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Comparative Free Radical Scavenging and Analgesic Activity of Ethanolic Leaves and Stem Extracts of *Solanum nigrum*

[1]Rajeswary Hari, [2]R. Vasuki, [3]J.Anbu, [1]B. Muralikrishna, [1]G. Manasa and [1]Geethanjali

In the present investigation a comparative analysis of the Free radical scavenging potential and analgesic activity of the Ethanolic Leaf (ELS) and stem extracts (ESS) of *Solanum nigrum* was performed. The extracts were evaluated for its DPPH and hydroxyl free radicals scavenging effect and inhibitory potential on protein carbonyl formation. Total phenolic and flavonoid content of the extracts were also determined by a colorimetric method. The ethanolic extracts of *Solanum nigrum* were evaluated for its peripheral analgesic activity by Acetic-acid induced writhing response and central analgesic activity by Tail flicking method and Hot plate method in mice. Both the plant extracts scavenged the free radicals in a dose dependent manner. However the scavenging effect was more pronounced in ELS extract when comparable to ESS extract. Both the extract possessed considerable quantity of phenols and flavonoids. In Tail flicking and Hot plate methods the ELS extract of *Solanum nigrum* showed higher mean basal latency time when comparable to ESS extract suggesting its central analgesic activity. Similarly in Acetic acid induced writhing response the ELS extract exhibited a significant inhibition of writhing 53.28% when comparable to ESS which exhibited an inhibition of 46.53%. The positive control Diclofenac sodium showed 70.66% of writhing inhibition. The analgesic activity of the plants extracts is probably due to its free radical scavenging activity.

Key words: Analgesic, free radicals, flavonoids, phenols, protein carbonyl

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For further information about this article or if you need reprints, please contact:

Rajeswari Hari
Department of Biotechnology,
Dr. MGR Educational and
Research Institute University,
Maduravoyal, Chennai, India

Tel: 09840774705

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[1]Department of Biotechnology, Dr. MGR Educational and Research Institute University, Maduravoyal, Chennai, India
[2]Department of Biomedical Engineering, Bharath University, Selaiyur, Chennai, India
[3]Department of Pharmacology, Vels college of Pharmacy, Chennai, India

INTRODUCTION

Herbal medicine is a form of alternative treatment that includes a use of different plants and plant extracts. In recent years, herbal drugs are being effectively tried in crude forms like expressed juice, powder, decoction or infusion to treat a variety of patho-physiological states. India has the long history of using plants as medicines in the world and Ayurveda, the ancient healing system of India, is one of the richest medicinal systems among those available around the world. According to the World Health Organization about 80% of population in the world countries still uses traditional medicine (e.g., medicinal plants) for their primary health care, due to poverty and lack of access to modern medicine (Kumar *et al*., 2006). The Plants contain a wide variety of bioactive secondary metabolites, such as Alkaloids, Tannins, Glycosides, Terpenoids, Carotenoids , Flavonoids and other Phenolic compounds that produce a definite physiological action on the human body (Edeoga *et al*., 2005).

Pain is an unpleasant sensation. The International Association for the Study of Pain's widely used definition states: "Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". It can also occur as a consequence of brain or nerve injury (Laurence and Bennett, 1992). Painful stimuli may be of physical stimuli such as pressure or heat or of chemical stimuli from the products of inflammation. A variety of naturally occurring inflammatory compounds are capable of eliciting pain e.g. Histamine, Acetylcholine, Bradykinin, Prostaglandin and 5- hydroxyl tryptamine. One or more of these substances are present in the products of inflammation. Among these Prostaglandins are ubiquitous substances present throughout the body not only involved in maintaining normal function of the cardiovascular, pulmonary, renal and gastrointestinal systems but also cause pain by direct action upon nerve endings (Gupta *et al*., 2006). Oxygen-centered free radicals, also known as Reactive Oxygen Species (ROS), play a vital role in energy production, phagocytosis, regulation of cell growth and intracellular signaling but their excess formation or incomplete removal is implicated in the pathogenesis of a large number of diseases (Halliwell and Gutteridge, 1999). Reactive Oxygen Species (ROS) and free transition metal ions cause extensive oxidative damage to cellular bio molecules such as DNA, Proteins and lipids. Analgesics are a group of drugs used to relieve pain without affecting the consciousness. They exert their action either by blocking pain signals going to the brain or by interfering with the brain's interpretation of the signals. The gastrointestinal ulceration and bleeding is the common side effect for the most of the analgesics drugs which come under NSAIDS So, in order to overcome, these complications there is need to focus on the scientific exploration of herbal drugs for the treatment of various diseases and having fewer side effects. *Solanum nigrum* is otherwise called as Black nightshade. The whole plant of *Solanum nigrum* was extensively used as herbal medicine in India and other parts of the world to cure liver disorders, fever, diarrhea, antiseptic, ant-inflammatory, expectorant, cardiotonic, diuretic, laxative, analgesic, sedative, cough and asthma and hydrophobia (An *et al*., 2008). In the present investigation a comparative analgesic and free radical scavenging capabilities of ethanolic extract of leaves of *Solanum nigrum* (ELS) and ethanolic extract of stem of *Solanum nigrum* (ESS) was studied.

MATERIALS AND METHODS

Chemicals: All routine chemicals such as Ascorbic acid, Gallic acid, Catechin, Vitamin E, Sodium nitroprusside, Ferric chloride Potassium thiocyanate were obtained from SD Fine Chemicals Ltd., India. All other chemicals and reagent used were of analytical grade.

Plant collection and authentification: The whole plant of *Soalnum nigrum* were collected from koyembedu vegetable market, Chennai and was authenticated Dr. Sankaranarayanan, Assistant Director, Department of Research and Development, Sairam Siddha Medical College and Research Centre, Chennai. India. The voucher specimen is also available in herbarium file of the same centre.

Extraction: The leaves and stems of *Solanum nigrum* (100 g each) were shade dried and pulverized to a coarse powder and passed through a 40- mesh sieve exhaustively extracted with 90% (v/v) ethanol by cold maceration process. They are prepared by soaking 100 g of each leaf and stem powder in 250 mL of ethanol in different flasks and kept for 72 h. The extract was evaporated under pressure till all the solvent had been removed and further removal of water was carried out by freeze drying. The solid residues were collected; yield was calculated and stored in refrigerator condition until analysis. Yield was found to be 5.5% w/w for ethanolic leaf extract of *Solanum nigrum* (ELS) and 2.26% w/w for ethanolic stem extract (ESS) of *Solanum nigrum.* The solid residues were collected stored in refrigerator condition until analysis.

Animals: Adult albino mice of weighing 30 to 40 g were used in the pharmacological studies. The inbred animals were taken from animal house in Vel's Pharmacy College,

Chennai, India. The animals were maintained in well ventilated room temperature with natural 12±1 h day-night cycle in the propylene cages. They were fed balanced rodent pellet diet from Poultry Research Station Nandam, Chennai, India and tap water *ad libitum* was provided throughout the experimental period. The animals were sheltered for one week and prior to the experiment they were acclimatized to laboratory temperature. The protocol was approved by Animal Ethics Committee constituted for the purpose as per CPCSEA Guideline.

Preliminary phytochemical screening: Ethanolic extracts of both leaves and stems are subjected to preliminary Phyto-chemical screening for identification of its active constituents by the method of Kokate *et al*. (2002).

Acute toxicity studies: Acute toxicity studies were conducted with the ELS and ESS extracts in adult albino mice by staircase method of Ghosh (1984). Albino mice of either sex were selected and segregated in to 10 groups of 6 animals each. Single dose of ELS and ESS extracts dissolved in 0.5% aqueous Tween 80, starting from the minimal dose of 50 up to 3000 mg kg^{-1} administered orally. The drug treated animals were observed carefully for its toxicity signs and mortality. LD_{50} doses were selected for the evaluation of analgesic activity. From the maximum dose, 1/10th of the concentration was considered as therapeutic dose for further study. All the animals were also observed for further 14 days for various clinical symptoms and mortality.

Free radical scavenging activity

DPPH radical scavenging activity: DPPH radical scavenging activity of plant extracts were determined by Shimada *et al*. (1992). One mililiter of crude extracts of ELS and ESS at variable concentrations ranging from 100 to 1000 μg in ethanol were mixed in 1 mL of freshly prepared DPPH solution and 2 mL of 0.1 M acetate buffer at pH 5.5. The resulting solutions were then incubated at 37°C for 30 min and measured spectrophotometrically at 517 nm. Standard antioxidants like L-Ascorbic acid was used as positive control under the same assay conditions. Negative control was without any inhibitor or extract. Lower absorbance at 517 nm represents higher DPPH scavenging activity. DPPH radical scavenging activity of the extracts was calculated accordingly from the decrease in absorbance at 517 nm in comparison with the negative control. Percentage inhibition was calculated.

Hydroxyl radical scavenging activity: Hydroxyl radical scavenging activity of plant extracts were determined by the method of Halliwell *et al*. (1987). Briefly for the non site-specific hydroxyl radical system, the reaction mixture containing 0.1 mL deoxyribose, 0.1 mL $FeCl_3$, 0.1 mL ascorbic acid, 0.1 mL EDTA and 0.1 mL H_2O_2 were mixed with or without various concentrations of the two extracts in 1 mL of final volume made with KH_2PO_4-KOH buffer pH 7.4 and was incubated in a water bath at 37°C for 1 h. The extent of deoxyribose degradation was measured by thiobarbituric acid (TBA) method. One mililiter of TBA and 1 mL trichloroacetic acid were added to the mixture and heated at 100°C for 20 min. After cooling to room temperature the absorbance was measured at 532 nm. Mannitol, a classical hydroxyl radical scavenger was used as positive control. The hydroxyl radical scavenging activity was calculated.

Assay of protein oxidation: Effects of the plant extracts on protein oxidation were carried out according to the method of Wang *et al*. (2006). Bovine serum albumin (BSA) was oxidized by a Fenton-type reaction. The reaction mixture (1.2 mL), containing sample extract (100-1000 μg mL^{-1}), potassium phosphate buffer, BSA, Ferric chloride, hydrogen peroxide and ascorbic acid were incubated for 30 min at 37°C. For determination of protein carbonyl content in the samples, 1 mL of 2, 4-dinitrophenylhydrazine (DNPH) was added to the reaction mixture. Samples were incubated for 30 min at room temperature. Then, 1 mL of ice cold TCA was added to the mixture and centrifuged at 3000 g for 10 min. The protein pellet was washed three times with 2 mL of ethanol/ethyl acetate (1:1 v/v) and dissolved in 1 mL of guanidine hydrochloride. The absorbance of the sample was read at 370 nm. The data were expressed in terms of percentage inhibition, calculated from a control measurement of the reaction mixture without the test sample. Vitamin E under same assay condition was used as standard.

Assay of Antioxidant chemicals

Total Flavonoids content: Total flavonoid content of extracts were determined by colorimetric method as described in literature of Zhishen *et al*. (1999). Aliquots of sample containing 100 mg of test drugs (0.5 mL) were mixed with 2 mL of distilled water and subsequently with 0.15 mL of sodium nitrite solution were added. After 6 min, 0.15 mL of aluminium chloride solution was added and allowed to stand for 6 min, then 2 mL of NaOH solution (4%) was added to the mixture. Immediately, water was added to bring the final volume to 5 mL and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then determined at 510 nm versus prepared water blank. Results were expressed as catechin equivalents (mg catechin/g dried extract).

Total phenolic content: The total phenolic content of extracts were determined by Folin-ciocalteu reagent (FCR) according to a published method of (Slinkard and Singleton, 1977). About 100 mg of the sample was dissolved in 0.5 mL of water, mixed with 2.5 mL Folin-Ciocalteu's reagent (diluted 1:10, v/v) followed by 2 mL of sodium carbonate solution. The absorbance was then measured at 765 nm after incubation at 30°C for 90 min. Results were expressed as gallic acid equivalents (mg gallic acid/g dried extract).

Evaluation of analgesic activity

Eddy's Hot plate method: The hot-plate was used to measure response latencies according to the method described by Eddy and Leimbach (1953) with minor modifications. The animals were divided into four groups of 6 animals each and were given dose schedule as Group I: Animals were given a single administration of 0.5 mL vehicle (0.5% (v/v) aqueous Tween 80 p.o. once. This group served as control. Group II Animals were treated with 1 mL of 300 mg kg^{-1} of ELS in vehicle (0.5% v/v of Tween 80) orally once. Group III Animals were treated with 1 mL of 300 mg kg^{-1} of ESS in vehicle (0.5% v/v of Tween 80) orally once. Group IV Animals served as positive control and were treated with 1 mL of 100 mg kg^{-1} of morphine in vehicle. (0.5% v/v of Tween 80) orally.

The animals were placed on the hot plate maintained at 55°C, one hour after their respective drug treatments. The response time was noted at 0, 30, 60 and 120 min at which animals reacted to the pain stimulus either by paw licking or jump response which ever appeared first. The cut off time for the reaction was 15 sec.

Tail flick test: The central analgesic activity of the plant material was studied using the tail flick test which is based on the pain response in animals, similar to the hot plate test. It is used in basic pain research and to measure the effectiveness of analgesics, in mice to heat stress applied to their tails by using a Medicraft Analgesiometer Mask-N (D'Amour and Smith, 1941) and described previously (Saha *et al*., 2007).The animals were divided into four groups of 6 animals each and were given dose schedule as the above experiment. Basal reaction time of animals to radiant heat was recorded by placing the tip (last 1-2 cm) of the tail on the radiant heat source. The tail withdrawal from the heat (flicking response) is taken as the end point. The animals which showed flicking response within 3-5 sec, were selected for the study. A cut off period of 15 sec is observed to avoid damage to the tail. The measurements of withdrawal time using the tail flick apparatus was conducted at 30, 60, 120 and 180 min after administration of drugs.

Acetic acid induced writhing test: The peripheral analgesic activity of leaf (ELS) and stem (ESS) of *Solanum nigrum* was measured by the acetic acid induced writhing test as described by Saha *et al*. (2007). The animals were divided into four groups of 6 animals each as described in Table 7. Diclofenac sodium (50 mg kg^{-1}, p.o.) was used as standard. 0.5% v/v Tween -80 was used as control and the ELS and ESS extracts (300 mg kg^{-1}, p.o.) were used as treatment for other two groups. Then 1% v/v aqueous acetic acid was administered intraperitonially to all the groups to produce writhe. Test substances were administered 30 min before injection of acetic acid. Animals were kept individually under glass jar for observation immediately after acetic acid injection for 20 min period. Onset on writhes was noted and the number of abdomen constrictions, trunk twist response and extension of hind limbs were recorded. The mean writhing scores in control, standard (Diclofenac sodium) and test groups were calculated and the data represent the total numbers of writhes observed during 20 min and are expressed as writhing percentage.

Statistical analysis: Values reported are Mean±SE. The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnet's't' test. The p values <0.05 were considered as significant.

RESULTS

Preliminary phytochemical screening: The phyto-chemical screening of the ELS and ESS extracts reveals the presence of alkaloids, carbohydrates, proteins, tannins, steroids, flavonoid, Saponins, Glycosides and phenols. However Gums, mucilage, fixed oils and fats were absent all the extracts of *Solanum nigrum.*

Acute toxicity studies: No toxic symptoms were observed after administration of different dose levels of extracts up to maximum of 3000 mg kg^{-1} p.o. according to OECD guideline 423. From this experiment the maximum therapeutic dose level of was fixed as 300 mg kg^{-1} for both the ELS and ESS extracts of the *Solanum nigrum.*

Free radical scavenging activity

DPPH radical scavenging activity: The hydrogen donating activity of the plant extract was measured by using DPPH test. The DPPH scavenging effects of various extracts of *Solanum nigrum* were illustrated in Table 1. Both the extracts (ELS and ESS) of the plant had significant scavenging effects on DPPH radical which increased with increasing concentrations. However, the Ethanolic leaf extract of *S. nigrum* (ELS) showed highest

percentage of inhibition that is 90.6% and Ethanolic stem extract of *S. nigrum* (ESS) showed 88.0% inhibition respectively at 1000 μg concentration. This inhibition was found to be more than the positive control ascorbic acid which showed only 85.6% inhibition at the same concentration. The ELS and ESS extracts contained 296 and 287 mg of ascorbic acid equivalents/g extract of activity, with EC_{50} value of 186 μg mL^{-1} and 179 mg mL^{-1}, respectively. The EC_{50} value of the standard ascorbic acid was found to be 168 μg mL^{-1}.

Hydroxyl radical scavenging activity: We examined the inhibitory action of our various extracts of *Solanum nigrum* on deoxyribose degradation which gives an indication of hydroxyl radical Scavenging action. When hydroxyl radical generated by the Fenton reaction (ferric+ascorbate-EDTA-H_2O_2) attacks deoxyribose and degrades it in to fragments that react with TBA on heating at low pH to form pink colour. The ELS and ESS extracts of *Solanum nigrum* were capable of reducing DNA damage at all concentrations (Table 2).The highest inhibition on deoxyribose degradation was shown (76.6%) by the ethanolic stem extract of *Solanum nigrum* (ESS) when comparable to the ethanolic extract of leaf extract (ELS) which showed only 67.6% inhibition at the same concentration. The standard drug Mannitol showed 82.7% at 1000 μg mL^{-1} concentration. With this assay, the ELS and ESS were found to contain 765 and 534 mg of Mannitol equivalent/g extract in non site-specific model of hydroxyl radical scavenging.

Table 1: Effect of *Solanum nigrum* plant extracts on DPPH radical scavenging activity

Concentration (μg mL^{-1})	Inhibitory activity of ELS (%)	Inhibitory activity of ESS (%)	Inhibitory activity of Ascorbic acid (%)
100	15.67±2.76a*	19.97±2.80b*	13.82±1.82
200	32.86±1.72a*	37.84±1.60b*	31.76±2.84
400	56.25±1.32a*	60.50±2.1b*	54.64±2.23
500	71.6±0.60 a **	73.80±2.4b*	62.35±2.34
1000	90.6±0.90a*	88±0.00b*	85.6±0.11

Values are expressed in Mean±SD (n = 3), statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t" test, comparison between: a: L-Ascorbic acid vs ELS b: L-Ascorbic acid vs. ESS *p<0.05, **p<0.01

Table 2: Hydroxyl radical scavenging activity of various extracts of *Solanum nigrum*

Concentration (μg mL^{-1})	Inhibitory activity of ELS (%)	Inhibitory activity of ESS (%)	Inhibitory activity of Mannitol (%)
100	21.41±0.98a*	22.84±1.82b*	26.20±2.30
200	37.84±0.18a*	34.46±1.27b**	35.60±2.41
400	46.41±1.80a*	50.51±2.06b*	56.40±3.82
500	54.71±1.64a*	62.84±1.10b*	68.80±1.72
1000	67.6±0.55a**	76.6±0.20 b*	82.70±0.17

Values are expressed in Mean±SD (n = 3), statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t" test comparison between: a:Mannitol vs. ELS b: Mannitol vs ESS *p<0.05, **p<0.01

Protein oxidation: The oxidative protein damages, provoked by free radicals, have been demonstrated to play a significant role in aging and several pathological events including inflammation and arthritis. Protein oxidation was measured in terms of protein carbonyl formation which is sensitive assay for oxidative damages of proteins. As shown in Table 3 the plant extracts showed significant inhibition of protein carbonyl formation in a dose dependent manner. A highly significant ($p<0.05$) inhibition of 69.6 % was produced by the Ethanolic Leaf Extract (ELS) which was comparable to the positive control vitamin E which showed almost the same value of inhibition 70.7% at the same concentration. At the same time the ESS extract exhibited lower inhibitory effects of PCO formation at the same concentration.

Assay of antioxidant chemicals: The free radical activity of the plant extract is probably due to its phenolic contents. It is well known that phenolic compounds are constituents of many plants and they have attracted a great deal of public and scientific interest because of their health promoting effects as antioxidants. Flavonoids are a class of secondary plant phenolics with powerful antioxidant properties. The total phenolic and flavonoid contents of the plant extracts were expressed in terms of gallic acid and catechin equivalents. Table 4 shows the concentration of phenols and flavonoids. The ethanolic extract of stem of *Solanum nigrum* (ESS) extract showed the highest phenol and flavonoid concentration when comparable to the ethanolic extract of leaves (ELS).Total phenolic and flavonoid contents of each gram of dried ESS were estimated and found to be equivalent to 55.6 mg gallic acid and 78.6 mg catechin.

Table 3: Inhibitory effect of protein carbonyl formation by different extracts *Solanum nigrum*

Concentration (μg mL^{-1})	Inhibitory activity of ELS (%)	Inhibitory activity of ESS (%)	Inhibitory activity of vitamin E (%)
100	28.46±1.38a*	22.89± 0.04b*	32.15±1.07
200	31.34±1.23a*	30.98±0.03b*	41.68±1.24
400	45.24±1.17a*	42.89±0.03b*	53.22±1.04
500	55.14±2.01a*	54.67±0.02b*	60.18±1.05
1000	69.6 ±0.75a^{NS}	66.0 ±0.50b*	70.7±1.70

Values are expressed in Mean±SD (n = 3), statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t" test, comparison between: a: Vitamin E vs. ELS, b: Vitamin E vs. ESS *p<0.05, **p<0.01 and NS: non significant

Table 4: Total phenolic and flavonoid content of ELS and ESS extracts of *Solanum nigrum*

Extracts	Total phenolic content (mg g^{-1})	Total flavonoid content (mg g^{-1})
ELS	49.8±1.24	74.8±1.24
ESS	55.6±1.23	78.6±0.08

Values are expressed in Mean±SD (n = 3), Total Phenols are expressed in mg g^{-1} of gallic acid equivalents, Total Flavonoids are expressed in mg g^{-1} of catechin equivalents

Evaluation of analgesic activity

Thermal method: The anti-nociceptive activity of ELS and ESS were evaluated using both Hot plate and Tail flick test method of nociception in mice (Table 5, 6). In both the methods, there was no significant difference in the mean pre drug reaction time between the different groups. In Hot plate method the administration of the plant extracts increased latency time of the pain stimulus significantly ($p<0.01$) when comparable to normal control animals. The paw licking or jump response were seen only after a period 7.89, 8.45, 8.23 and 8.56 sec after 30, 60, 120 and 180 min of drug administration for 300 mg kg^{-1} b.wt. of ethanolic leaf extract of *S. nigrum* (ELS) against the control animals which showed these response with a less latency time of 4.34, 4.23, 4.28 and 4.12 sec after 30, 60, 120 and 180 min of 0.5% v/v of tween 80 administration. The animals that received ethanolic stem extract of *S. nigrum* (ESS) showed the paw licking or jump response after a latency period of 5.23, 6.45, 6.23 and 6.26 sec, respectively after 30, 60, 120 and 180 min of drug administration which is found to be less than that of the ethanolic leaf extract of *Solanum nigrum* (ELS) but, in both the cases it was found to be less than that of morphine treated group which was considered as a positive drug in the present study which showed a maximum latency time of more than 10 sec after 30, 60, 120 and 180 min of drug administration.

In Tail flick method the Basal reaction time to withdraw the tail (flicking response) from radiant heat is taken as the end point. Like the Hot plate method there was no significant difference in the mean pre drug basal reaction time between the different groups. There was a significant increase ($p<0.01$) basal tail withdrawal reaction time in group II animals that received 300 mg kg^{-1} b.wt. of ethanolic leaf extract of *S. nigrum*. They showed a basal tail withdrawal time of 9.69, 9.15, 9.22 and 8.69 sec after 30, 60, 120 and 180 min of drug administration when comparable to the control group I animals that showed the basal tail withdrawal time of 5.34, 5.13, 4.28 and 4.62, respectively after 30, 60 120 and 180 min after the vehicle administration. It is observed in the present investigation that the ethanolic leaf extract of S. *nigrum* (ELS) possess pronounced antinociceptive activity when comparable (**$p<0.01$) to the ethanolic stem extract of *S. nigrum* (Table 6).

Chemical method: Pain was induced by injection of irritants (acetic acid) into the peritoneal cavity of mice and the animals react with a characteristic stretching behavior which is called writhing behavior (contraction of abdomen, turning of trunk and extension hind limb).This is considered as a model for chemically induced pain stimuli. In acetic acid induced writhing test both the extracts of *Solanum nigrum* reduced the writhing count significantly (Table 7). Similar to the anti-nociceptive activity exhibited by the plant extracts in thermal methods the ethanolic extracts of leaves of *Solanum nigrum* (ELS) exhibited maximum inhibition of writhing movements that

Table 5: Analgesic activities of the different extracts of *Solanum nigrum* on latency time of mice exposed to Hot plate test

		Post drug reaction time in sec			
Treatment	Pre-drug reaction time (sec)	30 min	60 min	120 min	180 min
Group-I(control)	3.25 ±0.24	4.34±0.23	4.23±0.54	4.28±0.44	4.12±0.38
Group-II ELS (300 mg kg^{-1} treated)	3.12±0.14	7.89±1.56a**	8.45±0.61a**	8.23±0.14a**	8.56±0.33a**
Group-III ESS (300 mg kg^{-1} treated)	3.15±0.16	5.23±3.56b*	6.45±0.39b*	6.23±0.29b*	6.26±1.24b*
Group-IV Morphine (100 mg kg^{-1} treated)	3.33±0.34	10.23±1.24	10.34±0.54	10.27±0.55	10.13±0.62

Values are expressed in Mean±SD (n = 6), Statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t" test, comparison between: a: Control vs. ELS, b: Control vs. ESS *$p<0.05$,**$p<0.01$

Table 6: Analgesic activity of the different extracts of *Solanum nigrum* on Tail flick response in rats

		Post drug reaction time in sec			
Treatment	Pre-drug reaction time (sec)	30 min	60 min	120 min	180 min
Group-I(control)	5.15 ±0.42	5.34±0.23	5.13±0.64	4.28±0.44	4.62±0.28
Group-II ELS (300 mg kg^{-1} treated)	5.62±0.24	9.69±1.16a**	9.15±0.31a**	9.22±0.44a**	8.69±0.53a**
Group-III ESS (300 mg kg^{-1} treated)	5.35±0.16	6.30±1.26b*	6.43±0.21b*	6.47±0.34b*	6.66±0.20b*
Group-IV Morphine (100 mg kg^{-1} treated)	5.43±0.24	11.03±1.07	10.94±0.64	10.56±0.43	10.33±0.64

Values are expressed in Mean±SD (n = 6), Statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t" test, comparison between: a: Control vs. ELS, b: Control vs. ESS *$p<0.05$,**$p<0.01$

Table 7: Analgesic activities of the different extracts of *Solanum nigrum* on acetic acid-induced Writhing response in rats

Treatment	No. of writhing	(%) inhibition of writhing
Group-I (control)	76.56±2.67	-
Group-II ELS (300 mg kg^{-1} treated)	46.17±2.38a**	53.28
Group-III ESS (300 mg kg^{-1} treated)	53.47±2.89b*	46.53
Group-IV Diclofenac sodium (50 mg kg^{-1} treated)	29.34±4.35	70.66

Values are expressed in Mean±SD (n = 6), Statistical significant test for comparison was done by ANOVA, followed by Dunnet's "t" test, comparison between: a: Control vs. ELS, b: Control vs. ESS *$p<0.05$,**$p<0.01$

is 53.28% when comparable ($p<0.01$) to the ethanolic stem extracts of *Solanum nigrum* (ESS)which showed 46.53% inhibition at the same concentration. The standard drug Diclofenac sodium exhibited 70.66 % inhibition of writhing movements in the present investigation.

DISCUSSION

Reactive Oxygen Species (ROS) such as singlet oxygen ($^{\circ}O_2$), hydrogen peroxide (H_2O_2) and hydroxyl ($^{\circ}OH$) radical are often generated as byproducts of biological reactions or from exogenous factors. The involvement of these species in the pathogenesis of a large number of diseases including rheumatoid arthritis, atherosclerosis, aging, nephrites, reperfusion injury, inflammation, asthma, diabetes mellitus and carcinogenesis are well documented (Stadtman and Oliver, 2005; Freig *et al*.,1994). In situations of increased free radical generation the reinforcement of endogenous antioxidants via intake of dietary antioxidants may be of particular importance in attenuating the cumulative effects of oxidatively damaged molecules. The phytochemical screening of chemical constituents of the leaf and stem extracts of *Solanum nigrum* were rich in alkaloids, Flavonoids, tannins and steroids. It is generally accepted that a synergistic relationship amongst phytochemical has been adduced to be responsible for the overall beneficial effect derivable from plants. It was stated by Huseini *et al.* (2005) that medicinal values of plants and vegetables are indicated by their phyto chemicals.

The antioxidant activity was studied in terms of its free radical scavenging potential. The radicals such as DPPH and Hydroxyl radical scavenging potential of the plant extracts namely ELS and ESS were investigated. In the DPPH test, the antioxidants reduce the DPPH radical to a yellow-colored compound, diphenyl picrylhydrazine and the extent of the reaction will depend on the hydrogen donating ability of the antioxidants (Bondet *et al*., 1997). The scavenging effect of plant extracts of *Solanum nigrum* on DPPH radical shows the significant effect on its scavenging potential which increased with increasing concentration. Rollet-Labelle *et al.* (1998) in his work stated that hydroxyl radicals are known to be the most reactive of all the reduced forms of dioxygen and are thought to initiate cell damage in vivo, capable of modifying almost every molecule in the living cells. This radical has the capacity to cause strand damages in DNA leading to carcinogenesis, mutagenesis and cytotoxicity. Moreover, according to Aruoma (1998) hydroxyl radicals are capable of quickly initiating the lipid peroxidation process as by abstracting hydrogen atoms from unsaturated fatty acids. Both the plant extracts ELS and ESS scavenged the hydroxyl radicals considerably. The maximum scavenging effect on DPPH and hydroxyl radical-scavenging is observed for ethanolic leaf extract (ELS) when compared to ethanolic stem extract of *Solanum nigrum*. This inhibition was found to be higher than the positive control ascorbic acid used in the present study which indicates the possible presence of high ascorbic content in the leaves and stem of the plant extract. Lin *et al*. (2008) demonstrated that the water extract of *Solanum nigrum* contains several antioxidants, such as gallic acid, PCA, catechin, caffeic acid, epicatechin, rutin and narigenin and possesses strong antioxidative activity *in vitro*. The presence of these above said phyto-chemicals in our ethanolic extracts of *Solanum nigrum* may be attributed to their free-radical scavenging potential observed in the present study.

The oxidative protein damages, provoked by free radicals, have been demonstrated to play a significant role in aging and several pathological events (Reznick and Packer, 1994).Radical mediated damages to proteins might be initiated by electron leakage, metal-ion dependent reactions and autoxidation of lipids and sugars. Major molecular mechanisms, leading to structural changes in proteins are free-radical mediated protein oxidation characterized by Protein Carbonyl formation (PCO).The protein oxidation was determined in terms of inhibition of Protein Carbonyl Formation (PCO). The ethanolic leaf and stem extracts of *Solanum nigrum* exhibited dose dependent inhibition of protein carbonyl formation. According to Lee *et al.* (2005) the glycoprotein isolated from *Solanum nigrum* has a strong scavenging activity against lipid peroxyl radicals and has hypolipidemic activity by increasing the detoxicant enzymes activity through the inhibition of hepatic HMG-CoA reductase in mice. Also it possesses antioxidant potential of which has been evaluated by several methods like DPPH, superoxide radical and hydroxyl radical assay. The inhibitory activity of the plants extracts on protein carbonyl formation is probably due to its antioxidant contents.

There is an increasing interest in natural antioxidants, namely phenols and flavonoids present in medicinal and dietary plants that might help to prevent oxidative damage. Dietary flavonoid and phenols have generally been considered as non nutrients as their possible beneficial effect on human health have only recently been recognized. Therefore it could be valuable to determine the total phenolic and flavonoid content of all the plant extracts. All the extracts showed the presence of considerable phenolic and flavonoid content which confirms the free radical scavenging activity through their reaction as hydrogen ion as electron donating agents and metal ion chelating property. All Substances termed

antioxidants can influence the oxidation process through simple or complex mechanism including prevention of chain initiation, binding of transitional metal ion catalysts, decomposition of peroxides prevention of continued hydrogen abstraction and radical scavenging (Rice-Evans *et al*., 1996). The antioxidant activities of various extracts of S*olanum nigrum* are probably due to its phenol and flavonoid contents.

The classification of anti-nociceptive drugs is usually based on their mechanism of action either on the central nervous system or on the peripheral nervous system (Planas *et al*., 2000). Thermal induced nociception indicates narcotic involvement (Besra *et al*., 1996). Thermal nociceptive tests are more sensitive to opioid μ receptors. The Eddy's hot plate and tail-flick tests are considered to be selective to examine compounds acting through opioid receptor (Elisabetsky *et al*., 1995). In the present investigation both the extracts of *Solanum nigrum* increased mean basal latency suggesting its central analgesic activity.

Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids (Pal *et al*., 1999). The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells (Ribeiro *et al*., 2000), acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways (Hossain *et al*., 2006). The analgesic activity of ELS and ESS extracts of *Solanum nigrum* against acute inflammatory pain was moderate as compared to potent inhibitory activity of Diclofenac sodium. Diclofenac sodium and Indomethacin offer relief from inflammatory pain by suppressing the formation of pain substances in the peripheral tissues, where prostaglandins and bradykinin were suggested to play an important role in the pain process. In the present investigation it can concluded that the active constituents present in these plant extracts might suppress the formation of these substances or antagonize the action of these substances and thus exerts its analgesic activity in acetic acid-induced writhing test.

Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain. In our present study the ethanolic leaf and stem extracts of *Solanum nigrum* inhibited both mechanisms of pain, suggesting its central as well as peripheral antinociceptive activity. Such a mode of action is proposed for opioid analgesic such as morphine. It is also reported by De Campos *et al*. (1997) that the inhibition of pain could arise not only from the presence of opioids and/or opiodiomimetics but could also arise from the presence of phenolic constituents. There are also reports from Rajnarayana *et al*. (2001) on the role of flavonoid in analgesic activity primarily by targeting prostaglandins. According to Ramaswamy *et al*. (1985), flavonoids are known to inhibit prostaglandin synthetase enzyme responsible for synthesis of prostaglandins. Since prostaglandins are involved in pain perception and are inhibited by flavonoids, it could be suggested that reduced availability of prostaglandins by flavonoids of ELS and ESS extracts might be responsible for its analgesic effect. Quantitative estimation of our plant extract revealed the presence of considerable quantities of phenols and flavonoids. Phyto chemical investigation of whole plant is reported by Saleem *et al*. (2009) reveals the alkaloids, flavonoids, tannins, saponins, glycosides, proteins, carbohydrates, coumarins and phytosterols. The free radical scavenging and analgesic activity of our plant extracts may be due to the cumulative effects of these phyto-chemicals also.

CONCLUSION

The herbal medicines are getting more importance in the treatment of many diseases because of their less toxic effect comparable to synthetic drugs. The ethanolic leaf and stem extracts of *Solanum nigrum* exhibited significant analgesic effect against the physical and chemical methods of pain induction in rats. This effect is probably mediated through its significant antioxidant activity and it justifies the traditional use of this plant in the treatment of various types of pains.

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