WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 8, Issue 12, 1251-1263. Research Article ISSN 2277– 7105

EVALUATION AND PRELIMINARY PHYTOCHEMICAL SCREENING OF HYDROETHANOLIC ROOT EXTRACT OF *SOLANUM ANGUIVI* LAM. (SYN: *S. INDICUM* AUCT) FOR ANALGESIC ACTIVITY

Ameena Mehaboob* and Binulal C.

Department of Pharmacology, KMCT College of Pharmaceutical Sciences, Kallanthode, Kozhikode.

Article Received on
13 Sept. 2019,

Revised on 03 Oct. 2019,
Accepted on 24 Oct. 2019,

DOI: 10.20959/wjpr201912-16133

***Corresponding Author**
Ameena Mehaboob
Department of Pharmacology, KMCT College of Pharmaceutical Sciences, Kallanthode, Kozhikode.

ABSTRACT

The present study focused on the preliminary phytochemical screening and evaluation of analgesic activity of hydroethanolic root extract of *Solanum anguivi* Lam (Syn: *S.indicum* auct) belongs to the family Solanaceae. The hydroethanolic extract was prepared by hot continuous extraction (soxhlet). The roots of *Solanum anguivi* Lam (Syn: *S.indicum* auct) were collected, dried and powdered. Adult Albino mice of either sex were used for the studies and subdivided into four groups (n=4). The extract was evaluated for analgesic activity using Tail immersion test, Eddy's hot plate method and Haffner's tail clip method. The statistical analysis was done through one way ANOVA followed by post hoc Tukey's multiple comparison tests. The results indicated that the extract depicts statistically significant analgesic activity ($P<0.001$). Preliminary phytochemical screening of the ethanolic root extract revealed the presence of carbohydrates, saponins, alkaloids, flavonoids, phenols, tannins, steroids & terpenoids.

KEYWORDS: Phytochemical screening, Analgesic activity, *Solanum anguivi* Lam.

INTRODUCTION

Herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herbal medicine is the oldest form of healthcare known to mankind. Herbs have been used by all cultures throughout history. Herbal medicines have become popular nowadays because of their fewer side effects, adverse or toxic effects as compared to synthetic medicines. The

natural products obtained from plants and animals are used as such or will be modifies using the basic molecule as lead compound. The lead compounds are modified accordingly to show affinity and efficacy.[1] Natural products have been the single most productive source of leads for the development of drugs. Over a 100 new products are in clinical development, particularly as anti-cancer agents and anti-infectives. Application of molecular biological techniques is increasing the availability of novel compounds that can be conveniently produced in bacteria or yeasts, and combinatorial chemistry approaches are being based on natural product scaffolds to create screening libraries that closely resemble drug-like compounds. Various screening approaches are being developed to improve the ease with which natural products can be used in drug discovery campaigns, and data mining and virtual screening techniques are also being applied to databases of natural products. It is hoped that the more efficient and effective application of natural products will improve the drug discovery process.[2]

Solanum anguivi Lam. is a non-tuberous and widely distributed plant that possesses various medicinal properties. Mostly, the plant prefers to grow in humid temperature and commonly found as weed in gardens. It is a rare ethnomedicinal herb belonging to the family Solanaceae. The plant is used as a therapeutic agent for various diseases. *Solanum anguivi* Lam. roots are used traditionally in the treatment of nervous disorders and to relieve pain. The different parts (fruits, leaves, roots) of this plant used by the traditional practitioners in the treatment of loss of appetite and anorexia, blood disorders, rhinitis, cough, asthma, sore throat and hiccup, nervous disorders, sexual disorders, abdominal pain and worm infestation, pain and fever, inflammation, insomnia, urinary complications, cardiac weakness.[3]

Various extracts of *Solanum anguivi* Lam. **(**Syn**:** *S. indicum* auct**)** berries showed hypoglycemic, antiperoxidative, antihyperlipidimic, antioxidant, CNS depressant and analgesic activities.[4-6] The present study was initiated to evaluate the analgesic activity of hydroethanolic root extract of *Solanum anguivi* Lam.

MATERIALS AND METHODS

Collection and identification of plant materials

The plant material was collected during the month of November 2016, from Kollam district in Kerala, India. The plant was taxonomically identified and authenticated by Dr. Nileena C.B, Assistant Professor in charge of HOD Botany, Sree Narayana College, Cherthala. A

voucher specimen was also deposited at the herbarium of Department of Botany with accession number SNCCH 094 for future reference and further study.

Preparation of the extract

The roots were washed thoroughly with water to remove the soil particles, shade dried and grounded. The ground powder was extracted using hydroethanol (Ethanol: Water = 80:20) solvent in a soxhlet apparatus. The extraction was continued for about 1 week until the solvent in the thimble was clear. The extract was then concentrated under reduced pressure using rotary evaporator at 40ºC until extraction solvent was completely dried. The yield of hydroethanolic extracts was 6.4w/w. The extract was stored in the refrigerator at 4ºC until further use. This extract was suspended in 5% Carboxymethyl cellulose (CMC) for animal studies. Preliminary phytochemical screening was conducted using chemical tests.

Phytochemical screening of the extract

The phytochemical screening was performed using standard procedures.[7-8]

Test for carbohydrates

Molisch's test: To 2ml of test extract, few drops of alcoholic α- naphthol was added and shaken well. Then through sides of test tube few drops of concentrated sulphuric acid was added. A purple to violet colour ring at the junction indicated the presence of carbohydrates.

Benedict's test: To 1ml of extract 2ml of Benedict's reagent was added and heated in a boiling water bath for 3-5 minutes. An orange to brick red colouration indicated the presence of reducing sugars.

Fehling's test: To 2ml of the extract equal volumes of Fehling A and Fehling B were added and placed in a boiling water bath for a few minutes. The production of reddish brown precipitate indicated the presence of reducing sugars.

Test for proteins and amino acids

Biuret test: To a small quantity of extract 2ml sodium hydroxide and 4-5 drops of 1% copper sulphate were added. The mixture was warmed for 5 minutes. Bluish violet colouration indicated the presence of proteins.

Ninhydrin test: To 2ml of extract 3-4 drops of Ninhydrin solution was added and the contents were boiled. Intense blue colouration confirmed the presence of proteins.

Test for alkaloids

0.5 g of extract was diluted with 5 ml water and dilute hydrochloric acid was added, boiled and filtered. With filtrate the following tests were performed.

Mayer's test: 2-3 ml filtrate with Mayer's reagent (Potassium mercuric iodide solution) gives cream colour precipitate.

Wagner's test: 2-3 ml filtrate with few drops of Wagner's reagent gives reddish brown precipitate.

Hager's test: filtrate with Hager's reagent (Saturated Solution of picric acid) gives yellow precipitate.

Dragendorff's test: 2-3 ml filtrate with few drops Dragendorff's reagent (Potassium bismuth iodide solution) gives reddish brown precipitate.

Test for glycosides

Keller-Killiani test: To the extract, 3 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This solution was transferred to the surface of 2 ml concentrated sulphuric acid. A reddish brown ring formed at the junction of two liquids and the upper layer slowly become bluish-green.

Legal's test: To the extract 1ml pyridine and 1ml sodium nitroprussside were added. Pink to red colour appears.

Baljet's test: Extract with sodium picrate shows yellow to orange colour.

Test for flavonoids

Alkaline reagent test (Sodium hydroxide test): To the test solution few drops of sodium hydroxide were added, intense yellow colour is formed, which decolourises after addition of dil. HCl indicated the presence of flavonoids.

Lead acetate test: Treated the extract with a few drops of lead acetate. The formation of yellow precipitate indicates the presence of flavonoids.

Shinoda Test: To the extract 5ml of 95% ethanol, magnesium turnings and few drops of concentrated hydrochloric acid were added. Pink colour indicates the presence of flavonoids.

Test for phenolic compounds

Ferric chloride test: Dissolved a little extract in distilled water and add 2ml of 5% ferric chloride solution. Formation of green or violet colour indicates the presence of phenolic compounds.

Test for tannins

Ferric chloride test: A few drops of 0.1% $FeCl_3$ was added to the extract and observed for intense green, purple, blue or black coloration.

Lead acetate test: To a few drops of the test extract, a few drops of 10% lead acetate were added. Formation of a precipitate indicates the presence of tannins.

Gelatin test: A few drops of extract were mixed with a few drops of 1% solution of gelatin containing 10% sodium chloride solution. White precipitate indicates the presence of tannins.

Test for saponins

Froth formation test: To 0.5 g of each plant extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth.

Experimental animals and exposure conditions

Healthy wistar rats and albino mice of either sex were obtained from Animal house, Gov. Veterinary College Mannuthy. They were housed in polypropylene cages in a conducive environmental situation i.e. temperature (22±2ºC), humidity (45±5ºC) and 12h of dark and light cycle. The animals were fed ad libitum with normal laboratory chow standard pellet diet. The animals were allowed to acclimatize for 7 days before commencing the experiments. All the studies were conducted in accordance with the Animal ethical committee of the institute (IAEC, proposal number SJCP/IAEC/2016-2/1), St. Joseph's College of Pharmacy, Cherthala.

Drugs and chemicals

Hydroethanolic extract of *Solanum anguivi* Lam roots at two different doses (200 and 400mg/kg p.o) were suspended in a vehicle. Here 5% CMC was used as a vehicle. The animals received Diazepam 4mg/kg (tab. Valium, Abbott Healthcare Pvt. Ltd.) which was used as anxiolytic, depressant, skeletal muscle relaxant and Tramadol 25mg/kg (tab. Anatram, Hiral Labs Ltd.) was used as a standard analgesic agent. The standard drugs were dispersed in distilled water containing vehicle. Drugs were given orally in a volume 1ml/100g

body weight of mice and 0.5ml/100g body weight of rat using an oral feeding tube. Fresh drug solutions were prepared on each day of the experiment.

Evaluation of analgesic activity

Hydroethanolic extract of *Solanum anguivi* Lam. roots (henceforth SAHER extract) was evaluated for analgesic activity using Eddy's hot plate method, tail immersion method and Haffners tail clip method.[9]

Selection of dose of the extract

Dose of the extract was selected by acute oral toxicity studies. Prashanta Kr. Deb et al; (2014) and other researchers already conducted acute toxicity studies on *Solanum anguivi*. They reported that *Solanum anguivi* extract was nontoxic up to dose 2000 mg/kg body weight and extract did not produce any mortality**.** Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further studies.[10]

Eddy's hot plate method

Experimental animals of either sex were randomly selected and divided into four groups designated as group-I, group-II and group-III, group IV consisting of four mice in each group for Control (5% CMC. p.o.), Standard (4mg/kg Tramadol, p.o.), and test samples (hydroethanolic extract of 200mg/kg p.o. and 400mg/kg p.o. respectively). The animals were placed on the hot plate heated to 55-56°C and the time until either licking or jumping occurs is recorded by a stop-watch. A cut off period of 15sec was observed to avoid damage to the paw. The reaction times were recorded was recorded before and after 60, 90 and 120 min following oral administration of the standard or the test compound.

Tail immersion method

Experimental animals of either sex were randomly selected and divided into four groups designated as group-I, group-II and group-III, group IV consisting of four mice in each group for Control (5% CMC. p.o.), Standard (4mg/kg Tramadol, p.o.), and test samples (hydroethanolic extract of 200mg/kg p.o. and 400mg/kg p.o. respectively). The lower portions of the tails of the animals were marked. This part of the tail was immersed in a cup of freshly filled water of exactly 55 °C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time was recorded by a stopwatch. After each determination the tail was carefully dried. The reaction time was determined before and periodically before and after 60,

90 and 120 minutes of oral administration of the test substance. A cut off time of 15sec was observed to avoid damage to the tail.

Haffner's tail clip method

Experimental animals of either sex were randomly selected and divided into four groups designated as group-I, group-II and group-III, group IV consisting of four mice in each group for Control (5% CMC. p.o.), Standard (4mg/kg Tramadol, p.o.), and test samples (hydroethanolic extract of 200mg/kg p.o. and 400mg/kg p.o. respectively). An artery clip was applied to the root of the tail of mice and the reaction time was noted. The test compounds were administered orally to fasted animals. An artery clip was applied to the root of the tail (approximately 1 cm from the body) to induce pain. The animal quickly responds to these noxious stimuli by biting the clip or the tail near the location of the clip. The time between stimulation onset and response is measured by a stopwatch. The reaction time is noted before and 60, 90 and 120 minutes after the administration of the test drug. A cut off time of 15sec was observed to avoid damage to the tail.

Statistical analysis

The results were expressed as mean ± SEM. Statistical analyses of all the data obtained were evaluated using one-way Analysis of Variance (ANOVA), followed by Tukey post –hoc multiple comparison test with SPSS Program; Version 20. All the results were also expressed as graph by Graph Pad Prism software (v.5). P values < 0.05 were considered as statistically significant.

RESULTS

Phytochemical screening

The extract revealed the presence of Carbohydrates, Saponins, Alkaloids, Flavonoids, Phenols, Tannins, Steroids & terpenoids (Table 1).

Table 1: Results of Phytochemical screening of hydroethanolic root extract of S. anguivi.

Sl. No	Test	Inference
1	**Carbohydrates**	
a.	Mollisch's test	+
b.	Benedict's test	+
c.	Fehling's test	+
2	**Proteins and aminoacids**	
a.	Biuret test	-
b.	Ninhydrin test	-

3	**Alkaloids**	
a.	Mayer's test	+
b.	Wagner's test	+
c.	Hager's test	+
d.	Dragendorff's test	+
4	**Glycosides**	
a.	Keller kiliani test	-
b.	Legal's test	-
c	Baljet test	-
5	**Flavonoids**	
a.	Alkaline reagent test	+
b.	Lead acetate test	+
c.	Schinoda test	+
6	**Phenols**	
a.	Ferric chloride test	+
7	**Tannins**	
a.	Ferric chloride test	+
b.	Lead acetate test	+
c.	Gelatin test	+
8	**Saponins**	
a.	Froth formation test	+
9	**Steroids and triterpenoids**	
a.	Salkowski's test	+
b.	Liebermann Buchard's test	+

Key: + indicates the presence of constituents

- indicates the absence of constituents

Eddy's hot plate method

The acute treatment of SAHER extract significantly increased the time taken for paw licking and jumping when exposed to heat in mice. The extract at doses of 200mg/kg p.o. and 400 mg/kg p.o. significantly increased the paw licking/ jumping latencies at ***P<0.001. The groups treated with *S. anguivi* extract 400mg/kg showed an increase in paw licking/ jumping latencies than the group treated with 200mg/kg. The responses were taken before and after 60, 90 and 120 minutes of drug administration. The results showed maximum increase in paw licking/ jumping latencies at 120 minutes after the administration of doses. (Table 2).

Table 2: Analgesic activity by Eddy's hot plate method in mice.

Sl. No	Groups	Pre-treatment	Paw licking/ Jumping latency (s)		
			60 minutes	90 minutes	120 minutes
1	Control	4.42±0.16	4.41±0.17	4.38±0.17	4.43±0.17
2	Standard	4.30±0.13	10.30±0.15***	12.37±0.16***	14.27±0.17***
3	Test 200 mg/kg	4.07±0.09	8.05±0.13***	10.11±0.12***	12.22±0.09***
4	Test 400 mg/kg	4.25±0.15	9.39±0.16***	11.35±0.21***	13.49±0.18***

Results are expressed as Mean ± S.E.M. The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA), followed by Tukey. The results were considered statistically significant when *P<0.05, **P<0.01, ***P<0.001 compared with positive control.

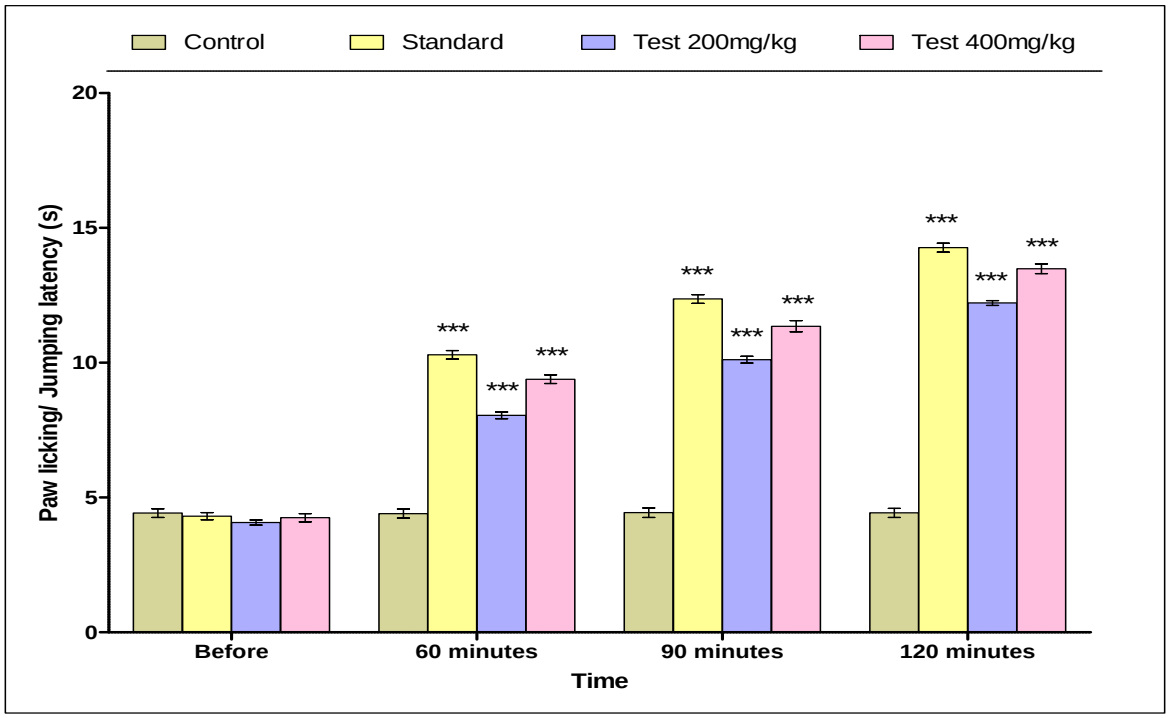

Figure 1: Analgesic activity by Eddy's hot plate method in mice.

Tail immersion method

The acute treatment of *S. anguivi* root extract significantly increased the time taken for tail withdrawal when immersed in hot water (55°C) in mice. The extract at doses of 200mg/kg p.o. and 400 mg/kg p.o. significantly increased the tail withdrawal latencies at ***P<0.001. The groups treated with the extract 400mg/kg showed an increase in tail withdrawal latencies than the group treated with 200mg/kg. The responses were taken before and after 60, 90 and 120 minutes of drug administration. The results showed maximum increase in tail withdrawal latencies at 120 minutes after the administration of doses.

Table 3: Analgesic activity by Tail immersion method in mice.

Sl. No.	Groups	Pre-treatment	Tail withdrawal latency (s)		
			60 Minutes	90 Minutes	120 Minutes
1	Control	6.62±0.43	6.62±0.49	6.68±0.47	6.61±0.48
2	Standard	6.31±0.45	12.43±0.23***	13.74±0.10***	14.78±0.10***
3	Test 200 mg/kg	5.80±0.61	10.09±0.33***	11.84±0.24***	13.70±0.14***
4	Test 400 mg/kg	5.85±0.61	10.39±0.54***	12.39±0.21***	14.36±0.17***

Results are expressed as Mean ± S.E.M. The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA), followed by Tukey. The results were considered statistically significant when (*P<0.05, **P<0.01, ***P<0.001) compared with positive control.

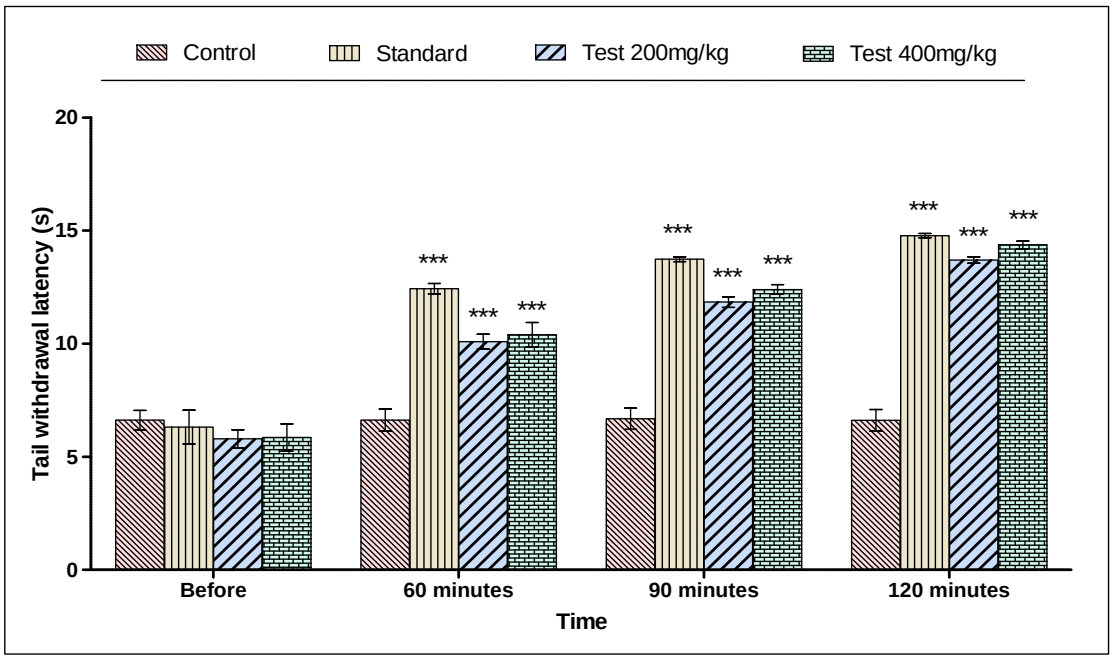

Figure 2: Analgesic activity by Tail immersion method in mice.

Haffner's tail clip method

The acute treatment of *S. anguivi* hydroethanolic root extract significantly increased the latency to attack the clip in mice. The extract at doses of 200mg/kg p.o. and 400 mg/kg p.o. significantly increased the latency to attack clip at ***P<0.001. The groups treated with the extract 400mg/kg showed an increase in latency to attack the clip than the group treated with 200mg/kg. The responses were taken before and after 60, 90 and 120 minutes of drug administration. The results showed maximum increase in latency to attack clip at 120 minutes after the administration of doses.

Table 4: Analgesic activity by Haffner's tail clip method in mice.

Sl. No.	Groups	Pre-treatment	Latency to attack clip (s)		
			60 Minutes	90 Minutes	120 Minutes
1	Control	1.92±0.14	1.97±0.27	1.91±0.15	1.93±0.13
2	Standard	1.87±0.12	6.76±0.34***	8.98±0.16***	10.94±0.14***
3	Test 200 mg/kg	1.94±0.11	4.38±0.36***	6.40±0.20***	9.37±0.21***
4	Test 400 mg/kg	1.94±0.14	5.68±0.23***	7.87±0.09***	10.91±0.06***

Results are expressed as Mean ± S.E.M. The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA), followed by Tukey. The results were considered statistically significant when *P<0.05, **P<0.01, ***P<0.001.Compared with positive Control.

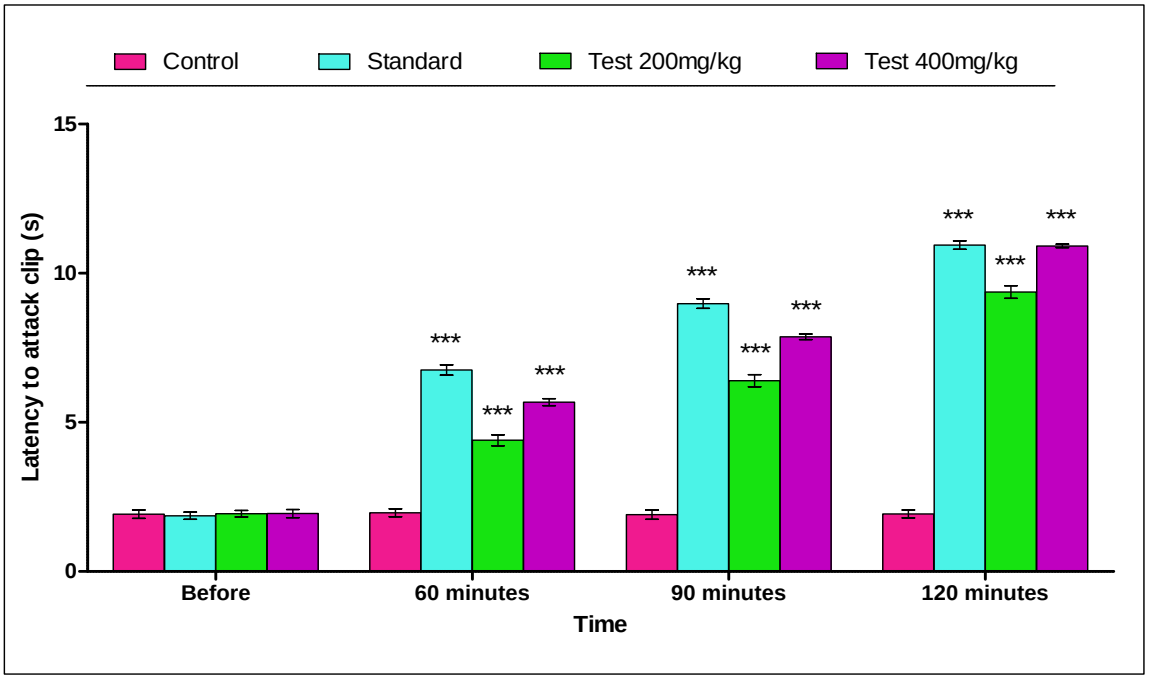

Fig. 15: Analgesic activity by Haffner's tail clip method in mice.

DISCUSSION

In the present study, *S. anguivi* produced analgesic activity in a dose dependent manner and the maximum effect was observed at 400 mg/kg. *Solanum anguivi* root extract protected mice against mechanical and thermal induced noxious stimuli, which were evidenced from their effects on tail-clip, hot-plate and tail immersion tests. Analgesic effect mediated through central mechanism indicates the involvement of endogenous opioids, peptides and biogenic amines like 5HT. In tail-clip, hot-plate and tail immersion studies, the pre-treatments with the test extract significantly increased the reaction time which may indicate central mechanism in analgesic action. The analgesic effect of the extract may be due to the inhibition at the central level of the transmission of painful message.[11] number of flavonoids have been reported to produce analgesic activity. Also, there are few reports on the role of tannins in analgesic activity. Hence, the present analgesic activity of *S. anguivi* may be attributed to the presence of alkaloids, flavonoids and tannins.[12]

CONCLUSION

It can be concluded that *Solanum anguivi* Lam roots contain carbohydrates, saponins, alkaloids, flavonoids, phenols, tannins, steroids & terpenoids which are responsible for these pharmacological effects. The hydroethanolic root extract of *S. anguivi* Lam showed potential analgesic activity in different established models which supports its use in traditional medicine as an analgesic and in the treatment of various nervous disorders. But these findings need to be validated by further studies. It is important to isolate and characterize the active compounds responsible for these activities through advanced techniques.

ACKNOWLEDGEMENTS

The authors are very grateful to KMCT College of Pharmaceutical Sciences, Kozhikode, St Joseph's College of Pharmacy, Cherthala, Sree Narayana College Cherthala, Govt Veterinary College Mannuthy for the collection, identification, authentication and supply and provision of the plant material and facilities to complete this research work.

REFERENCES

1. Li JW, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? Science, 2009; 325(5937): 161–165.
2. Alan LH. Natural products in drug discovery. Drug Discovery Today, 2008; 13(19-20): 894-901.
3. Oyeyemi SD, Ayeni, Adebiyi, Ademiluyi, Tedela, Osuji. Nutritional Quality and Phytochemical Studies of (*Solanum anguivi* Lam.) Fruits. Journal of Natural Sciences Research, 2015; 5(4): ISSN 2225-0921.
4. Elekofehinti OO, Kamdem JP, Kade IJ, Rocha JBT, Adanlawo IG. Hypoglycemic, antiperoxidative and antihyperlipidemic effects of saponins from *Solanum anguivi* Lam. fruits in alloxan-induced diabetic rats. South African Journal of Botany, 2013; 88: 56-61.
5. Elekofehinti OO, Kamdem JP, Bolingon AA, Athayde ML, Lopes SR, Waczuk EP et al. African eggplant (*Solanum anguivi* Lam.) fruit with bioactive polyphenolic compounds exerts *in vitro* antioxidant properties and inhibits Ca^{2+}-induced mitochondrial swelling. Asian Pacific Journal of Tropical Biomedicine, 2013; 3(10): 757–766.
6. Prashanta KD, Ranjib Ghosh, Raja Chakraverty, Rajkumar Debnath, Lakshman Das, Tejendra Bhakta. Phytochemical and Pharmacological Evaluation of Fruits of *Solanum indicum* Linn. International Journal of Pharmaceutical Sciences Review and Research, 2014; 25(2): 28-32.

7. Khandelwal KR. Text book of Practical Pharmacognosy, New Delhi: Nirali Prakashan, 2008; 149-153.
8. Trease GE, Evans WC. Phytochemical screening; A Textbook of Pharmacognosy, 15th edition: London: Bailiere Tindal Limited, 1989; 191: 223,336.
9. Robert Turner A. Screening Methods in Pharmacology. Volume I; New York: Academic Press, 1965; 110-117.
10. Prashanta KD, Ranjib Ghosh, Raja Chakraverty, Rajkumar Debnath, Lakshman Das, Tejendra Bhakta. Phytochemical and Pharmacological Evaluation of Fruits of *Solanum indicum* Linn. International Journal of Pharmaceutical Sciences Review and Research, 2014; 25(2): 28-32.
11. Satoskar RS, Nirmala NR, Bhandarkhar SD. Pharmacology and Pharmacotherapeutics. Revised 23rd edition: Mumbai: Popular Prakashan, 2013; 150-151.
12. Mutalik S, Paridhavi K, Mallikarjuna C, Udupa N. Antipyretic and analgesic effect of leaves of *Solanum melongena* Linn. in rodents. Indian Journal of Pharmacology, 2003; 35(1): 312-315.