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RESEARCH ARTICLE

INVESTIGATION OF RENOPROTECTIVE EFFECT OF *EUGENIA CARYOPHYLLATA* IN STREPTOZOTOCIN- INDUCED DIABETIC NEPHROPATHY IN WISTAR RATS

Chand Kaur[1], Tapan Behl*[2], Rupinder Sodhi[1]

[1]Department of Pharmacy, Chandigarh College of Pharmacy, Mohali, Punjab, India

[2]Department of Pharmacology, Vallabhbhai Patel Chest Institute,University of Delhi, India

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ABSTRACT

Essential oil of *Eugenia caryophyllata* has been proven to show antioxidant, antihyperglycemic, antihyperlipidemic, anti-inflammatory and antidiabetic properties. However its effect on diabetic nephropathy was unexplored. Therefore the present study was designed to investigate the renoprotective effect of *Eugenia caryophyllata* in streptozotocin induced diabetic nephropathy in Wistar rats. The diabetic rats showed a significant alteration in serum ($p<0.05$) biochemical parameters such as increase in level of serum glucose, blood urea nitrogen (BUN), creatinine, glycated hemoglobin and decrease in content of serum albumin. Moreover the level of TBARS was increased whereas glutathione level decreased significantly. The lipid profile of diabetic rats was also altered significantly due to increase in serum cholesterol, triglycerides and decline in high density lipoprotein (HDL). Henceforth, from results, it has been concluded that *Eugenia caryophyllata* has the ability to reduce kidney damage in diabetic rats and the renoprotective effect of *Eugenia caryophyllata* may be due to its anti-oxidant, hypolipidemic and anti- diabetic activities.

INTRODUCTION:

Diabetic nephropathy (DN) is considered as the major complication of diabetes mellitus. DN is the most common cause of end stage renal disease and accounts for significant morbidity and mortality in diabetic patients [1]. A series of ultra structural changes occur in renal compartments such as, excessive accumulation of extracellular matrix (ECM) e.g. fibronectin and collagen with the thickening of tubular basement membrane and glomerular basement membrane. Subsequent increased amount of mesangial matrix results in tubulointerstitial fibrosis, glomerulosclerosis and renal endothelial dysfunction which ultimately lead to DN [2]. Additionally, an increase in excretion of proteins in urine, increase in systemic blood pressure, and decrease in renal function also occurs [3]. However most of the kidney cellular elements, i.e., mesangial cells, glomerular endothelia, tubular epithelia, podocytes are targets of hyperglycemic injury. Hyperglycemia induced hemodynamic and metabolic pathways are considered as mediators of diabetic nephropathy. Hemodynamic factors that contribute to the progression of DN include increased systemic and intraglomerular pressure, activation of vasoactive hormonal pathway involving renin angiotensin aldosterone system (RAAS) that effects efferent and arteriole tone, mesangial contractility, proximal tubular solute transport and endothelin. These hemodynamic pathways activate intracellular second messengers such as Protein kinase C, mitogen activated protein kinase (MAPK) [4], nuclear transcription factor (NF-kB) and various fibrotic factors such as, transforming growth factor β (TGF- β) [5], connective tissue growth factor (CTGF) and vascular endothelial growth factor (VEGF) [6].

The reactive oxygen species (ROS) are the common activators of various signaling cellular pathways implicated in diabetic nephropathy. Most of the ROS are generated through mitochondrial oxidative phosphorylation and small amounts through NADPH-oxidase system [2]. ROS activates protein kinase C (PKC), mitogen activated protein kinase (MAPK), (JAK/STAT) and transcription factors NF-κB, activating protein-1 (AP-1), and up regulates transforming growth factor- β1 (TGF-β1) leading to deterioration of the functions of diabetic kidney. Antioxidants very efficiently inhibit high glucose-induced over expression of TGF-β and decrease the oxidative stress by enhancing the level of intracellular antioxidants e.g. superoxide dismutase, catalase etc [7].

Eugenia caryophyllus (Laung clove) is an unopened flower bud growing on a tree belonging to the family Myrtaceae. *Eugenia caryophyllata* was found to be effective against

*Corresponding author: Tapan Behl | E-mail: tapanbehl31@gmail.com

egg and adult of Pediculus capitis [8].It has antiseptic as well as bactericidal activities against several bacteria including *Escherichia coli* and *Staphylococcus aureus* [9]. Moreover clove also has anti-inflammatory activity [10] anti-pyretic activity [11], analgesic effect [12]. As reported in earlier studies *Eugenia caryophyllata* has antioxidant properties [13]. There are evidences that clove reduces the level of malondialdehyde (MDA), and increase the level of superoxide dismutase (SOD) and reduced glutathione (GSH) in the body [14]. Clove also prevent inflammation by down regulating the expression of COX-2 enzyme and other proinflammatory mediators like TGF-β, NF-KB, AGEs, protanoids and leucotrienes [15].

MATERIALS AND METHODS:

Chemicals and reagents:

All regents used in this study were of analytical grade and were freshly prepared. *Eugenia caryophyllata* was purchased from Aggrawal Pharmaceuticals, Delhi, India. Streptozotocin (STZ) was obtained from (Oswal-Scientific store, India), DTNB (5, 5-dithiobis-2-nitrobenzoic acid), Folin-Ciocalteu phenol reagent, Bovine serum albumin, n-butanol, pyridine, GSH from Loba Chemicals (Mumbai, India). Trichloroacetic acid was procured from Nice Chem. Pvt. Ltd. (Cochin, India) and Glucose estimation kit GOD-POD from (Reckon diagnostic Pvt. Ltd). Thiobarbituric acid, BUN, total cholesterol (CHOD-PAP), triglycerides (GOD-PAP) and HDL kits from (Magus chemical& scientific equipment India) and creatinine and albumin kits from (Recombigen Laboratories Pvt. Ltd.) were employed for various biochemical estimations.

Experimental design:

Adult Wistar rats of either sex weighing 200-350g were used for this study. Food and water were provided *ad libitum* throughout the experimental period. They were housed in departmental animal house facility of Chandigarh College of pharmacy Landran, Punjab in different plastic cages with husk bedding. All animals were maintained as per the guidelines for the care and use of laboratory animals. All the experiments were carried out between 09.00 and 15.00 hr. The experimental protocol used in the present study was approved by the Institutional Animal Ethics Committee (1201/a/08/CPCSEA).

Induction of experimental diabetic nephropathy: Streptozotocin (STZ) at a dose of 45 mg**/**kg body weight dissolved in citrate buffer was injected intraperitonially to induce diabetes [16].The animals were fasted for overnight before the STZ injection, and after the injection, 5% sucrose was supplemented for 24hrs in order to prevent the animals from fatal hyperglycemia. One week after injection, blood glucose level was evaluated. The animals with a blood glucose level of more than 240 mg/dl were considered diabetic and included in the study. The physiological changes start after 4 weeks of STZ administration and diabetic nephropathy develops after 8 weeks of STZ administration [17].

Biochemical parameters:

Collection of samples:

At the end of the experimental protocol, the blood samples were collected by retro-orbital sinus under light ether anesthesia and serum was separated. The serum samples were stored at 4°-6°C until the estimation of biochemical parameters

a) Assessment of STZ-induced Diabetes:

Estimation of Serum Glucose: The glucose concentration was estimated by Glucose-peroxidase (GOD-POD) method [18] using commercially available Kit. 1000 µl of working glucose reagent was added to 10 µl of serum, 10 µl of standard glucose (100 mg/dl) and 10 µl of purified water to prepare test, standard and blank respectively. All the test tubes were incubated at room temperature for 10 min. The absorbance of test and standard samples were noted against blank at 505 nm spectrophotometrically (Thermo Double Beam spectrophotometer). The intensity of the colour formed is directly proportional to the amount of glucose present in the sample.

b) Assessment of Diabetic Nephropathy:

The extent of diabetic nephropathy was estimated biochemically by estimating BUN, serum creatinine, serum albumin, Glycated Hb.

Estimation of Blood urea nitrogen (BUN):

The BUN was estimated by Berthelot method [19] using the commercially available kit. 1000 µl of working reagent (1) was added to 10 µl of serum, 10 µl of standard and 10 µl of purified water to prepare test, standard and blank respectively. All the test tubes were mixed well and incubated for 5 min at 37°C in incubator. Then 1000 µl of working reagent (2) was added to all the test tubes. All the test tubes were again mixed well and incubated for 10 min at 37°C and nitroprusside to yield a blue green coloured compound (indophenols). The intensity of colour produced was directly proportional to the concentration of urea in the sample.

Estimation of serum creatinine:

The serum creatinine concentration was estimated by alkaline picrate method [20] using commercially available kit. 2 ml of picric acid reagent added to 0.2 ml of serum for deproteinization of specimen. The content were mixed well and centrifuged at 3000 rpm to obtain clear supernatant, 0.1 ml of standard creatinine and 0.1 ml of purified water were added to prepare test, standard and

blank, respectively. 1.0 ml of picric acid reagent was added to blank and standard. The content was mixed well and test tubes were kept at room temperature (37^{o} C) for 20 minutes. The absorbance of test and standard samples was noted against the blank at 520 nm spectrophotometrically.

Estimation of serum albumin:

The serum albumin concentration was estimated by bromocresol green (BCG) dye binding method using commercially available kit. 0.01 ml of buffered dye reagent was added to 0.01 ml of water, 0.01 ml of standard and 0.01 ml of serum to prepare blank, standard, and test respectively. The content was mixed well and absorbance of test and standard samples were noted immediately against blank at 630 nm spectrophotometrically.

Determination of glycated hemoglobin:

Glycated hemoglobin level was measured by ion-exchange resin method as described in the product insert of Euro Diagnostic ltd. Chennai [16]. Briefly homolysate was prepared by lysing 50µl of blood with lysing reagent and the obtained homolysate was added to the ion exchange resin tube, the tubes were vortexed after inserting the resin separator into it, such that the rubber sleeve is 1 cm above the resin suspension. Finally the resin separator was pushed in completely to remove the supernatant and its absorbance was measured at 415 nm against distilled water to obtain ΔGHb 20 µl of homolysate was added to 5 ml of distilled water to measure the Δ Total Hb at 415 nm. The percentage glycated hemoglobin level was calculated by using the formula-

GHb% = (ΔGHb/Δ total Hb) × 4.61 assay factor.

c) Assessment of renal hypertrophy and renal fibrosis:

Estimation of kidney weight / body weight (%):

Both left and right kidneys were isolated, renal fascia were removed and kidneys were weighed individually. Kidney weight/ body weight (%) was calculated according to following formula: Left Kidney weight (gm) + Right kidney weight (gm)/ Body weight (gm) ×100 [22].

d) Assessment of serum lipid profile:

Estimation of serum total cholesterol:

The total cholesterol was estimated by the cholesterol oxidase peroxidase (CHOD-PAP) method [18] using commercially available kit. 1000 µl of cholesterol reagent was added to 10 µl of serum, 10 µl of standard cholesterol (200 mg/dl) and 10 µl of purified water to prepare test, standard and blank, respectively. All the test tubes were incubated at room temperature for 15 min. The absorbance of test and the standard samples were noted against blank at 505 nm spectrophotometrically.

Estimation of Serum Triglyceride:

The serum triglycerides were estimated by glycerophosphate oxidase peroxidase (GOD-PAP) method [18] using commercially available kit. 1000 µl of enzyme reagent was added to 10 µl of serum, 10 µl of standard (200 mg/dl) and 10 µl of purified water to prepare test, standard and blank, respectively. All the test tubes were incubated at room temperature for 15 min. The absorbance of test and standard samples was noted against blank at 505 nm spectrophotometrically.

Estimation of serum high density lipoprotein (HDL):

The HDL was estimated by PTA [23] method as described by using commercially available kit (Burstein *et al*., 1970). 1000 µl of working cholesterol reagent was added to 50 µl of supernatant, 50 µl of HDL cholesterol standard (50 mg/dl) and 50 µl of purified water to prepare test, standard and blank, respectively. The content was mixed well and all the test tubes were incubated at room temperature for 10 min. All the test tubes samples were noted against blank at 505 nm spectrophotometrically.

e) Assessment of renal oxidative stress:

The development of oxidative stress in kidney was assessed by estimating renal thiobarbituric acid reactive substance (TBARS) and reduced glutathione content (GSH).

Preparation of renal homogenate:

Each kidney was dissected and washed with ice cold isotonic saline and weighed. The kidney was then minced, and a homogenate (10% w/v) was prepared in chilled 1.15% KCl, was utilized for the biochemical estimations.

Estimation of lipid-peroxidation:

Concentration of Thiobarbituric acid reactive substances (TBARS) was determined as an index of lipid peroxidation as described by Niehius and Samuelson. In this method, 0.1 ml of renal supernatant was treated with 2 ml of (1:1:1) thiobarbituric acid- trichloroacetic acid-hydrochloric acid (TBA-TCA-HCL) reagent. TBARS reagent was prepared by mixing equal volumes of TBA (37%), TCA (15%) and HCL (0.25N). Then the mixture was boiled for 15 min, followed by centrifugation at 1000 g for 10 min. Finally the absorbance was measured at 532 nm (UV-1700 Spectrophotometer, Shimadzu, Japan) against blank and the values are expressed as µmole per g tissue [24].

Estimation of reduced glutathione:

The GSH level in the kidney was estimated by the method as described by (Ellman, 1959). Briefly ,the renal homogenate was be mixed with 10% w/v trichloroacetic acid in ratio of 1:1 and centrifuged at 4°C for 10 minutes at 5000 rpm. The supernatant obtained (0.5 ml) was mixed with 2 ml of 0.3 M disodium hydrogen phosphate

buffer (pH 8.4) and 0.4 ml of distilled water. Then 0.25 ml of 0.001M freshly prepared DTNB [5, 5'-dithiobis (2-nitrobenzoic acid) dissolved in 1% w/v sodium citrate] was added. The reaction mixture was incubated for 10 minutes and absorbance of yellow colored complex was noted spectrophotometrically at 412 nm. A standard curve was plotted using reduced form of glutathione and the results were expressed as nmol/mg of protein [25].

Estimation of total protein:

The renal total protein was determined by the method of Lowry *et al.* (1951) [26] with slight modifications using bovine serum albumin (BSA) as a standard. 0.15 ml of supernatant of tissue homogenate was diluted to 1 ml with distilled water and then 5 ml of Lowry reagent was added. The contents were mixed thoroughly. The mixture was allowed to stand 15 min at room temperature (37° C). Then 0.5 ml of 1:1 v/v diluted Folin-Ciocalteu reagent was added. The content was vortexed vigorously and incubated at room temperature (37°C) for 30 min. The protein content was determined spectrophotometrically at 750 nm against suitably prepared blank. A standard curve using 0.2-2.4 mg/ ml of BSA was plotted. The amount of total protein was expressed in mg/ml.

Statistical analysis:

All values were expressed as mean ± S.D. The data obtained from various biochemical parameters were statistically analyzed by one way ANOVA followed by Tukey's multiple comparison test. The p value <0.05 was considered statistically significant.

RESULTS:

Effect of Eugenia caryophyllata low dose, intermediate dose and high dose on serum glucose level in diabetic rats:

The glucose level in diabetic rats accentuated after the administration of STZ (45 mg/kg *i.p.*), in comparison the untreated control group. Furthermore, there was a significant decline in the glucose concentration of rats treated with *Eugenia caryophyllata* low dose (0.025 ml/kg/day), intermediate dose (0.050 ml/kg/day) and high dose (0.1 ml/kg/day) in comparison to the diabetic control rats.

Effect of Eugenia caryophyllata low dose, intermediate dose and high dose on BUN, serum creatinine, total protein, Glycated hemoglobin and albumin in diabetic rats:

The BUN, serum creatinine, total protein, and glycated hemoglobin were noted to be significantly accentuated and serum albumin level was decreased in diabetic rats, as compared to normal control rats. However treatment with low (0.025 ml/kg/day), intermediate dose (0.050 ml/kg/day) and high dose (0.1 ml/kg/day) of *Eugenia caryophyllata* significantly decreased BUN, serum creatinine, total protein, glycated hemoglobin, and increased serum albumin in STZ treated rats. Furthermore, there was a significant decline in the serum creatinine and increase in serum albumin of rats treated with *Eugenia caryophyllata* as compared with normal control rats.

Effect of Eugenia caryophyllata low dose, intermediate dose and high dose on lipid profile in diabetic rats:

The increase in serum total cholesterol, triglycerides and decreased in HDL were observed in diabetic rats. Treatment with low (0.025 ml/kg/day), intermediate dose (0.050 ml/kg/day) and high dose (0.1 ml/kg/day) of *Eugenia caryophyllata* significantly decreased in case of lipid profile as compared to diabetic rats.

Effect of Eugenia caryophyllata low dose, intermediate dose and high dose on Kidney weight/Body weight ratio in diabetic rats:

A significant increase in Kidney weight/Body weight ratio was noted in diabetic rats as compared to normal control. However treatment with low (0.025 ml/kg/day), intermediate dose (0.050 ml/kg/day) and high dose (0.1 ml/kg/day) of *Eugenia caryophyllata* significantly decreased Kidney weight/Body weight ratio in STZ treated rats.

Effect of Eugenia caryophyllata low dose, intermediate dose and high dose on oxidative stress parameters like TBARS, glutathione observed in diabetic rats:

A significant increase in TBARS and significant decrease in glutathione was observed in diabetic rats. However treatment with low (0.025 ml/kg/day), intermediate dose (0.050 ml/kg/day) and high dose (0.1 ml/kg/day) of *Eugenia caryophyllata* produced significant decrease in TBARS and increase in glutathione level in STZ treated rats as compared to diabetic rats.

Table 1: Effect of *Eugenia caryophyllata* on metabolic and serum biochemistry in diabetic rats

Parameters	Groups					
	Con	*EC per se*	*Dia*	*Dia+ EC (LD)*	*Dia+ EC (MD)*	*Dia+ EC (HD)*
Blood Glucose (mg/dl)	92.6±2	97.8±2.6	532.1±1.3	418±3	416±3	359±2.8
Glycated Hemoglobin (%)	4.69±1	4.2±1.2	6.9±2	5.1±0.8	5±2.1	4.9±1.1
Blood Urea Nitrogen (mg/dl)	11.3±0.4	10.21±0.3	96.8±0.6	82.07±0.5	80.03±0.1	63.93±0.3
Serum Creatinine (mg/dl)	0.92±0.1	1.0±0.3	2.47±0.07	1.51±0.3	1.48±0.06	1.22±0.09
Serum Albumin (mg/dl)	3.4±0.2	3.61±0.05	2.25±0.3	2.7±0.1	2.5±0.06	3.61±0.04
Body Wt Vs Kidney Wt (%)	0.58±0.03	0.59±0.2	1.0±0.04	0.7±0.07	0.6±0.01	0.68±0.02

The table represents the metabolic parameters and serum biochemistry of the animals from the six groups; control (con), EC *per se* (*Eugenia caryophyllata per se*), Diabetic (Dia), Diabetic rats treated with low dose of *Eugenia caryophyllata* (Dia+ EC [LD]), Diabetic rats treated with middle dose of *Eugenia caryophyllata* (Dia+ EC [MD]), Diabetic rats treated with high dose of *Eugenia caryophyllata* (Dia+ EC[HD]). values represent the mean± standard deviation (n=8) of the samples. Significant difference between all groups: $p<0.05$.

Table 2: Effect of *Eugenia caryophyllata* on lipid profile in diabetic rats

Parameters	Groups					
	Con	*EC per se*	*Dia*	*Dia+ EC (LD)*	*Dia+ EC (MD)*	*Dia+ EC (HD)*
Total Cholesterol (mg/dl)	116±2.0	112±3.0	210±1.4	166±2.7	144±1.12	133±1.48
Triglycerides (mg/dl)	97.5±1.0	96±1.2	136±0.9	104±1.1	103.3±0.7	100±0.5
High Density Lipoproteins (mg/dl)	37.6±3	35.3±2.2	20.4±1.4	27.2±1.0	29.0±1.3	33.9±1.0

The table represents the lipid profile of the animals from the six groups; control (con), EC *per se* (*Eugenia caryophyllata per se*), Diabetic (Dia), Diabetic rats treated with low dose of *Eugenia caryophyllata* (Dia+ EC [LD]), Diabetic rats treated with middle dose of *Eugenia caryophyllata* (Dia+ EC [MD]), Diabetic rats treated with high dose of *Eugenia caryophyllata* (Dia+ EC [HD]). Values represent the mean± standard deviation (n=8) of the samples. Significant difference between all groups $p<0.05$.

Table 3: Effect of *Eugenia caryophyllata* on renal oxidative stress markers in diabetic rats

Parameters	Groups					
	Con	EC per se	*Dia*	*Dia+ EC (LD)*	*Dia+ EC (MD)*	*Dia+ EC (HD)*
Lipid- peroxidation (nmol/mg tissue)	0.42±0.04	0.29±0.02	1.67±0.4	1.04±0.01	1.1±0.05	0.80±0.01
Reduced Glutathione (nmol/mg tissue)	33±2.0	38.6±0.6	17.25±0.4	19.5±0.3	21.21±0.4	27.3±0.8

The table represents the oxidative stress of the animals from the six groups; control (con), EC *per se* (*Eugenia caryophyllata per se*), Diabetic (Dia), Diabetic rats treated with low dose of *Eugenia caryophyllata* (Dia+ EC [LD]), Diabetic rats treated with middle dose of *Eugenia caryophyllata* (Dia+ EC [MD]), Diabetic rats treated with high dose of *Eugenia caryophyllata* (Dia+ EC [HD]). Values represent the mean± standard deviation (n=8) of the samples. Significant difference between all groups $p<0.05$.

DISCUSSION:

The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels and may lead to the occurrence of complications such as retinopathy, nephropathy, neuropathy and cardiomyopathy respectively [27]. Diabetic nephropathy is characterized by excessive accumulation of extracellular matrix (ECM), the thickening of tubular basement membrane and glomerular. Subsequent increase in the amount of mesangial matrix results in tublointerstitial fibrosis and glomerulosclerosis, renal endothelial dysfunction which ultimately lead to renal failure [2].

The present study was undertaken to investigate the renoprotective effect of *Eugenia caryophyllata* in

streptozotocin- induced diabetic nephropathy in Wistar rats.

Diabetes mellitus was induced by single administration of streptozotocin (STZ) (45 mg/kg) [16, 17]. Streptozotocin-induced diabetes is a well accepted model for induction of diabetic nephropathy in 4-8 weeks after single injection of STZ [17, 28]. STZ produces cytotoxocity to β-cells of islets of langerhans in a selective manner [29] by enhancing the activity of xanthine oxidase and poly (ADP-ribose) polymerase (PARP), which further causes apoptotic and necrotic death of pancreatic β-cells of langerhans [30]. STZ is a nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells and it partially mediates restriction of mitochondrial ATP generation. Furthermore, NO bind to the iron-containing aconitase and inhibits enzyme activity [31].

STZ was used in the present study because of its properties such as selective β-cells cytotoxicity and minimum toxicity to other organs as compared to alloxan. Moreover the $t_{1/2}$ of single dose of STZ is about 15 min, which is higher than the 1.5 minutes $t_{1/2}$ of alloxan [32]. Single administration of STZ at a dose of 40 mg/kg, 50 mg/kg, 55 mg/kg, and 60 mg/kg *i.p.* induces hyperglycemia in rats [33]. Persistent hyperglycemia leads to development of diabetic nephropathy in 4-8 weeks, assessed in terms of an increase in serum creatinine, BUN, proteinuria, glycated hemoglobin and decrease in serum albumin level, extracellular matrix deposition, dyslipidemia and consequent development of glomerulosclerosis and tubulointerstitial fibrosis [28]. STZ induces hyperglycemia and activates PKC, aldose reductase, NADPH oxidase, leads to the formation of AGEs and increases Ang II levels [34]. Therefore, streptozotocin induced diabetic nephropathy is a well accepted model for successful induction of diabetic nephropathy.

In the present study STZ- treated rats developed hyperglycemia. A significant alterations related to diabetic nephropathy such as increase in serum BUN, serum creatinine, glycated haemoglobin, and decreased serum albumin were also observed. These observations are in accordance with the other studies performed [16, 35, 36].

It has been well reported that increase in lipid level is a major contributor for the progression of diabetic nephropathy. In the present study increase in total cholesterol, triglycerides, and decrease in HDL level were observed as a result of dyslipidemia. Furthermore these results are similar to the results obtained in other studies [37].

Kidney weight/body weight (%) is reported to be a marker of renal hypertrophy in diabetic nephropathy [22]. The results of our study revealed that diabetes produced a significant reduction in weight of STZ- treated rats as compared to control. Moreover there was a significant increase in kidney weight of diabetic rats as compared to the untreated control animals. The results of present study are in consistent to the results of other studies [38].

The reactive oxygen species (ROS) are the common activators of various signaling cellular pathways implicated in diabetic nephropathy [2]. ROS activates protein kinase C (PKC), mitogen activated protein kinase (MAPK), Janus kinase Signal Transducer and Activator of Transcription (JAK/STAT), transcription factors NF-κB, activating protein-1 (AP-1), and up regulates transforming growth factor- β1 (TGF-β1) leading to deterioration of the functions of diabetic kidney. Advanced glycation end products (AGE) leads to formation of ROS directly or through RAGE, and ROS in turn, lead to the formation of AGEs. Moreover it has been well documented that ROS regulated signaling pathways lead to disposition of ECM in diabetic kidney. The key modulators of oxidative stress in diabetic nephropathy are marked increase in renal thiobarbituric acid reactive substances (TBARS) and decrease in renal glutathione (GSH). In the present study there was a significant increase in the level of renal TBARS whereas a marked decrease in the level of renal glutathione was observed in STZ-treated diabetic rats. These parameters are in accordance with the results obtained from the studies performed in other laboratories [39].

In *Eugenia caryophyllata* (0.025 ml/kg/day, 0.1 ml/kg/day *p.o.* for 4 weeks) treated diabetic rats, a significant decrease in serum glucose level was observed in comparison to the STZ treated diabetic rats. Furthermore *Eugenia caryophyllata* prevented STZ induced rise in serum creatinine, serum blood urea nitrogen, Glycated Hb and fall in serum albumin level. Kidney weight and body weight ratio was also decreased. A significant decline in serum triglyceride and total cholesterol levels was observed, with a marked rise in serum high density lipoprotein levels. Moreover oxidative damage was prevented by lowering TBARS and increasing GSH levels.

Eugenia caryophyllus (Laung, clove) is an unopened flower bud growing on a tree belonging to the family Myrtaceae. *Eugenia caryophyllata* possesses strong antioxidant activity, comparable to the activity of the synthetic antioxidant BHA (butylated hydroxyl toluene) and pyrogallol [39].Reports show that clove has the capacity to give off hydrogen and reduce lipid peroxidation. It is also found to have a significant inhibitory effect against hydroxyl radicals and acts as an iron chelator [40]. The antioxidant activity of clove bud

extract and its major aroma components, eugenol acetate and eugenol were comparable to that of the natural antioxidant α-tocopherol [41].

Reports indicate that *Eugenia caryophyllata* also possesses anti-diabetic property. It has been well studied that clove extract acts like insulin in hepatocytes and hepatoma cells by reducing phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) gene expression. The global analysis of gene expression by DNA microarray analysis revealed that clove and insulin regulated the expression of many of the same genes in a similar manner [42].

Segaey *et al* studied that clove reduced the level of total lipid, triglycerides, and total cholesterol which were raised due to the administration of ethanol in white albino rats. Moreover, there are evidences that clove reduces the level of MDA, and increases the level of SOD and GSH in the body [14]. Clove also prevent inflammation by down regulating the expression of COX-2 enzyme and other proinflammatory mediators like TGF-β, NF-KB, AGEs, protanoids and leucotrienes. The primary component of clove's volatile oil, eugenol functions as an anti-inflammatory agent. A variety of flavanoids, including kaempferol, rhamnetin and β-caryophyllene are present in clove's essential oil and contributes anti-inflammatory and antioxidant properties of clove [43].

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