ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

Screening Of Neuroprotective Role Of Hydroethanolic Extract Of *Clematis Buchananiana* Leaves On Diabetic-Induced Neuropathy

Yadav Kumar Sanjay*, Virmani Tarun
School of Pharmaceutical Sciences, MVN University, Palwal 121105, India
Corresponding Author: Mr. Sanjay Kumar Yadav
E-Mail id: sanjaykumar.yadav153@gmail.com

ABSTRACT

Diabetic neuropathy is the most dangerous complication of diabetes which is very difficult to treat. Its diagnosis in the early stage is very essential for its control and prevention of the illness. The protective effect of hydroethanolic extract of *Clematis buchananiana* leaves were investigated for evaluation of the neuroprotective effect in diabetic-induced neuropathy in Wistar rats. Streptozotocin induces diabetes within 3 days by destroying the beta cells of the pancreatic gland. For the assessment of diabetes, blood glucose level was measured with the help of a glucometer. Two different pain models i.e., Eddy's hot plate and Tail flick analgesiometer were used for the measurement of analgesic activity in Wistar albino rats. As a result of the study, it was discovered that the animals treated with a standard drug (Gabapentin, 100mg/kg) had the maximum neuroprotective effect, followed by the higher dose of hydroethanolic extract of *C. buchananiana* leaves. After this study, it can be concluded that hydroethanolic extract of *C. buchananiana* has exhibited a protective effect in diabetic-induced neuropathy and it can be further explored and investigated for benefits of humans in the management of several ailments.

Keywords: Streptozotocin, tail-flick latency, beta cells, hyperglycemia, neuropathic pain, hyperalgesia.

INTRODUCTION

Humans are supposed to be the most powerful living species on earth. But, on the other side, it cannot be denied the fact that even the smallest living organism either an invisible micro-organism to an insect or other big animals can cause several health disorders and even cause death. The various diseases occurring from time to time in an individual had made life more complicated. Unfortunately, diabetic neuropathy has emerged as one of the severe health issues and affects almost every person who is suffering from diabetes. Diabetes itself is a serious issue that causes delayed wound healing, diabetic retinopathy, diabetic nephropathy, and several body problems[1]. Diabetic neuropathy is the most dangerous complication of diabetes which is very difficult to treat. Its diagnosis in the early stage is very essential for its control and prevention of the illness[2]. Current examinations recommend that the hazard factors for diabetic neuropathy are the duration of diabetes, age, glycosylated hemoglobin A1c (HbA1c) assay, diabetic retinopathy (DR), smoking, and body weight Index (BMI)[3]. In

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

any case, the greater part of the previously mentioned examinations is cross-sectional, and the example sizes are restricted, so it can be optimized that diabetic-induced neuropathy is a serious concern for all. According to WHO estimates, 80 percent of people in various Asian and African nations now utilize herbal medicine as part of their basic health care. More and more research is showing that their usage is less widespread outside the therapeutic environment, but scientific proof concerning herbal medicine's efficacy becomes more publicly accessible. Their use in the management of various clinical manifestations related to several diseases is becoming more popular. Researchers have urged for a standard nomenclature for neuropathic pain since the categories have changed over time. It's now widely accepted that neuropathic pain is "a pain caused by illness or injury of the somatosensory system[4]. Neuropathic pain is diagnosed and treated differently than other types of chronic pain. This is the reason that makes the process more complicated and uncertain for the management of neuropathic pain[5]. Neuropathic pain is linked to an increase in prescriptions for painkillers and visits to the doctor[6]. Individuals with neuropathic pain have frequent and severe sleep disruptions, anxiety, and depression, and their quality of life is worse than that of patients with chronic non-neuropathic pain[7]. The disorder is marked by increased blood glucose levels in both fasting sugar 70-90 mg/dL and after meal 90-140 mg/dL along with increased sensitivity to various stimuli which the body faces[8]. Diabetic neuropathy is related to poor health and causes of many future ailments. As a result, diabetes complications like microvascular damage and macrovascular changes in organs can occur. It may also increase the risk of ischemic heart disease, stroke, and diabetic-induced neuropathy. The food is we eat releases energy in form of fats, protein, or carbohydrates. Food that is rich in carbohydrates like potatoes, rice, etc. plays a vital role in increasing blood sugar. Blood glucose is maintained by insulin (secreted from Beta cells of the pancreas). But if the sugar intake and carbohydrates are much more than usual followed by a poor lifestyle i.e., lacking physical activities are more likely to be affected by diabetes.

Type-1 diabetes is also known as insulin-dependent diabetes mellitus. In this type of diabetes, there is no insulin secretion (or there is a very very low amount of insulin production that occurs in the body). It affects almost five percent of the total population[9].Type-2 diabetes is also known as non-insulin-dependent diabetes mellitus. In this type of diabetes, there is delayed release of insulin, or the sensitivity of the receptors decreases over time leading to improper release of insulin in the body. It affects almost most of the population i.e., approximately 95% of the total population. Gestational diabetes (Gestational diabetes mellitus in short GDM) has been classified as the third type of Diabetes Mellitus[10]. Studies show that a mother expecting a baby (with no cases of diabetes earlier) is diagnosed with hyperglycemia at the time of delivery. It may be due to human placental lactogen or other physiological changes that take place mainly at the time of pregnancy[11]. Over half of diabetic patients are affected by diabetic neuropathy. The damages done on micro-vascular levels are the main threats associated with diabetic neuropathy. Diabetic neuropathy is among the most troublesome kinds of health threat for the most part[12]. The main reason behind this is the unknown etiology and lack of proper pharmacotherapy available or long-term patient

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

compliance needed during the treatment. The current study focuses on free radical oxidative damage done on a nerve in diabetic rodents. The study suggests that there is a decrease in tail-flick responses caused by mechanical allodynia in rodents provided with pure concentrated drug extract prepared from leaves of *C. buchananiana*[13]. Due to increased sensitivity caused by diabetic neuropathy, it has been found that diabetic individuals are more susceptible to nociceptive stimuli. Even though C. buchananiana's hepatoprotective action has been scientifically shown, the study seeks to uncover the potent pharmacological actions of the plant's protection against diabetic neuropathy, which may be used for the benefit of people.

MATERIALS & METHODS

Animals

Adult Wistar male rats (8-12 weeks old, 150–200 g) were obtained from the institute animal house, NIET (Pharmacy Institute). As per the standard protocol, all the animals were acclimatized for a period of 7 days under standard environmental conditions (temperature =25 ± 2º C; relative humidity = 35-60% and 12 hr dark/light cycle). All animals were allowed free access to water *ad libitum* and a pellet diet. After the acclimatization period, the animals are divided into various groups as per the design of the experiment. All experimental animals were approved by Institute Animal Ethics Committee, NIET (Pharmacy Institute).

Drugs and chemicals

The chemicals used during the conduction of the experiment were of analytical grade. Gabapentin as a standard drug was procured from Vitrag Pharma, Surat, India. A glucometer from Accucheck was used for the determination of blood glucose levels in animals. All the solvents and chemicals were of analytical grade which was procured from CDH Pvt. Ltd.

Acquisition and preparation of plant sample

C. buchananiana leaves had been collected in October 2021 from the exterior of Dhanaulti, Uttarakhand. The taxonomic authentication of plant material was done by Dr. Anjula Pandey, Scientist, ICAR-National Bureau of Plant Genetic Resources, New Delhi, India. Furthermore, the leaves of ***C. buchananiana*** were put in the shade to dry before crushing them into a coarse powder. The ethanolic extract of coarse powder (leaves) was prepared and subjected to extraction through the soxhlet apparatus. The obtained ethanolic extract was concentrated in a water bath and dried.

RESULTS & DISCUSSION

Acute toxicity Test

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

Studies on the acute toxicity of chemicals i.e, acute oral toxicity tests were performed using healthy Wistar albino male rats sex weighing 120-180 g under regular laboratory settings following OEC recommendations 423 (**Table 1**).

Table 1: Outcomes of acute toxicity studies for Hydroethanolic Extract of Leaves of *C buchananiana*

S.N.	Treatment	Dose (mg/kg)				
		5	**50**	**300**	**500**	**1000**
1.	Control	0/3	0/3	0/3	0/3	0/3
2.	Standard	0/3	0/3	0/3	0/3	2/3
3.	Test 1	0/3	0/3	0/3	0/3	1/3
4.	Test 2	0/3	0/3	0/3	1/3	2/3
5.	Test 3	0/3	0/3	0/3	1/3	3/3

After dosing, animals were watched daily for three days in a row, with at least one observation every 30 minutes for the first 30 minutes, and then every 24 hours after that (with a particular focus on the first 4 hours) (OECD, 423). A variety of measurements were taken daily to look for changes in the animal's skin and hair as well as eyes, nasal mucus membranes, respiratory rates, circulatory signals (heart rates), and autonomic effects (salivation, lacrimation, sweat, urine incontinence, and feces. *C. buchananiana* was administered to six animals at a dosage of 2 gm/kg body weight and the animals' behavior was monitored for 14 days to determine mortality and overall health. Until the completion of the research, none of the rats had died. Up to a dosage of 2 gm/kg body weight, the plant extract was deemed safe. As a consequence of these findings, the safer doses which can be used for the evaluation of diabetic neuropathy are 100, 200, 300, 400, 500, and 600 mg/kg of the total body weight.

Induction of Diabetes Mellitus

A dose of 70 mg/kg was given by intravenous route in all the male Wistar albino rats of the five groups for inducing diabetes. The administration of streptozotocin by intravenous route induces diabetes within a period of nearly of 72 hours[15]. For confirmation of diabetes, the rats in each group were tested for blood sugar by using a diagnostic kit. After confirmation of diabetes, the animals were isolated and divided into groups for the development of neuropathy. Generally, neuropathy develops around 14-21day in rodents[16]. Finally, after 21 days animals were assessed for neuroprotective effects by using experimental methods- Eddy's hot plate and Tail flick analgesiometer.

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

Induction of Diabetic Neuropathy

Diabetes was induced, and 12 weeks after the experimentation, its effects were evaluated on the nerves to find out the possibilities of generation of diabetic neuropathy. The neuropathic development was assessed with the help of various parameters like locomotor tests and examination of the nerve tissue morphologically on weeks first, second, third, and fourth week in every group respectively.

Estimation of Fasting Blood Glucose

The rats in each experimental group were kept on fast (overnight fasting i.e. 12 hours) and fed with water only before the estimation of glucose on days 7, 14, 21 & 28 In each group. Rats were kept on fast for one day before testing their glucose levels on days 7, 14, 21, and 28 of experimentation. Glucose levels were obtained by collecting a small amount of blood from the tail and tested via glucometer Accu-check (**Table 2**).

Table 2: Determination of fasting blood glucose

S.N.	Treatment	Fasting blood glucose level (mg/dl)			
		Week 1	Week 2	Week 3	Week 4
1.	Control	95.4±0.18	96.35 ± 1.76	97.24 ± 2.04	97.03 ± 2.19
2.	Standard	154.01 ± 014*	153.13 ± 1.91*	141.32 ± 1.41*	121.18 ± 3:23*
3.	Test 1	152.14 ± 1.62*	159.37 ± 0.37*	149.38 ± 3.27*	129.01 ± 3.45*
4.	Test 2	152.53 ± 1.61*	152.16 ± 2.81*	135.14 ± 2.47*	117.12 ± 1.35*
5.	Test 3	152.79 ± 1.83*	152.46 ± 1.71*	123.16 ± 2.71*	115.18 ± 2.05*

Values are expressed as the mean ± SEM; n = 6. One-way ANOVA; followed by Dunnet's test; P < 0:05 in comparison with normal control and ∗P < 0:05 in comparison with control.

Determination of neuroprotective activity

The evaluation of neuroprotective activity was carried out by utilizing standard animal models ie hot plate and tail flick analgesic method.

Hot plate method

The hot plate test was carried out for evaluation of the neuroprotective effect[16]. The experimental animals were divided into five different groups (n = 6) in each group. The first group (control) was fed and treated orally with saline, the second group standard was fed with gabapentin (100mg/kg) and the remaining groups (Test 1, 2, and 3) were fed with HEELCB 100, 200, and 400 mg/kg respectively. For hot plate latency, the animals were placed into a

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

glass cylinder on a hot plate & temperature was adjusted to 54.5 ± 1°C to induce thermal hyperalgesia. The percentage in response (licking of the hind paw or jumping) time was calculated after the specified period (15sec). Initial readings were taken before treatment of the groups with drug solutions and then readings of different drugs and at time intervals of 0,15, 30, 60, and 90 minutes i.e. post-treatment. Results were compared and the difference between the basal reaction time and the responses of the experimental animals after administration of the drug at various time intervals were measured and the observations have been shown in **Table 3.**

Table 3: Screening of neuroprotective effect of hydroethanolic extract of C. Buchananiana leaves by eddy's hot plate

S.No	Treatment & dose		Mean Latency (Sec) Before & After Drug Administration				
			0 min	15 min	30 min	60 min	90 min
1	Control (Saline 2ml/kg)	Week 1	3.33±0.21	3.16±0.16	3.00±0.12	3.00±0.00	3.00±0.00
		Week 2	3.50±0.22	3.16±0.16	3.00±0.10	3.08±0.08	3.08±0.08
		Week 3	3.33±0.21	3.16±0.16	3.16±0.10	3.25±0.17	3.08±0.08
		Week 4	3.14±0.15	3.25±0.17	3.33±0.10	3.41±0.15	3.16±0.10
2	Standard (Gabapentin) (100 mg/kg)	Week 1	3.33±0.21 NS	3.16±0.16 NS	5.00±0.10 **	5.33±0.21 **	4.91±0.08 **
		Week 2	3.33±0.21 NS	3.16±0.16 NS	5.52±0.17 **	5.50±0.18 **	5.08±0.15 **
		Week 3	3.90±0.08 NS	3.16±0.16 NS	5.75±0.17 **	6.16±0.30 **	5.16±0.10 **
		4.08±0.15 NS	3.58±0.15 NS	5.91±0.20 **	7.00±0.12 **	5.58±0.08 **	4.08±0.15 NS
3	Test-I (HEELCB)	Week 1	3.33±0.21 NS	3.16±0.16 NS	3.00±0.12 NS	3.00±0.00 NS	3.00±0.00 NS

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

	(100 mg/kg)	Week 2	3.33±0.21 NS	3.16±0.16 NS	3.08±0.08 NS	3.08±0.08 NS	3.08±0.08 NS
		Week 3	3.33±0.21 NS	3.16±0.16 NS	3.08±0.08 NS	3.08±0.08 NS	3.08±0.08 NS
		Week 4	3.50±0.18 NS	3.16±0.16 NS	3.16±0.10 NS	3.08±0.08 NS	3.25±0.17 NS
4	Test-II (HEELCB) (200 mg/kg)	Week 1	3.33±0.21 NS	3.16±0.16 NS	3.33±0.16 NS	3.50±0.18 NS	3.16±0.16 NS
		3.33±0.21 NS	3.16±0.16 NS	3.33±0.21 NS	3.33±0.21 NS	3.25±0.11 NS	3.33±0.21 NS
		Week 3	3.33±0.21 NS	3.16±0.16 NS	4.08±0.15 **	4.83±0.10 **	3.83±0.10 ** NS
		Week 4	3.50±0.22 NS	3.25±0.17 NS	4.08±0.20 *	5.16±0.10 **	4.41±0.15 ** NS
5	Test-III (HEELCB) (400 mg/kg)	Week 1	3.33±0.21 NS	3.16±0.16 NS	3.50±0.12 NS	3.33±0.21 NS	3.25±0.17 NS
		Week 2	3.50±0.76 NS	3.16±0.16 NS	3.41±0.20 NS	3.41±0.20 NS	3.33±0.16 NS
		Week 3	3.33±0.21 NS	3.33±0.60 NS	4.41±0.20 **	5.50±0.18 **	4.83±0.10 **
		Week 4	3.66±0.21 NS	3.41±0.15 NS	4.50±0.18 **	5.75±0.11 **	4.83±0.10 **

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

*The values are represented as mean ± standard error of the mean (SEM). Statistical significance was analyzed by One way ANOVA with Dunnett's T-test. ns= non-significant value, *= significant value and **= more significant value where P values < 0.05.*

Tail flick method

Before starting of the experiment, Wistar rats were screened for sensitivity tests by placing the tip of the tail on the radiant heat source and by observing the tail-flick response in standard time i.e. around 5secs. For this study, all the selected animals were divided into five groups i.e. 6 rats in each group (n = 6). The first group (control) was fed and treated orally with saline (2ml/kg); the second (standard) was fed with gabapentin (100mg/kg) and the remaining groups (Test 1, 2, and 3) were fed with HEELCB 100, 200 and 400 mg/kg respectively. The determination of the analgesic effect was done with the help of tail flick apparatus i.e. digital analgesiometer (manufactured by Inco instruments & chemicals limited Ambala City). In this method, an electric current (6 amp) was passed through the naked nichrome wire for 10 seconds. The heat source and the tail skin were placed 1 cm apart and the tail-flick latency was measured in seconds[17]. At the beginning of the experiment, basal reaction time was measured. And then variations in response time was calculated after administration of drugs at 0,15, 30, 60, and 90 minutes time interval. A cut-off period of 10 seconds was considered as standard response time so that the tails of the animals were not damaged. The results are tabulated in **Table 4**.

Table 4: Screening of neuroprotective effect of hydroethanolic extract of C. Buchananiana leaves by Tail flick analgesiometer

S.No	Treatment & dose		Mean Latency (Sec) Before & After Drug Administration				
			0 min	15 min	30 min	60 min	90 min
1	Control (Saline 2ml/kg)	Week 1	4.25±0.17	4.25±0.17	4.25±0.17	4.08±0.20	4.08±0.83
		Week 2	3.50±0.22	3.16±0.16	3.00±0.10	3.08±0.08	3.08±0.08
		Week 3	3.33±0.21	3.16±0.16	3.16±0.10	3.25±0.17	3.08±0.08
		Week 4	3.14±0.15	3.25±0.17	3.33±0.10	3.41±0.15	3.16±0.10
2	Standard (Gabapentin) (100 mg/kg)	Week 1	3.33±0.21 NS	3.16±0.16 NS	5.00±0.10**	5.33±0.21**	4.91±0.08**
		Week	3.33±0.21	3.16±0.16	5.52±0.17**	5.50±0.18**	5.08±0.15**

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

		2	NS	NS			
		Week 3	3.90±0.08 NS	3.16±0.16 NS	5.75±0.17**	6.16±0.30**	5.16±0.10**
		Week 4	4.08±0.15 NS	3.58±0.15 NS	5.91±0.20**	7.00±0.12**	5.58±0.08**
3	Test-I (HEELCB) (100 mg/kg)	Week 1	3.33±0.21 NS	3.16±0.16 NS	3.00±0.12 NS	3.00±0.00 NS	3.00±0.00 NS
		Week 2	3.33±0.21 NS	3.16±0.16 NS	3.08±0.08 NS	3.08±0.08 NS	3.08±0.08 NS
		Week 3	3.33±0.21 NS	3.16±0.16 NS	3.08±0.08 NS	3.08±0.08 NS	3.08±0.08 NS
		Week 4	3.50±0.18 NS	3.16±0.16 NS	3.16±0.10 NS	3.08±0.08 NS	3.25±0.17 NS
4	Test-II (HEELCB) (200 mg/kg)	Week 1	3.33±0.21 NS	3.16±0.16 NS	3.33±0.16 NS	3.50±0.18 NS	3.16±0.16 NS
		Week 2	3.33±0.21 NS	3.16±0.16 NS	3.33±0.21 NS	3.33±0.21 NS	3.25±0.11 NS
		Week 3	3.33±0.21 NS	3.16±0.16 NS	4.08±0.15**	4.83±0.10**	3.83±0.10** NS
		Week 4	3.50±0.22 NS	3.25±0.17 NS	4.08±0.20*	5.16±0.10**	4.41±0.15** NS
5	Test-III (HEELCB) (400 mg/kg)	Week 1	3.33±0.21 NS	3.16±0.16 NS	3.50±0.12 NS	3.33±0.21 NS	3.25±0.17 NS
		Week 2	3.50±0.76 NS	3.16±0.16 NS	3.41±0.20 NS	3.41±0.20 NS	3.33±0.16 NS

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

		Week 3	3.33±0.21 NS	3.33±0.60 NS	4.41±0.20**	5.50±0.18**	4.83±0.10**
		Week 4	3.66±0.21 NS	3.41±0.15 NS	4.50±0.18**	5.75±0.11**	4.83±0.10**

*The values are represented as mean ± standard error of the mean (SEM). Statistical significance was analyzed by One way ANOVA with Dunnett's T-test. ns= non-significant value, *= significant value and **= more significant value where P values < 0.05.*

RESULTS

Acute toxicity Test

All the animals were kept under regular observation for 14 days from the date of administration of the various doses of the test drug i.e. HEELCB. For toxicity, parameters like animal's skin and hair as well as eyes, nasal mucus membranes, respiratory rates, circulatory signals (heart rates), and autonomic effects (salivation, lacrimation, sweat, urine incontinence, and feces) were observed after regular time intervals. After the specified period of 14 days, the animals were sacrificed and various organs (like liver, brain, etc.) were isolated for confirmation of any signs of toxicity like neurological abnormalities, morphological changes, or autonomic reflexes.

Fasting Blood Glucose

The levels of glucose were measured in all groups (Table 2) where it was found that Groups III, IV, and V have shown 129.01 ± 3:45, 117.12 ± 1.35, and 115.18 ± 2.05 mg/dl concentrations, respectively, which shows improvement in glucose levels compared with the control group on day 28 on the experiment. Group II has shown demonstrated improvement in glucose levels with 121.18 ± 3.23 mg/dl concentration on the 28th day.

Estimation of Neuroprotective action by using Eddy's Hot Plate

The hydroethanolic extract of the leaf of *C. buchananiana* (HEELCB) showed a significant effect. The increase in basal reaction time for Eddy's hot plate (Paw licking & hind paw withdrawal latency) was observed after 14 days since treatment in the experimental Group-IV and Group-V when compared with that of Control Group-I. The research also reveals that the treatment with the Standard drug Gabapentin in Group-II (Dose: 100mg/kg) showed improvement in symptoms after the 7th day only after administration of the drug. Whereas, Test Group-III (Dose: 100mg/kg) did not show any significant results after the completion of 28 days also. The results were found to be statistically significant ($p<0.01$).

The study suggests that the presence of alkaloids, triterpenoids, tannins, flavonoids, glycosides, etc. in the plant may be explored further for identifying various pharmacological

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

actions. The experimental results showed a significant decrease in pain. The test drug at a dose of 200mg/kg & 400mg/kg exhibited a significant reduction in paw withdrawal latency as compared to that of the control group of rats. Whereas the standard group which was given Gabapentin (100 mg/kg) gave the maximum analgesic effect in between 3rd week and 4th week [Table:3]. It showed significant (P<0.05) improvement in paw withdrawal latency (assessed by the hot plate method test in Streptozotocin-induced neuropathy in rats, N=6). All data were subjected to ANOVA followed by Dunnett's test, the observation is mean±SEM. *P<0.05 as compared to a normal control group, standard group, and # P<0.05 as compared to the diabetic group of the test compound.

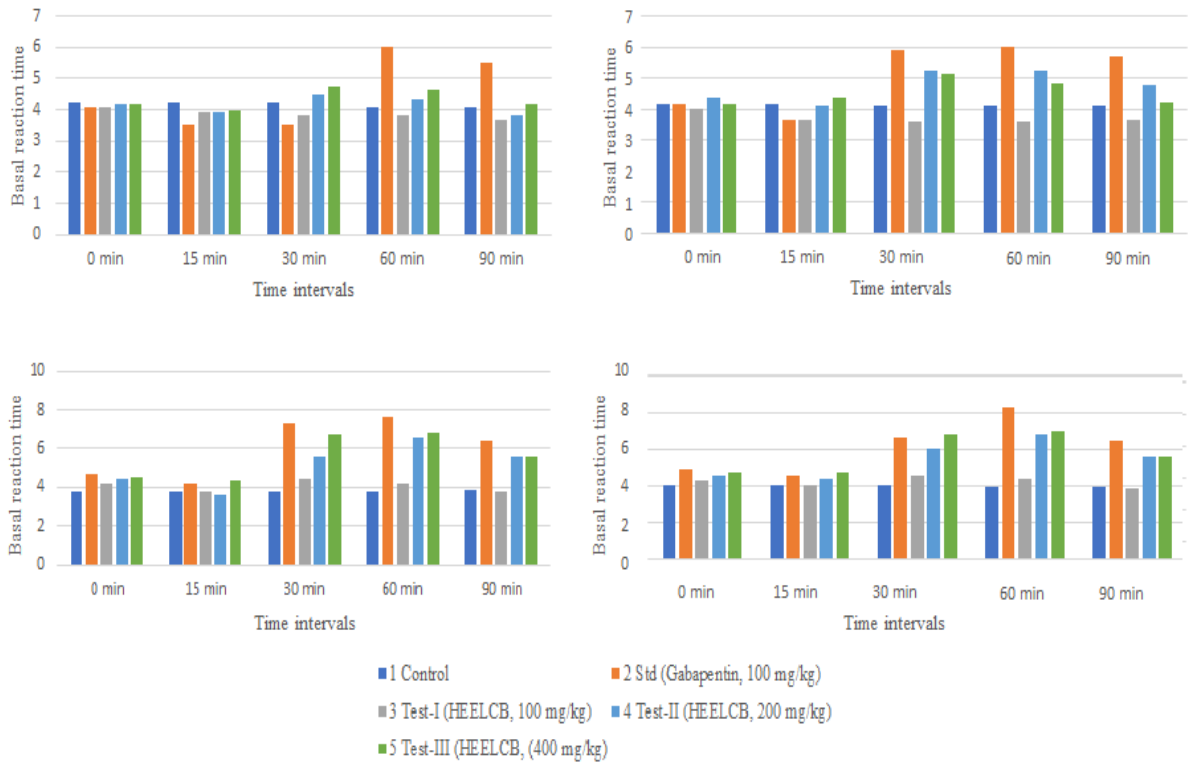

Fig. 1: Screening of neuroprotective effect of hydroethanolic extract of *C. buchananiana* leaves on diabetic-induced neuropathy by Eddy's Hot plate for Week I-IV

Estimation of Neuroprotective action by using Tail-flick Analgesiometer:

For estimation of analgesic activity by tail flick analgesiometer animals were screened for a sensitivity test. For analyzing the sensitivity of the experimental animals, animals were previously tested for sensitivity by placing the tip of the tail on the radiant heat source. The animals were observed for 5 sec and only those animals were treated as fit which showed a response within 5 sec. Animals were divided into five groups Group-I, Control (2ml/kg normal saline), Group II, Standard (Gabapentin:100mg/kg), Group III, Test-I (HEELCB: 100 mg/kg), Group IV, Test-II (HEELCB: 200 mg/kg) and Group-V, Test-III (HEELCB: 400 mg/kg) respectively. The analgesic response was carried out by using Tail Flick Analgesiometer. The basal readings were recorded at time intervals of 0 sec (Initial reading)

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

i.e., before administration of the drug and at intervals of 15 min, 30 min, 60 min & 90 min after administration of dug orally. During the research, study animals were fed once daily for 28 days daily before taking responses. The Group-I Control (Normal Saline Treated) did not show any improvement in basal reaction time after 28 days of observation completion. In the meantime, the Group-II Standard (Gabapentin: 100 mg/kg) exhibited analgesic action since the administration of the drug from Week-I and the basal time response increased week by week and it was observed maximum in Week IV. Whereas, Group-III, Test-I (HEELCB: 100mg/kg) did not show any significant effect when compared with the Control group after all weeks of completion. But, the results in Group IV i.e. Test-II (HEELCB: 200mg/kg) showed significant responses after 3rd and 4th week when compared with Group-I Control. The significant maximum values for the Test compound were observed for the higher doses of HEELCB i.e. Group-V, Test-III (HEELCB: 400mg/kg.

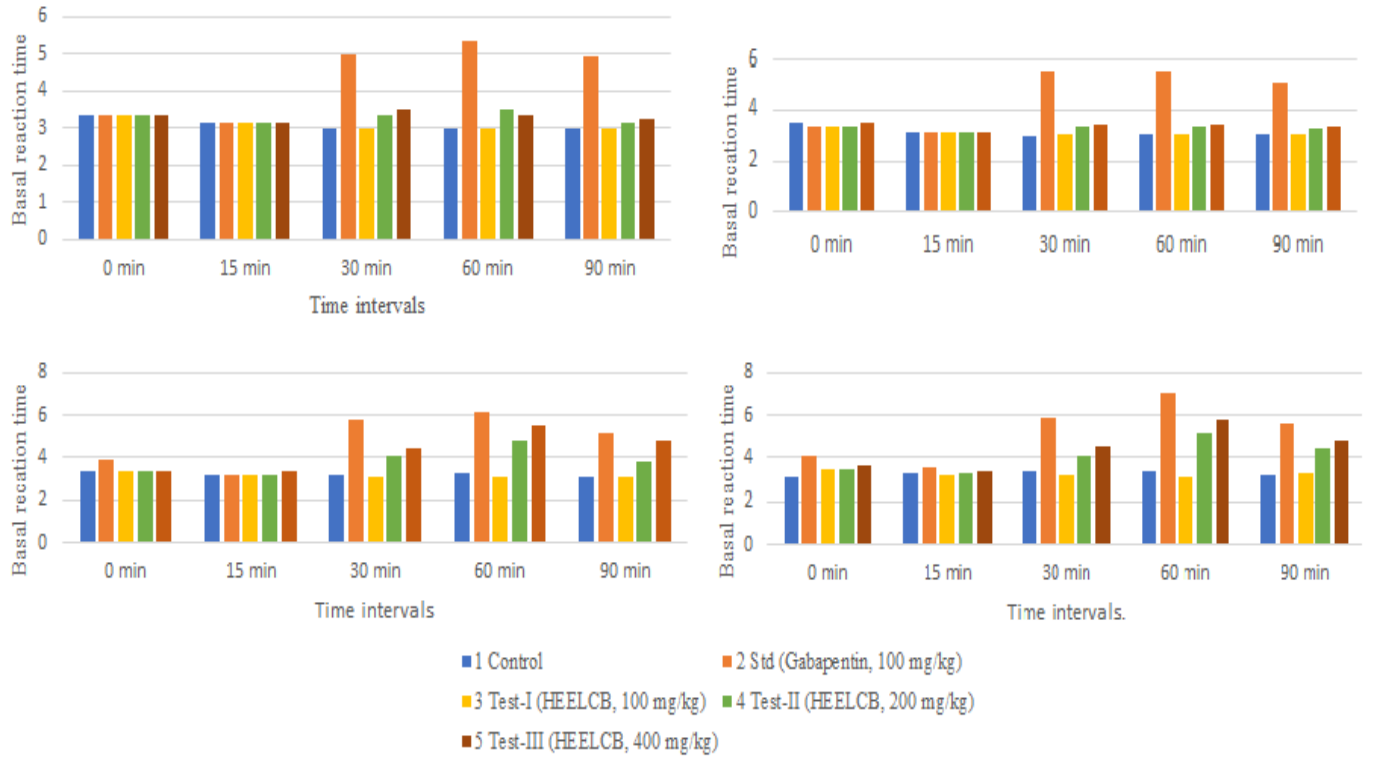

Fig. 2: Screening of neuroprotective effect of hydroethanolic extract of *C. buchananiana* leaves on diabetic-induced neuropathy by tail flick analgesiometer for Week I-IV

CONCLUSION

The experimental results of the various parameters evaluated during the study suggest that hydroethanolic extract of *C. buchananiana* leaves in diabetic-induced neuropathy.in Wistar albino rats possessed significant neuroprotective action. By this study, it can be concluded

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

that the hydroethanolic extract of *C. buchananiana* is a very potential herb which had proven to exhibit a protective effect in diabetic-induced neuropathy, and it can be further explored and investigated for benefits of humans in the management of several ailments. As the results show a significant difference in the standard Group while comparing it with the Controlled Groups. The plant is having several active chemical constituents, which can be used for the investigation of other pharmacological actions.

ACKNOWLEDGEMENTS

The authors are highly thankful to the management of MVN University, Palwal, for providing laboratory facilities for the study.

REFERENCES

1. Jain SE, Gupta SP. Effects of Cinnamomum zeylanicum bark extract on nociception and anxiety like behavior in mice. Asian J Pharm Clin Res. 2019;12(9):236-41.
2. Drinić Sm, Vukadinović J, Tosti T, Srdić J, Nikolić A, Anđelković V, Milojković-Opsenica D. Sweet Corn as Functional Food. Book Of.:589.
3. Sultana B, Anwar F, Przybylski R. Antioxidant activity of phenolic components present in barks of Azadirachta indica, Terminalia arjuna, Acacia nilotica, and Eugenia jambolana Lam. trees. Food chemistry. 2007 Jan 1;104(3):1106-14.
4. Baron R, Binder A, Wasner G. Neuropathic pain: Diagnosis, pathophysiological mechanisms, and treatment. The Lancet Neurology [Internet]. 2010;9(8):807–19.
5. Jensen TS, Baron R, Haanpää M, Kalso E, Loeser JD, Rice ASC, et al. A new definition of neuropathic pain. Pain [Internet]. 2011;152(10):2204–5.
6. Waldfogel JM, Wilson LM, Robinson KA. Author response: Pharmacotherapy for diabetic peripheral neuropathy pain and quality of life: A systematic review. Neurology. 2018;90(24):1123–5.
7. Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D, Freeman R, Truini A, Attal N, Finnerup NB, Eccleston C. Neuropathic pain. Nature reviews Disease primers. 2017 Feb 16;3(1):1-9.
8. Bellou V, Belbasis L, Tzoulaki I, Evangelou E. Risk factors for type 2 diabetes mellitus: an exposure-wide umbrella review of meta-analyses. PloS one. 2018 Mar 20;13(3):e0194127.
9. Berge OG, Garcia-Cabrera I, Hole K. Response latencies in the tail-flick test depend on tail skin temperature. Neuroscience Letters. 1988;86(3):284–8.
10. Rojas DR, Kuner R, Agarwal N. Metabolomic signature of type 1 diabetes-induced sensory loss and nerve damage in diabetic neuropathy. Journal of molecular medicine. 2019 Jun;97(6):845-54.
11. Gupta M, Sharma S, Gautam AK, Bhadauria R. Momordica charantia linn. (Karela): Nature's silent healer. International Journal of Pharmaceutical Sciences Review and Research. 2011;11(1):32–7.

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

12. Tirilomis T, Tempes T, Waldmann-Beushausen R, Ballat C, Bensch M, Schoendube FA. Histological changes in neonatal kidneys after cardiopulmonary bypass and deep hypothermic circulatory arrest. Thoracic and Cardiovascular Surgeon. 2009;57(1):7–9.
13. Saxena A, Tripathi RM, Zafar F, Singh P. Green synthesis of silver nanoparticles using aqueous solution of Ficus benghalensis leaf extract and characterization of their antibacterial activity. Materials Letters [Internet]. 2012;67(1):91–4.
14. Kaya ST, Bozdogan O, Ozarslan TO, Taskin E, Eksioglu D, Erim F, et al. The protection of resveratrol and its combination with glibenclamide, but not berberine on the diabetic hearts against reperfusion-induced arrhythmias: the role of myocardial K ATP channel. Archives of Physiology and Biochemistry [Internet]. 2019;125(2):114–21.
15. Pushparaj PN, Low HK, Manikandan J, Tan BKH, Tan CH. Anti-diabetic effects of Cichorium intybus in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology. 2007;111(2):430–4.
16. Pitcher GM, Ritchie J, Henry JL. Paw withdrawal threshold in the von Frey hair test is influenced by the surface on which the rat stands. Journal of Neuroscience Methods. 1999;87(2):185–93.
17. Dong W, Zhen Y. Nephro-Protective Effect of Cortex Lycii Radicis Extracts in Streptozotocin-Induced Diabetic Rats. Current Signal Transduction Therapy. 2014 Aug 1;9(2):106-11.