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Research Article
Solanum nigrum Leaf: Natural Food Against Diabetes and its Bioactive Compounds

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Abstract
In the present study, antidiabetic activity of the leaf extract of *Solanum nigrum* (SN), a traditional edible plant was investigated in streptozotocin-induced diabetic rats. Novel microwave extraction method was used to get more activity and bioactive compound. The SN leaf extract indicated significant inhibition for glucose diffusion and also for other pancreatic enzymes such as α-amylase (IC_{50}-39±0.06 μg mL^{-1}) and α-glucosidase (IC_{50}-78.8±0.7 μg mL^{-1}). The antidiabetic activity of the SN leaf extract (125, 250 and 375 mg kg^{-1}) was investigated in streptozotocin-induced diabetic rats. The oral administration of leaf extract (375 mg kg^{-1}) significantly decreased hyperglycemia, urine sugar level, total cholesterol and triglycerides. It also reduced and increased water and food intake respectively. Furthermore, it had an effect on increased kidney function, which reflected in decreased urine output and urine sugar compared to diabetic. *Solanum nigrum* contained polyphenols (14.72±0.37% GAE) and major bioactive molecules such as p-coumaric acid, quercitin, catechol, caffeic acid, gallic acid and protocatechuic acid was analyzed by High Performance Liquid Chromatography (HPLC) and its beneficial effects on diabetic status were evaluated. The results demonstrate the beneficial effect of the SN leaf extract as functional food or as adjuvant in streamlining diabetes by consuming as daily or frequent intake in the form of natural food.

Key words: *Solanum nigrum*, leaf extracts, microwave assisted extraction, antidiabetic, streptozotocin, wistar rats

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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

Diabetes mellitus is a metabolic disorder in the endocrine system with hyperglycemia, carbohydrate, protein and fat metabolism disturbances due to absolute or relative deficiency of insulin secretion (Cao *et al*., 2012a, b). It is chronic, multifaceted, dynamic expression of pathological disequilibria, resulting in various micro and macro vascular complications (Chung *et al*., 2011; Marin *et al*., 2011). The chronic hyperglycemia is associated with long term damage, dysfunction and failure of various organs/organ systems. Various drugs are in practice for the treatment of diabetes, but growing evidence suggests that long term use of these may result in number of side effects, raising the concern over the use of these drugs for the treatment of diabetes (Prabhakar and Doble, 2008).

Exploration for new drugs from natural source and also failure of most of the antidiabetic drugs created a vacuum for the researchers to identify a novel potent, safe drug of natural origin. The plants are being used for medicinal purpose for further elaboration of drugs. Extraction and characterization of several active phyto-compounds have contributed to high profile drugs. The SN (black nightshade) is a medicinal plant, member of the Solanaceae family. This family comprises many genera, well known for their therapeutic properties. The *Solanum nigrum* is been traditionally used to treat various ailments such as pain, inflammation and fever (Zakaria *et al*., 2006). It has various activities such as anti-inflammatory, antioxidant, antipyretic, antitumor, antiulcerogenic, cancer chemopreventive, hepatoprotective and immunomodulatory effects (Jain *et al*., 2011). *Solanum nigrum* is widely used in many traditional systems of medicine worldwide for ailments but has not garnered attention for modern therapeutic use. The various active compounds present in the plant impart therapeutic activities. The major active compounds include steroidal glycoalkaloids, steroidal saponins, tannins and avonoids (Kang *et al*., 2011). It also contains polyphenolic compounds such as gallic acid, catechin, protocatechuic acid, caffeic acid, epicatechin, rutin and naringenin. Conventional techniques for the extraction of active constituents are time and solvent consuming and analysis of numerous constituents in plant material is limited by the extraction step. Microwave Assisted Extraction (MAE) is a novel technique with high and fast extraction performance ability with less solvent consumption and protection offered to thermolabile constituents (Latha, 2007; Mandal *et al*., 2007).

In the present study, antidiabetic potential of *Solanum nigrum* leaf extract was explored in streptozotocin (STZ)-induced diabetic rats. The extract was evaluated for its inhibitory effect against pancreatic enzymes such as alpha-amylase, alpha-glucosidase enzyme and inhibitory effect on glucose diffusion. The extract was fed in three different doses and the bioactive molecules were identified by HPLC method.

MATERIALS AND METHODS

Chemicals: All the chemicals used in the experiment are as per the National Institute of Standards and Technology (NIST). All chemicals, drugs and solvents were of analytical grade and procured from S. D. Fine Chem. Ltd. (India). Streptozotocin was procured from Merck India Ltd., India. All the kits used were from M/s Agappe Diagnostics Ltd., India.

Sample preparation: The fresh, whole plant materials of SN was collected from the local market in Mysore (Karnataka, India) in July 2012 and specimen voucher was deposited in the herbarium (PPSFT-PSM/SN-2-2012) in Plantation Products, Spices and Flavour Technology Department, Central Food Technological Research Institute, Mysore, India.

***Solanum nigrum* leaf extracts preparation:** Fresh leaves of SN were harvested, washed in clean water and dried in the oven at 40°C and grinded. Powdered leaves (50 g) was mixed with ethanol and water in the ratio 1:1 and made up to 1000 mL and extracted at 55°C, 700 W for 2 min by microwave extractor, (Milestone lab station, model Start S, Italy). Extract were filtered at room temperature (28°C). Ethanol and water were removed completely by using rotary evaporator (model: Rotavapor RE–111, M/s. Buchi, Switzerland) and lyophilizer (Model: FD3, Heto-Holten, Allerad, Denmark), respectively. The dried powder obtained was stored in an air tight container at 4°C and used for further experiments.

Determination of total polyphenol content: The Total Polyphenol Content (TPC) was determined by spectrophotometry, using gallic acid as standard, according to the method followed by Anesini *et al*. (2008). Concisely, 1.0 mL of the sample extract was transferred in duplicate to separate tubes containing 5.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 mL of a sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperature for 60 min before absorbance at 765 nm was measured against water. The TPC was expressed as gallic acid equivalents (GAE) in g/100 g material. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 10-50 μg mL^{-1}.

HPLC analysis: The SN extract (10 g) was dissolved in 10 mL of distilled water for identification of phenolic compounds. The organic acids were analyzed on a C_{18} (25 cm×4.6 mm, 5 µm) column by HPLC system (Waters, Model LC-10A) using a dual lambda detector (operating at 268 nm). The mobile phase was chosen as per Kersten *et al.* (2006) with slight modification in the ratio of mobile phase (Kersten *et al.*, 2006). A solvent system consisting of water/methanol/acetic acid (69.5:29.5:1) was used as mobile phase (isocratic) at a flow rate of 1 mL min^{-1}. Ten microliter of sample and standard was injected into the column and their retention time was recorded and compared. The known concentration of the organic acid standards was used for the quantification of organic acids present in the water soluble extract. The calibration graph was obtained by injecting the organic acid standards of different concentrations (5, 10, 15 and 20 µL). The area of the standard peak was compared with that of the sample to quantify the amount of organic acids present in the extract and in the plant material.

Effect of extract on *in vitro* inhibitory glucose diffusion: A modest model system was used to evaluate the effects of SN leaf extracts on glucose movement in vitro as described by Edwards *et al.* (1987) which involved the use of a sealed dialysis tube into which 15 mL of a solution of glucose and sodium chloride (0.15 M) was introduced and the appearance of glucose in the external solution was measured (Edwards *et al.*, 1987). The model used in the present experiment consisted of a dialysis tube (6 cm×15 mm) into which 1 mL of 10 mg mL^{-1} plant extract in 1% CMC and 1 mL of 0.15 M sodium chloride containing 0.22 M D-glucose was added. The dialysis tube was sealed at each end placed in a 50ml centrifuge tube containing 45 mL of 0.15 M sodium chloride. The tubes were placed on an orbital shaker and kept at room temperature. The movement of glucose into the external solution was monitored at set time intervals.

Inhibition of α-amylase activity: Anti-diabetic activity was determined by α-amylase inhibitory assay as described by Oboh *et al.* (2013). The 0.5 mg mL^{-1} amylase was prepared by adding 50 mg amylase in 100 mL 0.02 M sodium phosphate buffer (pH 6.9) containing 0.006 M NaCl. The 500 µL extract concentration of (10-100 µg mL^{-1}) were added to 500 µL of α-amylase (0.5 mg mL^{-1}) and incubated for 10 min at 25°C. Later 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer was added and incubated at 25°C for 10 min. Reaction was terminated by adding 1 mL DNSA (3,5-Dinitrosalicylic acid). Further, this mixture was incubated boiling water bath for 5 min. Samples were diluted with 10 mL double distilled water after cooling to room temperature. The absorbance was measured at 540 nm using spectrophotometer (UV-VIS Spectrophotometer UV-1800 SHIMADZU).

The α-amylase inhibitory activity was calculated by following equation:

$$\text{Inhibition (\%)} = \frac{Ab_{control} - Ab_{extract}}{Ab_{control}} \times 100$$

The IC_{50} values were calculated, which denotes the concentration of sample required to inhibit 50% α-amylase activity.

Inhibition of α-glucosidase activity: The α-glucosidase inhibitory assay was conducted according to (Oboh *et al.*, 2013). Sample (0.1 mL from 10-100 µg mL^{-1}) was added to a test tube containing 0.1 mL of 20 mM pNPG (p-Nitrophenyl α-D-glucopyranoside) and 2.2 mL of 100 mM phosphate buffer at pH 7.0 and incubated for 5 min at 37°C. The reaction was initiated by addition of 0.1 mL of enzyme solution (1 mg/0.1 mL) and incubated for 15 min at 37°C. The reaction was stopped by addition of 2.5 mL of 200 mM sodium carbonate. The absorbance of p-nitrophenol released from pNPG at 400 nm was measured with a spectrophotometer. Percentage of inhibition on the α-glucosidase activity was calculated by the equation:

$$\text{Inhibition (\%)} = \frac{Ab_{control} - Ab_{extract}}{Ab_{control}} \times 100$$

The IC_{50} value denotes the concentration of sample required to inhibit 50% α-glucosidase activity.

Animals and diet: Animal experiments were performed after due clearance from the Institutional animal ethics committee (IAEC NO.230/12) and conducted at the institute's animal house. Male Wistar rats weighing 120±5 g were obtained from animal house of Central Food Technological Research Institute, Mysore. Animals were maintained on standard pellet feed (M/s Sai Durga Feed, Bangalore) and water *ad libitum*. All animal experiments were carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision on Experiments of Animals (CPCSEA), Govt of India.

Induction of diabetes and animal treatment: The animals were fasted for 12 h and diabetes was induced by a single intraperitoneal injection with streptozotocin dissolved in citrate buffer (0.1 M, pH 4.5) at a dosage of 45 mg kg^{-1} b.wt., while controls were injected with only citrate buffer. Rats were treated with 5% glucose solution for two days to overcome the drug induced hypoglycemia followed by drinking water (Pandit *et al*., 2010). After one, week, rats with Blood Glucose Level (BGL) or fasting blood sugar above 200 mg dL^{-1} were considered diabetic and selected for the experiment.

Experimental design: The animals were placed into five groups (n-5) such as control, diabetic, high dose SN fed diabetic (375 mg kg^{-1} b.wt.), medium dose of SN diabetic (250 mg kg^{-1} b.wt.) and low dose of SN fed diabetic (125 mg kg^{-1} b.wt.). The change in body weight, blood glucose, urine sugar, serum total cholesterol, triglycerides, urine volume, diet and water intake was measured after every week.

Biochemical parameters: The rats were treated with the extract orally; the glucose (blood and urine) and lipid levels were monitored after every week. The blood from the retro-orbital plexus of the rats was collected in tubes containing EDTA (1.5 mg mL^{-1} of blood). The plasma was obtained from blood by centrifuging the tubes for 3000 rpm for 10 min (Vessal *et al*., 2003). The glucose level was estimated by glucose oxidase and peroxidase (GOD-POD) method, serum cholesterol by CHOD-PAP method, triglycerides by GPO-PAP method and HDL by enzyme selective protection method by using estimation kit. For urine sugar estimation, the urine was collected under toluene layer for 24 h by keeping animals in metabolic cages. The urine collected was centrifuged at 4000 rpm for 10 min at 4°C. The supernatant was collected and estimated for glucose level (Kumar *et al*., 2005). After completion of the work, the rats were given anaesthesia and sacrificed by cervical dislocation.

Statistical analysis: Data were statistically evaluated using one way ANOVA and expressed as Mean±SEM. The $p \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Various plants have been reported to possess antidiabetic activity by potentiating the insulin effect, either by stimulating the pancreatic secretion of insulin from the cells of islets of langerhans or its release from bound insulin; while others act through extra pancreatic mechanisms by preventing production of hepatic glucose or by enhancing glucose transporter translocation to membrane which facilitates the increased glucose uptake independent of insulin. *Solanum nigrum* has been explored for its nutraceutical potential in a wide array of health foods and drinks (Jairajpuri and Qadri, 2015). In the present study, the extract was observed to demonstrate significant antidiabetic activities in streptozotocin-induced diabetic rats, which supports the findings of earlier researches of antidiabetic activity of ethanolic extract of SN (Tiwari and Jain, 2012). In another study, alloxan-induced diabetic rats, when administrated with SN leaves extract, showed significant anti-hyperglycemic and hypolipidemic effects (Poongothai *et al*., 2010). However, little is known about the mechanism of action, active ingredients present or safety of these treatments in diabetic and healthy individuals. The leaf extract may have acted through one of the above-mentioned mechanisms.

Bioactivity of a plant extract also depends upon the method of extraction and the solvent used. Since microwaves are electromagnetic in nature, they possess both electric and magnetic field. The electric field causes heating via two simultaneous mechanisms, dipolar rotation and ionic conduction. Dipolar rotation causes oscillation in solvent as well as solid sample. As such, molecules collide with each other which produce heat in the medium, which is directly proportional to the dielectric constant of the solvent. The larger the solvent dielectric constant, the more optimal is the heating.

Unlike classical conductive heating methods, microwaves heat the whole sample simultaneously by disruption of weak hydrogen bonds promoted by the dipole rotation of the molecules. The migration of dissolved ions increases solvent penetration into the matrix and thus facilitates the solvation of the analyte. Ionic currents are also induced in the solution by the electric field. As the medium resists these currents, frictions occur and heat is liberated by a Joule effect. This phenomenon depends on the size and charge of the ions present in the solution. The effect of microwave energy is strongly dependent on the nature of both the solvent and the solid matrix. Most of the time, the chosen solvent possesses a high dielectric constant and strongly absorbs microwave energy, however, the extracting selectivity and the ability of the medium to interact with microwaves can be modulated by using mixtures of solvents. Microwaves interact selectively with the polar molecules present in glands, trichomes or vascular tissues. Localised heating leads to the expansion and

rupture of cell walls and is followed by the liberation of active constituents into the solvent (Kaufmann and Christen, 2002). Also, microwave extraction increased extraction yield and decreased solvent amount. A range of solvents are used for extraction purpose but the water-ethanol mixture was found to give the best results (Kaufmann and Christen, 2002).

Since MAE shows better bioactivity than conventional and soxhlet method (Kaufmann and Christen, 2002; Latha, 2007; Mandal *et al*., 2007), it was chosen for the extraction method for SN leaves. SN leaf extract was obtained by MAE method using water and ethanol as solvent and was further used for assessing the bioactivity.

Determination of total polyphenol content: The Folin-Ciocalteu assay is one of the oldest methods developed to determine the content of total phenols. A linear calibration curve of gallic acid with r^2 value of 0.99 was obtained which was used to determine the total polyphenol content of the SN leaf extract. The total polyphenol content was calculated to be 14.72±0.37% GAE. Further, identification and quantification of some phenolic compounds have also been done.

The phenolic groups in polyphenols can accept an electron to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components. It is well established that polyphenol-rich foods and beverages may increase plasma antioxidant capacity. This increase in the antioxidative capacity of plasma following the consumption of polyphenol-rich food may be explained either by the presence of reducing polyphenols and their metabolites in plasma, by their effects upon concentrations of other reducing agents (sparing effects of polyphenols on other endogenous antioxidants) or by their effect on the absorption of pro-oxidative food components, such as iron (Pandey and Rizvi, 2009). There is growing evidence suggesting that dietary intake of polyphenol-rich foods and supplementation with these bioactive components could have protective effects against diabetes-induced cardiovascular pathogenesis; the mechanisms involved in these properties mainly include regulation of lipid metabolism, attenuation of oxidative damage and scavenging of free radicals, improvement of the endothelial function and vascular tone, enhancement the production of vasodilating factors such as nitric oxide and inhibition the synthesis of vasoconstrictors such as endothelin-1 in endothelial cells (Bahadoran *et al*., 2013).

Identification and quantification of phenolic compounds: The HPLC profile revealed various polyphenols and its composition in leaves of SN. Higher content of p-coumaric acid was observed in the leaf extract. The retention time and quantity tabulated is shown in Table 1. One of the most widely distributed phenolic acids in plants is p-coumaric acid. It has been reported by Roy and Prince (2013a) that p-coumaric acid reduces the risk of stomach cancer (Roy and Prince, 2013b). The dietary supplementation of a crude extract of p-coumaric acid isolated from pulses reduces the ester cholesterol levels, providing a protective mechanism against the development of atherosclerosis. It also prevents lysosomal dysfunction against cardiac damage induced by isoproterenol and brings back the levels of lipid peroxidation products and activities of lysosomal enzymes to near normal levels (Roy and Prince, 2013a). Apart from p-coumaric acid, other polyphenols such as quercitin, catechol, caffeic acid, gallic acid and protocatechuic acid was also identified. Quercetin affects immunity and inflammation by acting mainly on leukocytes and targeting many intracellular signaling kinases and phosphatases, enzymes and membrane proteins often crucial for a cellular specific function (Chirumbolo, 2010). Catechol is a colorless compound that occurs naturally in trace amounts. Synthetic catechol is used as a precursor to pesticides, flavors and fragrances. Caffeic acid is observed to have inhibitory effect on cancer cell proliferation by oxidative mechanism (Prasad *et al*., 2011). Gallic acid exerts its beneficial effect by inhibiting lipogenesis or by increase in activity of lipoprotein lipase that increases peripheral insulin sensitivity in rat adipose tissue (Patel and Goyal, 2011). In a study, a protocatechuic acid treatment was observed to significantly lower plasma glucose and increase insulin levels. It also significantly lowers triglyceride content in plasma, heart and liver; elevates glutathione level and the retention of glutathione peroxidase and catalase activities in heart and kidney (Lin *et al*., 2009). The chromatogram also showed several other unidentified peaks as shown in Fig. 1.

Table 1: Polyphenols present in *Solanum nigrum* extract

Compound	Retention time	Fresh leaves (mg g^{-1})
p-Coumaric acid	3.3	1.74
Quercitin	5.2	1.08
Catechol	6.3	0.82
Caffeic acid	11.3	0.52
Gallic acid	4.0	1.06
Protocatechuic acid	5.7	0.46

Effect on SN extract on glucose diffusion: Extract concentration at 10 mg mL^{-1} showed varying glucose inhibition at different time interval (Fig. 2). The extract caused a significant decrease in glucose diffusion ($p \leq 0.05$). At the end of 27 h, glucose movement of control (without plant extract) in the external solution had reached a plateau with a mean glucose concentration above 0.31 mg mL^{-1}. It is evident from the graph that the extract has significant inhibitory potential of glucose diffusion ($p \leq 0.05$).

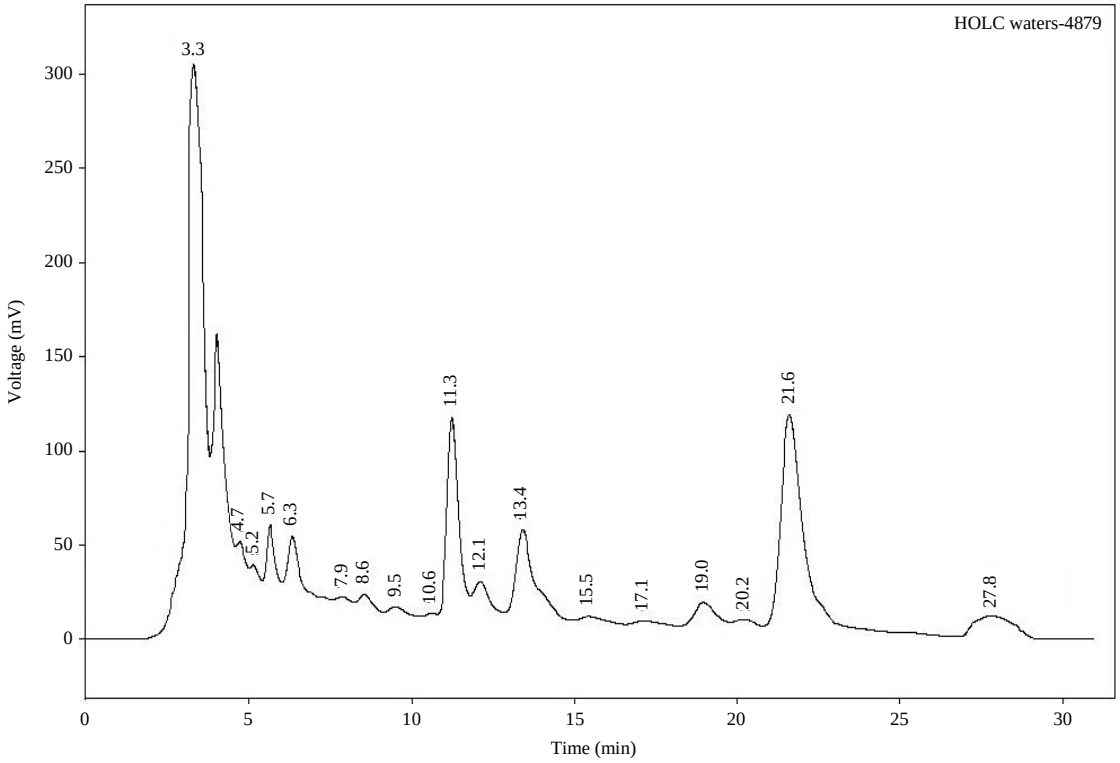

Fig. 1: HPLC profile of SN leaves extract at a concentration of 0.01 g mL^{-1}

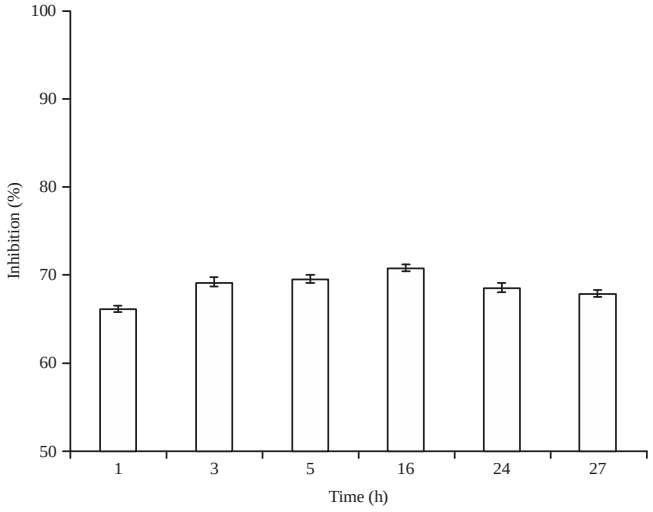

Fig. 2: Effect of SN leaves extracts (10 mg mL^{-1}) on the movement of glucose out of dialysis tube over 27 h incubation period

Effect of SN extracts on α-amylase inhibition: There was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme at lower concentration but at higher concentration the inhibition was uniform. At a concentration 100 µg mL^{-1} of extract, it showed a highest percentage inhibition of 73.15±0.212% and for 10 µg mL^{-1} it was 22.15±0.495% (Table 2). The extract gave an IC_{50} value of 39.725±0.06 µg mL^{-1}.

Effect of SN extracts on α-glucosidase inhibition: The SN leaves extract revealed a significant inhibitory action on α-glucosidase enzyme ($p \leq 0.05$). The percentage inhibition at

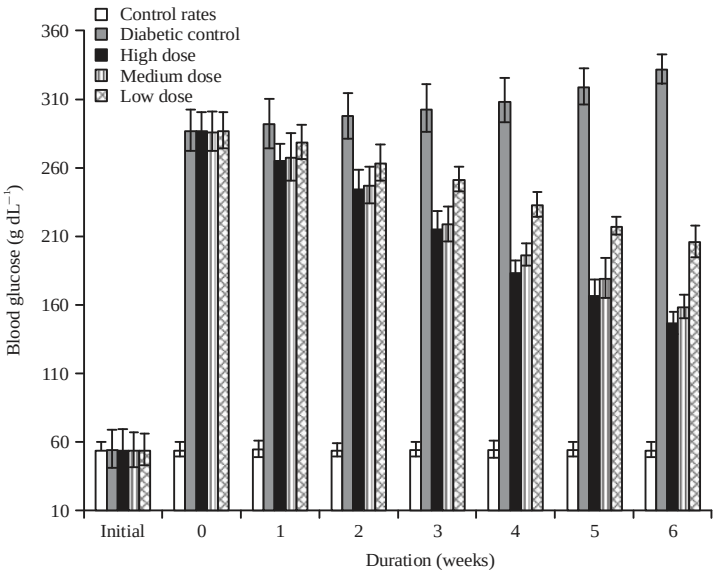

Fig. 3: Effect of SN extracton fasting plasma glucose level units (mg dL^{-1}) in rats. Values are Mean±SEM of 6 animals in each group ($p<0.05$). One-way ANOVA revealed that there were significant differences in the experimental group compared to normal group

Table 2: α-amylase inhibitory activity of *Solanum nigrum* leaves extract

Sample (μg mL^{-1})	Inhibition (%)
10	22.15±0.495
20	28.65±0.354
30	39.60±0.141
40	51.90±0.424
50	62.65±0.636
60	67.55±0.495
70	70.80±0.990
80	72.75±0.212
90	73.05±0.354
100	73.15±0.212

Table 3: α-glucosidase inhibitory activity of *Solanum nigrum* leaves extract

Sample (μg mL^{-1})	Inhibition (%)
10	10.45±0.354
20	16.50±0.283
30	20.90±0.707
40	26.80±0.566
50	29.80±0.735
60	37.05±0.636
70	43.50±0.566
80	51.20±0.539
90	57.80±0.757
100	64.50±0.424

10-100 μg mL^{-1} concentrations of SN extract showed a concentration dependent increase in percentage inhibition. The percentage inhibition varied from 10.45±0.354 to 64.5±0.424% for lowest concentration to the highest concentration of 100μg mL^{-1} (Table 3). The concentration required for 50% inhibition (IC_{50}) was found to be 78.8±0.707 μg mL^{-1}.

***In vivo* studies of SN extract on blood glucose, cholesterol and triglycerides on STZ induced diabetic rats:** A general symptom of STZ induced diabetes is the elevation of blood glucose levels and increased cholesterol. A decrease in blood glucose level was observed in rats treated with extract in a dose dependent manner and this reduction may be due to antioxidant activity of SN. Research has indicated that plants with high antioxidant activity exerts a protective effect on cytotoxic action of STZ by scavenging free radicals, as hydrogen donating sources or as singlet oxygen quenchers and metal ion chelators and causes only minor damage to β-cells of pancreas (Kumar *et al*., 2011; Kumar *et al*., 2012).

The present study demonstrated the antidiabetic and hypolipidemic activity of SN. Figure 3 represents the changes in blood glucose level in normal and experimental diabetic rats. SN lowered the blood glucose level significantly in diabetic rats as an index to normal rats in the study ($p\leq0.05$). From the first week onwards, a significant antihyperglycemic effect was noticed. The higher dose was effective in lowering the blood glucose levels.

Leaf extract of SN caused decreases in the levels of serum total cholesterol and triglycerides of STZ induced diabetic rats treated with them. These observed changes were statistically significant ($p<0.05$). The effect on treated groups was observed on every week for all the parameters. An initial cholesterol level of 84 mg dL^{-1} was observed, while the diabetic control with 272 mg dL^{-1} decreased to 117 mg dL^{-1} in higher dose group, 136 mg dL^{-1} in medium dose group and

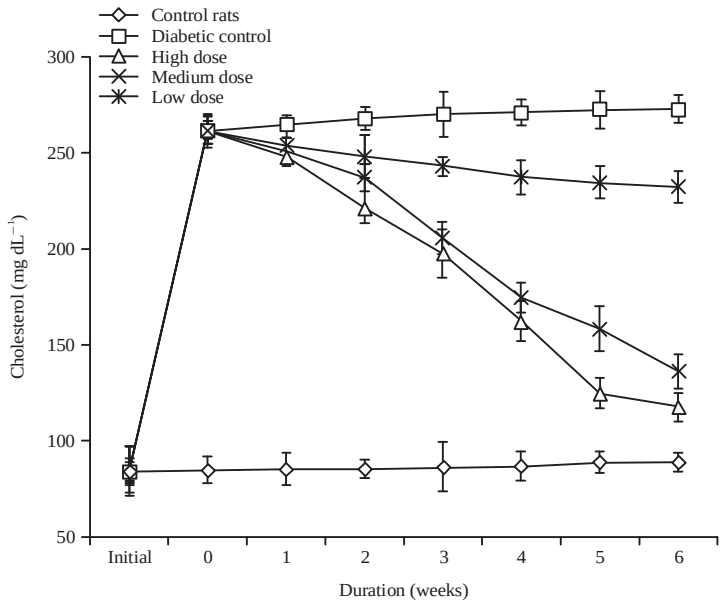

Fig. 4: Effect of *S. nigrum* leaves extract on cholesterol in control and diabetic rats. Values are Mean±SEM of 6 animals in each group ($p<0.05$). One-way ANOVA revealed that there were significant differences in the experimental group compared to normal group

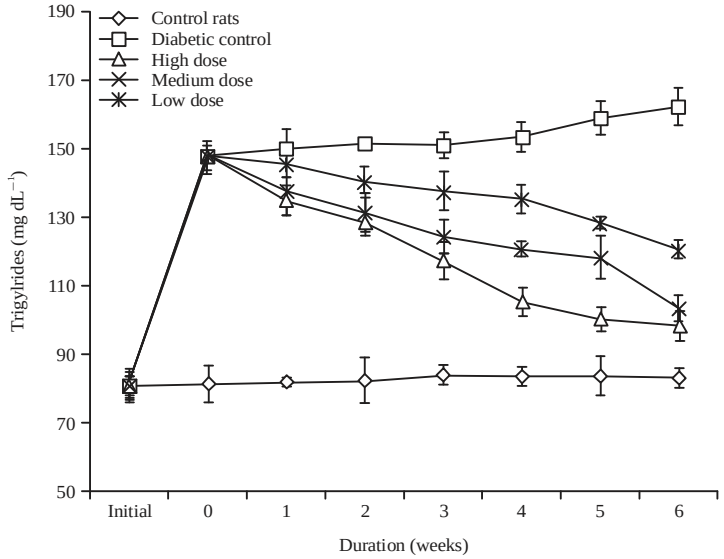

Fig. 5: Effect of *S. nigrum* leaves extract on triglycerides in control and diabetic rats. Values are Mean±SEM of 6 animals in each group ($p<0.05$). One-way ANOVA revealed that there were significant differences in the experimental group compared to normal group

232 mg dL^{-1} in lower dose as depicted in Fig. 4. Triglycerides are the major ones in which fats are present in the body. They accumulate from foods and are also produced by the liver. A high triglyceride level contributes to atherosclerosis, a build-up of plaque on the inner lining of the arteries that can cause them to harden and reduce blood flow. The treated groups showed an improvement of 98, 103 and 124 mg dL^{-1} in higher medium and lower dose, respectively. A significant improvement is observed in the high dose treated group as summarized in Fig. 5 ($p \leq 0.05$).

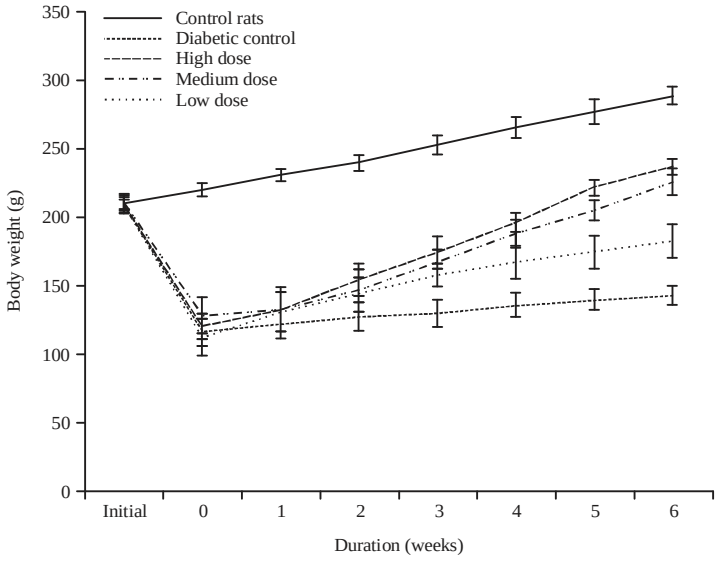

Fig. 6: Effect of *S. nigrum* leaves extract on body weight in control and diabetic rats. Values are Mean±SEM of 6 animals in each group ($p<0.05$). One-way ANOVA revealed that there were significant differences in the experimental group compared to normal group

Effect of body weight, water and diet on SN treated in diabetic rats: The characteristic loss of body weight associated with diabetes is due to loss of muscle protein (Shirwaikar *et al*., 2004, 2005). The decrease in body weight observed in diabetic rats could be the result of protein loss due to unavailability of carbohydrate for utilization as an energy source. An improvement in body weight of treated rats as compared to diabetic control suggests a protective role of SN on repair of muscle protein. Improved body weight to a certain extent indicates that control over muscle wasting resulted from glycemic control (Gandhi and Sasikumar, 2012).

Diabetes generally causes nephropathy, neuropathy and retinopathy to occur. In nephropathy state, the basal lamina of the Bowman's capsule is affected. Thus, urine is passed in diabetic patients without filtration. As such, water intake and urine excreted increases drastically in the diabetic state. The decrease in water intake observed may be due to regeneration of tubular epithelium of the kidney tissues (Advani and Gilbert, 2012).

A significant loss in body weight was observed as compared to normal rats during the study ($p\leq0.05$). Diabetic control continued to lose weight till the end of the study while SN treated group at all the three doses showed significant improvement in body weight compared to diabetic control group (Fig. 6). The normal rats maintained the average body weight of 289.2±6.46 g while for diabetes induced rats the average weight dropped to 132.7±16.3 g. After treating rats with the SN extract, the body weight of the treated groups improved. At the end of the study, the group with the high dose of extract had an average body weight of 237.6±5.72 g.

Diabetic rats showed increased water intake compared to control and significantly ameliorated in SN fed groups ($p\leq0.05$). High water intake and urine output are a characteristic symptom of diabetes. The control rats consumed 25-30 mL day^{-1} of water compared with 103 mL day^{-1} for the diabetic control rats. The treated groups consumed around 40-70 mL day^{-1}, which was statistically significant ($p\leq0.05$) (Fig. 7). Excretion of sugar in urine was followed on a weekly basis. The control rats excreted sugar in mg level while the diabetic rats excreted sugar in g level (glucose urea) throughout the experimental period. The excretion of sugar in the urine was decreased to 0.03 mg dL^{-1} in high dose followed by 0.01 mg dL^{-1} in medium dose and 0.38 mg dL^{-1} in low dose. All SN groups ameliorated urine sugar compared to control (Table 4). Diet consumption was followed in both control and diabetic rats (Fig. 8). The control rats consumed 12 g day^{-1}. The diet intake considerably decreased in all the treated groups. While the higher dose consumed 13 g day^{-1} the medium and lower dose consumed 15 and 18 g day^{-1}, respectively.

Effect of SN extract on urine volume in control and diabetic rats: Excretion of urine was monitored on a weekly basis. The control rats excreted around 25 mL day^{-1}. More excretion

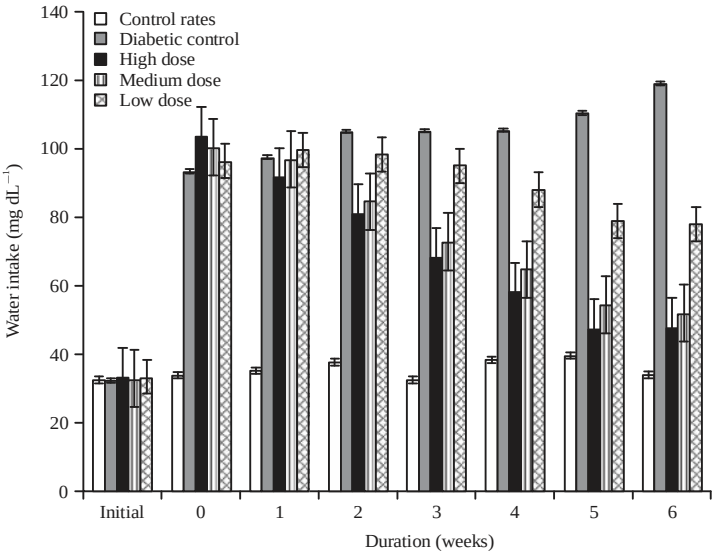

Fig. 7: Effect of *S. nigrum* leaves extract on water intake in control and diabetic rats. Values are Mean±SEM of 6 animals in each group ($p<0.05$). One-way ANOVA revealed that there were significant differences in the experimental group compared to normal group

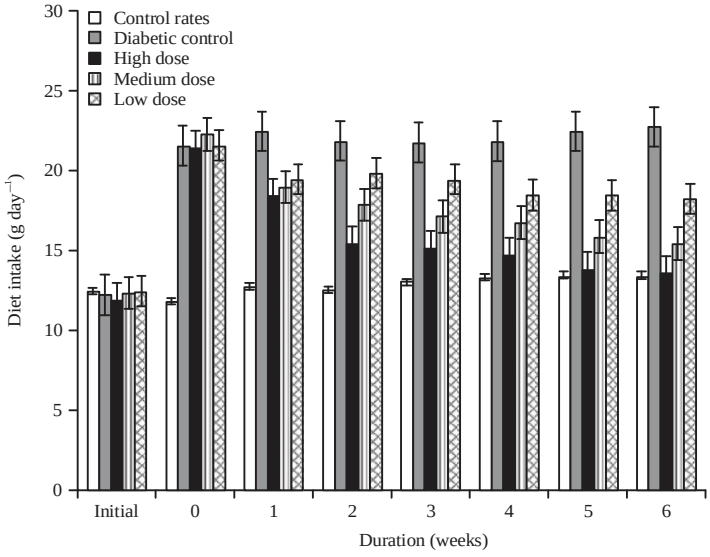

Fig. 8: Effect of *S. nigrum* leaves extract on diet intake in control and diabetic rats. Values are Mean±SEM of 6 animals in each group ($p<0.05$). One-way ANOVA revealed that there were significant differences in the experimental group compared to normal group

Table 4: Effect of *S. nigrum* leaves extract on urine sugar (mg dL^{-1}) in control and diabetic rats

Groups	Initial	Diabetic (0 day)	Final (week 6)
Control rats	0.0013±0.0001[a]	0.0017±0.001[a]	0.0015±0.0002[a]
Diabetic control	0.0012±0.0002[a]	3.77±0.06[c]	4.31±0.12[c]
Treated group (high dose)	0.0013±0.0001[a]	3.76±0.03[c]	0.03±0.007[a]
Treated group (medium dose)	0.0013±0.0001[a]	3.81±0.06[c]	0.01±0.002[a]
Treated group (low dose)	0.0013±0.0002[a]	3.78±0.08[c]	0.38±0.009[ab]

Superscript indicates that the values bearing the same superscript did not differ significantly ($p<0.05$), values are Mean±SEM of 6 animals in each group, one-way ANOVA revealed that there were significant differences in the experimental group compared to normal group

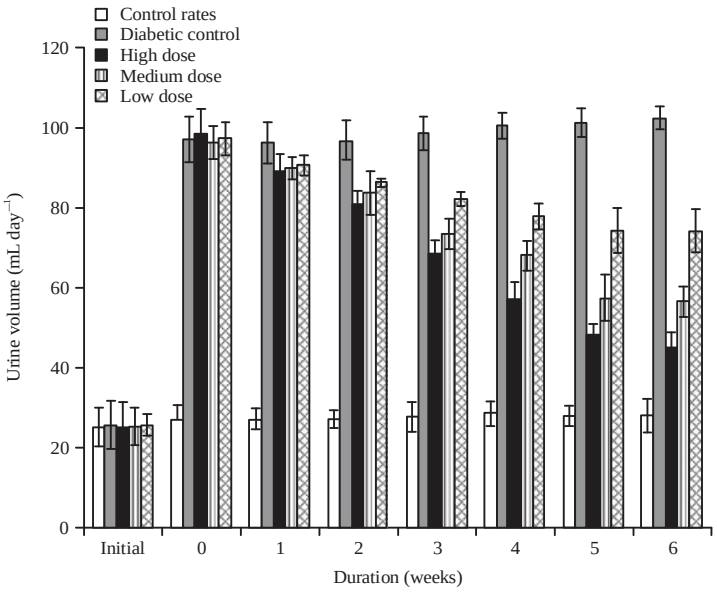

Fig. 9: Effect of *S. nigrum* leaves extract on urine volume in control and diabetic rats. Values are Mean±SEM of 6 animals in each group ($p<0.05$). One-way ANOVA revealed that there were significant differences in the experimental group compared to normal group

of urine (polyurea) prevailed in diabetic rats around 100 mL day^{-1} while the treated groups showed an improved to 42, 56 and 74 mL day^{-1} in high, medium and low dose, respectively (Fig. 9).

Hyperlipidaemia is a common complication of diabetes mellitus. A reduction in insulin secretion causes a variety of abnormalities in metabolic and regulatory mechanisms leading to accumulation of lipids. Serum lipids are been implicated in the development of atherosclerosis (Akpan *et al*., 2012). The serum lipid levels of the treated diabetic rats reduced in a dose dependent manner after treatment as against the untreated diabetic rats in this study. Diabetes-induced hyperlipidemia is contributed to increase mobilization of fat from the adipose due to the under utilization of glucose. Lipid peroxide mediated tissue damage has been observed in the development of diabetes. Insulin secretion is impaired during diabetes and this invokes lipid peroxidation in biological system. Improved levels of triglycerides indicate enhanced levels of thiobarbituric acid reactive substances (TBARS) and activation of lipid peroxidative system. The regression of the diabetic state due to the administration of the leaves extract may have increased utilization of glucose, thereby depressing the mobilization of fat. There are many reports implicating some phytochemical compounds in plants as being responsible for their antidiabetic activities. Some of these phytochemical compounds revealed present in this extract include flavonoids, saponins, alkaloids, phytosterols (Javed *et al*., 2011). Certain class of compounds viz., flavonoids, triterpenoids, sterols, alkaloids and phenolics are known to be bioactive antidiabetic principles (Narender *et al*., 2011). The antidiabetic effect of SN may be due to the presence of more than one hyperglycemic principles and their synergistic property. This may in part be responsible for both the antidiabetic and hypolipidemic activities of this leaf extract and fractions (Karthic *et al*., 2012). Further, these medicinal property of SN can be used in the nanoemulsion form for the better delivery of its bioactive compounds (Ranjan *et al*., 2014; Dasgupta *et al*., 2015).

CONCLUSION

In conclusion, the present study reveals that SN extracts yielded 14.42% by MAE with ethanol/water (1:1 ratio). The total polyphenol contents found in SN leaf extracts by MAE is 14.72±0.37% GAE. Total of six polyphenols have been identified namely p-coumaric acid, quercitin, catechol, caffeic acid, gallic acid and protocatechuic acid out of which major polyphenol was p-coumaric acid (1.74 mg g^{-1}). The SN could be used in the treatment of diabetes. The SN leaf extract significantly reversed STZ induced rise in glucose levels, indicating STZ induced diabetes is type 1 diabetes model. The antidiabetic activity may be due to improvement in glucose tolerance, restoration of liver glycogen and antioxidant activity

of SN thus, reducing the risk of secondary complications associated with diabetes. Detailed fractionation studies of SN may unveil the constituents responsible for the antidiabetic activity.

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REFERENCES

Advani, A. and R.E. Gilbert, 2012. The endothelium in diabetic nephropathy. Semin. Nephrol., 32: 199-207.

Akpan, E.J., J.E. Okokon and E. Offong, 2012. Antidiabetic and hypolipidemic activities of ethanolic leaf extract and fractions of *Melanthera scandens*. Asian Pac. J. Trop. Biomed., 2: 523-527.

Anesini, C., G.E. Ferraro and R. Filip, 2008. Total polyphenol content and antioxidant capacity of commercially available tea (*Camellia sinensis*) in Argentina. J. Agric. Food Chem., 56: 9225-9229.

Bahadoran, Z., P. Mirmiran and F. Azizi, 2013. Dietary polyphenols as potential nutraceuticals in management of diabetes: A review. J. Diabetes Metab. Disord., Vol. 12.

Cao, J., C. Li, P. Zhang, X. Cao, T. Huang, Y. Bai and K. Chen, 2012a. Antidiabetic effect of burdock (*Arctium lappa* L.) root ethanolic extract on streptozotocin-induced diabetic rats. Afr. J. Biotechnol., 11: 9079-9085.

Cao, J.F., P.Y. Zhang, C.W. Xu, T.T. Huang, Y.G. Bai and K.S. Chen, 2012b. Effect of aqueous extract of *Arctium lappa* L.(burdock) roots on the sexual behavior of male rats. BMC Complementary Altern. Med., Vol. 12. 10.1186/1472-6882-12-8

Chirumbolo, S., 2010. The role of quercetin, flavonols and flavones in modulating inflammatory cell function. Inflamm. Allergy Drug Targets, 9: 263-285.

Chung, I.M., E.H. Kim, M.A. Yeo, S.J. Kim, M.C. Seo and H.I. Moon, 2011. Antidiabetic effects of three Korean sorghum phenolic extracts in normal and streptozotocin-induced diabetic rats. Food Res. Int., 44: 127-132.

Dasgupta, N., S. Ranjan, D. Mundekkad, C. Ramalingam, R. Shanker and A. Kumar, 2015. Nanotechnology in agro-food: From field to plate. Food Res. Int., 69: 381 -400.

Edwards, C.A., N.A. Blackburn and L. Craigen, P. Davison and J. Tomlin *et al*., 1987. Viscosity of food gums determined *in vitro* related to their hypoglycemic actions. Am. J. Clin. Nutr., 46: 72-77.

Gandhi, G.R. and P. Sasikumar, 2012. Antidiabetic effect of *Merremia emarginata* Burm. F. in streptozotocin induced diabetic rats. Asian Pac. J. Trop. Biomed., 2: 281-286.

Jain, R., A. Sharma, S. Gupta, I.P. Sarethy and R. Gabrani, 2011. *Solanum nigrum*: Current perspectives on therapeutic properties. Altern. Med. Rev., 16: 78-85.

Jairajpuri, D.S. and S. Qadri, 2015. Fortification of orange juice with *Withania somnifera Solanum nigrum* extract-a potential functional fruit beverage and its quality evaluation. J. Food Sci. Technol., 25: 58-65.

Javed, T., U.A. Ashfaq, S. Riaz, S. Rehman and S. Riazuddin, 2011. *In-vitro* antiviral activity of *Solanum nigrum* against hepatitis C virus. Virol. J., Vol. 8, 10.1186/1743-422X-8-26

Kang, H., H.D. Jeong and H.Y. Choi, 2011. The chloroform fraction of *Solanum nigrum* suppresses nitric oxide and tumor necrosis factor-α in LPS-stimulated mouse peritoneal macrophages through inhibition of p38, JNK and ERK1/2. Am. J. Chin. Med., 39: 1261-1273.

Karthic, R., S. Nagaraj, P. Arulmurugan, S. Seshadri, R. Rengasamy and K. Kathiravan, 2012. *Gymnema sylvestre* R. Br. Suspension cell extract show antidiabetic potential in Alloxan induced diabetic albino male rats. Asian Pac. J. Trop. Biomed., 2: S930-S933.

Kaufmann, B. and P. Christen, 2002. Recent extraction techniques for natural products: Microwave-assisted extraction and pressurised solvent extraction. Phytochem. Anal., 13: 105-113.

Kersten, P.J., B.J. Kopper, K.F. Raffa and B.L. Illman, 2006. Rapid analysis of abietanes in conifers. J. Chem. Ecol., 32: 2679-2685.

Kumar, G.S., A.K. Shetty, K. Sambaiah and P.V. Salimath, 2005. Antidiabetic property of fenugreek seed mucilage and spent turmeric in streptozotocin-induced diabetic rats. Nutr. Res., 25: 1021-1028.

Kumar, R., D.K. Pate, S.K. Prasad, K. Sairam and S. Hemalatha, 2011. Antidiabetic activity of alcoholic leaves extract of *Alangium lamarckii* Thwaites on streptozotocin-nicotinamide induced type 2 diabetic rats. Asian Pac. J. Trop. Med., 4: 904-909.

Kumar, R., D.K. Patel, S.K. Prasad, K. Sairam and S. Hemalatha, 2012. Antidiabetic activity of alcoholic root extract of *Caesalpinia digyna* in streptozotocin-nicotinamide induced diabetic rats. Asian Pac. J. Trop. Biomed., 2: S934-S940.

Latha, C., 2007. Microwave-assisted extraction of embelin from *Embelia ribes*. Biotechnol. Lett., 29: 319-322.

Lin, C.Y., C.S. Huang, C.Y. Huang and M.C. Yin, 2009. Anticoagulatory, antiinflammatory and antioxidative effects of protocatechuic acid in diabetic mice. J. Agric. Food Chem., 57: 6661-6667.

Mandal, V., Y. Mohan and S. Hemalatha, 2007. Microwave assisted extraction: An innovative and promising extraction tool for medicinal plant research. Pharmacogn. Rev., 1: 7-18.

Marin, D.P., A.P. Bolin, R.D.C.S. MacEdo, S.C. Sampaio and R. Otton, 2011. ROS production in neutrophils from alloxan-induced diabetic rats treated *in vivo* with astaxanthin. Int. Immunopharmacol., 11: 103-109.

Narender, T., T. Khaliq and G. Madhur, 2011. Naturally Occurring Antihyperglycemic and Antidyslipidemic Agents. In: Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry, Tiwari, V.K. and B.B. Mishra (Eds.). Research Signpost, Kerala, India, ISBN-13: 9788130804484, pp: 155-185.

Oboh, G., A.O. Ademosun, O.V. Odubanjo and I.A. Akinbola, 2013. Antioxidative properties and inhibition of key enzymes relevant to type-2 diabetes and hypertension by essential oils from black pepper. Adv. Pharmacol. Sci. 10.1155/2013/926047

Pandey, K.B. and S.I. Rizvi, 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Med. Cell. Longevity, 2: 270-278.

Pandit, R., A. Phadke and A. Jagtap, 2010. Antidiabetic effect of *Ficus religiosa extract* in streptozotocin-induced diabetic rats. J Ethnopharmacol., 128: 462-466.

Patel, S.S. and R.K. Goyal, 2011. Cardioprotective effects of gallic acid in diabetes-induced myocardial dysfunction in rats. Pharmacog. Res., 4: 239-245.

Poongothai, K., K.S.Z. Ahmed, P. Ponmurugan and M. Jayanthi, 2010. Assessment of antidiabetic and antihyperlipidemic potential of *Solanum nigrum* and *Musa paradisiaca* in alloxan induced diabetic rats. J. Pharm. Res., 9: 2203-2208.

Prabhakar, P.K. and M. Doble, 2008. A target based therapeutic approach towards diabetes mellitus using medicinal plants. Curr. Diabetes Rev., 4: 291-308.

Prasad, N.R., A. Karthikeyan, S. Karthikeyan and B.V. Reddy, 2011. Inhibitory effect of caffeic acid on cancer cell proliferation by oxidative mechanism in human HT-1080 fibrosarcoma cell line. Mol. Cell Biochem., 349: 11-19.

Ranjan, S., N. Dasgupta, A.R. Chakraborty, S.M. Samuel, C. Ramalingam, R. Shanker and A. Kumar, 2014. Nanoscience and nanotechnologies in food industries: opportunities and research trends. J. Nanopart Res., Vol. 16. 10.1007/s11051-014-2464-5

Roy, A.J. and P.S.M. Prince, 2013a. Preventive effects of p-coumaric acid on cardiac hypertrophy and alterations in electrocardiogram, lipids and lipoproteins in experimentally induced myocardial infarcted rats. Food Chem. Toxicol., 60: 348-354.

Roy, A.J. and P.S.M. Prince, 2013b. Preventive effects of p-coumaric acid on lysosomal dysfunction and myocardial infarct size in experimentally induced myocardial infarction. Eur. J. Pharmacol., 699: 33-39.

Shirwaikar, A., K. Rajendran, C.D. Kumar and R. Bodla, 2004. Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin-nicotinamide type 2 diabetic rats. J. Ethnopharmacol., 91: 171-175.

Shirwaikar, A., K. Rajendran and I.S.R. Punitha, 2005. Antidiabetic activity of alcoholic stem extract of *Coscinium fenestratum* in streptozotocin-nicotinamide induced type 2 diabetic rats. J. Ethnopharmacol., 97: 369-374.

Tiwari, V.K. and S.K. Jain, 2012. Hypoglycemic Activity of ethanolic extract of *Solanum nigrum* Linn. Leaves on alloxan induced diabetes mellitus in rats. Int. J. Pharmaceut. Phytopharm. Res., 2: 26-28.

Vessal, M., M. Hemmati and M. Vasei, 2003. Antidiabetic effects of quercetin in *Streptozocin*-induced diabetic rats. Comparat. Biochem. Physiol. Part C. Toxicol. Pharmacol., 135: 357-364.

Zakaria, Z.A., H.K. Gopalan, H. Zainal, N.H.M. Pojan, N.A. Morsid, A. Aris and M.R. Sulaiman, 2006. Antinociceptive, anti-inflammatory and antipyretic effects of *Solanum nigrum* chloroform extract in animal models. Yakugaku Zasshi, 126: 1171-1178.