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Hydroalcoholic extract of fruit of *Terminalichebula*alleviate Diabetic Neuropathy in Alloxan induced diabetic rats.

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ABSTRACT

In the present study we investigated the effect of hydroalcoholic extract of fruit of *Terminalia chebula*(TCE) in alleviating the diabetic neuropathy in rats. Male wistar albino rats were classified into two groups as normal control and diabetic group. Normal control group rats were injected with distilled water (1ml/kg) while in diabetic group, diabetes mellitus was induced by intraperitoneal injection of Alloxan monohydrate (120 mg/kg) freshly dissolved in normal saline solution.21 days after diabetes induction, development of neuropathy was assessed in control and diabetic animals by evaluation of pain thresholds. Diabetic rats showed a significant decline in the nociceptive threshold as compared to the normal control groups. Following this, the diabetic rats were divided into five groups and treated orally as: diabetic control group with distilled water 1 ml/day, TCE-200 group with TCE 200 mg/kg, TCE-400 group with TCE 400 mg/kg, TCE-800 group with 800 mg/kg and standard group with Gabapentine 100 mg/kg for 21 days. Pain threshold responses for all rats were recorded on 0, 2^{nd}, 4^{th}, and 6^{th} week of treatment. The study revealed dose dependent improvement in pain threshold against diabetic control group, reflecting increased potency. These results suggest that TCE have beneficial activity against diabetic neuropathy in rats.This observed activity of TCE may be due to the presence of phytoconstituents like tannins , alkaloids, saponins, flavonoids. Further studies required to determine the exact molecular mechanism of action of *Terminalia chebula*.

KEYWORDS: *Terminalia chebula*; diabetes mellitus; Neuropathy.

INTRODUCTION: Diabetic neuropathy is a nerve disorder.Neuropathy is a common and costly complication of both type 1 (T1DM) and type 2 diabetes (T2DM) which is related to

duration and severity of hyperglycaemia. The prevalence of neuropathy is estimated to be about 8% in newly diagnosed patients and greater than 50% in patients with long-standing(Boulton *et al.*, 1983).Apart from hyperglycemia, there may be multiple etiologies which result in development of various neuropathic syndromes in diabetics. Various pathways responsible for microvascular complications following glucose flux include: Polyol pathway; the Hexosamine pathway; Protein Kinase C (PKC) pathway; Advanced Glycation Endproduct (AGE) pathway, Poly (ADP-ribose) polymerase (PARP) pathway. All of these pathways are related to the metabolic and/or redox state of the cell. While each pathway may be injurious alone, collectively they cause an imbalance in the mitochondrial redox state of the cell and lead to excess formation of reactive oxygen species (ROS). Increased oxidative stress within the cell leads to activation of the Poly (ADP-ribose) polymerase (PARP) pathway, which regulates the expression of genes involved in promoting inflammatory reactions and neuronal dysfunction.(James *et al*., 2008, Sharma, 2011)

Use of analgesics in painful diabetic neuropathy has poor efficacy and adverse effects. Long term NSAID ingestion causes hepatotoxicity, while narcotic analgesics reported to cause addiction and worsening of autonomic neuropathic symptoms. The dried ripe fruit of Terminalia chebula Retz. (Combretaceae), is used extensively in Ayurveda and is widely distributed throughout India. It is commonly known as black myroblans in English and has traditionally been used in the treatment of diabetes, asthma, sore throat, vomiting, hiccough, diarrhoea, bleeding piles, gout and heart and bladder diseases. It contains proteins ,carbohydrates, saponins ,tannins, alkaloids, flavonoids, triterpenoids, glycosides. Tannins is the chief medicinal compound present in the fruit (Thomas R.et al.,2012).The *Terminalia chebula* have been reported to possess the antihyperglycemic and antioxidant activity in different animal species (Rao NK et al.,2006 & Lee S.I et al.,2005).However, its role in diabetic neuropathy has not been studied. In light of this, the present investigation was carried out to study the effect of hydroalcoholic extract of fruit of *Terminaliachebula*in alleviating the diabetic neuropathy in Alloxan induced male diabetic rats .

MATERIALS AND METHODS

Plant Extract

Hydroalcoholic extract of *Terminalia chebula* fruit received from InnoconFoods,Pune.The*Terminalia chebula* sample provided by Innocon foods was authenticated from Botanical Survey of India, Pune. (BSI/WRC/Tech/2012)

CHEMICALS AND DRUGS

Alloxan monohydrate,Hypnoket (Ketamine injection) Gabapin-300, 5, 5-dithiobisnitrobenzoic acid, thiobarbituric acid (TBA), nicotinamide adenine dineuleotide phosphate (NADPH), glutathione oxidized, glutathione reduced, phenazine methosulphate (PMS), nitroblue tetrazolium, NADH, folinCiocalteau reagent,Framycetin cream (Soframycin) were purchased from the local market.

INSTRUMENTS USED

Accu check Glucometer ,Digital Randall–Selittoapparatus,Centrifuge,Homogeniser

ANIMALS

Wistar albino rats of either sex were used. They were maintained at 25 ± 2°C and relative humidity of 45 to 55% and under standard environmental conditions (12 hour light: 12 hour dark cycle). Animals were allowed to take specified amount of standard laboratory feed (Amrut feed, Pune) and water ad libitum. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of AISSMS College of Pharmacy, Pune which is constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), approval no.is CPCSEA/IAEC/PC-07/12-2K11. Ethical guidelines were strictly followed during all the experiments.

ACUTE TOXICITY STUDY

Healthy adult female Wistar albino rats (200-250g) were subjected to acute toxicity studies as per guidelines (AOT 423) suggested by the OECD-2000. The maximum upper limit dose 2000 mg/kg of hydroalcoholic extract of fruit of *Terminalia chebula* was administered orally to three female Wistar rats. Animals were observed individually after dosing for the presence of any clinical signs, such as changes in skin fur, lacrimation, salivation, piloerection, diarrhea, and mortality. The gross behaviors, e.g. body positions, locomotion, rearing and tremors were observed. Survived animals were observed for outcomes for a period of 24 hours. The animals were under supervision upto14 days for any sign of toxicity or mortality.

EXPERIMENTAL INDUCTION OF DIABETES MELLITUS

The Alloxan monohydrate (AL) (120 mg/kg) freshly dissolved in normal saline was injected intraperitoneally (i.p.) to male Wistar rats after overnight fasting. After AL treatment, all animals were given free access to food and water. Blood glucose levels were measured after three days of AL injection and only animal with blood glucose levels higher than 200mg/dL

were considered diabetic and included in study. (Leite*et al*., 2007; Ndiaye *et al*., 2008)

EXPERIMENTAL DESIGN

A total of 36 male wistar rats (30 diabetic surviving and 6 normoglycemic rats) were used. After 21 days of diabetes induction, rats in diabetic group were subdivided into five groups (n=6) groups and treated orally for six weeks as: diabetic control group with distilled water 1 ml/day, TCE-200 group with TCE 200 mg/kg, TCE-400 group with TCE 400 mg/kg, TCE-800 group with 800 mg/kg and standard group with Gabapentine 100 mg/kg for 21 days. Development of neuropathy was assessed in control and diabetic animals by evaluation of pain thresholds by evaluation of mechanical ,thermal hyperalgesia (Kuhad and Chopra) , and cold allodynia (Kastrup *et al*., 1987)after 21 days of diabetes induction. Body weight of all animals was recorded on 0, 2^{nd}, 4^{th} and 6^{th} week of treatment. At the end of experimental protocol, all rats were scarified by cervical dislocation under ether anesthesia. Sciatic nerves were removed, washed with physiological saline and cleared of fatty tissues.

BLOOD GLUCOSE ESTIMATION

Blood samples from the tail vein were collected for the measurement of blood glucose .Blood glucose level of all animal were measured on 0, 14^{th} and 21^{st} day of treatment by using Accu check glucometer (Umar *et al*., 2010).

STATISTICAL ANALYSIS

The results are expressed as mean $\pm$ SEM. Comparison between the control and diabetic group was made with unpaired Students *t* test. Comparison between test groups and diabetic control was made with one way analysis of variance (ANOVA) followed by Dunnett's t test.

RESULT

1.Acute toxicity study

OECD guidelines AOT-423 was followed for estimation of acute toxicity study in rats. Symptoms like lethargy and sleep were seen in TCE treated group animals. Any other toxic symptom or death was not seen in both groups for first four hours and thereafter for 14 days. All rats were free of any toxicity as per acceptable range given by the OECD guidelines upto the dose of 2000 mg/kg.

2.Effect of alloxan induced diabetes mellitus on nociceptive threshold of rats

After 21 days of diabetes induction, the nociceptive threshold was significantly (P<0.0001)

lower in diabetic rats as compared with control animals. Thermal hyperalgesia,Cold allodynia and Mechanical hyperalgesia was evident in alloxan treated animals after the third week of diabetes induction.

Table 1 Screening of animals for Diabetic Neuropathy

a) Reaction time (sec)- Tail immersion (hot water) test

Group	Mean ± SEM	
	Before diabetes induction	**21 days after diabetes induction**
Control	8.888 ± 0.3340	9.001 ± 0.1761
Diabetic	8.1653 ± 0.1313	3.173 ± 0.0976$^{\#}$

b) Reaction time (sec)- Tail immersion (cold water) test

Group	Mean ± SEM	
	Before diabetes induction	**21 days after diabetes induction**
Control	11.343 ± 0.3633	11.586 ± 0.3352
Diabetic	11.176 ± 0.1526	4.110 ± 0.1365$^{\#}$

c)Paw withdrawal pressure (gm)-paw pressure withdrawal test

Group	Mean ± SEM	
	Before diabetes induction	**21 days after diabetes induction**
Control	243.66 ± 2.362	243.16 ± 2.212
Diabetic	240. 86 ± 1.134	138.03 ± 0.8476$^{\#}$

The results were expressed as Mean ± SEM (n=6 for control group, n=24 for diabetic group). The data was analyzed using unpaired student 't' test. Statistically significant at $^{\#}p<0.0001$ compared to control.

3.Effect of TCE treatment on alloxan induced diabetic neuropathy in wistar albino rats

3.1.Effect of TCE treatment on thermal hyperalgesia

Thermal hyperalgesia was evident in alloxan/vehicle-treated animals since the tail withdrawal latency was significantly shorter than that of control/vehicle-treated animals after the third week of diabetes induction. After 4 weeks of respective treatment, TCE 400 , 800 and Gabapentin 100 treated animals showed significant increase in reaction time upto 3.986 ±

0.2097 (p<0.05) , 4.01 ± 0.2551 (p<0.05) and 4.81±0.3014 (p<0.01)respectively as compare to diabetic control animals (2.996 ± 0.2058). After 6 weeks of treatment, TCE 400,800 and Gabapentin 100 treated animals showed very significant (p<0.01) increase in pain threshold as compare to diabetic control male rats (2.746 ± 0.2259)(Table 2, Figure1).

Table 2 Effect of TCE treatment on thermal nociceptive threshold

Test	Group	Reaction time (seconds)			
		Week of treatment			
		0	2nd	4th	6th
Tail-immersion (hot water) test	Control	9.001 ± 0.1761	8.904 ± 0.2518	8.8 ± 0.1714	9.121 ± 0.2004
	Diabetic control	2.983 ± 0.2572#	3.006 ± 0.2526#	2.996 ± 0.2058#	2.746 ± 0.2259#
	TCE 200	3.276± 0.2047	3.426 ± 0.2306	3.286 ± 0.2319	3.831 ± 0.3118*
	TCE 400	3.231 ± 0.1894	3.568 ± 0.2176	3.986 ± 0.2097*	6.34 ± 0.2699**
	TCE 800	3.31 ± 0.2181	3.575 ± 0.1474	4.01 ± 0.2551*	7.02 ± 0.3176**
	Gabapentin 100	3.063 ± 0.2622	3.99 ± 0.3039	4.81 ± 0.3014**	8.631 ± 0.3074**

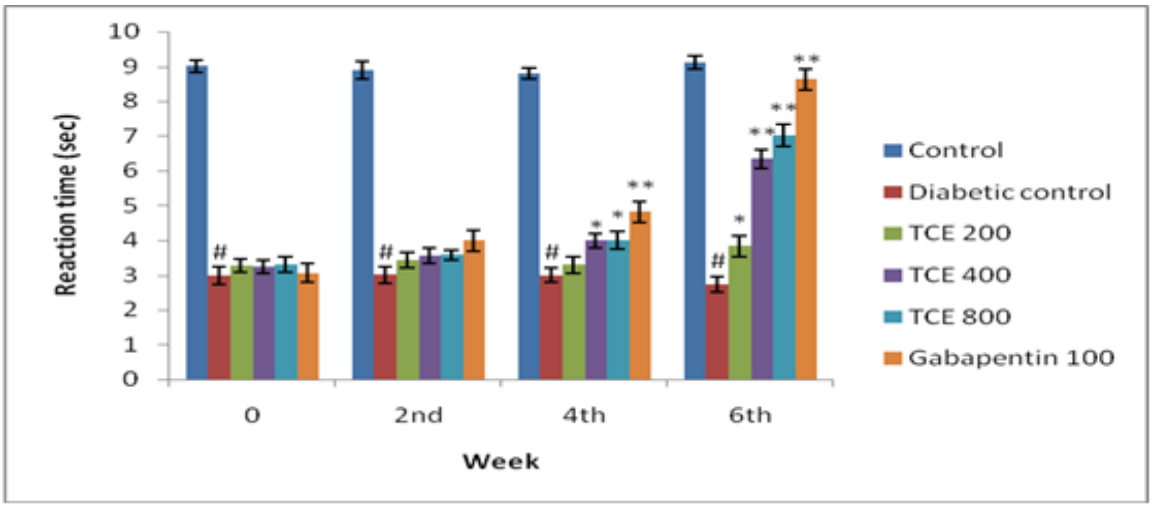

Figure1 Effect of TCE treatment on tail withdrawal latency

Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between Control and Diabetic control group, whereas multiple comparisons were done by using One-way Analysis of Variance (ANOVA) followed by *Dunnett's 't' test*. #p<0.0001 compared with control; *p<0.05, **p<0.01 compared with Diabetic control.

3.2 Effect of TCE treatment on mechanical hyperalgesia (paw pressure withdrawal test)

Mechanical hyperalgesia was evident in alloxan/vehicle-treated animals since the paw pressure withdrawal threshold was significantly (p<0.0001) shorter than that of control/vehicle-treated animals after the third week of diabetes induction. After 4 weeks of respective treatment, TCE 400, 800 and Gabapentin 100 treated animals showed significant increase in paw pressure withdrawal threshold upto 150.00 ± 2.781 (p<0.05) , 159.66 ± 2.539 (p<0.01) and 163.83 ± 1.922 (p<0.01) respectively as compare to diabetic control animals (139.66 ± 2.499). After 6 weeks of treatment, TCE 400, 800 and Gabapentin 100 treated animals showed very significant (p<0.01) increase in pain threshold as compare to diabetic control male rats(Table 3 , Figure2).

Table 3. Effect of TCE treatment on paw pressure withdrawal thresholds

Group	Paw withdrawal thresholds (gm)			
	Week of treatment			
	0	2nd	4th	6th
Control	243.16 ± 2.212	244.00 ± 2.352	242.83 ± 2.926	243.66 ± 1.944
Diabetic control	139.83 ± 2.056#	141.33 ± 1.909#	139.66 ± 2.499#	131.33 ± 3.730#
TCE 200	138.00 ± 1.653	138.16 ± 2.072	136.16 ± 2.120	143.83 ± 2.056*
TCE 400	136.33 ± 2.290	137.33 ± 2.753	150.00 ± 2.781*	188.66 ± 2.431**
TCE 800	135.66 ± 1.520	149.83 ± 2.713	159.66 ± 2.539**	226.00 ± 2.805**
Gabapentin 100	140.33 ± 1.706	150.16 ± 2.301	163.83 ± 1.922**	232.16 ± 2.651**

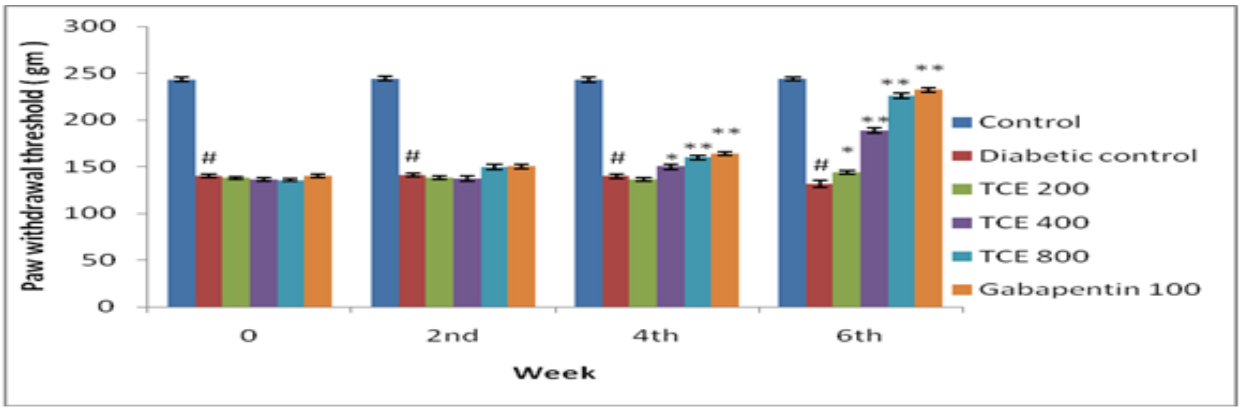

Figure 2. Effect of TCE treatment on paw pressure withdrawal thresholds

Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between Control and Diabetic control group, whereas multiple comparisons were done by using One-way Analysis of Variance (ANOVA) followed by *Dunnett's 't' test*. #p<0.0001 compared with control; *p<0.05, **p<0.01 compared with Diabetic control.

3.3 Effect of TCE treatment on Cold allodynia

Tail immersion (cold water) test

Cold allodynia was evident in alloxan/vehicle-treated animals since the tail withdrawal latency was significantly (p<0.0001) shorter than that of control/vehicle-treated animals after the third week of diabetes induction. After 4 weeks of respective treatment, TCE 400 and 800 treated animals showed significant increase in reaction time upto 4.895 ± 0.3079 (p<0.05) and 5.056 ± 0.3051 (p<0.01) respectively as compare to diabetic control animals (3.593 ± 0.3080). After 6 weeks of treatment, TCE 400 and 800 treated animals showed very significant (p<0.01) dose dependent increase in pain threshold as compare to diabetic control male rats (3.658 ± 0.3333). (Table 4, Figure 3).

Table 4. Effect of TCE treatment on Tail withdrawal latency in Tail immersion (cold water) test

Group	Reaction time (sec)			
	Week of treatment			
	0	2	4	6
Control	11.586 ± 0.3352	11.378 ± 0.2505	11.483 ± 0.1858	11.295 ± 0.2312
Diabetic control	4.383 ± 0.3206#	3.926 ± 0.3874#	3.593 ± 0.3080#	3.658 ± 0.3333#
TCE 200	4.393 ± 0.2888	3.936 ± 0.2846	4.133 ± 0.2608	4.793 ± 0.1767*
TCE 400	3.843 ± 0.3228	3.778 ± 0.1611	4.895 ± 0.3079*	6.716 ± 0.3006**
TCE 800	4.083 ± 0.3030	4.52 ± 0.3553	5.056 ± 0.3051**	8.298 ± 0.3466**
Gabapentin 100	3.885 ± 0.3256	4.47 ± 0.3022	5.706 ± 0.3738**	9.83 ± 0.3584**

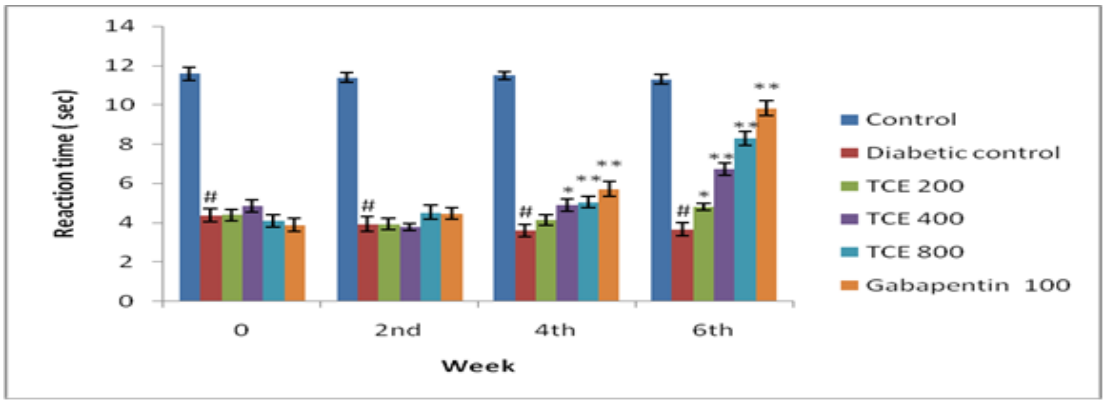

Figure3. Effect of TCE treatment on tail withdrawal latency in Tail immersion (cold water)

test

Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between Control and Diabetic control group, whereas multiple comparisons were done by using One-way Analysis of Variance (ANOVA) followed by *Dunnett's 't' test*. #p<0.0001 compared with control; *p<0.05, **p<0.01 compared with Diabetic control.

Effect of TCE treatment on serum glucose level

Rats in diabetic control group showed significant (p<0.0001) increase in serum glucose level on 2nd, 4th and 6th week of treatment as compare to normal control animals. TCE 200, 400 and 800 treated animals showed significant (p<0.01) reduction in serum blood glucose level after 2nd week of treatment as compare to diabetic control. Dose and time dependent decrease in serum glucose level was observed till 6 weeks of treatment.Gabapentin 100 treated animals showed no any significant change in serum glucose level till 6 weeks of treatment as compare to diabetic control animals. (Table 5, Figure 4).

Table 5 Effect of TCE treatment on serum glucose level

Group	**Blood glucose concentration (mg/dl)**			
	0 Week	**2nd Week**	**4th Week**	**6th Week**
Control	98.66 ± 2.539	99.16 ± 2.482	99.33 ± 3.127	100.83 ± 2.600
Diabetic control	262.00 ± 3.194#	278.16 ± 3.198#	287.5 ± 5.784#	303.66 ± 4.006#
TCE 200	266.33 ± 2.076	232.33 ± 2.951**	211.16 ± 3.591**	181.66 ± 2.963**
TCE 400	259.33 ± 4.716	210.00 ± 3.055**	178.16 ± 2.982**	164.5 ± 3.243**
TCE 800	261.00 ± 3.933	172.83 ± 3.945**	159.66 ± 3.221**	141.66 ± 2.472**
Gabapentin 100	260.00 ± 3.204	262.83 ± 2.272	270.5 ± 2.802	296.5 ± 5.841

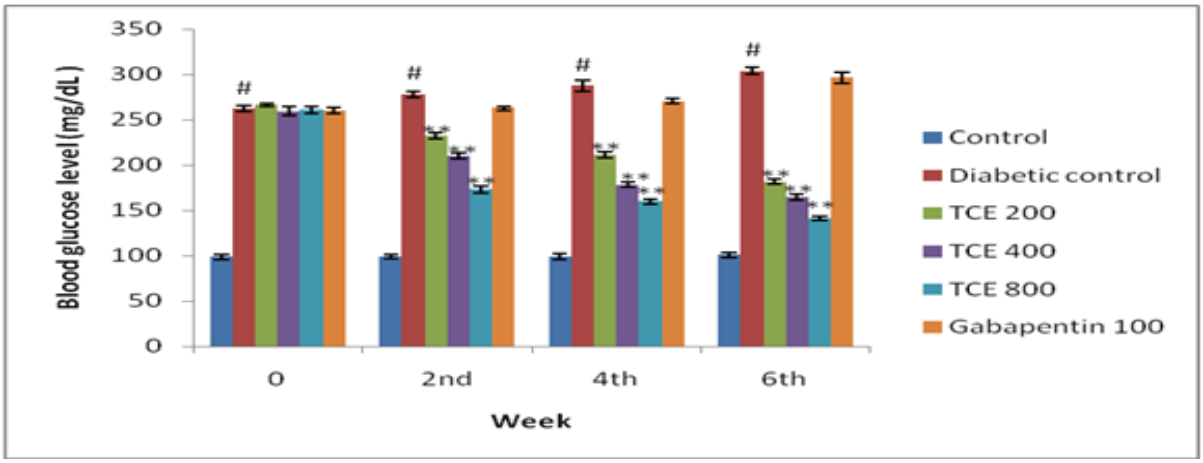

Figure 4. Effect of TCE treatment on serum glucose level

Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between Control and Diabetic control group, whereas multiple comparisons were done by using One-way Analysis of Variance (ANOVA) followed by *Dunnett's 't' test*. #p<0.0001 compared with control; **p<0.01 compared with Diabetic control.

5.Effect of TCE treatment on body weight

Rats in diabetic control group showed significant (p<0.0001) decrease in body weight on 2nd, 4th and 6th week of treatment as compare to normal control animals. TCE 200, 400,800 and Gabapentin 100 treated animals showed significant (p<0.01) increase in body weight after 4th week of treatment as compare to diabetic control. Dose and time dependent increase in body weight was observed till 6 weeks of treatment. (Table 6, Figure 5)

Table 6. Effect of TCE treatment on body weight

Group	Body weight (gm)			
	0 Week	2nd Week	4th Week	6th Week
Control	244.16 ± 3.219	252.00 ± 4.980	258.00 ± 3.587	266.66 ± 2.201
Diabetic control	202.66 ± 2.704#	195.66 ± 3.051#	190.66 ± 2.974#	185.00 ± 2.595#
TCE 200	200.5 ± 3.481	204.5 ± 3.085	208.83 ± 2.903**	213.83 ± 2.833**
TCE 400	200.66 ± 3.955	203.66 ± 3.490	215.33 ± 3.283**	223.66 ± 3.273**
TCE 800	200.16 ± 3.572	207.83 ± 3.016	219.83 ± 3.250**	229.16 ± 2.937**
Gabapentin 100	201.66 ± 3.667	203.5 ± 3.253	206.66 ± 3.461**	210.33 ± 3.242**

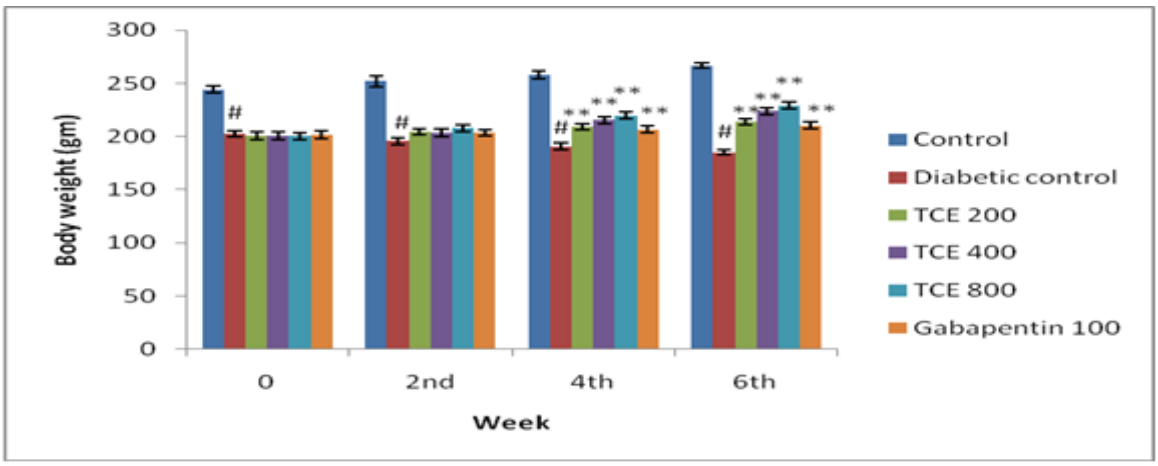

Figure 5. Effect of TCE treatment on body weight

Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between Control and Diabetic control group, whereas multiple comparisons were done by using One-way Analysis of Variance (ANOVA) followed by *Dunnett's 't' test*. #p<0.0001 compared with control; **p<0.01 compared with Diabetic control.

6. Effect of TCE treatment on biochemical parameters of sciatic nerve homogenate

a) Malondialdehyde (MDA)

Rats in Diabetic control group showed a significant (p<0.0001) increase in MDA concentrations as compared to control group. Treatment with TCE 800 and Gabapentin 100 for 6 weeks significantly (p<0.01) attenuated increase in MDA concentration as compare to diabetic contro (Table 7, Figure 6).

b) Reduced glutathione (GSH)

Rats in Diabetic control group showed a significant (p<0.0001) decrease in GST concentrations as compared to control group. Treatment with TCE 800 for 6 weeks significantly (p<0.01) attenuated decrease in CAT concentration as compare to diabetic control (Table 7, Graph 7).

c) Catalase (CAT)

Rats in Diabetic control group showed a significant (p<0.0001) decrease in CAT concentrations as compared to control group. Rats in Diabetic control group showed a significant (p<0.0001) decrease in CAT concentrations as compared to control group. Treatment with TCE 800 for 6 weeks significantly (p<0.01) attenuated decrease in CAT concentration as compare to diabetic control (Table 7, Figure8).

d) Superoxide dismutase (SOD)

Rats in Diabetic control group showed a significant (p<0.0001) decrease in SOD concentrations as compared to control group. Treatment with TCE 800 for 6 weeks significantly (p<0.01) attenuated decrease in SOD concentration as compare to diabetic control (Table 7, Figure 9)

Table7. Effect of TCE on oxidative markers in sciatic nerve homogenate against alloxan induced diabetic neuropathy in rats

Groups	MDA nM of MDA/ gm of tissue	GSH µgm of GSH/gm of tissue	CAT µM of H_2O_2/ gm of tissue/min	SOD Units / gm of tissue
Control	13.65± 1.318	212.83± 6.311	3.905± 0.3169	0.625± 0.03085
Diabetic control	32.378± 1.459#	116.83± 6.279#	2.661± 0.2378#	0.308± 0.01815#
TCE 200	30.031± 1.366	139.83± 3.683*	3.196± 0.2021	0.403± 0.01406*
TCE 400	27.096± 1.324*	139.66± 3.844*	3.521± 0.1416*	0.408± 0.02167*
TCE 800	15.55± 0.8448**	208.5± 4.884**	3.788± 0.1774**	0.611± 0.02587**
Gabapentin 100	24.146± 1.101**	140.16± 4.693*	3.493± 0.2064*	0.496± 0.02486*

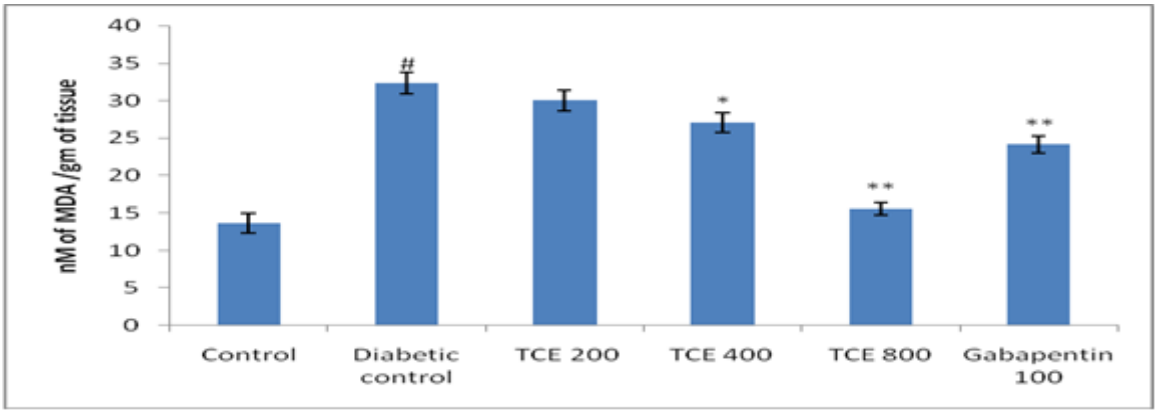

Figure 6. Effect of TCE treatment on MDA in sciatic nerve homogenate against alloxan induced diabetic neuropathy in rats

Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between Control and Diabetic control group, whereas multiple comparisons were done by using One-way Analysis of Variance (ANOVA) followed by *Dunnett's 't' test*. #p<0.0001 compared with control; *p<0.05, **p<0.01 compared with Diabetic control.

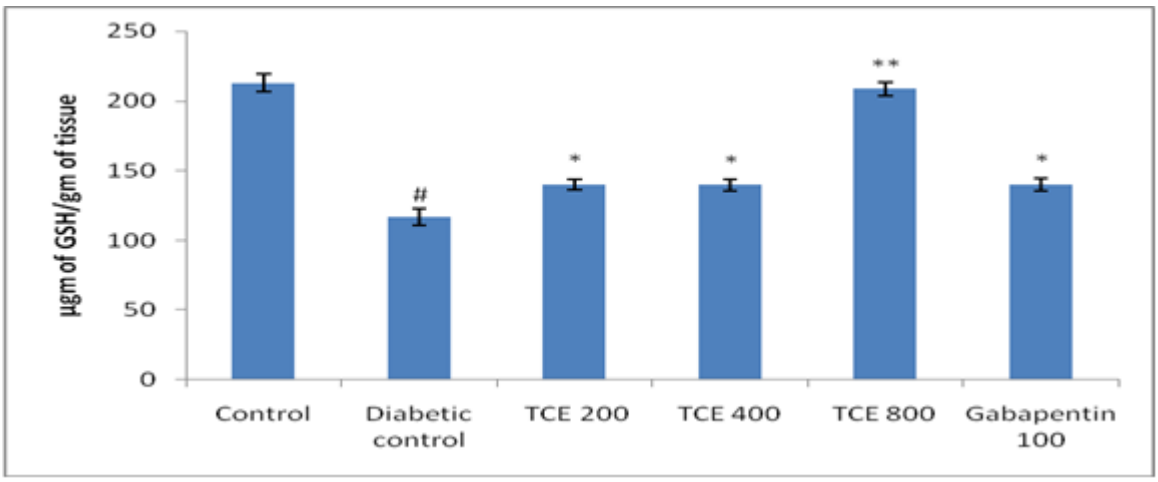

Figure 7. Effect of TCE treatment on GSH level in sciatic nerve homogenate against alloxan induced diabetic neuropathy in rats

Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between Control and Diabetic control group, whereas multiple comparisons were done by using One-way Analysis of Variance (ANOVA) followed by *Dunnett's 't' test*. #p<0.0001 compared with control; *p<0.05, **p<0.01 compared with Diabetic control

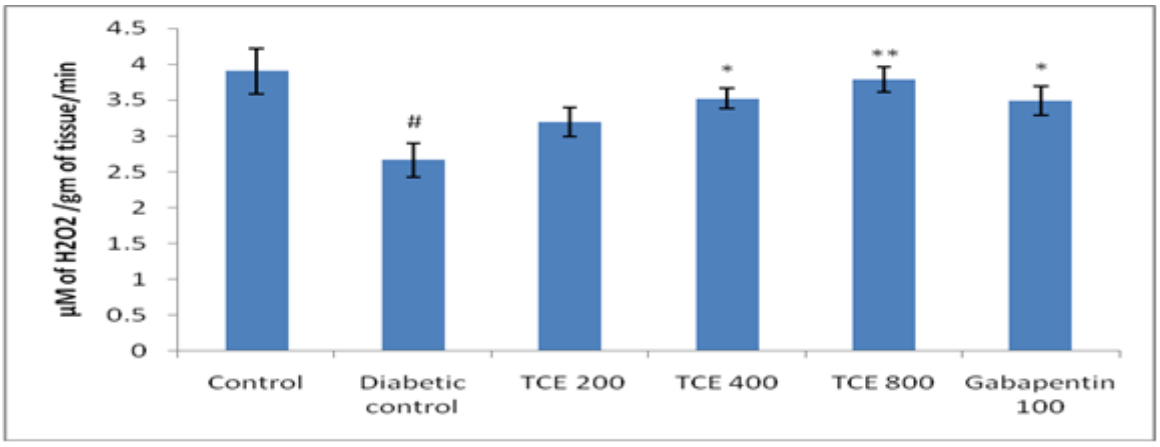

Figure8. Effect of TCE treatment on CAT level in sciatic nerve homogenate against alloxan induced diabetic neuropathy in rats

Results are expressed as Mean ±SEM (n=6). The unpaired Student's t-test was used for analyzing the data between Control and Diabetic control group, whereas multiple comparisons were done by using One-way Analysis of Variance (ANOVA) followed by *Dunnett's 't' test.*#p<0.0001 compared with control; *p<0.05, **p<0.01 compared with Diabetic control.

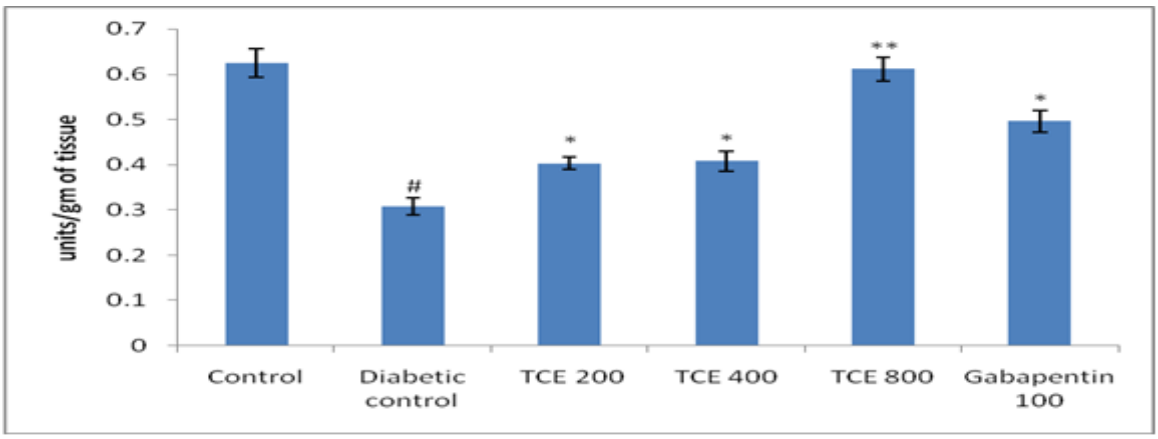

Figure9. Effect of TCE treatment on SOD level in sciatic nerve homogenate against alloxan induced diabetic neuropathy in rats

Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between Control and Diabetic control group, whereas multiple comparisons were done by using One-way Analysis of Variance (ANOVA) followed by *Dunnett's 't' test*. #p<0.0001 compared with control; *p<0.05, **p<0.01 compared with Diabetic control.

DISCUSSION

In diabetes, chronic and persistant hyperglycemia coupled with associated risk factors, participates in the development of neuropathy. Diabetes and its complications is usually screened by using different preclinical models of diabetes. Streptozotocin and alloxan are common diabetogenic agents and are useful to study the multiple aspects of the diabetes. Alloxan is chemically unstable pyrimidine derivative , which is toxic to pancreatic β cells because it generate toxic free oxygen radicals during redox cycling in the presence of reducing agent such as glutathion and cystein.In our study, the alloxan-induced diabetic rats showed high blood glucose level.This hyperglycemia was evident throughout the entire experimental period indicating state of diabetes(Ashok BS.,et al 2006).

In the present study, alloxan-injected rats showed significantly higher blood glucose level, decreased body weight as compare to normal control rats. The nociceptive threshold of diabetic rats was significantly lower than non-diabetic rats in tail immersion hot water test, paw pressure withdrawal test and tail immersion cold water test,indicating that diabetic animals exhibit thermal hyperalgesia, mechanical hyperalgesia and cold allodynia. In the present study, hydroalcoholic extract of *Terminalia chebula* fruit (TCE) treatment restored body weight, blood glucose, along with pain threshold in diabetic rats to that of normal control rats.

The Fruits of *Terminalia chebula* have been previously reported to have antinociceptive activity in rats (Sarabjit *et al.*, 2010) which can further assist in protecting against diabetic neuropathy. The TCE 400 and 800 mg/kg treated diabetic animals showed significant increased pain threshold after 4 weeks of treatment; whereas TCE 200 mg/kg increased pain threshold after 6 weeks of treatment in diabetic animals. So, the doses 400 and 800 mg/kg can be used effectively to have comparatively quick relief from pain associated with diabetic neuropathy. Gabapentin 100 mg/kg showed significant effect to limit the painful symptoms like cold allodynia, thermal and mechanical hyperalgesia.

Hyperglycemia is reported to induce oxidative stress through multiple pathways. We observed a significant increase in lipid peroxides (MDA) and reduction in endogenous antioxidant enzymes like GSH, CAT, SOD activity in sciatic nerves of diabetic rats. Treatment with TCE 400 and 800 mg/kg for six weeks, in dose dependent manner restored above mentioned biochemical parameters in diabetic rats. Phytoconstituents present in *Terminalia chebula* like chebulic acid, chebulanin, ellagic acid and tannins having antioxidant potential may be responsible for restoring above parameters. The improvement in diabetic state by *Terminalia chebula* treatment along with the antioxidant activity could be the probable way by which it had alleviated diabetic neuropathy. However further investigation is required to establish the exact mechanism of protective effect of *Terminalia chebula* in diabetic neuropathy.

In diabetes mellitus , catabolism and dehydration of proteins and fats result in the loss of body weight (Tripathi,2008). In the present investigation there was observed significance decrease in body weight of diabetic rats compared with control rats.Upon treatment with TCE 200, 400 ,800 mg/kg and Gabapentin 100, the body weight was improved but the effect was more pronounced in TCE treated rats than gabapentin as compare to diabetic control rats which may be due to better glycemic control in TCE treated rats, however this part need to be studied seperatly.

TCE 200, 400 and 800 mg/kg treated animals showed significant reduction in blood glucose level after 2nd week of treatment as compare to control. Our study supported the reported and traditionally claimed antidiabetic activity of *Terminalia chebula*,as the test doses 200, 400 and 800 mg/kg showed dose dependent antihyperglycemic activity in alloxan induced diabetic rats. The ability of Hydroalcoholic extract of Terminalia chebula fruit in significantly increasing the body weight and effectively controlling the increase in the blood glucose level

in diabetic groups of rats may be attributed to its antihyperglycemic effect.Further antihyperglycemic activity of *Terminalia chebula* may be associated with an increase in plasma insulin level , suggesting an insulinogenic activity of TCE. *Terminalia chebula* stimulate insulin secretion from remnant β cells or regenerated β cells (Gandhipuram PSK *et al*., 2006).

CONCLUSION

hydroalcoholic extract of *Terminalia chebula* fruit (TCE) at the dose of 200, 400,800 mg/kg showed dose dependant reduction in pain threshold and elevated blood glucose level in diabetic test animals. Hence TCE showed beneficial activity against diabetic neuropathy in male rats.

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