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Full Length Research Paper

Phytochemical and pharmacological evaluation of *Solanum nigrum* Linn.

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The present investigation designed to evaluate the phytochemical and pharmacological activity of ethanolic extract of *Solanum nigrum* in experimental animal models. The ethanolic extract of *S. nigrum* was used in three different doses (100, 200 and 300 mg/kg bw) to evaluating anti-inflammatory and anti-convulsant activity by employing carrageenan paw edema and Supramaximal electric shock (MES) methods. Ethanolic extract of *S. nigrum* produced significant anti-inflammatory ($P < 0.01$) and anti-convulsant ($P < 0.05$) effect in dose dependent manner. The flavonoids present in the berries might be a responsible active constituent for this activity.

Key words: *Solanum nigrum*, anti-inflammatory activity, anti-convulsant, flavonoids

INTRODUCTION

Nature has provided a complete store-house of remedies to cure all aliments of mankind (Kokate et al., 2002). This is where, nature provides us drugs in the form of herbs, plants and algae's to cure the incurable diseases without any toxic effect (Trees and Evans, 1989). There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown (Gupta et al., 2005). Epilepsy (sometimes referred to as a seizure disorder) is a common chronic neurological condition that is characterized by recurrent unprovoked epileptic seizures. These seizures are transient signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. It affects approximately 50 million people worldwide (Fisher et al., 2005). Inflammation is a disorder involving localized increases in the number of leukocytes and a variety of complex mediator molecules. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflamemation. Their biosynthesis has also been implicated in the pathophysiology of cardiovascular diseases, cancer, colonic adenomas and Alzheimer's disease (Gupt et al., 2006). Because of increasing side effects of available synthetic drugs for epilepsy and inflammation, there is need to focus on the scientific exploration of herbal drugs having fewer side effects. *Solanum nigrum* Linn. (Solanacea) is a thorny shrub widely distributed in Sikkim, Uttar Pradesh, Southern India and Sri Lanka in moist places. This plant is well known in English and Tamil system as 'Black night shade' and 'Kakamachi', respectively (Nadkarni, 1976). The berries of *S. nigrum* (Solanaceae) have been reported in the ancient Indian medicinal literature with beneficial effects in inflammation, tuberculosis, diuretics etc (Chopra et al., 1956). This research was aimed to investigating the possible anti-convulsant and anti-inflammatory activities of *S. nigrum* in order to support or refute the claims by traditional herbalists in India.

MATERIALS AND METHODS

Animals

Albino strain of rats, weighing about 150 – 200 g were obtained from institute animal house, KMCP, Madurai and used in the experiments. The protocol was approved by the Institute's Animal Ethical Committee. Animals were kept in animal house at an ambient temperature of 25°C and 45 – 55% relative humidity, with 12 h each of dark and light cycles. Animals were fed pellet diet and

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water *ad*-libitum.

Preparation of plant extract

Berries of *S. nigrum* were collected from the medicinal garden of institute in November, 2007 and identified by Dr. D. Stephen, Department of botany, American College, Madurai. The shade dried berries were powdered to get a course granule. About 750 g of dried powder was extracted with various solvents in increasing polarity by continuous hot percolation, using soxhlet apparatus. The resulted dark – brown extract was concentrated up to 100 ml on Rota vapour under reduced pressure. The concentrated crude extracts were lyophilized in to powder and used for the study.

Phytochemical screening

The extracts obtained were subjected to preliminary phytochemical screening, to identify the chemical constituents. The methods of analysis employed were those described by (Trease and Evans, 1989; Harbone and Baxter, 1993).

Anti-inflammatory activity by Carrageenan induced rat paw edema method

Anti-inflammatory activity was assessed by the method described by (Winter et al., 1962). Albino rats of either sex weighing 150 – 200 g were divided in 5 groups (N = 6). Group-I received 0.5% CMC suspension (control), Group- II, III and IV received ethanolic extract (100, 200, 300 mg/kg, P.O) of *S. nigrum* respectively. Group-V received Indomethacin (reference standard 10 mg/kg, P.O). Animals were treated with drugs by oral route and subsequently 1 h after treatment; 0.1 ml of 1% suspension of carrageenan in normal saline was injected into the subplanter region of left hind paw to induce edema.

The paw volume was measured initially at 0, 1, 2, 3 and 4 h after Carrageenan injection using digital paw edema meter (520-R, IITC Life Science - USA). The difference between the initial and subsequent values gave the actual edema volume which was compared with control. The inhibition of inflammation was calculated using the formula, % inhibition = 100 (1-Vt/Vc), Where 'Vc' represents edema volume in control and 'Vt' edema volume in group treated with test extracts.

Anti-convulsant activity

Maximum electroshock (MES) induced seizure, (Fisher, 1989): The animals were divided into 5 groups of 6 numbers each and were administered as follows: Group I received vehicle, Group II received Phynytoin 25 mg/kg, Group- III, IV and V received ethanolic extract (100, 200, 300 mg/kg, P.O) of *S. nigrum* respectively. All these were administered by oral route 30 min before application of electroshock (150 MA, 0.2 sec) using corneal electrode. The duration tonic hind leg extension was noted.

Statistical analysis

The results are presented as Mean ± SEM. One way Analysis of Variance (ANOVA) followed by Dunnett's t-test for multiple comparisons were used for statistical evaluation. P-values less than 0.05 were considered significant.

RESULTS

Phytochemical investigation

Hexane, benzene extracts showed the presence of saponin, phytosterols, tanins and fixed oils and fats. The ethanolic and aqueous extracts showed the presence of carbohydrates, coumarins, phytosterols and flavonoids. The phytochemical investigation of extracts of *S. nigrum* is shown in Table 1.

Anti-inflammatory activity

The extract was found to have a significant ($P < 0.01$) inhibitory effect on the carrageenan-induced edema in rats at all the doses (100, 200 and 300 mg/kg body weight) tested in rats when compared to the normal saline control and standard Indomethacin. The activity resides more at the higher dose of 300 mg/kg with 63% inhibition after 4 h of extract administration. Also in regard to the other doses 100 and 200 mg/kg there was also significant decrease with 28 and 52% decrease after 4 h of extract administration when compared with standard drug 38%. The effect of ethanolic extracts of *S. nigrum* in Carrageenan induced paw edema in rats was shown in Table 2.

Anti-convulsant activity

The extracts was found to have a significant effect ($P< 0.05$ on supra maximal electric shock in rats. The percentage inhibitory activity of extract (100, 200, 300 mg/kg) when compared with standard drug phenytoin was 36.01, 25.21 and 38.86% respectively? The effects of ethanolic extracts of *S. nigrum* in supra maximal electric shock method in rats shown in Table 3.

DISCUSSION

Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1 - 2 h) The significant inhibitory activity shown by the EESN 100, 200, and 300 mg/kg) over a period of 4 h in carragenan-induced inflammation was quite similar to that exhibited by the group treated with Indomethacin. The highest percentage inhibition activity was found in the dose of 300 mg/kg with the mean percentage inhibition of 63% after 4 h of extract administration. Previous study with some other plants likes *S. trilobatum* (Pandurangan et al., 2008; 2009), *Plumeria acuminate* (Gupta et al., 2006) and *Thesium chinense* (Parveen et al., 2007) also showed the same effect in this model. These results indicate that the extract acts in later phases in dose de-

Table 1. Phytochemical screening of extracts of *Solanum nigrum.*

Name of the extract	CH	GS	SP	TN	FO	AK	PS	FN	CM
Hexane	-	-	+	-	+	-	+	-	-
Benzene	-	-	+	+	+	-	+	-	-
Chloroform	-	-	-	+	-	+	+	-	+
Ethanol	+	-	-	+	-	+	+	+	+
Water	+	-	-	+	-	+	+	+	+

CH-Carbohydrate, GS-Glycoside, SP-Saponins, TN-Tanins, FO-Fats and Oils, AK-Alkaloids, PS-Phytosterols, FN-Flavonoids, CM-Coumarin.
+ Indicates positive test result, - Indicates negative test result.

Table 2. Effect of ethanolic extract of berries of *Solanum nigrum* on Carrageenan induced paw edema in rats.

Treatment groups (n = 6)	Dose (mg/kg)	Paw volume in ml (Mean ± SEM)					% Inhibition
		0 h	1 h	2 h	3 h	4 h	
Normal Saline	10ml/kg	0.680±0.033	0.700±0.013	0.723±0.23	0.749±0.019	0.723±0.022	----------
Indomethacin	10	0.693±0.025	0.629±0.025	0.651±0.020	0.592±0.023	*0.524±0.014	38 %
EESN	100	0.682±0.015	0.689±0.020	0.66±0.023	0.588±0.024	*0.563±0.012	28 %
EESN	200	0.714±0.020	0.688±0.012	0.626±0.033	0.564±0.019	**0.476±0.021	52 %
EESN	300	0.612±0.012	0.641±0.013	0.572±0.007	0.542±0.015	**0.444±0.017	63 %

* Values are significantly different from control (P< 0.05).
** Values are significantly different from control (P<0.01).
One way ANOVA followed by Dunnet Multiple comparison test.

Table 3. Effect of ethanolic extract of berries of *Solanum nigrum* on supra maximal electric shock in rats.

Treatment Groups (n = 6)	Dose (mg/kg)	Duration of extension phase in seconds (Mean ± SEM)	% Inhibition
Saline	0.5 ml	11.58 ± 0.35	----------
Phenytoin	10	2.83 ± 0.44	75.56
EESN	100	7.41 ± 0.45	* 36.01
EESN	200	8.66 ± 1.07	* 25.21
EESN	300	7.08 ± 0.62	* 38.86

*Values are significantly different from control (P< 0.05).
One way ANOVA.

pendent manner, probably involving arachidonic acid metabolites, which produce an edema dependent neutrophils mobilization (Just et al., 1998). This anti-inflammatory effect of the extract observed might be due to the presence of flavonoids in the plant.

The data generated during present study indicated that ethanolic extract of *S. nigrum* (EESN) possesses anticonvulsant activity against seizures induced by MES, in a dose dependent manner. It was effective against MES induced seizures, since inhibition of the MES test predicts activity against generalized tonic-clonic and cortical focal seizures. Anticonvulsant activity of EESN in inhibiting seizures may be by regulating GABA – mediated synaptic inhibition through action at distinct sites of the synapse. It is hasty to attribute the effect of EESN to the GABAergic system (Kumaresan and Saravanan, 2009). The results suggest a possible anti- convulsant effect of EESN in animal-model. The precise mechanisms of possible anticonvulsant effect of EESN are not clear. The benzodiazepine site in the GABAA receptor and T-type Ca2+ currents could be targets for future studies to know the mechanisms of action of EESN.

Conclusion

From this preliminary investigation it has been concluded that the berries of *S. nigrum* having significant anti-

inflammatory and anti-convulsant activity, the flavonoids present in the berries might be a responsible active constituent for this activity. Further research is in progress to isolate the compound responsible for this activity.

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