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Research Article

Protective Effect of *Commiphora Mukul* in Experimental Paradigm in Streptozotocin–Induced Diabetic Neuropathy and Nephropathy

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Abstract

Diabetic neuropathy (DN) is the most common and intractable microvascular complication of diabetes. *Commiphora mukul,* known for its anti-diabetic, anti-oxidant and anti-inflammatory activities, can be used for diabetic neuropathy. Single injection of streptozotocin (STZ, 55 mg/kg, intraperitoneal (i.p.)) in male Wistar rats was used to induce diabetes. *Commiphora mukul* (50 and 100 mg/kg, per oral (p.o)) and Ramipril (0.2 and 2.3 mg/kg, p.o.) were administered for 8 weeks. All the behavioral parameters were evaluated weekly and after 8 weeks, blood was collected for protein, creatinine, albumin, urea levels and the assessment of mean nerve conduction velocity was done. The animals were sacrificed, biochemical estimations and histopathological examination of isolated kidneys were performed. Streptozotocin significantly resulted in neuropathic pain in comparison to naïve treatment. In contrast, Commiphora mukul and ramipril individually for 8 weeks significantly protected all the biochemical, behavioral, histopathological and electrophysiological anomalies against diabetes induced neuropathic pain. The combination of ramipril significantly improved the protective effect of *Commiphora mukul* which was significant as compared to their per se. The current study elaborates the protective effect of *Commiphora mukul* and ramipril which reflects upon its anti-inflammatory action and anti-diabetic action.

Keywords: *Diabetic Neuropathy; Guggul; Oxidative Stress; Ramipril*

Abbreviations

ACE: Angiotensin Converting Enzyme; Ages: Advanced Glycation End Products; Ang I And II: Angiotensin I and II; AT1: Angiotensin Receptor 1; COX: Cyclooxygenase; DN: Diabetic Neuropathy; ERK: Extracellular Signal-Regulated Kinases; IL: Interleukin; I.P.: Intraperitoneal; MAPK: Mitoge-Activated Protein Kinase; NF kβ: Nuclear Factor Kappa Beta; PKC: Protein Kinase C; P.O.: Per Oral; PPAR α and γ : Peroxisome Proliferator-Activated Receptor α and γ ; RAS – Renin Angiotensin System; STZ: Streptozotocin; TGF B1: Transforming Growth Factor B1; TNF α: Tumor Necrosis Factor α

Introduction

Diabetic neuropathy (DN) is the most widespread and intractable microvascular complication of diabetes [1]. The neuropathy progresses with decreasing nerve functionality and nerve blood perfusion which may result in malnourished nerve and leads to permanent nerve damage [2]. Diabetic nephropathy (DN) is a progressive damage of the kidney associated with elevated albuminuria and arterial blood pressure [3].

Commiphora mukul, commonly known as guggul, belongs to Burseraceae family. The anti-inflammatory activity of guggul enables it to be used for the treatment of rheumatoid arthritis, gout and hyperlipidaemia. Guggul has found as an important candidate for hypoglycaemic action. *Commiphora mukul* has antidiabetic activity owing to its action on PPAR α and γ [4].

Essential oil of guggulsterone and guggul were established as an effective antioxidant [5]. Besides, being an antioxidant, it has shown to reduce the levels of several hepatic enzymes such as aspartate amino transaminase and alanine amino transaminase [6]. Researchers reported an inhibitory effect of guggul on both COX-1 and COX-2 and lipid peroxidation [7]. Both lipid-lowering and antioxidant properties are beneficial against atherogenesis [8]. It suppresses phosphorylation of MAPK namely NFκβ, p38 and ERK that play a main role in modulating various inflammatory responses and also decreases transcription of IL-2, TNF-α and IL-1β [9]. It also reduces NO formation thus impacting on oxidative-nitrosative stress [10].

Ramipril is a second-generation ACE inhibitor, which prevent conversion of Ang I to Ang II. Ang-II is sufficient to induce injury of primary neurons via reduction in blood flow which disturbs the metabolic needs and activity of neurons resulting in neuronal damage and pain [11]. Further, the role of RAS has been revealed in pain processing because of increase in AT1 receptors accompanying sciatic nerve injury [12]. Inhibition of RAS could affect diabetic complications through modulation of PKC activation and AGEs formation [13] converging to the activation of both oxidative stress and inflammatory pathways.

Therefore, the present study has been proposed to explore the protective effect of *Commiphora mukul* and its interaction with ramipril against STZ – induced diabetic neuropathy and nephropathy.

Materials and Methods

Animals

Male Wistar rats (180-250g) obtained from Central Animal House, Panjab University Chandigarh were used in the current study. All the animals were acclimatized to laboratory conditions prior to experimentation. They were maintained on a 12-hour light/dark cycle and water and standard laboratory diet were available ad libitum. All the experiments were performed between 9:00 and 17:00h in order to avoid any circadian effect. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of Panjab University (PU/IAEC/S/05/29, 15/09/2015) and carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), Government of India and Indian National Science Academy Guidelines for the use and care of experimental animals.

Induction and assessment of diabetes

Induction of diabetes occur by only one intraperitoneal (i.p.) injection of streptozotocin (STZ, 55 mg/kg) dissolved in 0.1M citrate buffer (pH 4.5) to overnight fasted rats. Glucose (5%w/v) supplementation was given for first 8 hrs to prevent hypoglycaemic shock. Blood samples were collected after 48 hrs of STZ injection from retro-orbital plexus under light anaesthesia and blood glucose was measured. Animals with blood glucose level more than 250 mg/dl are considered diabetic and are used for further studies.

Method of glucose estimation

Blood glucose was measured using glucose estimation kit (AMS analysers, Italy).

Drugs and Treatment Schedule

Different groups (n = 6) were made (Table 1). Naive and diabetic rats were randomly selected. *Commiphora mukul* (50 and 100 mg/kg, p.o) (Dabur Research Foundation, Sahibabad, UP) and Ramipril (0.2 and 2.3 mg/kg, p.o.) (IPCA, Mumbai) was prepared in 0.25% w/v sodium carboxy methyl cellulose. All drugs are freshly prepared and administered in a constant volume of 0.5 ml/100g. Each group received drug treatment daily in the morning for 8 weeks at 10:00 am to avoid influence of circadian rhythm. Experimental protocol is described below (Figure 1).

S.No	Treatment Group (mg/kg)	Treatment given
1	Naïve	No treatment (Vehicle administered)
2	Control	Streptozotocin (55mg/kg; i.p.)
3	CM (50)	*Commiphora mukul* (50 mg/kg; p.o.) daily for a period of 8 weeks, 48 h after streptozotocin treatment
4	CM (100)	*Commiphora mukul* (100 mg/kg; p.o.) daily for a period of 8 weeks, 48 h after streptozotocin treatment
5	Ram (0.2)	Ramipril (0.2 mg/kg; p.o.) daily for a period of 8 weeks, 48 h after streptozotocin treatment
6	Ram (2.3)	Ramipril (2.3 mg/*kg*; p.o.) daily for a period of 8 weeks, 48 h after streptozotocin treatment
7	CM (50) +Ram (0.2)	*Commiphora mukul* (50 mg/kg; p.o.) + Ramipril (0.2 mg/kg; p.o.) daily for a period of 8 weeks, 48 h after streptozotocin treatment
8	CM (100) +Ram (2.3)	*Commiphora mukul* (100 mg/kg; p.o.) + Ramipril (2.3 mg/kg; p.o.) daily for a period of 8 weeks, 48 h after streptozotocin treatment

Table 1: *Treatment groups.*

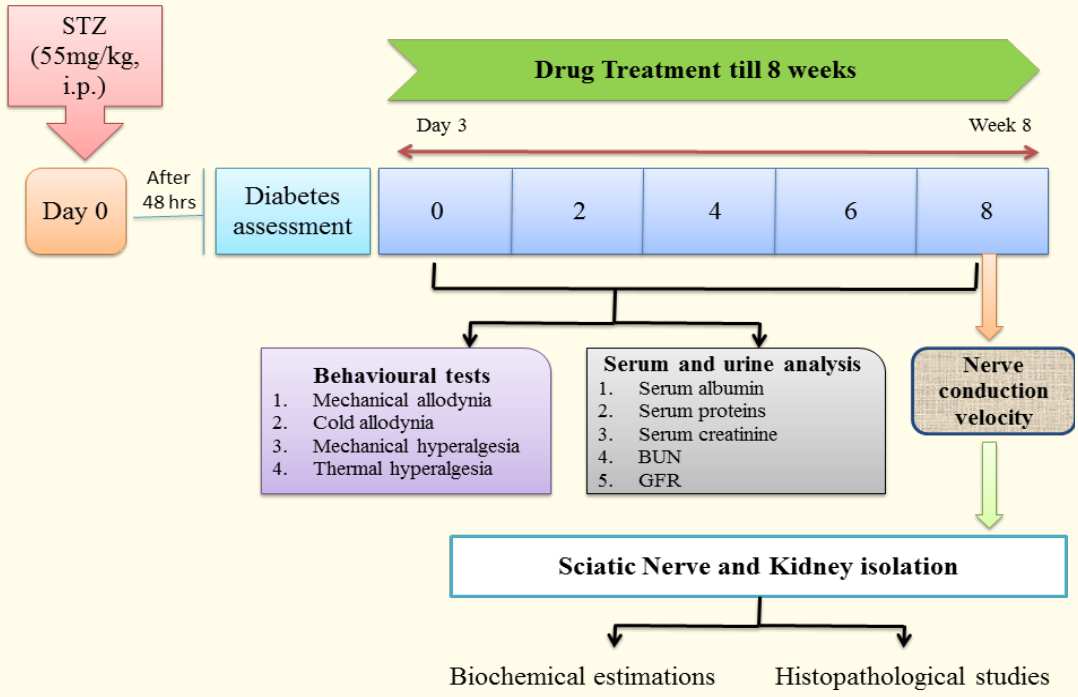

Figure 1: *Experimental design.*

Behavioural Assessments

Measurement of body weight

Body weights of all the animals were also recorded on weekly intervals week 0 to week 8 before behavioural examinations. The mean weight of the animals was recorded as compared to the naive treatment.

Food and water intake

Food and water intake of each individual group of animals was recorded on week 0 to week 8 using metabolic cages.

Urine Output

Urine output of each individual group of animals was recorded on weekly intervals from week 0 to week 8 using metabolic cages.

Mechanical allodynia

Allodynia was measured in rats by using an automated von-frey apparatus (IITC Life Sciences, USA). Animals were placed individually in a clear plastic cage containing mesh (1 cm2 perforations). They were well adapted to the testing environment for 30 minutes prior to testing. A polypropylene rigid tip of 0.5 mm diameter was used to apply force to the plantar region of the left hind paw. The tip was capable of applying a maximum force of 90 gm. The pressure at which the animal withdraws its paw was recorded. The test was repeated three times at the interval of 15 min and the mean value was finally calculated and recorded [14].

Cold allodynia

Individual animals were placed on a cold plate maintained at 5˚C ± 1˚C which was surrounded by a Plexiglas counter. 3 minutes cut off time was maintained. Time latency to paw licking/lifting was observed and recorded [15].

Mechanical hyperalgesia

Paw withdrawal threshold was assessed by Randall–Selitto paw pressure analgesia meter (IITC Life Science, Woodland Hills, CA). Increasing pressure was applied at the plantar region of hind paw and the pressure at which animal withdraws its paw was recorded. Cut off threshold was 250g to avoid potential tissue damage. Test was repeated three times at the interval of 10 min and mean value was finally recorded [16].

Thermal hyperalgesia

Animals were placed individually on the Eddy's hot plate maintained at 55 ± 0.5^{0}C. Time latency for paw licking/Jumping was noted. Cut off time is 10 secs was maintained to avoid tissue damage [17].

Blood collection and processing

Blood samples were collected from each animal through retro-orbital route after behavioural assessments and serum was separated by centrifugation at 5000 rpm for 5 minutes. Overnight urine was collected using metabolic cages. Both urine and serum samples were stored at -20^{0}C until further analysis.

Assessment of urine and serum parameters

Assessment of total proteins

The total protein content is assessed by reaction of protein with copper ions in alkali using total protein estimation kit (AMS analysers, Italy).

Assessment of serum albumin level

Serum albumin levels are assayed by Bromo cresol green reagent reaction with albumin in presence of citrate buffer with serum albumin estimation kit (AMS analysers, Italy).

Assessment of serum/urinary creatinine

Serum/urinary creatinine levels were assayed by following the reaction with picric acid using the creatinine estimation kit (AMS analysers, Italy).

Assessment of serum urea nitrogen

Serum/urinary urea and nitrogen levels were assayed by urease method using the urea estimation kit (AMS analysers, Italy).

Electrophysiological examination

After last set of behavioral assessments, animals were anaesthetized with thiopentone sodium 30 mg/kg. An incision was made on the left thigh and the sciatic nerve was exposed. Recording electrodes were placed superficially into the plantar region of the foot and sciatic nerve was stimulated with 3V proximally at sciatic notch and distally at tibial notch. Mean nerve conduction velocity (MNCV) was measured using the Power Lab instrument (AD Instruments, Power Lab Chart, Australia) [18].

$$MNCV\ \left(m/s\right) = \frac{Distance\ between\ sciatic\ and\ tibial\ nerve\ stimulation\ point}{Sciatic\ M\ wave\ latency\left(s\right)\ -\ tibial\ M\ wave\ latency\left(s\right)}$$

Dissection and homogenization

The animals were sacrificed by cervical dislocation immediately after electrophysiological examination at the end of 8th week. Sciatic nerve (starting from the point of emergence from the spinal cord to its trifurcation) was isolated and stored at -20˚C for further biochemical estimations to be carried out the next day. Kidney was also isolated and stored in formalin for histopathological estimations.

Biochemical estimations

First, 10 % (w/v) tissue homogenates of sciatic nerves and kidney were prepared in 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000g at 4˚C for 15 min and the supernatants so formed were separated and used for biochemical estimations.

Lipid peroxidation

The extent of lipid peroxidation in sciatic nerve and kidney were determined by performing the procedure as previously described by Wills [19]. Malondialdehyde (MDA) is taken to be a marker of lipid peroxidation. Its reaction with thiobarbituric acid (TBA) was measured using a PerkinElmer Lambda 20 Spectrophotometer (Norwalk, CT, USA) at 532 nm. The values were calculated using molar extinction coefficient of chromophore (1.56 x 105 M-1 cm-1) and expressed as nanomoles of malondialdehyde per milligram of protein.

Superoxide dismutase activity

Superoxide dismutase activity estimation was based on its ability to inhibit the reduction of nitro blue tetrazolium (NBT). This is initiated by the addition of hydroxylamine hydrochloride to the reaction mixture containing NBT and the sample. The results were expressed as unit/mg protein, where one unit of enzyme is defined as the amount of enzyme inhibiting the rate of reaction by 100 %. The readings were recorded using a spectrophotometer at 560 nm [20].

Reduced glutathione (GSH) estimation

GSH activity in the sciatic nerve and kidney was measured according to the method previously described by Ellman. 1 ml supernatant was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested at 4˚C for 1h. The sample was centrifuged at 1200 rpm for 15 min at 4˚C. To 1 ml of this supernatant, 2.7 ml of 0.1 M phosphate buffer (pH 8) and 0.2 ml of 5,5-dithiobis 2-nitrobenzoic acid (DTNB) were added. The absorbance was read immediately at 412 nm, and the results were calculated using molar extinction coefficient of chromophore (1.36 x l04 M-1 cm-1) and expressed as micromole GSH per milligram protein.

Catalase estimation

Catalase estimation is carried out by measuring the breakdown of hydrogen peroxides. The assay mixture consists of 3 ml of H_2O_2 phosphate buffer and 0.05 ml of supernatant of tissue homogenate (10%), and the change in absorbance is recorded at 240 nm. The results are expressed as micromole H_2O_2 decomposed per milligram of protein/min [21].

Protein estimation

Protein estimation was done by biuret method utilizing bovine serum albumin as standard.

Histological Studies

Kidneys were isolated according to the previously described method. Tissues were fixed in formalin and embedded in paraffin. 5µm sections were transversely cut and stained with hematoxylin and eosin (H and E staining) and observed under microscope at 50x magnification.

Statistical analysis

Statistical analysis was performed by using GraphPad Prism (Graph Pad Software, San Diego, CA). A group of six animals (n = 6) were assigned to a specific drug treatment. Results were expressed as mean ± S.E.M. The data were analyzed by two analysis of variance (ANOVA) followed by Bonferroni post tests for behavioral parameters and one way analysis of variance (ANOVA) followed by Tukey's test for biochemical estimations. $P < 0.05$ was considered to be statistically significant.

Results

Effect of *Commiphora mukul*, ramipril and their combination on body weight in STZ- induced diabetic rats

STZ injection (55 mg/kg) induced diabetes exhibited significant fall in body weight from 2nd to 8th week as compared to naïve group. 8 weeks treatment with *Commiphora mukul* (50 and 100 mg/kg) and ramipril (0.2 and 2.3 mg/kg) significantly restored the body weight from 2nd to 8th week as compared with diabetic control group. Further, combinations of *Commiphora mukul* (50 and 100 mg/kg) with ramipril (0.2 and 2.3 mg/kg) respectively did not exhibited any significant influence on body weight as compared to their effect per se in STZ induced diabetic rats (Table 2).

Treatment group (mg/kg)	Change in body weight (g) on weekly intervals				
	0	2	4	6	8
Naive	223.3 ± 12.0	231.66 ± 5.8	243.33 ± 8.8	250 ± 15.2	265 ± 15.3
STZ (55)	235.1 ± 5.6	170.3 ± 18.9[a]	165.8 ± 18.0[a]	161 ± 19.0[a]	148 ± 14.9[a]
CM (50)	230 ± 5.0	235.8 ± 8.2[b]	240 ± 7.7[b]	240.8 ± 10.6[b]	245 ± 10.1[b]
CM (100)	237.5 ± 8.0	241.6 ± 9.3[b]	251.6 ± 9.4[b]	260.8 ± 11.2[b]	260.8 ± 10.8[b]
Ram (0.2)	229.1 ± 5.8	245 ± 5.9[b]	260.5 ± 8.5[b]	268.3 ± 6.5[b]	272.5 ± 1.3[b]
Ram (2.3)	240 ± 6.8	245 ± 5.6[b]	260 ±7.3[b]	259 ± 6.6[b]	261.66 ± 4.7[b]
CM (50) + Ram (0.2)	260 ± 7.1	255.8 ± 9.4[b]	262.5 ± 9.4[b]	273.3 ± 9.9[b]	278.3 ± 9.9[b]
CM (100) + Ram (2.3)	250.3 ± 2.6	245 ± 5.6[b]	265.8 ± 3.2[b]	277.5 ± 4.7[b]	286.6 ± 4.2[b]

Table 2: *Effect of Commiphora mukul, ramipril and their combination on body weight in diabetic rats.*

Data are expressed as mean ± S.E.M. [a]p<0.05 as compared to naive group; [b]p<0.05 as compared to control group. (Two-way ANOVA followed by Bonferroni post tests). CM (50): Commiphora mukul (50mg/kg); CM (100) Commiphora mukul (100mg/kg); Ram (0.2): ramipril (0.2 mg/kg); Ram (2.3): ramipril (2.3 mg/kg).

Effect of *Commiphora mukul*, ramipril and their combination on food intake in STZ- induced diabetic rats

STZ injection (55mg/kg) significantly increased food intake (polyphagia) from 2nd to 8th week as compared to naïve group. 8 weeks treatment with *Commiphora mukul* (50 and 100 mg/kg) and ramipril (0.2 and 2.3 mg/kg) significantly decreased food intake from 2nd to 8th week as compared to diabetic control group. Further, combinations of *Commiphora mukul* (50 and 100 mg/kg) with ramipril (0.2 and 2.3 mg/kg) respectively significantly decreased food intake from 2nd to 8th week as compared to their effect per se in STZ induced diabetic animals (Table 3).

Treatment group (mg/kg)	Change in food intake (g) for weekly intervals				
	0	2	4	6	8
Naive	23.7 ± 1.1	22.5 ± 0.5	21.3 ± 1.1	23.1 ± 0.6	21.8 ± 0.5
STZ (55)	22.7 ± 1.7	44.5 ± 0.5[a]	49.2 ± 0.7[a]	52.6 ± 0.8[a]	57.3 ± 1.1[a]
CM (50)	22.8 ± 0.9	39.1 ± 0.4[b]	35.1 ± 0.8[b]	31.9 ± 0.6[b]	27 ± 0.3[b]
CM (100)	22.3 ± 0.6	34.6 ± 0.5[b,c]	30.9 ± 0.4[b,c]	26.2 ± 1.1[b,c]	22.2 ± 0.4[b,c]
Ram (0.2)	21.5 ± 0.6	39.3 ± 0.2[b]	38.85 ± 0.3[b]	37.7 ± 0.6[b]	29.5 ± 0.3[b]
Ram (2.3)	23 ± 0.4	32.5 ± 0.3[b]	29.1 ± 0.2[b]	24.1 ± 0.8[b]	21.5 ± 0.3[b]
CM (50) + Ram (0.2)	22.8 ± 0.5	36.8 ± 0.3[b,e]	33.4 ± 0.6[b,e]	28 ± 0.2[b,e]	20.8 ± 0.4[b,c]
CM (100) + Ram (2.3)	22 ± 0.7	28.4 ± 0.3[b,d,f,g]	24.9 ± 0.4[b,d,g]	23.3 ± 0.4[b,d,g]	20 ± 0.2[b,d]

Table 3: *Effect of Commiphora mukul, ramipril and their combination on food intake in diabetic rats.*

Data are expressed as mean ± S.E.M. [a]p<0.05 as compared to naive group; [b]p<0.05 as compared to control group; [c]p<0.05 as compared to CM (50); [d]p<0.05 as compared to CM (100); [e]p<0.05 as compared to ram (0.2); [f]p<0.05 as compared to ram (2.3); [g]p<0.05 as compared to CM (50) + ram (0.2) (Two-way ANOVA followed by Bonferroni post tests). CM (50): Commiphora mukul (50mg/kg); CM (100) Commiphora mukul (100mg/kg); Ram (0.2): ramipril (0.2 mg/kg); Ram (2.3): ramipril (2.3 mg/kg).

Effect of *Commiphora mukul*, ramipril and their combination on water intake in STZ- induced diabetes

STZ (55 mg/kg) administration significantly increased water intake (polydipsia) from 2nd to 8th week as compared to naïve group. 8 weeks treatment with *Commiphora mukul* (50 and 100 mg/kg) and ramipril (0.2 and 2.3 mg/kg) significantly ($p < 0.05$) attenuated an increase in water intake from 2nd to 8th week as compared to diabetic control group. However, combination of *Commiphora mukul* (100 mg/kg) with ramipril (2.3 mg/kg) significantly decreased water intake from 4th to 8th week as compared to their effect per se group in STZ induced diabetic rats (Table 4).

Treatment group (mg/kg)	Change in water intake (ml) on weekly intervals				
	0	2	4	6	8
Naive	29.5 ± 0.5	27.4 ± 1	28.5 ± 0.5	27.6 ± 0.5	27.5 ± 0.5
STZ (55)	29.7 ± 0.3	73.5 ± 0.5[a]	89 ± 1.0[a]	90.6 ± 0.6[a]	102 ± 1.1[a]
CM (50)	28.1 ± 1.0	51.1 ± 1.1[b]	48.4 ± 0.4[b]	240.1 ± 0.6[b]	38.5 ± 0.6[b]
CM (100)	29.4 ± 0.7	47.7 ± 0.6[b,c]	43.9 ± 1.0[b,c]	39.7 ± 1.1[b]	32.6 ± 0.8[b,c]
Ram (0.2)	29.2 ± 0.8	50.8 ± 0.6[b]	47.2 ± 0.8[b]	41.2 ± 0.4[b]	36.3 ± 1.3[b]
Ram (2.3)	29.2 ± 0.3	45.4 ± 0.8[b,e]	41.1 ± 0.5[b,e]	35.9 ± 0.6[b,e]	30.1 ± 0.7[b,e]
CM (50) + Ram (0.2)	30.2 ± 0.3	47.1 ± 0.6[b,c,e]	44.5 ± 0.3[b,e]	38.2 ± 0.4[b,e]	35. ± 0.1[c,e]
CM (100) + Ram (2.3)	30.5 ± 0.5	45.1 ± 0.4[b]	39.7 ± 0.6[b,d,f,g]	36.1 ± 0.4[b,d,f]	28.7 ± 0.4[b,d,f,g]

Table 4: *Effect of Commiphora mukul, ramipril and their combination on water intake in diabetic rats.*

Data are expressed as mean ± S.E.M. [a]p<0.05 as compared to naive group; [b]p<0.05 as compared to control group; [c]p<0.05 as compared to CM (50); [d]p<0.05 as compared to CM (100); [e]p<0.05 as compared to ram (0.2); [f]p<0.05 as compared to ram (2.3); [g]p<0.05 as compared to CM (50) + ram (0.2) (Two-way ANOVA followed by Bonferroni post tests). CM (50): Commiphora mukul (50mg/kg); CM (100): Commiphora mukul (100mg/kg); Ram (0.2): Ramipril (0.2 mg/kg); Ram (2.3): Ramipril (2.3 mg/kg).

Effect of *Commiphora mukul*, ramipril and their combination on urine output in STZ- induced diabetes

Intraperitoneal STZ (55 mg/kg) has significantly increased urine output (polyuria) from 2nd to 8th week as compared to naïve group. 8 weeks treatment with *Commiphora mukul* (50 and 100 mg/kg) and ramipril (0.2 and 2.3 mg/kg) significantly decreased urine output from 2nd to 8th week as compared to control (diabetic) group. However, combination of *Commiphora mukul* (50 mg/kg) with ramipril (0.2 mg/kg) respectively significantly decreased urine output in 2nd, 4th and 6th week as compared to their effect per se in STZ induced diabetic rats (Table 5).

Treatment group (mg/kg)	Change in urine output (ml) on weekly intervals				
	0	2	4	6	8
Naive	15.5 ± 0.5	14.5 ± 0.5	14 ± 1.0	13.5 ± 0.5	13.5 ± 0.5
STZ (55)	16.5 ± 1.5	35.5 ± 1.5 [a]	38.5 ± 0.3 [a]	53.5 ± 1.5 [a]	59.5 ± 0.2 [a]
CM (50)	14.2 ± 1.1	32.9 ± 1.7 [b]	26.6 ± 0.8 [b]	22.1 ± 0.7 [b]	19.0 ± 1.1
CM (100)	15.5 ± 0.6	26.5 ± 1.1 [b,c]	22.8 ± 0.9 [b,c]	17.1 ± 0.5 [b]	16.1 ± 0.9 [b]
Ram (0.2)	14.2 ± 0.6	31.4 ± 0.7 [b]	28.5 ± 1.3 [b]	24.6 ± 1.7 [b]	20.5 ± 0.5 [b]
Ram (2.3)	14.7 ± 0.6	27.9 ± 0.6 [b,e]	25 ±1.0 [b,e]	20.9 ± 0.3 [b,e]	16.1 ± 1.0 [b,e]
CM (50) + Ram (0.2)	14.2 ± 0.6	27.4 ± 0.4 [b,c,e]	23.4 ± 0.3 [b,c,e]	22.4 ± 0.3 [b]	14.5 ± 0.5 [b,c,e]
CM (100) + Ram (2.3)	15.6 ± 0.2	23.6 ± 0.6 [b,f,g]	20.9 ± 0.4 [b,f]	18.8 ± 0.3 [b,g]	14 ± 0.8 [b]

Table 5: *Effect of Commiphora mukul, ramipril and their combination on urine output in diabetic rats.*

Data are expressed as mean ± S.E.M. [a]p<0.05 as compared to naive group; [b]p<0.05 as compared to Control group; [c]p<0.05 as compared to CM (50mg/kg); [e]p<0.05 as compared to Ram (0.2mg/kg); [f]p<0.05 as compared to Ram (2.3); [g]p<0.05 as compared to CM (50) + Ram (0.2); [g]p<0.05 as compared to CM (100) + Ram (2.3) (Two-way ANOVA followed by Bonferroni post tests). CM (50): Commiphora mukul (50mg/kg); CM (100) Commiphora mukul (100mg/kg); Ram(0.2): Ramipril (0.2 mg/kg); Ram (2.3): Ramipril (2.3 mg/kg).

Effect of *Commiphora mukul,* ramipril and their combination on mechanical allodynia in STZ- induced diabetic rats

Single STZ (55 mg/kg) administration significantly increased mechanical allodynia (decreased paw withdrawal threshold) from 2nd to 8th week as compared to naïve group. 8 weeks treatment with *Commiphora mukul* (50 and 100 mg/kg) and ramipril (0.2 and 2.3 mg/kg) significantly raised the paw withdrawal threshold from 4th to 8th week as compared to control group. However, combination of *Commiphora mukul* (50 and 100 mg/kg) with ramipril (0.2 and 2.3 mg/kg) did not influence significantly paw withdrawal threshold as compared to their effect per se in STZ induced diabetic rats (Figure 2).

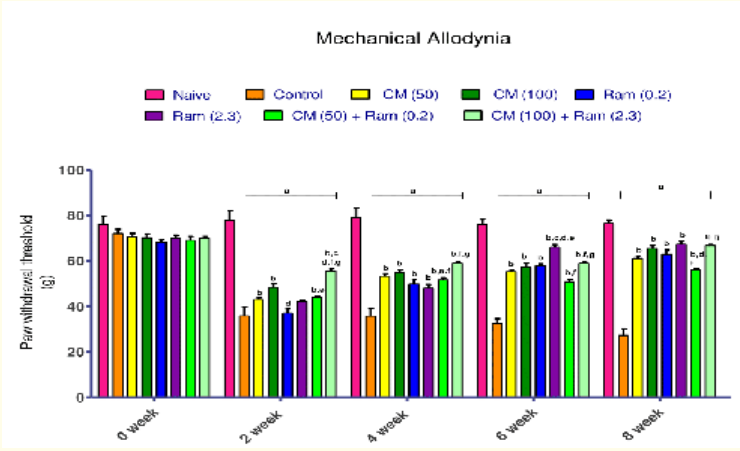

Figure 2: *Effect of Commiphora mukul, ramipril and their combination on mechanical allodynia in diabetic rats. Data are expressed as mean ± S.E.M. [a]p < 0.05 as compared to naive group; [b]p < 0.05 as compared to control group; [d]p < 0.05 as compared to CM (100); [e]p < 0.05 as compared to ram (0.2); [f]p < 0.05 as compared to ram (2.3); [g]p < 0.05 as compared to CM (50) + ram (0.2) (Two-way ANOVA followed by Bonferroni post tests). CM (50): Commiphora mukul (50mg/kg); CM (100) Commiphora mukul (100mg/kg); Ram (0.2): ramipril (0.2 mg/kg); Ram (2.3): ramipril (2.3 mg/kg).*

Effect of *Commiphora mukul*, ramipril and their combination on cold allodynia in STZ- induced diabetes

Single intraperitoneal injection of STZ (55 mg/kg) significantly delayed time latency to paw licking/ lifting to cold stimuli (increased total duration of the hind paw licking and lifting) from 2nd to 8th week as compared to the naïve group. 8 weeks treatment with *Commiphora mukul* (50 and 100 mg/kg) and ramipril (0.2 and 2.3 mg/kg) significantly attenuated time latency to paw licking/ lifting to cold stimuli from 4th to 8th week as compared to the control group. However, ramipril (0.2 and 2.3 mg/kg) in combination with *Commiphora mukul* (50 and 100 mg/kg) did not show any significant influence on cold allodynia as compared to their effect per se in STZ induced diabetic rats (Figure 3).

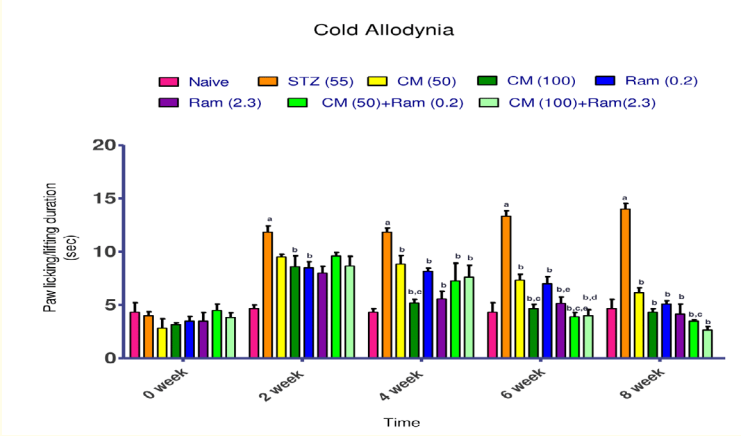

Figure 3: *Effect of Commiphora mukul, ramipril and their combination on cold allodynia in diabetic rats. Data are expressed as mean ± S.E.M. [a]p < 0.05 as compared to naive group; [b]p < 0.05 as compared to Control group; [c]p < 0.05 as compared to CM (50); [d]p < 0.05 as compared to CM (100); [e]p < 0.05 as compared to Ram (0.2) (Two-way ANOVA followed by Bonferroni post test). CM (50): Commiphora mukul (50mg/kg); CM (100) Commiphora mukul (100mg/kg); Ram (0.2): Ramipril (0.2 mg/kg); Ram (2.3): Ramipril (2.3 mg/kg).*

Effect of *Commiphora mukul*, ramipril and their combination on mechanical hyperalgesia in STZ- induced diabetes

STZ (55 mg/kg) injection significantly reduced paw withdrawal threshold from 2nd to 8th week as compared to the naïve group. 8 weeks treatment with *Commiphora mukul* in a dose of 50 and 100 mg/kg significantly improved paw withdrawal threshold from 4th to 8th week as compared to the naïve group. Treatment with ramipril (0.2 and 2.3 mg/kg) significantly increased paw withdrawal effect from 4th to 8th week as compared to the control group. However, combinations of *Commiphora mukul* (50 and 100 mg/kg) with ramipril (0.2 and 2.3 mg/kg) respectively did not show any influence on mechanical hyperalgesia as compared to their effect per se in STZ induced diabetic rats (Figure 4).

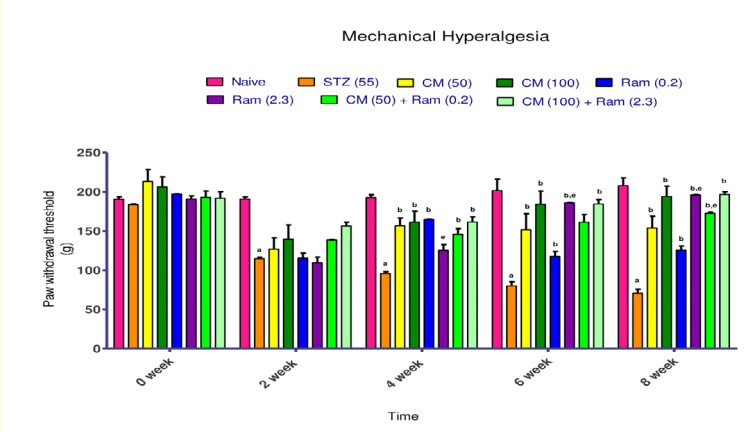

Figure 4: *Effect of Commiphora mukul, ramipril and their combination on mechanical hyperalgesia in diabetic rats. Data are expressed as mean ± S.E.M. [a]p<0.05 as compared to naive group; [b]p<0.05 as compared to Control group; [e]p<0.05 as compared to Ram (0.2) (Two-way ANOVA followed by Bonferroni post test). CM (50): Commiphora mukul (50mg/kg); CM (100) Commiphora mukul (100mg/kg); Ram (0.2): Ramipril (0.2 mg/kg); Ram (2.3): Ramipril (2.3 mg/kg).*

Effect of *Commiphora mukul*, ramipril and their combination on thermal hyperalgesia in STZ- induced diabetes

Single STZ injection (55 mg/kg) significantly decreased paw withdrawal latency from 2nd to 8th week as compared to naïve group. 8 weeks treatment with *Commiphora mukul* (50 and 100 mg/kg) and ramipril (0.2 and 2.3 mg/kg) significantly improved paw withdrawal latency as compared to control group. However, combinations of *Commiphora mukul* (50 and 100 mg/kg) with ramipril (0.2 and 2.3 mg/kg) respectively did not show any significant improvement on increased paw withdrawal threshold as compared to their effect per se in STZ- induced diabetic animals (Figure 5).

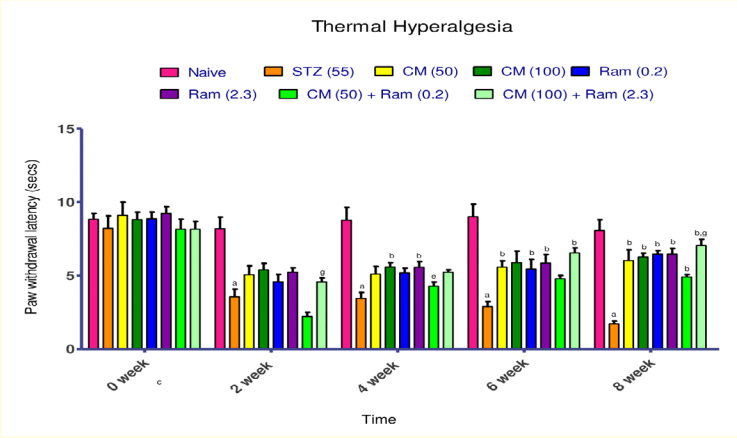

Figure 5: Effect of Commiphora mukul, ramipril and their combination on thermal hyperalgesia in diabetic rats. Data are expressed as mean ± S.E.M. [a]p<0.05 as compared to naive group; [b]p<0.05 as compared to control group; [g]p<0.05 as compared to CM (100) + ram (2.3) (Two-way ANOVA followed by Bonferroni post test). CM (50): Commiphora mukul (50mg/kg); CM (100) Commiphora mukul (100mg/kg); Ram (0.2): ramipril (0.2 mg/kg); Ram (2.3): ramipril (2.3 mg/kg).

Effect of *Commiphora mukul*, ramipril and their combination on serum albumin in STZ- induced diabetic rats

Single intraperitoneal injection of STZ (55 mg/kg) has significantly decreased serum albumin level as compared to naïve group. 8 weeks treatment with *Commiphora mukul* (50 and 100 mg/kg) and ramipril (0.2 and 2.3 mg/kg) significantly restored the decrease in serum albumin level from 6th to 8th week as compared to diabetic control group. However, combinations of *Commiphora mukul* (50 and 100 mg/kg) with ramipril (0.2 and 2.3 mg/kg) respectively did not show any significant effect on serum albumin as compared to their effect in per se in STZ induced diabetic rats (Table 6).

Treatment group (mg/kg)	Change in serum albumin (g/dl) level on weekly intervals				
	0	2	4	6	8
Naive	5.8 ± 0.2	5.7 ± 0.1	5.1 ± 0.8	5.2 ± 0.4	5.4 ± 0.7
STZ (55)	5.1 ± 0.3	4.1 ± 0.05	3.9 ± 0.1 [a]	3.8 ± 0.3 [a]	3.1 ± 0.08 [a]
CM (50)	5.4 ± 0.07	2.8 ± 0.01	4.7 ± 0.1	5.3 ± 0.08 [b]	4.9 ± 0.02 [b]
CM (100)	5.1 ± 0.1	2.9 ± 0.05	4.9 ± 0.01 [b]	5.6 ± 0.06 [b]	5.3 ± 0.02 [b]
Ram (0.2)	5.3 ± 0.1	3.7 ± 0.1	4.5 ± 0.2	4.6 ± 0.1	5.4 ± 0.4 [b]
Ram (2.3)	5.5 ± 0.1	4.0 ± 0.1	5.2 ± 0.04 [b]	5.7 ± 0.04 [b]	5.8 ± 0.06 [b]
CM (50) + Ram (0.2)	5.3 ± 0.1	3.3 ± 0.03	4.7 ± 0.1	5.3± 0.1 [b]	5.4 ± 0.05 [b]
CM (100) + Ram (2.3)	5.3 ± 0.2	4.1 ± 0.2	4.8 ± 0.1 [b]	5.5 ± 0.2 [b]	6.3 ± 0.1 [b]

Table 6: *Effect of Commiphora mukul, ramipril and their combination on serum albumin in diabetic rats. Data are expressed as mean ± S.E.M. [a]p<0.05 as compared to naive group; [b]p<0.05 as compared to control group; (Two-way ANOVA followed by Bonferroni post test). CM (50): Commiphora mukul (50mg/kg); CM (100) Commiphora mukul (100mg/kg); Ram (0.2): ramipril (0.2 mg/kg); Ram (2.3): ramipril (2.3 mg/kg).*

Effect of *Commiphora mukul*, ramipril and their combination on blood urea nitrogen in STZ- induced diabetic rats

Single STZ (55 mg/kg) administration significantly increased blood urea nitrogen level from 2nd to 8th week as compared to naïve group. 8 weeks treatment with *Commiphora mukul* (50 and 100 mg/kg) and ramipril (0.2 and 2.3 mg/kg) decreased the raised blood urea

nitrogen level from 2nd to 8th week as compared to diabetic control group. Combinations of Commiphora mukul (50 and 100 mg/kg) with ramipril (0.2 and 2.3 mg/kg) respectively did not show significant influence on blood urea nitrogen as compared to their effect per se in STZ induced diabetic rats (Table 7).

Treatment group (mg/kg)	Change in blood urea nitrogen (mg/dl) level on weekly intervals				
	0	2	4	6	8
Naive	32.2 ± 2.4	26.4 ± 0.2	29.4 ± 1.8	31.2 ± 3.6	24.9 ± 0.5
STZ (55)	37.7 ± 0.7	41.8 ± 3.3[a]	52.4 ± 1.9[a]	52.7 ± 1.3[a]	51.5 ± 1.01[a]
CM (50)	32.5 ± 1.6	44.1 ± 2.7[b]	42.9 ± 1.2[b]	40.0 ± 2.2[b]	38.2 ± 1.4[b]
CM (100)	33.2 ± 1.6	42.7 ± 2.5[b]	39.3 ± 4.7[b]	39.1 ± 0.8[b]	35.3 ± 0.5[b]
Ram (0.2)	35.4 ± 0.4	40.5 ± 0.9[b]	37.6 ± 0.9[b]	36.4 ± 0.8[b]	35.3 ± 0.8[b]
Ram (2.3)	33.2 ± 0.9	41.5 ± 0.8[b]	40.6 ± 0.8[b]	39.6 ± 0.8[b]	37.8 ± 0.9[b]
CM (50) + Ram (0.2)	33.2 ± 0.9	41.5 ± 0.8[b]	40.6 ± 0.8[b]	36.6 ± 0.8[b]	35.8 ± 0.9[b]
CM (100) + Ram (2.3)	34.2 ± 0.9	39.5 ± 0.9[b]	38.4 ± 0.8[b]	36.1 ± 0.5[b]	33.2 ± 0.4[b]

Table 7: *Effect of Commiphora mukul, ramipril and their combination on blood urea nitrogen in diabetic rats.*

Data are expressed as mean ± S.E.M. [a]p<0.05 as compared to naive group; [b]p<0.05 as compared to control group. (Two-way ANOVA followed by Bonferroni post test). CM (50): Commiphora mukul (50mg/kg); CM (100) Commiphora mukul (100mg/kg); Ram (0.2): ramipril (0.2 mg/kg); Ram (2.3): ramipril (2.3 mg/kg).

Effect of *Commiphora mukul*, ramipril and their combination on serum total proteins in STZ- induced diabetic rats

Intraperitoneal STZ (55 mg/kg) injection significantly decreased serum total proteins from 2nd to 8th week as compared to naïve group. The fall in serum creatinine level of STZ injected rats were significantly attenuated with *Commiphora mukul* (50 and 100 mg/kg) and ramipril (0.2 and 2.3 mg/kg) from 6th to 8th week. *Commiphora mukul* (100 mg/kg) in combination with ramipril (2.3 mg/kg) significantly increased serum total proteins level as compared to their effect per se in STZ induced diabetic rats (Table 8).

Treatment group (mg/kg)	Change in serum total proteins (g/dl) levels on weekly intervals				
	0	2	4	6	8
Naive	7.4 ± 0.3	7.3 ± 0.4	7.2 ± 0.1	7 ± 0.9	7.1 ± 0.1
STZ (55)	7.2 ± 0.2	4.9 ± 0.3[a]	4.8 ± 0.2[a]	4.1 ± 0.2[a]	3.1 ± 0.5[a]
CM (50)	7.2 ± 0.03	3.9 ± 0.1	4.1 ± 0.01	4.47 ± 0.08[b]	4.8 ± 0.06[b]
CM (100)	7.3 ± 0.03	3.9 ± 0.02	4.3 ± 0.02	5.07 ± 0.1[b]	4.9 ± 0.1[b]
Ram (0.2)	7.3 ± 0.01	3.8 ± 0.06	3.9 ± 0.02	4.2 ± 0.08	5.1 ± 0.3[b]
Ram (2.3)	7.3 ± 0.05	3.9 ± 0.06[c]	4.4 ± 0.02[c]	5.1 ± 0.1	5.5 ± 0.1[b]
CM (50) + Ram (0.2)	7.3 ± 0.07	3.9 ± 0.04[b]	4.3 ± 0.03	5.4 ± 0.03	5.8 ± 0.1[c]
CM (100) + Ram (2.3)	7.4 ± 0.1	3.9 ± 0.1	4.8 ± 0.04	5.5 ± 0.1[b]	6.4 ± 0.2[b,d,f]

Table 8: *Effect of Commiphora mukul, ramipril and their combination on serum total proteins in diabetic rats.*

Data are expressed as mean ± S.E.M. [a]p<0.05 as compared to naive group; [b]p<0.05 as compared to control group; [c]p<0.05 as compared to CM (50); [d]p<0.05 as compared to CM (100); [f]p<0.05 as compared to ram (2.3). (Two-way ANOVA followed by Bonferroni post test). CM (50): Commiphora mukul (50mg/kg); CM (100) Commiphora mukul (100mg/kg); Ram (0.2): ramipril (0.2 mg/kg); Ram (2.3): ramipril (2.3 mg/kg).

Effect of *Commiphora mukul,* ramipril and their combination on serum creatinine in STZ- induced diabetic rats

Single STZ (55 mg/kg) injection significantly increased serum creatinine level from 2nd to 8th week as compared to naïve group. Treatment with *Commiphora mukul* (50 and 100 mg/kg) and ramipril (0.2 and 2.3 mg/kg) for a period of 8 weeks significantly reduced serum creatinine level from 2nd to 8th week as compared to control diabetic group. However, *Commiphora mukul* (50 and 100 mg/kg) in combinations with ramipril (0.2 and 2.3 mg/kg) respectively did not show any significant effect on serum total protein level as compared to their effect per se in STZ induced diabetic rats (Table 9).

Treatment group (mg/kg)	Change in serum creatinine (mg/dl) on weekly intervals				
	0	2	4	6	8
Naive	0.66 ± 0.11	0.61 ± 0.15	0.67 ± 0.15	0.52 ± 0.02	0.66 ± 0.09
STZ (55)	0.58 ± 0.05	1.6 ± 0.04 [a]	1.4 ± 0.07 [a]	1.1 ± 0.19 [a]	1.2 ± 0.08 [a]
CM (50)	0.67 ± 0.004	1.11 ± 0.03 [b]	1.0 ± 0.02 [b]	0.95 ± 0.01	0.93 ± 0.01
CM (100)	0.66 ± 0.003	1.04 ± 0.02 [b]	0.93 ± 0.04 [b]	0.81 ± 0.02 [b]	0.80 ± 0.01 [b,c]
Ram (0.2)	0.66 ± 0.04	1.1 ± 0.02 [b]	1 ± 0.03 [b]	0.9 ± 0.02 [b]	0.8 ± 0.11 [b]
Ram (2.3)	0.6 ± 0.01	0.98 ± 0.05 [b]	0.89 ± 0.04 [b]	0.79 ± 0.01 [b]	0.6 ± 0.03 [b]
CM (50) + Ram (0.2)	0.64 ± 0.03	1.01 ± 0.02 [b]	0.71 ± 0.06 [b]	0.67 ± 0.03 [b]	0.65 ± 0.02 [b]
CM (100) + Ram (2.3)	0.66 ± 0.01	0.97 ± 0.04 [b]	0.67 ± 0.01 [b]	0.66 ± 0.03 [b]	0.58 ± 0.02 [b]

Table 9: *Effect of Commiphora mukul, ramipril and their combination on serum creatinine in diabetic rats.*

Data are expressed as mean ± S.E.M. [a]p<0.05 as compared to naive group; [b]p<0.05 as compared to control group; [c]p<0.05 as compared to CM (50). (Two-way ANOVA followed by Bonferroni post test). CM (50): Commiphora mukul (50mg/kg); CM (100) Commiphora mukul (100mg/kg); Ram (0.2): ramipril (0.2 mg/kg); Ram (2.3): ramipril (2.3 mg/kg).

Effect of *Commiphora mukul*, ramipril and their combination on urine parameter (glomerular filtration rate) in STZ- induced diabetic rats

Single intraperitoneal injection of STZ (55 mg/kg) significantly decreased glomerular filtration rate from 2nd to 8th week as compared to naïve group (Figure 6). 8 weeks treatment with *Commiphora mukul* (50 and 100 mg/kg) and ramipril (0.2 and 2.3 mg/kg) significantly restored decrease in glomerular filtration rate from 4th to 8th week as compared to control group. However, combination of *Commiphora mukul* (100 mg/kg) with ramipril (2.3 mg/kg) significantly increased glomerular filtration rate on 8th week as compared to their effect per se in STZ induced diabetic rats.

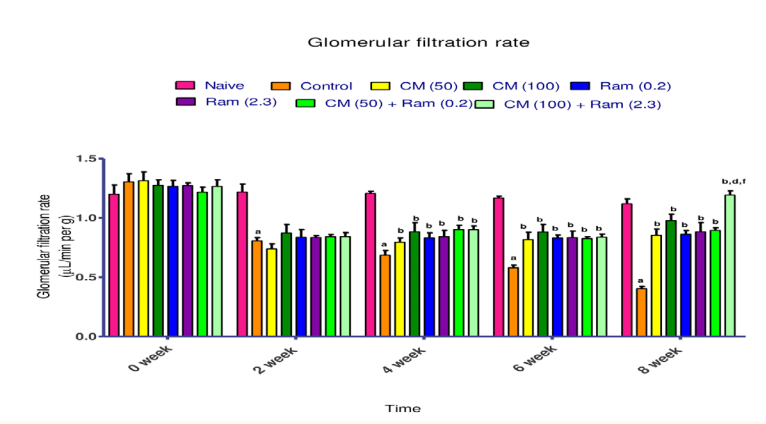

Figure 6*: Effect of Commiphora mukul, ramipril and their combination on urine parameter (glomerular filtration rate) in diabetic rats. Data are expressed as mean ± S.E.M. ap<0.05 as compared to naive group; bp<0.05 as compared to control group; dp<0.05 as compared to CM (100); fp<0.05 as compared to CM (50) + ram (0.2) (Two-way ANOVA followed by Bonferroni post test). CM (50): Commiphora mukul (50mg/kg); CM (100) Commiphora mukul (100mg/kg); Ram (0.2): ramipril (0.2 mg/kg); Ram (2.3): ramipril (2.3 mg/kg).*

Effect of *Commiphora mukul*, ramipril and their combination on motor nerve conduction velocity in STZ- induced diabetic rats

Streptozotocin administration (55 mg/kg) significantly reduced motor nerve conduction velocity on 8th week as compared to the naïve group. Treatment with *Commiphora mukul* (50 and 100 mg/kg) and ramipril (0.2 and 2.3 mg/kg) significantly improved motor nerve conduction velocity of sciatic nerve as compared to control group (Figure 7). However, combination of *Commiphora mukul* (50 mg/kg) and ramipril (0.2 mg/kg) significantly improved motor nerve conduction velocity as compared to their effect per se in STZ induced diabetic rats.

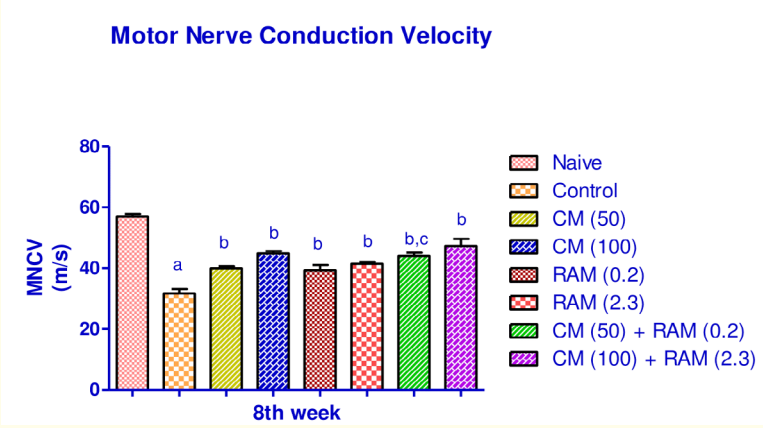

Figure 7: *Effect of Commiphora mukul, ramipril and their combination motor nerve conduction velocity in diabetic rats. MNCV: motor nerve conduction velocity.Data are expressed as mean ± S.E.M. ap<0.05 as compared to naive group; bp<0.05 as compared to control group; cp<0.05 as compared to CM (50). (One- way ANOVA followed by Bonferroni post test). CM (50): Commiphora mukul (50mg/kg); CM (100) Commiphora mukul (100mg/kg); Ram (0.2): ramipril (0.2 mg/kg); Ram (2.3): ramipril (2.3 mg/kg).*

Effect of *Commiphora mukul*, ramipril and their combination on oxidative stress in STZ induced diabetic rats

Intraperitoneal administration of streptozotocin significantly increased lipid peroxidation and depleted SOD, catalase and reduced glutathione levels in sciatic nerves and kidney as compared to the naïve group (Table 10). Treatment with *Commiphora mukul* (50 and 100 mg/kg) and ramipril (0.2 and 2.3 mg/kg) for 8 weeks significantly attenuated lipid peroxidation, and restored GSH and catalase enzyme activities in both sciatic nerve and kidney as compared to the control group. However, combination of *Commiphora mukul* (100 mg/kg) and ramipril (2.3 mg/kg) significantly caused antioxidant like effect as compared to their effect per se in STZ induced diabetic rats.

Treatment (mg/kg)	MDA (nmol of MDA/ mgpr) (% of naive)		Reduced glutathione (nmol of GSH/mgpr) (% of naive)		Superoxide dismutase (U/min/mgpr) (% of naive)		Catalase (µmol of H_2O_2) / min/mgpr) (% of naive)	
	Sciatic nerve	Kidney	Sciatic nerve	Kidney	Sciatic nerve	Kidney	Sciatic nerve	Kidney
Naive	1.3 ± 0.1 (100)	0.4 ± 0.02 (100)	30.3 ± 0.5 (100)	23.5 ± 1.3 (100)	15.51 ± 1.22 (100)	11.1 ± 0.3 (100)	4.7 ± 0.6 (100)	49.8 ± 1.4 (100)
Control	3.6 ± 0.4[a] (273.1)	0.8 ± 0.07 [a] (100)	12.5 ± 1.3[a] (41.3)	5.8 ± 0.2[a] (24.7)	2.9 ± 0.3[a] (18.6)	3.3 ± 0.1[a] (30)	0.4 ± 0.1[a] (9.5)	16.3 ± 1.4[a] (32.7)
CM (50)	1.9 ± 0.2[b] (145.5)	0.6± 0.02[b] (132)	17.9 ± 1.7 (59.1)	14.7 ± 1[b] (62.4)	5.6 ± 0.3[b] (36.2)	5.9 ± 0.7[b] (53.5)	0.7 ± 0.1 (14.8)	30.7 ± 1.4[b] (61)
CM (100)	1.5 ± 0.1[b] (116.4)	0.5 ± 0.02[b] (110)	20.8 ± 1.7[b] (68.7)	19.6 ± 1.4[b] (83.4)	6.5 ± 0.3[b] (41.9)	7.9 ± 1.0[b] (71)	1.31 ± 0.1[b] (27.8)	33.5 ± 1.8[b] (67.3)
Ram (0.2)	2.4 ± 1.4[b] (182)	0.7 ± 0.08 (154.3)	17 ± 1.4 (56.1)	13.5± 0.9[b] (57.5)	4 ± 0.5 (26.04)	4.2 ± 0.3 (37.9)	0.6 ± 0.04 (14.6)	27.7 ± 1.7[b] (55.5)
Ram (2.3)	1.5 ± 0.1[b] (115.6)	0.4 ± 0.01[b] (104.3)	23.6±1.6[b,e] (98.2)	20 ± 0.7[b,e] (98.2)	5.4 ± 0.6 (35.3)	6.8± 0.5[b,e] (61.8)	0.8 ± 0.08 (17.1)	34.8 ± 1.2[b] (69.9)
CM (50) + Ram (0.2)	1.4 ±0.1[b,e] (108)	0.5 ± 0.1 (120.8)	22.9 ± 0.6[b] (75.4)	19.7±1.1[b,c] (83.9)	6.0 ± 0.4[b] (38.6)	5.9 ± 0.6[b] (52.9)	1 ± 0.03 (21.4)	34.9 ± 1.6[b] (70)
CM (100) + Ram (2.3)	1.3 ±0.1[b,e] (99.2)	0.5 ± 0.11 (110.4)	26.2 ± 1.3[b] (86.4)	21.2 ± 1.1 [b,d] (90.2)	7.9 ± 1.1[b] (50.9)	7.1 ± 0.3[b] (64)	1.5 ± 0.1 (31.8)	40.1±1.8[b,d] (80.3)

Table 10: *Effect of Commiphora mukul, ramipril and their combination on oxidative stress parameters in sciatic nerves and kidney following STZ-induced diabetic neuropathy and nephropathy.*

Data are expressed as mean ± S.E.M. ap<0.05 as compared to naive group; bp<0.05 as compared to control group; cp<0.05 as compared to CM (50); dp<0.05 as compared to CM (100); dp<0.05 as compared to ram (0.2); ep<0.05 as compared to ram (2.3). One- way ANOVA followed by Bonferroni post test). CM (50): Commiphora mukul (50mg/kg); CM (100) Commiphora mukul (100mg/kg); Ram (0.2): ramipril (0.2 mg/kg); Ram (2.3): ramipril (2.3 mg/kg).

Effect of *Commiphora mukul*, ramipril and their combination on histopathological changes in kidney of STZ induced diabetic rats

The morphologic lesions in type 1 diabetes, predominantly affect the glomeruli, with thickening of glomerular basement membrane and mesangial expansion, although also the podocytes, renal tubules, interstitium and arterioles undergo substantial changes. Histological sections from Kidney in naïve group seemed to be normal. The glomerulus size appeared normal along with the tubules both distal and proximal tubules appeared normal. Intraperitoneal administration of streptozotocin (55 mg/kg) treatment showed cast formation in scattered tubules as compared to the naïve group. Treatment with of Commiphora mukul (50 and 100 mg/kg) attenuated the sign of cast formation as compared to control group. Ramipril (0.2 mg/kg) did not show any significant effect on glomeruloatrophy, cast formation and interstitial fibrosis as compared to control diabetic group. The combination of *Commiphora mukul* (50 and 100 mg/kg) and Ramipril (0.2 and 2.3 mg/kg) significantly decreased cast formation, glomeruloatrophy and hyalinosis as compared to their effect per se in STZ diabetic treated animals (Figure 8).

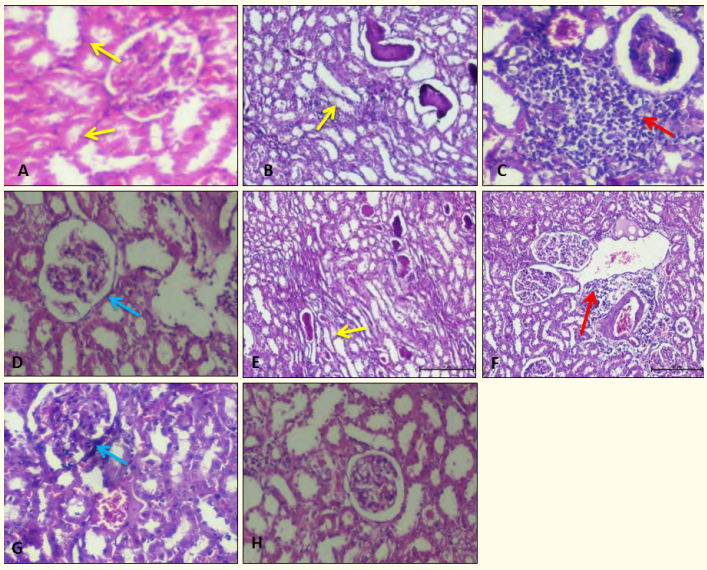

Figure 8: *Effect of Commiphora mukul, ramipril and their combination on cross sections of Kidney (Haematoxylin & Eosin staining). A: naïve; B: control; C: CM (50); D: CM (100); E: ram (0.2); F: ram (2.3); G: CM (50) + Ram (0.2); H: CM (100) + Ram (2.3). Yellow arrow indicates tubules, red arrow indicates lymphocytes cluster, and blue arrow indicates hyalinosis and glomeruloatrophy.*

Discussion

Diabetic neuropathy and nephropathy develops in eight weeks in animals with STZ as indicated by decreased motor nerve conduction velocity, decreased serum albumin, serum total proteins, glomerular filtration rate and increased serum creatinine, and blood urea nitrogen level. Along with this all signs of diabetes including decreased body weight, polyphagia, polydipsia and polyuria have also been observed. The sensory alteration i.e. allodynia and hyperalgesia is well known which develops in diabetic animals. Apart from functional and structural alteration there exist many biochemical changes like depletion of anti-oxidant enzyme and enhanced lipid peroxidation in sciatic nerve and kidney that accompanies diabetic neuropathy and nephropathy.

Hyperglycemia induced oxidative stress plays a vital role in the precipitation of both diabetic neuropathy and nephropathy and reduced glutathione directly through scavenging ROS as well as indirectly through functioning as a co-factor of anti-oxidant enzymes plays a central role in defense mechanism [22].

In the present study, STZ administration leads to the development of neuropathic pain and nephropathy. STZ administration produces symptoms of neuropathic pain like allodynia and hyperalgesia that were demonstrated by decreased paw threshold for mechanical stimuli as in mechanical allodynia, cold allodynia and mechanical hyperalgesia; and shortened paw withdrawal latency in thermal hyperalgesia. Furthermore, decreased serum albumin, serum total proteins, glomerular filtration rate and increased serum creatinine, and blood urea nitrogen level suggest the development of nephropathy. Besides, marked lipid peroxidation and decreased endogenous antioxidant levels in sciatic nerves and kidney have also been observed.

Chronic treatment with *Commiphora mukul* significantly resulted in the diminishing of behavioral, biochemical, electrophysiological and histopathological anomalies accompanied by changes in kidney histopathology and serum and urine parameters. It also leads to improvement in the diabetic potential of animals as seen by improvement in body weight, food intake, water intake and urine output. Besides, increase in the reduced glutathione level, catalase and superoxide dismutase activity in addition to decrease in the malionaldehyde level has been seen. On the other hand inhibitory effect of *Commiphora mukul* could be secondary to its anti-oxidant mechanism. Recent

studies suggested its therapeutic importance as an antihyperalgesic and antiallodynic agent in chronic constriction injury (CCI) as well as in spinal nerve injury induced neuropathic pain models [23].

Ang-II, TGF-β1 and collagen IV have been reported as major mediators that cause many pathophysiological changes in diabetic neuropathy and nephropathy. Ang-II has shown to stimulate TGF-β1 expression that directly acts on renal glomeruli [24] or indirectly by enhancing the development of proteinuria. Ramipril is a potent ACE inhibitor that possesses antioxidant activity [25]. Apart from its anti-oxidant and anti-hypertensive effect it also demonstrated anti-inflammatory activity. Furthermore, Ang-II has been shown to induce MAPK signaling pathway leading to activation of different immune and inflammatory cells; also increases expression of pro-inflammatory cytokines [26].

Besides, ramipril in a dose of 0.2 mg/kg alone partly restore the behavioral biochemical, electrophysiological and histopathological changes as compared to ramipril in a higher dose of 2.3 mg/kg and its combination with the rutin has shown significant reversal of all these changes indicating the possible interaction between two drugs in STZ-induced diabetic neuropathy and nephropathy.

STZ administration also produces histological changes in renal structure. Cast formation has been seen in histological sections accompanied by hyalinosis, glomeruloatrophy, tubulointerstitis. Treatment with *Commiphora mukul* causes reduction in the histological anomalies. However, combination of *Commiphora mukul* and ramipril has shown to ameliorate the structural and functional anomalies.

Conclusion

In conclusion, *Commiphora mukul* is a promising candidate for the management of diabetic neuropathy and nephropathy. Its combination with ramipril further potentiates its efficacy. However, further investigations are required to delineate the underlying mechanism.

Conflicts of interest

The authors have no competing financial interests to declare. There is no conflict of interest between any of the authors.

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