **p<0.01 highly significant as compared to normal control

Table 8: Effect of *Hedera helix* L. extract on HbA_1c

	Blood glucose (mg dL^{-1})			
Time (min)	Diabetic control	Standard	*H. helix* L. (500 mg kg^{-1})	*H. helix* L. (650 mg kg^{-1})
60	8.68±0.16	5.65±0.38**	4.44±0.27**#	5.70±0.41**

Values are expressed as Mean±SEM, n = 10, **p<0.01 highly significant as compared to diabetic control, #p<0.05 significant as compared to standard

Chronic effect on post-prandial blood glucose: Table 7 shows the chronic effect of *Hedera helix* L. aqueous leaf extract on post-prandial blood glucose level of diabetic control, standard and treated groups in alloxan induced diabetic rats.

Aqueous leave extract of *Hedera helix* L. at 500 mg kg^{-1} following administration for 15, 30, 45 and 60 days exhibited highly significant fall in post-prandial blood glucose level which was similar to the effect of standard drug gliclazide on the same days. The fall in postprandial blood glucose level by *Hedera helix* L. at 650 mg kg^{-1} was also highly significant on all days except after 30 days which was significant.

Effect on HbA_1c: Table 8 shows the HbA_1c levels of diabetic control, standard and treated groups on 60th day of study. Animals of treated and standard groups both revealed highly significant reduction in HbA_1c as compared to diabetic control group however, the effect at 500 mg kg^{-1} was even better than standard drug gliclazide.

Histopathological examination: The histopathological examination of renal tissue in normal control group showed normal parenchyma with intact cortex and medullary architecture (Fig. 1).

In the diabetic control group which received only alloxan the renal tissue revealed parenchyma with focal edematous renal tubules. Foci of tubular degeneration were also noted with congested capillary loops in a few glomeruli (Fig. 2). The examination of renal tissue in animals received gliclazide was found to be intact with cortex and medullary architecture (Fig. 1). However, animals received *Hedera Helix* L. aqueous leave extract at both doses 500 and 650 mg kg^{-1} showed a few foci of tubular degeneration and congested glomeruli (Fig. 3).

Histological examination of hepatic tissue in normal control group revealed normal parenchyma with intact hepatic architecture, central vein and portal tracts (Fig. 4). Hepatic tissue in the diabetic control group which received only alloxan showed hepatic degeneration and focal necrosis.

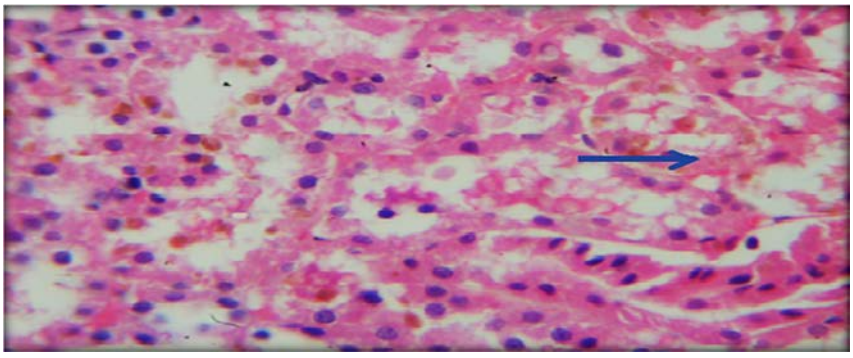

Fig. 2: Renal tubules showing foci of tubular degeneration (40×)

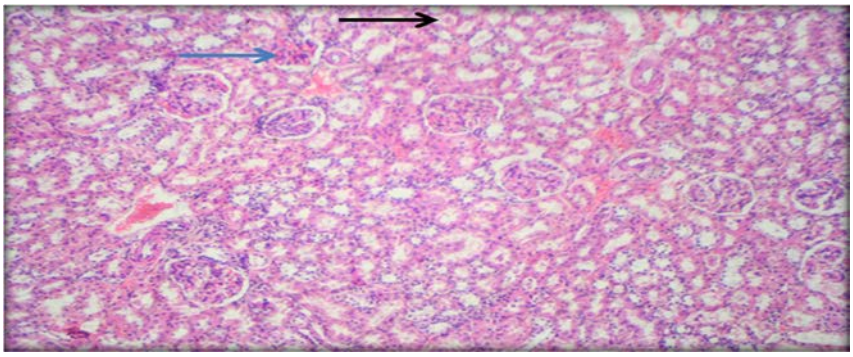

Fig. 3: Renal tissue with intact architecture and few foci of congested glomeruli (20×)

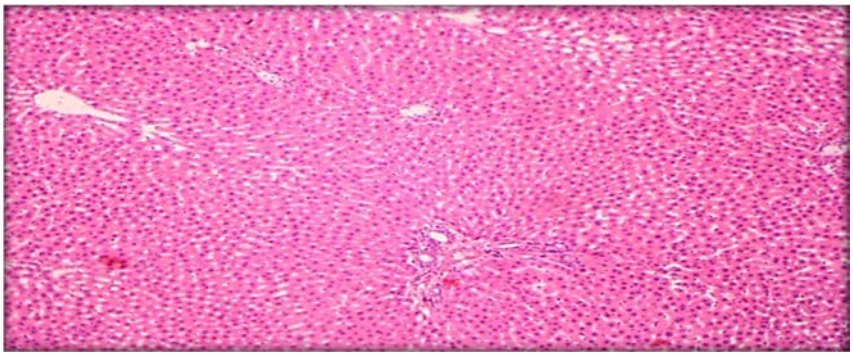

Fig. 4: Hepatic tissue with an intact architecture (20×)

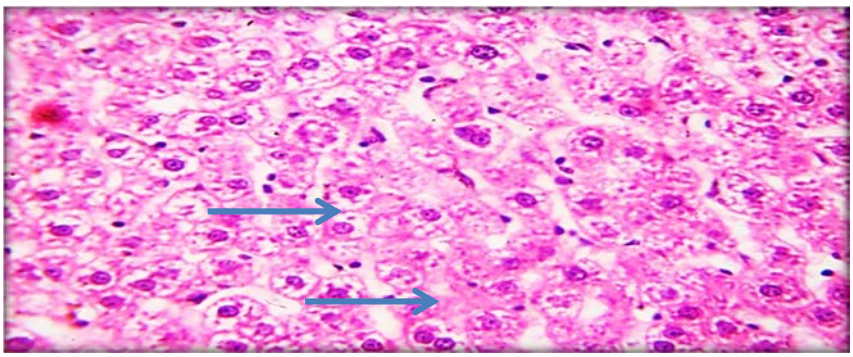

Fig. 5: Hepatic tissue showing ballooning degeneration and focal necrosis (40×)

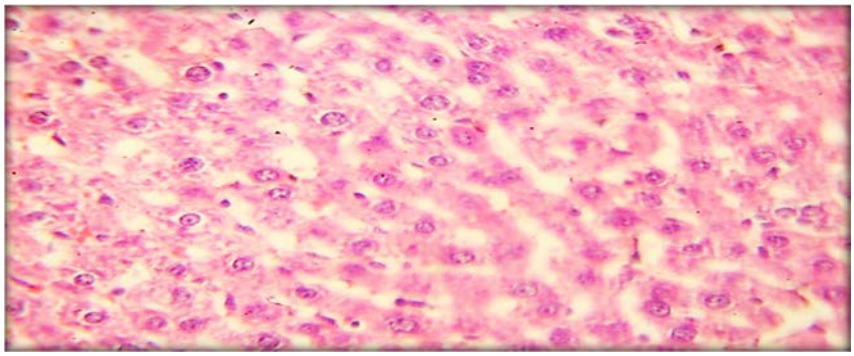

Fig. 6: Hepatic tissue showing intact cellular architecture (40×)

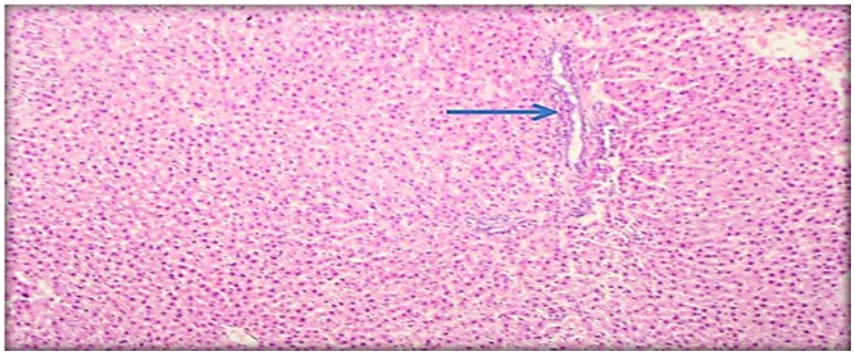

Fig. 7: Hepatic tissue with intact architecture but single focus of mild inflammation (20×)

Chronic inflammatory infiltrate was also seen in portal areas (Fig. 5). Hepatic tissue of animals received standard drug gliclazide revealed normal parenchyma with intact architecture (Fig. 4).

The hepatic tissue of animals received *H. Helix* L. aqueous leave extract at the doses of 500 and 650 mg kg^{-1} showed intact architecture with single foci of mild chronic inflammation. No evidence of degenerative changes, edema or necrosis was noted in Fig. 6 and 7.

DISCUSSION

Diabetes Mellitus (DM) is an endocrinological disorder manifested by chronic elevated blood sugar levels with altered carbohydrate, protein and fat metabolism either due to defected insulin action and/or secretion[19]. Alloxan-induced diabetes in rats represents well-studied animal models of Type I Insulin dependent diabetes mellitus. It is well known that reactive oxygen species are engaged in the diabetogenic action of alloxan[20] and antioxidants have been shown to be effective in preventing the pancreatic islets against the cytotoxic effect of alloxan thus producing beneficial effects in the management of diabetes[21]. Elevated oxidative stress has strong potential in the development of DM and its related micro and macro vascular complications[22,23]. The DM is a metabolic syndrome which is usually manifested by marked generation of ROS that contributes to cellular and tissue deterioration provoked by glucolipotoxicity in diabetes[24] or by altered antioxidant defense mechanisms. Elevated blood glucose levels in insulin secreting pancreatic beta cells increase mitochondrial ROS, which in turn suppresses the initial step of glucose-induced insulin secretion[25].

Traditional herbal remedies have been employed for centuries in the management of diabetes[26] but only a few have been precisely evaluated. A ten year literature review of herbal medicinal plants reveals that nine plant families including 5 species of Araliaceae had potential antidiabetic activity. This review also established that there are several merits of using medicinal plants in the management of diabetes mellitus[27]. Therefore, *Hedera helix* L. was chosen to evaluate its antidiabetic activity.

The enzyme α-Glucosidase, which is distributed in small intestinal epithelial cells, play a role in liberating glucose molecules by decomposing 1,4-α-glucopyranoside bonds in carbohydrate[28]. Thus, α-glucosidase plays an important role in regulating blood glucose levels after meals and keeping blood

glucose levels within normal ranges. Reducing postprandial glucose level by delay glucose absorption after meal is the prominent benefit from α-glucosidase inhibitor. In small intestine, the inhibition of α-glucosidase may delay the hydrolysis of carbohydrates and induce glucose uptake. Therefore, the inhibition of α-glucosidase is one of the important approaches in oral antidiabetic medication. In general, carbohydrates are converted to simple sugars and absorbed from the intestine. Thus, α-glucosidase inhibitors reduce blood sugar by inhibiting the conversion of carbohydrates to simple sugar[29]. As shown in Table 1, at 500 μg mL^{-1} concentration, ivy leaf extract exhibited highly significant inhibition of α-glucosidase enzyme than the standard Acarbose group. Ivy aqueous leaf extract showed better inhibition of α-glucosidase enzyme than acarbose which is commercially used antidiabetic drug to manage type 2 diabetes mellitus.

In the present study antidiabetic potential have been investigated in normoglycemic rats, OGTT in normal rats as well as acute and chronic effects in alloxan induced diabetic rats. Results showed that *Hedera helix* L. leaves extract after acute administration exert response similar to control animals on blood glucose levels of normoglycemic rats. This finding indicated that *Hedera helix* L. leave extract does not have any effect on normal blood glucose levels. However the significant rise in blood glucose levels in normoglycemic rats after 30 min time interval may be attributed to the presence of carbohydrates, glycosides and reducing sugars in *Hedera helix* L. extract[2].

Highly significant decline in blood glucose levels of animals were seen subjected to OGTT test after 120 min at 500 and 650 mg kg^{-1} dose of *Hedera helix* L. leave extract respectively and confirmed that the aqueous extract of *Hedera helix* L. has a remarkable hypoglycemic effect at 500 mg kg^{-1} and 650 mg kg^{-1} as indicated by improvement of the glucose tolerance test. This effect could be due to the presence of trace elements (Cr, Mn and Zn) in *Hedera helix* L. leaves as revealed by Atomic Absorption Spectrophotometer analysis by Ibrar *et al.*[12]. Chromium is a vital component of an organo-chromium complex, known as Glucose Tolerance Factor (GTF), which potentiates insulin action by facilitating the attachment of insulin to cell membranes and ultimately enhances the uptake of glucose by the cells. Ranasinghe *et al.*[30] have reported that Zinc depletion leads to abnormalities in glucose utilization. Manganese also plays a crucial role in glucose metabolism. Barbagallo and Dominguez[31] have demonstrated that in absence of adequate manganese in diet the body is unable to utilize glucose and precipitates diabetes mellitus. Therefore, the hypoglycemic activity of *Hedera helix* L. might be attributed due to the presence of these trace elements in sufficient amount.

The results of acute hypoglycemic effect of *Hedera helix* L. indicates that both doses of *Hedera helix* L. leaves extract i.e. 500 and 650 mg kg^{-1} produced highly significant decline in FBS in alloxan induced diabetic rats as compared to diabetic control. These results were almost comparable to standard drug gliclazide. The reduction in Post prandial glucose level was highly significant at all dosing intervals with both doses of *Hedera helix* L. as compared to diabetic control while these results were comparable to standard drug gliclazide. The leaf of *Hedera helix* L. contains numerous antioxidants such as triterpene, saponins (α-Hederin, Hederagenin and oleanolic acid), flavonoids (quercetin, isoquercetin), vitamins E, C and A[32]. Alpha-Hederin and vitamins (E and C) has protective effects against H_2O_2 mediated DNA degradation either by capturing free radicals or by augmenting the catalase activity[33]. Oleanolic acid, a potent antioxidant has been described to retain structure related hypoglycemic action[34]. Furthermore, Kim *et al.*[14] also reported that hederagenin markedly dropped the blood glucose levels in streptozotocin induced diabetic animals. Quercetin, a flavonoid present in the leaves of *Hedera helix* L. has been reported to inhibit the enzyme glycogen phosphorylase which in turn reduces glycogen degradation, thus producing hypoglycemia[35].

Furthermore, the results of chronic administration of *H. helix* L. aqueous leave extract showed a consistent highly significant hypoglycemic effect throughout the study period on FBS levels, post prandial and better results of glycosylated hemoglobin (HbA_1c) levels were attained by both doses i.e. 500 and 650 mg k^{-1} as compared to diabetic control group. The results were also comparable to standard drug gliclazide at both doses in both FBS and post prandial levels and particularly (HbA_1c) levels were significantly reduced at 500 mg kg^{-1} dose. This significant results could be due to its antioxidant potential as Süleyman *et al.*[36] reported *Hedera Helix* L. leaves possess Alpha-hederin and hederagenin compounds which exert antioxidant effect. Oxidative stress is a potential contributor to chronic hyperglycemia in diabetes. Diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defenses which ultimately worsen the diabetes and results in diabetic complication[37].

The histopathological studies of vital organs liver and kidney show that *Hedera helix* L. has protective effect at both

doses. Results obtained from the present study are very much promising and comparable with gliclazide, a standard hypoglycemic drug used to treat DM. These results further confirmed that *Hedera helix* L. is safe when used for the management of diabetes mellitus.

The data revealed that *Hedera helix* L. is not only effective in DM but is also been extensively used throughout the world for its therapeutic effects to manage a number of diseases. This may be due to the fact that the plant possesses over a number of various medicinal constituents[32]. Marked production of Reactive Oxygen Species (ROS) have been linked to the onset of diabetes and its associated complications. Thus consistent hypoglycemic effect of this herb may be partly due to its antioxidant or free radical scavenging properties as *Hedera helix* L. leaf extract has been reported to possess potent antioxidant properties for treating bronchial and other inflammatory disorders[11]. Hence *Hedera helix* L. may serve as a potent nutraceutical in future for treating diabetes and its associated complications.

It is widely accepted that the rapidly increasing incidence of diabetes mellitus has become a major health problem worldwide. The modern oral hypoglycemic agents such as sulphonylureas, biguanides, thiazolidinediones and α-glucosidase inhibitors are commonly used for the treatment of type 2 diabetes. However, it is well known that they can produce side effects associated with their applications[38]. Moreover, a progressive decline in their effectiveness, termed secondary failure has been reported[39]. During the past decade, there is a growing interest in alternative herbal medicine due to their efficacy, less side effects in clinical practice and relatively low costs. It has been estimated that about 800 plants have antidiabetic potentials[40]. Most of them have been used as folk medicines in many countries around the world. *Hedera helix* L., commonly called ivy in English, is one of medicinal plants which have long been prescribed by local practitioners for traditional treatment of diabetes mellitus. However, there is a paucity of scientific evidence that confirms its antidiabetic activity. Herein, we first evaluated the antidiabetic effect of the aqueous extract from *Hedera helix* L. leaf to confirm its benefits according to the use of this plant as an antidiabetic herb.

CONCLUSION

In conclusion, this research study discovered the effectiveness of *Hedera helix* L. leave extract at the doses of both 500 and 650 mg kg^{-1} that can be beneficial in diminishing the abnormally raised blood glucose level of alloxan-induced diabetic rats. The 500 mg kg^{-1} dose has shown even more promising results as compared to normal control and gliclazide. However, there is still a need for further studies to explore and isolate the active principle(s) from the extracts and to establish exact molecular mechanism of action.

SIGNIFICANCE STATEMENT

This study will also assist in unfolding the critical aspects of herbal antidiabetic therapy that many researchers were not able to explore. Thus a new theory on the consideration of *Hedera helix* L. as an adjuvant therapy in the management of diabetes mellitus owing to its multiple mechanism may be arrived at.

ACKNOWLEDGMENT

We would like to thanks The Department of Pharmacology, University of Karachi for providing financial and technical assistance. Researcher availed the research grant No.ASRB/03368/Pham which was awarded to the supervisor for research work.

REFERENCES

1. Gruenwald, J., T. Brendler and C. Jaenicke, 2000. PDR for Herbal Medicine. 2nd Edn. Medical Economics Company, Montvale, N.J., USA, pp: 194-195.
2. Al-Snafi, A.E., 2018. Pharmacological and therapeutic activities of *Hedera helix*-a review. IOSR J. Pharm., Vol. 41.
3. Popa, D., 2008. European Pharmacopoeia. 7th Edn., Monograph, Pages: 1273.
4. Eguale, T., G. Tilahun, A. Debella, A. Feleke and E. Makonnen, 2007. *Haemonchus contortus*: *In vitro* and *in vivo* anthelmintic activity of aqueous and hydro-alcoholic extracts of *Hedera helix*. Exp. Parasitol., 116: 340-345.
5. Majester-Savornin, B., R. Elias, A. Diaz-Lanza, G. Balansard, M. Gasquet and F. Delmas, 1991. Saponins of the ivy plant, *Hedera helix* and their leishmanicidic activity. Planta Med., 57: 260-262.
6. Danloy, S., J. Quetin-Leclercq, P. Coucke, M.C. De Pauw-Gillet and R. Elias *et al*., 1994. Effects of α-hederin, a saponin extracted from *Hedera helix*, on cells cultured *in vitro*. Planta Med., 60: 45-49.
7. Sieben, A., L. Prenner, T. Sorkalla, A. Wolf, D. Jakobs, F. Runkel and H. Ha¨berlein, 2009. α-Hederin, but not hederacoside C and hederagenin from *Hedera helix*, affects the binding behavior, dynamics and regulation of β2-adrenergic receptors. Biochem., 48: 3477-3482.

8. Favel, A., M.D. Steinmetz, P. Regli, E.V. Olivier, R. Elias and G. Balandsard, 1994. *In vitro* antifungal activity of triterpenoid saponins. Planta Med., 60: 50-53.
9. Villani, P., T. Orsiere, I. Sari-Minodier, G. Bouvenot and A. Botta, 2001. *In vitro* study of the antimutagenic activity of alphahederin. Ann. Biol. Clin., 59: 285-289.
10. Moulin-Traffort, J., A. Favel, R. Elias and P. Regli, 1998. Study of the action of α hederin on the ultrastructure of *Candida albicans*. Mycoses, 41: 411-416.
11. Lutsenko, Y., W. Bylka, I. Matlawska, O. Gavrylyuk, M. Dworacka, A. Krawczyk and V. Brytska, 2017. Anti-Inflammatory, antimicrobial activity and influence on the lungs and bronchus of *Hedera Helix* leaves extracts. Acta Pol. Pharm. Drug Res., 4: 1159-1166.
12. Ibrar, M., I.H. Ilahi and F.A. Hussain, 2004. Hypoglycemic activity of *Hedera helix* L. leaves and the possible mechanism of action. Pak. J. Bot., 35: 805-810.
13. Zafar, I., P.M. Asadullah, M. Ismail, B. Ahmad, S. Zakir and G. Saima, 2002. Study of the hypoglycemic activity of *Hedera helix* L. in alloxan-induced diabetic rabbits. J. Med. Sci., 2: 206-208.
14. Kim, M.A., H.U. Son, E.K. Yoon, Y.H. Choi and S.H. Lee, 2012. Comparison of anti-diabetic activities by extracts of grape cultivar. Korean J. Food Preserv., 19: 400-405.
15. Elya, B., R. Handayani, R. Sauriasari, Azizahwati , U.S. Hasyyati, I.T. Permana and Y.I. Permatasari, 2015. Antidiabetic activity and phytochemical screening of extracts from indonesian plants by inhibition of alpha amylase, alpha glucosidase and dipeptidyl peptidase IV. Pak. J. Biol. Sci., 18: 279-284.
16. El-Demerdash, F.M., M.I. Yousef and N.I.A. El-Naga, 2005. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem. Toxicol., 43: 57-63.
17. Du Vigneaud, V. and W.G. Karr, 1925. Carbohydrate utilization I. Rate of disappearance of dglucose from the blood. J. Biol. Chem., 1: 281-300.
18. Adebayo, G.I., O.T. Alabi, B.V. Owoyele and A.O. Soladoye, 2009. Anti-diabetic properties of the aqueous leaf extract of Bougainvillea glabra (Glory of the Garden) on alloxan-induced diabetic rats. Rec. Nat. Prod., Vol. 3, No. 4.
19. American Diabetes Association, 2014. Diagnosis and classification of diabetes mellitus. Diabetes Care, 37: S81-S90.
20. Raut, N.A. and N.J. Gaikwad, 2006. Antidiabetic activity of hydro-ethanolic extract of *Cyperus rotundus* in alloxan induced diabetes in rats. Fitoterapia, 77: 585-588.
21. Johansen, J.S., A.K. Harris, D.J. Rhychly and A. Ergul, 2005. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. Cardiovasc. Diabetol., 4: 5-9.
22. Baynes, J.W. and S.R. Thorpe, 1999. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. Diabetes, 48: 1-9.
23. Ceriello, A., 2000. Oxidative stress and glycemic regulation. Metabolism, 49: 27-29.
24. Newsholme, P., E.P. Haber, S.M. Hirabara, E.L.O. Rebelato and J. Procopio *et al*., 2007. Diabetes associated cell stress and dysfunction: Role of mitochondrial and non-mitochondrial ROS production and activity. J. Physiol., 583: 9-24.
25. Nishikawa, T. and E. Araki, 2007. Impact of mitochondrial ROS production in the pathogenesis of diabetes mellitus and its complications Antioxide. Redox Signal, 9: 343-353.
26. Ahmad, M., R. Qureshi, M. Arshad, M.A. Khan and M. Zafar, 2009. Traditional herbal remedies used for the treatment of diabetes from district Attock (Pakistan). Pak. J. Bot., 41: 2777-2782.
27. Patel, D.K., S.K. Prasad, R. Kumar and S. Hemalatha, 2012. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian. Pac. J. Trop. Biomed., 2: 320-330.
28. Flanagan, P.R. and G.G. Forstner, 1978. Purification of rat intestinal maltase/glucoamylase and its anomalous dissociation either by heat or by low pH. Biochem. J., 173: 553-563.
29. Sørensen, S.H., O. Norén, H. Sjöström and E.M. Danielsen, 1982. Amphiphilic pig intestinal microvillus maltase/glucoamylase. structure and specificity. Eur. J. Biochem., 126: 559-568.
30. Ranasinghe, P., S. Pigera, P. Galappatthy, P. Katulanda and G.R. Constantine, 2015. Zinc and diabetes mellitus: understanding molecular mechanisms and clinical implications. DARU J. Pharm. Sci., 10.1186/s40199-015-0127-4
31. Barbagallo, M., 2015. Magnesium and type 2 diabetes. World J. Diabetes, Vol. 6, No. 10. 10.4239/wjd.v6.i10.1152
32. Lutsenko, Y.U., W.I. Bylka, I. Matlawska and R.O. Darmohray, 2010. *Hedera helix* as a medicinal plant. Herba Pol., 56: 83-96.
33. Gulcin, I., V. Mshvildadze, A. Gepdiremen and R. Elias, 2004. Antioxidant activity of saponins isolated from ivy: A-Hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F. Planta Med., 70: 561-563.
34. Castellano, J.M., A. Guinda, T. Delgado, M. Rada and J.A. Cayuela, 2013. Biochemical basis of the antidiabetic activity of oleanolic acid and related pentacyclic triterpenes. Diabetes, 62: 1791-1799.
35. Kato, A., Y. Minoshima, J. Yamamoto, I. Adachi, A.A. Watson and R.J. Nash, 2008. Protective effects of dietary chamomile tea on diabetic complications. J. Agric. Food Chem., 56: 8206-8211.

36. Süleyman, H., V. Mshvildadze, A. Gepdiremen and R. Elias, 2003. Acute and chronic antiinflammatory profile of the ivy plant, *Hedera helix*, in rats. Phytomed., 10: 370-374.
37. Hilliard, M.E., J.P. Yi-Frazier, D. Hessler, A.M. Butler, B.J. Anderson and S. Jaser, 2016. Stress and A1c among people with diabetes across the lifespan. Curr. Diab. Rep., 10.1007/s11892-016-0761-3
38. Fowler, M.J., 2007. Diabetes treatment, part 2: oral agents for glycemic management. Clin. Diabetes, 25: 131-134.
39. Groop, L.C., R. Pelkonen, S. Koskimies, G.F. Bottazzo and D. Doniach, 1986. Secondary failure to treatment with oral antidiabetic agents in non-insulin dependent diabetes mellitus. Diabetic Care, 9: 129-133.
40. Grover, J.K., S. Yadav and V. Vats, 2002. Medicinal plants of India with anti-diabetic potential. J. Ethnopharmacol., 81: 81-100.