Effects of *Cleome viscosa* on hyperalgesia, oxidative stress and lipid profile in STZ induced diabetic neuropathy in Wistar rats

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Abstract: Diabetes mellitus is a severe devastating epidemic has an effect on both developing and developed countries. Diabetic neuropathy (DN) is a secondary microvascular complication of diabetes causing damage to the nerves and is characterized by fall in nerve conduction velocity, severe pain, impaired sensation and degeneration of nerve fibres. In the present study, we investigated the neuroprotective effect of ethanolic extract of *Cleome viscosa* (EECV) against streptozotocin (STZ) induced diabetic neuropathy in Wistar rats. Intraperitoneal injection of STZ resulted in significant increase in thermal hyperalgesia and hyperlipidemia after four weeks. Antioxidant enzyme [superoxide dismutase (SOD), glutathione (GSH) and catalase) levels were reduced and malondialdehyde (MDA) level was increased significantly in diabetic rats as compared to the vehicle control rats. Four weeks of chronic treatment with EECV (100, 200 and 400 mg/kg) attenuated the level of nociceptive threshold significantly and dose dependently. It also decreased the elevated levels of lipids, lipid peroxidation, and oxidative stress significantly and dose dependently. The present study provides investigational evidence of the protective effect of EECV on nociception; hyperlipidemia and oxidative stress in STZ induced diabetic neuropathy.

Keywords: Diabetic neuropathy, *Cleome viscosa*, streptozotocin, oxidative stress, hyperalgesia.

INTRODUCTION

Diabetes mellitus has become one of the greatest epidemics of our time, projected that by 2050 it will affect approximately 366 million individuals. The most frequent pathological features of diabetic complications appearing in around half of the diabetic population are diabetic retinopathy, neuropathy, nephropathy and cardio-myopathy (Pirart, 1977; Wild *et al*., 2004). Diabetes is a heterogeneous group of syndromes characterized by an increased fasting blood glucose caused by a comparative or complete deficiency in insulin. Prolonged diabetes leads to microvascular and macrovascular complications. Diabetic neuropathy is one of the most frequently occurring microvascular complications of diabetes, of which the most common type is distal symmetrical neuropathy. This results in significant disability and morbidity (Chacra *et al.*, 2005). Severe pain, loss of ambulation and greater risk of foot ulceration are the common symptoms associated with diabetic neuropathy (Vinik *et al.*, 2004). Among different hypotheses that have been suggested to explain the various modes of development of diabetic neuropathy, hyperglycemia and oxidative stress are crucial role players. Hyperglycemia has been reported to result in increased activity of polyol pathway, oxidative stress, advanced glycation end product (ALE) formation, nerve ischemia, protein C and reduction of nerve growth factor support (Feldman, 2003; Obrosova, 2003; Sima *et al.*, 1999; van Dam, 2002; Vincent *et al.*, 2004). Oxidative stress plays a crucial role in contributing to neural and vascular complication because once the reactive oxygen species are formed they deplete antioxidant defenses such as SOD, catalase and GSH, rendering the affected cells and tissues more prone to oxidative damage. Hyperglycemia and oxidative stress increases lipid peroxidation and accelerated advanced lipoxidation end products formation may play a crucial role in the progression of neurovascular complications in diabetes (Sima *et al.*, 1999; Vincent *et al.*, 2004).

At present there is no definitive course of treatment available for diabetic neuropathy, as it is not clearly understood. Tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRI), anticonvulsants, opioids and topical capsaicin have been used in the management of painful neuropathy. The use of all these classes of drugs is restricted by their cost and side effects. Furthermore, these are only moderately effective, produce potential toxicity and develop tolerance, so need for newer, better tolerated and efficacious treatment is in high demand (Jacob *et al.*, 1999; Jamal *et al.*, 1990; Ziegler, 2008).

Herbal medicine is currently enjoying reinforcement in popularity and in fact, it is the major form of medicine in several parts of the world. Till date more than 1200 medicinal plants have been described to be experimentally or ethno pharmacologically tried in the management of diabetes mellitus. Prior to the introduction

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of insulin injections and other pharmaceutical preparation, healers relied greatly upon medicinal plants and herbs to treat diabetes (Bever, 1980). For centuries people have been trying to relieve and treat disease with different plant extracts and formulations. It is therefore vital that efforts must be made to introduce new medicinal plants to develop cheaper drugs. With the greater reliance on this type of medicine, it becomes important to look for potent, effective and relatively safe plant medicines, as well as scientific justification of the success claims about plants previously in use by conventional medicine practitioners so as to improve their safety and effectiveness. Screening of the plants for their biological activity is made on the basis of their chemotaxonomic investigation and ethno botanical analysis for a particular disease. Discovery of a particular compound against a specific disease is a difficult process. Significance of the plant lies in the biological activity of their phytoconstituents. There are two types of plant chemicals, primary metabolites such as proteins, amino acids, sugars etc. The other category of chemicals is called secondary metabolites, which includes alkaloids, terpenoids, saponins and phenolic compounds. These chemicals exert a significant physiological effect on the mammalian system (Ahmad *et al.*, 1992; Cowan, 1999).

Cleome viscosa, belongs to the Capparidaceae family, often called as " dog mustard," is an annual, sticky herb with yellow flowers and lengthy slender pods containing seeds which bear a resemblance to those of mustard with strong penetrating odour found as a common weed all over the plains of India and all over the tropics of the world (Parimala Devi *et al.*, 2004). The plant contains lignans, flavonoids, saponins, ascorbic acid, and polyunsaturated fatty acid. Some other chemical constituents isolated from *C. viscosa* are glucosinolates, cleomeolide, Stigmasta-5, 24(28)-diene-3β-*O*-α-L-rhamnoside, kaempferide-3-glucuronide, and naringenin glycoside (Sudhakar *et al.*, 2006). Traditionally described medicinal uses of *C. viscosa* are laxative, anti-helminthic, stomachic, diuretic (Gupta *et al.*, 2009; Rukmini, 1978) and hypoglycemic (Yaniv *et al.*, 1987). Furthermore, it can be used in treatment of malarial fevers, skin diseases, leprosy and fever due to indigestion, blood disorders and uterine complications. Earlier pharmacological reports of *C. viscosa* indicated that it acts as hepatoprotective, anthelminthic, analgesic, anti-inflammatory, antioxidant, immunomodulatory and antimalarial agent (Devi *et al.*, 2003; Parimaladevi *et al.*, 2003).

It is therefore important to carry out a research on the effect of *Cleome viscosa* in STZ-induced diabetic neuropathy. The result obtained from this study will go a long way to serve the poor and rural folks who have a propensity to favor herbal therapy to conventional medicine to deal with diabetes at lower cost.

MATERIALS AND METHODS

Plant material

The plants of *Cleome viscosa* were collected from various parts of Guwahati, Assam from October to November 2011. They were authenticated from the department of botany, Ayurvedic Research and Hospital, Vetapara, Guwahati, India. The selected parts of the plants were dried in shade at a temperature ranging from 21 to 30°C for 15-30 days. Afterwards, the selected plant parts were chopped and grounded. Extraction was carried out by the following method.

Preparation of ethanolic extract

Dried powdered material of the aerial parts of *Cleome viscosa* were extracted with ethanol (yield 16.65%) in a Soxhlet apparatus. The ethanolic extract was then distilled, evaporated, and dried in vacuum. Preliminary qualitative investigation of the ethanolic extract showed the presence of steroids, triterpenoids, flavonoids and tannins. The ethanolic extract of *Cleome viscosa* (EECV) was used for the present study.

Animals and drugs

Healthy male Wistar rats (weighing between 180–200 g, 8-10 weeks old) were obtained from the central animal facility, Gauhati Medical College (GMC). Animals were provided with standard diet and water *ad libitum*. They were housed in cages (three in each) and maintained at a constant temperature (24±1°C) and humidity (55±5%), with 12-hour light and dark cycle. All experimental protocols were approved by the institutional animal ethics committee of Gauhati Medical College.

Sterptozotocin, DMSO, 1, 1, 3, 3-tetramethoxy propane, 2-thiobarbituric acid, trizma, 5, 5-dithiobis (2-nitro-benzoic acid), were purchased from Sigma chemicals (Saint Louis, MO, USA). Reduced Glutathione, Triton-X 100, EDTA and HBSS buffer were obtained from Hi-media Laboratories Ltd., Mumbai, India. All other chemicals used were of analytical grade purity.

Induction and assessment of diabetes

Diabetes was induced in Wistar rats, with freshly prepared solution of single dose of 55 mg/kg STZ in citrate buffer (pH 4.4, 0.1 M) which was injected intraperitoneally to those animals which were kept on fasting for 24 hrs prior to injection to induce diabetes (Bhatt *et al.*, 2010). The vehicle control rats received an equal volume of citrate buffer and were used along with diabetic induced animals. Diabetes was confirmed 48 h after streptozotocin injection for which the blood samples were collected via rat tail vein technique using heparinized capillary glass tubes and plasma glucose levels were estimated by the enzymatic GOD-POD (Accurex®, India) diagnostic kit method. The rats were previously (one day before STZ injection) kept on 5%

glucose solution in the cages to avoid hypoglycemia (Dandu *et al.*, 2009). After two days, the rats with fasting blood glucose levels more than 250 mg/dl were considered as diabetic and were used in the subsequent experimental procedures (Kandhare *et al.*, 2012).

Experimental design

All the animals were divided into six groups of six animals in each as follows: (fig. 1)

[A] Non-diabetic animals

Group I Normal non-diabetic (ND): Animals received a single injection of citrate buffer (vehicle) and oral gavage of 0.5 % Carboxy methyl cellulose (CMC).

[B] Diabetic animals

Group II Diabetic (STZ) control: Animals received oral gavage of 0.5 % CMC daily from 5-8 weeks.

Group III Diabetic (STZ) + SC (5mg/kg): Animals received Glibenclamide (5mg/kg, p.o.) daily from 5-8 weeks.

Group IV Diabetic (STZ) + CV1 (100): Animals received oral gavage of EECV (100 mg/kg) in 0.5% CMC daily from 5-8 weeks.

Group V Diabetic (STZ) + CV2 (200): Animals received oral gavage of EECV (200 mg/kg) in 0.5% CMC daily from 5-8 weeks.

Group VI Diabetic (STZ) + CV3 (400): Animals received oral gavage of EECV (400 mg/kg) in daily from 5-8 weeks.

The *Cleome viscosa* extracts were freshly prepared in three different doses (100, 200 and 400 mg/kg) and administered for 4 weeks (once daily, per-orally) starting from week 5 after streptozotocin injection. After 8 weeks, rats were killed under deep anesthesia and sciatic nerves were immediately isolated, tissue homogenates were prepared in phosphate buffer solution (pH 7.0) and used for the estimation of biochemical parameters.

Thermal hyperalgesia

Tail immersion method

Hyperalgesia was assessed by tail immersion method (Courteix *et al.*, 1993). Rat tail was immersed in warm water (45°C) and the tail flick latency (withdrawal response of tail) was observed as the end point response.

Hot-plate method

Hyperalgesic response on the hot plate method is considered to be produced from a combination of central and peripheral mechanism (Kanaan *et al.*, 1996). Animals were individually placed into a glass chamber (Eddy's Hot plate) with the temperature adjusted to 55±1°C. The latency of first reaction [licking, moving the paws, little leaps or a jump to escape the heat] was recorded, with the cut off time kept at 12 sec in order to avoid paw damage. The test was repeated three times within thirty minutes.

Biochemical assays

Nerve collection and preparation of homogenate

After 8 weeks of experiment, sciatic nerve was removed bilaterally from the inguinal ligament to its trifurcation and incubated in triton X 100 for 20 min to remove blood from sciatic nerve. Sciatic nerve was homogenized with phosphate buffer solution (pH 7.0) by using homogenizer. The homogenate was then centrifuged in a cold centrifuge (Thermo Scientific) at 4°C, 4000×g for 10 min. Nerve homogenate was used for the measurement of SOD, GSH,

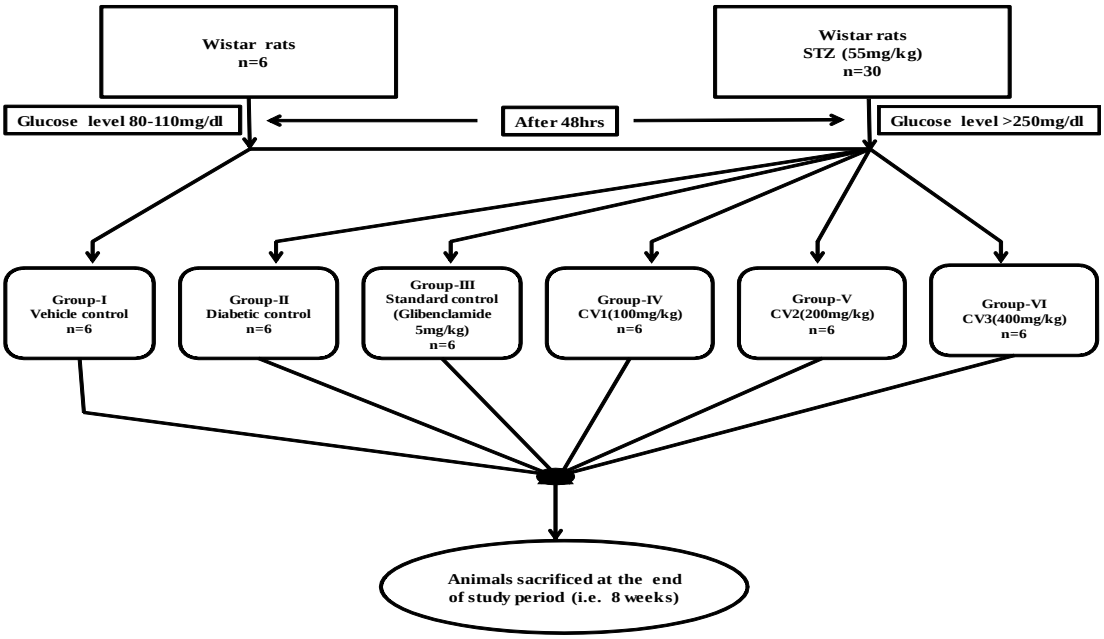

Fig. 1: Experimental design of the study.

lipid peroxidation and catalase activity. All procedures were carried out in cold conditions (Sayyed *et al.*, 2006).

Estimation of reduced glutathione

Reduced glutathione was measured as described by Ellman (Ellman, 1959). Equal quantity of sciatic nerve homogenate was mixed with 10% trichloroethanoic acid; subsequently proteins were separated by centrifugation. To 0.01 ml of the above resultant supernatant, 2ml of phosphate buffer, 0.5ml of 5, 5' dithio-bis-2-nitrobenzoic acid and 0.4 ml of double distilled water was added. Mixture was vortexed and the absorbance was measured at 412 nm within 15min. The concentration of reduced glutathione was expressed as µg/mg of protein.

Measurement of lipid peroxidation

Sciatic nerve was detached bilaterally from the inguinal ligament to its trifurcation. Nerve was then homogenized in 2.5% of 50 mM PBS buffer (pH 7.0) using polytron homogenizer after incubation in triton X 100 for 20 min. Homogenate was used for the measurement of thiobarbituric acid reactive substance at an absorbance of 535 nM according to the method of Zhang, 2004 (Zhang *et al.*, 2004). Amount of thiobarbituric acid reactive substance was calculated using an extinction coefficient of 156 mmol/cm and the values were expressed as malondialdehyde levels in µM/mg protein.

Measurement of SOD and catalase activity

Sciatic nerve homogenate was centrifuged at 4°C, 17,500×g for 10 min, resulted supernatant was used for the measurement of SOD activity by hematoxylin auto oxidation method (Martin, 1990) and catalase activity by hydrogen peroxide (H_2O_2) degradation method (Stevens *et al.*, 2000).

Estimation of lipid profile

The plasma samples were analyzed for total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides using sigma diagnostic assay kit.

STATISTICAL ANALYSIS

All the results were expressed as mean ± S.E.M. For multiple comparisons, one-way analysis of variance (ANOVA) was used. When ANOVA showed significant difference, post hoc analysis was done with Tukey's test. $P<0.05$ was considered statistically significant. Statistical analysis was carried out using graph pad prism software (Version 5).

RESULTS

Fasting blood glucose level and body weight

After 48 hours of STZ injection, diabetic rats showed significant increase in fasting blood glucose level as compared to control rats. Fasting blood glucose level were unaffected in normal control rats while multiple doses of EECV exhibited a significant reduction in fasting blood glucose level at the end of the study (Table 1), CV3 treated diabetic rats showed maximum reduction in fasting blood glucose level ($p<0.001$). Body weight of 8th week diabetic rats were significantly ($P<0.001$) lower than non-diabetic control rats. Moreover, multiple doses of EECV showed significant improvement in the body weight loss of diabetic rats when compared to non-diabetic control rats ($p<0.001$). However, CV3 treated rats showed maximum improvement in the body weight loss (table 1).

Measurement of antinociceptive activity

The nociceptive threshold was significantly lower in diabetic rats as compared with the basal value tested in both the tail immersion and hot-plate test. Hyperalgesia is discernible in the tail immersion and hot-plate after one and two weeks respectively, and we found decrease in threshold at 4 weeks (data not shown) after STZ injection in rats as compared to non-diabetic rats and the level of significance was maintained until 8 weeks. EECV administration to diabetic rats produced a dose and time dependent increase in pain threshold level as compared to untreated diabetic rats. The maximum increase in pain threshold level was observed in a progressive manner in both tail immersion and hot-plate test (figs. 2 & 3). CV3 produced highest significant antinociceptive activity when compared with remaining two doses of EECV ($p<0.001$).

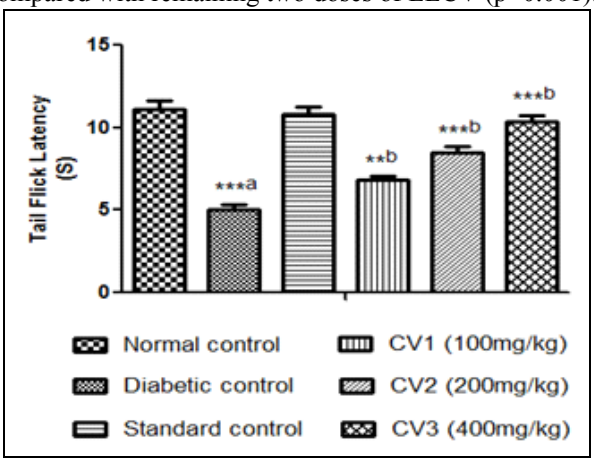

Fig. 2: Effect of EECV on tail flick latency in tail immersion test. All the values are expressed as mean ± SEM (n=6); a Vs Normal control, b Vs Diabetic control. $^{***}P<0.001$; $^{**}P<0.01$. Where CV1, CV2, CV3 are different doses of ethanolic extract of *Cleome viscosa*.

Effect of Cleome viscosa extracts on sciatic nerve lipid peroxidation and glutathione

Sciatic nerve malondialdehyde (MDA) levels were significantly higher in diabetic rats as compared to age matched non-diabetic rats. There was significant reduction in the levels of reduced glutathione in diabetic rats. Four weeks treatment of EECV appreciably reduced the sciatic nerve MDA content and raised the levels of reduced glutathione. CV3 treated rats showed highest significant reduction in sciatic nerve MDA content and

highest rise in the levels of reduced glutathione (figs. 4 & 5). However, total protein levels were not significantly altered (data not shown).

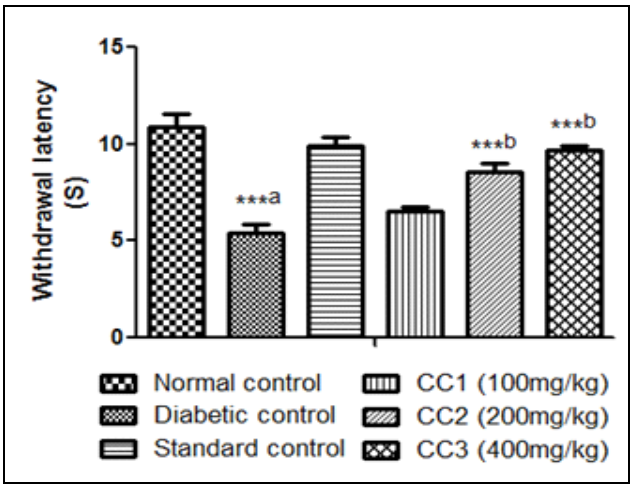

Fig. 3: Effect of EECV on paw withdrawal latency in hot plate method. All the values are expressed as mean±SEM (n=6); a Vs Normal control, b Vs Diabetic control. ***P<0.001; Where CV1, CV2, CV3 are different doses of ethanolic extract of *Cleome viscosa.*

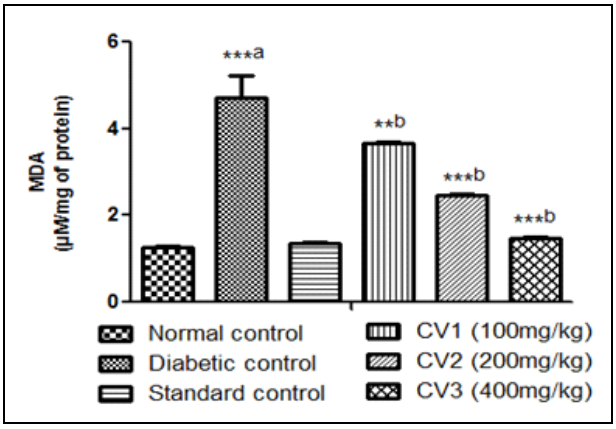

Fig. 4: Effect of EECV on sciatic nerve MDA level. All the values are expressed as mean ± SEM (n=6); a Vs Normal control, b Vs Diabetic control.
***P<0.001; **P<0.01. Where CV1, CV2, CV3 are different doses of ethanolic extract of *Cleome viscosa*.

Effect of Cleome viscosa extracts on sciatic nerve SOD and catalase activity

Sciatic nerve SOD activity was significantly lower in 8 weeks diabetic rats as compared to age matched non-diabetic rats. Four weeks treatment of *Cleome viscosa* extracts significantly restored SOD activity in the direction of normal control animals. CV3 treated rats showed highest improvement in the SOD activity after four weeks of treatment as compared to two left behind groups of EECV (fig. 6). Sciatic nerve catalase activity was significantly lower in 8 weeks diabetic rats as compared to age matched non-diabetic rats. Four weeks treatment of EECV significantly restored catalase activity to control animal values (fig. 7). CV3 treated rats showed highest improvement in the catalase activity after four weeks of treatment as compared to two left over groups of EECV (p<0.001).

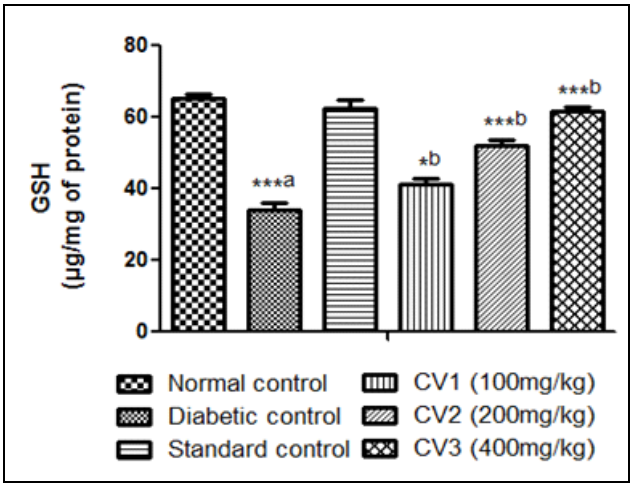

Fig. 5: Effect of EECV on sciatic nerve GSH activity. All the values are expressed as mean ± SEM (n = 6); a Vs Normal control, b Vs Diabetic control.
***P< 0.001; *P< 0.05. Where CV1, CV2, CV3 are different doses of ethanolic extract of *Cleome viscosa*.

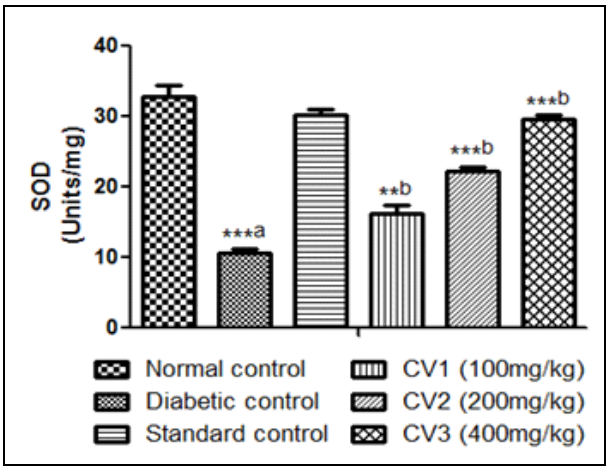

Fig. 6: Effect of EECV on sciatic nerve SOD activity. All the values are expressed as mean ± SEM (n=6); a Vs Normal control, b Vs Diabetic control.
***P<0.001; **P<0.01. Where CV1, CV2, CV3 are different doses of ethanolic extract of *Cleome viscosa*.

Effect of Cleome viscosa extracts on lipid profile

Oral administration of EECV showed dose dependent hypolipidemic activity. It reduced plasma cholesterol, triglyceride, LDL in STZ treated rats. In addition to hypolipidemic activity, EECV also produced a noteworthy dose dependent increase in level of high density lipoproteins (HDL). High density lipoprotein (HDL) is commonly referred to as good cholesterol possessing the ability to reverse cholesterol transport and

also protect LDL from oxidation, thereby minimizing the deleterious consequences of LDL oxidation. CV3 showed highest decrease in the levels of plasma cholesterol, triglyceride and LDL whereas increase in HDL level as compared to the left over groups of EECV (table 2).

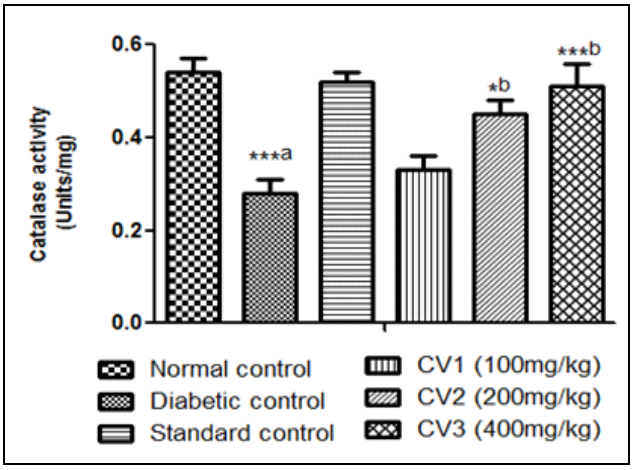

Fig. 7: Effect of EECV on sciatic nerve Catalase activity. All the values are expressed as mean ± SEM (n = 6); a Vs Normal control, b Vs Diabetic control.
***P<0.001; *P<0.05. Where CV1, CV2, CV3 are different doses of ethanolic extract of *Cleome viscosa*.

DISCUSSION

Diabetic neuropathy induced by streptozotocin is a reliable and reproducible animal model for preclinical evaluation of diabetic neuropathy for potential drug candidate (Baynes 1991; Gillery *et al.*, 1989). Hyperglycemia must be the originator in the pathogenesis of diabetic complications. Diabetes is a heterogeneous set of disorders with a range of pathologies and one of the most frequent complications of diabetes is peripheral neuropathy (Saini *et al.*, 2007). Diabetes is usually accompanied by excessive production of free radicals, and it was recently established that hyperglycemia induced mitochondrial ROS production could be a key episode in the progress of diabetic complications (Sayyed *et al.*, 2006). Oxidative stress has a detrimental effect on proteins, cellular lipids and DNA directly. Malondialdehyde levels (indicator of oxidative damage) were proven to rise in both *in vitro* & *in vivo* with diabetes (Anwer *et al.*, 2007; Iida *et al.*, 2004). In our study, development of diabetic neuropathy in STZ induced diabetic rats was evident from reduction in tail flick latency and paw withdrawal response. These results are consistent with the earlier reports, wherein comparable results of thermal hyperalgesia in STZ-induced diabetic rats were reported (Cameron *et al.*, 2002). The diabetic rats showed a significant increase in the plasma glucose level. Oral administration of EECV to diabetic rats at a dose of 400 mg/kg showed a significant drop in the plasma glucose level as compared to STZ treated rats. Treatment of diabetic rats with EECV reduced the plasma glucose level significantly as compared to the diabetic control rats. The effect of the CV3 was close to as that of standard drug glibenclamide**.** The capability of the EECV to defend body weight loss seems to be due to its ability to decrease hyperglycemia. It was reported that the increase in body weight is far less in poorly controlled diabetic rats as compared to good controlled diabetic rats (Latha *et al.*, 2004). Similar interpretations were made in this study. Hypoglycemic activity of the *Cleome viscosa* may be due to stimulation of insulin secretion from pancreatic β-cells.

Elevated plasma lipid levels, principally of cholesterol along with production of reactive oxygen species plays a main role in the progress of diabetic neuropathy. There is now enormous support that herbal drugs may be helpful in the cure and control of hyperlipidemia and this may translate directly or indirectly to the management of diabetic complications. The unusually elevated plasma lipids are mainly due to enhanced recruitment of free fatty acids from the peripheral fat deposits, as insulin inhibits the hormone sensitive lipase generation. Hence, the elevated level of plasma lipids in diabetes causes the risk of diabetic neuropathy (Georg *et al.*, 2000; Januszewski *et al.*, 2003). The administration of EECV to diabetic rats tends to bring the values close to normal**.** *Cleome viscosa* is already proved to have antioxidant properties, and this may trim down the susceptibility of lipids to oxidation and cause the membrane lipids stabilization, thus reducing oxidative stress. Thus, 4 weeks EECV treatment exhibited hypocholesterolaemic, hypotriglyceridaemic, effects while at the same time increasing the HDL.

Table 1: Effect of EECV on the final body weight and blood glucose level in STZ induced diabetic rats. All the values are expressed as mean ± SEM (n=6); ^{a}P<0.001 Vs Normal control; ^{b}P<0.001, ^{c}P<0.01 Vs Diabetic control. Where CV1, CV2, CV3 were different doses of ethanolic extract of *Cleome viscosa*.

Treatment	Body weight (gm)	Blood glucose (mg/dl)
Normal control	249.54±7.56	86.64±8.81
Diabetic control	126.23±9.48^{a}	387.19±11.58^{a}
Standard control	208.74±8.64^{b}	126.64±13.12^{b}
CV1(100mg/kg)	156.23±6.22	226.70±11.26^{b}
CV2(200mg/kg)	171.34±9.23^{c}	174.59±12.16^{b}
CV3(400mg/kg)	204.14±7.42^{b}	138.65±9.12^{b}

Neuropathic pain is usually connected with diabetic neuropathy. We observed decline in tail flick latency in tail immersion test and paw withdrawal response in hot plate method in diabetic rats, this shows thermal hyperalgesia. Several researchers have reported comparable hyperalgesia in diabetic rats (Anjaneyulu *et al.*, 2004; Ciruela *et al.*, 2003; Wuarin-Bierman *et al.*, 1987). Mechanisms, for instance tissue injury due to ischemia, sensitization of peripheral receptors, changes in

Table 2: Effect of EECV on the plasma lipid levels in STZ induced diabetic rats.

Treatment	HDL(mg/dl)	LDL (mg/dl)	TC (mg/dl)	TG (mg/dl)
Normal control	51.45 ± 3.98	62.64 ± 7.81	113.2 ± 4.05	66.47 ± 6.77
Diabetic control	28.21 ± 4.26[b]	232.19 ± 6.58[a]	244.7 ± 3.43[a]	132.27 ± 5.18[a]
Standard control	49.76 ±4.23[d]	69.64 ± 5.42[c]	120.22 ± 2.66[c]	67.34 ± 4.84[c]
CV1 (100mg/kg)	33.49 ± 3.42	171.7 ± 4.26[c]	218.20 ± 3.78[c]	126.56 ± 5.34
CV2 (200mg/kg)	41.60 ± 4.65	114.59 ± 5.16[c]	169.24 ± 5.13[c]	97.49 ± 6.16[d]
CV3 (400mg/kg)	49.35 ± 3.46[d]	85.65 ± 7.18[c]	129.42 ± 4.25[c]	69.65 ± 4.12[c]

All the values are expressed as mean ± SEM (n = 6); [a]P<0.001, [b]P<0.01 Vs Normal control; [c]P<0.001, [d]P<0.01 Vs Diabetic control. Where CV1, CV2, CV3 were different doses of ethanolic extract of *Cleome viscosa*.

dorsal root ganglia cells and ectopic activity in nascent fibres, are reported to give transform nociception (Jensen *et al.*, 2003). In the present study, we observed a significant enhancement in tail flick latency in hot immersion test and paw withdrawal time in hot plate method upon EECV treatment, thus suggesting the role of reactive oxygen species mediated oxidative stress in nociceptive changes in STZ induced diabetic rats.

Oxidative stress that leads to an enlarged production of ROS and finally cellular lipid peroxidation (LPO) has been found to play significant role in the advancement of diabetes mellitus and its complications. Lipid peroxidation is an indicator of cellular oxidative injury generated by reactive oxygen species (Singab *et al.*, 2005). Streptozotocin induced diabetes rats showed an enhance in lipid peroxidation (TBARS), an indirect proof of intensified free radical production (Maritim *et al.*, 2003). Most of the tissue damage took place by these free radicals destroying membranes through peroxidation of unsaturated fatty acids. In diabetic induced rats, we observed a significant increase in MDA levels and fall in antioxidant enzymes activity. In our study, lipid peroxidation was significantly reduced by EECV treatment. SOD & GSH activities were significantly improved on subsequent four weeks treatment with EECV. Catalase activity in EECV treated diabetic rats was close to normal rats, however we do observed maximum restoration of antioxidant enzymes by CV3 treated diabetic rats. Collectively, our study suggests the function of oxidative stress in diabetic neuropathy as reported by previous studies.

CONCLUSION

In conclusion, present study shows that the administration of EECV produces significant protection in diabetic neuropathy as apparent from amelioration in oxidative stress, thermal hyperalgesia, hyperlipidemia and lipid peroxidation in diabetic treated rats. This outcome might be due to prevention of free radical production in diabetic rats. This study suggests the role of oxidative stress in diabetic neuropathy and beneficial effects of *C. viscosa* in diabetic neuropathy. Further research is in progress to determine the exact phytoconstituents that are accountable for the above reported pharmacological effects of the ethanolic extract of *Cleome viscosa*.

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