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Research Article

Anti Diabetic Neuropathy and Pharmacological Evaluation of the Indian Traditional Herb *Gymnema sylvestre*

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ABSTRACT

Diabetic neuropathy (DN) is an important complication of diabetes mellitus resulting in a great deal of morbidity. The prevalence of diabetic neuropathy is about 26.1% in Indian population. Peripheral diabetic neuropathy is characterised by peripheral demyelination, decrease in the nerve conduction and degeneration of myelinated and demyelinated sensory nerve fibres. Aim and objective: The present study will be undertaken with an objective to evaluate the effect of aqueous extract of leaves of Gymnema sylvestre on hyperalgesia in streptozotocin induced diabetic rats and in- vitro aldose reductase inhibition. Materials and methods: Wistar albino rats, rendered diabetic with streptozotocin, were divided into 5 groups, namely the diabetic control treated with vehicle (DC), standard control which received glibenclamide+metformin (SC), test groups treated with 100, 200and 400 mg/kg b.w. of Gymnema sylvestre (GS1, GS2 and GS3 respectively). A group of five normal animals served as normal control (NC). Fasting blood glucose, body weight and reaction time to tail flick were measured one week after induction of diabetes. The animals were then treated orally for two weeks after which the same parameters were repeated. In-vitro aldose reductase inhibition assay was carried out at concentrations of 5, 10, 25, 50, 100 and 200 mcg/ml of Gymnema sylvestre using rat lens from normal rats. The in-vivo results were analysed with Mann Whitney test. Results: The DC group demonstrated a decrease in the reaction time (hyperalgesia) compared to NC while a significant increase in the reaction time was observed with SC, GS2 and GS3 groups ($p<0.05$) as compared to the DC group. GS1 and GS2 showed a significant reduction in body weight compared to their baseline values ($p<0.05$). There was no significant change in the fasting blood glucose (FBS) in any of the groups. In-vitro aldose reductase inhibition was observed with GS with an IC 50 of 103 mcg/ml. Conclusion: Hence this study form the basis of an effort of Gymnema sylvestre reduece the hyperalgesia in experimental diabetic neuropathy. It has an aldose reductase inhibitory activity in-vitro which may contribute to the beneficial effects.

Key words: Diabetic neuropathy, Gymnema sylvestre, streptozotocin, hyperalgesia

INTRODUCTION

Diabetic Neuropathy is a demonstrable disorder, either subclinical or clinically evident, that occurs in both peripheral and the autonomic nervous systems. Neuropathies are the most common complication of diabetes mellitus (DM) affecting upto 50% of patients with Type 1 and Type 2 diabetes. In type 1 diabetes, distal polyneuropathy becomes symptomatic after several years of diagnosis; in contrast, type 2 diabetes patients may have neuropathy at the time of diagnosis. The Important biochemical mechanisms of Diabetic Neuropathy are polyol pathway, advanced glycation and oxidative stress.

Polyol Pathway

Hyperglycemia causes increased level of intracellular glucose in nerves, leading saturation of normal glycolytic pathway. Extra glucose shunted to polyol pathway and converted to sorbitol and fructose by the enzyme aldose reductase and sorbitol dehydrogenase. Accumulation of sorbitol and fructose lead to reduced myoinositol, decreased membrane Na+/K+ - ATPase activity, impaired axonal transport and structural breakdown of nerves. This is the rationale for using aldose-reductase inhibitors as treatment to improve nerve function.

Advanced glycation end products (AGE)

Excess glucose in hyperglycemia can lead to nonenzymatic glycation of proteins, nucleotides and lipids resulting in production of advanced glycation end products (AGE) that may have role in disrupting neuronal integrity and repair mechanisms.

Oxidative Stress

The increased production of free radicals in diabetes may be detrimental via several mechanisms. They may directly damage small blood vessels, supplying nerves, leading to nerve ischemia. Use of antioxidant alpha-lipoic acids may hold promise for improving neuropathic symptoms.

Aldose reductase inhibitors: Aldose reductase inhibitors block the rate-limiting enzyme in the polyol pathway that is activated by chronic hyperglycemia. Epralrestat is available in India, reduces intracellular sorbitol accumulation, which has been implicated in the

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Table:1 Effect of Gymnema sylvestre on the tail fl ick reaction time in Wistar albino rats.

S.No	Group(n=6**)	Mean reaction time (sec) on day 0 ±SEM	Mean reaction time (sec) on day 15± SEM
1	Normal controle(NC)	8.2±0.5	9.5±0.6
2	Diabetic controle(DC)	7.5±0.4	7.6±1.5
3	Standard controle(SC)	8.81±0.6	12.81±0.3*
4	Gymnema sylvestre 100mg/Kg(GS1)	9.1±1.43	13.2±0.85
5	Gymnema sylvestre 200mg/Kg(GS2)	7.5±0.9	13.5±1.05*
6	Gymnema sylvestre 400mg/Kg(GS3)	8.5±0.5	15.6±0.21

*P<0.05 compared to the diabetic controle. **except in normal controle where n=5

Table 2: Effect of Gymnema sylvestre on body weight in Wistar albino rats

S.No	Group(n=6**)	Mean body weight (grms) on day 0 ±SEM	Mean body weight (grms) on day 15 ±SEM
1	Normal controle(NC)	185±9.38	198±12.01
2	Diabetic controle(DC)	138±4.365	130±9.56
3	Standard controle(SC)	120±5.628	122±2.613
4	Gymnema sylvestre 100mg/Kg(GS1)	148±6.69	106.65±6.2*
5	Gymnema sylvestre 200mg/Kg(GS2)	140±5.965	125.16±6.75*
6	Gymnema sylvestre 400mg/Kg(GS3)	128.5±2.0	128.6±7.16

*P<0.05 compared to the baseline values within the group** exept in normal controle where n=5

pathogenesis of diabetic neuropathy. Epralrestat 150mg/day for 12 wks improved motor and sensory nerve conduction velocity and vibration threshold in patients with diabetic neuropathy. Ubjective symptoms, including pain, numbness, hyperesthesia coldness in extremities, muscular weakness and orthostatic fainting were also improved.

Table 3: Effect of Gymnema sylvestre on aldose reductase enyme activity in-vitro

Sample	Conc tested ($\mu g/mL$)	% Inhibition	IC 50 ($\mu g/mL$)
GS/Lot 02	5	5.65	102.054
	10	10.52	81.74-136.15
	25	20.15	
	50	32.65	
	100	42.65	
	200	68.52	

GS- Gymnema sylvestre

Alpha lipoic acid: Alpha-lipoic acid has shown to improve symptomatic relief of neuropathy symptoms in type 2 diabetes with neuropathy.There is no definitive treatment for DN at present. Tricyclic antidepressants, SNRIs, anticonvulsants, opioids and topical capsaicin have been tried in the management of painful neuropathy of which duloxetine and pregabalin have been approved by the US FDA. The use of these drugs is limited by their cost and side effects.

Herbal medicines may be used as an alternative therapy in this condition as they are effectively used for the treatment of diabetes in Ayurveda and are generally well tolerated. Gymnema sylvestre (GS) is recommended for diabetes, burning sensation, fever, edema etc. It has been scientifically validated in various animal models for hypoglycemic, immunomodulatory, anti-inflammatory, antioxidant and other pharmacological activities. The aqueous and alcoholic extract of the plant has been shown to improve glucose tolerance in diabetic rats. A study by Grover et al showed amelioration of experimental diabetic neuropathy by TC at a dose of 400mg/kg b.w.

The leaves of Gymnema sylvestre have been used for centuries in the traditional Indian system of Ayurvedic medicine. It has been used in India for the treatment of diabetes for over 2,000 years. It was hypothesised that GS may reduce the symptoms of DN. The leaves mainly reduce the blood sugar levels. The hypoglycemic (blood sugarlowering) action of Gymnema leaves was first documented in the late 1920s . The leaves were also used for stomach ailments, constipation, water retention and liver disease. Extracts of Gymnema is not only claimed to curb sweet tooth but also for treatment of as varied problems as hyperglycemia, obesity, high cholesterol levels.

Hence the present work aimed at studying the effect of three different doses of standardized aqueous extract of stem of GS in animal model of diabetic neuropathy. Our primary objective was to evaluate the effect of GS extract on hyperalgesia in diabetic rats. We have also evaluated the effect of TC on aldose reductase inhibition in-vitro, to explor its probable mode of action.

MATERIALS AND METHODS

All the animals were obtained from Department of pharmacology, Geethanjali college of pharmacy, Keesara, and approved by Animal Ethics committee, Regd no : 648/PO/a/12/CPCSEA-GCOP-IAEC-03/2013.

Streptozotocin was obtained from Sigma Chemicals and glibenclamide (Bristol Mayer's squibb) with metformin (Franco Indian pharmaceuticals) were used as standard control. A standardised aqueous extract of Gymnema sylvestre was prepared. All the animals were obtained from Department of Pharmacology, Geethanjali College of Pharmacy, Keesara, and approved by Animal Ethics Committee, Regd no: 1648/PO/a/12/CPCSEA-GCOP-IAEC-03/2013. pharmaceuticals) were used as standard control.

Processing Of Plant Material

The leaves of G.sylvestre were collected from Geethanjali college of pharmacy, medicinal garden, cheeryal, Andhrapradesh. The plant leaves were air dried under shed at 25°C and the dried leaves were made in to a fine powder with an auto-mix blender. The powder was kept in deep freezer until the time of use.

Preparation of Aqueous Extract:

One hundred grams of dry fine powder was suspended in 250 ml of water for two hours and then boiled at 60°C to 65°C for 30 minutes (since boiled decoction of the leaf of this plant has been used as remedy for diabetes). The collected extract was pooled and passed through a fine cotton cloth. The filtrate upon evaporation at 40°C yielded 20 percent semi-solid extract.

Experimental Evaluation of Diabetic Neuropathy

Adult female Wistar albino rats, aged 10-12 weeks, weighing 130-200 g were obtained from Geethanjali college of pharmacy, animal house (Reg. No. 1648/PO/a/12/CPCSEA-GCOP-IAEC-03/2013). They were acclimatised to room temperature with a relative humidity 55±10% for 2 weeks during which time they were provided standard feed (from Hindustan lever) and filtered water ad libitum. They were housed in propylene cages.

An initial fasting blood sugar (FBS) was done by glucose oxidase method with the help of a well calibrated glucometer (Accucheck) after 14 hours of fasting. All the tests were carried out between 9.30 -10.30 a.m. We also recorded a reaction time to radiant heat using techno-analgesiometer (Incorp, India). The animal was put into a rat restrainer which had an opening for the tail at the rear wall and holes on the front wall for easy breathing. The restrainer was placed on the techno-analgesiometer. The tail was held gently by the investigator so that the middle portion of proximal one third was placed on the metal base, below which was the heating element (nichrome wire). The temperature was preset to allow a flow of 1.5 amperes of current. After allowing the animal to acclimatise in this position for 2-3 minutes, once the tail was found to be in a relatively stable position, the switch was put on. The time taken for the rat to flick its tail in response to radiant heat was considered as the reaction time. Animals showing FBS of 60-90mg/dl and reaction time of 7-12 seconds were selected for the final experiment.

The animals were made diabetic by an intraperitoneal injection of freshly prepared streptozotocin at a dose of 90 mg/kg b.w. after overnight fasting. Streptozotocin was prepared in ice-cold citrate buffer (pH 4.0) as per the international protocol of animal models of Diabetes Complications Consortium. [10] The animals with FBS more than 200 mg/dl after 48 hours of injecting streptozotocin, were randomized into 5 groups, with a minimum of 6 animals each and were given the following treatment

NC -Normal control -Vehicle only

DC- Diabetic control-Vehicle only

SC-Standard control- Glibenclamide 5mg/kg body weight+metformin 25 mg/kg

GS1- Gymnema sylvestre 100mg/kg

GS2- Gymnema sylvestre aqeous extract 200mg/kg

GS3- Gymnema sylvestre aqueous extract 400mg/kg

One week after the induction of diabetes, FBS was repeated and the medications were administered once a day orally for 15 days starting from the eighth day. The FBS, body weight and the reaction time were repeated once again on the 15 th day of dosing.

In-vitro Aldose-reductase Inhibition Assay

Aldose reductase activity was assayed according to the method described by Hayman and Kinoshita [11] with some modifications. The incubation mixture, in a final volume of 250µL, consisted of 67mM potassium phosphate buffer (pH-6.2), 0.4M lithium sulphate, 150µM NADPH, 300µM DL-glyceraldehyde, enzyme (50µL) and various concentrations of the test sample preparations (as mentioned in the table below). Quercetin 0.5µg/mL was used as standard inhibitor. Appropriate blanks were prepared for quercetin and the test samples without DL-glyceraldehyde. The reaction was initiated by adding NADPH. The absorbance was read at 340nm for 20 minutes using FLUOstar microplate reader (BMG Labtech) in a kinetic mode. The percent inhibition by test sample/quercetin was calculated by considering the control value as 100%, using the equation mentioned below. The median inhibitory concentration (IC 50) was calculated using Finney software. Similar procedure was carried out with TC at strengths of 5, 10, 25, 50, 100 and 200 mcg/ml. The percentage inhibition was calculated with the following formula:

$$\text{Percent Inhibition} = 100 - \left(\frac{\Delta A_{340}\ \text{Sample} * 100}{\Delta A_{340}\ \text{Controle}}\right)$$

Where Inhibition = Change in absorbance at 340 nm

Statistical Analysis

The results for experimental neuropathy are expressed as mean ± standard error of mean and analysed using Mann-Whitney test with SPSS software. $P<0.05$ was considered as statistically significant. IC 50 was calculated for the in-vitro aldose inhibitory activity of GS.

RESULT

As shown in [Table 1], the reaction time on the 15 th day was less in the DC group as compared to the normal animals (NC), indicating hyperalgesia. All the other groups showed an increase in the reaction time which was statistically significant in SC, GS2 and GS3 groups as compared to the diabetic group ($P<0.05$). The percentage increase in reaction time was calculated with reference to their base line values. The increase in the normal control was found to be 18.02%. This value was deducted from the observed percentage increase in the standard and the test groups. Accordingly, the percentage increase in the SC group was 25% and groups GS1, 2, and 3 showed an increase by 27%, 62% and 60% respectively.The actual values are shown in [Table 1].

Effect of Gymnema sylvestre on Fasting blood glucose

At the end of 15 days of treatment, the FBS in the NC group remained in the range of 61-84mg/dl while the animals in diabetic control showed very high values

(>500mg/dl). In the SC group, there was no change in FBS values except in one animal where it was 140mg/dl (from a value of 416gms/ dl). The GS1, GS2 and GS3 groups showed no response except in one animal in the GS2 group, where an improvement from 352mg/ dl to 263 mg/dl was observed.

Effect of Gymnema sylvestre on Body weight

There was weight loss in the DC group as compared to their baseline values (7.8%) which was not statistically significant. The SC group, however, did not show any weight loss. GS1 and GS2 showed significant reduction in body weight compared to their base line values, the mean reduction being 26%, and 09% respectively ($P<0.05$). There was no reduction in the feed intake in any of the groups [Table 2].

Effect of Gymnema sylvestre on aldose reductase

Quercetin at a concentration of 0.5µg/mL exhibited 45.18% inhibition of aldose reductase activity. Percent inhibition of aldose reductase enzyme by the GS samples at different concentrations was calculated and plotted on a graph. IC 50 of GS was 102 µg/mL [Table 3].

Effect of Gymnema sylvestre on experimental diabetic neuropathy

DISCUSSION

The model chosen in the present study was based on the principle that at an increased level of lood sugar in a diabetic rat, hyperalgesia can be detected as a reduced latent period (reaction time) for tail flick in response to radiant heat by the analgesiometer. A drug beneficial in DN will reverse the hyperalgesia. G. sylvestre is a potent antidiabetic plant and used in folk, ayurvedic and homeopathic systems of medicine.

Diabetic neuropathy is characterized by an increased sensitivity to touch and pain due to neuronal loss or alteration in the neurotransmitters. It is reported that significant hyperalgesia i.e. a reduction in the tail flick latency to radiant heat develops 3 weeks after induction of diabetes. A similar observation was made in the present study. Aqueous extract of Gymnema sylvestre reversed the hyperalgesia at all three doses, with significant effects at 200 and 400 mg/kg b.w.. This is in contrast with the study by Grover et al where there was a non significant response at 400 mg/kg. The response increased with increase in the dose although a typical dose response relationship was not observed with the three doses.We chose a dose of 90 mg/kg of streptozotocin as we were unable to induce hyperalgesia at lesser doses in our pilot studies.

A significant reduction in the body weight was also observed with GS1 and GS2 inspite of normal feed intake and absence of any behavioural abnormalities. Clinical trials with aldose reductase inhibitor, significant improvement in the pain relief, motor and sensory nerve conduction velocities with minimum toxicity in patients with DN. These observations suggest that aldose reductase inhibitors may be important in the treatment of symptomatic, somatic and autonomic neuropathies complicating diabetes. Hence the in-vitro effect of GS on aldose reductase inhibition was observed. It showed an inhibitory effect although the IC50 was higher as compared to the standard (quercetin). Thus, the beneficial effect of GS on diabetic neuropathy appears to be due to its analgesic effect and unrelated to its anti-hyperglycemic effect. An anti-oxidant role of GS cannot be ruled out as oxidative damage contributes to the causation of diabetic neuropathy and GS is proven to be an anti-oxidant in experimental models.

Limitations of the study

The study gives only preliminary evidence about the role of GS in diabetic neuropathy. Experiments involving nerve conduction studies would be more confirmatory. Moreover, a true dose response relationship of the action of GS on hyperalgesia Could not be demonstrated, which may be because of a small size of a sample.

CONCLUSION

Based on study We observed A significant reduction in the body weight with GS1 and GS2 inspite of normal feed intake and absence of any behavioural abnormalities. And also Aqueous extract of Gymnema sylvestre reversed the hyperalgesia at all three doses, with significant effects at 200 and 400 mg/kg b.w.. This is in contrast with the study by Grover et al where there was a non significant response at 400 mg/kg. The response increased with increase in the dose although a typical dose response relationship was not observed with the three doses.We chose a dose of 90 mg/kg of streptozotocin as we were unable to induce hyperalgesia at lesser doses in our pilot studies. This study gives study gives only preliminary evidence about the role of GS in diabetic neuropathy. Experiments involving nerve conduction studies would be more confirmatory.

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