AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Holarrhena antidysenterica Extract Promotes Recovery of Peripheral Neuropathy In Diabetic Rats

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ABSTRACT

This study explored the effect of bark of *Holarrhena antidysenterica* Linn in management of diabetic neuropathy in experimental animals. Adult Wistar rats (either sex; 250-275 g) were injected with streptozotocin (50 mg/kg; i.p.) to induce diabetes. Methanol extract of bark of *Holarrhena antidysenterica* was administered in 3 doses (200, 400 and 600 mg/kg; p.o.) to rats for 28 successive days daily after 4 weeks of STZ administration. After 8 weeks, the neuropathic activity was evaluated using Open field test, Tail Flick test, Cold Allodynia and Formalin test. Afterwards, sciatic nerve was used for TBARS, GSH, Nitrite, Catalase and protein estimations. STZ induced diabetic neuropathy caused decrease in tail-flick latency time in radiant heat test and decreased allodynic response in tail-immersion (cold water) test. STZ caused increase in blood glucose, Glycosylated Haemoglobin and blood Cholesterol levels. Furthermore, activity of endogenous antioxidants like GSH and catalase significantly decreased; however, TBARS and nitrite levels were increased. Administration of MEHA for 28 days prevented the development of diabetic neuropathy as evident from reversed ($p< 0.05$) cold allodynia and tail flick latency ($p< 0.05$) as compared to diabetic control group. Glycosylated haemoglobin and cholesterol levels were significantly decreased ($p< 0.05$) in rats as compared to diabetic control group. MEHA treated rats showed significant decreased TBARS and nitrite levels and increased GSH and Catalase level. Thus, *Holarrhena antidysenterica* not only improved the diabetic condition but also reversed neuropathic pain through modulation of oxidative–nitrosative stress.

Keywords: *Holarrhena antidysenterica*, neuropathy, diabetes, streptozotocin, oxidative stress

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Received 14 July 2016, Accepted 01 August 2016

Please cite this article in press as: Bansal N *et al.*, Holarrhena Antidysenterica Extract Promotes Recovery Of Peripheral Neuropathy In Diabetic Rats . American Journal of PharmTech Research 2016.

INTRODUCTION

Diabetes mellitus describes is a metabolic disorder characterized by chronic hyperglycaemia due to defects in insulin deficiency, secretion or action. Diabetic people are more prone to get affected with cardiovascular and cerebrovascular diseases[1]. The long term complications of diabetes mellitus include retinopathy, nephropathy and/or neuropathy and autonomic dysfunction. These complications are also found in non-diabetic population, but have a two to five-fold increase in diabetic subjects. The treatment options for diabetes include insulin and oral hypoglycemic agents such as sulfonylureas, biguanides and α-glucosidase inhibitors[2]. International Diabetes Federation and WHO report says that the worldwide prevalence of diabetes is expected to increase from 382 million people in 2013 to 592 million by 2035[3].

Neuropathic disorders are one of complications of diabetes and are result of microvascular injury involving blood capillaries that supply blood to nerves are injured. The main conditions, which are associated with diabetic neuropathy, include third nerve palsy, autonomic neuropathy, mononeuropathy multiplex, a painful polyneuropathy, thoraco-abdominal neuropathy and diabetic amyotrophy. In diabetic patients, glucose dysmetabolism plays an important role in the development of diabetic neuropathy. Hyperglycemia causes accumulation of polyols in nerves, leading to neuropathy. Furthermore, hyperglycemic neurons are the source of production of reactive oxygen species, which can cause damage of DNA and membranes, impairment of cell functions and might lead to nerve cell degeneration[4]. The neuropathy progresses with decreasing nerve functionality and nerve blood perfusion which may result in malnourished nerve and leads to permanent nerve damage[5]. Painful symptoms such as burning, tingling (‘pins and needles ‘or paraesthesia), shooting (like electric shock) or lancing (stab-bing) are present in around one third of patients with DN and around one-fifth of all diabetic patients[6]. DN starts in the toes and gradually moves proximally. Once it is well established in the lower limbs, it affects the upper limbs, with sensory loss following the typical ‘glove and stocking’ pattern of distribution[7]. Significant motor deficits are not common in the early stages of DN. These symptoms are generally worse at night and disturb sleep[8].

Treatment of DN is based on either pathogenetic mechanisms or symptomatic relief. A number of clinical trials have established symptomatic treatment but for pathogenetic mechanisms, the only proven treatment strategy is strict glycemic control. As glycemic variability leads to oxidative stress, therefore the therapeutics having ability to control glucose levels, holds potential for attenuating DN. Other drugs like anticonvulsants, antidepressants, topical agents,

and opioid based therapies are used to treat the neuropathy[2]. Lamotrigine is a new anticonvulsant which blocks voltage gated sodium channels, decreases presynaptic calcium currents to inhibit the release of glutamate, and increases GABA levels in the brain[9]. Gabapentin is widely used for neuropathic pain due to its effectiveness and relatively fewer side effects than TCA and other anticonvulsants. Gabapentin produces analgesia via binding to the α2-δ site of L-type voltage gated calcium channels and decreasing calcium influx[2]. Plants are rich source of secondary metabolites like flavonoids, alkaloids, terpenoids, tannins etc. and that have been implicated in several therapeutic approaches. Plants and parts of plants including the active chemical constituents, mechanism of action of active constituents responsible for attenuation of hyperglycemia, oxidative stress and amelioration of diabetic complications[6]. The chief lines of therapy for treatment of diabetic neuropathy are alpha-lipoic acid, acetyl-L-carnitine, benfotiamine and methylcobalamin[10].

Holarrhena antidysenterica Linn is commonly known as Tellicherry Bark (English) and Kurchi (Hindi) and belongs to family Apocynaceae. The plant is found in tropical and subtropical regions of Asia and Africa. In India, it can be found throughout the country, especially in deciduous forests of tropical Himalayas, at altitudes ranging from 900 to 1250 m[11]. The stem bark of *Holarrhena antidysenterica* Linn contains conessine ($C_{24}H_{40}N_2$), isoconessine ($C_{24}H_{40}N_2$), conessimine/ isoconessimine ($C_{23}H_{38}N_2$), conarrhimine ($C_{21}H_{34}N_2$)[12]. The bark *Holarrhena antidysenterica* is indicated to be used as an astringent, anthelmintic, stomachic and diuretic. It is a well known drug for chest infections, amoebic dysentery, diarrhea, indigestion, flatulence and colic[13]. Ethanol extract of seeds of *H. antidysenterica* Linn in castor oil induced diarrhea in rats in vivo has shown a significant increase in the dry weight of their faeces and reduction in defecation drops[14]. Aqueous and alcoholic bark extracts are also known to act against *enteroinvasive E. coli (EIEC), Shigella flexneri, Shigella boydii and Salmonella enteritidis*[12]. *H. antidysenterica* Linn methanol leaf extracts was found to scavenge superoxide ions and hydroxyl ions[15]. Methanol extract of bark of *Holarrhena antidysenterica* Linn has shown inhibition of acetylcholinesterase activity in the brain of Swiss albino mice[16].

However, no sufficient studies have been carried out to explore the role of *Holarrhena antidysenterica* Linn as in the treatment of diabetic neuropathy, to best of our knowledge. Therefore, the present study was undertaken to elucidate the Effect of *Holarrhena antidysenterica* Linn on Experimental Diabetic Neuropathy in Rats.

MATERIALS AND METHOD

Preparation of extract

The bark of *Holarrhena antidysenterica* Linn was shade dried and it was powdered with gridding process using grinder. Then, coarse powder was separated with sieve and 250 g of powdered bark was packed in Soxhlet apparatus and defatted with petroleum ether (40-60ºC) for 72 hours. Then, the defatted bark powder was extracted with methanol as a solvent in Soxhlet apparatus. The temperature was maintained on an electric heating mantle with thermostat control. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was concentrated to syrupy consistency using water bath. The concentrated extract was then air dried at room temperature and stored in air tight container at 2–8ºC until used[17].

Animals

Adult Wistar rats (either sex), weighing between 250-275 g, were procured from the Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Science Hisar. The animals were kept in quarantine section till monitoring of health status of animals and subsequently transferred to the housing area. The animals were acclimatized for seven days to the housing conditions of Central Animal House Facility of ASBASJSM College of Pharmacy, BELA prior to experiments. Animals were housed in polypropylene cages with dust free rice husk as a bedding material and maintained under standard laboratory conditions with controlled temperature (23 ± 2°C), humidity (40 ± 10%) and natural (12 h each) light-dark cycle. The animals were fed with standard rodent pellet diet (Ashirwad Industries, Mohali) and water *ad libitum.* The experiment was carried out between 09:00 and 18:00 h. The care of laboratory animals was done following the guidelines of CPCSEA, Ministry of Forests & Environment, Government of India.

Drugs and Chemicals

Holarrhena antidysenterica was procured from the Rajesh chemicals, Mumbai. Streptozotocin was purchased from SRL, Mumbai. Gabapentin was procured from INTAS Pharmaceuticals, Sikkim. DTNB, Thiobarbituric acid purchased was from Himedia laboratories, Mumbai. Reduced glutathione, 5,5′-dithiobis (2-nitrobenzoic acid), bovine serum albumin, thiobarbituric acid, Tris buffer, sucrose, trichloroacetic acid, citric acid monohydrate, sodium nitrate, copper sulfate, sodium potassium tartarate, tri-sodium citrate, Folin's phenol reagent, sodium hydroxide procured from Hi-media.

Induction & assessment of diabetes

A single intraperitoneal injection of streptozotocin (50 mg/kg body weight freshly dissolved in 0.1 mol/L citrate buffer, pH 4.4) was given to rats for induction of diabetes[18]. The blood glucose was measured after 48 h of streptozotocin injection, the blood samples were collected via retro-orbital plexus technique using heparinized capillary glass tubes. Animals showing blood glucose more than 250 mg/dl were considered as diabetic and were maintained for 8 weeks (56 days) for diabetic neuropathy studies. Control rats received vehicle equal volume of citrate buffer. The body weight and blood glucose levels were measured before and at the end of the experiment[19].

Experimental Design

All the animals were divided into six groups of six animals. Four weeks after the neuropathy induction on 29th day the treatment with *Holarrhena antidysenterica* was given for 4 weeks orally.

Group I – (Normal Control): Normal saline was given for 28 successive days.

Group II – (Diabetic (STZ) Control): STZ 50 mg/kg i.p. + Citrate buffer was administered for 28 successive days.

Group III – (STZ + MEHA200): STZ 50 mg/kg i.p. + Methanol extract of *Holarrhena antidysenterica* (200 mg/kg/p.o) for 28 days.

Group IV – (STZ + MEHA400): STZ 50 mg/kg i.p. + Methanol extract of *Holarrhena antidysenterica* (400 mg/kg/p.o) for 28 days.

Group V – (STZ + MEHA600): STZ 50 mg/kg i.p. + Methanol extract of *Holarrhena antidysenterica* (600 mg/kg/p.o) for 28 days.

Group VI – (STZ + Gabapentin): STZ 50 mg/kg i.p. + Gabapentin (100 mg/kg/i.p) for 28 days.

After the 4 weeks of the treatment at the 8th week (56th day), the animals were subjected to behavioral assessments. The animals were sacrificed by decapitation and sciatic nerve was isolated for estimating the biochemical parameters.

Estimation of blood glucose level

Blood was collected from retro-orbital of rats for the determination of the blood glucose levels. After the sample collection the blood glucose was determined by Accu-Check Active Strips[20].

Glycosylated Haemoglobin (HbA1c)

Blood was collected by puncturing retro-orbital plexus under mild ether anaesthesia by using fine glass capillary in ependorff tubes. Serum was separated by centrifugation and buff coat (packed blood cells) was taken, Wash the packed cells by using normal saline at least six times

and these packed blood cells were used to measure HbA1c at absorbance 532 nm by using U.V/Visible spectrophotometer (Shimadzu 1700, Singapore)[21,22].

Estimation of Cholesterol level

The samples were analyzed for plasma Cholesterol levels using Cholesterol solution kit provided by Erba mannchim Pvt. Ltd. Mumbai, India.

BEHAVIORAL ASSESSMENT

Tail flick test

Acute nociception was induced by tail flick apparatus. Briefly, each rat placed in a restrainer and the tail flick latency (TFL) was determined by placing the tail of rat near to red hot wire and the time taken to remove the tail from the noxious thermal stimulus. Cut off time was kept at 10-12 sec for each animal, 2 to 3 recordings were made at an interval of 15 min; the mean value was used for statistical analysis[23].

Cold Allodynia (Tail-immersion test)

The animals were trained for 3 days prior to test. Rat tail was immersed in cold (10 ± 0.5^0C) water and the tail flick response latency (withdrawal response) or any signs of struggle were observed as the end point response. Cut off time was kept at 15 sec. The shortening of tail withdrawal time indicates hyperalgesia. The test was repeated 3 times within 30 min and mean was taken as final response[18].

Open field test

The apparatus consisted of a square arena [56 × 56 cm] made of wood, side walls height of 20 cm and its floor was divided by lines into 16 equal squares forming central and peripheral parts. At the beginning of the session, each rat was placed in the centre of the arena and its activity was recorded for 5 min and the following behavioral parameters were then scored: Number of squares crossed; immobility time [s]; frequency of rearing and grooming. At the end of each session, rats were returned to home cage and the apparatus was cleaned and dried [24].

Formalin Test

Behavioral responses to noxious chemical stimuli were measured using the formalin test. Briefly, rats received a subcutaneous injection of freshly-prepared formalin (50 µ1 of 0.5% solution in sterile saline) into the dorsal surface of the right hind paw. This concentration of formalin induces sub-maximal behavioral responses in control rats and allows detection of hyperalgesia in diabetic rats during Q phase and 2 phase. Animals were transferred to an observation chamber constructed to allow continuous visualization of the paws. The number of flinches during one minute periods were counted at 5 min intervals for the next 60 min[25].

BIOCHEMICAL ESTIMATIONS

Sciatic nerve homogenate preparation

All animals were sacrificed at the end of study i.e. 8th week and sciatic nerves were immediately isolated. Sciatic nerve was removed bilaterally from the inguinal ligament to its trifurcation.[18] Tissue homogenates were prepared with 0.1 M Tris-HCl buffer (pH 7.4) and supernatant of homogenates was employed to estimate thiobarbituric acid reactive substances (TBARS)[26], reduced glutathione (GSH)[27], Catalase[28], nitrite level[29], protein estimation[30].

Measurement of lipid peroxidation

Sciatic nerve was removed bilaterally from the inguinal ligament to its trifurcation. Nerve then homogenized with 0.1 M Tris-Hcl buffer (pH 7.4) and supernatant was used for the measurement of thiobarbituric acid reactive substances (TBARS) at absorbance 532 nm by using U.V/Visible spectrophotometer (Shimadzu 1700, Singapore)[26].

Measurement of GSH

The GSH assay was performed by the method Ellman et al. Supernatant was used for the measurement of GSH at absorbance 412 nm by using U.V/Visible spectrophotometer. The concentrations were determined using a standard curve of reduced glutathione and the results were expressed as µM/ml[27].

Measurement of catalase activity

The assay mixture consisted of 3 ml of H_2O_2, phosphate buffer and 0.05 ml of supernatant of tissue homogenate (10%), and the change in absorbance was recorded at 240 nm. The results were expressed as micromoles of H_2O_2 decomposed per milligram of protein/min[28].

Estimation of nitrite level

The nitrite levels were estimated by the acidic Griess reaction after reduction of nitrate to nitrite by vanadium trichloride according to the method described by Green et al. The concentrations were determined using a standard curve of sodium nitrate and the results were expressed as µM/ml[29].

Protein estimation

Protein concentration was estimated according to the method of Lowry et al. using BSA (bovine serum albumin) as a standard[30].

Statistical analysis

All the results are expressed as Mean ± SEM. The data of all the groups were analyzed by one-way ANOVA followed by Tukey's test using software Graph Pad Prism 6 (Graph Pad Software Inc., USA). A value of $P<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Streptozotocin induced diabetic neuropathy is a very common and intrinsic model of neuropathy. The cytotoxic action of streptozotocin is mediated by reactive oxygen species, liberation of the toxic amounts of NO, alkylation and damage of DNA which result in rapid destruction of pancreatic β-cells[32]. It is an excellent model to study the molecular, cellular and morphologic changes in brain and sciatic nerve induced by free radicals generation[33]. In the present study, the administration of STZ lowered ($p < 0.05$) the body weight of diabetic control rats when measured after 8 weeks (56 days). STZ administration caused significantly ($p < 0.05$) higher blood glucose level of diabetic control rats (514.37 ± 6.87 mg/dl) as compared to normal control rats (96.57 ± 1.05 mg/dl) (Table 1). Diabetic rats also showed higher glycosylated haemoglobin (HbA1c) (7.99 ± 0.07%) and cholesterol levels (218.44 ± 2.51 mg/dl) as compared to non-diabetic rats (Table 1).

Table: 1 Effect of MEHA on general characteristic in STZ-induced diabetic neuropathic rats

Treatment	Body weight (gm)	Blood Glucose (mg/dl)	HbA1c (%)	Cholesterol (mg/dl)
Normal Control	267.2 ± 4.71	96.57 ± 1.05	4.31 ± 0.07	112.93 ± 3.30
Diabetic Control	158.5 ± 2.26 [a]	514.37 ± 6.87 [a]	7.99 ± 0.07 [a]	218.44 ± 2.51 [a]
STZ + MEHA200	162.2 ± 2.09	491.55 ± 9.82	7.59 ± 0.05	212.96 ± 3.67
STZ + MEHA400	175.5 ± 2.10 [b]	363.97 ± 2.1 [b]	6.66 ± 0.15 [b]	155.33 ± 3.01 [b]
STZ + MEHA600	185.8 ± 2.54 [b]	306.04 ± 2.58 [b]	5.84 ± 0.03 [b]	124.5 ± 3.66 [b]
STZ + Gabapentin	164.7 ± 2.63	515.12 ±1.37	7.38 ± 0.03	214.86± 2.66

Values are expressed as mean ± S.E.M by one way ANOVA followed by Tukey's Test. MEHA200 (methanol extract of Holarrhenaantidysenterica 200 mg/kg); MEHA 400 (methanol extract of Holarrhenaantidysenterica 400 mg/kg); MEHA600 (methanol extract of Holarrhenaantidysenterica 600 mg/kg). [a]denotes $p < 0.05$ vs. Normal Control; [b] denotes $p < 0.05$ vs. Diabetic control

Chronic hyperglycemia leads to reduced threshold of pain due to increased oxidative stress, advanced glycated end product (AGE), voltage gated Na^+ channels, and inflammatory mediators. STZ administered rats showed ($p < 0.05$) the decrease in tail flick latency (Figure 1) and tail withdrawal latency (Figure 2) after 8 weeks as compared to normal rats, indicating induction of diabetic neuropathy.

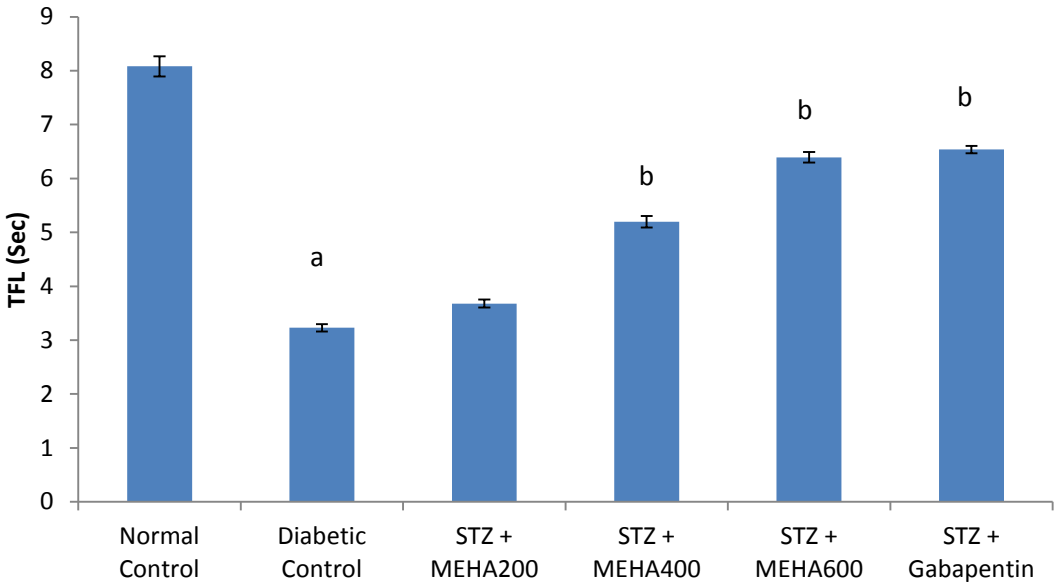

Figure 1: Effect of MEHA on Tail Flick latency (TFL) in rats

Values are expressed as mean ± S.E.M by one way ANOVA followed by Tukey's Test. [a] denotes p< 0.05 vs. Normal Control; [b] denotes p< 0.05 vs. Diabetic control.

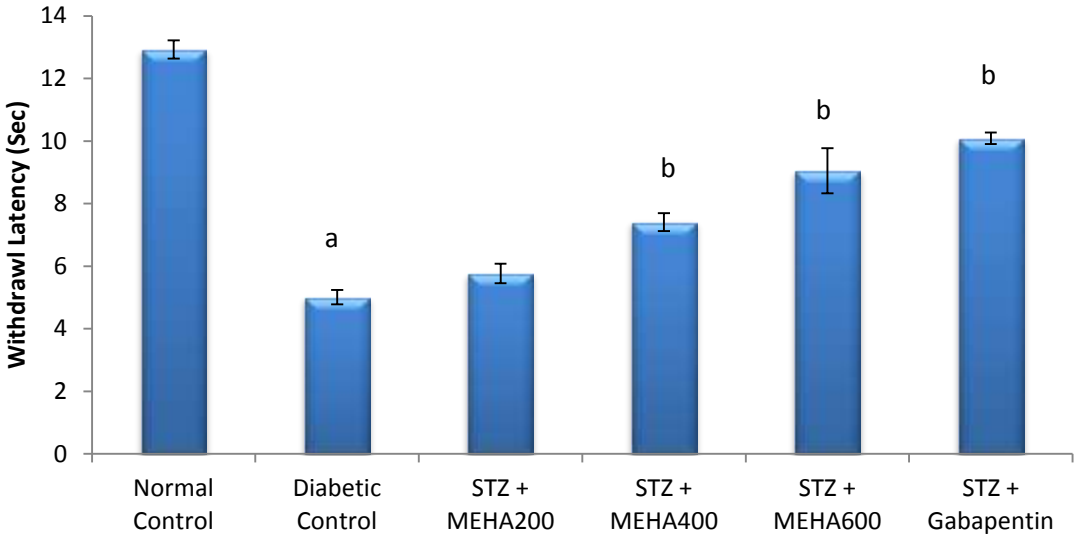

Figure 2: Effect of MEHA on Tail immersion (Cold Allodynia) Test in rats

Values are expressed as mean ± S.E.M by one way ANOVA followed by Tukey's Test.

[a] denotes p< 0.05 vs. Normal Control; [b] denotes p< 0.05 vs. Diabetic control.

Furthermore, STZ administration exhibited (p<0.05) the decrease in number of squares crossed and frequency of rearing score and caused the higher immobile duration as compared to normal control group as shown in figure 3. However, there was no statistically significant difference in grooming score.

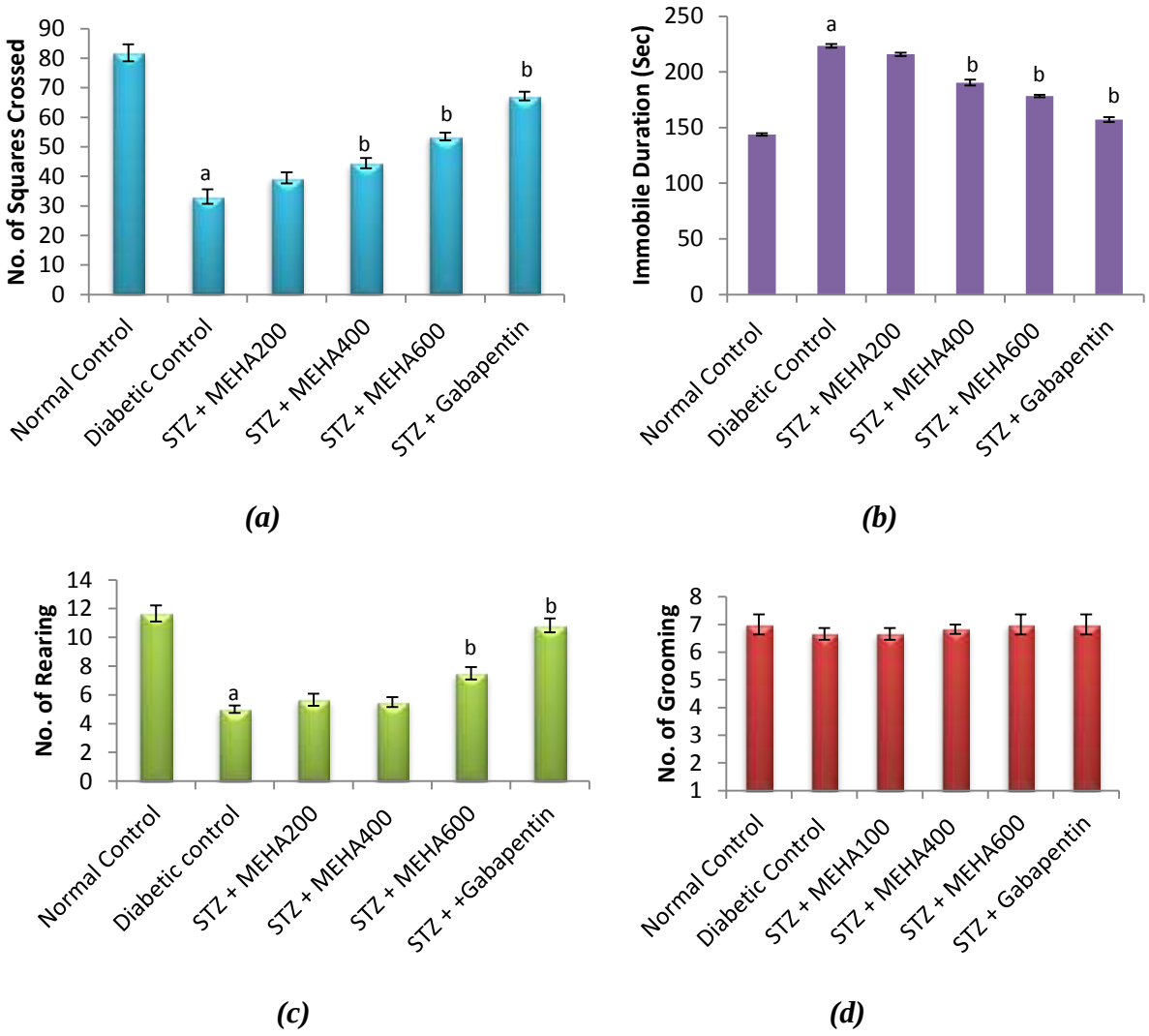

Figure 3: Effect of MEHA on Locomotor activity in Open field test

(a) Squares crossed, (b) immobile duration, (c) rearing and (d) grooming. Values are expressed as mean ± S.E.M by one way ANOVA followed by Tukey's Test. [a] denotes $p < 0.05$ vs. Normal Control; [b] denotes $p < 0.05$ vs. Diabetic control.

The formalin test is used to investigate spinal sensitization in animals and allows investigation of sensory processing beyond peripheral nociceptive pathways[36]. All Phases of the formalin test in normal rats is driven by spinal prostaglandin release, while the increased flinching in diabetic rats has been attributed to elevated COX-2 protein and prolonged prostaglandin release[37]. The mean of flinches in three phases (Phase 1, Q phase and Phase 2) of the formalin test in diabetic control were significantly ($p < 0.05$) greater (42 ± 2.47, 72.33 ± 2.21 and 171 ± 2.01 respectively) than normal control rats (12.5 ± 0.76, 21 ± 0.57 and 38.66 ± 1.14 respectively). These observations indicate the development of diabetic neuropathy.

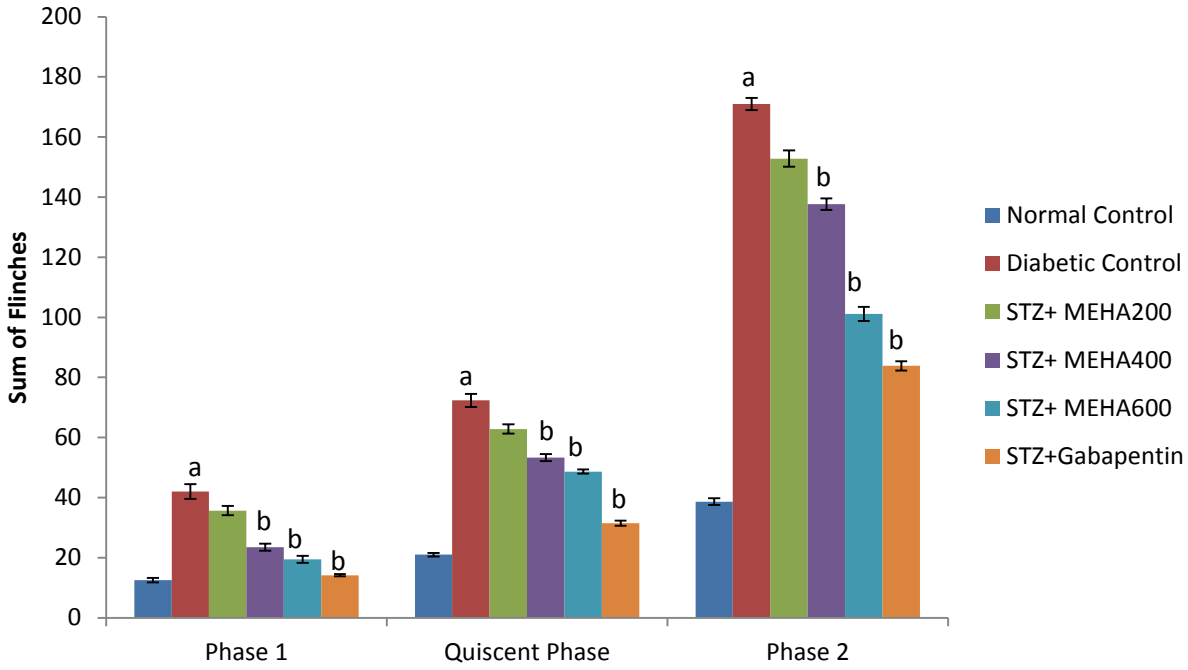

Figure 4: Effect of MEHA on Formalin test

Values are expressed as mean ± S.E.M by one way ANOVA followed by Tukey's Test. [a] denotes $p< 0.05$ vs. Normal Control; [b] denotes $p< 0.05$ vs. Diabetic control.

Uncontrolled hyperglycemia may lead to generation of high levels of free radicals[34]. Which leads to failure of natural antioxidant defense system and ultimately causes tissue injury or diabetes induced complications including diabetic neuropathy[35]. STZ treated rats exhibited ($p< 0.05$) the augmentation in sciatic nerve TBARS (22.24 ± 0.44) and nitrite (18.31 ± 0.24) levels (Table 2). Afterwards, STZ administration caused significant reduction in GSH (77.31 ± 2.05) and catalase (0.33 ± 0.02) levels. Treatment with MEHA (400 and 600 mg/kg) significantly ($p< 0.05$) prevented fall in body weight as compared to the diabetic control group. Table 1 depicts that, the elevated levels of blood glucose (363.97 ± 2.1 mg/dl and 306.04 ± 2.58 mg/dl) and plasma cholesterol (155.33 ± 3.01 and 124.5 ± 3.66) levels were significantly depleted with the treatment of MEHA (400 and 600 mg/kg) for 4 weeks. The HbA1C level was considered as a key indicator of AGEs and in the present investigation treatment with MEHA significantly inhibited this elevated level of HbA1c (6.66 ± 0.15% and 5.84 ± 0.03%) (Table 1). MEHA treated rats showed improvement in locomotor activity as compared to their non-treated counterparts indicate the prevention of diabetic neuropathy (Figure 3). The treatment with MEHA (400 and 600 mg/kg) improved the reduction in tail flick latency i.e. caused by the STZ administration (Figure 1). MEHA has an antioxidant and anti-inflammatory profile. In the present investigation, we observed, there was a significant decrease in nociception with MEHA

treatment for 28 days in diabetic rats (Figure 2). Furthermore, MEHA treatment for 28 days attenuated the development of hyperalgesia in formalin test as compared to diabetic control group (Figure 4). Treatment with MEHA prevented the rise in MDA and nitrite levels and fall in GSH and catalase levels in STZ-treated diabetic rats (Table 2).

Table: 2 Effect of MEHA on levels of various endogenous biomarkers in STZ-induced diabetic neuropathic rats

Treatment	TBARS (µM/ml)	GSH (µM/ml)	Nitrite (µM/ml)	Catalase (units/mg of protein
Normal Control	9.33 ± 0.28	145.73 ± 3.93	8.16 ± 0.33	0.85 ± 0.03
Diabetic Control	22.24 ± 0.44 [a]	77.31 ± 2.05 [a]	18.31 ± 0.24 [a]	0.33 ± 0.02 [a]
STZ + MEHA200	20.31 ± 0.31	82.29 ± 1.02	16.21 ± 0.2	0.46 ± 0.03
STZ + MEHA400	16.98 ± 0.37 [b]	95.95 ± 0.96 [b]	14.28 ± 0.33 [b]	0.55 ± 0.02 [b]
STZ + MEHA600	13.81 ± 0.39 [b]	109.4 ± 1.47 [b]	12.82 ± 0.63 [b]	0.67 ± 0.03 [b]
STZ + Gabapentin	11.88 ± 0.27 [b]	123.03 ± 1.46 [b]	11.11 ± 0.52 [b]	0.74 ± 0.03 [b]

Values are expressed as mean ± S.E.M by one way ANOVA followed by Tukey's Test. MEHA200 (methanol extract of Holarrhena antidysenterica 200 mg/kg); MEHA 400 (methanol extract of Holarrhena antidysenterica 400 mg/kg); MEHA600 (methanol extract of Holarrhena antidysenterica 600 mg/kg); [a] denotes $p < 0.05$ vs. Normal Control; [b] denotes $p < 0.05$ vs. Diabetic control.

CONCLUSION

In the light of above discussion, we conclude that MEHA comprises an antidiabetic and antioxidant properties. By virtue of these properties, MEHA may prove to be a potential remedy for the management of diabetic neuropathy. However, further studies are needed to better understand the mechanism of action by evaluating another parameters like motor nerve conduction velocity and cardiovascular parameters which exactly clear the mechanism of action of MEHA in diabetic neuropathy.

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