



CODEN [USA]: IAJPBB

ISSN: 2349-7750

**INDO AMERICAN JOURNAL OF  
PHARMACEUTICAL SCIENCES**<http://doi.org/10.5281/zenodo.833453>Available online at: <http://www.iajps.com>

Research Article

**INVESTIGATION OF NEUROPROTECTIVE EFFECT OF  
VALPROIC ACID IN DIABETIC NEUROPATHY IN  
STREPTOZOTOCIN INDUCED TYPE2 DIABETIC RATS****Neeraj Kumar\*, Suruti Rawat, Preeti Kothiyal**Department of Pharmaceutical Sciences, Shri Guru Ram Rai Institute of Technology and  
Science, Dehradun, Uttarakhand, India.**Abstract:**

**Objectives:** The aim of this study is to evaluate the effect of Valproic acid in diabetic neuropathy.

**Methods:** Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg). When STZ was administered to the albino wistar rats then it showed marked Hyperglycemia & reduces the thermal Cold allodynia, thermal hyperalgesia, motor co-ordination which was compared to the animals of control group. Effect of valproic acid on biochemical parameters (Serum glucose level, Lipid peroxidation, SOD & GSH and Oxidative stress) was also assessed in the brain tissue of albino wistar rat.

**Results:** After 28 days of treatment with valproic acid (150 and 300 mg/kg), rats showed significant reduction in serum glucose level. Valproic acid showed good results in different parameters such as Thermal Hyperalgesia, Thermal Allodynia (Hot and Cold) motor co-ordination in comparison with diabetic control group. The most profound effect was seen with 300mg/kg of valproic acid after 28<sup>th</sup> day of treatment. Treatment with valproic acid (150 & 300mg/kg) resulted significant decrease in serum glucose level, lipid peroxidation and increase in the SOD and GSH level as compared to diabetic control.

**Conclusion:** Thus valproic acid due to its antioxidant property can be concluded to be effective treatment or preventive option for diabetic neuropathy. STZ causes increase in the level of oxidative stress and lipid peroxidation and this is reverse by treatment with valproic acid and by reducing the generation of free radicals it also increase in SOD & GSH level significantly.

**Keyword:** Diabetic neuropathy, Oxidative stress, Free radicals, Valproic acid, Streptozotocin.

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Please cite this article in press as Neeraj Kumar *et al*, *Investigation of Neuroprotective Effect of Valproic Acid in Diabetic Neuropathy in Streptozotocin Induced Type2 Diabetic Rats*, *Indo Am. J. P. Sci*, 2017; 4(07).

**INTRODUCTION:****Diabetes Mellitus**

Diabetes is a long term metabolic disorder with a difficult pathogenesis due to participation of various cellular and molecular signaling [1]. Diabetes is characterized by metabolic abnormalities and hyperglycemia causing metabolic and physiological changes in various organs including brain due to decreased insulin level/action [2]. It is a metabolic disorder of lipid, protein and carbohydrate. The metabolic defects is occurs due to the insulin secretion, insulin action or both. Diabetes mellitus is also known as hyperglycemia and it leads to chronic complications such as micro vascular, macro vascular and neuropathic disorder [3]. These Type 1 and Type 2 are two major type of diabetes, although diabetes may occur during pregnancy and under other conditions like chemical toxicity or drug, genetic disorder, endocrinopathies, insulin receptor disorder and in association with pancreatic exocrine disease[4]. American diabetes association introduced a new classification system in which the terms IDDM and NIDDM also called Type 1 and Type 2 diabetes [5].

**Types of Diabetes****Type1 diabetes- (Pancreatic beta cells destruction and leading to absolute insulin deficiency)**

Occurs in younger people and termed as juvenile – onset diabetes, even if it can occur at any age [6]. Type-1 diabetes mellitus (T1DM) is a chronic autoimmune disorder characterized by hyperglycemia due to compromised insulin secretion from beta-cell and/or reduced beta-cell mass, which generally developed in genetically susceptible individuals by environmental factors [7-8]. In the pathogenesis of T1DM both genetic and epigenetic factors contributed equally [9-10].

**Type 2 diabetes- (Ranging from predominantly insulin resistance with relative insulin deficiency to predominantly insulin secretory defects with insulin resistance)**

It is also called non-insulin dependent diabetes mellitus, type2 diabetes, or adult onset diabetes. This account for 90-95% of those with diabetes .This type of diabetes occurs in individuals who have insulin resistant and have relative (rather than absolute) insulin deficiency[11]. The tissues which are having reduced insulin sensitivity include skeletal muscle, liver and adipose tissue due to the particular requirements for glucose uptake and metabolism at these sites. However in most subjects the relative decrease in insulin secretion is the final event leading to hyperglycemia. Genetic and environmental factor have important role in type 2 diabetes. High calorie

and dietary fat intake in the context of reduced exercise with an associated increase in body weight ultimately lead to type 2 Diabetes [12]

**Diabetic complications**

Diabetic complications are generally divided into macro vascular complications (disease of coronary artery, disease of peripheral arterial and stroke) and micro vascular complications (diabetic nephropathy, retinopathy and neuropathy) [13].

**Nephropathy**

Neuropathy is well-defined by proteinuria > 500 mg in 24 hours in the setting of diabetes but this is preceded through lower degrees of “micro albuminuria” or proteinuria [14]. In the European Diabetes Prospective Complications Study, the cumulative incidence of micro albuminuria patients with type 1 diabetes was ~ 12% throughout a period of 7 years [14-15]. Patients should be treated to the lowest safe glucose level that can be gained to prevent [14-16-17].

Valproic Acid is widely used in clinics as an anti-epileptic drug and for the therapy of bipolar disorders and migraine prophylaxis. The VA mechanism of action was primarily related to its effects on amino acid neurotransmitters and modulation of intracellular pathways. Recently, the pharma- cological characteristics similar to those of other anti- cancer agents, acting on cell growth, differentiation and apoptosis showed by VA Other studies [18]. VA treatment significantly decreased plasma glucose, beta-cell damage, and apoptosis as well as increased the beta-cell function, insulin level/expression [19].

**MATERIAL AND METHODS:****Animals**

Albino Wistar rats (250-350g) of either sex were procured from the departmental animal house of Division of Pharmaceutical Sciences of Shri Guru Ram Rai Institute of Technology and Science, Patel Nagar, Dehradun. Animals were acclimatized in the departmental animal house facility and housed (n=6 per cage) in 12 hr light/dark cycle. During the study animals was fed with a water ad libitum and standard diet. The protocol was approved by the Institutional Animal Ethics Committee (Registration No. 264/PO/ReBi/S/2002/CPCSEA) and was carried out in accordance with the CPCSEA guidelines.

**Experimental Design**

5 groups, each comprising of 6 animals

**Group 1: Control:** Citrate buffer was administered as a Vehicle.

**Group 2: Diabetic control:** Streptozotocin (60 mg/kg, i.p.) was administered after 15 minute of NAD (235mg/kg i.p) administration.

**Group 3: Active Control:** Pregabalin (300mg /kg i.p) was administered for 28 days in diabetic albino wistar rat as a standard.

**Group 4:** Diabetic neuropathy induced albino wistar rat + Valproic acid (150mg/kg/day i.p) was administered for 28 days.

**Group 5:** Diabetic neuropathy induced albino wistar rat + Valproic acid (300/mg/kg/day i.p) was administered for 28 day

**NAD-** Nicotinamide-adenine dinucleotide

#### Induction of diabetes [20, 21,22]

A Streptozotocin single dose (60mg/kg) was prepared with citrate buffer (pH 4.4, 0.1M) and administered intraperitoneally to overnight fasted animal to induce diabetes. NAD + 235 mg/kg were administered prior (15 minutes) administration of STZ. The control group albino wistar rats have given an equal volume of citrate buffer and were used along with diabetic animals. Serum glucose level was estimated on 0<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>th</sup> and 28<sup>th</sup> day respectively, after Streptozotocin administration by enzymatic GOD-POD (glucose oxidase peroxidase) diagnostic kit. The albino wistar rats having fasting plasma glucose levels more than 250 mg/dl were referred as diabetic and included in the study. STZ treated animal were given 10% glucose solution after 6 hrs. To prevent fatal hypoglycemia, since Streptozotocin is potent enough to cause fatal hypoglycemia due to massive pancreatic insulin release.

**Drugs-** Streptozotocin, Pregabalin, Valproic acid

#### Parameters evaluated

##### Biochemical Methods

##### Estimation of Serum Glucose Level [23]

Serum glucose level was assessed by glucose oxidase/peroxidase method using commercially presented enzymatic GOD-POD diagnostic kit.

##### Principle

Glucose is oxidized into gluconic acid and hydrogen peroxide by glucose oxidase enzyme (GOD). Hydrogen peroxide in the presence of Peroxidase enzyme (POD) oxidizes and converted into the chromogen-4-Aminoantipyrine/phenolic compound into a red coloured compound. The red colour so developed was measured spectro photometrically at 505 nm. The intensity is related to the concentration of the glucose present in the specimen.

##### Procedure

1000 µl of glucose reagent was added with 10 µl of the serum, 10 µl of the standard glucose (100 mg/dl) and 10 µl of purified water to prepared test sample, standard and blank sample respectively. The samples were incubated at 37°C for 15 minutes. 100 ml of purified water was added to each tube. The absorbance of the standard and test sample was measured spectrophotometrically at 505 nm against blank respectively.

**Concentration of Glucose in the sample can be calculated using the following formula:**

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard (100)}$$

##### Serum glucose level

The serum glucose level of all animal was recorded on 0<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>th</sup> and 28<sup>th</sup> post STZ administration. At end of experimental period the blood samples were collected from retro orbital plexus under mild anaesthesia for estimation of serum glucose level.

##### Estimation of Lipid peroxidation[24]

Lipid peroxidase levels were estimated by Thiobarbituric acid reaction method described by Ohkawa *et al*.

**Principle:** The estimation of malondialdehyde (MDA), a product of lipid peroxidation was done by this method. One molecule of malondialdehyde reacts with two molecules of thiobarbituric acid (TBA) under mildly acidic conditions to form a pink color chromogen, the intensity of whose was measured in spectrophotometer at 535nm.

##### Reagents

- Trichloroacetic acid (30%)
- Thiobarbituric acid (TBA) (0.8%)

**Procedure-** 2 ml of homogenate was taken, to this 2 ml of 30% of trichloroacetic acid was added, followed by the addition of 0.8% thiobarbituric acid reagent. The test tube was kept in cold water for half an hour. Then the homogenate was centrifuged at 3000 RPM for 15 min. Then the supernatant was separated and the absorbance was read at 535 nm against blank. The blank solution was consisting of 2 ml distilled water, 2 ml of 30% tricholoacetic acid and 2 ml of 0.8% thiobarbituric acid. The malondialdehyde expressed as n moles formed per mg of protein in the tissue. It was calculated by the following formula-

**Concentration=A x (V/E) x P**

Where A=Absorbance at 535 nm

V = Volume of solution

E = Extinction coefficient ( $1.56 \times 10^5 \text{ m}^{-1} \text{ cm}^{-1}$ )

P = mg of protein per g of tissue

The value was expressed in nM of MDA/mg of protein.

Reagents	Blank	Test
Ellman's Reagent	50µl	50µl
Reaction buffer	2.5µl	2.5µl
Tissue homogenate	-	250µl

**Estimation of Reduced glutathione[25]**

The Estimation of reduced glutathione was done by the method of Ellman GL.

**Principle**

Glutathione is a major non protein thiol and endogenous antioxidant that counters balance of free radical mediated damage. It is involved in the protection of normal cell structure and function by maintaining the redox homeostatic, quenching of free radicals groups. 5, 5 di thio 2-nitrobenzoic acid (DTNB), a disulphide compound gets easily attacked by tissue sulfhydryl group and forms a yellow coloured anion the intensity of which is measured at spectrophotometer at 412 nm.

**Reagents**

Reaction buffer:

(0.1M sodium phosphate (pH 8.0) containing 1mM EDTA)

Stock A=2.78 g sodium phosphate monobasic was dissolved in 100 ml water.

Stock B= 2.84 g anhydrous sodium phosphate dibasic was dissolved in 100 ml water

2.65 ml of stock A and 47.35 ml of stock B were mixed together and then volume was made up to 100 ml with distilled water. The 37.2 mg of EDTA was dissolved in above solution.

- Ellman's reagent solution:
- 4 mg of Ellman's reagent (5-5-di thio-2-nitro benzoic acid) was dissolved in 1 ml of reaction buffer.

**Procedure**

The homogenate was mixed with 10% trichloroacetic acid and centrifuged. Then it was mixed with DTNB

and phosphate buffer and estimation was done at 412 nm.

Mix well the above reagents and incubate at the room temperature for 15 min. Absorbance was measured at 412 nm.

**Calculation**

$$\text{GSH} = \frac{\text{Absorbance} \times 11.2 \times 10^9}{1.4550 \times 10^4}$$

Unit =n mol /mg of protein

**Estimation of Superoxide Dismutase [25]**

The superoxide dismutase activity in supernatant was measured by the method of Mishra and Fridovich.

**Principle**

Superoxide dismutase is involved in the antioxidant defense against ROS by lowering the steady state oxygen level. SOD scavengers the superoxide ions produced as cellular by-products. This enzyme is a major defense for aerobic cells combating the toxic effect of superoxide radicals. It has the ability to inhibit the auto oxidation of epinephrine to adrenochrome at pH 10.2. This inhibition can be measured with spectrophotometer at 480 nm. One unit of SOD is defined as the enzymes amount requires producing 50% inhibition of epinephrine auto-oxidation.

**Reagents**

- Carbonate buffer (100mM, pH 10.2)
- Epinephrine (3Mm)

**Procedure**

The homogenate (500 µl) was added to 0.800 ml of carbonate buffer (100 mm, pH 10.2) and 100µl of epinephrine (3 mM).Then the change in absorbance of each sample was recorded at 480 nm in spectrophotometer for 2 min at an interval of 15 sec. Parallel to this blank and standard were run for estimation of SOD activity.

Control	Blank	Test
0.900 ml of carbonate buffer	1.0 ml of carbonate buffer	0.800 ml of carbonate 0.1 ml of homogenate
0.1 ml of epinephrine	-	0.1 ml of epinephrine

The reaction mixture was diluted 1/10 just before taking the readings in the spectrophotometer.

$$\text{Calculation} = \% \text{ Inhibition} = \frac{A_{480/\text{min}}(\text{Control}) - A_{480/\text{min}}(\text{Test})}{A_{480/\text{min}}(\text{Control}) - A_{480/\text{min}}(\text{Blank})} \times 100$$

$$\text{Units/ml enzyme} = \frac{\% \text{ Inhibition} \times V_t}{(50\%) \times V_s}$$

V<sub>t</sub>=total volume (1ml)

V<sub>s</sub>=volume of the sample (0.1 ml)

The final value of superoxide dismutase is expressed in unit/mg of protein.

### Behavioural Method

#### Thermal hyperalgesia

Tail immersion (hot water) test- Rats were held in position in a suitable restrainer with the tail protruding out. The tail up to 5 cm was dipped in a beaker of water at 55 degree centigrade temperature. The time taken to withdraw the tail clearly out of water was taken as the reaction time [26]

#### Thermal allodynia

##### Eddy's Hot plate method

Animals were placed into a glass cylinder on a hot plate adjusted to 38 degree centigrade temp. The latency of first reaction was recorded (licking, moving the paws) with a cutoff time of 30 s [26]

#### Cold allodynia

Allodynia was estimated through tail in which the tail of the rat was dipped in cold water (5°C) and the tail withdrawal latency was noted. Cut off latency was fixed at 15 sec [27].

#### Motor co-ordination

The motor coordination and performance of each rat was evaluated using Rota-rod apparatus. Latency to fall from the rotating rod was registered in seconds [28].

#### Statistical Analysis

The data obtained from the results were analyzed by using two ways ANOVA followed by Bonferroni's post- test using Graph pad prism 7 software. All data were expressed as the mean +SEM of their parameters.

### RESULTS:

#### Effect of Streptozotocin on serum glucose level in STZ induced diabetic rats

Serum glucose level was estimated on 0<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 28<sup>th</sup> and 75<sup>th</sup> days in all STZ treated groups and the result was found significant in comparison to control

group. The diabetic rats were selected whose serum glucose level was found more than 250 mg/dl after administration of STZ. (**Fig no 5a**)

#### Effect of valproic acid on serum glucose level in STZ induced diabetic rat

*Valproic acid* in two different doses (150 mg/kg, 300mg/kg), were administered to diabetic rats and it was observed that the Serum Glucose level reduced as estimated after 10<sup>th</sup> and 28<sup>th</sup> days and effect was found significant (**Fig no. 5b**)

#### Effect of Streptozotocin on thermal Hyperalgesia and Allodynia in STZ induced diabetic rats

Thermal Hyperalgesia tests were performed after the administration of STZ in the albino wistar rats, Thermal Allodynia and Cold Allodynia tests were performed. The tail flick latency in case of thermal Hyperalgesia (**Fig no. 5b**) was found significant. The paw withdrawal latency in case of Thermal Allodynia (**Fig no. 5c**) was found significant  $p < (0.001)$  and tail flick latency in case of cold Allodynia (**Fig no. 5f**) was found significant  $p < (0.001)$  as compared to control. On the other hand these parameters remained constant throughout the experiment for albino wistar Rat of control group (n= 6).

#### Effect of Streptozotocin on motor-coordination in STZ induced diabetic rat

28 days of post administration of STZ the motor co-ordination test was performed and it exhibits those diabetic rats took less amount of time to stay at the Rota rod. On the other hand there was significant change observed for rats of control group (n=6). (**Fig no. 5e**)

#### Effect of Valproic Acid on level of lipid peroxidation of STZ induced rat

When STZ is compared with the control group the levels of MDA during lipid peroxidation in brain produced a significant difference ( $p < 0.001$ ). Administration of valproic acid (150 and 300 mg/kg) significantly ( $p < 0.001$ ) reduce the lipid peroxidation level as compare to disease control. Standard treatment with pregabalin significantly ( $p < 0.001$ ) reduce the level of lipid peroxidation in brain.

#### Effect of Valproic acid on level of superoxide dismutase in STZ induced rat

Administration of STZ showed a significant ( $p < 0.001$ ) decrease in SOD levels in comparison with control group. However administration of valproic acid (300 mg/kg) significantly ( $p < 0.001$ ) increase the level of SOD in brain while *valproic acid* at a dose of 150 mg/kg showed a less significant ( $p < 0.05$ ) effect.



Pregabalin also significantly ( $p < 0.001$ ) maintain the level of SOD.

#### Effect of Valproic Acidon level of Glutathione in STZ induced rat

Administration of STZ produce a significant ( $p < 0.001$ ) decrease in glutathione level in comparison with vehicle control group. However treatment with valproic acid significantly ( $p < 0.001$ ) increase the glutathione level in brain. In other side the Standard treatment with pregabalin significantly ( $p < 0.001$ ) increase the level of glutathione.

#### Effect of valproic acidon diabetic neuropathy in STZ induced diabetic rat

Thermal Hyperalgesia and Thermal Allodynia (Hot and Cold)

Thermal Hyperalgesia (**Fig no. 5b**) and Thermal Allodynia (**Fig no. 5c**) and Cold Allodynia (**Fig no. 5d**) test were performed 28 days after STZ administration and decrease in tail flick and paw withdrawal latency was observed. Administrations of valproic acid resulted in significant increase in the tail flick and paw withdrawal latency on 28 days treatment.

#### Motor co-ordination

It was observed that motor co-ordination in Streptozotocin treated rat after 28 days STZ administration significantly decreased.

Valproic acid 28 days administration resulted in improved motor co-ordination and positive effect was observed at the end of 8 weeks. (**Fig no. 5e**)

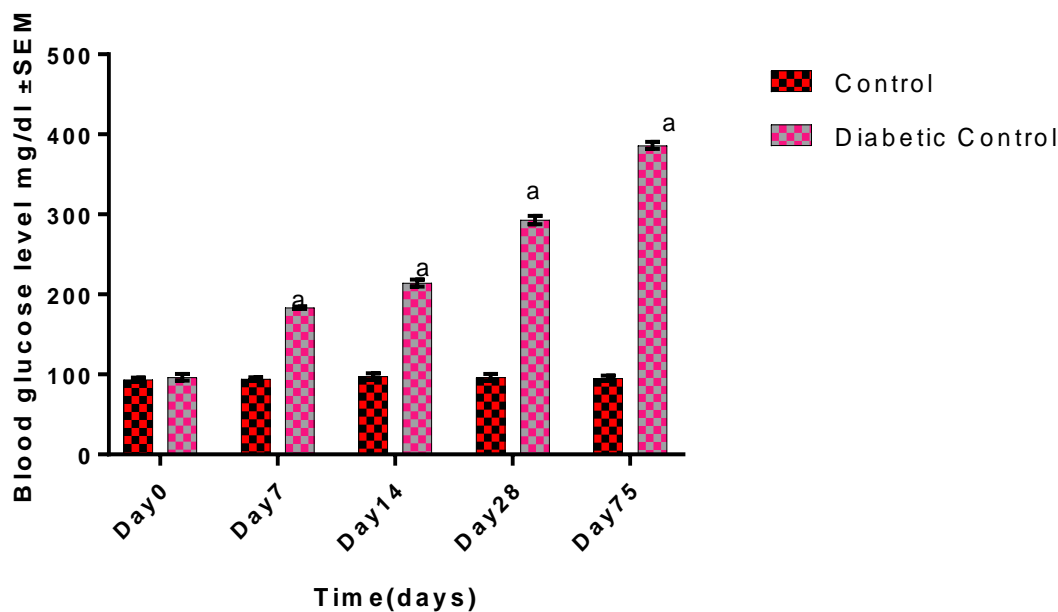
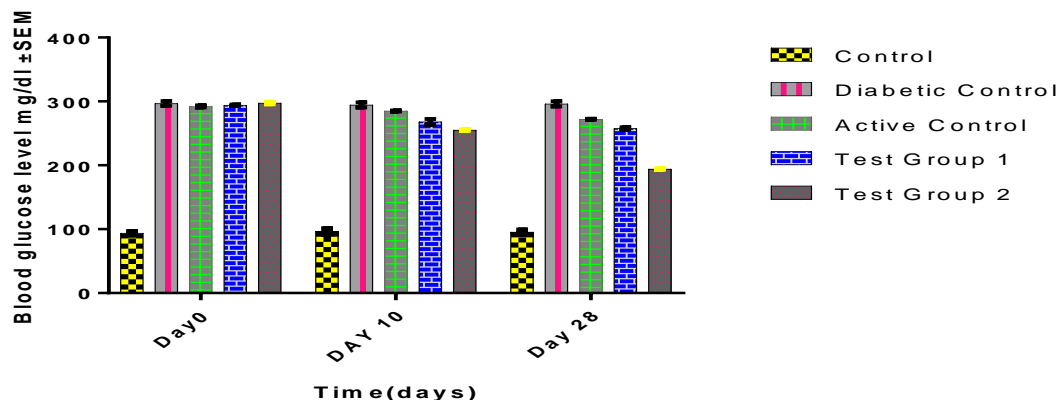


Fig 5a: Effect of STZ on blood glucose level in rats

Control group represents the administrations of citrate buffer. Diabetic control group represents the group treated with STZ (60mg/kg ip) + NAD<sup>+</sup> (235mg/kg) administered (15min) prior to STZ administration.

a=  $p \leq 0.001$  as compared to Control group

The statistical analysis was carried out using prism graph pad 7 software. All values are represented as mean  $\pm$  SEM (n=6). Results were compared by using two ways ANOVA and Bonferroni's post test



**Fig 5b: Effect of valproic acid on blood glucose level in rat**

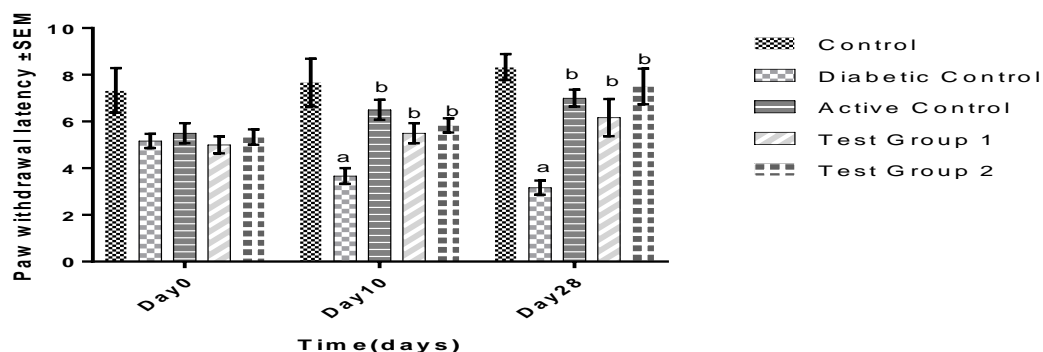
Control represents the group which is treated with citrate buffer. Diabetic control group represents the group treated with streptozotocin (60 mg/kg i.p) + NAD<sup>+</sup> (235mg/kg) administered (15min) prior to STZ administration. Active control group represents administration of pregabalin to diabetic animal. Test group I represents administration of test drug (150 mg/kg) valproic acid to the diabetic animals. Test group II represents administration of test drug (300 mg/kg) valproic acid to the diabetic animals.

a= $p \leq 0.001$ , compared to control group

b= $p \leq 0.05$ , compared to diabetic control group

The statistical analysis was carried out using prism graph pad 7 software. All values are represented as mean  $\pm$  SEM (n=6). Data were compared by using two-way ANOVA, followed by Bonferroni's test. The effect was found significant.

#### Thermal Hyperalgesia (in sec)



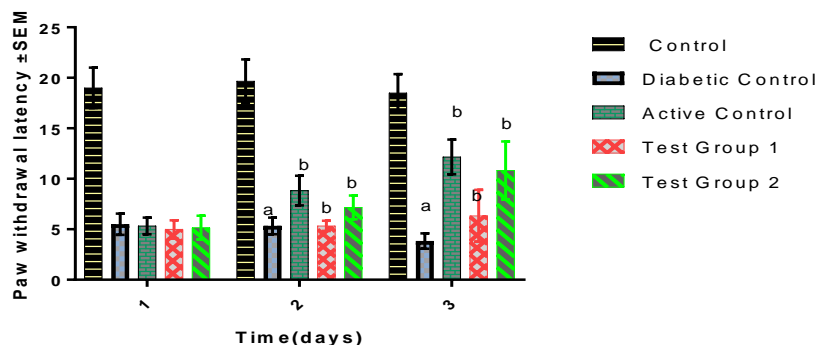
**Fig 5c: Effect of valproic acid on thermal hyperalgesia in rat.**

Control represents the group which is treated with citrate buffer. Diabetic control group represents the group treated with streptozotocin (60 mg/kg i.p) + NAD<sup>+</sup> (235mg/kg) administered (15min) prior to STZ administration. Active control group represents administration of pregabalin to diabetic animal. Test group I represents administration of test drug (150 mg/kg) valproic acid to the diabetic animal. Test group II represents administration of test drug (300 mg/kg) valproic acid to the diabetic animal.

a= $p \leq 0.001$ , as compared to control group

b= $p \leq 0.001$ , as compared to diabetic control group

The statistical analysis was carried out using prism graph pad 7 software. All values are represented as mean  $\pm$  SEM (n=6). Results were compared by using ANOVA and Bonferroni's post test

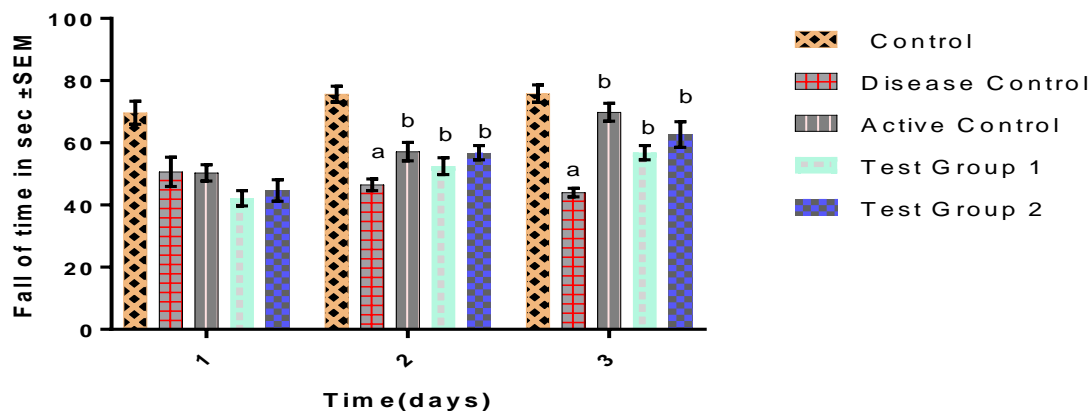
**Thermal Allodynia (in sec)****Fig 5d: Effect of Valproic Acid on Thermal Allodynia in rat**

Control represents the group which is treated with citrate buffer. Diabetic control group represents the group treated with streptozotocin (60 mg/kg i.p) + NAD<sup>+</sup> (235mg/kg) administered (15min) prior to STZ administration. Active control group represents administration of pregabalin to diabetic animal. Test group I represents administration of test drug (150 mg/kg) valproic acid to the diabetic animal. Test group II represents administration of test drug (300 mg/kg) valproic acid to the diabetic animal.

a=  $p \leq 0.0001$ , as compared to control group

b=  $p \leq 0.001$ , as compared to diabetic control group

The statistical analysis was carried out using prism graph pad 7 software. All values are represented as mean  $\pm$  SEM (n=6). Results were compared by using two ways ANOVA and Bonferroni's post test

**Motor Co-ordination****Fig 5e: Effect of valproic acid on rota rod apparatus in rat**

Control represents the group which is treated with citrate buffer. Diabetic control group represents the group treated with streptozotocin (60 mg/kg i.p) + NAD<sup>+</sup> (235mg/kg) administered (15min) prior to STZ administration. Active control group represents administration of pregabalin to diabetic animal. Test group I represents administration of test drug (150 mg/kg) valproic acid to the diabetic animal. Test group II represents administration of test drug (300 mg/kg) valproic acid to the diabetic animal.

a=  $p \leq 0.001$ , as compared to control group

b=  $p \leq 0.05$ , as compared to diabetic control group

The statistical analysis was carried out using prism graph pad 7 software. All values are represented as mean  $\pm$  SEM (n=6). Results were compared by using two way ANOVA and Bonferroni's post-test.



## Cold Allodynia

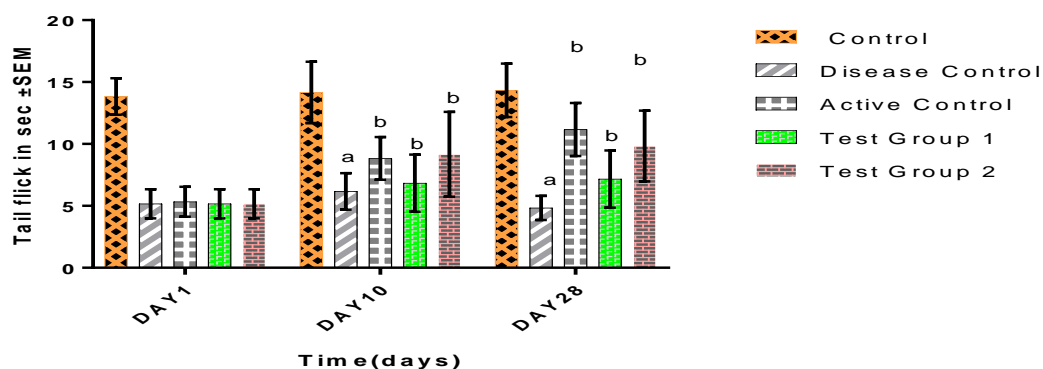


Fig 5f: Effect of *valproic acid* on cold allodynia in rat

Control represents the group which is treated with citrate buffer. Diabetic control group represents the group treated with STZ. Active control group represents administration of pregabalin to diabetic animal. Test group I represents administration of test drug (150 mg/kg) valproic acid to the diabetic animal. Test group II represents administration of test drug (300 mg/kg) valproic acid to the diabetic animal. All data are presented as mean  $\pm$  SEM.

a= $p \leq 0.0001$ , compared to control group

b= $p \leq 0.05$ , compared to diabetic control group

The statistical analysis was carried out using prism graph pad 7 software. All values are represented as mean  $\pm$  SEM (n=6). Data were compared by using two-way ANOVA, followed by Bonferroni's post test. The effect was found significant.

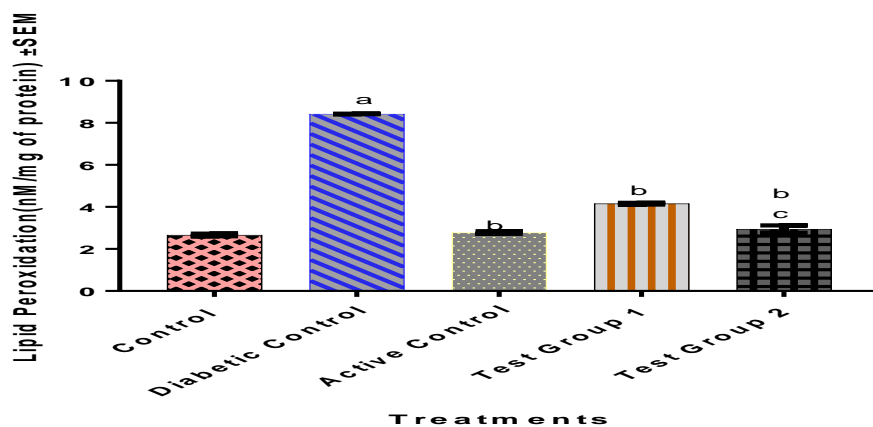


Fig 5.g Effect of *Valproic acid* on level of lipid peroxidation (nM/mgprotein) of Albino wistar rat

STZ- Streptozotocin

a indicates significance versus normal control

b indicates significance versus disease control

c indicates significance versus low dose of valproic acid (150 mg/kg).

The statistical analysis was carried out using prism graph pad 7 software. All values are represented as mean  $\pm$  SEM. Multiple comparison between different groups were performed using one way analysis of variance followed by Tukey's test.

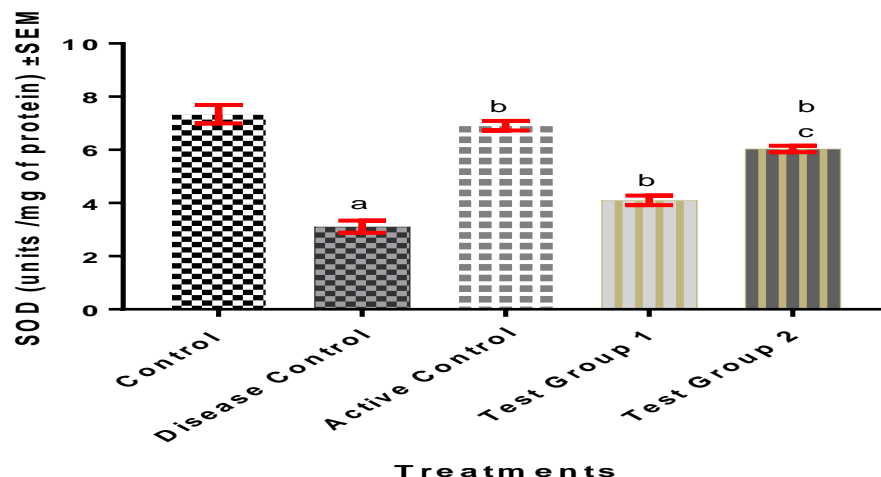


Fig 5.h Effect of *Valproic Acid* on level of Superoxide dismutase in Albino wistar rats.

STZ- Streptozotocin

VA- *valproic acid*

a indicates significant versus vehicle control  $p < 0.001$ .

b indicates significance versus disease control  $p < 0.01$

c indicates significance versus STZ+ VA (150 mg/kg)  $p < 0.05$ .

The statistical analysis was carried out using prism graph pad 7 software. All values are represented as mean  $\pm$  SEM (n=6). Multiple comparison between different groups were performed using one way analysis of variance followed by Tukey's test.

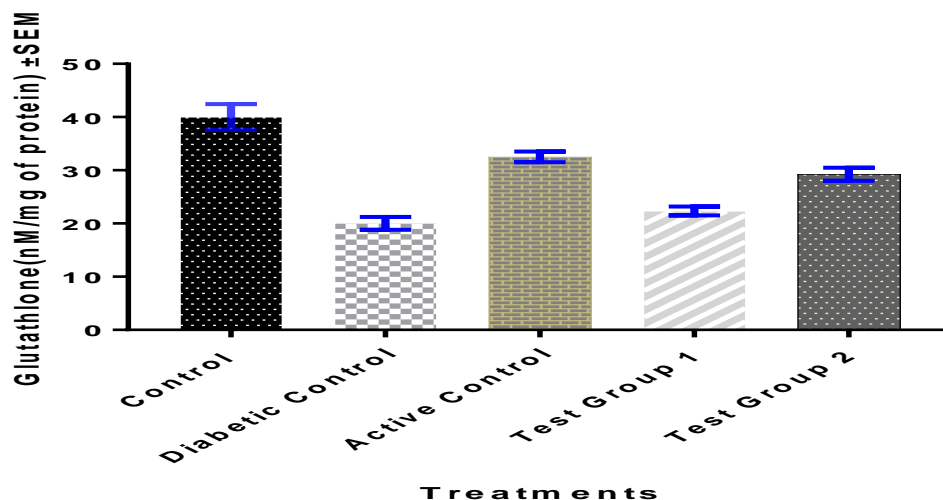


Fig 5.i Effect of *Valproic Acid* on level of Glutathione of Albino wistar rat

STZ=Streptozotocin

VA=Valproic Acid

a indicates significance versus normal control  $p < 0.0001$

b indicates significance versus disease control  $p < 0.001$

c indicates significance versus STZ+VA (150 mg/kg)  $p < 0.001$ ,

The statistical analysis was carried out using prism graph pad 7 software. All values are represented as mean  $\pm$  SEM (n=6). Multiple comparison between different groups was performed using one way analysis of variance followed by Tukey's test

**DISCUSSION:**

Diabetes mellitus is a global health problem and it is increasing constantly worldwide. Diabetes is referred as a metabolic disorder which is characterized by relative or absolute deficiency of insulin secretion or insulin resistance. Diabetes is said to be one of the foremost cause of mortality and morbidity in the world [3]. Diabetes can affect the quality of patient's life and it causes variety of symptoms like pain, weakness, ataxia, impotence and sensory loss. Diabetes is a progressive disease and results in various complications such as retinopathy, nephropathy, neuropathy etc.

Neuropathy is one of the most common complications of diabetes. There are various therapies which are available for the symptomatic treatment of neuropathy but therapies which can eradicate the root cause of disease are less available. Neuropathy is a nerve disorder which involve somatic and autonomic nervous system and it is characterized by hyperalgesia, allodynia etc. [29,30]. Hyperalgesia include reduced motor nerve conduction velocity, elevated nociceptive response, reduced threshold to painful stimuli and neuronal hypoxia. Almost half of diabetic individuals develop diabetic neuropathy. For the symptomatic relief of diabetic neuropathy certain medications are used such as tricyclic antidepressants (TCAs), antiepileptics, NSAIDS, opioids, protein kinase C inhibitors etc. herbal drugs like phenolic compound and evening primrose oil are also used in the symptomatic treatment of diabetic neuropathy.

There are several factors which lead to the development of diabetic neuropathy. Over production of reactive oxygen species or free radicals occurs due to hyperglycaemia and these free radicals leads to neuronal dysfunction and damage. Reactive oxygen species plays important role in development of diabetic neuropathy other factors like generation of advanced glycation end products, mitochondrial dysfunction, activation of NF-kB are also implicated in the development of disease [31].

Tissue damage takes place by free radicals which attack membranes through peroxidation of lipid or unsaturated fatty acid present in membrane. Lipid peroxidation leads to membrane damage and dysfunction. Improved antioxidant level contributes to decreased lipid per oxidation. Antioxidants play a key role in prevention and treatment of neuropathy [32].

Our present study showed rise in the blood glucose level of streptozotocin treated rats. The diabetic rats showed various symptoms of diabetic neuropathy like hyperalgesia, thermal allodynia, cold allodynia, decreased motor coordination as compared to control group.

*Valproic acid* has a antioxidants property. It showed significant improvement in the symptoms of neuropathy in a dose dependent manner. *Valproic acid* significantly reduces lipid peroxidation. This occurs due to antioxidant property of *valproic acid*. It is helpful in oxidative stress induced disease like diabetic neuropathy.

The most profound effect was observed at the dose of 300 mg/kg of valproic acid. valproic acid showed significant decrease in hyperglycemia, hyperalgesia, allodynia, improve motor coordination when compared to diabetic control group. Our study proves that *valproic acid* helps to treat diabetic neuropathy in dose dependent manner.

The neuronal damage lead to diabetic neuropathy and this is due to the excessive generation of free radicals or the ROS. In our body system antioxidant defence are present that inhibit the formation of free radicals that are SOD and GSH etc. Present study evaluate that the oxidative stress which was induced by STZ for 75 days in rat decrease the SOD and GSH and the treatment with the valproic acid increase the level of both the antioxidant enzymes and it may possibly decrease the formation of ROS and nerve cell death.

Lipid peroxidation level in brain of diabetic neuropathy rat is increased. The oxidative stress induced by STZ and it cause increase amount of lipid peroxidation. The administration of valproic acid is effective in reducing the level of lipid peroxidation.

Hyperglycemia induced the oxidative stress and it may lead to neuronal/damage and death. This damage is mainly caused by increase ROS formation, increase MDA level and decrease SOD and GSH level. This overall study shows the antioxidant property of valproic acid reduced the level of lipid peroxidation and increases the level of antioxidants (SOD, GSH) significantly.

**CONCLUSION:**

This experimental study was designed to evaluate the neuroprotective effect of *valproic acid* in diabetic neuropathy. For this study STZ treated albino wistar rat were used to evaluate the effect of drug by inducing type 2 Diabetes in rats by STZ.

The following findings were revealed in the study:

1. *Valproic acid* significantly reduces the symptoms of diabetic neuropathy and show the positive effect.
2. *Valproic acid* showed most profound effect at a dose of 300mg/kg on day 28 of treatment.
3. *Valproic acid* reduce Serum glucose level which effect produced by *valproic acid* (300 mg/kg) on

28 day of continuous treatment was very close to that produced by active control group.

Thus *valproic acid* & its antioxidant property can be concluded to be effective treatment or preventive option for diabetic neuropathy.

- STZ treated rats showed a significant increase in the level of lipid peroxidation which was reversed by the treatment with *valproic acid*. The activity of antioxidants like SOD and GSH in STZ treated group is decreased, the activity is restored after treated with *valproic acid* by reducing the generation of free radicals.

Valproic acid reduces the neuropathic pain because of its antioxidant property. Hence, it was concluded that valproic acid have a potential role in management of diabetic neuropathy.

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