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EVALUATION OF SINAPIC ACID AGAINST NEUROPATHIC PAIN IN ANIMAL MODEL

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ABSTRACT

Diabetic Neuropathy is one of the general and usual Complications of both type of diabetes, injury, or any lesion creating diseases of somatosensory neuronal system leads to generation of neuropathic pain which characterized by some kind of different type that is hyperalgesia, allodynia.. etc. which are severely painful. Apart from etiological reason, the oxidative stress has vital role in the pathogenesis of diabetic neuropathy. Sinapic acid is the phenolic compound and also one type of aromatic plant metabolite. Sinapic acid are proved to have potent antioxidant property, so on this basis this study was undertaken to evaluate the effect of Sinapic acid on Stz induced diabetic neuropathy on preclinical level; by assessing behavioral and biochemical alteration. Diabetes was induced in wistar rats by using single injection of STZ (55mg/kg i.p) after evolution of blood glucose level and confirmation of diabetes animal were treated with Gabapentin (300mg/kg p.o) and Sinapicacid (25, 50 &100mg/kg p.o) for next 28 days and weekly behavioral studies were carried out and on 28th day rats were scarified and sciatic nerve homogenate get evaluated for reduced glutathione antioxidant assay. Sinapicacid (50 and 100mg/kg p.o) treated rats showed significant increase in reduced glutamine level and also behavioral readings improve from 2nd week gradually as compare to positive control group. This study has suggested neuroprotective and antioxidant of Sinapic acid in Streptozotocine induced diabetic neuropathy.

INTRODUCTION

Neuropathic pain (NP) is chronic pain caused due to damage to nervous system either by injury or diseases. NP is distinguished by the neural sensory abnormalities viz., dysesthesia (unpleasant abnormal sensation), hyperalgesia (an elevated response to painful stimuli) and allodynia (pain to stimuli that normally does not provoke pain). (1) NP is complication of both types of diabetes. It occurs at about 8% in New patients and more than 50% in patients with long-standing disease. (2) Oxidative stress raised due to chronic hyperglycemia is responsible for diabetic complications like neuropathy. Apoptosis in supporting glial cells and neurons is also developed by this oxidative stress and could be the mechanism causing nervous system damage in diabetes.(3) Reduction in hyperglycemia mediated

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mitochondrial ROS by certain agents prevent production of advanced glycation end products, glucose-induced activation of protein kinase C, accumulation of sorbitol and activation of NF-B (nuclear factor B) and thus, prevent development of diabetic complications.(4) As most of pain producing stimuli produces neural injury, human experimentation to evaluate of NP is complex. So, animal experimentation is required to understand various mechanisms involved with NP.(5) STZ (Streptozotocine) induced neuropathy is widely accepted model that mimics the diabetic neuropathy. STZ is an anticancer antibiotic and chemically nitrosoureas analogue. STZ at dose of 45 to 60 mg/kg administered either i.v or i.p. selectively causes pancreatic beta cells destruction and in rats induces diabetes after three to four days.(5) This is one type of peripheral neuropathies identified by hyperalgesia, hyperesthesia and cold or hot allodynia, hyperglycemia induced nitrosative and oxidative stress which is a major mechanism in diabetic neuropathy. ROS can lead to afferent and efferent nerve conduction defects. (6) Consistent control on hyperglycemia is a challenge in many cases, and patients with good glucose control can experience neuropathy. Therefore, therapies that additionally target various pathways causing hyperglycemia-mediated complications are important to maintain long-term quality of life for diabetic patients (7) Also, hyperglycemia induced oxidative stress can mediate microvascular and neuronal deficits which are major contributors for diabetic complications. Until we can fully control blood glucose levels, antioxidants might therefore be helpful for treating diabeties and its complications (8)

Drugs from natural sources are safer therapeutic option to treat neuropathy instead of modern medicines as these modern medicines are with several adverse effects. Various plants phytoconstituents have been studied for the management of neuropathy in rats. Many flavonoids and polyphenols with promising antioxidant and anti-inflammatory activity have been evaluated in the treatment of neuropathic pain. Isolated plant bioactive moieties are promising free radical scavengers and play important role in management of neuropathy in animals.(9) Besides antioxidant activity and ability of phenolic acids to scavenge free radicals, recently many phenolic acids are studied for their neuroprotective role as those are protecting glial cells along with neurons. (10). Currently, many research studies have shown the role of various phenolic acids

Chlorogenic acid, CAPE (Caffeic acid phenethyl ester), Ferulic acid, Protocatechuic acid in treatment of neuropathic pain and other neurological disorders (10). Sinapic acid (3,5-Dimethoxy-4- hydroxycinnamic acid SINAPINATE) is a phenolic derivative of edible plants and fruits; its structure is given in Fig. 4. It is also an intermediate compound in the production of vanillin from ferulic acid. The largest amount of VA is found in the plant roots of Angelica sinensis. It has several medicinal properties including antifilarial, antibacterial, and antimicrobial activities, free- radical scavenging abilitity, and a.chemopreventive effect.Earlier reports from our lab showed the hepatoprotective effect of Vanillic Acid on actaminophen (APAP) induced toxicity in rats. Recently, there has been an upsurge of interest to explore the antihypertensive and antioxidant potential of natural products. Very few scientific reports are available on the antihypertensive and antioxidant effects of phenolic acids in hypertensive rats ([10) Phenolic acids (4-hydroxyl-3-methoxy benzoic acid) are reported to possess antimicrobial, anti-cancer, anti-DNA oxidation activities and hepatoprotective activity (11) Experimental profile provide evidence of effectiveness on cardiovascular (12), gastrointestinal (13) and liver disease (14). The beneficial activity on acute inflammatory processes has also been described (14). In view of the

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above literature, the current study was planned to evaluate effect of acid against STZ induced diabetic neurodegeneration / neuropathy.

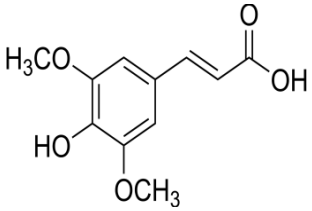

Figure 1: Chemical structure of S.A (3, 5-Dimethoxy-4-hydroxycinnamic acid Sinapinate)

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EXPERIMENTAL DESIGN

STZ- induced diabetic neuropathy: Animals were divided into 6 groups (n=6, either sex) each and treated for 4 weeks.

Group I Negative control: received vehicle as saline only (10 ml/kg)

Group II: Positive control: Rats treated with Streptozotocin (55 mg/kg; i.p +100mg/Kg Nicotinamide p.o) once only.

Group III Standard: Diabetic rats treated with Gabapentine (300 mg/kg; p.o.) once every day for 4weeks.

Group IV: Test drug 1: Diabetic rats treated with Sinapic acid (25 mg/kg; p.o.) once every day for 4weeks.

Group V Test drug 2: Diabetic rats treated with Sinapic acid (50 mg/Kg; p.o.) once an every day for 4weeks.

Group VI Test drug 3: Diabetic rats treated with Sinapic acid (100 mg/Kg; p.o.) once a every day for 4weeks.

During the treatment schedule, all groups of both animal models were subjected to behavioral tests once a week for 4 weeks. At the end of treatment schedule, antioxidant studies, blood glucose measurement in case of STZ diabetic neuropathy model and behavioral studies were performed (15).

STZ- induced diabeticneuropathy:

Experimental diabetes induced by single I.P injection of STZ (55mg/kg) and after 15 min Nicotinamide (100mg/Kg p.o) was administered. At 16 hrs before the induction of hyperglycemia rats were deprived of food, however they allow to drink water. After 72hrs rats with marked hyperglycemia (fasting blood glucose ≥ 200mg/dl) were selected and used for the study. After this all the animals were allowed free access to water and pellets diet & maintained at room temperature in plastic cages as per guidelines of IAEC of corresponding college (15).

Behavioral study

Cold allodynia (Cold platetest):

The cold plate test is one of the simplest assays to determine behavioral responses to both noxious and innocuous cold temperatures in both mice and rats. A number of endpoints can be obtained from the cold plate test, similar to the hot plate test. First, the response to a specific temperature (typically 5 to 15°C) can be recorded. Here, the rodent was placed on the plate after it has been cooled to the desired temperature(4°C) and the time taken to evoke nociceptive behavior such as shaking, jumping or licking in the animal is recorded as the response time. Cold chemical thermal sensitivity was assessed using cold plate method (15).

Motor coordination (Rota rodtest):

This test was conducted using rota rod apparatus by placing rats on 15 rpm rotating spindle. The fall off time of each rat from rotating spindle was recorded during 5 min period (16).

Hot plate method (Heat hyperalgesia):

The nociceptive threshold for heat is an index for thermal hyperalgesia. The plate was preheated and maintained at a temperature of 55±2°C. Rat was placed on the hot plate and nociceptive threshold with respect to licking of the hind paw or jumping was recorded in seconds. The onset for licking and jumping response was recorded. The cut off time of 20 sec was maintained (17).

Mechanical hyperalgesia (Von Frey hairtest):

Rats placed individually on elevated maze in a clear plastic cage and adopted to the testing environment for at least 15min. Filament (von frey hairs) was applied from below the mesh floor to the planter surface of left hind paw with sufficient force to cause slight bending against paw and hold for sec. Application repeated 5 times repeated at interval of 4-5 sec. Withdrawal of left hind limb robustly immediately was considered as a positive response (18, 19).

Locomotor activity (Open fieldtest):

Open field chamber of size 50 cm (length) x 50 cm (width) x 38 cm (height) and was made from white high density and non-porous plastic was used in test. The chamber was

Statistical analysis:

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

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RESULTS

Blood Glucose level Monitoring by Glucometer (Accusure)

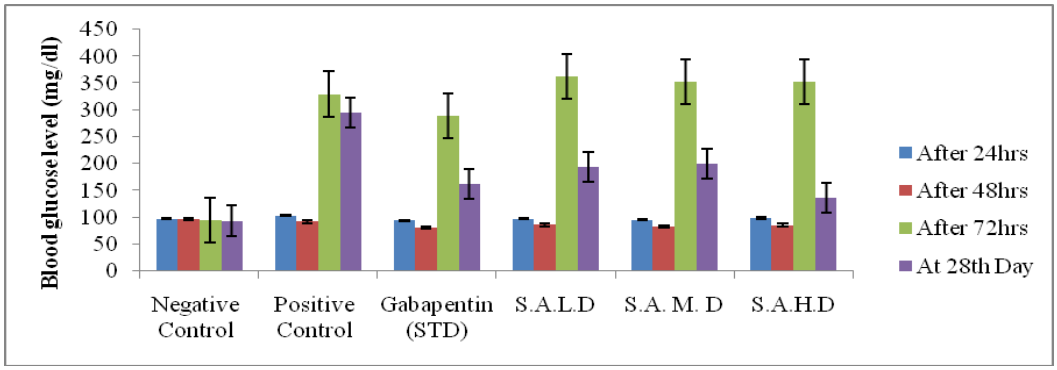

Fig.1: Effect of Sinapic acid (25, 50 and 100 mg/Kg p.o) and Gabapentin (300mg/Kgp.o) on blood glucose level of STZ-induced diabetic neuropathy in diabetic rats. The observations are Mean±SEM. N= 6, All data were subjected to ANOVA followed by Dunnett's test, * P≤0.05 as compared to positive control rats.

Assessment of cold allodynia by cold plate test

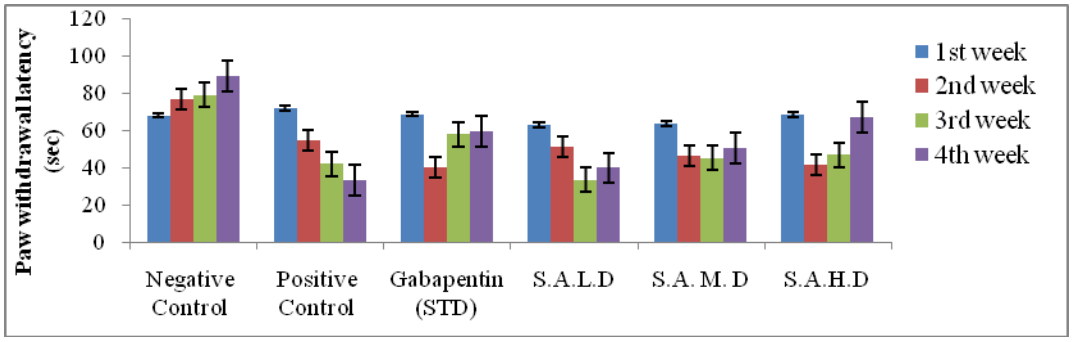

Fig.2: Effect of S.A (25,50and 100 mg/Kg p.o) and Gabapentin (300mg/Kg p.o)oncold allodynia assessed by the cold plate test in STZ - induced Diabetic neuropathy in rats. The observations are Mean±SEM. N= 6, All data were subjected to ANOVA followed by Dunnett's test.*P≤0.05 as compared to positive control group. S.A: Sinapic acid, STZ: Streptozotocine.

Assessment of motor in-coordination by Rota rod test

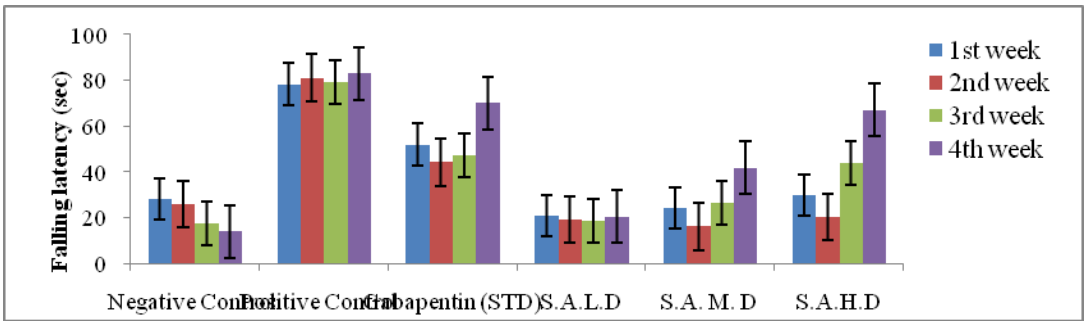

Fig.3: Effect of S.A (25, 50 and 100 mg/Kg p.o) and Gabapentin(300mg/Kg p.o)on motor coordination assessed by the rota rod test in STZ- induced diabetic neuropathy model of rats. The observations are Mean±SEM. N= 6, All data were subjected to ANOVA followed by Dunnett's test.* P≤0.05 as compared to positive control group. S.A: Sinapic acid, STZ: Streptozotocin.

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Heat hyperalgesia (Hot Plate Method):

Paw Lick Latency (sec)

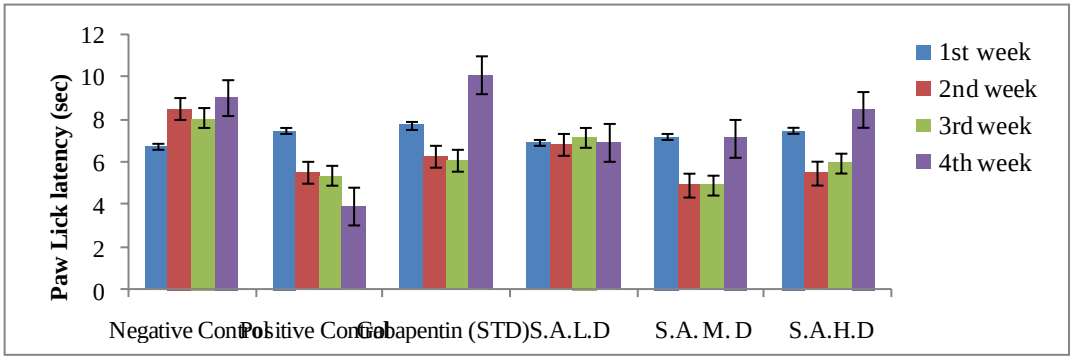

Fig.4: Effect of S.A (25,50 and 100mg/Kg p.o) and Gabapentin(300mg/Kg p.o) on heat hyperalgesia assessed by assessing paw lick response latency (sec) on hot plate in STZ- induced diabetic neuropathy model of rats. The observations are Mean±SEM,N= 6, All data were subjected to ANOVA followed by Dunnett's test.*P≤0.05 as compared to positive control group. S.A: Sinapic acid, STZ: Streptozotocin.

Jump Response (sec):

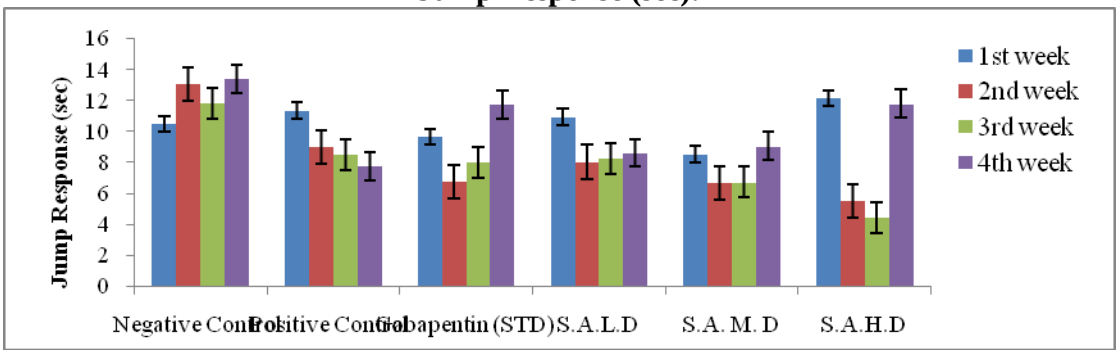

Fig.5: Effect of S.A (25, 50 and 100mg/Kg p.o) and Gabapentin(300mg/Kg p.o)on heat hyperalgesia assessed by assessing jump response latency (sec) on hot plate in STZ induced diabetic neuropathy model of rats. The observations are mean±SEM. N= 6, All data were subjected to ANOVA followed by Dunnett's test. *P≤0.05 as compared to positive control group. S.A:Sinapic acid, STZ:Streptozotocin.

Assessment of Tactile mechanical hyperalgesia by Von frey hair test:

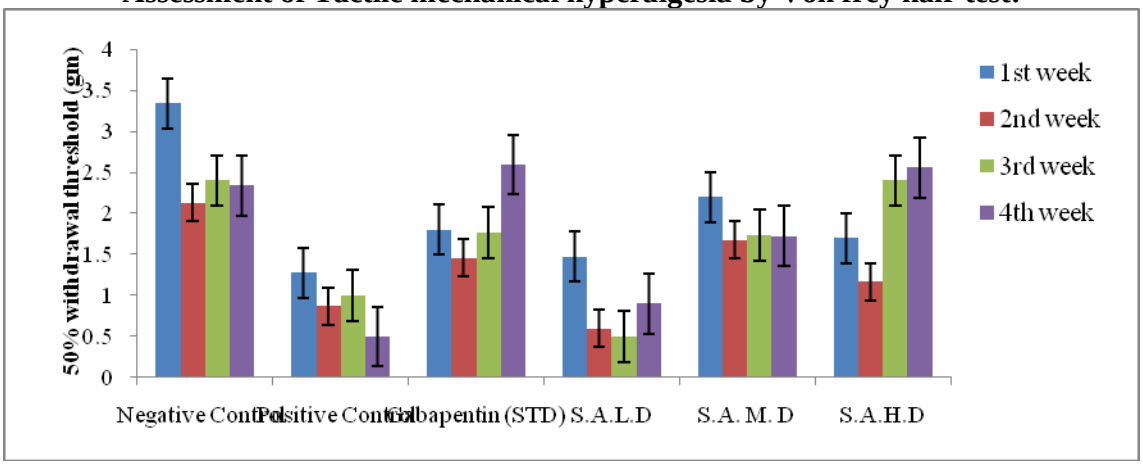

Fig.6: Effect of S.A (25, 50 &100 mg/Kg p.o) and Gabapentin(300mg/Kg p.o) on mechanical hyperalgesia assessed by the von frey hair test in STZ- induced diabetic neuropathy model of rats. The observations are Mean±SEM.N= 6, All data were subjected to ANOVA followed by Dunnett's test. * P≤0.05 as compared to Positive control group.S.A: Sinapicacid, STZ: Streptozotocin.

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Assessment of Locomotor/exploratory activity by open field test:

Assisted rearing

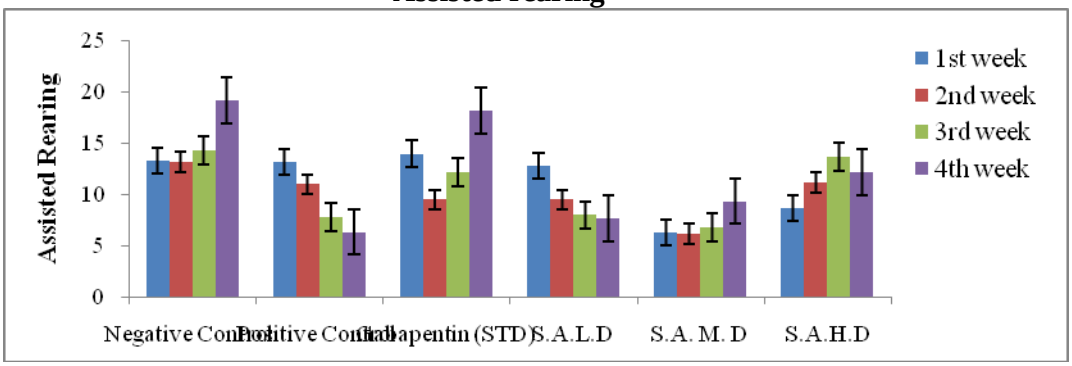

Fig.7: Effect of Sinapic acid (25, 50 and 100mg/Kg p.o) and Gabapentin(300mg/Kgp.o) on Locomotor activity assessed by (counting no.of assisted rearing) open field test in an induced diabetic neuropathy in diabetic rats. The observations are mean±SEM. N= 6, All data were subjected to ANOVA followed by Dunnett's test. * P<0.05 as compared to positive control group. S.A: Sinapic acid, STZ: Streptozotocin

Number of square crossed:

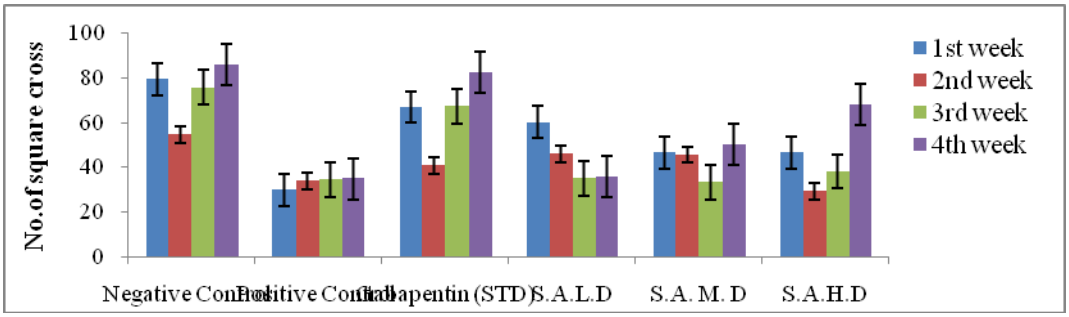

Fig.8: Effect of Sinapic acid (25, 50 and 100 mg/Kg p.o) and Gabapentin(300mg/Kg p.o) on Locomotor activity assessed by (No.of square cross) open field test in an induced diabetic neuropathy in diabetic rats. The observations are mean±SEM. N= 6, All data were subjected to ANOVA followed by Dunnett's test. * P≤0.05 as compared to positive control group. S.A: Sinapic acid, STZ: Streptozotocin.

Self assisted rearing:

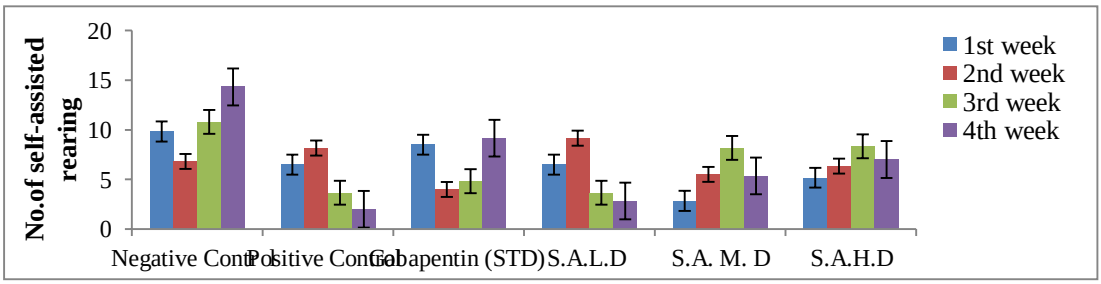

Fig.9: Effect of Sinapic acid (25, 50 and 100 mg/Kg p.o) and Gabapentin(300mg/Kg p.o) on locomotor activity assessed by (Self assisted rearing) open field test in an induced diabetic neuropathy in positive control rats. The observations are mean±SEM. N= 6, All data were subjected to positive control group. ANOVA followed by Dunnett's test. * P≤0.05 as compared to S.A: Sinapic acid, STZ: Streptozotocin

DISCUSSION

The present study is aimed to evaluate the effect of Sinapic acid in neuropathic pain. It has been evaluated by STZ induced diabetic neuropathy in rats by assessing its behavioral parameters and blood glucose level.Neuropathic pain is an underestimated socioeconomic health problem affecting millions of people worldwide, it has been recently redefined by the international Association for the study of pain as a "pain caused by lesion or disease of the somatosensory system" and it may appear in wide range of conditions; it can be divided into peripheral or central NP, depending on autonomic location of lesions or disease. Neuropathic pain is worldwide health issue that affect 6.9% to 10% in general population (20). Oxidative stress will eventually cause lots of neuropathic lesions by activation of many degenerative pathways such as reduction of intracellular antioxidant enzyme activity vascular damage, increased production of free radicals in mitochondria decreased nitric oxide induced endoneurial hypoxia apoptosis, degeneration of cellular components and increased expression of inflammatory factors. Reactive Oxygen species (ROS) are formed because of some biochemical and physiological processes. Environmental stress increases the level of the free radicals in uncontrolled manner there by disturbing the equilibrium between free radicals generation and the antioxidant level resulting in oxidative stress. Excess ROS, antioxidant depletion, or both with cellular generation of ROS cause damage to cellular macro component such as mutation in target cell and tissue resulting in cell death. This damage is associated with increase in risk of diseases such as diabetes and complications associated such as neuropathies, retinopathies nephropathy (21). A strong relationship exist between glycemia and diabetic microvascular complications in both type 1 and type 2 diabetes (22). Generation of superoxide due to oxidative stress in diabetes may be responsible for vascular and neuronal complications of painful neuropathy (23). Early in the course of diabetes,intra-cellular hyperglycemia causes abnormalities in blood flow and increased vascular permeability with microvascular cell loss in part as result of programmed cell death. Hyperglycemia may also decrease survival factors of endothelial and neuronal cell death. Together these changes results in edema, ischemia and hypoxia induced neovascularizton in retina, messengial matrix expansion, and glomerulosclerosis in kidney and multifocal axonal degeneration in peripheral nerves [22]. Oxidative stress related reduction in perfusion in thought to play role in cardiac autonomic dysfunction and also in small fibers sensory neuropathy (21). Generation of superoxide radicals because of oxidative stress in diabetic condition may be responsible for vascular and neuronal complications of painful neuropathy. Diabetic neuropathy is a very common complication in diabetes. The peripheral nerve injury is associated with neuropathic pain and is characterized by sensory abnormalities such as dysesthesia (unpleasant abnormal sensation), hyperalgesia (increased response to painful stimuli & allodynia (pain in response to stimulus that does not normally provoke pain) and with motor in co-ordination (3).Streptozotocin (2-desoxi-2(3-methyl-3nitro shureido)-n-glycopyranose is an anticancer agent I.V/I.P injection of STZ (45-60mg/Kg p.o) induces diabetes after 72hrs because STZ found to be selectively toxic to pancreatic beta cells as it preferentially accumulate in the beta cells as glucose analogue and hyperglycemia induces spontaneous oxidation of glucose through a variety of enzymatic and non-enzymatic activities and increased the advanced glycation end products (AGEs), protein kinase pathway activity (aldose reductase), poly ADP-ribose polymerase (PARP) pathway activity. hexosamine flow and decrease growth factors, all of which are the key components of the mentioned complex cascade. This pathway finally leads to oxidative stress of nerve cells through stimulating the production active oxygen and nitrogen species (24, 25).The role of oxidative stress in the pathogenesis and progression of diabetic neuropathy has

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been considered as one of the major cause of secondary complications (26). In view of prominent adverse effects of modern medicine, drugs from natural sources offer safetherapeutic option in the treatment of neuropathy several active phytoconstituents like caffeic acid, phenethyl ester flavonoids like bioflavone, polyphenols with reputable antioxidant and anti-inflammatory activity have been evaluated in the treatment of neuropathic pain. So, the present study is undertaken to evaluate effect of Sinapic acid in different animal models of neuropathic pain. Based on epidemiology and etiology of neuropathic pain STZ-induced diabetic neuropathy model were selected in the present study. In diabetic patient regulation of blood glucose level can prevent the various complications associated with diseases. Maintenance of blood glucose level for a long term under a variety of dietary conditions is the one of the most important and regulated process observed in mammalian species(27).In present study significant increase in blood glucose level (250mg/dl ≥) was observed in positive control rats compared with negative control rats after 72 h of STZ-injection(55mg/Kg i.p). While the diabetic rats treated with Gabapentin (300mg/kg p.o) and Sinapic acid (50&100mg/kg p.o) showed significant (*P<0.05) decrease in blood glucose level as compared with positive control rats on 4th week indicating the anti-hyperglycemic activity of Sinapic acid. Behavioral changes indicating onset of development of neuropathy were observed after 2nd week of induction of diabetes &peripheral nerve injury in experimental animals. The behavioral parameters such as heat and mechanical hyperalgesia, cold allodynia and motor coordination, locomotor activity was assessed by using hot plate method, von frey hair test, cold plate method, rota rod test and open field test respectively. In behavioral test neuropathic rats showed significant (*P≤0.05) reduction in paw withdrawal latency in cold plate, and significant decrease in paw lick latency and jump response (sec) in hot plate method .A significant decrease in 50 % paw withdrawal threshold in von frey test and significant (*P≤0.05) decrease in failing latency (sec) in rota rod test was observed in positive control rats as compared to normal rats. In case of exploratory activity and locomotor activity the positive control rats showed significant decrease in number of square cross, number of self-assisted rearing and assisted rearing as compared to negative control rats.Cold allodynia assessed by cold plate test. The rats treated with Gabapentin (300mg/kg) and Sinapic acid (50&100mg/Kg p.o) showed significant improvement in paw withdrawal latency (sec) as compare to positive control rats after 3rd week of treatment schedule. From the results of present study, four week treatment with Sinapic acid (50 &100mg/Kg p.o) improved cold allodynia in both experimental animals model. In case of motor coordination which is assessed by rota rod test, treatment with the Gabapentin (300mg/Kg p.o) and Sinapic acid (50 &100mg/kg) showed significant improvement in motor coordination indicated by increased in falling latency (sec) as compared to positive control rats after 3rd week of treatment schedule. Thus treatment of Sinapic acid prevents motor in coordination in diabetic neuropathy in experimental animals. Heat hyperalgesia assessed by hot plate method. The rats with neuropathy treated gabapentin (300mg/Kg p.o) and Sinapic acid (50 &100mg/Kg p.o) showed significant improvement in jump response and paw lick latency (sec) after 3rd week of treatment schedule. Open field was used to evaluate locomotor activity. Rats with neuropathy treated with gabapentin (300mg/Kg p.o) and Sinapic acid (100mg/Kg p.o) showed significant improvement in loco motor activity as indicated by significant increase in number of square crossed , number of self-assisted and assisted rearing after 3rd week of treatment schedule as compared to positive control rats. Thus treatment with Sinapic acid (100mg/Kg p.o) prevents loss of Locomotor activity of rats. Thus Sinapic acid treatment has shown significant protective effect in STZ-induced diabetic neuropathy possibly which may be due its

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antioxidant, antihyperglycemic and neuroprotective effect.

CONCLUSION

In present study, Sinapic acid has significantly reversed behavioral changes blood glucose level and antioxidant depletion. Thus sinapic acid treatment has shown significant protective effect in STZ-induced diabetic neuropathy, possibly which may be due its antioxidant, antihyperglycemic and neuroprotective effect.

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