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SCREENING OF ANALGESIC ACTIVITY OF CHLOROFORM EXTRACT OF *SOLANUM NIGRUM* IN SWISS ALBINO MICE

Parvathy RL[1], Madhavrao C[2]
[1]Senior Lecturer, Department of Pharmacology, PMS College of Dental Science and Research, Trivandrum, Kerala.
[2]Associate Professor, Department of Pharmacology, All India Institute of Medical Sciences, Mangalagiri, Andhra Pradesh.
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Corresponding author: Parvathy RL

Abstract:
Background: Pain is the one of the major symptom in any pathological condition. Various drugs are available to reduce the pain but they are associated with various adverse drug reactions. In Ayurveda *Solanmum nigrum* leaves are used to reduce the pain. The present study aimed to screen the analgesic activity of *Solanmum nigram* leaves in Swiss Albino mice.
Materials and Methods: The study was conducted in the Department of Pharmacology, Sree Mookambika Medical College, Tamil Nadu. Total of 24 mice were divided into 4 groups each of 6 rats. G-I (0.9% normal saline), G-II (Morphine 5 mg/kg), G-III (*Solanmum nigram* 100mg/kg) and G-IV (*Solanmum nigram* 200 mg/kg). The analgesic activity was evaluated by tail clip and tail flick methods. In both methods reaction time was noted after 30min of drug administration. Mean reaction time was calculated and compared between the groups by using SPSS (16.0 version) soft ware.
Results: The results of tail clip and flick methods are group-I showed significant difference compared group-II, III and IV. Group-II showed significant difference with group-III but not with group-IV. Group-III showed significant difference compared to group-IV. In both methods reaction time was increased compared to group-II with IV but not statistically significant.
Conclusion: High dose of chloroform extract of *Solanmum nigrum* showed significant analgesic activity compared to its low dose. It can be used as analgesic where synthetic drug are contraindicated.
Keywords: Analgesic activity, chloroform, *Solanum Nigrum,* tail clip, tail flick, reaction time

Introduction

Plants are considered to be one of the important sources of medicines for alleviating the sufferings caused by various diseases in humans. Traditional medicine (includes medicinal plants, minerals, organic matter) has been defined by World Health Organization (WHO) as those that utilizes the indigenous system of medicine that has been used since ancient times before the development of modern medicine and are still being used[1]. The use of plants by humans as medicines is broadly termed as Ethnomedicine and is probably the first and the oldest system of human health care[2]. Their wide use in developing countries like India can be attributed to the general belief of the people that herbal drugs are locally available, culturally acceptable, cheap and are having better safety profile[3]. Herbal drugs have given major contributions to the modern therapeutics too by paving the way to the discovery of many effective allopathic drugs when plants were identified as their important sources[4,5]. India is endowed with rich flora because of the extreme variation in geographic and climatic conditions and around 15000 medicinal plants have been recorded in India and is considered to be one of the richest source of medicinal plants[6,7].

Pain is one of the major symptoms is observed in all pathological conditions. There are various mediators are involved to induce the pain. Prostaglandins, interleukins and cytokines will be released from damaged are and produce the pain. Various drugs are used to reduce the pain. Majorly NSAIDs and opioids are commonly used to reduce the pain. But both classes of drugs have limitations to use and they will produce the adverse drug reactions. To overcome these limitations Ayurvedic drugs can be used. The present study is based on an important medicinal plant named *S. nigrum Linn* found abundantly in India which is being widely used for treating various diseases traditionally. *S. nigrum Linn* commonly known as black night shade is a member of a plant family called *Solanaceae* which are well known for their therapeutic properties[8]. *S. nigrum* (*Solanum nigrum*) is used worldwide to treat inflammation, pain and fever. Pharmacological studies on different parts of this plant have shown its significant antiproliferative, antioxidant, anti-inflammatory, antiseizure, hepatoprotective and antimicrobial effects[9-15]. Despite of its potential clinical effects *S. nigrum* has not grabbed much attention for its use in modern medicine. The literature findings suggest that there only a few studies done on evaluating the central effects of *S. nigrum.* Hence more studies are warranted to increase the strength of evidence. So this study attempts to investigate the analgesic effects of *S. nigrum* in mice.

Materials and Methods

Study settings and Period

The study conducted in the Department of Pharmacology, Sree Mookambika Medical College, Kulasekharam, Tamil

Nadu for the period of 3 months. The study was approved by Institutional Research Committee (IRC) and Institutional Animal Ethics Committee (IAEC).

Animals

Healthy adult Swiss Albino mice of either sex weighing between 20-30 g were procured from the central animal house of the institution. A total of 24 Swiss Albino mice were used for the study purpose. After the procurement of the animals they were transferred to the experimental room of the central animal house for a period of 4-5 hrs per day for a total of 7 days for acclimatization under standard husbandry conditions as (Room temperature : 26 ± 2oC, Relative humidity : 70 – 80% and Light: dark cycle : 12: 12hrs). All animals were fed with standard laboratory food pellets and water *ad libitum*. The animals that were assigned to receive drugs orally were fasted overnight in order to avoid food-drug interaction and to facilitate absorption. All the experiments were conducted during the day time between 10.00 AM and 3.00 PM to prevent the errors in the analysis of data obtained.

Collection and Preparation of chloroform extract of Solanum nigrum

S. nigrum Linn was identified and collected at a place called Kattakkada in Trivandrum district and the leaves were authenticated by Dr. P.C. Jessykutty (Associate Professor, Department of Plantation Corps and Spices, College of Agriculture, Trivandrum). The plant specimen is kept at the museum of the Pharmacology department of this institute. After separating the leaves, the remaining part of the plant was discarded. The leaves were then allowed to air dry for 2 days. The air dried leaves were powdered and it was then subjected to chloroform extraction using the Soxhlet apparatus. The solvent was then removed under reduced pressure which gave greenish-black coloured residue. It was then filtered using Whatman No: 1 filter paper. The filtrate was then evaporated to dryness and the weight of the crude chloroform extract obtained was measured and it approximately weighed 2gms.The dried extract was diluted in gum acacia (1:50 weight/volume) and considered as the stock solution of dose 200 mg/kg and it was further diluted to 100 mg/kg. The prepared extracts were then subjected to analgesic screening study.

Study groups

Total 24 rats were divided into 4 groups each of 6 rats
Group-I: Control (0.9% saline/orally)
Group-II: Morphine (5 mg/kg/BW/SC)
Group-III: Chloroform extract of *Solanum nigrum* (100 mg/kg/BW/i.p)
Group-IV: Chloroform extract of *Solanum nigrum* (200 mg/kg/BW/i.p)

Procedure

Analgesic activity of *Solanum nigrum* was evaluated by Haffner's tail clip and Radiant heat method (Tail flick) methods.

1. Haffner's tail clip method

The animals were screened by applying an artery clip to the base of the tail. The animal quickly responds to it by biting and trying to dislodge it. The pressure exerted by the clip were so adjusted that it was just sufficient to make all the mice to respond. The animals that did not attempt to dislodge the clip within 10 seconds were discarded from the experiment. The rest of the animals were divided into 4 groups with 6 animals each. Control group were given 0.9% normal saline 1 ml orally while the rest of the groups received either Morphine 5mg/kg BW (standard drug) subcutaneously or chloroform extract of *S. nigrum Linn* (test drug) in doses 100 mg/kg, 200 mg/kg BW i.p. The tail clip was applied 30 minutes after the administration of the above drugs to the respective groups and reaction time was noted by a stopwatch in seconds. In order to avoid tissue damage the cut off period was taken as 15 seconds. The reaction time was the time between the application of the clip and the response. The reaction time for each animal was noted and the mean reaction time was taken and compared among the groups.

2. Tail-flick method (Radiant heat method)

Mice were placed in restrainer and their tails were left exposed. In order to induce pain a thermal stimulus via electrically heated nichrome wire (6 mA) of an analgesiometer was used. The site of application of the heat was maintained at 3cm, measured from the root of the tail. The animal responded to it by withdrawing (flicking) the tail. The time taken by mouse to withdraw (flick) its tail from the hot wire was considered as the reaction time. Basal reaction time was noted for each animal. The animas with reaction time above 6 seconds were discarded. Rest of the mice was allotted into 4 groups each containing 6 animals each. Control group were given normal saline 1 ml orally while the rest of the groups received either morphine 5 mg/kg BW (standard drug) subcutaneously or chloroform extract of *S. nigrum Linn* (test drug) in doses 100 mg/kg, 200 mg/kg BW i.p. The pain threshold was noted at 30 minutes after the drug administration to their respective groups by noting the reaction time in seconds with the help of a stop watch. The cut off time to screen the pain threshold was taken as 10 seconds so as to avoid tissue damage. The reaction time for each animal was noted. The mean values of the reaction time was measured and compared among the groups.

Statistical analysis

The data was expressed in mean and standard deviation. Statistical Package for Social Sciences (SPSS 16.0) version used for analysis. One way ANOVA (Post hoc) followed by Tukeys's test. P value less than 0.05 ($p<0.05$) considered statistically significant at 95% confidence interval.

Results

Haffner's tail clip method

Tail clip method the mean reaction time in group-I was noted as 3.59±0.33 seconds. A significant ($p<0.05$) increase in the reaction time towards the mechanical stimulus was observed after 30 minutes of drug administration in Group-II (11.17±0.31 sec), group-III (8.17±0.48 sec) and in group-IV (12.16±0.56 sec) when compared to Group-I. The chloroform extract of *S. nigrum* showed a dose-dependent and significant ($p< 0.05$) increase in the pain threshold at 30 min post-treatment with doses of 100 and 200 mg/kg of the extract when compared to group-I. There was statistically no significant difference observed when group-II was compared to group -IV ($p>0.05$). There was an increase in the reaction time noted in group-IV when compared to group-II but it was not found statistically significant (Table-1 and Graph-1).

Tail-flick method (Radiant heat method)

In tail flick method the mean reaction time noted in the group-I was 2.33±0.33 seconds. The study showed a significant ($p<0.05$) increase in the reaction time towards the thermal stimulus after 30 minutes of drug administration in group-II (9.58±0.21 sec), group-III (8.55±0.33 sec) and in group-IV (9.45±0.37 sec) when compared to group-I. The chloroform extract of *S. nigrum* showed a dose dependent significant ($p< 0.05$) increase in the analgesic activity with doses of 100 and 200 mg/kg when compared to group-I. There was statistically significant difference was observed when group-I was compared to group-II and group-III ($p<0.05$). There was an increase in the reaction time noted in group-IV when compared to group-II and it was observed no statistical significant ($p>0.05$) (Table-2 and Graph-2)

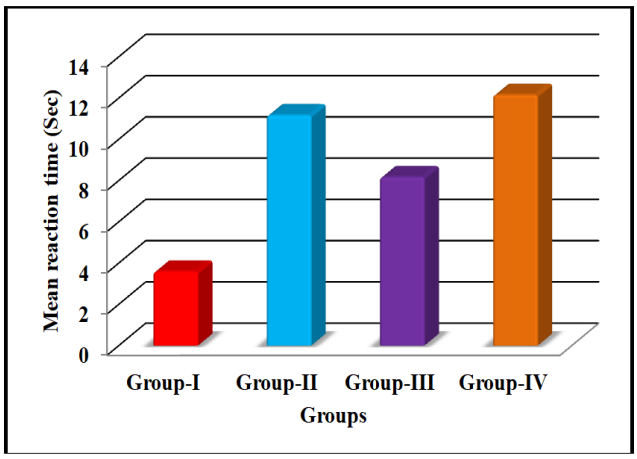
Table 1: Evaluation of analgesic effect of chloroform extract of *Solanum nigrum Linn* using tail clip method in Swiss Albino mice

Groups	Drug (Dose and route of administration)	Reaction time (Sec) (MEAN±SD)
Group-I	0.9% Normal saline (1ml/kg/oral)	3.59±0.33
Group-II	Morphine (5 mg/kg/BW/SC)	11.17±0.31*
Group-III	Chloroform extract pg of *Solanum nigrum* (100 mg/kg/BW/ip)	8.17±0.48*,#
Group-IV	Chloroform extract pg of *Solanum nigrum* (200 mg/kg/BW/ip)	12.16±0.56*,$

(*p<0.05 significant compared group-I with other groups,

#p<0.05 significant compared group-II with others,

$p<0.05 significant compared group-III with other groups)



Graph 1: Evaluation of analgesic effect of chloroform extract of *Solanum nigrum Linn* using tail clip method in Swiss Albino mice

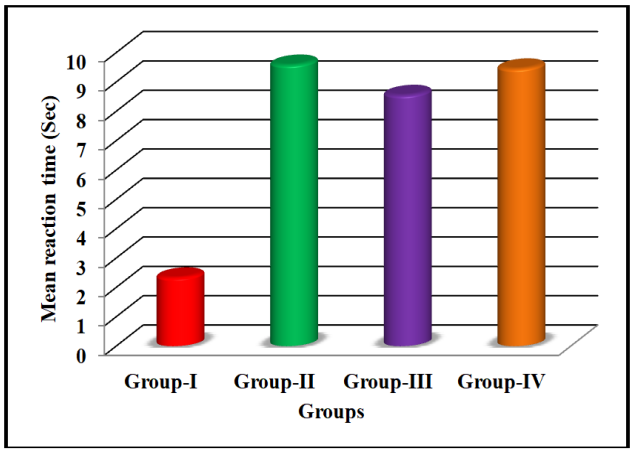
Table 2: Evaluation of analgesic effect of chloroform extract of *Solanum nigrum Linn* using tail clip method in Swiss Albino mice

Groups	Drug (Dose and route of administration)	Reaction time (Sec) (MEAN±SD)
Group-I	0.9% Normal saline (1ml/kg/oral)	2.33±0.33
Group-II	Morphine (5 mg/kg/BW/SC)	9.58±0.21*
Group-III	Chloroform extract pg of *Solanum nigrum* (100 mg/kg/BW/ip)	8.55±0.33*,#
Group-IV	Chloroform extract pg of *Solanum nigrum* (200 mg/kg/BW/ip)	9.45±0.37*,$

(*p<0.05 significant compared group-I with other groups,

#p<0.05 significant compared group-II with others,

$p<0.05 significant compared group-III with other groups)



Graph 2: Evaluation of analgesic effect of chloroform extract of *Solanum nigrum Linn* using tail clip method in Swiss Albino mice

Discussion

The present study was aimed to investigate the analgesic effect of the chloroform extract of the leaves of *S. nigrum Linn* in Swiss albino mice. In our study the chloroform extract of *S. nigrum* showed a dose dependent increase in the pain threshold after 30 min of post treatment with doses of 100 and 200 mg/kg of the extract. The analgesic effects noted with 200 mg/kg were comparable with that of the standard drug morphine used in the study. An anti-nociceptive screening of the chloroform extract of *S. nigrum* was done by Zakaria et al.[16] using abdominal constriction, hot plate and formalin tests in rodents. However in the above study the abdominal constriction test revealed the significant peripheral pain relieving effect of the drug and the central effects were confirmed by the other two tests they had chosen. But the main aim of our study was to evaluate the central analgesic property of the extract therefore we opted only the tail clip and flick methods as they are considered to be good models for studying the central properties[17]. In contrast to our findings Zakaria et.al., observed a dose independent analgesic activity with the preparation. In the same above study it was observed significant analgesic effect on all observations but the effects were lower when compared to that of the morphine. The results of the analgesic screening in our study is consistent to the observations made by Adnam et.al.,[18] who demonstrated a step by step increase in the analgesic property of the fruit extract of *S. nigrum*. As per our study findings both 100 and 200 mg/kg of the preparations showed significant analgesic effects with increase in doses but according to Adnam et.al., a statistical significant analgesic effect of the fruit preparation was observed only with 400 mg/kg. Kaushik et al.[19] demonstrated the central and peripheral analgesic effects of the *S. nigrum* by using Eddy's hot plate and acetic acid induced writhing tests respectively and they compared it with the standard drug diclofenac.

Their study was carried out at doses of 100 to 500 mg/kg body weight orally in rodents and the extract showed significant analgesic activity at 500 mg/kg when compared to diclofenac. Antinociceptive assays done on various plant members of Solanaceae family have shown significant analgesic property and thereby supporting our study findings. Sumalatha et.al.,[20] performed the tail flick and tail immersion in mice with the methanolic extract of *S. pubescens.* In the above study the animals were treated with 300 mg/kg of the extract and the analgesic effects were analyzed at various time intervals. The same study observed the marked analgesic activity of the extract at 2 hrs after the test drug administration in tail flick and immersion methods and the results were compared with pentazocin too. Additionally they performed the abdominal constriction test in rats and showed increased protection from the writhes on treatment with the methanolic preparation thereby proving involvement of both the spinal and supra spinal components. Results of a study conducted by Mwonjoria et.al.,[21] were in accordance with our study findings. The models they had chosen were tail flick and hot plate for assessing the analgesic effects of *S. incanum.* In the above study the extracts were given at doses 50 mg/kg, 100 mg/kg and 200 mg/kg and the reaction time were noted. The extract exhibited significant prolongation of the reaction time when compared to the control. They compared the test results with acetylsalicylic acid and morphine. The above study there were no significant differences noted between the test groups and the acetylsalicylic acid group where as morphine exhibited significant difference on comparison with the test groups.

The significant increase in pain threshold produced by the chloroform extract of *S. nigrum Linn* in these models suggests the involvement of central pain pathways. Pain is centrally modulated via a number of complex processes involving opiate, dopaminergic, noradrenergic and serotonergic pathways[22]. *S. nigrum* have also shown analgesic property when tested using acetic acid induced writhing model and hence showing the involvement of peripheral mechanisms too. Thus it could be suggested that the analgesic effect produced by the chloroform extract of *S. nigrum Linn* may be via the central mechanisms involving central receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leucotrienes, and other endogenous mediators involved in nociception.

Conclusion

The study results were concluded that *Solanum nigrum* leaves can be used as alternative to synthetic analgesic drugs. It will produce less adverse effects. There is requirement of more studies to indentify the phytochemical responsible for analgesic activity.

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