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RESEARCH ARTICLE

PHARMACOLOGICAL EVALUATION OF NEURO PROTECTIVE EFFECT OF FERULIC ACID IN ANIMAL MODEL OF NEUROPATHY

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Reduced glutathione.

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ABSTRACT

Aim: Pharmacological evaluation of neuro protective effect of Ferulic acid in animal model of neuropathy. ***Materials and Methods:*** Diabetes was induced in rats by intraperitoneal injection of single dose of STZ (60 mg/kg). Neuropathic pain was assessed in diabetic rats by pin prick method, cold allodynia and hot plate method. Pain was developed at 4th week. At the end of experiment animals were scarified and biochemical changes (Lipid peroxidation, SOD, reduced glutathione and CAT content) in sciatic nerve were evaluated. Animals were treated with Ferulic acid doses (50, 100 and 150 mg/kg i.p) for 4th week. ***Results:*** Treatment with Ferulic acid at doses of 50,100 and 150 mg/kg significantly restored the reduced body weight, food, water and elevated blood sugar level. Further the drug Ferulic acid showed dose dependent reduction in pain threshold tested by mechanical, cold and thermal hyperalgesia. The level of lipid peroxidation, reduced glutathione, SOD and CAT content was significantly prevented. The blood serum level on sodium, potassium, urea, uric acid and creatinine content was significant prevented. ***Conclusion:*** The result of present study suggests the antidiabetic, antioxidant and neuroprotective property of Ferulic acid in laboratory animals.

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INTRODUCTION

One of the major concerns with uncontroled diabetes is the development of microvascular complications such as neuropathy, cardiovascular, nephropathy, retinopathy and erectile dysfunction. Among these complications, symptoms of diabetic neuropathy have been observed to emerge at the early stages. These symptoms include hyperalgesia (exaggerated response to non-noxious stimuli) and allodynia (low threshold pain stimuli) have commonly been reported in diabetic patients (Bril, 2012). Streptozotocin is one of the most common complications of diabetes affecting more than 50% patient with diabetes. Streptozotocin (STZ) induced diabetic rat model has been widely used to mimics insulin-dependent diabetes mellitus and a number of abnormalities (Thiticornpong, 2011). A single does of STZ leads to the development of hyperglycemia, after three weeks pain was developed in rats which are similar to those observed in patients with painful diabetic neuropathy. Treatment of DN is always a challenging and expensive task. It beings with optimizing glycemic control first and then associated pain. As oxidative stress is an ancillary player in DN, compounds with antioxidant property can be used as supplement with the conventional treatment. Based on the above assumption the present study was de-signed (Hosseini and Mahammad, 2013).

Ferulic acid1 (FA), or 4-hydroxy-3-methoxycinnamic acid, is one of the most abundant HCAs in Nature. It was first isolated in 1866 by Hlasiwetz and Barth, from the plant Ferula foetida (Apiaceae family). Nowadays, FA is a well-known and well-studied compound, with many applications in the industry and as a phytochemical. As stated previously, ingestion of secondary metabolites through dietary intake is one of the most significant forms of human consumption of these substances (Judy *et al.,* 2003). As such, it is important to consider the distribution of FA in products present in human nourishment. FA can be found throughout the plant kingdom as a ubiquitous component of plants' tissues, particularly as a constituent of their cells' walls. Therefore, it is only natural it is widely present in foodstuffs, namely grains, fruits, and vegetables, but it can also be found in beverages such as coffee and beer (Yamaguchi *et al.,* 2006).

MATERIALS AND METHODS

Drug and Chemicals: Streptozotocin (PubChem CID-29327) (Sigma-aldrich chemical Pvt. Ltd., USA.), acetone(PubChem CID-180) (Sigma), TBA (Pubchem CID-2723628) (Sigma), TCA (PubChem-6421) (Modern Science Apparatus Pvt. Ltd.), DTNB (PubChem CID-6254) (Modern Science.), Pet ether 60-

80ºc (PubChem CID-3283) (Research lab), formalin (PubChem-712) (Research lab), Gabapentin(Sigma) (PubChem- 3446), Frulic acid (PubChem-445858) were used for study.

Experimental Animals: Wistar rats of either sex weighing around 150- 200 gm were purchased from Bombay Veterinary College, Parel. Animals were housed separately in groups of 6 per cage (Perpex) under laboratory conditions and kept in a temperature (22-24ºc) and humidity (50-60%) controlled central animal house facility with light and dark cycle of 12h each. The animals were acclimatized for at least five days before behavioral studies. All experiments were carried out during day time between 09.00 and 16.00 hours. All animals had proper access to standard food and water. Study protocol were approved by the Institutional Animal Ethics Committee (Project Approval number: MGV/PC/CPCSEA/XXXVI/ 01/2018/04), Government of India, New Delhi.

Experimental design for DN: Animals were divided into 6 groups of 6 rats (either sex) each and treated for 4 weeks.

1. **Group I** Normal control receive vehicle as saline only (10ml/kg)
2. **Group II** Rats treated with STZ (55-60 mg/kg; i.p.) twice a weekly for 4 week.
3. **Group III** Neuropathic rats treated with Ferulic acid (50 mg/kg,i.p.) once every day for 4 week.
4. **Group IV** Neuropathic rats treated with Ferulic acid (100mg/kg,i.p.) once every day for 4 week.
5. **Group V** Neuropathic rats treated with Ferulic acid (150mg/kg ,i.p.) once every day for 4 week.
6. **Group IV** Neuropathic rats treated with Standard Drug Gabapentin (30mg/kg,p.o) once every day for 4 week.

Assessment of neuropathic pain

Morphological Study: Body weight, water intake and food intake was monitored once in a week.

Behavioral Study

Mechanical Hyperalgesia (Von Frey Test): The mechanical hyperalgesia was assessed by the von frey test (Aesthesio, DanMic Global, LLC). Rats were individually placed in suspended acrylic chamber on mesh floor. After the acclimatization period for 30 min, planter surface of hind paw was tested with von frey hair. The latency of paw withdrawal was recorded. The paw withdrawal time was measured weekly after conformation of neuropathic pain (Veves *et al.,* 2008).

Hot Plate Method: The nociceptive threshold for heat is an index for thermal hyperalgesia (Orchid make). The plate was preheated and maintained at a temperature of 52.5±2ºC. Rat was placed on the hot plate and nociceptive threshold with respect to licking of the hind paw or jumping will be recorded in seconds. The onset for licking and jumping response were recorded. The cut off time 20sec was maintained (Kumar *et al.,* 2016).

Acetone Drop Method: Cold chemical thermal sensitivity was assessed using acetone drop method. Rats were placed in metal mesh cage and allowed to habituate for approximately 20 min. acetone drop (50µ) was applied gently on to the mid planter surface of the hind paw. It generates a cold chemical sensitivity reaction that is paw licking, shaking or rubbing the hind paw with brisk foot withdrawal after application (2-5 sec) of acetone which was considered as anti-nociceptive effect (Naik *et al.,* 2006).

Rota Rod Test: This test was conducted using rota rod apparatus by placing rats on 15 rpm rotating spindle. The fall off time of each rat form rotating spindle was recorded during 5 min period (Karki *et al.,* 2014).

Blood Glucose Levels: Rats were in restrainers; their tail was cleaned with warm soap solution using cotton. Blood samples were withdrawn from tail vein under mild isoflurane anesthesia. Blood glucose levels were monitored after 72 hrs of induction of diabetes by Streptozotocin (60 mg/kg, i.p.) Blood glucose levels were monitored by using glucometer (Accusure kit) to confirm hyperglycemia. Hyperglycemic rats (glucose level >200 mg/dl) were separated and selected for study. Blood glucose levels were also measured at the end of 9 weeks of treatment in all groups (Later and Sawyer, 1971).

Antioxidant Study

Reduced Gluthione (RGSH): 1ml homogenate (liver) was added to 1ml of 10% trichloroacetic acid, followed by centrifugation. 1ml supernatant was collected and 5ml Ellman's reagent in 100ml of 1% sodium citrate and 3ml of phosphate buffer (pH-8) was added. Change in colour was noted. Absorbance was taken at 412nm and result was expressed as nM/mg of wet tissue of RGSH activity (Moron *et al.,* 1979).

Lipid Peroxidation (LOP): It was estimated using the method described by Slater and Sawyer (1971). 2.0ml of the tissue homogenate (supernatant) was added to 2.0ml of freshly prepared 10% w/v trichloroacetic acid (TCA) and the mixture was allowed to stand in an ice bath for 15 minutes. After 15minutes, the precipitate was separated by centrifugation and 2.0ml of clear supernatant solution was mixed with 2.0ml of freshly prepared thiobarbituric acid (TBA). The resulting solution was heated in a boiling water bath for 10 minutes. It was then immediately cooled in an ice bath for 5 minutes. The colour developed was measured at 532 nm against reagent blank. Different concentration (0-23nM) of standard malondialdehyde was taken processed as above for standard graph. The values were expressed as nM of MDA/mg protein (Misera and Fridovich, 1972).

Superoxide dismutase (SOD)

Superoxide dismutase was estimated using the method developed by Misra and Fridovich (1972). 0.5ml of tissue homogenate was diluted with 0.5ml of distilled water, to which 0.25ml of ice-cold ethanol and 0.15ml of ice-cold chloroform was added. The mixture was mixed well using cyclo mixer for 5 minutes and centrifuged at 2500 rpm. To 0.5ml of supernatant, 1.5ml of carbonate buffer and 0.5ml of EDTA solution were added. The reaction was initiated by the addition of 0.4ml of epinephrine and the change in optical density/minute was measured at 480nm against reagent blank. SOD activity was expressed as units/mg protein. Change in optical density per minute at 50% inhibition of epinephrine to adrenochrome transition by the enzyme is taken as the enzyme

unit. Calibration curve was prepared by using 10-125 units of SOD (Guevara *et al.,* 1988).

Catalase (CAT): It was estimated by the method of Hugo Aebi as given by Colowick et al. To 2ml of diluted sample 1ml of hydrogen peroxide (30 mmol/l) was added to initiate the reaction. The blank was prepared by mixing 2ml of diluted sample with 1ml of phosphate buffer (50mmol/l; pH 7.0). The dilution should be such that the initial absorbance should be approximately 0.500. The decrease in absorbance was measured at 240nm. Catalase activity was expressed as µmoles of H2O2 consumed/min/mg protein (Aebi, 1984).

Assessment of Biochemical parameters

Removal and Processing of Serum and Tissues for Various Estimations: At the end of the treatment period, blood was collected from the retro-orbital plexus without any anti-coagulant and allowed to clot for 10 minutes at room temperature. It was then centrifuged at 2500 rpm for 20 minutes. The Serum Sample is used for ion estimation like a Na^+, K, Urea, Uric acid and Creatinine (Fukushima *et al.,* 2006).

Histopathlogical Analysis of Sciatic Nerve: Histopathological study was done at Histopathological lab (Dr. Vasantrao Pawar Medical College Hospital & res., Nashik). The left area was shaved and then prepared for surgery. The sciatic nerve was exposed through skin incision and gluteal muscle splitting. Then sciatic nerve was cut into 4 µmm thickness and then kept in the 10% formalin (fixation solution). Staining was done by using hematoxyline and eosin. Then cross sections were observed using light microscope (100×) for axonal degeneration and vascular defects (Vincent *et al.,* 2004).

Statistical Analysis

Data was analyzed using PRIMER statistical software and expressed as Mean±SEM. Statistical analysis was done using One-way ANOVA, followed by Dunnett's test. *P value<0.05 was considered statistically significant.

RESULT

Morphological test

Body weight - After second week of induction of diabetes, there was significant (P<0.05) decrease in body weight of diabetic control rats as compared to normal control rats. Diabetic rats treated with standard Gabapentin (30 mg/kg) and diabetic rats treated with ferulic acid (50,100 and 150 mg/kg) showed significant improvement in body weight as compared to diabetic control rats in the 4th week of treatment schedule.

Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on body weight in Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observation are mean±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared to diabetic control group.

Food Intake - After second week of induction of diabetes, there was significant (P<0.05) decrease in food intake of diabetic control rats as compared to normal control rats. Diabetic rats treated with standard Gabapentin (30 mg/kg) and diabetic rats treated with ferulic acid (50,100 and 150 mg/kg) showed significant improvement in body weight as compared to diabetic control rats in the 4th week of treatment schedule. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on food intake in Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observation are mean±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared to diabetic control group.

Water Intake - After second week of induction of diabetes, there was significant (P<0.05) decrease in water intake of diabetic control rats as compared to normal control rats. Diabetic rats treated with standard Gabapentin (30 mg/kg) and diabetic rats treated with ferulic acid (50,100 and 150 mg/kg) showed significant improvement in body weight as compared to diabetic control rats in the 4th week of treatment schedule. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on water intake in Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observation are mean ±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared to diabetic control group.

Behavioral Test

Von Frey test – Paw withdrawal threshold of STZ diabetic control rats was significantly decreased (P<0.05) as compared to normal control rats. Ferulic acid administration (150 mg/kg) result in a significant increase (P<0.05) in paw withdrawal threshold as compared to STZ diabetic control rats. Rats treated with Gabapentin (30 mg/kg) also showed the significant increase (P<0.05) in the mean paw withdrawal threshold as compared to STZ diabetic control rat. However, this inhibition in decrease in the mean paw withdrawal threshold by GBA (30 mg/kg) treatment was more significant (P<0.05) than ferulic acid (50,100 and 150 mg/kg) treatment. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on Paw withdrawal threshold assessed by the Von Frey test in Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observation are mean ±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared to diabetic control group.

Hot Plate Method- Heat hyperalgesia in diabetic rats was observed at the 3rd week of induction of diabetes. Heat hyperalgesia was indicated by significant reduction in paw withdrawal latency as compared to normal rats. Diabetes rats treated with Gabapentin (30 mg/kg) and ferulic acid (50,100 and 150 mg/kg) showed significant (P<0.05) improvement in paw withdrawal latency at 4th week of treatment schedule as compared to diabetic control rats. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on heat hyperalgesia assessed by the hot plate method test in Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observation are mean±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared to diabetic control group.

Acetone drop method – In these test, acetone drop applied to planter surface of hind paw of diabetic rats in cold allodyania, it was indicated by decrease in reaction time as compared to normal rats.

Animal treated with standard Gabapentin (30 mg/kg) showed significant (P<0.05) decrease in reaction time at 3rd and 4th of treatment schedule as compared to diabetic control rat. While animals treated with ferulic acid (150 mg/kg) showed significant (P<0.05) decrease in reaction time at 3rd and 4th week of treatment schedule as compared to diabetic controls rats. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on cold allodyania assessed by the acetone drop test in Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observation are mean ±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared to diabetic control group.

Rota rod test - diabetic rats showed motor in coordination as indicated by significant (P<0.05) decrease in fall time using rota rod apparatus. Diabetic rats treated with Gabapentin (30 mg/kg) and ferulic acid (50,100 and 150 mg/kg) showed significant (P<0.05) improvement in motor coordition as indicated by decrease in fall off time as compared to diabetic control rats after 2nd week of treatment schedule as compared to diabetic control rats. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on motor coordination assessed by the rota rod test in Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observation are mean±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared to diabetic control group.

Blood Glucose Level – After treatment glucose levels significantly (P<0.05) decrease in diabetic rats treated with Gabapentin (30 mg/kg) and diabetic rats treated with ferulic acid (150 mg/kg) as compared to diabetic control rats. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on Blood glucose level of Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observation are mean± SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared to diabetic control group.

Relative organ weight – There was significant (P<0.05) decrease in relative organ weight of liver of animals of diabetic control rats as compared with normal control rats, and significantly (P<0.05) increase in relative organ weight of liver in diabetic rats, treated with standard Gabapentin (30 mg/kg) and in diabetic rats treated with ferulic acid (50,100 and 150 mg/kg) as compared to diabetic control rats. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on relative organ weight of Streptozotocin induced diabetic rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observation are mean ±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared to diabetic control group.

Antioxidant Studies – In –vivo antioxidant studies like RGSH, LPO, CAT, SOD level were estimated by performing various standard procedures.

Reduced glutathione – RGSH is primary antioxidant in the cell. Significant (P<0.05) decrease in the amount of RGSH was observed in diabetic control group compared with normal control group, while in diabetic rats treated with Gabapentin and ferulic acid (50,100 and 150 mg/kg) showed significant increase in RGSH level as compared with diabetic control group.

Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on reduces glutathione in liver homogenate of Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observation are mean ±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared to diabetic control group.

LPO – LPO level in liver homogenate of STZ treated diabetic control rats was significant (P<0.05) elevated as compared to normal control rats. LPO level in ferulic acid treated rats at different dose (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) was significantly (P<0.05) decreased as compared to diabetic neuropathy rats. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on Lipid peroxidation in liver homogenate of Streptozotocin induced neuropathy in rats.

N=6, all data were subjected to ANOVA followed by Dunnett's test, the observation are mean ±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared to diabetic control group.

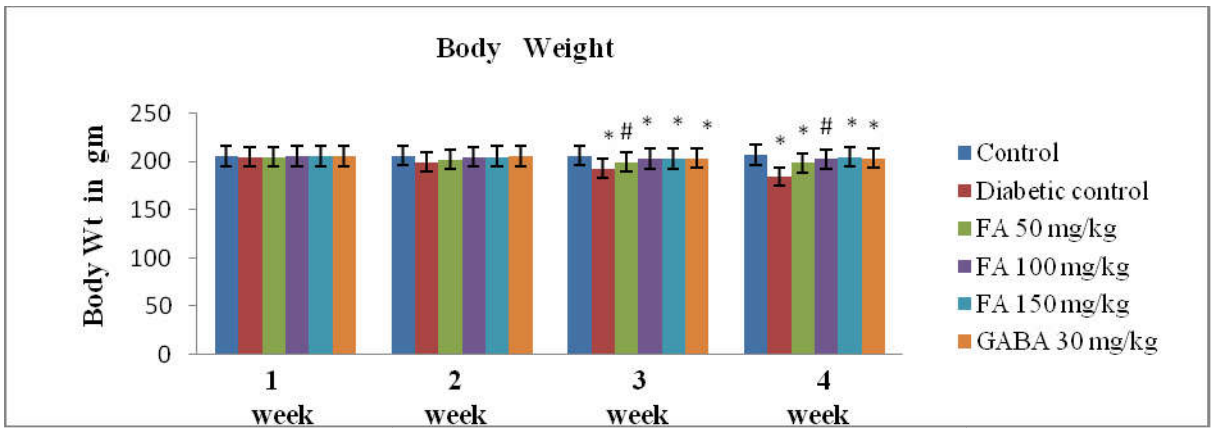
Superoxide dismutase – SOD in liver homogenate of STZ treated diabetic rats was significantly (P<0.05) reduced as compared to normal control rats. Treatment with ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) showed significant (P<0.05) increase in the activity of SOD as compared to STZ induced diabetic neuropathy rats. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on Superoxide dismutase in liver homogenate of Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observation are mean ±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared to diabetic control group.

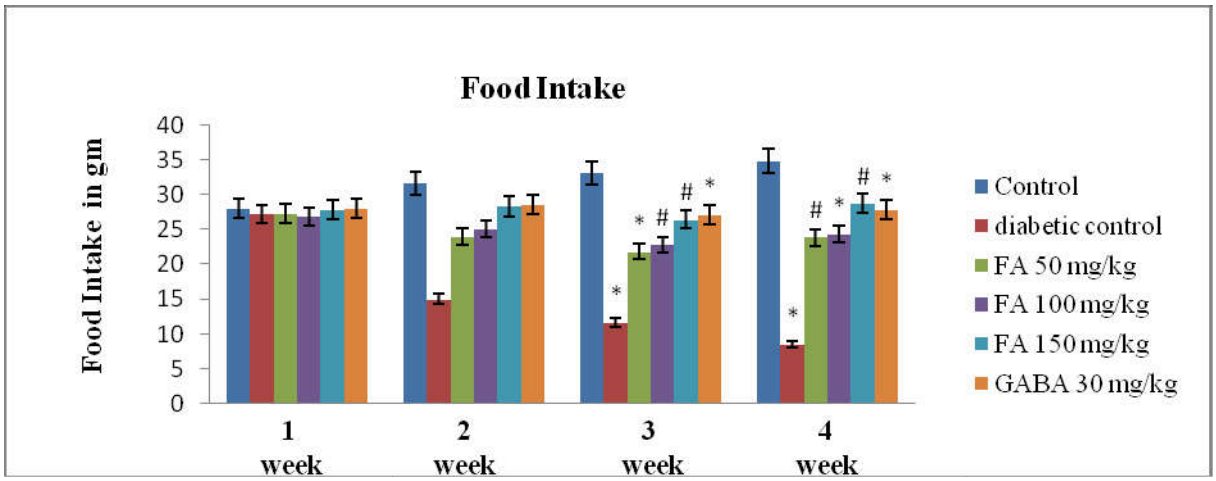
Catalase – CAT in liver homogenate of STZ treated diabetic rats was significantly (P<0.05) reduced as compared to normal control rats. Treatment with ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) showed significant (P<0.05) increase in the activity of CAT as compared to STZ induced diabetic neuropathy rats. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on Catalase in liver homogenate of Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observation are mean ±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared to diabetic control group.

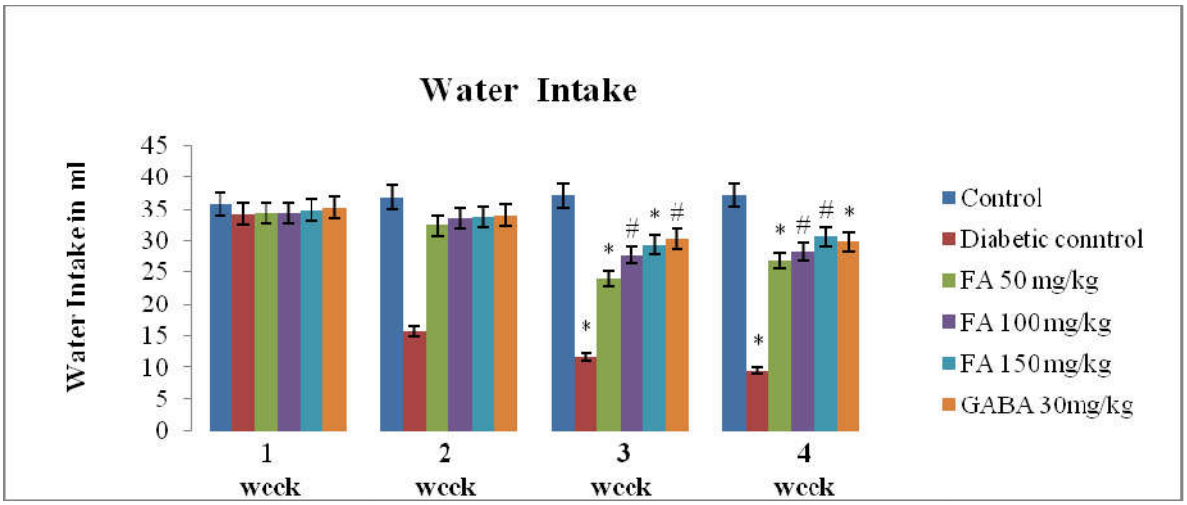
Blood serum estimation

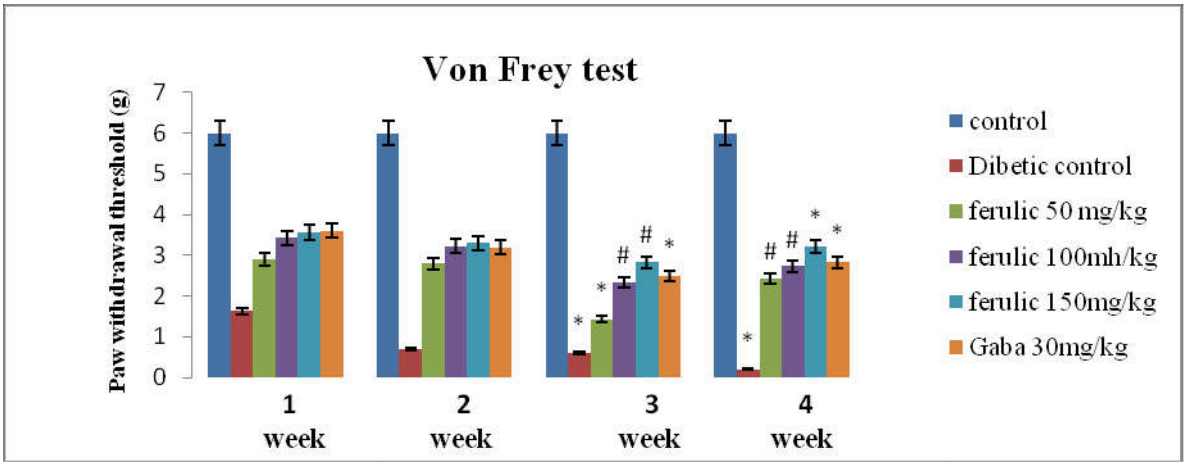
Ion Estimation

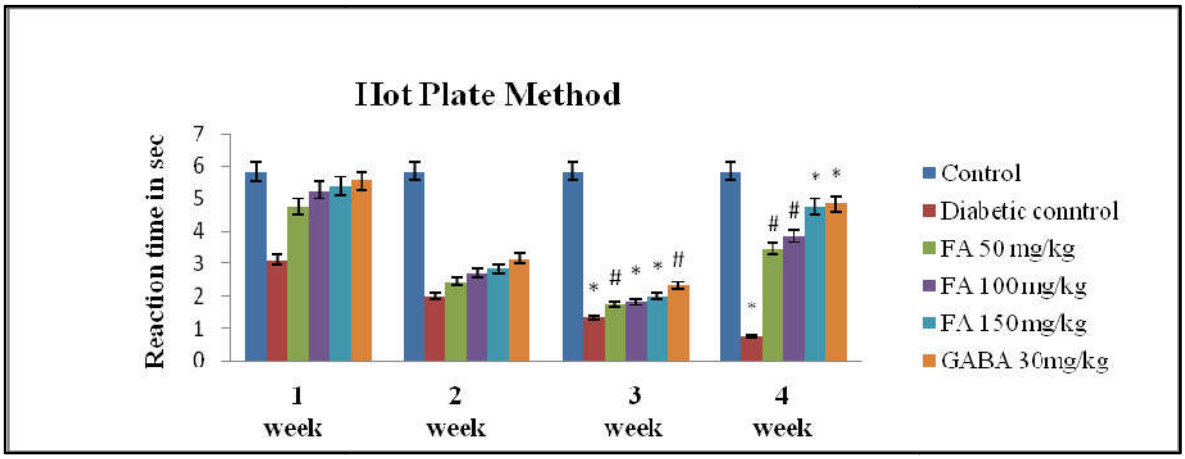
Potassium- Diabetic neuropathy rats for potassium was decreased (P<0.05) when compared with control. Ferulic acid (50,100 and 150 mg/kg) pretreated rats showed slightly decreased potassium level (P<0.05) when compared with diabetic control while changes were observed in alone Gabapentin (30 mg/kg) treated rats. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on Blood serum level in potassium of Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean ±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared diabetic control group.

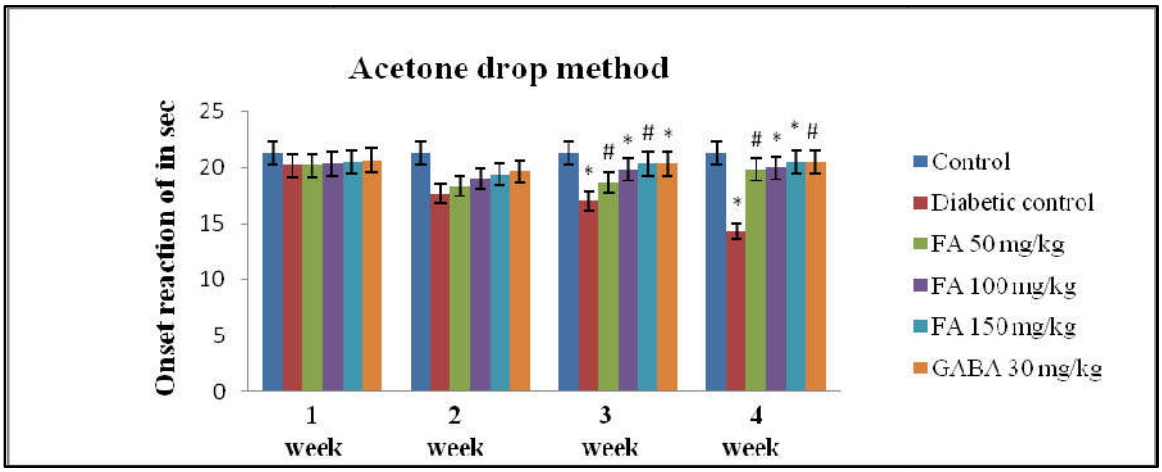

Body Weight
Body Wt in gm
250
200
150
100
50
0
1 week
2 week
3 week
4 week
Control
Diabetic control
FA 50 mg/kg
FA 100 mg/kg
FA 150 mg/kg
GABA 30 mg/kg

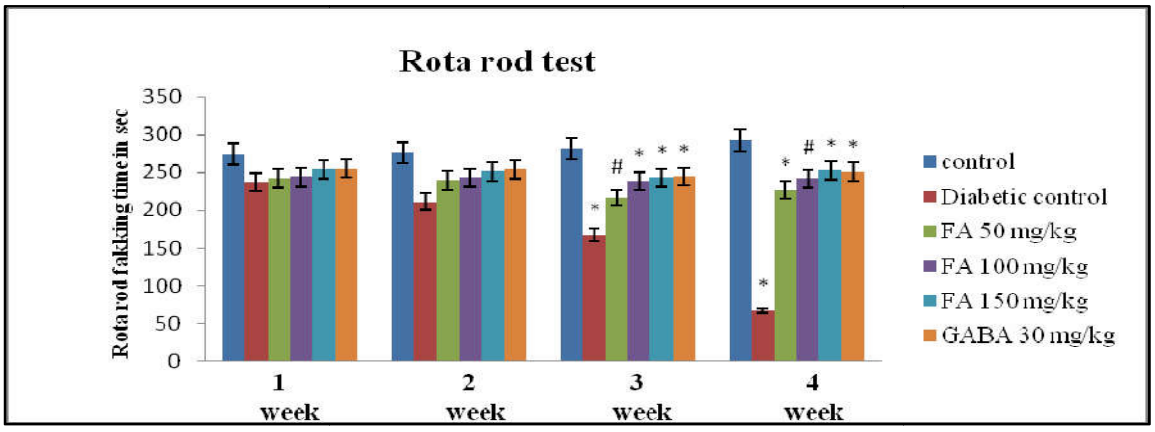

Food Intake
Food Intake in gm
40
35
30
25
20
15
10
5
0
1 week
2 week
3 week
4 week
Control
diabetic control
FA 50 mg/kg
FA 100 mg/kg
FA 150 mg/kg
GABA 30 mg/kg

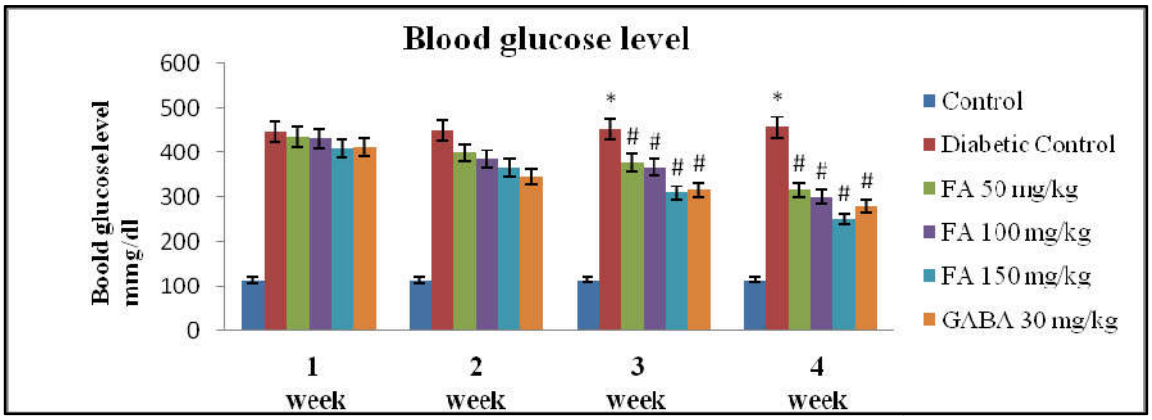

Water Intake
Water Intake in ml
45
40
35
30
25
20
15
10
5
0
1 week
2 week
3 week
4 week
Control
Diabetic conntrol
FA 50 mg/kg
FA 100 mg/kg
FA 150 mg/kg
GABA 30mg/kg

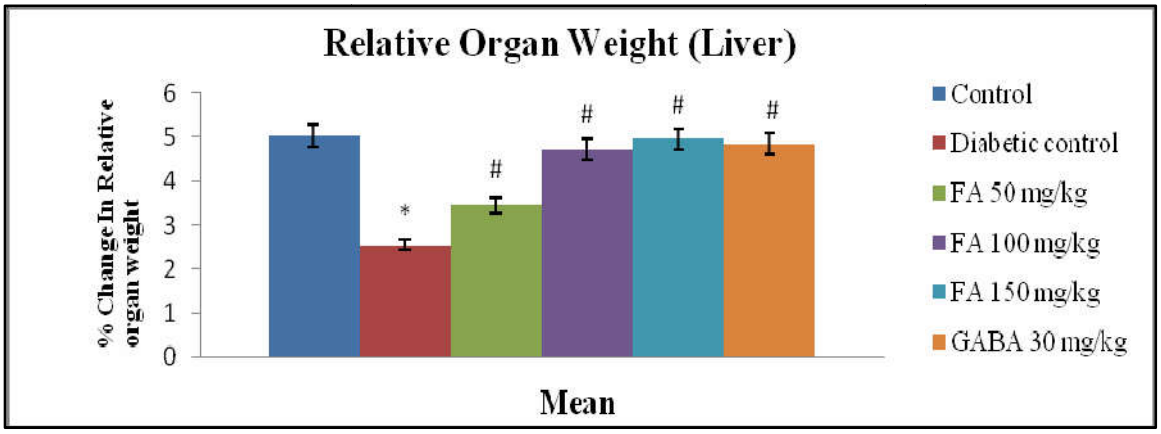

Von Frey test
Paw withdrawal threshold (g)
7
6
5
4
3
2
1
0
1 week
2 week
3 week
4 week
control
Dibetic control
ferulic 50 mg/kg
ferulic 100mh/kg
ferulic 150mg/kg
Gaba 30mg/kg

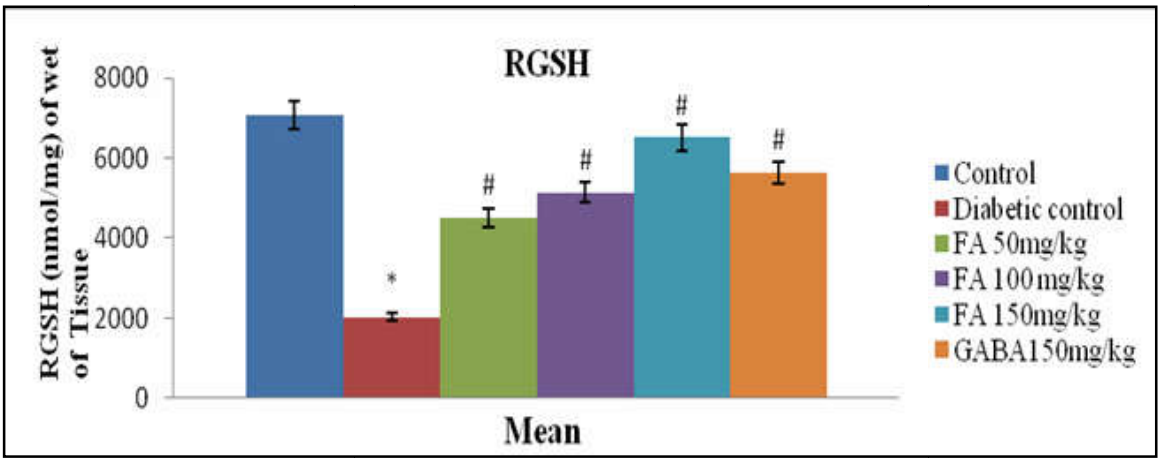

IIot Plate Method
Reaction time in sec
0
1
2
3
4
5
6
7
1 week
2 week
3 week
4 week
Control
Diabetic conntrol
FA 50 mg/kg
FA 100mg/kg
FA 150mg/kg
GABA 30mg/kg

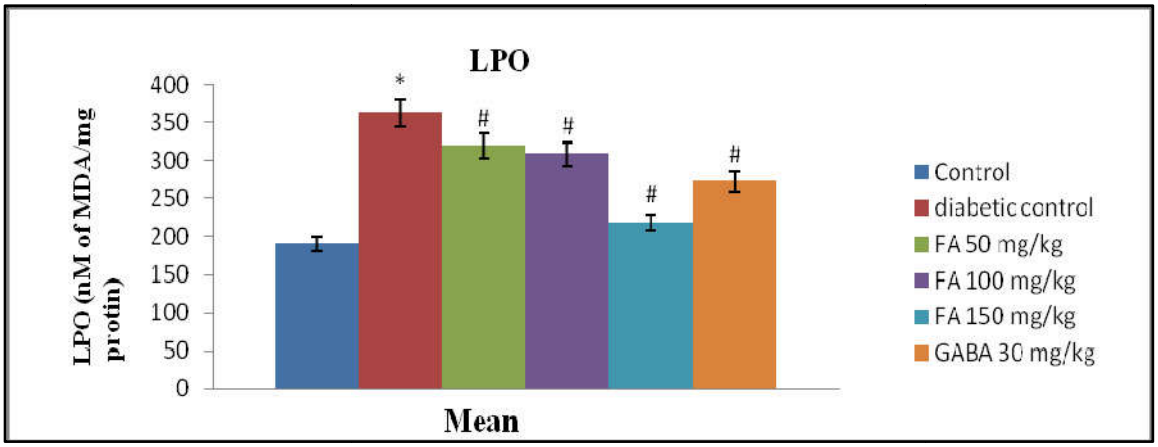

Acetone drop method
Onset reaction of in sec
0
5
10
15
20
25
1 week
2 week
3 week
4 week
Control
Diabetic control
FA 50 mg/kg
FA 100 mg/kg
FA 150 mg/kg
GABA 30 mg/kg

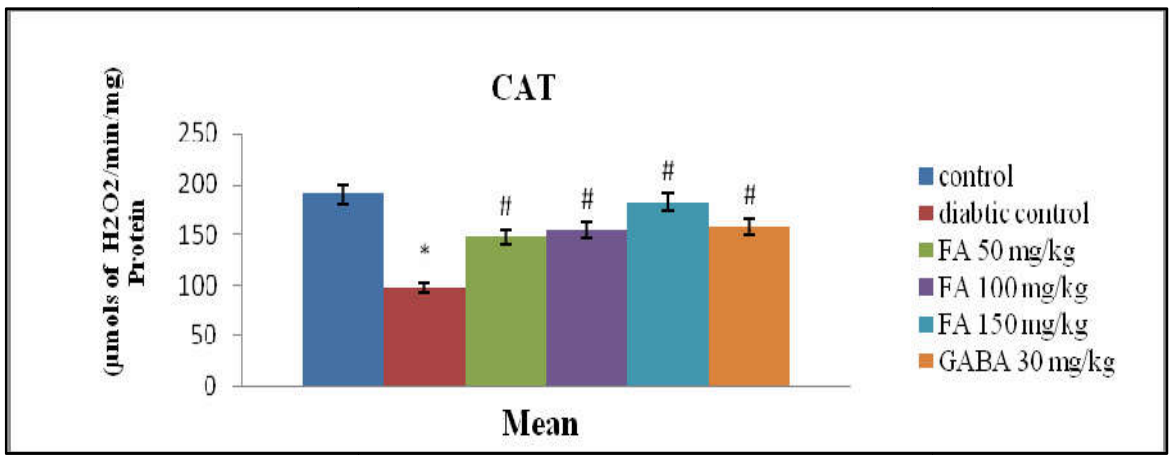

Rota rod test
Rota rod fakking time in sec
0
50
100
150
200
250
300
350
1 week
2 week
3 week
4 week
control
Diabetic control
FA 50 mg/kg
FA 100 mg/kg
FA 150 mg/kg
GABA 30 mg/kg

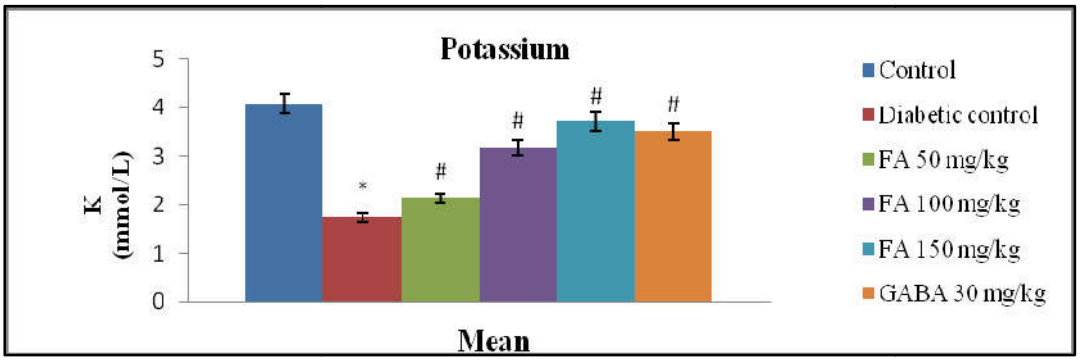

Blood glucose level
Boold glucose level mmg/dl
0
100
200
300
400
500
600
1 week
2 week
3 week
4 week
Control
Diabetic Control
FA 50 mg/kg
FA 100 mg/kg
FA 150 mg/kg
GABA 30 mg/kg

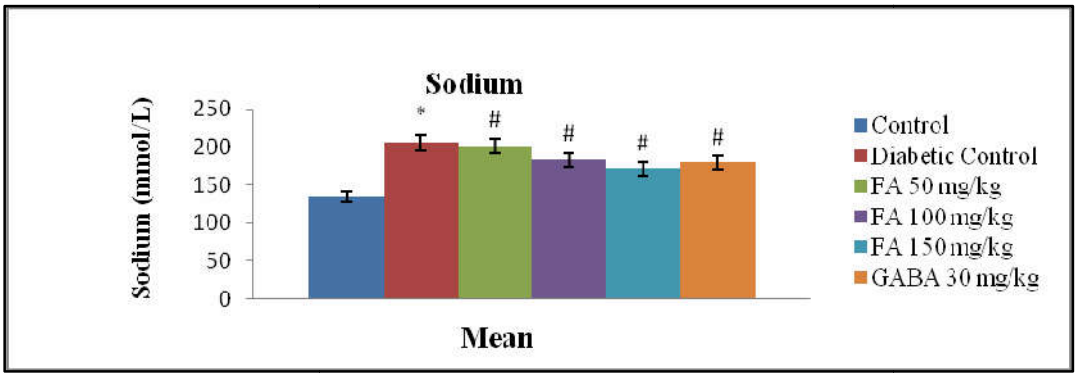

Relative Organ Weight (Liver)
% Change In Relative organ weight
6
5
4
3
2
1
0
*
#
#
#
#
Control
Diabetic control
FA 50 mg/kg
FA 100 mg/kg
FA 150 mg/kg
GABA 30 mg/kg
Mean

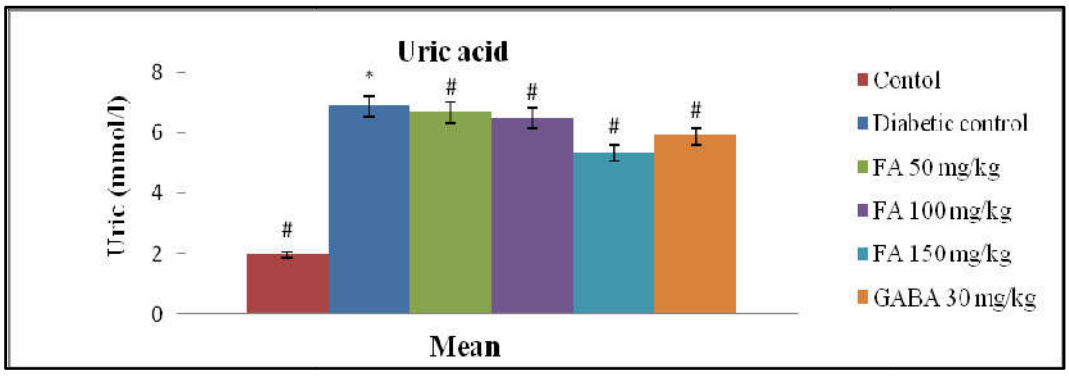

RGSH
RGSH (nmol/mg) of wet of Tissue
8000
6000
4000
2000
0
*
#
#
#
#
#
Control
Diabetic control
FA 50mg/kg
FA 100mg/kg
FA 150mg/kg
GABA150mg/kg
Mean

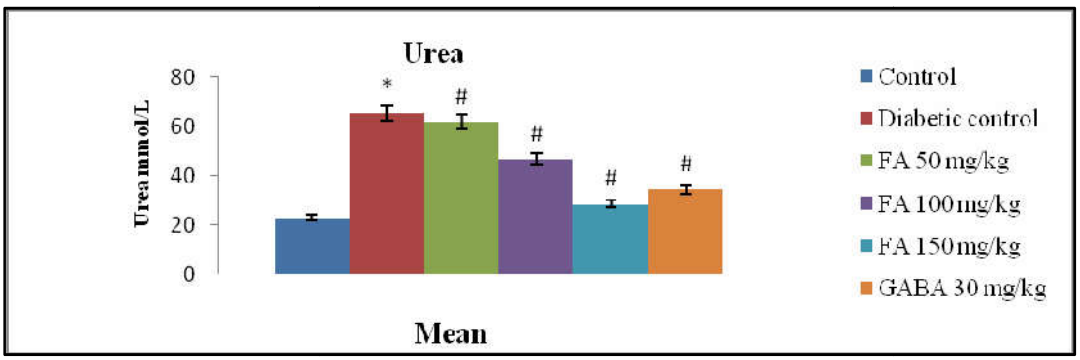

LPO
LPO (nM of MDA/mg protin)
400
350
300
250
200
150
100
50
0
*
#
#
#
#
Control
diabetic control
FA 50 mg/kg
FA 100 mg/kg
FA 150 mg/kg
GABA 30 mg/kg
Mean


CAT
(µmols of H2O2/min/mg) Protein
250
200
150
100
50
0
*
#
#
#
#
control
diabtic control
FA 50 mg/kg
FA 100 mg/kg
FA 150 mg/kg
GABA 30 mg/kg
Mean


Potassium
K (mmol/L)
5
4
3
2
1
0
*
#
#
#
#
Mean
Control
Diabetic control
FA 50 mg/kg
FA 100 mg/kg
FA 150 mg/kg
GABA 30 mg/kg


Sodium
Sodium (mmol/L)
250
200
150
100
50
0
*
#
#
#
#
Mean
Control
Diabetic Control
FA 50 mg/kg
FA 100 mg/kg
FA 150 mg/kg
GABA 30 mg/kg


Uric acid
Uric (mmol/l)
8
6
4
2
0
*
#
#
#
#
#
Mean
Contol
Diabetic control
FA 50 mg/kg
FA 100 mg/kg
FA 150 mg/kg
GABA 30 mg/kg


Urea
Urea mmol/L
80
60
40
20
0
*
#
#
#
#
Mean
Control
Diabetic control
FA 50 mg/kg
FA 100 mg/kg
FA 150 mg/kg
GABA 30 mg/kg

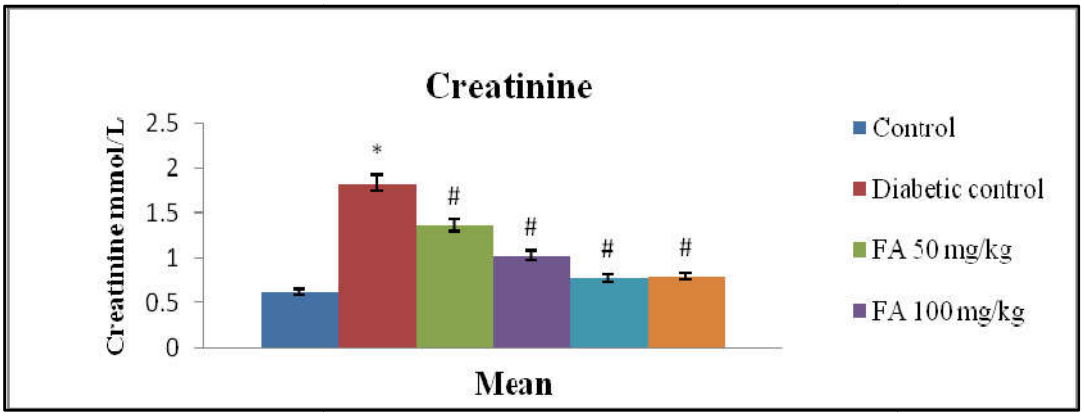
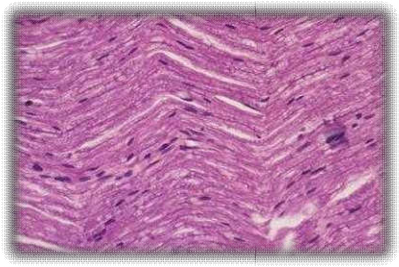
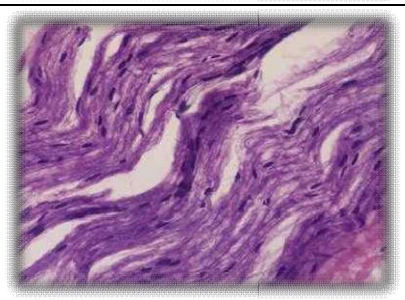
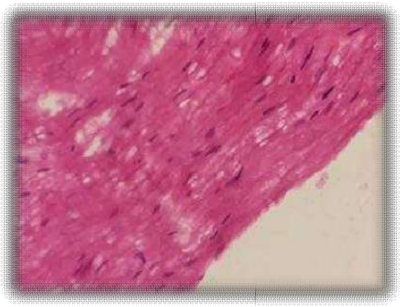
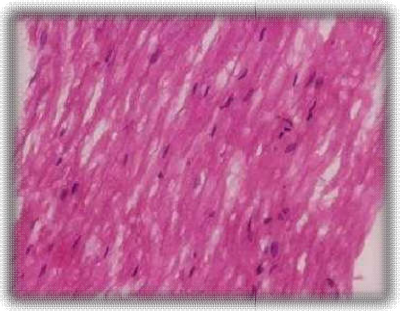
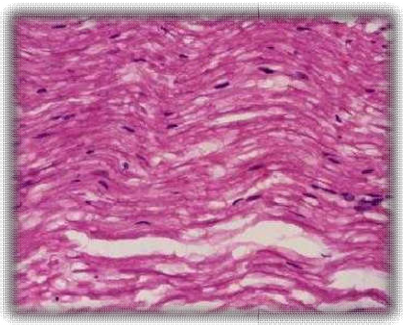
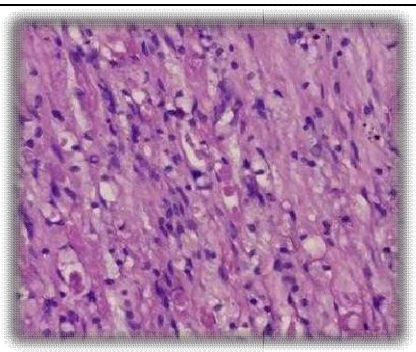

Creatinine
Creatinine mmol/L
2.5
2
1.5
1
0.5
0
*
#
#
#
#
Mean
Control
Diabetic control
FA 50 mg/kg
FA 100 mg/kg

Table 1. Histopathological examination of sciatic nerve

Treatment	Image
1] Control Section of H& E stained sciatic nerve of normal rat's showing normal sciatic nerve fibers and outer membrane blood vessel.	
2] Diabetic control Section of H& E stained sciatic nerve of diabetic control control rats showed edema around the epineurium and infiltration of neutrophils around the blood vessel and swelling of never fibers.	
3] Group (1) - 50 mg/kg Ferulic acid Section of H& E stained sciatic nerve of diabetic rats treated with ferulic acid (50 mg/kg) showed accumulation of macrophages and monocytes around the Schwann cells.	
4] Group (2) - 100 mg/kg Ferulic acid Section of H& E stained sciatic nerve of diabetic rats treated with ferulic acid (100 mg/kg) showed mild edema around the epineurium and few infiltrating neurofiles around the blood vessels and minor swelling of nerve fibers.	
5] Group (3) - 150 mg/kg Ferulic acid Section of H& E stained sciatic nerve of diabetic rats treated with ferulic acid (150 mg/kg) showed mild edema around the epineurium and few infiltrating neurofiles around the blood vessels and minor swelling of nerve fibers.	
6] Group (4) - 30 mg/kg GABA Section of H&E stained sciatic nerve of Cisplatin rats treated with Gabapentin (30 mg/kg) showed swelling of never fibers and demylination of nerve fiber.	

Sodium- Diabetic induced neuropathy rats for sodium levels were increased in diabetic treated rats (P<0.05). Ferulic acid (50,100 and 150 mg/kg) treated rats showed decreased level when compared with diabetic control (P<0.05) while change were observed in Gabapentin pretreated rats. When compared with diabetic control. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on Blood serum level in Sodium of Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean ±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared diabetic control group.

Uric Acid- Diabetic induced neuropathy rats for uric acid levels were increased in diabetic treated rats (P<0.05). Ferulic acid (50,100 and 150 mg/kg) treated rats showed decreased level when compared with diabetic control (P<0.05) while change were observed in Gabapentin pretreated rats when compared with diabetic control. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on Blood serum level in uric acid of Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared diabetic control group.

Urea- Diabetic induced neuropathy rats for urea levels were increased in diabetic treated rats (P<0.05). Ferulic acid (50,100 and 150 mg/kg) treated rats showed decreased level when compared with diabetic control (P<0.05) while change were observed in Gabapentin pretreated rats when compared with diabetic control. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on Blood serum level in urea of Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared diabetic control group.

Creatinine - Diabetic induced neuropathy rats for Creatinine levels were increased in diabetic treated rats (P<0.05). Ferulic acid (50,100 and 150 mg/kg) treated rats showed decreased level when compared with diabetic control (P<0.05) while change were observed in Gabapentin pretreated rats when compared with diabetic control. Effect of ferulic acid (50,100 and 150 mg/kg) and Gabapentin (30 mg/kg) on Blood serum level in Creatinine of Streptozotocin induced neuropathy in rats. N=6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. *P<0.05 as compared to normal control group and # P<0.05 as compared diabetic control group.

The sciatic nerve of diabetic rats developed severe pathological changes as compared with the groups treated with Gabapentin and Ferulic acid (50,100 and 150 mg/kg). Diabetic control groups showed edema round the epineurium and infiltration of neutrophils around the blood vessels and showed swelling of nerve fibres. Diabetic rats treated with Gabapentin (30 mg/kg) showed swelling of nerve fibers and demylination of nerve fibers. Macrophases and monocytes were observed around the Schwann cells of diabetic rats treated with ferrulic acid (50 and 100 mg/kg),while diabetic rats treated with ferulic acid (150 mg/kg) showed mild edema around the epineurium, few infiltrating neutrophils around the blood vessels and only minor swelling of nerve fibers.

DISCUSSION

The present study shows the effect of Ferulic acid on diabetic neuropathy in Streptozotocin induced diabetic rats as assessed by its morphological, behavioural, biochemical, blood serum test and Histopathological parameters. A strong relationship exists between glycemia and diabetic microvascular complications in both Type-1 and Type-2diabets. Generation of superoxide due to oxidative stress in diabetes may be responsible for vascular and neuronal complications of painful neuropathy. Early in the course of diabetes, intracellular hyperglycemia causes abnormalities in blood flow and increased vascular permeability (Kandhare *et al.,* 2011). Quantitative abnormalities of extracellular matrix contribute to an irreversible increase in vascular permeability. With time Hyperglycemia may also decrease production of factor for endothelial and neuronal cell death. Together, these changes lead to odema, ischema and hypoxia induced neovacularization in the retina, protein urea, messengial matrix expansion, glomerulosclerosis in the kidney and multifocal axoal degeneration in peripheral nerves. Oxidative stress related reduction in perfusion is thought to play role in cardiac autonomic dysfunction and also in small fiber sensory neuropathy. Generation of superoxide due to oxidative stress in diabetes may be responsible for vascular and neuronal and neuronal complication of painful neuropathy (Negi *et al.,* 2010). In the present study ferulic acid (FA), 4-hydroxy-3-methoxycinamic acid is an antioxidant which neutralizes free radicals (superoxide, nitric oxide and hydroxyl radical) which could cause oxidative damage to cell membrane and DNA. Exhibits good antidiabetic, antioxidant activity when administered at a dose 150 mg/kg i.p. At this high doses of Ferulic acid (150 mg/kg), shows a similar effect as that of Gabapentin (30 mg/kg, oral antiepileptic agent) (Anjaneyulu and Chopra, 2004). The development of diabetes was observed after 72 hrs of induction of diabetes by Streptozotocin (55-60 mg/kg). The development of neuropathy was observed after 2nd week of induction of diabetes. Regulation of blood glucose level in diabetic patient can prevent the various complication associated with the diseases. Maintenance blood glucose level for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species (Mahesh and Menon, 2004). The morphological study such as body weight, food intake and water intake was assessed once in a week Diabetic rats treated with Gabapentin (30 mg/kg) and Ferulic acid (50,100 and 150 mg/kg) showed significant improvement in body weight as compared to diabetic control rats in the 3rd and 4th week of treatment schedule. Food intake and Water intake was decreased in diabetic control rats as compared with normal control, Gabapentin and Ferulic acid treated STZ induced diabetic rats (Sharma *et al.,* 2006). The behavioural parameters such as mechanical hyperalgesia, hot plate method, cold allodyania in acetone drop test and motor coordination are rota rod test respectively. Behavioral tests, STZ induced diabetic rats showed significant reduction in paw withdrawal latencies in tests for the mechanical hyperalgesia was assessed by the von frey test and hot plate method respectively. A significant decreased in reaction time for acetone drop method and significant decreased in fall off time for rata rod test was observed in diabetic rats as compared to normal control (Klein *et al.,* 2007). In case of cold allodyania assessed by acetone drop test, diabetic rats treated with Gabapentin (30 mg/kg) showed significant decreased in reaction time at week diabetic 3rd and 4th week of diabetic treated rats. Diabetic in treated

with ferulic acid (100 and 150 mg/kg) showed significant decrease in reaction time 3rd ad 4th week of treatment schedule. As evident from the results of the present study, Diabetic are 4th week treatment week Ferulic acid (150 mg/kg) improved cold allodyania and thermal hyperalgesia in experiment animal (Park *et al.,* 2011). Motor in coordination assessed by using rota rod test. Diabetic rats treated with Gabapentin (30 mg/kg) and Ferulic acid (50, 100 and150 mg/kg) showed significant improvement in motor coordination as indicated by decreased in fall off time as compared to diabetic control rats after 2nd week of treatment schedule as compared to diabetic control rats, thus treatment with Ferulic acid, prevent motor in coordination in diabetic rats. Heat hyperalgesia assed by hot plate method, diabetic rats treated with Gabapentin (30 mg/kg) and Ferulic acid (150 mg/kg) showed significant improvement in paw withdrawal latency at 3rd, 4th week of treatment schedule as compared to Diabetic control rat (Muller *et al.,* 2008). After treatment, blood glucose levels significantly decrease in diabetic rats treated with Gabapentin (30mg/kg) and diabetic rats treated with Ferulic acid (150mg/kg) as compared to diabetic control rats. As evident from the result of the present study, four week treatment with ferulic acid (150mg/kg) improved hyperalgesia in experimental animals. There was significant decrease in relative organ weight of liver of animal of diabetic control rats compared with normal control rats, and significant increase in relative organ weight of liver in diabetic rats treated with standard Gabapentin (30 mg/kg) and diabetic rats treated with Ferulic acid (50 mg/kg) as compared to diabetic control rats.

The purpose of relative organ weight analysis is to detect any indirect effects caused by the treatment on body weight (Saha *et al.,* 2009). Glutathione (GSH) is highly abundant in all cell compartments and is the major soluble antioxidant. Reduced GSH/Oxidized GSH ratio is a major determinant of oxidative stress. GSH shows its antioxidant effects in several ways. It detoxifies hydrogen peroxide and lipid peroxide action of GSH peroxide. RGSH, SOD, CAT is primary antioxidant in the cell. Significant decrease in the amount RGSH, SOD, CAT was observed an diabetic control group compared with normal control group, while in diabetic rats treated with Gabapentin (30 mg/kg) and Ferulic acid (50,100 and 150 mg/kg) showed significant increase in RGHS, SOD, CAT level as compared with diabetic control group. In case of LPO, there was significant increase in LPO level in the diabetic control rats as compared to normal rats, while diabetic rats treated with Gabapentin , Ferulic acid (50,100 and 150 mg/kg) showed significant decreased in LPO level as compared with diabetic control rats, indicating the antioxidant activity of STME (Miura *et al.,* 2004). The Streptozotocin induced diabetic rats are estimation in blood serum diabetic control rats shoed increased sodium, urea, uric acid and decreased potassium was also observed with Gabapentin (30 mg/kg) and ferulic acid (50,100 and 150 mg/kg). Showed the significant data. It is reported that sciatic nerve of diabetic rats produces severe pathological changes as compared with groups treated with Gabapentin and Ferulic acid (50,100 and 150 mg/kg). Diabetic control group showed edema round the epineurium and infiltration of neutrophils around the blood vessels and showed swelling of nerve. Diabetic rats treated with Gabapentin (30mg/kg) showed swelling of nerve fibers and demyelization of nerve fibers. Macrophages and monocytes were observed around the Schwann cell of diabetic rats treated with Ferulic acid (50 mg/kg), while diabetic rats treated with Ferulic acid (100and 150 mg/kg) showed mild edema around the epineurium, few infiltrating neutrophils around the blood vessels and only minor swelling of nerve fibers, thus Ferulic acid treated animals showed sciatic nerve stability than diabetic control animals. Treatment with Ferulic acid (150 mg/kg) shows significant neuroprotective activity in Streptozotocin induced diabetic rats form the above experimental data. Thus the Ferulic acid ameliorates diabetic neuropathy in Streptozotocin induced diabetic induced diabetic rats by its possible antioxidant, antidiabetic and neuroprotective effect (Naik *et al.,* 2006).

Conclusion

In conclusion, chronic treatment of Ferulic acid in diabetes neuropathic pain significantly reversed mechanical and thermal hyperalgesia, allodynia and algesia in rat. Ferulic acid treatment significantly and dose dependently reduces the development of oxidative stress during pain by scavenging free radicals. These neuroprotective effects of Ferulic acid might be due to its strong antidiabetic and antioxidant activity. This is the first report of Ferulic acid which showed protective effect in Diabetic neruopathy.

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