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EVALUATION OF ANTINOCICEPTIVE ACTIVITY OF AQUEOUS EXTRACT OF *SAPINDUS TRIFOLIATUS* IN RODENTS

[1]Rohit Dixit, [2]S Ubedulla Iqbal, [3]N Chandrasekhar [4]Prakash M, [3]Shankar Jagan, [5]VS Manohar

[1]Asst Prof, Dept of Pharmacology, Navodaya Medical College, Raichur, Karnataka, India.
[2]Asst Prof, Dept of Pharmacology, Mamata Medical College, Khammam, Andhra Pradesh, India.
[3]Post Graduate, Dept of Clinical Pharmacology, NIMS, Hyderabad, Andhra Pradesh, India.
[4]Post Graduate, Dept of Pharmacology, Mamata Medical College, Khammam, Andhra Pradesh, India.
[5]Pharmacovigilance Physician, Quintiles, Bangalore, Karnataka, India.

ABSTRACT

Aims & Objectives: The aim of the study was to evaluate the antinociceptive activity of aqueous extract of *Sapindus trifoliatus* in albino rats and albino mice. Materials & Methods: Laboratory bred albino mice and albino rats of either sex were used for the study. The animals were maintained under standard laboratory conditions. The study was done using various methods like Haffner's tail clip method in mice, Radiant heat method in rats and 0.6% Acetic acid induced writhing methods in mice. The statistical analysis of data was done using one way analysis of variance (ANOVA) followed by Dunnett's test. P value less than 0.05 was considered to be significant. Results: The test animals treated with Aqueous Extract of *Sapindus trifoliatus* (AEST) have shown an increase in the reaction time at 30mins, 60mins and 90mins post administration compared to pretreatment levels. In the writhing test there was a dose dependent reduction in the number of writhes in the test animals pretreated with aqueous extract of *Sapindus trifoliatus*. Conclusion: The Aqueous extract of *Sapindus trifoliatus* (AEST) has potent antinociceptive activity which was evident in all three pain models used in this study. Further isolation and purification of the crude aqueous extract and phytochemical studies may lead to compounds with potential analgesic activity.

Keywords: Pain models, Analgesic, Pre clinical, Sapindus, Tramadol, New drug.

INTRODUCTION

Western medicine can be traced back to the Greek physician Hippocrates, who believed that diseases had natural causes and used various herbal remedies in his treatments. In India, herbal medicine dates back several thousand years to the Rig-Veda, the collection of Hindu sacred verses. Many of the remedies employed by the herbalists provided effective treatments. It is estimated that 25% of prescriptions written in the U.S. contain plant-derived ingredients (close to 50% if fungal products are included); an even greater percentage are based on semi-synthetic or wholly synthetic ingredients originally isolated from plants. [1] Humans have always sought relief from pain. The act of relieving pain probably is as old as the medical profession itself. Today, the impact of pain on society is still great, and indeed pain is the most common reason for physician consultation. [2] When one thinks of "conventional" pain treatment, one thinks of opioids which have been used historically for millennia, non-steroidal anti-inflammatories and local anaesthetics which have their genesis over one hundred years ago and even the tricyclic antidepressants which are now over 40 years old. With an aging population and patients recovering from previously irrecoverable illness, but with pain sequelae, the need for effective pain treatment has never been greater and yet the fundamental question remains as to whether we have the ability to effectively treat all pain. Even if currently available drugs were effective in all cases, which they are not, the side effects produced by these drugs are frequently unacceptable to the patient. [3] So, the search for an effective analgesic with least side effects still continues. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs.[4, 5] There is a great deal of interest in and support for the search for new and useful drugs from higher plants in many countries. The random collection and broad screening method is a reasonable approach that eventually should produce useful drugs [6-8]. The lack of potent analgesic and anti-inflammatory drugs now actually in use prompted the present study with the aim to evaluate the antinociceptive activity of aqueous extract of *Sapindus trifoliatus* in albino rats and albino

Corresponding Author: **Dr. Rohit Dixit** Email:- rohitdixit.mbbsmd@gmail.com

mice. The objective was to compare the antinociceptive activity of aqueous extract of *Sapindus trifoliatus* with that of control and standard analgesic drugs aspirin and tramadol.

MATERIALS AND METHODS

Animals

Laboratory bred albino mice of either sex, weighing between 18-22g and albino rats of either sex, weighing between 175-250g were used for the study. The animals were maintained under standard laboratory conditions at 25°C, commercial pellet diet with water ad libitum and normal photo period (12hr dark/12hr light). Experimental protocol has been approved by the Institutional Animal Ethics Committee (IAEC).

Drugs and Chemicals: Tab Acetyl salicylic acid, Cap Tramadol and 0.6% solution of acetic acid

Instruments: Modified artery clips and Tail flick analgesymeter – Inco, Ambala, India.

Animal dose calculation from human dose [9]

Aspirin (Human dose=600mg)

- For mice=600x0.0026=1.56/20gm mice=78mg/kg
- For rats=600x0.018=10.8/200gm rat=54mg/kg

Tramadol (Human dose=100mg)

- For mice=100x0.0026=0.26/20gm mice=13mg/kg
- For rats=100x0.018=1.8/200gm rat=9mg/kg

Plant Material and Extraction Procedure

The dried pericarps of the fruits of *Sapindus trifoliatus* were collected from the local market and were authenticated by Professor and Head, Department of Botany, Government Degree College, Khammam. The extract was obtained by Continuous hot percolation process or Soxhlet extraction or Soxhelation [10]

Acute toxicity study and establishment of dose of the extract [11]

According to literature, toxicity studies have already been carried out in female albino rats as per staircase method according to OECD guidelines 425, 2005. There was no mortality, no signs of toxicity and found to be safe up to 2,000 mg/kg/ body weight.

Experimental Design

The following three methods were used to evaluate the antinociceptive activity of Aqueous Extract of *Sapindus trifoliatus* (AEST).

Haffner's Tail Clip Method in mice

In this method, 60 albino mice of either sex were taken for the study and were divided into 6 groups of 10 animals each. A modified artery clip was applied to the root of the mouse tail (approximately 1 cm from the body) and the reaction time in seconds was noted in all the animals. Group 1 (control) received 0.2ml of normal saline. Group 2 and 3 served as standard and were given Aspirin (78 mg/kg) and Tramadol (13 mg/kg) respectively. Group 4, 5 and 6 (Test) were given aqueous extract of *Sapindus trifoliatus* (AEST) in the dose of 25, 50 and 100 mg/kg respectively. Again the test was repeated in all the group of animals after 30, 60 and 90 minutes. Results were tabulated and statistical analysis was done.

Radiant Heat Method in rats (Tail Flick Analgesymeter)

In this test, 60 albino rats of either sex were used and were divided into 6 groups of 10 animals each. The animal was put into a small cage (restrainer) with an opening for the tail at the rear wall. The proximal third of the tail was then exposed to the radiation using analgesymeter. The response of the animal by pulling the tail away or turning the head to one side was noted. Before administration of the test compound or the standard the normal reaction time was determined. Group 1 (control) received 0.2ml of normal saline. Group 2 and 3 (standard) were given Aspirin (54 mg/kg) and Tramadol (9 mg/kg) respectively. Group 4, 5 and 6 (Test) were given aqueous extract of *Sapindus trifoliatus* (AEST) in the dose of 25, 50 and 100 mg/kg respectively. Feeding of the standard drugs and the extract was done using gavage tube. Again the test was repeated and the reaction time was noted in all the group of animals after 30, 60 and 90 minutes. Results were tabulated and statistical analysis was done.

Writhing Tests (0.6% Acetic Acid Induced Writhing In Mice)

In this study, 60 albino mice of either sex were taken for the study and were divided into 6 groups of 10 animals each. All the animals were fasted overnight but were freely accessible to water. All the animals in Group 1 were pretreated with 0.2ml of normal saline, Group 2 and 3 with standard drug Aspirin-78 mg/kg and Tramadol-13 mg/kg respectively and Group 4, 5 and 6 with aqueous extract of *Sapindus trifoliatus* (AEST) in the dose of 25, 50 and 100 mg/kg respectively before injecting acetic acid. 1ml/100g body weight of 0.6 % Acetic acid was injected intraperitoneally which then produced writhing/stretching syndrome. The mice were placed individually into glass beakers and five minutes were allowed to elapse. The mice were then observed for a period of ten minutes and the number of writhes was recorded for each animal. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The results were tabulated and statistical analysis was done.

The percentage inhibition of writhes in the standard and test groups was also calculated using the formula:

Percentage inhibition in standard group animals = (1-Ws/Wc) x 100, where Ws and Wc represent the number of writhes in standard and control groups respectively.

Percentage inhibition in test group animals = (1-Wt/Wc) x 100, where Wt and Wc represent the number of writhes in test and control groups respectively.

Statistical Analysis

The statistical analysis of data was done using one

way analysis of variance (ANOVA) followed by Dunnett's test using the software **"Primer of Biostatistics".** P value less than 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Haffner's tail clip method in mice:

The reaction times after application of modified artery clip to all the mice at various time intervals are represented in table 1.

Cut off time and number of positive responders

Cut off time = Average reaction time

+ 3SD of combined latencies of the control mice at all time periods

Cut off time = 6.4+3x1.3 = 10.3sec

Positive responders are those mice which show a reaction time >10.3sec and are represented in graph 2.

Radiant Heat Method in rats (Tail Flick Analgesymeter)

The reaction times after exposing the rat's tail to radiation using analgesymeter at various time intervals are represented in table 2.

Cut off time and number of positive responders

Cut off time = Average reaction time

+ 3SD of combined latencies of the control rats at all time periods

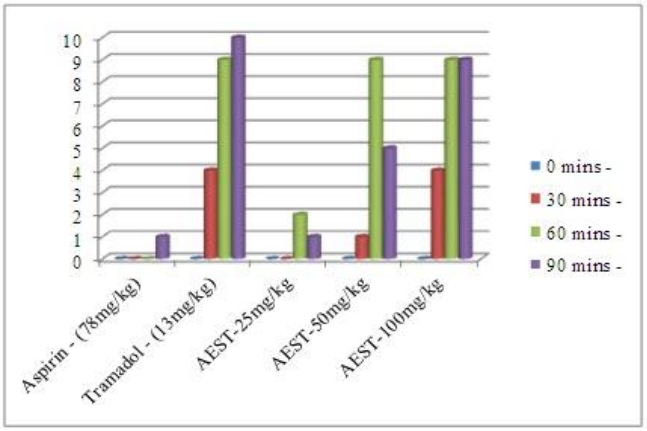
Cut off time = 6.15+3x0.29 = 7.1sec

Positive responders are those rats which show a reaction time >7.1sec

Writhing Tests (0.6% Acetic Acid Induced Writhing In Mice)

In this method the average number of writhes a rat develops after injecting acetic acid i.p was counted in all the groups and is shown in table 3.

Graph 1. Number of mice showing positive response in tail clip method at various time intervals in different groups.



Graph 2. Number of rats showing positive response in tail flick method at various time intervals in different groups.

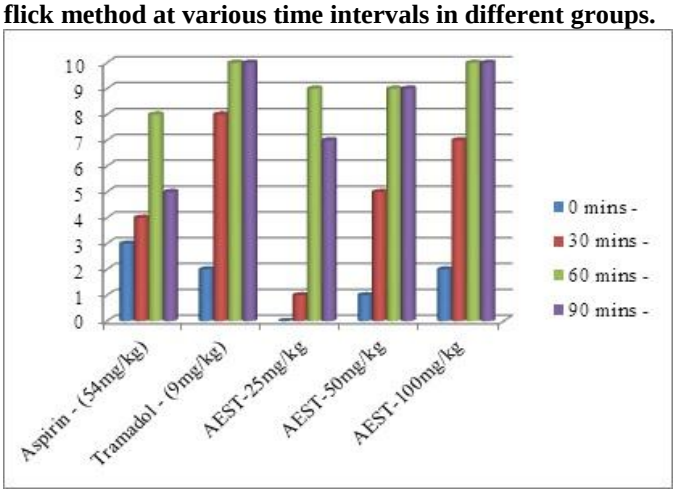


Table 1. Effect of aqueous extract of *Sapindus trifoliatus* (AEST) in tail clip method in albino mice

Group	Reaction times in seconds at various time intervals			
	0 minute	30 minute	60 minute	90 minute
Control	6.2±1.31	6.3±1.34	6.6±1.17	6.5±1.51
Aspirin - (78mg/kg)	6.4±1.43	7±0.94$^{\#}$	7.9±0.88*	9.2±0.92$^{\#}$
Tramadol - (13mg/kg)	6.1±1.29	10.6±1.07$^{\#}$	13.5±2.22$^{\#}$	20.1±2.13$^{\#}$
AEST-25mg/kg	6.2±1.32	7.8±1.32*	9.2±1.55$^{\#}$	8.5±1.27*
AEST-50mg/kg	6.4±1.07	9.2±1.14$^{\#}$	12.3±1.34$^{\#}$	10.3±1.27$^{\#}$
AEST-100mg/kg	5.9±0.99	10.5±1.51$^{\#}$	13.8±2.20$^{\#}$	12.7±1.89$^{\#}$

All values are expressed as Mean±SEM, n=10, *P<0.05, #P<0.001

Table 2. Effect of aqueous extract of *Sapindus trifoliatus* (AEST) in tail flick method in albino rats

Groups	Reaction times in seconds at various time intervals			
	0 mins	30 mins	60 mins	90 mins
Control	5.7±1.34	6.1±0.74	5.9±1.11	5.9±1.11
Aspirin - (54mg/kg)	6.3±1.64	7.1±1.38	8.3±1.49$^{\#}$	7.1±1.45*
Tramadol - (9mg/kg)	6.2±1.32	8.3±1.70*	16.2±2.35$^{\#}$	19.1±1.73$^{\#}$
AEST-25mg/kg	6±1.05	6.4±0.84*	8.7±0.95$^{\#}$	7.8±0.79$^{\#}$
AEST-50mg/kg	6.3±0.95	7.2±1.14*	10.5±2.32$^{\#}$	9.4±2.01$^{\#}$
AEST-100mg/kg	6.4±1.07	7.7±1.06	12.6±2.18$^{\#}$	11.1±1.06$^{\#}$

All values are expressed as Mean±SEM, n=10, *P<0.05, #P<0.001

Table 3. Effect of aqueous extract of *Sapindus trifoliatus* (AEST) in acetic acid induced writhing test in albino mice

Groups	Average number of writhes	Percent inhibition (%)
Control	25±3.53	-
Aspirin - (78mg/kg)	7.1±4.20#	71.6
Tramadol - (13mg/kg)	9.5±6.36*	62
ST-25mg/kg	13.2±2.78*	47.2
ST-50mg/kg	10.8±4.83*	56.8
ST-100mg/kg	7.7±6.25#	69.2

Values are Mean±SEM, n=10, *<0.05, #<0.01

DISCUSSION

Any injury or tissue damage is associated with pain and inflammation. Analgesics can act on peripheral or central nervous system. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptors site of pain, while centrally acting analgesics not only raise the threshold for pain, but also alter the physiological response to pain [12]. The current study was done to evaluate the antinociceptive activity of aqueous extract of *Sapindus trifoliatus* (AEST) using various methods like Haffner's tail clip method in mice, Radiant heat method in rats and 0.6% Acetic acid induced writhing methods in mice.

Haffner's Tail Clip Method in Mice

In this method, the average reaction time in all the mice was between 5-7seconds. The test animals treated with Aqueous Extract of *Sapindus trifoliatus* (AEST) have shown an increase in the reaction time at 30mins, 60mins and 90mins post administration compared to pretreatment levels. Further this increase was dose dependent and it was highly significant (P<0.001) when compared to the control group indicating antinociceptive activity of AEST. The maximum increase in the reaction time was seen with 100mg/kg dose at 60mins which was 13.8±2.20 seconds. In the standard Group (2 and 3) the increase in the reaction time was seen only in those animals treated with tramadol and not with aspirin. The reason being, the tail flick method is used only to evaluate analgesics acting through central mechanism (Tramadol). [13] Maximum increase in the reaction time in animals treated with tramadol (13mg/kg) was seen at 90mins which was 20.1±2.13seconds (P<0.001). Since the test animals treated with AEST has shown a positive response in this test, we can conclude that the AEST has central mechanism of antinociceptive activity similar to tramadol.

The Radiant Heat Method in Rats (Tail Flick Analgesymeter)

The average reaction time in all the rats was between 5-7seconds. The rats treated with AEST have shown a highly significant (P<0.001) increase in the reaction time compared to the control group. Maximum increase in the reaction time in these animals treated with AEST was at 100mg/kg dose and at 60mins which was 12.6±2.18seconds, whereas the animals treated with tramadol have shown still higher reaction time of 19.1±1.73seconds which was seen at 90mins post administration. Since the Radiant heat method is very effective to estimate the efficacy and potency of centrally acting analgesics, [13] we can conclude that AEST has an antinociceptive activity similar to tramadol (i.e. Central mechanism).

Writhing Tests (0.6% Acetic Acid Induced Writhing in Mice)

In this test, the average number of writhes in control group was 25±3.53. The mice pretreated with 25mg/kg, 50mg and 100mg/kg of AEST produced 13.2±2.78, 10.8±4.83, 7.7±6.25 writhes respectively. All these were significant (P<0.05) when compared to the control group. We can see that there was a dose dependent reduction in the number of writhes in the test animals pretreated with AEST. The percentage inhibition in number of writhes was 47.2%, 56.8% and 69.2% in the animals pretreated with 25mg/kg, 50mg and 100mg/kg of AEST respectively. The mice pretreated with Aspirin and Tramadol have also shown reduced number of writhes which was 7.1±4.20 and 9.5±6.36 respectively. But the percentage inhibition was maximum with Aspirin (71.6%) compared to Tramadol (62%). Since this test detects both centrally and peripherally acting analgesics, [14] we can conclude that AEST has both peripheral and central mechanisms of antinociception.

Acetic acid causes inflammatory pain by increasing capillary permeability and liberating endogenous substances that excite pain nerve ending. Acetic acid is also known to increase PGE_1 and PGE_2 peripherally. [15] NSAIDs can inhibit COX in peripheral tissues and therefore interfere with the mechanism of transduction of primary afferent nociceptors. [15] The mechanism of antinociceptive activity of AEST could be probably attributed to the blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of Aspirin and other NSAIDs. Thus, the reduction in the number of writhing indicates that AEST might exert antinociceptive activity by inhibition of prostaglandin synthesis or action of prostaglandins.

Sapindus trifoliatus is a rich source of saponins. It contains saponin A, saponin C, sapindosid A, sapindosid B and hederagin 3-o - acetyl-beta–D-xylose [16,17]. Further, the saponins are known to have analgesic, anti-inflammatory and anti-rheumatic effect [18-20]. It is therefore probable that the saponin component of the extract may contribute in part for the observed antinociceptive activity.

CONCLUSION

The results of this study indicate that the Aqueous

extract of *Sapindus trifoliatus* (AEST) has potent antinociceptive activity which was evident in all three pain models used in this study namely, Haffner's tail clip method, Radiant Heat Method (Tail Flick Analgesymeter) and 0.6% Acetic acid induced writhing test. The AEST elicits antinociceptive activity by both peripheral and central mechanisms which are comparable to Aspirin and Tramadol. Further isolation and purification of the crude aqueous extract and phytochemical studies may lead to compounds with potential analgesic activity.

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