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Research Article

PHARMACOLOGICAL EVALUATION OF CENTRAL ANALGESIC ACTIVITY OF *ACACIA CATECHU* BY HOT PLATE AND TAIL IMMERSION METHOD

VIMLA AHIRWAR*, MANOJ KUMAR SAHU, LOKESH VERMA, JITENDRA BANWEER

Department of Pharmacy, Sagar Institute of Research and Technology-Pharmacy, Bhopal, Madhya Pradesh, India.
Email: vimlaahirwar1998@gmail.com

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ABSTRACT

Objective: The aim of the study was to evaluate the analgesic activity of *Acacia catechu* heartwood extracts on Swiss albino mice using hot plate and tail immersion method.

Methods: In this study, we used the aqueous and methanol extracts of heartwood of *A. catechu* to evaluate its analgesic activity. The aqueous extract was achieved by doubled distillation method and the methanol extract was achieved by cold maceration method. The mice were divided into four groups, each group consisting of six animals. Doses of 50, 100, and 300 mg/kg, p.o. of both the extracts of *A. catechu* were given to the mice. The responses were noted at 0, 15, 30, and 60 min.

Results: The results of both the methods were quick similar but differ based on extracts. The aqueous extract of *A. catechu* showed a much better yield of analgesic activity as compared to the methanol extract. The data were analyzed by graph using two-way analysis of variance, which showed the dose-dependent pain resistance.

Conclusion: The study confirmed the potency of *A. catechu* in pain management and points the future development to be conducted on it.

Keywords: *Acacia catechu*, Analgesic, Hot plate, Tail immersion, Mice.

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INTRODUCTION

From ancient time, India is the center of all the herbal medicinal plants. The herbal medicinal plants have the potency to cure various types of health problems such as – cancer, heart diseases, diarrhea, and constipation, various infectious diseases, acute and chronic pain, etc. *Acacia catechu* is one of the medicinal plants that bring various chemical constituents, which proved to exist in different pharmacological activities. *A. catechu* belongs to the family- Leguminosae. In the local language, it is known as Babool, and its resin part is called "kattha" which has been used with betel by people from ancient times. The aqueous extract of heartwood of catechu is prepared by boiling chips of heartwood to form a dark brownish-colored product called "kattha."

Various studies have been reported on the catechu which makes it important as a medicinal plant. The other species of *Acacia* like *Acacia hydaspica* showed antipyretic, anti-inflammatory, and analgesic activity [1,2]. The study reported the anti-microbial activity of the resin part of *A. catechu* [3,4]. The chief chemical constituent of *A. catechu* is (+) - catechin, which is a polyphenol derivative [5]. The heartwood extract of *A. catechu* has been reported to have anti-oxidant, anti-inflammatory, and chemoprotective properties [6], anti-oxidant [7] and anti-diarrheal [8], hepatoprotective [9,10], anti-cancer [11,12], hypoglycemic [13,14], osteoarthritis [15,16], and leprosy [17], it is effective to trigger apoptosis [18,19]. The study of anti-hyperglycemic and anti-nociceptive activity of wood *A. catechu* was carried out by Rahmatullah *et al*. 2013 [20], the study was carried out on acetic acid-induced gastric pain models. Yimam *et al*., in 2012 [21], studied the analgesic effects of *Scutellaria baicalensis* and *A. catechu* in a composition. However, no single study has been carried out on the *A. catechu* for the analgesic activity, that is, why we carried out this study.

METHODS

Collection of the plant material

The kattha or catechu was taken from the local market during May and identified by the botanist. The catechu was powdered, sieved, and stored in an airtight container for further use.

Preparation of extracts

Aqueous extraction

For aqueous extraction, doubled distillation method (Patel *et al*., 2009) was used; 50 g of powered catechu was placed in 1000 ml round bottom flask and 500 ml distilled water added into it. It was subjected to boiling until the total volume of water was reduced to one-fourth. The extract was filtered through a filter paper, cooled, and transferred to screw-capped glass bottles.

Methanol extraction

The methanol extract was achieved by cold maceration method; 10 g. of powered catechu was dissolved in methanol for 24 h and then filtered through the Whatman filter paper. The extract was concentrated near to dryness by a rotary evaporator. For the experimentation, this was diluted with the solvent.

Drugs and chemicals

Diclofenac sodium was taken from the local medical store and other solvents and chemicals were taken from the institute. All the chemicals were taken from the store of SIRT-Pharmacy, which were the laboratory-grade chemicals used in the study.

Ethical clearance

The study protocols of ethical clearance were approved by the Institutional Animal Ethics committee of the institute before the study was carried out on animals.

Animals

All experiments were carried out on male/female Swiss albino mice (20–25 g). The animals were housed in plastic cages (n=6) in an animal room maintained at 23±2°C for 12 h light/dark cycle (light on 07:00–19:00 h). Food and water were available at all times except during the experiments [22].

Animal experimental design

Central analgesic activity

Hot plate method

Albino mice of either sex (n=6) weighing 20–25 g were used. The animals were pretested on a hot plate, where the temperature was maintained at 52±0.1°C. During pretesting, animals on a hot plate those having a latency time of more than 15 s were rejected. All the animals divided randomly into four groups were each group containing six mice. Group I called the control group was treated with saline (10 ml/kg), Group II was treated with diclofenac sodium as a standard drug (15 mg/kg, i.p.), Group III was treated with oral doses of 50, 100, and 300 mg/kg, p.o. of aqueous extract of *A. catechu,* and Group IV treated with oral doses of 50, 100, and 300 mg/kg, p.o. of methanol extract of *A. catechu,* respectively. After 0, 15, 30, and 60 min of drug treatment, mice were kept on the hot plate in the cylinder and the latency time (without jumping and licking or flicking of hind limb the mice stayed on the hot plate) was recorded. For all animals, a cutoff time of 45 s was fixed to avoid any tissue injury. Using this formula percent, analgesia was calculated,

$$\%\text{Analgesia} = \frac{(\text{Test latency} - \text{control latency})}{(\text{Cut off time} - \text{control latency})} \times 100$$

Tail immersion test

Albino mice of either sex (n=6) weighing 20–25 g were used. All the animals divided randomly into four groups were each group containing six mice. Group, which I called the control group was treated with saline (10 ml/kg), Group II was treated with diclofenac sodium as a standard drug (15 mg/kg, i.p.), Group III was treated with oral doses of 50, 100, and 300 mg/kg, p.o. of aqueous extract of *A. catechu,* and Group IV treated with oral doses of 50, 100, and 300 mg/kg, p.o. of methanol extract of *A. catechu,* respectively. After 0, 15, 30, and 60 min of drug treatment, each mice was placed in a holder with its tail protruding. The tail was placed in a water bath at 55°C until the tail whipped or a flinch of the whole body occurred, and a cutoff time of 7 s was imposed. The latency time of the reaction was recorded for each group after treatment with the drug. Percent analgesia was calculated using the following formula-

$$\%\text{antinociception} = \frac{T - C}{7 - C} \times 100$$

Statistical analysis

All the data obtained from both the hot plate and tail immersion test were analyzed by the graph pad prism by two-way analysis of variance (ANOVA) using Bonferroni multiple comparison test. p≤0.05 was taken as significant.

RESULTS

Preliminary phytochemical studies revealed the presence of alkaloids, carbohydrates, tannins, phenols, saponins, triterpenoids, and steroids in *A. catechu*. The *A. catechu* heartwood extracts (both water and methanol extract) were found to be non-toxic when administered orally to the Swiss albino mice at doses in the range of 50–500 mg/kg, p.o. and the LD50 was found to be safe at the highest dose. The mice treated with *A. catechu* had significantly (p<0.05) reduced pain-related indices compared to the saline-treated group.

Effects of aqueous extract of *A. catechu* by hot plate method

In this study, *A. catechu heartwood aqueous extract* showed a dose-dependent increase in latency time of paw licking and blocked the pain sensation in a way similar to standard drug; diclofenac sodium (15 mg/kg, i.p.), whereas the effect of the extract was shown at 50, 100, and 300 mg/kg, p.o. The mice treated with *A. catechu* had significantly (p<0.05) reduced pain-related indices compared to the saline-treated group. Two-way ANOVA revealed that administration of aqueous extract significantly affects the paw licking time as compared to the saline-treated group on the hot plate [paw licking (F 3,15)=p<0.0001]. *Post hoc* indicates that aqueous extract (50, 100, and 300 mg/kg, p.o.) significantly increases paw licking time (p<0.05) as compared to the saline-treated group. These results showed in Fig. 1.

Effects of aqueous extract of *A. catechu* by tail immersion test

The aqueous extract *of A. catechu* heartwood showed a dose-dependent increase in latency time of tail flicking and blocked the pain sensation in a way similar to standard drug; diclofenac sodium(15 mg/kg, i.p.). The effect showed at 50, 100, and 300 mg/kg, p.o. The mice treated with *A. catechu* had significantly (p<0.05) reduced pain-related indices compared to the saline-treated group. Two-way ANOVA revealed that administration of aqueous extract significantly affects the tail flicking time as compared to the saline-treated group on the tail immersion test [tail flicking (F 4,20)=p<0.0001]. *Post hoc* indicates that aqueous extract (50, 100, and 300 mg/kg, p.o.) significantly increases tail flicking time (p<0.05) as compared to the saline-treated group. These results showed in Fig. 2.

Effects of methanol extract of *A. catechu* by hot plate test

In this study, *A. catechu* heartwood *methanol extract* exhibited a dose-dependent increase in latency time of paw licking and blocked pain sensation in a way similar to standard drug; diclofenac sodium (15 mg/kg, i.p.), while the effect of the extract was shown at 50, 100, and 300 mg/kg, p.o. Two-way ANOVA revealed that administration of methanol extracts significantly affects the paw licking time as compared to the saline-treated group on the hot plate [paw licking (F 3,13)=p<0.0001]. *Post hoc* indicates that methanol extract (50, 100, and 300 mg/kg, p.o.) significantly increases paw licking time (p<0.05) as compared to the saline-treated group. These results showed in Fig. 3.

Effects of *A. catechu methanol extract* by tail immersion test

The methanol extracts *of A. catechu* heartwood showed a dose-dependent increase in latency time of tail flicking and blocked the pain sensation in a way similar to standard drug; diclofenac sodium(15 mg/kg, i.p.). The effect showed at 50, 100, and 300 mg/kg, p.o. The mice treated with *A. catechu* had significantly (p<0.05) reduced pain-related indices compared to the saline-treated group. Two-way ANOVA revealed that administration of methanol extract significantly affects the tail flicking time as compared to the saline-treated group on the tail immersion test [tail flicking (F 4,18)=p<0.0001]. *Post hoc* indicates that methanol extract (50, 100, and 300 mg/kg, p.o.) significantly increases tail flicking time (p<0.05) as compared to the saline-treated group. These results showed in Fig. 4.

DISCUSSION

The present study was performed to evaluate the analgesic activity and therapeutic potency of *A. catechu*. To evaluate the analgesic activity, hot plate and tail immersion test models were used on Swiss albino mice. Both the aqueous and methanol extracts of *A. catechu* showed significant efficacy in these models. The hot plate method is a convenient and easy method that involves the paw licking and jumping of mice (Afsar *et al*., 2015). Doses of 50, 100, and 300 mg/kg, p.o. of both the extracts of *A. catechu* were given to the mice. Only one animal is treated for one dose, no mice were used for multiple doses. The effect of the test was analyzed by the graph by two-way ANOVA.

The tail immersion test involved the flicking of the tail of mice. The tail of mice is very sensitive to heat; the time of tail flicking before and after the treatment of mice with standard drug and plant extract was recorded as the pain resistance time in the second [23]. The doses of both the aqueous and methanol extracts of *A. catechu* were given at doses of 50, 100, and 300 mg/kg, p.o. A similar result was found in the

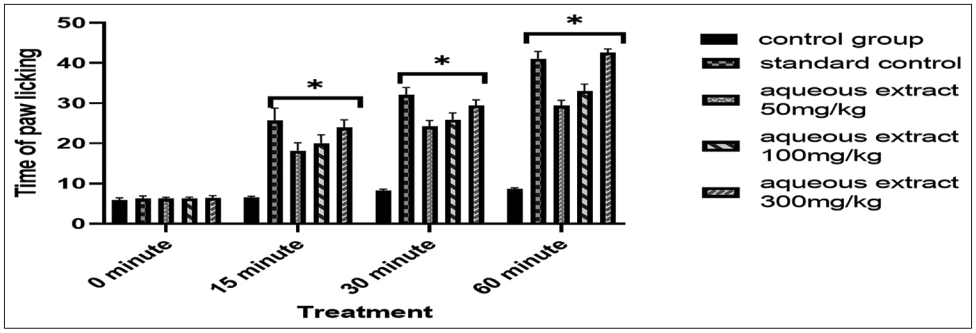

Fig. 1: Effects of *A. catechu heartwood aqueous extracts* on pain in hot plate test. Different groups (n=6) of animals by administered with saline (10 ml/kg, i.p.) or aqueous extract for 0, 15, 30, and 60 min. These animals were subjected to a hot plate test for 45 s and pain-related indices were measured, each representing mean±SEM of 6 mice in each group p*<0.05 versus control [Two-way ANOVA] followed by the Bonferroni comparison test

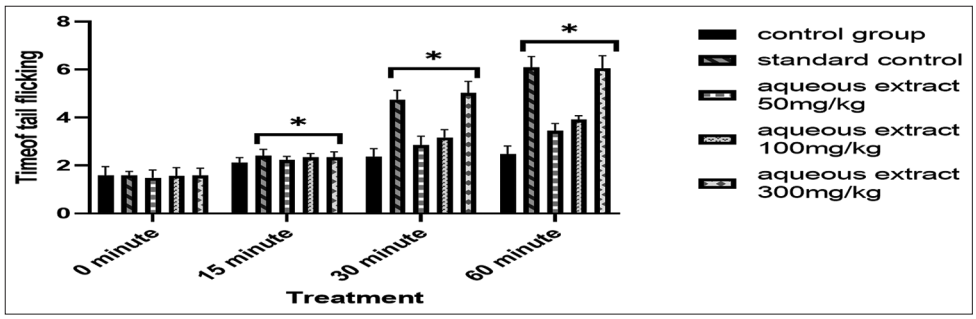

Fig. 2: Effect of *A. catechu heartwood aqueous extracts*, on pain in tail immersion test. Different groups (n=6) of animals by administered with saline (10 ml/kg, i.p.) or aqueous extract for 0, 15, 30, and 60 min. These animals were subjected to a tail immersion test for 7 s and pain-related indices were measured, each representing mean±SEM of six mice in each group p*<0.05 versus control (Two way ANOVA) followed by the Bonferroni comparison test

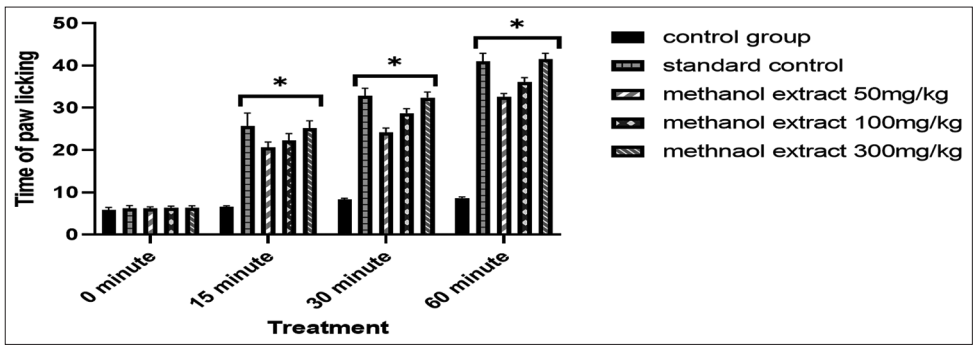

Fig. 3: Effects of *A. catechu heartwood methanol extract*, on pain in hot plate test. Different groups (n=6) of animals by administered with saline (10 ml/kg, i.p.) or methanol extract for 0, 15, 30, and 60 min. These animals were subjected to a hot plate test for 45 s and pain-related indices were measured, each representing mean±SEM of six mice in each group p*<0.05 versus control [Two-way ANOVA] followed by the Bonferroni comparison test

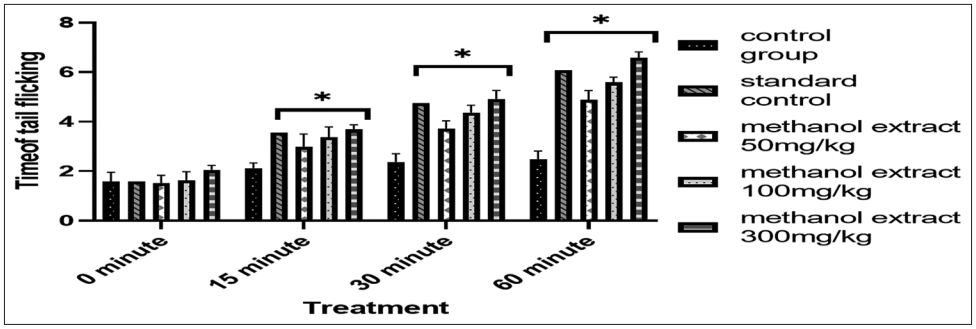

Fig. 4: Effects of *A. catechu heartwood methanol extract*, on pain in tail immersion test. Different groups (n=6) of animals by administered with saline (10 ml/kg, i.p.) or methanol extract for 0, 15, 30, and 60 min. These animals were subjected to a tail immersion test for 7 s and pain-related indices were measured, each representing mean±SEM of 6 mice in each group p*<0.05 versus control (Two way ANOVA) followed by the Bonferroni comparison test

tail immersion test as shown in the hot plate test, and the data were analyzed by graph using two-way ANOVA, which showed the dose-dependent pain resistance. The study showed the significant effect of the *A. catechu* and points the future development to be conducted on it.

CONCLUSION

The study involved the investigation of analgesic activity of different extracts of *A. catechu*, using Swiss albino mice. The aqueous extract was achieved by the doubled distillation method, while the methanol extract was achieved by the cold maceration process. Hot plate and tail immersion tests were used to evaluate the analgesic activity of these extracts. Diclofenac sodium was used as a standard drug which is a known pain-resisting drug that belongs to the class of NSAIDs. To conclude, the analgesic activity of *A. catechu* analyzed by two-way ANOVA showed a dose-dependent effect on pain resistance and has significant pain resisting properties. Several pharmacological activities of *A. catechu* have already been reported, but the analgesic study is not conducted alone on *A. catechu* using these models. The study concluded that the plant has a potent analgesic activity that makes its value a safe remedy in pain management.

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AUTHORS' CONTRIBUTIONS

Supervised and conceived the experimental design: Manoj Sahu. Performed the experiments: VimlaAhirwar. Analysis of data and result: Dr. LokeshVerma, VimlaAhirwaar. Wrote the manuscript: Vimla Ahirwar, with some contribution from Dr. Jitendra Banweer.

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Nil.

CONFLICTS OF INTEREST

The authors do not have conflicts of interest.

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