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H. Spinosa T. Anders Ameliorates Diabetic Neuropathy in Wistar Albino Rats

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Abstract

Diabetic neuropathic pain, an important microvascular complication in diabetes, is recognised as one of the most difficult types of pain to treat. The development of tolerance, inadequate relief, and potential toxicity of classical antinociceptives warrant the investigation of the newer agents to relieve this pain. Reactive oxygen/nitrogen species, increased oxidative stress, cytokines, and apoptosis are implicated in the pathogenesis of diabetic neuropathy. The aim of the present study was to explore the effect of methanolic extract of aerial parts of *H. spinosa* (HSME) on alloxan induced diabetic neuropathy in Wistar rats. Diabetic rats developed neuropathy after the third week of diabetes induction. Chronic treatment with HSME (250, 500, and 750 mg/kg body weight; p.o.) for 6 weeks starting from the 3rd week of alloxan injection showed significant increase in the pain threshold levels as compared to diabetic rats. HSME treated diabetic animals showed significant decrease in blood glucose level and increase in body weight as compared to diabetic control animals. The changes in lipid peroxidation status and antioxidant enzymes levels observed in sciatic nerve of diabetic rats were significantly restored by HSME treatment. Thus, the results suggest therapeutic potential of *H. spinosa* in treatment of diabetic neuropathy.

KEYWORDS: H. spinosa, diabetic neuropathy, hyperalgesia, allodynia, oxidative stress

1. Introduction

Diabetes mellitus has now become an epidemic with a worldwide incidence of 5% in the general population. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025 (Jarald et al., 2008). Much of the burden of diabetes mellitus for both patients and society comes from the complications of the disease.

Diabetic peripheral neuropathy is the most common complication of long-standing diabetes mellitus which frequently results in clinically significant morbidities e.g. pain, foot ulcers and amputations (Said, 2007). A large number of neuroanatomical, neurophysiological and neurochemical mechanisms are thought to contribute to the development and maintenance of diabetic neuropathic pain (Edwards et al., 2008; Gidal and Billington, 2006). The most common precipitating cause of neuropathic pain is diabetes particularly where blood glucose control is poor (Morley et al., 1984). Approximately 20–24% of diabetes patients experience neuropathic pain (Schmader, 2002). Diabetic neuropathic pain can occur either spontaneously, as a result of exposure to normally mildly painful stimuli (i.e. hyperalgesia), or to stimuli that are not normally perceived as being painful (i.e. allodynia) (Brown and Asbury, 1984). The cause of painful diabetic neuropathy, like other neuropathic pain states, is still unclear (Calcutt, 2002; Sommer, 2003).

Currently, the only agents approved for the treatment of painful diabetic neuropathy are lidocaine patches 5%, duloxetine, gabapentin, and pregabalin (Gidal and Billington, 2006). Despite the use of these agents, the successful therapy of diabetic neuropathy remains a challenge. Due to pathogenic complexity of diabetic neuropathy, new therapeutic interventions targeting primary mechanisms contributing to nerve damage are critical for the future treatment of this complication (Kuhad and Chopra, 2009).

Hygrophila spinosa (K. Schum.) Heine, syn. *Asteracantha longifolia* Nees (Acanthaceae) is a wild herb commonly found in moist places on the banks of tanks, ditches and paddy fields throughout India commonly known as Talimkhana, Kokilaksha. Aerial parts of the plant are used ethnobotanically for the treatment of body pain, jaundice and malaria, while the seeds are used for treatment of impotence and thus as aphrodisiac (Jain 1991). Aerial parts of *H. spinosa* have been reported to contain lupeol, stigmasterol and butelin. *H. spinosa* has been shown to possess hypoglycemic activity (Fernando et al., 1989), hepatoprotective (Singh A and Handa, 1999; Shailajan et al., 2005), antitumor (Mazmudar et al., 1997), anabolic and adrogenic activities (Jayatilak et al., 1976). *Hygrophila spinosa* seeds have been reported for antinociceptive activity and are traditionally claimed as nervine tonic. Aerial parts of the plant are also reported for its hypoglycemic and antioxidant activity (Vijayakumar et al., 2006; Patra et

al., 2009). The literature survey revealed that there are no scientific studies carried out regarding the effect of aerial parts of *Hygrophila spinosa* in diabetic neuropathy to substantiate their traditional therapeutic claim. Hence in the present study, the methanolic extract of aerial parts was evaluated for its possible benefits in alloxan-induced diabetic neuropathy in Wistar albino rats.

2. Materials and methods

2.1. Animals

The healthy adult Wistar albino rats (5-6 months) of either sex weighing 200–250g were used for the study. All animals were housed in polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle, 25 ± 5^0C, 35–60 % relative humidity). The animals were fed with standard rat pellet diet (Amrut feed, Sangali) and water *ad libitum*. All the procedures were performed in accordance with the institutional animal ethical committee (IAEC) constituted as per the directions of CPCSEA, approval no. CPCSEA/IAEC/PC-07/06-2K9.

2.2. Chemicals

Alloxan monohydrate was purchased from Ozone International, India. Biochemical kit for estimation of serum glucose was purchased from Biolab Diagnostic (I) Pvt. Ltd., India.

2.3. Preparation of extract

The shade dried aerial parts of *H. spinosa* (HS) were subjected for size reduction to coarse powder. The powder was defatted with petroleum ether (60-80°C) and then extracted with 80% methanol using five times of solvent in soxhlet apparatus at 80°C under vacuum. Contents were filtered and filtrate (A) was collected. Marc was extracted once again using 4 times of 80% methanol as mentioned earlier. Contents were filtered again; the filtrate (B) was collected and marc was discarded. Solvent from filtrate A & B was distilled at around 60°C under vacuum. Thick paste obtained thereafter was dried in vacuum drier at a temperature below 50°C. The dry mass was homogenised to make fine free flowing powder of 60 mesh sieve. The percentage yield of methanolic extract of *H. spinosa* (HSME) was found to be 10-12% (w/w). The HSME was then subjected to phytochemical screening (Kokate, 1994).

2.4. 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 HS methanolic extract was administered at a dose of 2000mg/kg orally to three female Wistar rats. Animals were observed individually for the first four hours 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 kept under supervision upto 14 days for any sign of toxicity or mortality (OECD Guideline, 2000).

2.5. Induction and assessment of diabetes

Alloxan monohydrate freshly dissolved in normal saline was injected to male Wistar rats intraperitoneally (150 mg/kg, i.p.) after overnight fasting. After alloxan treatment, all animals were given free access to food and water. Blood glucose levels were measured after three days of alloxan injection and only animals with blood glucose levels higher than 200 mg/dL were considered diabetic and included in the study (Leite et al., 2007).

2.6. Treatment schedule and Experimental protocol

A total of thirty male Wistar rats (24 diabetic surviving and 6 normoglycemic rats) were used. Group 1 (control group) was the non-diabetic/normoglycemic animals which received distilled water (n=6). After 21 days of diabetes induction, rats in diabetic group were subdivided into four groups (n=6). Group 2 (diabetic control) consisted diabetic animals which received distilled water (1ml/kg, p.o.). The diabetic animals from group 3, 4 and 5 received HSME 250, 500 and 750 mg/kg/day (p.o.) for six weeks respectively and served as test groups.

Body weight of all animals was recorded on 0, 2^{nd}, 4^{th} and 6^{th} week of treatment. Blood of all animals was collected through retro-orbital route initially and on 2^{nd}, 4^{th} and 6^{th} week of treatment to measure the serum glucose levels. Development of neuropathy was assessed in control and diabetic animals from all groups by evaluation of pain thresholds on 0, 2^{nd}, 4^{th} and 6^{th} week of respective treatment by assessment of thermal/mechanical hyperalgesia and thermal allodynia. At the end of treatment, the animals were sacrificed under deep anesthesia by cervical dislocation, and sciatic nerves were rapidly removed and weighed. A tissue homogenate 10% (w/v) was prepared in 0.1 M phosphate buffer (pH 7.4). Homogenates were centrifuged at 200 g for 10 min, at 4 ^{0}C and

supernatant was used for estimation of lipid peroxidation, catalase, glutathione, glutathione peroxidase, glutathione S transferase, glutathione reduced (Kuhad and Chopra, 2009 ; Kuhad and Chopra, 2008; Bhatt et al., 2009; Beyreuther et al., 2006).

2.7. Assessment of thermal hyperalgesia

2.7.1. Tail-immersion (hot water) test

The hyperalgesic response in tail immersion test is considered to result from central mechanism. Tail of rat was immersed in a hot water bath (52.5 ± 0.5^0C) until tail withdrawal (flicking response) or signs of struggle were observed (cut-off 12 seconds). Shortening of the tail withdrawal time indicates induction of hyperalgesia (Kuhad and Chopra, 2009).

2.7.2. Hot-plate test

In this test, animals were individually placed on a hot-plate analgesiometer (Columbus instruments, USA) with the temperature adjusted to 55 ± 1^0C. The latency to the first sign of paw licking or jump response to avoid the hot surface was taken as an index of the pain threshold; the cut-off time was 10 seconds in order to avoid damage to the paw (Kuhad and Chopra, 2009).

2.8. Assessment of mechanical hyperalgesia (Paw pressure withdrawal test)

Nociceptive flexion reflexes were quantified using the Digital Randall–Selitto apparatus (IITC Life Science, USA). Linearly increasing pressure, with the cut-off of 250g to avoid tissue injury, was applied to the center of hindpaw. When animal displayed pain by withdrawal of the paw, vocalization or overt struggling; the applied paw pressure was registered and expressed in mass units (grams). Five tests separated by at least 15 min were performed for each animal, and the mean value of these tests was calculated (Kuhad and Chopra, 2009; Beyreuther et al., 2006). Rats were trained by using repeated paw withdrawal tests on the previous 3 days before experimentation.

2.9. Assessment of thermal allodynia (warm plate test)

Animals were individually placed on a hot plate analgesiometer (Columbus Instruments, USA), with the temperature adjusted to 38°C. The latency of the first reaction was recorded (licking, moving the paws, little leaps or a jump to escape the heat) with a cut-off time of 30 seconds (Beyreuther et al., 2006).

2.10. Biochemical estimation

The total protein content was estimated (Lowry et al., 1951). The malondialdehyde content, a measure of lipid peroxidation, was assessed in the form of thiobarbituric acid reactive substances (Ohkawa et al., 1979). Reduced glutathione (GSH) levels (Ellman, 1959), glutathione peroxidase (GPx) activity (Flohe and Gunzler, 1984), catalase (CAT) activity (Claiborne, 1991), glutathione-S-transferase (GST) activity (Habig and Jakoby, 1981) and glutathione reductase (GR) activity (Carlberg and Mannervik, 1975) were assayed by using standard biochemical procedures. The MDA, GSH, GPx, CAT, GST and GR were expressed as units per mg protein.

2.11. Statistical analysis

The results are expressed as mean ± SEM. Statistical analysis was done using INSTAT graphpad software. Comparison between the control and diabetic control group was made with unpaired Student's *t* test. Comparison between diabetic control and test groups was made with one way analysis of variance (ANOVA) followed by *Dunnett's* test.

3. Results

3.1. Phytochemical analysis

Phytochemical analysis of the methanolic extract of *H. spinosa* revealed the presence of steroids, glycosides, triterpenoids, carbohydrates, saponins, proteins, alkaloids and flavonoids.

3.2. Toxicity profile

All the animals did not showed any sign of toxicity or mortality in the first four hours after dosing and thereafter upto next 14 days.

3.3. Effect of HSME of body weight and blood glucose levels

The alloxan-treated animals had significantly reduced body weight than the control rats. The average blood glucose level of the alloxan-treated animals was significantly higher as compared to the control animals. Treatment with HSME 250, 500 and 750 mg/kg in diabetic animals showed significant increase in body weight (Table 1) and significant decrease in serum glucose (Table 2) as compare to vehicle treated diabetic animals in dose and time dependent manner.

Table 1. Effect of HSME treatment on body weight

Group	***Body weight (g)***			
	0 Week	***2nd Week***	***4th Week***	***6th Week***
Control	236.27 ± 1.990	243.42 ± 2.122	256.70 ± 1.585	263.87 ± 1.104
Diabetic control	161.60 ± 1.990#	155.90 ± 2.172#	154.67 ± 0.7684#	150.53 ± 1.242#
HSME 250	159.97 ± 2.456	165.63 ± 1.994*	177.80 ± 1.883**	183.68 ± 1.694**
HSME 500	159.13 ± 2.322	177.32 ± 2.815**	186.75 ± 1.624**	204.02 ± 2.992**
HSME 750	163.77 ± 2.157	186.35 ± 3.414**	196.60 ± 1.923**	217.32 ± 1.599**

Results are expressed as Mean ± SEM (n=6). The data was analysed using Student t test and One-way Analysis of Variance (ANOVA) followed by Dunnett's- test.
#p<0.01 compared with control; **p<0.01 compared with diabetic control.
Where, HSME is methanolic extract of aerial parts of *H. spinosa*.

Table 2. Effect of HSME treatment on serum glucose level

Group	***Blood glucose concentration (mg/dl)***			
	0 Week	***2nd Week***	***4th Week***	***6th Week***
Control	98.000 ± 2.436	97.000 ± 2.017	97.333 ± 2.124	98.167 ± 1.922
Diabetic control	265.33 ± 2.642#	261.33 ± 2.777#	288.00 ± 5.686#	306.83 ± 3.877#
HSME 250	267.83 ± 1.778	228.83 ± 2.982**	212.83 ± 2.880**	184.50 ± 2.391**
HSME 500	260.17 ± 6.118	208.83 ± 2.522**	177.83 ± 2.892**	166.50 ± 2.262**
HSME 750	256.67 ± 4.341	175.50 ± 3.649**	157.50 ± 1.928**	141.67 ± 2.108**

Results are expressed as Mean ± SEM (n=6). The data was analysed using Student t test and One-way Analysis of Variance (ANOVA) followed by Dunnett's- test.
#p<0.01 compared with control; **p<0.01 compared with diabetic control.
Where, HSME is methanolic extract of aerial parts of *H. spinosa*.

3.4. Effect of HSME treatment on thermal hyperalgesia

The nociceptive threshold was significantly lower in diabetic rats as compared with control animals tested in both the tail-immersion (Fig. 1) and hot-plate assays (Fig. 2). Thermal hyperalgesia was evident in alloxan/vehicle-treated animals since the paw withdrawal latency was significantly shorter than that of control/vehicle-treated animals after the third week of diabetes induction. Treatment of diabetic rats with HSME 500 and 750 mg/kg induced a statistically significant increase in pain threshold after four weeks of treatment, which was further increased after six weeks of treatment in dose dependant manner. The HSME 250 mg/kg treatment showed significant increase in pain threshold level as compare to diabetic control only after six weeks of treatment.

Fig 1. Effect of HSME treatment on tail withdrawal latency.

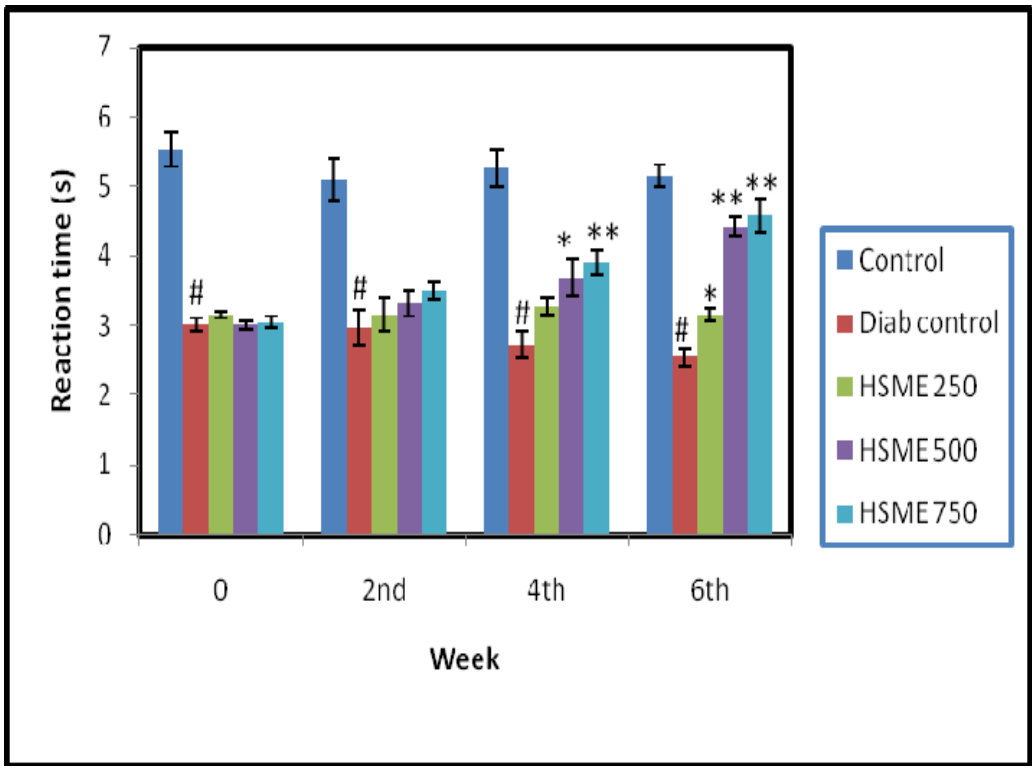

Fig 1. Results are expressed as Mean ± SEM (n=6). The data was analysed using Student t test and One-way Analysis of Variance (ANOVA) followed by Dunnett's- test. #p<0.01 compared with control; *p< 0.05, **p<0.01 compared with diabetic control. Where, HSME is methanolic extract of aerial parts of *H. spinosa*.

Fig 2. Effect of HSME treatment on paw withdrawal latency in hot-plate test.

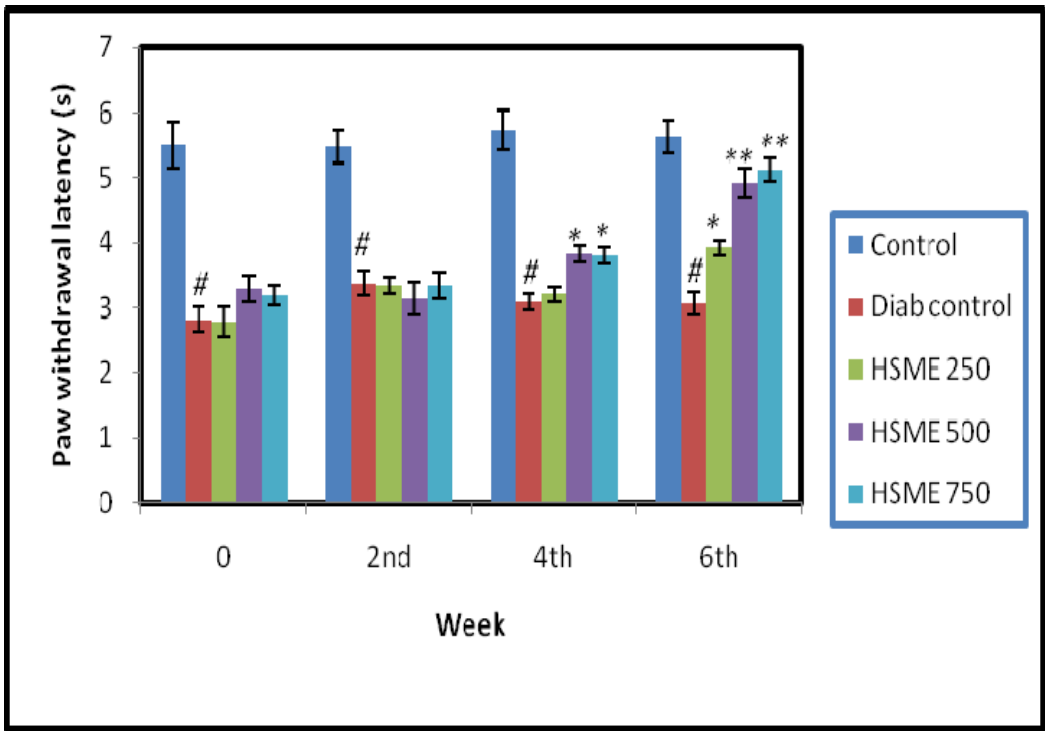

Fig 2. Results are expressed as Mean ± SEM (n=6). The data was analysed using Student t test and One-way Analysis of Variance (ANOVA) followed by Dunnett's- test. #p<0.01 compared with control; *p< 0.05, **p<0.01 compared with diabetic control. Where, HSME is methanolic extract of aerial parts of *H. spinosa*.

3.5. Effect of HSME treatment on mechanical hyperalgesia

There was a marked mechanical hyperalgesia as evidenced by a reduction in the paw pressure withdrawal thresholds in the alloxan/vehicle-treated animals compared to control/vehicle-treated animals (Fig. 3). Treatment of diabetic rats with HSME 500 and 750 mg/kg induced a significant increase in paw pressure withdrawal threshold compared to alloxan/vehicle-treated animals after four weeks of treatment which was further increased after six weeks of treatment in dose dependant manner. Whereas HSME 250 mg/kg induced a significant increase in paw pressure withdrawal threshold only after six weeks of treatment compare to diabetic control animals.

Fig 3. Effect of HSME treatment on paw pressure withdrawal thresholds

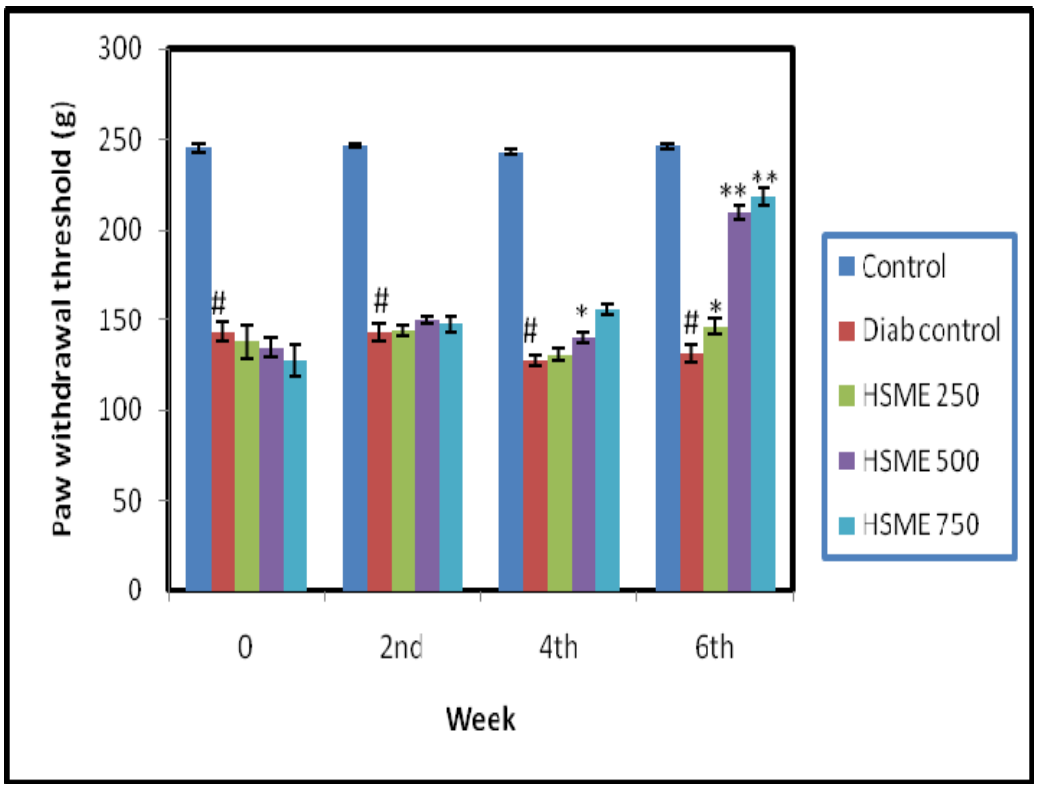

Fig 3. Results are expressed as Mean ± SEM (n=6). The data was analysed using Student t test One-way Analysis of Variance (ANOVA) followed by Dunnett's- test.
#p<0.01 compared with control; *p< 0.05, **p<0.01 compared with diabetic control.
Where, HSME is methanolic extract of aerial parts of *H. spinosa*.

3.6. Effect of HSME treatment on thermal allodynia

Marked thermal allodynia was observed in the alloxan/vehicle-treated animals as evidenced by a reduction in the pain thresholds compared to control/vehicle-treated animals (Fig. 4). Treatment of diabetic rats with HSME 500 and 750 mg/kg induced a significant increase in pain threshold compared to alloxan/vehicle-treated animals after four weeks of treatment which was further increased after six weeks of treatment in dose dependant manner. Whereas HSME 250 mg/kg treatment showed significant increase in paw withdrawal reaction time after six weeks of treatment compare to diabetic control animals.

Fig 4. Effect of HSME treatment on paw withdrawal latency in warm plate test

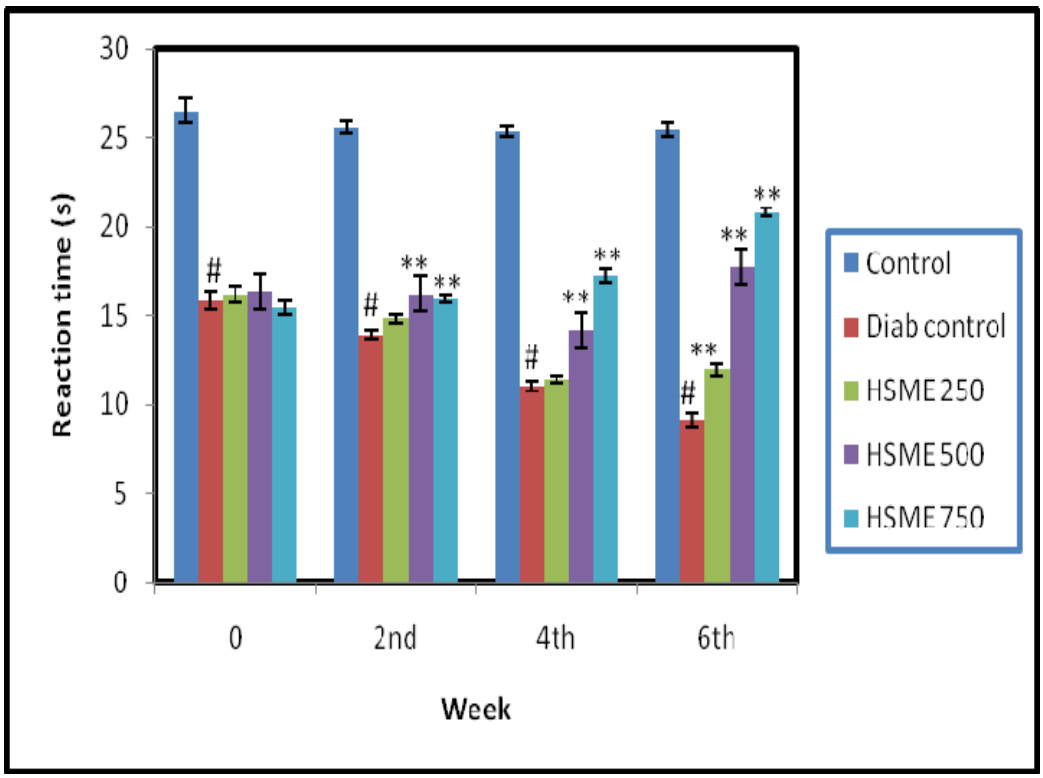

Fig 4. Results are expressed as Mean ± SEM (n=6). The data was analysed using Student t test and One-way Analysis of Variance (ANOVA) followed by Dunnett's- test. #p<0.01 compared with control; *p< 0.05, **p<0.01 compared with diabetic control. Where, HSME is methanolic extract of aerial parts of *H. spinosa*.

3.7. Biochemical estimation

A significant increase in lipid peroxides (MDA) and reduction in endogenous antioxidant enzymes like CAT, GR, GPx, GST and GSH activity was observed in sciatic nerve homogenates of diabetic rats. Treatment with HSME at all doses for six weeks in diabetic rats, restored above mentioned biochemical parameters in dose dependent manner compare to vehicle treated diabetic animals as shown in Table 3.

Table 3. Effect of HSME on oxidative markers in sciatic nerve homogenate against alloxan induced diabetic neuropathy in rats

Parameter	*Control*	*Diabetic control*	*HSME 250*	*HSME 500*	*HSME 750*
MDA	2.465 ± 0.07818	7.220 ± 0.4191#	6.328 ± 0.1934*	4.888 ± 0.1102**	3.360 ± 0.1325**
CAT	26.688 ± 0.5344	12.172 ± 0.3584#	13.898 ± 0.3626*	17.770 ± 0.3639**	21.660 ± 0.3891**
GSH	9.913 ± 0.2128	3.665 ± 0.1314#	4.532 ± 0.1796*	5.855 ± 0.2150**	7.697 ± 0.2268**
GPX	15.078 ± 0.2726	5.403 ± 0.07279#	6.183 ± 0.1657*	8.720 ± 0.1580**	12.768 ± 0.1288**
GST	28.777 ± 0.7303	6.490 ± 0.1339#	8.503 ± 0.4160*	16.342 ± 0.4962**	20.822 ± 0.3712**
GR	6.527 ± 0.1459	1.580 ± 0.09726#	2.037 ± 0.05090*	4.005 ± 0.05915**	5.290 ± 0.1176**

Results are expressed as Mean ± SEM (n=6). The data was analysed using One-way Analysis of Variance (ANOVA) followed by Dunnett's- test.
#$p<0.01$ compared with control; *$p<0.05$, **$p<0.01$ compared with diabetic control.
Where, MDA is malondialdehyde content, GSH is reduced glutathione, GPx is glutathione peroxidase, CAT is catalase, GST is glutathione-S-transferase, Gr is glutathione reductase and HSME is methanolic extract of aerial parts of *H. spinosa*

4. Discussion

Neuropathic pain is the most common symptom associated with diabetic neuropathy. Due to this, individuals with diabetic neuropathy were shown to have significantly lower quality of life scores. Overall, approximately 10% of patients with diabetes experience persistent pain from neuropathy (Vinik and Mehrabyan, 2004). The conventional drugs used in the management of diabetic neuropathy are associated with a variety of side effects. When compared with well-defined synthetic drugs, herbal medicines exhibit some marked difference as are relatively safe and have lesser side effects when used in chronic diseases (Kamboj, 2000).

The extensive literature survey indicated that aerial parts of *H. spinosa* have been traditionally claimed for their use in treatment of diabetes, impotency,

renal complications, pain and is claimed as nervine tonic (Patra et al., 2009; Nadkarni, 2007; Kirtikar and Basu, 2005; Khare, 2004). The *H. spinosa* have been reported to possess the hypoglycemic, androgenic, aphrodisiac, diuretic, antinociceptive and antioxidant activity in different animal species (Vijayakumar et al., 2006; Chauhan et al., 2009; Hussain et al., 2009; Shanmugasundaram and Venkataraman, 2005; Shanmugasundaram and Venkataraman, 2006). *H. spinosa* has not been yet scientifically documented so far for its effect in diabetic neuropathy. In light of which the present investigation was planned.

A synthetic or herbal drug with potential beneficial activity against diabetes and its complications can be tested by using different animal models of diabetes (Frode and Medeiros, 2008). In our study, the alloxan-induced diabetic rats showed evident hyperglycemia throughout the entire experimental period indicating state of diabetes.

It is a well-established fact that diabetic rats display exaggerated hyperalgesic behavior in response to noxious stimuli that model the aspects of painful diabetic neuropathy (Freshwater et al., 2002). While thermal hypoalgesia is reported in diabetic rats by some researchers (Akunne and Soliman, 1987; Calcutt et al., 2003; Kolta et al., 1996), others have reported hyperalgesic behavior (Forman et al., 1986; Ohsawa and Kamei, 1999; Simon and Dewey, 1981; Vijayvargia et al., 2000). Although evaluation of mechanisms causing these symptoms is complicated because of the overlap between the systemic effects of hyperglycemia and its toxic effects within the peripheral nervous system, direct functional toxicity of hyperglycemia in the peripheral nervous system (Dobretsov et al., 2001), an increased activity of primary afferent fibers leading to an increased excitatory tone within the spinal cord, increased release of glutamate and activation of the NMDA receptor, reduced activity of both opioidergic and GABAnergic inhibitory systems (Malcangio and Tomlinson, 1998), decreased activity of nNOS–cGMP system in neurons of dorsal root ganglion (Sasaki et al., 1998), altered sensitivity of the dopaminergic receptors and altered responsiveness of the dopaminergic system, possibly through the enhancement and/or deactivation of the endogenous Met-enkephalinergic system (Takeshita and Yamaguchi, 1998; Rutledge et al., 2002), and alterations in L-type Ca^{2+} channels (Gullapalli et al., 2002) could be involved in the modulation of nociception in diabetic rats.

Recently, it has been reported that the aerial parts of *H.spinosa* reduced the elevated serum glucose level in diabetic Sprague–Drawley rats (Vijayakumar et al., 2006). Our results are in agreement to these finding as the HSME in all dose levels significantly reduced the elevated blood glucose level near to normal.

Hyperglycemia is reported to induce oxidative stress through multiple pathways such as redox imbalances secondary to enhanced aldose reductase activity (Yagihashi et al., 2001), increased advanced glycation end products

(Brownlee et al., 1988), altered protein kinase C activity, especially b-isoforms (Cameron et al., 1999), prostanoid imbalances (Kellogg and Pop-Busui, 2005; Pop-Busui et al., 2002) and mitochondrial overproduction of superoxide (Brownlee, 2003). This increased oxidative stress leads to many biochemical changes and is an important causative factor in the development of peripheral neuropathy. (Vinik and Mehrabyan, 2004). It has been postulated that the etiology of the complications of diabetes involves oxidative stress perhaps as a result of hyperglycemia (Vijayakumar et al., 2006). We observed a significant increase in lipid peroxides (MDA) and reduction in endogenous antioxidant enzymes like CAT, GR, GPx, GST activity along with significant decreased levels of GSH in sciatic nerves of diabetic rats. Treatment with HSME at all doses for six weeks, restored above mentioned biochemical parameters in diabetic rats in dose dependent manner. Phytoconstituents present in *H. spinosa* like flavonoids and triterpenoids having antioxidant potential may be responsible for restoring above parameters.

In the present study, nociceptive threshold of diabetic control rats was found to be significantly lowered than control rats in warm plate, hot plate, tail immersion and paw pressure withdrawal tests, indicating that diabetic animals exhibit allodynia, thermal and mechanical hyperalgesia. HSME treated diabetic rats had shown restored pain threshold responses as compared to diabetic animals.

Recent years have witnessed a renewed interest in plants as pharmaceuticals because they synthesize a variety of secondary metabolites with antioxidant potential which can play a major role in protection against molecular damage induced by reactive oxygen species (ROS) (Vijayakumar et al., 2006). The improvement in diabetic state after *H. spinosa* treatment along with the antioxidant activity could be the probable way by which it had alleviated diabetic neuropathy. Since hyperglycemia in diabetic state could induce some functional alterations in the nervous system (Dobretsov et al., 2001), this extract may have attenuated the hyperalgesia and allodynia. Further the aerial parts of *H. spinosa* have been previously reported to have antinociceptive activity in rats (Shanmugasundaram and Venkataraman, 2005) which may have further assist in management of painful diabetic neuropathy. However further investigation is required to establish the exact mechanism of protective effect of *hygrophila spinosa* in diabetic neuropathy.

5. Conclusion

To conclude, administration of methanolic extract of aerial parts of hygrophila spinosa extract could attenuate the diabetic neuropathy in Wistar rats and this may be of potential benefit in clinical practice for the management of diabetic neuropathy.

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