Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 3213-3218
Received: 22-03-2019
Accepted: 24-04-2019

DK Patil
Sarvepalli Radhakrishnan University, NH-12 Hoshangabad Road, Misrod, Bhopal, Madhya Pradesh, India

Alok Pal Jain
Sarvepalli Radhakrishnan University, NH-12 Hoshangabad Road, Misrod, Bhopal, Madhya Pradesh, India

Correspondence
Alok Pal Jain
Sarvepalli Radhakrishnan University, NH-12 Hoshangabad Road, Misrod, Bhopal, Madhya Pradesh, India

In-vivo antidiabetic activity of methanolic extract of *Corchorus olitorius* for the management of type 2 diabetes

DK Patil and Alok Pal Jain

Abstract
Management of type 2 diabetes with an agent having no side effects is still a challenge for the researchers, however if the side effects are lessened & there may be a chance for reduced adverse reactions or severe side effects due to drug interaction. These interactions may be due to either any concomitant drug therapy or any dietary supplements taken together with the drugs. This study sought to evaluate the antidiabetic potentials of methanolic extract of jute leaf (*Corchorus olitorius*) in diabetic rats. Diabetes was induced in rats by streptozotocin (60mg/kg; *i.p.*) administration. Oral treatment of methanolic extract of *C. olitorius* using rat oral needle at 100 and 200mg/kg doses significantly (P<0.001) decreased blood glucose and cholesterol levels in diabetic rats than control rats. In this study, Body weight, blood glucose, HDL, Total Protein, SGOT, SGPT, cholesterol and triglyceride were measured. The results showed that there was a significant decrease (P<0.01) in the blood glucose, cholesterol, total protein, SGOT, SGPT and triglyceride levels in diabetic rats when compared to the normal (control) rats and increase the level of HDL and body wt. In conclusion, the above actions might be responsible for the antidiabetic activity of extract due to presence of gallic acid and other biomarkers.

Keywords: Type 2 diabetes, *Corchorus olitorius*, Streptozotocin, cholesterol, gallic acid

Introduction
Diabetes mellitus (DM) is one of the most significant chronic metabolic disorders characterized by hyperglycemia. The vast majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. In the other, much more prevalent category, type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin-secretory response [1].The overall prevalence of diabetes mellitus in the global population is approximately 6%, of which 90% is type 2 diabetes. India had 32 million diabetics in 2000, and this number is expected to increase to 80 million by 2030 [2, 3]. Characteristic of diabetes is associated with disturbances in the metabolism of carbohydrates, lipids and proteins due to defects in insulin secretion, insulin action or both [4]. Diabetic complications are nephropathy, retinopathy, neuropathy, atherosclerosis and fatty liver. In all these cases continual hyperglycemia plays a significant role in the induction of oxidative stress by increasing glucose autooxidation, nonenzymatic protein glycation and activation of polyol pathway [5]. Also hyperglycemia induced stress sensitive signaling pathways including nuclear factor (NF)-kB. Activation of NF-kB increased cytokine concentrations such as tumor necrosis factor-a (TNF-α). The renal cells are capable to synthesis TNF- α moreover the sensitive to changes of serum's TNF- α level. This process suggests a causal role for hyperglycemia in the immune activation of diabetes [6]. Since ancient times, consumption of medicinal herbs has considered in treatment of several diseases [7]. In recent years this kind of treatment has received growing attention because it is natural and has a few side effects [8]. Many medicinal plants extracts such as Bougainvillea spectabilis, Moringa oleifera, Curcuma longa, Cynodon dactylon and Trichosanthes dioica were used for treatment of diabetes mellitus due to having hypoglycaemic effects [9-13]. Corchorus olitorius is a plant from the Tiliaceae family from the Mediterranean region, its leaves have been found to be rich in antioxidants, such as vitamin C, vitamin E, β-carotene, α-tocopherol, glutathione and phenols [14]. The leaves also contain fatty acids, minerals, other vitamins and mucilaginous polysaccharides, and have been used as traditional folkmedicine. Yokoyama *et al.* [15] reported C. olitorius leaves to ameliorate atopic dermatitis in NC/Nga mice [15]. It is called 'ewedu' in Yoruba Language and is a common source of vegetable among Yoruba tribe in Nigeria. Therefore, the aim of the present study is to determine the antidiabetic activity of methanolic extract of *C. Olitorius*.

Material and method

Plant material

Whole plant material of *Corchorus olitorius* were collected from ruler area of Nagpur (Maharashtra) in the month of August, 2017.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade. Metformin was used as standard drugs. Streptozotocin (Sigma-Aldrich), Lipid profile estimation kit (Transasia Bio Medical Limited, Mumbai, India) and other chemicals and solvent obtained from Qualigens, India were used.

Extraction procedure

Defatting of plant material

Powdered plant material of *C. Olitorius* was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place

Extraction

50gm of dried plant material were exhaustively extracted with different solvent using maceration method for 48 hrs. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts.

Animals

Wistar rats (180–250 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity

Acute toxicity study of the prepared extracts was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines-423 [16] the animals were fasted for 4 h, but allowed free access to water throughout. As per the OECD recommendations, the starting dose level should be that which is most likely to produce mortality in some of the dosed animals; and when there is no information available on a substance to be tested in this regard; for animal welfare reasons, The dose level to be used as the starting dose is selected from one of three fixed levels 5, 300 and 2000 mg/kg body weight. Acute toxicity was determined as per reported method [17].

Induction of Diabetes in Rat

Streptozotocin was dissolved in 100 mM citrate buffer (pH 4.5) and calculated amount of the dose (60 mg/kg) of the fresh solution was injected intraperitoneally to overnight fasted rats. Blood glucose was checked 48 h later and animals showing blood glucose value more than 250 mg/dl were included in the experiments and termed as diabetic.

Experimental

Five groups of rats were employed in the present study and each group contains six animals, as follows

Group I- Normal

Group II- Diabetic rats received only distilled water (negative control)

Group III- Diabetic rats was treated with Metformin (500mg/kg p.o.)

Group IV- Diabetic rats received *Corchorus olitorius* (100 mg/kg/day p.o.)

Group V- Diabetic rats received *Corchorus olitorius* (200 mg/kg/day p.o.).

Blood sampling and glucose estimation

For blood glucose determination, blood was withdrawn by tail snipping technique. For various lipid profile and biochemical parameters estimation, blood was collected from cardiac puncture is a suitable technique. Blood was collected in plain micro centrifuge tube at every second week throughout the study period from all the overnight fasted (16-20 hr.) animals, under anesthesia. Serum was separated from blood sample by centrifugation at 4000 r.p.m. for 10 minutes. Biochemical parameters were studied by using automated biochemistry analyzer Hitachi-902.

Estimation of oral glucose tolerance test

Glucose and the oxygen react in the presence of glucose oxidase producing gluconic acid and hydrogen peroxide subsequently. Hydrogen peroxide oxidizes the dyes in a reaction mediated by peroxidase resulting to a blue colour of the dyes. However instead of conventional and lengthy procedure of other diagnostic kits, the glucometer was found to be suitable diagnostically in term of test accuracy, where a drop of blood is sufficient to get the results and easy to access at ambient temperature >95° F and hence used.

Statistical Analysis

All the values of blood sugar, lipid profile and biochemical estimations were expressed as Mean ± S.E.M. (Standard Error of Mean) for six rats in each group and analyzed with one way analysis of variance (ANOVA) followed by Dunnett's test. Differences between groups were considered significant at $P<0.050$ & P 0.001 levels.

Results of anti-diabetic activity

The various results obtained from different experiments carried out were compiled here under

As represented in Table 1 body weights of animals in all groups were performed at the initial and end of the study. Body weight of animals was significantly ($p<0.05$) maintained in all treatment groups (Metformin 500 mg/kgp.o., *C. olitorius* 100 and 200mg/kg/p.o., 190.40±8.26; 199.00±5.50 and 196.00 ± 9.70) during study as compared to control group (190.00±10.00).

Table 1: Mean body weight change

Group	Drug	Dose	Body weight (g)	
			Onset of study	End of study
I	Normal	Normal saline	200.15±6.83	220.18±6.83
II	Control	Normal saline	210.20± 10.00	190.00±10.00
III	Metformin	500 mg/kg p.o.	220.22± 8.26	190.40±8.26*
IV	*Corchorus olitorius*	100 mg/kg p.o.	230.10± 5.50	199.00±5.50
V	*Corchorus olitorius*	200 mg/kg p.o.	227.10± 5.00	196.00 ± 9.70*

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at p<0.05 vs. Control group respectively (One-way ANOVA followed by Dunnett's test).

As shown in Table 2 Blood glucose level of animals in all groups was recorded at 0, 8th and 21th day. Progressive decrease in blood glucose level was found in all treatment groups during study. At the end of experiment Metformin 500 mg/kgp.o., *C. olitorius* 100 and 200 mg/kg/p.o. (112.00 ± 6.50; 118.00 ± 6.00 and 120.00 ± 5.50) treated group blood glucose level was decrease significantly (p<0.05) at 21st days, respectively.

Table 2: Antidiabetic activity of *Corchorus olitorius* on blood glucose level in STZ-induced diabetic rats

Groups	Treatment	Dose	Blood glucose (mg/dl)		
			Days 0	Days 8	Days 21
I	Normal	Normal saline	80.00 ± 4.00	85.00 ± 4.00	92.00 ± 4.00
II	Control	Normal saline	299.00 ± 7.00	380.00 ± 7.00#	397.00± 7.00#
III	Metformin	500 mg/kg p.o.	250.00± 6.50	140.00 ± 6.50**	112.00 ± 6.50**
IV	*Corchorus olitorius*	100 mg/kg p.o.	265.00± 6.00	155.10 ± 6.00*	118.00 ± 6.00**
V	*Corchorus olitorius*	200 mg/kg p.o.	270.00 ± 5.50	157.00 ± 5.50 *	120.00 ± 5.50*

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at p<0.05 vs. Negative control group respectively (One-way ANOVA followed by Dunnett's test).

In *C. olitorius* 100 and 200mg/kg/p.o. (138.0 ± 6.00; 125.0 ± 5.00) treated group total cholesterol decreased significantly (p<0.05). In 500 mg/kg metformin (110.0 ± 5.00) treated group total cholesterol decreased significantly (p<0.05), respectively as compared with control group (210.0 ± 5.00), as shown in Table 3.

Table 3: Effect of *Corchorus olitorius* on total cholesterol level in STZ-induced diabetic rats

Group	Drug	Dose	Total Cholesterol (mg/dl)
I	Normal	Normal saline	80.00 ± 6.00
II	Control	Normal saline	210.0 ± 5.00
III	Metformin	500 mg/kg p.o.	110.0 ± 5.00***
IV	*Corchorus olitorius*	100 mg/kg p.o.	138.0 ± 6.00
V	*Corchorus olitorius*	200 mg/kg p.o.	125.0 ± 5.00**

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test).

In *C. olitorius* 100 and 200 mg/kg/p.o. (96.00 ± 7.00; 86.50 ± 5.00) treated group triglyceride decreased significantly (p<0.05). In 500 mg/kg metformin (82.00 ± 5.00) treated group triglyceride decreased significantly (p<0.05), respectively as compared with control group (150.5 ± 6.00), as shown in Table 4

Table 4: Effect of *Corchorus olitorius* on triglyceride level in STZ -induced diabetic rats

Group	Drug	Dose	Triglyceride (mg/dl)
I	Normal	Normal saline	72.00 ± 5.00
II	Control	Normal saline	150.5 ± 6.00
III	Metformin	500 mg/kg p.o.	82.00 ± 5.00**
IV	*Corchorus olitorius*	100 mg/kg p.o.	96.00 ± 7.00*
V	*Corchorus olitorius*	200 mg/kg p.o.	86.50 ± 5.00*

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test).

As shown in Table 5 *C. olitorius* 100 mg/kg (35.00±1.80) treated group high density lipoprotein (HDL) increased significantly (p<0.05), and *Corchorus olitorius* 200 mg/kg (46.00±1.90) treated group HDL also increased significantly (p<0.001). In 500 mg/kg p.o. metformin (50.50±2.00) treated group HDL increased significantly (p<0.001), respectively as compared with control group (28.40±2.70).

Table 5: Effect of *Corchorus olitorius* on HDL in STZ-induced diabetic rats

Group	Drug	Dose	HDL (mg/dl)
I	Normal	Normal saline	51.80±1.10
II	Control	Normal saline	28.40±2.70
III	Metformin	500 mg/kg p.o.	50.50±2.00***
IV	*Corchorus olitorius*	100 mg/kg p.o.	35.00±1.80*
V	*Corchorus olitorius*	200 mg/kg p.o.	46.00±1.90**

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test).

As shown in Table 6 *C. olitorius* 100 mg/kg (98.00 ± 5.00) treated group total protein (TP) significantly decreased, and *C. olitorius* 200 mg/kg (85.00 ± 5.00) treated group TP also decreased significantly (p<0.01). In 500 mg/kg p.o. metformin (85.00 ± 7.00) treated group TP was significantly decreased (p<0.001), respectively as compared with control group (140.00± 6.00).

Table 6: Antidiabetic effect of *Corchorus olitorius* on serum lipid profile i.e. total protein (TP) level in STZ-induced diabetic rats

Group	Drug	Dose	TP (g/dl)
I	Normal	Normal saline	76.00± 5.00
II	Control	Normal saline	140.00± 6.00
III	Metformin	500 mg/kg p.o.	85.00 ± 7.00***
IV	*Corchorus olitorius*	100 mg/kg p.o.	98.00 ± 5.00
V	*Corchorus olitorius*	200 mg/kg p.o.	85.00 ± 5.00**

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test).

Table 7: Effect of *Corchorus olitorius* on SGOT in STZ-induced diabetic rats

Group	Drug	Dose	SGOT (IU/L)
I	Normal	Normal saline	58.00 ± 5.00
II	Control	Normal saline	122.0± 7.00
III	Metformin	500 mg/kg p.o.	67.00 ± 4.00**
IV	*Corchorus olitorius*	100 mg/kg p.o.	77.50 ± 5.50*
V	*Corchorus olitorius*	200 mg/kg p.o.	71.00 ± 5.00*

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test).

Similarly, at the end days of experiment the serum transaminase such as SGPT level was significantly (p<0.001) elevated in diabetic control group. As shown in Table 8 *C. olitorius* 100 mg/kg (71.00 ± 5.00) treated group SGPT significantly decreased, and *C. olitorius* 200 mg/kg (60.00 ± 5.00) treated group SGPT also decreased significantly (p<0.01). In 500 mg/kg p.o. metformin (58.00 ± 5.00) treated group SGPT was significantly decreased (p<0.001), respectively as compared with control group (117.0 ± 6.00).

Table 8: Effect of *Corchorus olitorius* on SGPT in STZ-induced diabetic rats

Group	Drug	Dose	SGPT (IU/L)
I	Normal	Normal saline	47.00 ± 5.00
II	Control	Normal saline	117.0 ± 6.00
III	Metformin	500 mg/kg p.o.	58.00 ± 5.00**
IV	*Corchorus olitorius*	100 mg/kg p.o.	71.00 ± 5.00*
V	*Corchorus olitorius*	200 mg/kg p.o.	60.00 ± 5.00**

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test).

Discussion

Normalization of increased blood glucose by *C. olitorius* extract suggests that it may enhance glucose transport across the cell membranes and stimulate glycogen synthesis or enhance glycolysis. The extract might possess insulin like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis. In the present study a single intra-peritoneal administration of STZ significantly increased the level of blood glucose. Similar increase in the blood glucose level has been reported in STZ-induced-diabetic-rats. The blood glucose level was significantly reduced when hyperglycemic rats were treated with *C. olitorius* @100 and 200 mg/kg B.W indicating the hypoglycaemic activity of *C. olitorius*. The higher number of type 2 diabetics is directly linked to higher incidence of diabetic complications. The growing number of patients with complications such as diabetes, cardiomyopathy and neuropathy increase significantly the medical costs. Therefore, it is a global interest to discover new therapeutic drugs to diminish or prevent these disabling conditions. To construct the diabetes model in type-2 diabetic rats it should be based on the functional and structural lesion of human DNA as well as metabolic abnormalities. Recently, the therapeutic drug has some drawbacks such as hepatotoxicity, vascular complications, neuronal and cardiotoxicity side effect. The chemotherapeutic drug still has a challenge for the management of diabetes in the medical system. This has led to an increase in the demand for natural products with antihyperglycemic activity and fewer side effects. Traditional plant medicines and its bioactive compounds are used in the worldwide for the diabetic disease. The study of medicines field might offer a new key to unlock a diabetic pharmacy for the future. Streptozotocin injection resulted in diabetes mellitus, which may be due to the destruction of beta cells of Islets of Langerhans as proposed by others. Diabetes arises from irreversible destruction of pancreatic beta cells, causing degranulation and reduction of insulin secretion. STZ - induced diabetes is characterized by a severe loss in body weight and may exhibit most of the diabetic complications such as, myocardial, cardiovascular, nervous, kidney and urinary bladder dysfunction through oxidative stress [18]. The hypoglycemic effects of polyphenols/flavonoids are mainly attributed to the reduced intestinal absorption of dietary carbohydrate, modulation of the enzymes involved in glucose metabolism, improvement of β-cell function and insulin action, and stimulation of insulin secretion. Several researchers have reported that antioxidants have hypoglycaemic effects. Overproduction of glucose using excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental bases of hyperglycemia in diabetes mellitus. Intraperitoneal administration of STZ effectively induced diabetes mellitus in normal rats as reflected by blood glucose level and body weight loss compared with normal rats. The results demonstrate that *C. olitorius* have

antidiabetic activity as evaluated reduced the blood glucose level in STZ -induced diabetic rats. In our study, there was decrease in blood glucose level was found in all treatment groups during study. Progressive decrease in blood glucose level was found in all treatment groups during study. At the end of experiment Metformin 500 mg/kgp.o., *C. olitorius* 100 and 200 mg/kg/p.o. (112.00 ± 6.50; 118.00 ± 6.00 and 120.00 ± 5.50) treated group blood glucose level was decrease significantly (p<0.05) at 21st days, respectively. Impaired carbohydrate metabolism and developing insulin resistance is the main metabolic disorder in non-insulin dependent diabetes mellitus leading to hyperglycemia. Altered digestion and absorption of dietary carbohydrate, depletion of glycogen storage, increased gluconeogenesis, β-cell dysfunction, insulin resistance of peripheral tissue and defect in insulin signaling pathways are important causes of hyperglycemia. However, changes in initial and final body weight of normal control and experimental groups are shown in Table 1. Marked body weight loss was observed in diabetic rats. The data obtained from this study showed that the treatment of *C. olitorius* protects the diabetic rats from massive body weight loss, when given orally. *C. olitorius* treated rats showed a recovery in final body weight which was close to that of normal control rats. Moreover, body weights of animals in all groups were performed at the initial and end of the study. Body weight of animals was significantly (p<0.05) maintained in all treatment groups (Metformin 500 mg/kgp.o., *C. olitorius* 100 and 200 mg/kg/p.o., 190.40±8.26; 199.00±5.50 and 196.00 ± 9.70) during study as compared to control group (190.00±10.00). Dehydration and loss of body weight have been associated with diabetes mellitus. In diabetic rats, increased food consumption and decreased body weight were observed. This indicates the polyphagic condition and loss of weight due to excessive breakdown of tissue proteins. The decrease in body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins [19]. Therefore, a detailed understanding of the mechanisms that govern the balance between lipid deposition and lipid mobilization is fundamentally important for the treatment of obesity. It has been reported that less than 5% of TGs are synthesized in the liver and adipose tissue themselves. Most FFAs that are stored as TG in adipose tissue is derived from the diet. Therefore, agents that regulate food intake and food absorption target the first important step in the process of body fat regulation. Pancreatic lipase is important for the digestion and absorption of fat. Lipoprotein lipase, which is secreted by a variety of cell types, is an indicator of the anabolic pathway that provides FFA to the adipocytes directly from the plasma lipoproteins and indirectly from the diet. At its extracellular location on the capillary endothelium in various tissues, the enzyme hydrolyzes the triglyceride component of these particles to glycerol and FFA. There is overwhelming evidence that obese individual has a substantially higher risk of developing many diseases such as type 2 diabetes, hyperlipidemia, cardiovascular disease and hypertension [20]. The present study has generated many interesting outcomes which are discussed below. In this present study shows initial the animals treated with STZ showed marked glucose intolerance which indicated that they developed diabetes. While on 21st days, *C. olitorius* showed its hypoglycemic activity compare to negative control with the unexplained mechanism.

Conclusion

On the basis of blood biochemical parameters, it has been concluded that *C. olitorius* @ 100 and 200 mg/kg B.W has antidiabetic potential in STZ induced diabetes. However, the study needs further investigations and further validations. Diabetes mellitus (DM) is a metabolic disease with abnormal glucose homeostasis, due to defects in secretion or action of insulin. It is a potentially morbid condition with high prevalence worldwide and has become a major medical concern. Animal models play an important role in understanding such type of diseases. Diabetes in experimental animal develops either spontaneously or by using chemical, surgical, genetic or other techniques, and depicts many clinical features or related phenotypes of the disease. Streptozotocin (STZ) is a widely used chemical for the induction of experimental diabetes in animals. Other findings of the study suggest that the *C. Olitorius* is also produced beneficial effects in complications of the diabetes mellitus. The present study findings indicated that the usefulness of the *C. olitorius* streptozotocin-induced diabetic rats. Our study suggested that *C. olitorius* dose-dependently produced antidiabetic activity. In this study might be helpful to understand the role of *C. olitorius* in the clinical treatment of diabetes mellitus. The *C. olitorius* has also seems have potent hypolipidemic activity, which lowers the cholesterol, triglyceride, total proteins and increasing high-density lipoproteins levels. The mechanism has pointed towards, inhibiting cholesterol and triglyceride synthesis. Our study indicates the role of antioxidant activities of *C. olitorius* and active constituents play a role in preventing diabetic complications at 200 mg/kg of the dose was found to be more effective than 100 mg/kg of dose, its produced effects dose-dependently.

References

1. American Diabetes Association, Diagnosis and classification of diabetes mellitus. Diabetes Care. 2005; 28:37-42.
2. Ramchandran A, Snehalatha C, Vijay V. Burden of type 2 diabetes and its complications – the Indian scenario. Cur Sci. 2002; 83:1471-1476.
3. Cowie CC, Eberhardt MS, editors. Diabetes 1996: Vital Statistics. Alexandria: American Diabetes Association, 1996.
4. Sivakumar S, Palsamy P, Subramanian SP. Impact of D-pinitol on the attenuation of proinflammatory cytokines, hyperglycemiamediated oxidative stress and protection of kidney tissue ultrastructure in streptozotocin-induced diabetic rats. Chem Biol Interact. 2010; 188(1):237-45.
5. Saravanan G, Ponmurugan P. S-allylcysteine improves streptozotocin- induced alterations of blood glucose, liver cytochrome P450 2E1, plasma antioxidant system, and adipocytes hormones in diabetic rats. Int J Endocrinol Metab. 2013; 11(4):e10927.
6. Ingaramo PI, Ronco MT, Frances DE, Monti JA, Pisani GB, Ceballos MP *et al*. Tumor necrosis factor alpha pathways develops liver apoptosis in type 1 diabetes mellitus. Mol Immunol. 2011; 48(12-13):1397-407.
7. Nasiri A, Ziamajidi N, Behrouj H, Abbasalipourkabir R, Dehghan A. The effects of aqueous extract of chicory root on steatosis, lipid profile and liver damage enzyme markers in tamoxifentreated rats. Mol Biochem Diagn J. 2014; 1(3):185-94.

8. Behrouj H, Ziamajidi N, Abbasalipourkabir R, Nasiri A, Solemani Asl S. Therapeutic effect of *Silybum marianum* plant extracton tamoxifen-induced fatty liver in rats. Avicenna J Med Biochem. 2015; 3(1):e27160.
9. Chauhan P, Mahajan S, Kulshrestha A, Shrivastava S, Sharma B, Goswamy HM, Prasad GB. *Bougainvillea spectabilis* exhibits antihyperglycemic and antioxidant activities in experimental diabetes. J Evid Based Complement Altern Med. 2016; 21(3):177-85.
10. Jaiswal D, Rai PK, Mehta S, Chatterji S, Shukla S, Rai DK *et al*. Role of *Moringa oleifera* in regulation of diabetes-induced oxidative stress. Asian Pac J Trop Med. 2013; 6(6):426-32.
11. Rai PK, Jaiswal D, Mehta S, Rai DK, Sharma B, Watal G. Effect of *Curcuma longa* freeze dried rhizome powder with milk in STZ induced diabetic rats. Indian J Clin Biochem. 2010; 25(2):175-81.
12. Rai PK, Jaiswal D, Rai DK, Sharma B, Watal G. Antioxidant potential of oral feeding of *Cynodon dactylon* extract on diabetes induced oxidative stress. J Food Biochem. 2010; 34(1):78-92.
13. Rai PK, Jaiswal D, Rai DK, Sharma B, Watal G. Effect of water extract of *Trichosanthes dioica* fruits in streptozotocin induced diabetic rats. Indian J Clin Biochem. 2008; 23(4):387-90.
14. Zeghichi S, Kallithraka S, Simopoulos AP. Nutritional composition of molokhia (*Corchorus olitorius*) and stamnagathi (*Cichorium spinosum*). World Rev Nutr Diet. 2003; 91:1-21
15. Yokoyama S, Hiramoto K, Fujikawa T, Kondo H, Konishi N, Sudo S et al. Topical application of *Corchorus olitorius* leaf extract ameliorates atopic dermatitis in NC/ Nga mice. Dermatol Aspects. 2014; 2:3
16. Guideline Document on Acute oral Toxicity Testing, Series on Testing and Assessment No. 423. Paris: Organization for Economic Co-Operation and Development, OECD Environment, Health and Safety Publications, 1996. Available from: http//www.oecd.org/ehs.
17. Jonsson M, Jestoi M, Nathanail AV, Kokkonen UM, Anttila M, Koivisto P *et al*. Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin. Food Chem Toxicol. 2013; 53:27-32.
18. Rajasekaran S, Sivagnanam K, Subramanian S. Antioxidant effect of *Aloe vera* gel extract in streptozotocin-induced diabetes in rats. Pharmacol Rep. 2005; 57(1):906.
19. Wang J, Ryu HK. The effects of Momordica charantia on obesity and lipid profiles of mice fed a high-fat diet. Nutr Res Pract. 2015; 9(5):489-95.
20. Sullivan PW, Ghushchyan VH, Ben-Joseph R. The impact of obesity on diabetes, hyperlipidemia and hypertension in the United States. Qual Life Res. 2008; 17:1063-71.