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INVESTIGATION OF NEUROPROTECTIVE EFFECT OF *SAPINDUS MUKROOSSI* EXTRACT ON TYPE1 DIABETE INDUCED NEUROPATHIC PAIN PERCEPTION IN RAT

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ABSTRACT

Diabetic Neuropathy is a most common Microvascular diabetic complication, associated with neuropathic disorder causing nerve injury it mainly depends upon duration of hyperglycemia, elevation in glycosylated haemoglobin. Good Neuropathic pain perception can improve Neuropathy. Single intraperitoneal injection of STZ (60 mg\kg) was administered to the albino wistar rats induced Diabetes, it showed marked hyperglycemia. Hyperglycemia effect Glycated haemoglobin level in blood and reduce and reduce thermal hyperalgesia (Eddy's Hot plate, Tail flick), motor- coordination, as compared to the animals of control group. Effect of test drug Sapindus Mukroosi was assessed by behavioural and biochemical parameters (Serum Glucose Level, Lipid peroxidation, reduced Gluthathione) in the brain tissue of wistar rats. After 28 days treatment of Sapindus Mukroosi with low (250 mg\kg) and higher (500 mg\kg) dose in diabetic rats showed antidiabetic action by reduction in hyperglycemia. Sapindus showed good result also in behavioural and biochemical parameters such as Thermal hyperalgesia, motor coordination in comparison with diabetic control group. The most promising effect of Sapindus seen with 500mg\kg after treating period. It significantly decrease serum glucose level, Glycated haemoglobin, lipid peroxidation and improving reduced gluthathione level as compared to diabetic control rats. Thus Sapindus Mukroosi due to its antioxidant and antidiabetic property can be concluded as a effective or preventive treatment for Diabetic Neuropathy. Streptozotocin induce hyperglycemia and causes increase in oxidative stress and this

conditions are oppose by treatment with Sapindus Mukroosi by inhibit the generation of free radicals and improving self antioxidant GSH level significantly.

KEYWORDS: Diabetic Neuropathy, Glycated Haemoglobin, Neuropathic Pain.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. Diabetes mellitus is a serious health problem in developing as well as developed countries. The world health organization stated it as an epidemic disease of the 21st century, due to its rising population of 382 million in 2013, and May projected to rise up to 592 million by the year 2035. Uncontrolled diabetes leads to microvascular and macrovascular complications. The most common type of microvascular complication of diabetes is peripheral neuropathy. The prevalence rate is 7% in one year of freshly diagnosed diabetes and 50% in long standing diabetes history patients aged more than 25 years. In them 12% patients experience painful diabetic neuropathy with generating the symptoms of allodynia-stimulus that normally doesn't provoke pain, hyperalgesia increased the response to a non painful stimulus. Patients explain their symptoms as a sharp electric shock shooting their legs, and feeling of walking on broken glass. Hyperglycemia induces fatal changes in nerve tissue, a verity classical metabolic pathways; polyol pathway resulting in accumulation of sorbitol and glucose, increased hexosamine shunt; excess/inappropriate activation of protein kinase C isoforms; resulting in accumulation of advanced glycation end products; weaken neurotrophic support and disrupt the repair mechanism; and stimulation of poly (ADP-ribose) polymerase (PARP); result in imbalanced nerve myo-inositol. Although all of these pathways fatal in the generation of reactive oxygen species through oxidation and reduction reactions in mitochondria lead to aggravation of neuropathy.[1,2,3,4]

Several drugs such as tricyclic antidepressants and antionvulsant drugs are presently available to reduce the neuropathic pain. However, these drugs were reported to exhibit a wide spectrum of adverse effects in the management of painful neuropathy. Hence, there are a limited number of ideal medicines to treat diabetes neuropathy and its generating pain. Researchers, health care professionals and educated people acknowledge their interest towards new alternative medicines to treat diabetes associated neuropathy.[5,6]

The present chosen trailing herb bearing a Latin name Sapindus Mukroosi (Sapindaecae) is a creeping herb grown in tropical regions of, India. Pharmacological studies on Sapindus fruit

pericarp were proven its antioxidant, antidiabetic, hepatoprotective, antifibrinolytic and anti-inflammatory activities in experimental animals, due to presence of various phytochemicals like flavonoids, amino acids, liriodendrons (lignans), β-sistosterol and stetracosanoic acid, ecosanoic, steroic and urosolic acid have proven capacity to cure and control disease prognosis. Lack of scientific data on neuroprotective activity of Sapindus Mukroosi against diabetic neuropathy was gaining my attention towards this experiment.[7,8]

MATERIAL AND METHOD

Plant Material

Collection and Authentification

Plant Material were collected from the wild area of Garhwal in the month of and authenticated from Botanical Survey of India (BSI), Dehradun. The specimen authentication No. is BSI\NRC Tech.\Herb (Ident.)\2016-17\830.

Method of Extraction The roots of Boerhaavia diffusa were collected and dried under shade and grinded into powder. Ethanolic extract of Boerhaavia diffusa roots was done in the department of Pharmacology, Geetanjali Medical College, Udaipur using cold maceration The Fruits of Sapindus Mukroosi were collected and washed under running tap water for removal of dust. Pericarp (separated from fruits) and leaf was shade dried, oven dried at 40–45◦C for 2h, and then grinded in mechanical grinder to make coarse powder. Aqueous extract of Sapindus Mukroosi pericap was done in department of pharmacology S.G.R.R.I.T.S Dehradun using hot percolation extraction process.[11,13]

Drugs and Chemicals: Streptozotocin was obtained from Sisco research laboratories Pvt. Ltd, Mumbai, India and Duloxetine was perchased from Swapnaroop drugs & pharmaceuticals, Aurangabad, Maharastra, India. All other chemicals and reagents used were of analytical grade.[15,17]

Procurement of Animals

Wistar rats weight 250-300gm of either sex was procured from the departmental animal house Department of Pharmaceutical Sciences of Shiri Guru Ram Rai Institute of Technology and Sciences, Patel Nagar, Dehradun. The Animal were acclimatized in the departmental animal house facility and housed n=6 per cage under standard laboratory conditions 23±2oC with 12hr light dark cycle and had free accesses to water with standard chow diet. Animal care should be taken as per guidelines of the Committee for the Purpose of Control and

Supervision of Experiments on Animals (CPCSEA). Approval was taken from the Institutional Animal Ethics Committee264/ReBi/2002/CPCSEA) for the study.

METHODOLOGY

Experimental Design

2 Experimental Design

5 groups each comprising of 6 animals

Group 1: Control: Normal Saline was administered as a Vehicle.

Group 2: Diabetic control: Streptozotocin (60 mg/kg) was administered.

Group 3: Active Control: Duloxetine was administered for 28 days in diabetic wistar rat as standard.

Group 4: Test Group: Diabetic neuropathy induced wistar rat + SME (250 mg/kg) was administered for 28 days.

Group 5: Test Group: Diabetic neuropathy induced wistar rat + SME (500 mg/kg) was administered for 28 days.

Streptozotocin induced diabetes diabetic neuropathy

Healthy wistar strain albino rats of either sex weighing about 150-200 grams were taken. Animals were deprived to food for 16 hours but allowed free access to water after that blood sample was collected from tail of rats and measure blood glucose level by using GOD-POD kit method. Then they were injected with streptozotocin dissolved in 0.1M sodium citrate and citric acid at a dose of 55 mg/kg body weight intraperitoneally. Then animals were kept for 21 days during which food and water was allowed. After 21days of streptozotocin administration blood glucose level, body weight, grip strength and pain sensation measurements were taken. The animals showed fasting blood glucose level above 250 mg/dl considered diabetic rats and after that they were divided into five groups in which each group contained six animals. the blood glucose level, body weight, measurements of each rat were taken at the start and at the end of experiment. After the 4th week of the experiment administered the control, standard and test drug orally daily for 8 weeks.[20,25]

Physical parameters

I. Grip-strength (Rota-rod) test

Grip strength used for evaluation of muscle strength during Diabetic Neuropathy. The test was being used to assess muscular strength or neuromuscular function with the help of rota rod apparatus in which the rats were placed on a horizontal rod rotating at a speed of 25 rpm.

The rats which were capable of remaining on the top for 25 sec or more, in three successive trials were selected for the study. The selected animals were divided into five groups (n= 6). The time of stay at the rod was calculated.[26,27]

II. Tail Flick

All groups of animals were experimented for this test. Animals are placed into individual restraining cages leaving the tail hanging out freely. The animals are allowed to adapt to the cages for 30 min before testing. The lower 5 cm portion of the tail is marked and the tail of the rat was immersed in hot water maintained at 55±0.2°C. the basal tail flick latency (withdrawal response of tail) or signs of struggle were observed. The cut off time was 10 sec.[28,29]

III. Hot Plate Method

In this Hot Plate Method animals from the each group were placed on the hot plate which is commercially available, consists of an electrically heated surface. Temperature of this hot plate is maintained at 55-56 ˚C and observation is done up to the time until paw licking or jumping was noted the cut-off time was 10 sec. Then the average basal reaction time was noted after the oral administration of the drugs and test compounds.[30,31]

Bio-chemical Parameters

I. Blood Glucose Level

The animal blood was collected from tail of rats for the determination of the blood glucose levels. The plasma was obtained after centrifugation (3000 g for 10 min, 4°C). Blood glucose was estimated by GOD-POD kit method.[32,33]

II. Lipid Peroxidation Assay

This assay was used to determine thiobarbituric acid-reactive substances as described by Slater and Sawyer (1971). In 2.0 mL of the tissue homogenate (supernatant) was added 2.0 mL of freshly prepared 10% w/v trichloroacetic acid (TCA) and the mixture was allowed to stand in an ice bath for 15 min, followed by centrifugation at 2500 rpm for another 15 min at 4°C. Two milliliter of clear supernatant solution was mixed with 2.0 mL of freshly prepared 0.67% w/v TBA. The resulting solution was heated in a boiling water bath for 10 min. It was then immediately cooled in an ice bath for 5 min. The absorbance of colour developed was measured by UV/VIS double beam spectrophotometer (systraonic Japan) at 532 nm using 1, 1, 3, 3-tetraethoxypropane as a standard.[34,36]

III. Estimation of Glutathione

The assay of GSH was determined by method described by Moron et al. (1979). One milliliter of tissue homogenate (supernatant) and 1.0 mL of 20% TCA were mixed and centrifuged at 2500 rpm for 15 min at 4°C. In 0.25 mL of supernatant, 2 mL of DTNB (0.6 M) reagent was added. The final volume was made up to 3 mL with phosphate buffer (pH 8.0). The colour developed was read at 412 nm against reagent blank. Different concentrations (10-50 µg) of standard glutathione were processed as mentioned above for constructing standard curve. The amount of reduced glutathione was expressed as µg of GSH /mg of protein.[40,41]

IV. Estimation of Glycosylated Heamoglobin (Ion Exchange Method)

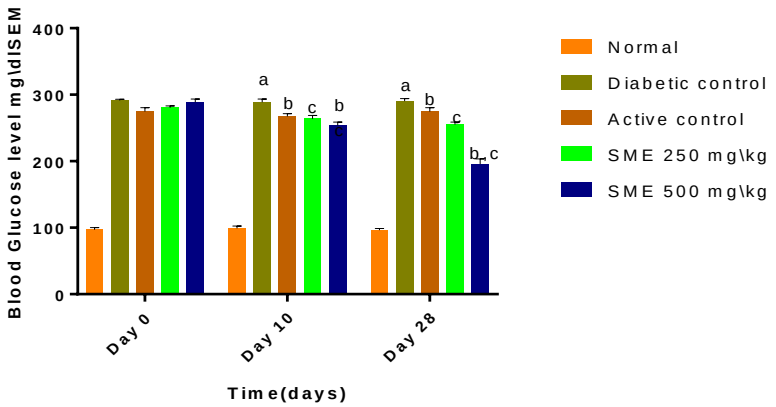
Whole blood is Mixed with lysine reagent to prepare a hemosylate. It then mixed with a weakely binding cation exchange resin. Resin bind with non-glycosylated hemoglobin and forming GHb free in supernatant. This percentage is examined by calculating the absorbance of fraction & of the total Hb level.[42,44]

Statistical Analysis: The data obtained from the result was analyzed by using twp ways ANOVA followed by Bonferroni's post test using graph pad prism 7 software. All data were expressed as the mean SEM of their parameter.[45,50]

RESULTS

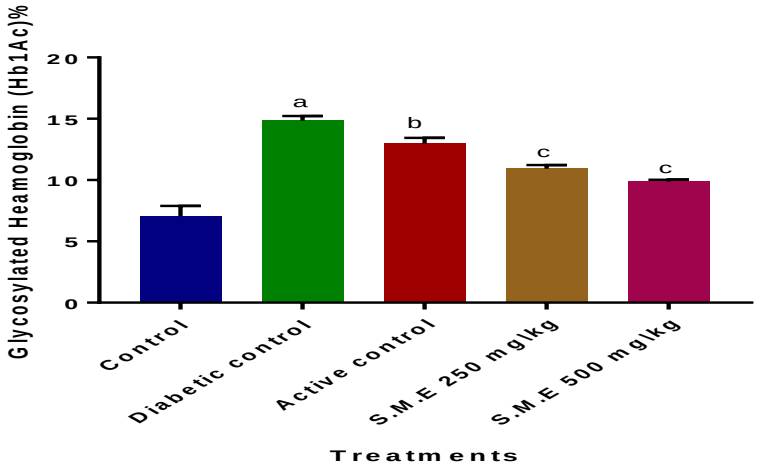
- **Effect of Sapindus Mukroosi on serum glucose level in STZ induced diabetic rats**

Serum glucose level was estimated on 0^{th}, 7^{th}, 14^{th}, 28^{th}, and 75^{th}, 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. Sapindus Mukroosi in two different doses 250 mg\kg, 500 mg\kg was administered to diabetic rat and it was observed that the Serum Glucose level reduced as estimated after 10^{th} and 28^{th} days and effect was found significant.



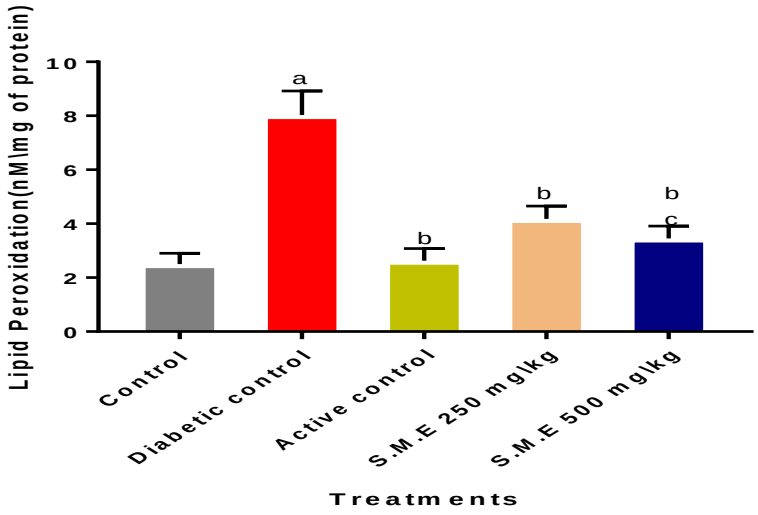
- **Effect of Sapindus Mukroosi on Glycosylated Heamoglobin in STZ induced diabetic rats**

Hyperglycemia produces a significant increase in Glycosylated Heamoglobin level in comparison with vehicle control group. However treatment with Sapindus Mukroosi significantly decreases Glycosylated Heamoglobin level and effect was found significant.



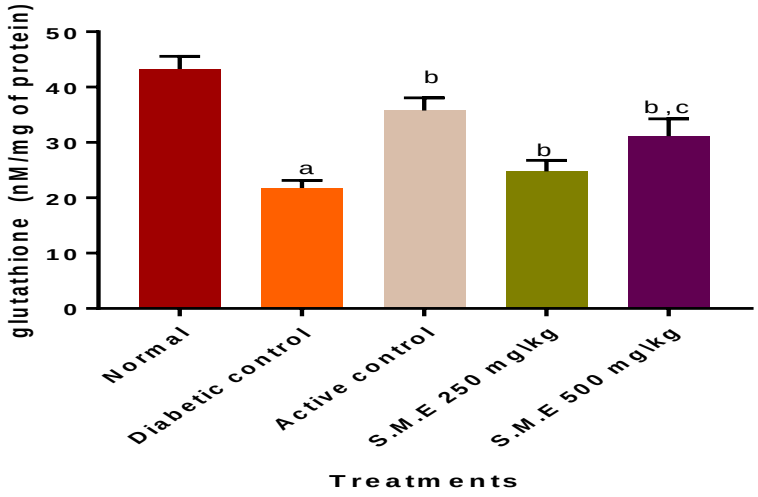
- **Effect of Sapindus Mukroosi on level of lipid peroxidation in STZ induced diabetic rats**

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 SM (250 & 500 mg\kg) significantly reduces the lipid peroxidation level as compare to disease control. Standard treatment with Duloxetine significantly reduces the level of lipid peroxidation in brain.



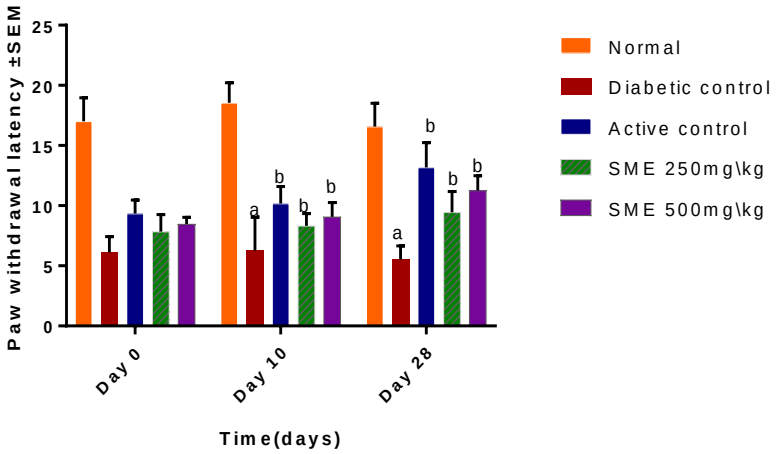
➢ **Effect of Sapindus Mukroosi on level of Gluthathione in STZ induced diabetic rats**

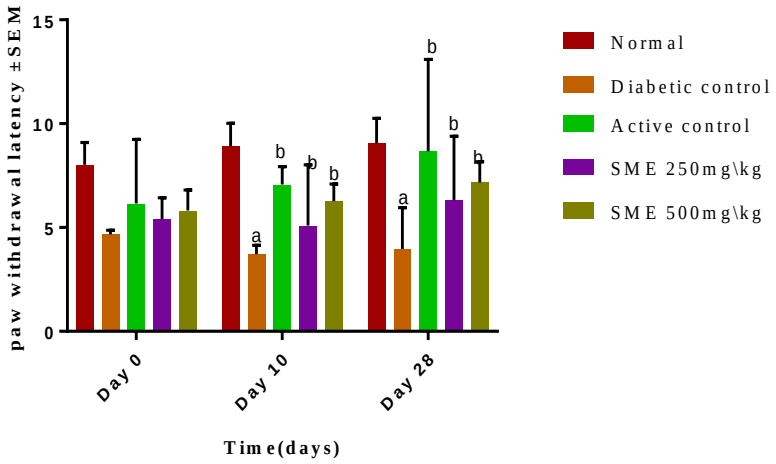
Administration of STZ produces a significant decrease in gluthathione level in comparison with vehicle control group. However treatment with Sapindus Mukroosi significantly increases the gluthathione level in brain. In other side the Standard treatment with Duloxetine significantly increase the level of gluthathione.



➢ **Effect of Sapindus Mukroosi on thermal allodynia\ hyperalgesia (Hot plate, tail flick) STZ induced diabetic rats**

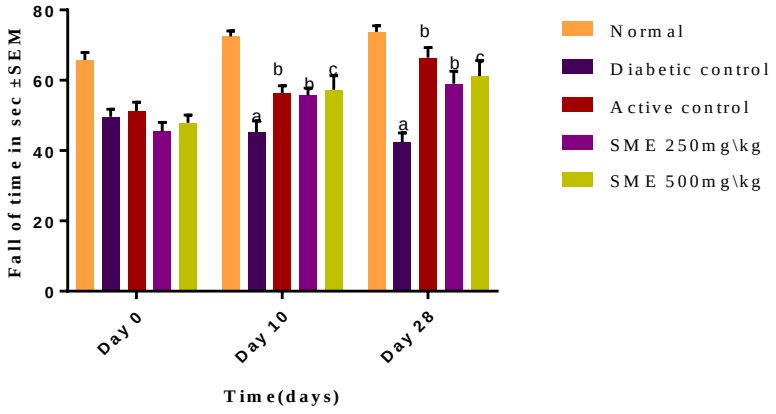
Thermal Hyperalgesia test were performed 28 days after STZ administration and decrease in tail flick and paw withdrawal latency was observed. After Administration of Sapindus Mukroosi resulted in significant increase in tail flick and paw withdrawal latency on 28 days treatment as compared to Diabetic control group.





➢ **Effect of Sapindus Mukroosi on motor-coordination in STZ induced diabetic rats**

Motor coordination in Streptozotocin treated rat after 28 days STZ administration significantly decrease. Sapindus Mukroosi administration resuled in improved Motar coordination grip strength and positive effect was observed as compared to Standard drug (Duloxetine) treated group.



DISCUSSION

Diabetes mellitus (DM) is a group of metabolic disease which is characterized by persistent hyperglycemia, glycosuria, hyperlipidemia, and negative nitrogen balance and also frequently ketonaemia on account of defects in the insulin secretion and insulin action, or both of these leading to impaired functioning in the carbohydrate, lipid and protein metabolism which furthermore leads to increase fasting and postprandial blood glucose levels.Diabetic neuropathy is a multifaceted and possibly severe complication of diabetes affecting more than 50% of the diabetic individuals.

Present study showed rise in blood glucose level of STZ treated rats.the aqueous root extract of Sapindus Mukroosi (250, 500 mg\kg) showed significant reduction in serum glucose level and immobility time in pain perception test of diabetic rats. Antioxidant Sapindus Mukroosi extract improved brain gluthathione level And diminished the lipid peroxidation and Glycosylated Heamoglobin level as compared to control group. It showed significant improvement in signs of neuropathy. Essentially the most profound effect was observed at the dose of 500mg\kg of SME.

The experimental study was designed to evaluate the Neuroprotective effect of Sapindus Mukroosi in diabetic neuropathy. For this study STZ treated albino wistar rat were used to evaluate the effect of drug in Type1 diabetes induce neuropathic pain perception.

CONCLUSION

The following finding was revealed in the study.

- Sapindus Mukroosi significantly reduces the symptoms of diabetic neuropathy and show the positive effect. its antioxidant property reduced the blood glucose level as well as Glycated Heamoglobin & increase pain perception in Streptozotocin-induced diabetic rats to prevent progression of diabetic neuropathy.
- Sapindus Mukroosi showed most effect at a dose of 500 mg\kg on day 28 of treatment.
- STZ treated rats showed a significant increase in the level of lipid peroxidation which was reversed by the treatment with SME. The activity of antioxidants like GSH in STZ treated group is decreased, the acitivity is restored after treated with Sapindus Mukroosi by reducing generation of free radicals. So it can be concluded that SM used as ideal drug which could offer a better preventive option for diabetic neuropathy. This can act by, either preventing the nerve damage or by providing symptomatic pain control along with good glycemic control.

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