LABORATORY STUDY

Renoprotective effect of Bacopa monnieri via inhibition of advanced glycation end products and oxidative stress in STZ-nicotinamide-induced diabetic nephropathy

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ABSTRACT
Hyperglycemia and oxidative stress are involved in the development of diabetic nephropathy (DN). This study was designed to evaluate the effect of alcohol and hydroalcohol extract of Bacopa monnieri and stigmasterol isolated from B. monnieri in the treatment of DN. Diabetes was induced in male wistar rats by streptozotocin (65 mg/kg i.p.) 15 min after nicotinamide (230 mg/kg, i.p.) administration. After 30 days, the rats were treated with different doses of extracts (100, 200, and 400 mg/kg) and stigmasterol (5 and 10 mg/kg) for 45 days to analyze their nephroprotective effect and produced significant attenuation in the serum glucose level, uric acid, creatinine, and lipid levels. Moreover, there is improvement in the level of superoxide dismutase (SOD), glutathione (GSH), and decrease in lipid peroxidation in terms of TBARS. The formation of AGEs in kidneys was also significantly reduced. These findings suggest that B. monnieri and its isolate (stigmasterol) might inhibit the progression of DN.

ARTICLE HISTORY
Received 2 June 2016
Revised 20 July 2016
Accepted 18 August 2016
Published online 26 October 2016

KEYWORDS
Advanced glycation end products; Bacopa monnieri; diabetic nephropathy; hyperglycemia; oxidative stress; stigmasterol

1. Introduction
Diabetes mellitus is characterized by hyperglycemia due to low insulin secretion or insulin resistance and is a major provider of morbidity and mortality to society. Diabetic nephropathy (DN) is a commonly occurring and precarious microvascular complication of diabetes mellitus. DN is also known as diabetic glomerulosclerosis or diabetic kidney disease and has been accepted as one of the major and prominent cause of end stage renal disease (ESRD) worldwide which leads to renal failure. The changes in dietary habits, living as well as working styles has directed to brisk amplification of diabetes patients in developed and emerging countries. Patients with chronic diabetes have the prospect of getting DN. Thus, DN has become a mounting global menace. DN is characterized by a succession of renal structure abnormalities, including tubulointerstitial fibrosis, thickening of diffuse glomerular basement membrane, glomerulosclerosis, glomerular hypertrophy, mesangial matrix expansion and occurrence of pathological quantities of urine albumin, creatinine, and protein excretion, and abnormal glomerular filtration rate (GFR). However, in the present scenario there is no superlative therapy available for DN, particularly in the late period. Hyperglycemia is a pre-requisite for the development of diabetic complications and prolonged exposure to high glucose concentrations (hyperglycemia) promotes the development of complications associated with diabetes mellitus.

Oxidative stress also plays a key role in the pathogenesis of diabetes and diabetic complications. The excessive free radicals generation occurs in diabetes due to glucose oxidation, nonenzymatic glycation of proteins etc. These high levels of free radicals can lead to lipid peroxidation, damage of enzyme system, and cellular organelles. It can also lead to insulin resistance. These consequences of oxidative stress can uphold the development of complications of diabetes mellitus.

Bacopa monnieri (L.) Wettst. (Scrophulariaceae), commonly known as “Brahmi” is a medicinal herb, found throughout the Indian subcontinent in wet and marshy places. It has been used for centuries in Ayurvedic system of medicine as a brain tonic, memory enhancer, revitalizer of sensory organs, antianxiety, cardio-tonic, diuretic, antidepressant, and anticonvulsant agent. The major chemical constituents of B. monnieri are dammarane-type triterpenoid Saponins such as bacoside A.
and bacoside B, which are mainly responsible for the pharmacological activity of this plant.\textsuperscript{13} Other compounds include phenylethanoid glycosides, flavonoids, and alkaloids such as brahmine and herpestine.\textsuperscript{14} \textit{Bacopa monnieri} also exhibits potent antioxidant,\textsuperscript{15} anti-cancer,\textsuperscript{16} anti-ulcer,\textsuperscript{17} calcium antagonist,\textsuperscript{18} vasodilatory, mast cell stabilizing,\textsuperscript{19} anti-inflammatory,\textsuperscript{20} and anti-stress\textsuperscript{21} properties. Saponins isolated from medicinal plants are found to be renoprotective as they reduce fasting blood glucose and albuminuria, reverses the glomerular hyperfiltration state and ameliorates proliferative glomerular pathological changes during the early stages of DN in rat models.\textsuperscript{22} Flavonoids are the most widespread polyphenolic compounds with hypoglycemic and antidiabetic properties. Flavonoid also leads to the regeneration of pancreatic $\beta$-cells, reduces necrosis and degeneration, attenuates oxidative stress and thus, may be effective in treating hyperglycemia thereby preventing DN.\textsuperscript{23} A study conducted by Ghosh et al.,\textsuperscript{24} proved that Bacosine, a triterpenes from \textit{B. monnieri}, increase in peripheral glucose consumption as well as protection against oxidative damage in alloxanized diabetes. Taking into consideration the promising constituents present in \textit{B. monnieri} and its ameliorating effect on diabetes and oxidative stress, present study involves the evaluation of \textit{B. monnieri} on DN.

2. Materials and methods

2.1. Chemicals

Streptozotocin (STZ) was obtained from Sigma Aldrich (Milwaukee, WI), USA and Nicotinamide (NAD) from Finar India Ltd. and diagnostic kits for the biochemical estimations were obtained from Reckon Diagnostics Pvt. Ltd., New Delhi, India. All the other chemicals used were of analytical grade.

2.2. Collection of plant material

Aerial parts of \textit{B. monnieri} Linn. (Scrophulariaceae) were procured from Green earth Pvt. Ltd., New Delhi and identified by Dr Sunita Garg, NISCAIR, New Delhi. A voucher specimen (NISCAIR/RHMD/Consult/2013/2296/76) was deposited in the herbarium of NISCAIR, New Delhi for future reference. Botanical name of the plant was verified from published literature and database.\textsuperscript{25}

2.3. Preparation of extract

The aerial parts were dried in the shade, powdered, and then used for the extraction of potential antidiabetic constituents into different solvents (petroleum ether, chloroform, ethanol, and hydroalcohol). Aerial parts were sequentially extracted with solvents in the order of increasing polarity i.e., petroleum ether 60–80°C, chloroform, ethanol, and hydro-alcohol (40%) by Soxhlet extraction method.\textsuperscript{26} The extraction procedure was continued until the extract gave no coloration. The extracts were distilled and concentrated under reduced pressure and finally freeze dried. Ethanol (BA) and hydro-alcohol (BHA) extracts were used for further studies.

2.4. Phytochemical screening

BA and BHA extracts were subjected to phytochemical screening for the identification of chemical constituents, including alkaloids, carbohydrates, fixed oils and fats, terpenoids, phenols, tannins, glycosides, saponins, proteins, amino-acids, and flavonoids.\textsuperscript{27,28}

2.5. Determination of total phenol content

Total phenolic content in the BA and BHA was determined using Folin–Ciocalteau reagent using gallic acid as a standard phenolic compound. Briefly, aliquot of 1 ml of sample solution (with appropriate dilution to obtain absorbance in the range of the prepared calibration curve) was mixed with 1 ml of Folin–Ciocalteau reagent. Three minutes later, 3.0 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 3 h with intermittent shaking. The absorbance of the blue color that developed was measured at 760 nm. The concentration of total phenolic compounds in the extract was determined as mg of gallic acid equivalent (GAE) per gram dry weight.\textsuperscript{29}

2.6. AGE’s inhibitory assay

\textit{In vitro} antiglycation activity of BA and BHA was examined by testing their ability to inhibit the fluorescence of BSA in accordance with a previous method.\textsuperscript{30} The reaction mixture of BSA (10 mg/ml), 1.1 M fructose in 0.1 M phosphate buffered-saline (PBS), pH 7.4 containing 0.02% sodium azide with or without extract (BA and BHA; dissolved in PBS; 50–500 $\mu$g/ml) was incubated in darkness at 37 °C for 1, 2, 3, and 4 weeks. AGE formation was measured by fluorescent intensity at an excitation wavelength of 355 nm and emission wavelength of 460 nm. Aminoguanidine (AG) was used as a positive control for this study. The percentage inhibition of AGE formation was determined by the following
formula:

\[
\% \text{Inhibition} = \frac{\text{Fluorescence of control} - \text{Fluorescence of test}}{\text{Fluorescence of control}}
\]

2.7. Aldose reductase (ALR1) enzyme inhibition

Purification of ALR1 from rat kidney: ALR1 was partially purified from rat kidney following the previously described methods. Briefly, freshly obtained bovine kidney was homogenized in 3 volume of 10 mM sodium phosphate buffer, pH 7.2 containing 0.25 M sucrose, 2.0 mM EDTA, 2.5 mM 2-mercaptoethanol. The homogenate was centrifuged at 10,000 g for 20 min and the supernatant was subjected to ammonium sulfate precipitation. Precipitate obtained between 45% and 75% saturation was dissolved in the above buffer and dialyzed extensively against the same buffer. DEAE-52 resin was added to the dialyzed material and then removed by centrifugation. The supernatant was used as the source of ALR1.

ALR1 assay: The activity of ALR1 was measured spectrophotometrically by monitoring the oxidation of NADPH at 340 nm as a function of time at 37°C using glyceraldehyde as substrate. The 1 ml of assay mixture contained 50 mM sodium phosphate buffer of pH 7.2, 0.2 M ammonium sulfate, 10 mM D,L-glyceraldehyde, 5 mM \( \beta \)-mercaptoethanol, and 0.1 mM NADPH.

Inhibition studies: For inhibition studies stock solutions of BA and BHA prepared in distilled water were used. Various concentrations (2–10 \( \mu \)g/ml) of BA and BHA were added to assay mixtures of ALR1 and incubated for 5 min before initiating the reaction by NADPH as described above. The percentage inhibition was calculated considering the activity in the absence of BA and BHA as 100%. The \( IC_{50} \) values were determined by linear regression analysis of the plot of percent inhibition versus inhibitor concentration.

2.8. Isolation of active compounds

The dried alcohol extract (BA; 5 g) was then subjected to column chromatography (silica gel packed column, Molychem 100–200 mesh, 100 g) by pre-absorbing with silica gel (5 g) and was eluted using pet ether and ethyl acetate (80:20). The fractions (100 ml each) obtained from the column were collected and combined on monitoring TLC. A total of 43 fractions were collected and out of which the fractions 10–17 were found to be significant. The isolated compound was purified using methanol to obtain white crystalline powder, which was characterized as Stigmasterol (ST) on the basis of spectroscopic analysis (IR and 1H NMR) and data from the literature.

2.9. Experimental design and animal

The male Wistar rats weighing 260–280 g were kept in clean polypropylene cages and maintained at the local animal house conditions of temperature 24 ± 2°C, humidity 45 ± 5%, and 12 h day and 12 h night cycle. The animals were fed with a standard pellet diet (Ashirwad Industries, Mohali, Punjab, India) and water ad libitum. The experimental protocol was approved by the Institutional Animal’s Ethics Committee and by the regulatory body of the government (MMCP/IAEC/13/09).

Diabetic mellitus was induced by single intraperitoneal injection of freshly prepared streptozotocin (single dose of 65 mg/kg, bw) in 0.1M citrate buffers (pH 4.5) 15 min after nicotinamide (230 mg/kg, i.p.), in a volume of 1 ml/kg body weight. The control animals were treated with citrate buffer (pH 4.5). After 7 days of STZ administration, rats with a fasting blood glucose level of 300–350 mg/dl were selected for the study. DN symptoms typically develops after 30–45 days. At the end of 30 days, the levels of serum urea, uric acid, creatinine, and BUN were significantly high suggesting the development of DN.

In the experiment, a total of 66 rats (6 normal; 60 STZ-diabetic rats) divided into 11 groups consisting of 6 rats each were used. Group 1 was normal control; Group 2 was diabetic control; Group 3–5 consisted of DN rats receiving 100, 200, and 400 mg/kg of BA; Group 6 to 8 consisted of DN rats receiving 100, 200, and 400 mg/kg of BHA; Group 9 and 10 consisted of DN rats receiving 5 and 10 mg/kg of ST; Group 11 consisted of DN rats receiving 10 mg/kg glimepride. Treatment with extracts and glimepride were started after 30 days of STZ-administration and continued to next 45 days.

Blood glucose level and body weight were estimated on 30th, 45th, 60th, and 75th day of STZ induction. All biochemical estimation was carried out using commercially available kits of Reckon Diagnostics Pvt. Ltd. Animals were sacrificed at the end of the study and the liver, kidney, and pancreas were removed and stored at −70°C until use for histopathological study.

2.10. Body weight, blood glucose, and serum insulin estimation

Body weight of each animal was measured at the start of the study, and animals with similar weight were grouped together. Body weight of each group was measured periodically till the end of the study. Fasting
blood glucose level was estimated at an interval of 15 days using commercial enzymatic kits purchased from Reckon Diagnostics Pvt. Ltd., New Delhi, India throughout the study. Plasma insulin was determined by Insulin ELISA kit (DRG, Marburg, Germany) in blood collected into tubes containing anticoagulant.

2.1.1. Lipid profiles assay
Serum total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), very low density lipoproteins (VLDL), and high density lipoproteins (HDL) were measured by the commercial enzymatic kits purchased from Reckon Diagnostics Pvt. Ltd., New Delhi, India.

2.1.2. Renal function tests
Serum creatinine, urea, uric acid and BUN were measured using commercial enzymatic kits purchased from Reckon Diagnostics Pvt. Ltd., New Delhi, India. The animals were placed in individual metabolic cages for 12 h to collect urine samples before the experimental rats were killed. Urinary creatinine was assessed using kits purchased from Reckon Diagnostics Pvt. Ltd., New Delhi, India. Creatinine clearance was calculated according to the standard formula:

$$C_{cr} = \frac{\text{urinary creatinine (mg/ml)} \times \text{urine volume (ml)} \times \text{serum creatinine (mg/ml)}}{\text{100/body weight (g)} \times \frac{1}{720} (\text{min})}$$

2.1.3. Biochemical analysis
For biochemical estimations, a 10% (w/v) tissue homogenate (kidney, pancreas, and liver) was prepared in chilled phosphate-buffered saline (pH 7.4) using a Teflon homogenizer. The homogenate was centrifuged at 1000g for 10 min at 4°C to remove nuclei and unbroken cells. The pellet was discarded and clear supernatant thus obtained was used to assay thiobarbituric acid reactive substances (TBARS) and level of antioxidant enzymes, namely, superoxide dismutase (SOD) and reduced glutathione (GSH).

2.1.3.1. Estimation of superoxide dismutase
The superoxide anion generation was estimated in terms of measuring reduced nitroblue tetrazolium (NBT). Weighed amount of tissue (25 mg) was taken in 5 ml phosphate-buffered saline containing 100 μM of NBT and incubated at 37°C for 1.5 h. The NBT reduction was stopped by adding 5 ml of 0.5 M HCl. Then, the left ventricular tissue was taken out and was minced and homogenized in a mixture of 0.1 M sodium hydroxide and 0.1% sodium dodecyl sulfate in water containing 40 mg/l diethylene triamine penta acetic acid. The mixture was centrifuged at 20,000g for 20 min and the resultant pellet was suspended in 1.5 ml of pyridine and kept at 80°C for 1.5 h to extract formazan, an adduct formed after reaction of NBT with superoxide anions. The mixture was again centrifuged at 10,000g for 10 min and absorbance of formazan was determined spectrophotometrically at 540 nm. The amount of reduced NBT was calculated using the following formula:

$$\text{Amount of reduced NBT} = \frac{A \times V}{T \times Wt \times E \times 1}$$

2.1.3.2. Estimation of reduced glutathione
The reduced GSH content in tissues was estimated using the method described by Beutler et al. The supernatant of homogenate was mixed with trichloroacetic acid (10% w/v) in 1:1 ratio. The tubes were centrifuged at 1000g for 10 min at 4°C. The supernatant (0.5 ml) was mixed with 2 ml of 0.3 M sodium hydrogen phosphate. Then, 0.25 ml of 0.001 M freshly prepared DTNB [5,5'-dithiobis (2-nitrobenzoic acid)] dissolved in 1% w/v citric acid was added and absorbance was noted spectrophotometrically at 412 nm. A standard curve was plotted using 5–50 μM of reduced form of GSH and results were expressed as micromoles of reduced GSH per mg of protein.

2.1.3.3 Estimation of TBARS
The quantitative measurement of TBARS, a measure of lipid peroxidation, was assayed by the method described by Ohkawa et al. Aliquot of 0.2 ml of supernatant of homogenate was pipetted out in a test tube, followed by the addition of 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 30% acetic acid (pH 3.5), 1.5 ml of 0.8% thiobarbituric acid and the volume was made up to 4 ml with distilled water. The test tubes were incubated for 1 h at 95°C, then cooled and added 1 ml of distilled water followed by the addition of 5 ml of n-butanol-pyridine mixture (15:1 v/v). The tubes were then centrifuged at 4000g for 10 min. The absorbance of developed pink color was measured spectrophotometrically at 532 nm. A standard calibration curve was prepared using 1–10 nM of 1,1,3,3-tetra methoxy propane. The TBARS values were expressed as nanomoles per mg of protein.

2.1.4. AGEs estimation in kidneys
AGEs levels in the kidneys were determined by the method as previously described by Sensi et al.
Briefly, perfused kidneys were homogenized in 2 ml of 0.25 M sucrose followed by centrifugation at 900g at 5°C and the supernatant was separated. The pellet was resuspended in 2 ml sucrose solution and centrifuged and the supernatant obtained was mixed with the previous one. The proteins present were precipitated by adding equal volume of trichloroacetic acid (TCA). Following centrifugation at 4°C, 900g, the protein pellet obtained was mixed with 1 ml methanol twice to remove the lipid fraction. The insoluble protein, after washing with 10% cooled TCA was centrifuged and the residue was solubilized in 1 ml of 1 N NaOH and the protein concentration was estimated by measuring the absorbance at 280 nm against BSA standard curve. The AGEs content was then measured fluorometrically with an emission at 440 nm and excitation at 370 nm, and the results were expressed as relative fluorescence units (RFU)/mg of protein.

2.15. Histopathology

The liver, kidney, and pancreatic tissues were obtained from the sacrificed animals and fixed in 10% neutral buffered formalin solution, dehydrated in ethanol, and embedded in paraffin. Sections of 5 μm thickness were prepared using a rotary microtome and stained with hematoxylin and eosin (H & E) dye for microscopic observations.

2.16. Statistical analysis

Statistical analysis was performed using Graphpad Prism 6. Values were expressed as mean ± SEM and one-way analysis of variance (ANOVA) was used for statistical analysis. ANOVA was followed by Tukey’s post hoc multiple comparison test. The results were considered significant if p ≤ .05.

3. Results

3.1. Phytochemical screening

Preliminary phytochemical screening of BA showed the presence of carbohydrates, flavonoids, tannins, and terpenoids whereas carbohydrates, polyphenolic compounds, flavonoids, saponins, tannins, and terpenoids were found to be present in BHA. Total phenol content present in BA and BHA contained 700 and 846 mg/g GAE of phenol content, respectively.

3.2. Effect of B. monnieri on AGEs formation

In the present study, the formation of AGEs was monitored weekly by measuring the fluorescence intensity of BSA-fructose solutions for 4 weeks. A significant inhibition of AGEs formation (93.37%) was observed in fructose-induced glycated BSA plus aminoguanidine (500 μg/ml). At the fourth week of incubation, the percentage inhibitions of AGEs formation by BA (50–500 μg/ml) was 31.34–92.29%, respectively and for BHA was found to be 31.88–93.37%, respectively.

3.3. Aldose reductase (ALR1) enzyme inhibition

The present study was conducted to evaluate the ALR enzyme inhibitory effect of BA and BHA, which might have potential uses in the treatment of diabetic complications. Results revealed that BA and BHA had good inhibitory effects with IC50 values 6.057 and 6.189 μg/ml, respectively. Inhibitory effect of extracts was compared with quercetin having an IC50 value of 5.17 μg/ml.

3.4. Stigmasterol from B. monnieri

Chemical test for sterol was found positive. Colorless powder; mp 165–170°C; the IR spectrum showed a broad strong band at 3185 cm⁻¹ which was due to the presence of a hydroxyl group. The absorption band at 1747 cm⁻¹ was typical of C–C double bond. Shift of this band from 1645 cm⁻¹ must be due to the ring tension, which would cause bathochromic shift. The stretching and bending vibrations for methyl group were noticed by an intense band at 2891 cm⁻¹ and a less intense band at 1445 cm⁻¹. Band at 3185 cm⁻¹ showed a CH alkene stretch and a bend at 1354 cm⁻¹. The moderately intense band at 1445 cm⁻¹ was due to rocking movement of methylene part. These bands are characteristic for this type of compounds. 1H NMR (400 MHz, CDCl3) δ 0.89 (2H, t, H-1), δ 1.44 (2H, q, H-2), δ 3.5 (1H, m, H-3), δ 1.9 (2H, d, H-4), δ 5.3 (1H, t, H-6), δ 1.92 (2H, t, H-7), δ 1.45 (1H, t, H-8), δ 1.44 (1H, q, H-9), δ 0.9 (2H, q, H-11), δ 1.37 (2H, t, H-12), δ 1.4 (3H, s, H-18), δ 0.98 (3H, s, H-19), δ 2.3 (1H, t, H-20), δ 1.32 (3H, d, H-21), δ 5.0 (1H, t, H-22), δ 5.1 (1H, t, H-23), δ 2.0 (1H, q, H-24), δ 1.7 (1H, m, H-25), δ 0.78 (6H, d, H-26,27), δ 0.6 (3H, t, H-29), δ 2.1 (s, OH). Therefore, the compound is structurally Stigmasterol (ST) (Figure 1).

![Structure of Stigmasterol (ST).](image-url)
3.5. Effect of BA, BHA, and ST on body weight

Administration of STZ-NAD injection decreased body weight of diabetic animals by 37.05% at the end of the study as compared to the initial body weight. At the end of study BA, BHA (400 mg/kg), and ST (10 mg/kg) increased the body weight significantly by 14.1, 21.96, and 9.82%, respectively. Glimepride also increased the body weight by 16.43% in DN rats (Figure 2(a)). Further, a significant increase in food and water intake in diabetic rats was observed in comparison to the normal rats. Chronic treatment with BA, BHA, and ST significantly ameliorated these physiological parameters.

3.6. Effect of BA, BHA, and ST on blood glucose and serum insulin level

Fasting blood glucose level of all animals was within the normal range initially and STZ-NAD induction increased blood glucose level significantly with respect to time compared to normal control animals (Figure 2(b)). Chronic treatment with BA, BHA (100, 200, and 400 mg/kg), and ST (5 and 10 mg/kg) from 30th–75th day produced significant reduction in fasting blood glucose level compared with diabetic control group. A significant decrease in fasting insulin level (7.00 ± 0.12 μU/ml) was observed in STZ-treated DN rats in comparison to normal control rats (15.07 ± 0.08 μU/ml). Administration of BA, BHA, and ST significantly increased the level of serum insulin in comparison to diabetic control (Figure 2(c)).

3.7. Effect of BA, BHA, and ST on renal function

In diabetic control rats a significant increase in urea (89.33 ± 1.418 mg/dl), uric acid (15.10 ± 0.378 mg/dl), creatinine (3.92 ± 0.062 mg/dl), and BUN (41.72 ± 0.662 mg/dl) levels was observed in STZ-nicotinamide-induced DN rats over the period of study. Treatment from BA, BHA, and ST attenuated the increased renal parameters in a dose-dependent manner (Table 1). Increased kidney weight/body weight ratio was significantly ameliorated by the treatment with BA, BHA, and ST. 12 h urine volume and urinary creatinine significantly increased in diabetic control rats in comparison to the normal control. Treatment with BA, BHA (100, 200, and 400 mg/kg), and ST (5 and 10 mg/kg) significantly reduced the levels of urine volume and urine creatinine level. In addition, treatment with and ST significantly improved creatinine clearance (Table 1).

3.8. Effect of BA, BHA, and ST on lipid profile

After 30 days of STZ induction, serum total cholesterol (TC), triglyceride (TG), low density lipoproteins (LDL), and very low density lipoproteins (VLDL) significantly increased in DN rats. BA, BHA (100, 200, and 400 mg/kg), and ST (5 and 10 mg/kg) produced a dose- and time-dependent attenuation in the levels of both TC and TG. Moreover, a significant attenuation in the levels of LDL and VLDL was observed with BA, BHA (100, 200, and 400 mg/kg), and ST (5 and 10 mg/kg) treatment. Further, significant decrease in the level of HDL-c in STZ-NAD-induced diabetic rats was attenuated with BA, BHA, and ST treatment (Table 2).

3.9. Effect of BA, BHA, and ST on antioxidant enzymes and lipid peroxidation

The level of reduced GSH and SOD were found significantly low after 30 days of STZ induction in diabetic rats as compared to normal control group. Continuous administration of BA, BHA (100, 200, and 400 mg/kg), and ST (5 and 10 mg/kg) from 30th to 75th day significantly increased the level of these antioxidant enzymes in a dose-dependent manner.

TBARS levels were increased significantly in kidney, pancreas, and liver of diabetic rats as compared to normal control group. Chronic treatment with BA, BHA (100, 200, and 400 mg/kg), and ST (5 and 10 mg/kg) produced time- and dose-dependent reduction in the levels of TBARS (Table 3).

3.10. Effect of BA, BHA, and ST on AGEs in kidneys

STZ-NAD administration in rats led to a significant increase in the levels of AGEs in kidney of diabetic rats compared to normal animals. Administration of BA, BHA (100, 200, and 400 mg/kg), and ST (5 and 10 mg/kg) significantly prevented this increase in AGEs level as compared to control group. However, administration of glimepride (10 mg/kg) also produced significant change (p < .05) in AGEs levels when compared to control group (Figure 3).

3.11. Histopathology

The renal tissue of diabetic rats showed glomeruli with mesangiocapillary proliferation. Medullary tubules show vacuolation and dilation of the descending thin loop. Administration of STZ produced significant structural changes in the nephron. Administration of BA, BHA, and ST to the experimental animals significantly improved the structural changes in kidney (Figure 4). Pancreas of
Figure 2. (a) Effect of BA, BHA, and ST on body weight (g). Values are represented as Mean ± SEM (n = 6). Data were analyzed using one-way ANOVA followed by Tukey’s multiple test; a vs control; b vs Diabetic control. *p < .001, #p < .01, †p < .05. (b) Effect of BA, BHA, and ST on fasting blood glucose level (mg/dl). Values are represented as Mean ± SEM (n = 6). Data were analyzed using One-way ANOVA followed by Tukey’s multiple test; a vs control; b vs Diabetic control; c vs BA/BHA 100 mg/kg; d vs BA/BHA 200 mg/kg. *p < .001, #p < .01, †p < .05. (c) Effect of BA, BHA, and ST on serum insulin level (mg/dl). Values are represented as Mean ± SEM (n = 6). Data were analyzed by one-way ANOVA followed by Tukey’s multiple test; a vs control; b vs Diabetic control; c vs BA/BHA 100 mg/kg; d vs BA/BHA 200 mg/kg; e vs Glimepride 10 mg/kg. *p < .001, #p < .01, †p < .05.
the diabetic animals showed scanty and hypocellular islets along with small duct with mild inflammatory cells. These changes were ameliorated in BA, BHA, and ST administered DN rats (Figure 5). Liver of diabetic rats showed perivenular inflammatory collection and Kupffer cell hyperplasia with condensed nuclei. These pathological changes were reversed by the administration of BA, BHA, and ST to the experimental animals (Figure 6).

4. Discussion

STZ-induced diabetic animal represent a good experimental diabetic state with residual or remnant insulin production by the pancreatic β-cells. Moreover, STZ-induced diabetes in animals mimics early-stage clinical DN. Nicotinamide decreases the severity of streptozotocin-induced diabetes in animals due to its antioxidant effect. Therefore, STZ-NAD induced type 2 diabetes model was selected for the study. STZ does not possess nephrotoxic potential and all changes in the kidney function after STZ administration in rats can thus be attributed to altered metabolism in diabetes. Previously, B. monnieri and its isolate, Stigmasterol, have been reported as antihyperglycemic agents in alloxanized diabetic rat. The present study aims to evaluate chronic complication of diabetes i.e., DN using STZ-NAD model for type 2 diabetes.

It is reported that hyperglycemia-induced activation of polyol pathway leads to an increase in oxidative stress resulting in various diabetic complications. ALR is the principal and rate-controlling enzyme in the polyol pathway, which catalyzes the NADPH-dependent reduction of glucose to sorbitol. The over production of sorbitol, imbalance of NADPH/NADP⁺, and subsequent oxidative stress inside the cells are thought to be the major causes of cellular damage that develop diabetic complications. Moreover, excessive accumulation of sorbitol leads to increased osmotic stress, along with cross-linking with tissue proteins by nonenzymatic glycosylation. These glycated products generally called as advanced glycation end products (AGEs) readily accumulate in the tissues leading to cellular damage and there by diabetic complications. During the course of diabetes, hyperglycemia induced the formation of AGEs and generation of ROS play a major role in kidney damage. In diabetes, these reactions are greatly accelerated and are important in the pathogenesis of diabetic complications. AGEs induce direct injury to the mesangial cells leading to mesangial expansion and basement membrane thickening. Therefore, inhibition of AGEs formation and ALR activity seems to be an attractive therapeutic option that may alter the pathogenesis and delay in the progression of DN. The present study demonstrates that BA, BHA, and ST exhibits

![Graph showing serum insulin level](Figure 2. Continued.)
### Table 1. Effect of BA and BHA on renal function estimation in diabetic-nephropathy Wistar rats.

| Parameters       | Urea (mg/dl) | Uric acid (mg/dl) | Creatinine (mg/dl) | BUN (mg/dl) | Urine output (ml) | Urine creatinine (mg/dl) | Creatinine clearance (ml/min/kg) |
|------------------|--------------|-------------------|--------------------|-------------|------------------|--------------------------|----------------------------------|
| **Groups**       |              |                   |                    |             |                  |                          |                                  |
|                  | 30th day     | 5th day           | 30th day           | 5th day     | 30th day         | 5th day                  | 30th day                         |
| Normal           | 34.3 ± 0.672 | 35.83 ± 0.935     | 0.70 ± 0.016       | 16.03 ± 0.314 | 16.73 ± 0.445   | 14.77 ± 0.763           | 0.0702 ± 0.0016                  |
| Diabetic         | 79.33 ± 1.239 | 89.33 ± 1.418     | 5.07 ± 0.135       | 17.10 ± 0.378 | 17.62 ± 0.446   | 15.44 ± 0.575           | 0.0705 ± 0.0012                  |
| control          |              |                   |                    |             |                  |                          |                                  |
| BA 100 mg/kg     | 83.03 ± 1.172 | 93.33 ± 1.014     | 5.06 ± 0.159       | 17.10 ± 0.378 | 17.62 ± 0.446   | 15.44 ± 0.575           | 0.0705 ± 0.0012                  |
| BA 200 mg/kg     | 92.17 ± 1.830 | 102.67 ± 1.049    | 5.06 ± 0.159       | 17.10 ± 0.378 | 17.62 ± 0.446   | 15.44 ± 0.575           | 0.0705 ± 0.0012                  |
| BA 400 mg/kg     | 97.50 ± 2.493 | 108.00 ± 1.049    | 5.06 ± 0.159       | 17.10 ± 0.378 | 17.62 ± 0.446   | 15.44 ± 0.575           | 0.0705 ± 0.0012                  |
| BHA 100 mg/kg    | 87.17 ± 0.479 | 97.66 ± 0.471     | 5.06 ± 0.159       | 17.10 ± 0.378 | 17.62 ± 0.446   | 15.44 ± 0.575           | 0.0705 ± 0.0012                  |
| BHA 200 mg/kg    | 87.17 ± 0.479 | 97.66 ± 0.471     | 5.06 ± 0.159       | 17.10 ± 0.378 | 17.62 ± 0.446   | 15.44 ± 0.575           | 0.0705 ± 0.0012                  |
| BHA 400 mg/kg    | 87.17 ± 0.479 | 97.66 ± 0.471     | 5.06 ± 0.159       | 17.10 ± 0.378 | 17.62 ± 0.446   | 15.44 ± 0.575           | 0.0705 ± 0.0012                  |
| ST 5 mg/kg       | 85.30 ± 1.576 | 95.62 ± 0.739     | 5.06 ± 0.159       | 17.10 ± 0.378 | 17.62 ± 0.446   | 15.44 ± 0.575           | 0.0705 ± 0.0012                  |
| ST 10 mg/kg      | 82.48 ± 1.185 | 92.59 ± 0.514     | 5.06 ± 0.159       | 17.10 ± 0.378 | 17.62 ± 0.446   | 15.44 ± 0.575           | 0.0705 ± 0.0012                  |
| Glimipride       | 83.83 ± 1.176 | 95.50 ± 1.268     | 5.06 ± 0.159       | 17.10 ± 0.378 | 17.62 ± 0.446   | 15.44 ± 0.575           | 0.0705 ± 0.0012                  |

Each group (n = 6) represents Mean ± SEM. Data were analyzed using One-way ANOVA followed by Tukey’s multiple test.

- vs control.
- vs Diabetic control.
- vs B. monnieri extract 100 mg/kg.
- vs B. monnieri extract 200 mg/kg.
- vs standard.
- *p < .01.
- †p < .05.

### Table 2. Effect of BA and BHA on lipid profile in diabetic-nephropathy Wistar rats.

| Parameters       | TC (mg/dl) | TG (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) | HDL (mg/dl) |
|------------------|------------|-----------|-------------|--------------|-------------|
| **Groups**       | 30th day   | 5th day   | 30th day    | 5th day      | 30th day    |
|                  | 30th day   | 5th day   | 30th day    | 5th day      | 30th day    |
| Normal           | 104.8 ± 3.520 | 104.3 ± 2.02 | 77.17 ± 2.405 | 71.67 ± 1.418 | 34.90 ± 3.923 |
| Diabetic control | 255.5 ± 2.715 | 249.33 ± 2.10 | 168.00 ± 2.146 | 150.50 ± 1.629 | 34.90 ± 3.923 |
| BA 100 mg/kg     | 247.67 ± 2.255 | 243.33 ± 2.50 | 168.00 ± 2.146 | 150.50 ± 1.629 | 34.90 ± 3.923 |
| BA 200 mg/kg     | 257.67 ± 2.522 | 253.13 ± 2.90 | 168.00 ± 2.146 | 150.50 ± 1.629 | 34.90 ± 3.923 |
| BA 400 mg/kg     | 250.17 ± 1.859 | 156.00 ± 3.099 | 168.00 ± 2.146 | 150.50 ± 1.629 | 34.90 ± 3.923 |
| BHA 100 mg/kg    | 256.67 ± 2.482 | 188.00 ± 2.656 | 168.00 ± 2.146 | 150.50 ± 1.629 | 34.90 ± 3.923 |
| BHA 200 mg/kg    | 253.83 ± 2.821 | 168.33 ± 3.073 | 168.00 ± 2.146 | 150.50 ± 1.629 | 34.90 ± 3.923 |
| BHA 400 mg/kg    | 256.67 ± 2.551 | 194.33 ± 3.603 | 168.00 ± 2.146 | 150.50 ± 1.629 | 34.90 ± 3.923 |
| ST 5 mg/kg       | 253.78 ± 2.597 | 159.99 ± 1.143 | 152.12 ± 1.006 | 115.46 ± 1.516 | 34.90 ± 3.923 |
| ST 10 mg/kg      | 255.52 ± 1.471 | 130.51 ± 2.916 | 154.00 ± 1.461 | 102.64 ± 0.950 | 34.90 ± 3.923 |
| Glimipride       | 251.50 ± 2.627 | 151.83 ± 1.299 | 160.83 ± 1.435 | 103.83 ± 1.811 | 34.90 ± 3.923 |

Each group (n = 6) represents Mean ± SEM. Data were analyzed using One-way ANOVA followed by Tukey’s multiple test.

- vs control.
- vs Diabetic control.
- vs B. monnieri extract 100 mg/kg.
- vs B. monnieri extract 200 mg/kg.
- *p < .01.
- †p < .05.
Table 3. Effect of BA and BHA on the levels of antioxidant enzymes and lipid peroxidation (TBARS) in diabetic-nephropathy Wistar rats.

| Parameters | SOD (U/mg protein) | GSH (μM/mg protein) | TBARS (nmo/L mg protein) |
|------------|--------------------|---------------------|-------------------------|
|            | Kidney             | Pancreas            | Liver                   | Kidney             | Pancreas            | Liver                   |
| Normal     | 3.87 ± 0.083a      | 3.77 ± 0.146        | 4.22 ± 0.063            | 70.93 ± 0.472      | 67.62 ± 0.285       | 76.21 ± 0.476          | 0.54 ± 0.023           | 0.48 ± 0.003          | 0.64 ± 0.010          |
| Diabetic control | 1.24 ± 0.028a     | 1.24 ± 0.033        | 1.16 ± 0.035            | 35.26 ± 0.438      | 40.40 ± 0.465       | 41.36 ± 0.336          | 2.27 ± 0.026           | 2.24 ± 0.032          | 2.68 ± 0.023          |
| BA 100 mg/kg | 1.85 ± 0.013b     | 1.65 ± 0.011        | 1.34 ± 0.021            | 41.90 ± 0.80b      | 42.72 ± 0.49b       | 46.53 ± 0.30b          | 2.06 ± 0.021           | 1.92 ± 0.029          | 2.45 ± 0.01b          |
| BA 400 mg/kg | 2.39 ± 0.011c     | 2.57 ± 0.024        | 2.33 ± 0.021            | 53.08 ± 0.64b      | 53.14 ± 0.63b       | 52.53 ± 0.46b          | 1.92 ± 0.015           | 1.47 ± 0.026          | 2.03 ± 0.018          |
| BHA 100 mg/kg | 0.020 ± 0.006d   | 0.91 ± 0.014        | 0.62 ± 0.025            | 60.00 ± 0.33c      | 59.76 ± 0.46b       | 66.62 ± 0.48b          | 1.57 ± 0.028           | 1.13 ± 0.020          | 1.60 ± 0.022          |
| BHA 200 mg/kg | 2.58 ± 0.017d    | 2.72 ± 0.029        | 2.58 ± 0.034            | 54.78 ± 0.68d      | 43.05 ± 0.32d       | 50.03 ± 0.52d          | 1.89 ± 0.075           | 1.74 ± 0.009          | 2.15 ± 0.012          |
| BHA 400 mg/kg | 3.77 ± 0.010d    | 3.58 ± 0.026        | 3.37 ± 0.014            | 58.58 ± 1.02d      | 52.81 ± 0.65d       | 61.19 ± 0.54d          | 1.71 ± 0.003           | 1.30 ± 0.023          | 1.18 ± 0.019          |
| ST 3 mg/kg  | 3.77 ± 0.010d    | 3.58 ± 0.026        | 3.37 ± 0.014            | 64.50 ± 0.36d      | 60.10 ± 0.30d       | 69.12 ± 0.67d          | 1.37 ± 0.012           | 1.00 ± 0.016          | 1.32 ± 0.023          |
| ST 10 mg/kg | 2.05 ± 0.018    | 2.22 ± 0.017        | 2.09 ± 0.023            | 43.34 ± 0.52d      | 43.21 ± 0.319d      | 48.36 ± 0.317d         | 2.22 ± 0.018           | 1.86 ± 0.069          | 1.48 ± 0.067          |
| Glimepride  | 3.41 ± 0.026c    | 3.49 ± 0.044        | 3.55 ± 0.041            | 53.24 ± 0.78e      | 50.96 ± 0.471e      | 53.61 ± 0.492e         | 1.95 ± 0.015           | 1.36 ± 0.016          | 1.50 ± 0.011          |
| 10 mg/kg    | 2.62 ± 0.028d    | 2.22 ± 0.017        | 2.09 ± 0.023            | 60.50 ± 0.48b      | 57.75 ± 0.65b       | 61.59 ± 0.55b          | 1.69 ± 0.028           | 1.05 ± 0.032          | 1.53 ± 0.031          |

Each group (n = 6) represents Mean ± SEM. Data were analyzed using One-way ANOVA followed by Tukey’s multiple test.

a vs control,

b vs Diabetic control,
c vs B. monnieri extract 100 mg/kg,
d vs B. monnieri extract 200 mg/kg,
e p < .01,
f p < .05.
release from pancreas and reduces oxidative stress whereas phenolic compounds are potent radical scavengers which increase the levels of antioxidant enzymes and reduce lipid peroxidation.\textsuperscript{54,55} It has been reported that the alcoholic extract of \textit{B. monnieri} comprised various types of saponins including bacopasaponin A, B, C, D, pseudojujubogenin, bacopaside I, II, IV, and V.\textsuperscript{56,57} Saponins isolated from medicinal plants are found to be renoprotective as they reduce fasting blood glucose and albuminuria, reverses the glomerular hyperfiltration state and ameliorates proliferative glomerular pathological changes during the early stages of DN in rat models.\textsuperscript{22} Saponins produce a significant reduction in blood glucose and lipid profile. This hypoglycemic action is due to the nature of saponins to stimulate remnant \(\beta\)-cells to produce insulin.\textsuperscript{58}

Previous work by authors also reported that Stigmasterol was effective in diabetes, which is a supporting evidence for our present study.\textsuperscript{59,60} Phytosterols play an important role in the prevention of diabetic complications by ameliorating oxidative stress and altering antioxidant enzyme levels.\textsuperscript{61} Persistent hyperglycemia associated with diabetes has been shown to increase the production of free radicals through glucose auto-oxidation and protein glycation. High level of glucose is known to induce ROS and upregulate TGF-\(\beta\)1 and extracellular matrix expression in glomerular mesangial cells. Inhibition of these changes by antioxidants strengthens the role played by ROS in mediating glucose-induced renal injury. Antihyperglycemic and antioxidant effect of steroidal components of plants help in preventing renal complications associated with diabetes.\textsuperscript{61}

Abnormalities in lipid profile are one of the most common complications in diabetes mellitus. Hyperglycemia causes an increase in the cholesterol, triglycerides, LDL, and VLDL\textsuperscript{62} mainly due to the increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Administration of BA, BHA, and ST increased the level of serum HDL-cholesterol and decreased the levels of total cholesterol, triglyceride, and LDL-cholesterol. Hyperlipidemia has been reported to accompany hyperglycemic state. Hence, a compound which reduces blood cholesterol might be expected to reduce blood sugar as well. It was recognized that plant sterols such as stigmasterol, \(\beta\)-sitosterol etc. lower serum concentration of cholesterol by reducing the absorption of cholesterol from the gut by competing for the limited space for cholesterol in the mixed micelles and thereby it might reduce blood sugar level.\textsuperscript{63}

Oxidation plays an important role in the formation of AGEs, and the plant-derived agents with the antiglycation and antioxidant activities are highly important in preventing diabetic complications. AGEs have long been associated with increased oxidative stress both \textit{in vitro}\textsuperscript{64} and \textit{in vivo}.\textsuperscript{65} Free radicals are known to stimulate AGE production by autoxidation of sugars.\textsuperscript{66}
The ROS generated during glycation and glyoxidation are able to oxidize side chains of amino acid residues in protein to form carbonyl derivatives and also diminish an oxidative defense of protein by decreasing thiol groups. Thus, these phenomena are reflective of high oxidative stress, protein oxidative damage and formation of AGEs, which is the direct reflection of excess of free radical generation. Inhibition of the formation of

Figure 4. Histopathological changes in the kidney of normal and treated rats (H&E 100×). (A) Normal, (B) diabetic nephropathy control, (C) standard, (D) BA 100 mg/kg treated, (E) BA 200 mg/kg, (F) BA 400 mg/kg treated, (G), BHA 100 mg/kg treated, (H) BHA 200 mg/kg treated, (I) BHA 400 mg/kg treated, (J) ST 5 mg/kg treated, and (K) ST 10 mg/kg treated.
AGEs in kidneys of experimental rats is a potentially therapeutic avenue in DN. Administration of BA, BHA, and ST led to significant reduction in the level of AGEs in kidneys. Observed effect can be attributed to antihyperglycemic activity of BA, BHA, and ST.

Kidneys remove metabolic wastes such as urea, uric acid, creatinine, and ions, thereby optimum chemical composition of body fluids is maintained. The concentration of the metabolites increase in blood during renal diseases or renal damage associated with uncontrolled
diabetes mellitus. This may be due to metabolic disturbance in diabetes reflected in high activities of xanthine oxidase, lipid peroxidation, and increased triacylglycerol and cholesterol levels. Chronic hyperglycemia increased concentration of metabolic waste like urea, uric acid, BUN, and creatinine due to which renal function is compromised. After 30 days of STZ-induction, increased level of serum urea, uric acid, BUN, and creatinine levels suggested development of DN. In this study, the untreated diabetic rats showed signs of DN, including the increased urine volume, reduced level of creatinine in urine and creatinine clearance. Administration of BA, BHA, and ST for 45 days significantly decreased the level of all these parameters.

Figure 6. Histopathological changes in the liver of normal and treated rats (H&E 100×). (A) Normal, (B) diabetic nephropathy control, (C) standard, (D) BA 100 mg/kg treated, (E) BA 200 mg/kg, (F) BA 400 mg/kg treated, (G), BHA 100 mg/kg treated, (H) BHA 200 mg/kg treated, (I) BHA 400 mg/kg treated, (J) ST 5 mg/kg treated, and (K) ST 10 mg/kg treated.
At the onset of diabetes, the kidney grows large and the glomerular filtration rate (GFR) becomes disturbed.\(^{71}\)

Histopathological observations in the present study showed glomeruli with mesangiocapillary proliferation in the kidney of diabetic rats along with amelioration of hemodynamic parameters of kidney due to increased formation of AGEs. The ultrastructure of diabetic pancreas showed considerable reduction in the islet of Langerhans and depleted islets. BA and BHA administered DN rats showed amelioration in these changes. Liver showed perivenular inflammatory collection and Kupffer cell hyperplasia with condensed nuclei. Increased oxidative stress due to excess generation of free radicals, lipid peroxidation, and decreased level of antioxidant enzymes causes deleterious structural changes in the liver of DN rats.\(^{72}\)

5. Conclusion

Findings in the present study suggest that supplementation with *B. monnieri* and its isolate (stigmasterol) might be beneficial in chronic diabetics and thus may find application in DN via reducing the formation of AGEs and amelioration of oxidative stress.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

1. Satirapoj B. Nephropathy in diabetes. *Adv Exp Med Biol.* 2012;771:107–122.
2. Sun H, Yuan Y, Sun ZL. Cholesterol contributes to diabetic nephropathy through SCAP-SREBP-2 pathway. *Int J Endocrinol.* 2013;2013:592576.
3. Rossing P. Diabetic nephropathy: Worldwide epidemic and effects of current treatment on natural history. *Curr Diab Rep.* 2006;6:479–483.
4. Ayodele OE, Alebiosu CO, Salako BL. Diabetic nephropathy – a review of the natural history, burden, risk factors and treatment. *J Natl Med Assoc.* 2004;96:1445–1454.
5. Alsaad KO, Herzenberg AM. Distinguishing diabetic nephropathy from other causes of glomerulosclerosis: An update. *J Clin Pathol.* 2007;60:18–26.
6. Abe M, Okada K, Soma M. Antidiabetic agents in patients with chronic kidney disease and end-stage renal disease on dialysis: Metabolism and clinical practice. *Curr Drug Metab.* 2011;12:57–69.
7. Aronson D. Hyperglycemia and the pathobiology of diabetic complications. *Adv Cardiol.* 2008;45:1–16.
8. Kaur N, Kishore L, Singh R. Attenuating diabetes: What really works? *Curr Diabetes Rev.* 2016;12:259–278.
9. Singh R, Kaur N, Kishore L, Gupta GK. Management of diabetic complications: A chemical constituents based approach. *J Ethnopharmacol.* 2013;150:51–70.
10. Nadkarni KM. *Indian Materia Medica.* Mumbai: Popular Prakashan Pvt. Ltd; 1976: 624–625.
11. Monograph. *Bacopa monniera.* *Altern Med Rev.* 2004;9:79–85.
12. Chopra RN, Chopra IC, Verma BS. *Glossary of Indian Medicinal Plants.* New Delhi, India: Council of Scientific and Industrial Research (CSIR); 1969.
13. Deepak M, Amit A. The need for establishing identities of bacoside A and B, the putative major bioactive saponins of Indian medicinal plant *Bacopa monnieri.* *Phytomedicine.* 2004;11:264–268.
14. Russo A, Borrelli F. *Bacopa monniera,* a reputed nootropic plant: An overview. *Phytomedicine.* 2005;12:305–317.
15. Anbarasi K, Vani G, Balakrishna K, Desai CS. Creatine kinase isoenzyme patterns upon exposure to cigarette smoke: Protective effect of Bacoside A. *Vascular Pharmacol.* 2005;42:57–61.
16. Elangovan V, Govindasamy S, Ramamoorthy N, Balasubramanian K. In-vitro studies on the anticancer activity of *Bacopa monniera.* *Fitoterapia.* 1995;66:211–215.
17. Sairam K, Rao CV, Babu MD, Goel RK. Prophylactic and curative effects of *Bacopa monniera* in gastric ulcer models. *Phytomedicine.* 2001;8:423–430.
18. Dar A, Channa S. Calcium antagonistic activity of *Bacopa monniera* on vascular and intestinal smooth muscles of rabbit and guinea-pig. *J Ethnopharmacol.* 1999;66:167–174.
19. Samiulla DS, Prasanth D, Amit A. Mast cell stabilising activity of *Bacopa monniera.* *Fitoterapia.* 2001;72:284–285.
20. Kishore K, Singh M. Effect of bacosides, alcoholic extract of *Bacopa monniera* Linn. (brahmi), on experimental amnesia in mice. *Indian J Exp Biol.* 2005;43:640–645.
21. Chowdhary DK, Parmar D, Kakkar P, Shukla R, Seth PK, Srimal RC. Antistress effects of bacosides of *Bacopa monnieri:* Modulation of HSP70 expression, superoxide dismutase cytochrome P450 activity in rat brain. *Phytoter Res.* 2002;16:639–645.
22. Zhang J, Xie X, Li C, Fu P. Systematic review of the renal protective effect of Astragalus membranaceus (root) on diabetic nephropathy in animal models. *J Ethnopharmacol.* 2009;126:189–196.
23. Sefi M, Fetoui H, Makni M, Zeghal N. Mitigating effects of antioxidant properties of *Artemisia campestris* leaf extract on hyperlipidemia, advanced glycation end products and oxidative stress in alloxan-induced diabetic rats. *Food Chem Toxicol.* 2010;48:1986–1993.
24. Ghosh T, Maity TK, Singh J. Antihyperglycemic activity of Bacosine, a Triterpene from *Bacopa monnieri* in alloxan-induced diabetic rats. *Planta Med.* 2011;77:804–808.
25. The Plant List. 2015. Available at: http://www.theplant-list.org [last accessed 28 December 2015].
26. Raaman N, ed. *Pharmaceutical Techniques.* Vol. 1. New Delhi: New India Publishing Agency; 2006:19–24.
27. Trease GE, Evans WC. Text Book of Pharmacognosy. 12th ed. London, UK: Balliere Tindall; 1989.
28. Harbourne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London, UK: Chapman and Hall; 1998.
29. Liu J, Jia L, Kan J, Jin CH. In vitro and in vivo antioxidant activity of ethanolic extract of white button mushroom (Agaricus bisporus). Food Chem Toxicol. 2013;51:310–316.
30. Adisakwattana S, Sompong W, Meeprom A, Ngamukote S, Yibchok-anun S. Cinnamic acid and its derivatives inhibit fructose-mediated protein glycation. Int J Mol Sci. 2012;13:1778–1789.
31. Saraswat M, Muthenna P, Suryanarayana P, Petrash JM, et al. Dietary sources of aldose reductase inhibitors: Prospects for alleviating diabetic complications. Asia Pac J Clin Nutr. 2008;17:58–65.
32. Bhatjea PK, Singh R. Antidiabetic activity of Acacia tortilis (Forsk.) Hayne ssp. raddiana polysaccharide on streptozotocin-nicotinamide induced diabetic rats. BioMed Res Int. 2014;2014:572013.
33. Putt DA, Zhong Q, Lash LH. Adaptive changes in renal mitochondrial redox status in diabetic nephropathy. Toxicol Appl Pharmacol. 2012;258:188–198.
34. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides from the adventitia of the rat thoracic aorta inactivates superoxide production blocks three pathways of hyperglycaemic damage. Nature. 2000;404:787–790.
35. Yazdanparast R, Amin A, Jamshidi S. Experimental diabetes treated with Achillea santolina: effect on pancreatic oxidative parameters. J Ethnopharmacol. 2007;112:13–18.
36. Searle AJ, Wilson RL. Glutathione peroxidase: Effect of superoxide, hydroxyl and bromine free radicals on antioxidant responses in brain and kidney of diabetic rats. Environ Toxicol Pharmacol. 2009;27:62–69.
37. Dewanjee S, Das AK, Sahu R, Gangopadhyay M. Antidiabetic activity of Diospyros peregrina fruit: Effect on hyperglycemia, hyperlipidemia and augmented oxidative stress in experimental type 2 diabetes. Food Chem Toxicol. 2009;47:2679–2685.
38. Afolayan AJ, Sunmonu TO. Artemisia afra Jacq. ameliorates oxidative stress in the pancreas of streptozotocin-induced diabetic wistar rats. Biosci Biotech Biochem. 2011;75:2083–2086.
39. Chakravarty AK, Sarkar T, Masuda K, Shijoima K, Nakane T, Kawahara N. Bacopaside I and II: Two pseudojujubogenin glycosides from Bacopa monniera. Phytochemistry. 2001;58:553–556.
40. Sivaramakrishna C, Rao CV, Trimurtulu G, Vanisree M, Subbaraju GV. Triterpenoid glycosides from Bacopa monniera. Phytochemistry. 2005;66:1123–1137.
41. Swanston-Flat SK, Day C, Bailey CJ, Flatt PR. Traditional plant treatment for diabetes: Studies in normal and streptozotocin diabetic mice. Diabetologia. 1990;33:462–464.
42. Chatterjee MN, Shinde R. Text Book of Medical Biochemistry. New Delhi, India: Jaypee Brothers Medical Publisher; 2002:317.
43. Swanston-Flat SK, Day C, Bailey CJ, Flatt PR. Traditional plant treatment for diabetes: Studies in normal and streptozotocin diabetic mice. Diabetologia. 1990;33:462–464.
61. Kumar VL, Padhy BM. Protective effect of aqueous suspension of dried latex of Calotropis procera against oxidative stress and renal damage in diabetic rats. *Biocell*. 2011;35:63–69.

62. Soltani N, Keshavarz M, Dehpour AR. Effect of oral magnesium sulfate administration on blood pressure and lipid profile in streptozocin diabetic rat. *Eur J Pharmacol*. 2007;560:201–205.

63. Ghosh T, Ghosh S, Maity TK. Antihyperglycemic activity of stigmasterol isolated from *Bacopa monnieri* Linn. aerial parts against alloxan induced diabetic rats. *Int J Nat Prod Res*. 2014;4:40–46.

64. Scivittaro V, Ganz MB, Weiss MF. AGEs induce oxidative stress and activate protein kinase C-beta(II) in neonatal mesangial cells. *Am J Physiol Renal Physiol*. 2000;278:F676–F683.

65. Odetti P, Traverso N, Noberasco G, Pronzato MA, Marinari UM. Good glycaemic control reduces oxidation and glycation end-products in collagen of diabetic rats. *Diabetologia*. 1996;39:1440–1447.

66. Wolff SP, Jiang XY, Hunt JV. Protein glycation and oxidative stress in diabetes mellitus and ageing. *Free Radic Biol Med*. 1991;10:339–352.

67. Balu M, Sangeetha P, Murali G, Panneerselvam C. Age-related oxidative protein damages in central nervous system of rats: Modulatory role of grape seed extract. *Int J Dev Neurosci*. 2005;23:501–507.

68. Almdal TP, Vilstrup H. Strict insulin treatment normalizes the organic nitrogen contents and the capacity of urea–N synthesis in experimental diabetes in rats. *Diabetologia*. 1988;31:114–118.

69. Madinov IV, Balabolkin MI, Markow DS. Main causes of hyperglycemia in diabetes mellitus. *Ter Arkh*. 2000;72:55–58.

70. Anwar MM, Meki AR. Oxidative stress in streptozotocin-induced diabetic rats: Effects of garlic oil and melatonin. *Comp Biochem Physiol Part A Mol Integr Physiol*. 2003;135:539–547.

71. Dabla PK. Renal function in diabetic nephropathy. *World J Diabetes*. 2010;1:48–56.

72. Polavarapu R, Spitz DR, Sim JE, et al. Increased lipid peroxidation and impaired antioxidant enzyme function is associated with pathological liver injury in experimental alcoholic liver disease in rats fed diets high in corn oil and fish oil. *Hepatology*. 1998;27:1317–1323.