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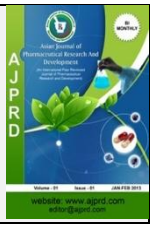

Open Access Research Article

Acute Toxicity Study and Anti-Nociceptive Activity of Ethanol Extract of *Aesculus Indica* Seeds on Experimental Animal Models

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A B S T R A C T

Aesculus indica, widely known as the horse chestnut tree, has long been used as antiangiogenic, antibacterial, antidiabetic, antiviral and antifungal. Ttraditionally it has been used as medicine for the treatment of skin diseases, rheumatism and different pain conditions. The current study was undertaken to investigate possible effects of ethanol extract of seeds of plant in experimentally produced pain in animals because there were no scientific publications on the use of *Aesculus indica* seeds for anti-nociceptive activity. Preliminary phytochemical screening revealed the presence of flavonoids, tannins, carbohydrates, Saponins, and phenolic substances in the extract. The OECD guideline 423 was followed for acute toxicity testing.At a dose of 2000 mg/kg, the extract was confirmed to be safe. The anti-nociceptive effect of three distinct dose levels of extract (100, 200, and 300 mg/kg) was tested in Swiss albino mice using a hot plate, tail immersion test, and acetic acid induced writhing. Extract had strong anti-nociceptive efficacy ($P < 0.001$) in a hot plate test. The extract significantly increased the tail withdrawal reaction in the tail immersion test ($P < 0.001$).The extract considerably reduced the number of writhes in the acetic acid writhing test ($P < 0.001$). The findings indicate that the extract has substantial anti-nociceptive effect.

Key Words-Anti-nociceptive, Acute toxicity, *Aesculus indica*, Phytochemical screening.

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INTRODUCTION

Pain is more than just a vexing sensation; it is a complicated sensory mechanism. Pain is caused by the activation of nociceptors at peripheral nerve terminals in response to tissue injury, which results in the release of a range of chemicals that change the local environment and cause pain.[1] Many pathological aches progress due to inflammatory responses in the central and peripheral nerve systems.[2] When nociceptive signals from the peripheral nerve system to the brain are regulated, pain is usually felt. In reaction to tissue injury or stimuli, a variety of chemicals such as histamine, bradykinin, and prostaglandins are released, resulting in nociception.[3]Also implicated in nociception are endogenous opioid and cannabinoid receptors[1]. Treatment of pain in chronic inflammatory disorders such as rheumatoid arthritis is a major issue for clinicians and the general public, as chronic use of current synthetic medications has dangerous side effects that cannot be ruled out. This needs the creation of a new nociceptive agent that is both safe and effective in eradicating or minimising unwanted effects. Herbal medications are used in several developing countries, despite the fact that they are not documented in science. Traditional folk remedies are used by about 80% of the population in some developing nations.[4]Pain has traditionally been treated using a variety of herbal medicines. Phytoconstituents have been scientifically verified for anti-nociceptive action.[5-8]The seeds of *Aesculus indica* (Family –Sapindaceae) arerich in saponins most specifically aescin.[9] Aescin has been reported to have anti-nociceptive activity via inhibition of oxidative stress and inhibition of prostaglandins.[10] Additionally with saponins*Aesculus indica*also contains flavonoids and tannins it may show anti-nociceptive activity. Moreover Anti-nociceptive activity forextract of *Aesculus indica* leaves has been reported in the literature.[11] Hence the

present research work was aimed to evaluate anti-nociceptive activity for ethanol extract of *Aesculus indica* seeds in experimental animal models.

MATERIALS AND METHODS

Drugs and reagents

Aspirin was purchased from USV Pharma, Mumbai, India and pentazocine was purchased from Themis medicare Ltd., Haridwar, India. All chemicals and reagents used for the experiments were of analytical grades.

Collection and authentication of plant material

Seeds of the *Aesculus indica* were procured from Royal Rifco Company, Shrinagar, India.After collection seeds were cleaned, washed to remove any dirt, dust and foreign particles. Botanical identity of plant specimen was authenticated by Dr. S. A. Mohite,Head, Department of Botany, Lal Bahadur Shastri College, Satara (MS), India.A voucher specimen of theseeds has been deposited in the department for future reference.Theseeds were coarsely powdered and further utilized for preparation ofethanol extract.

Preparation of ethanol extract

The ethanol extraction of seeds of *Aesculus indica* was carried out by soxhlet apparatus. The seeds were crushed and ground to powder and placed into extractor. The ethanol was poured on powder with three cycles. After that extraction process was started and continued till appearance of solvent in syphon tube turns brown to clear. Then brown colored solvent mixture from round bottom flask was collected and evaporated with the help of rotary evaporator to get a solid residue. The residue was placed in a vacuum desiccator and was further used for the experiments.

Prelminary phytochemical screening:

Prepared ethanol extract was subjected to preliminary phytochemical screening for presence of Alkaloids, Glycosides, Carbohydrates, Phenolic compounds, Flavonoids, Saponins, Reducing sugars.[12,13]

Experimental animals

Swiss albino mice (18–25 g) were provided byYashoda Technical Campus, Faculty of Pharmacy, Wadhe. Satara. Animals were housed and maintained according to standard guideline and procedures with animal facility at relative humidity 75 ± 5% temperature 22 ± 2° C, and a 12 h light/dark cycle. Animals were provided with standard diet and purified water ad libitum. Mice were allowed to acclimatize to the environment for seven days before start of the animal study. The experimental protocol was approved by Institutional Animal Ethics Committee. All the animal experimental procedures were performed according to the National Institutes of Health (NIH) guidelines on handling of experimental animals.

Acute oral toxicity study

The acute oral toxicity was performed as per the Organization for economic co-operation and development (OECD) guideline 423.[14]Acute toxicity study was performed in Swiss albino mice. The animals were grouped with three numbers in each. Ethanol extract of *Aesculus indica* seeds was given to animals with starting dose 300mg/kg in 0.1% CMC for first group.

According to observations of first group, study was carried out further on next group with dose 2000 mg/kg. From obtained results it was clear that no death as well as no toxicological signs in animals so, for confirmation of safety of extract study was repeated with dose 2000mg/kg on third group. After administration of extract, animals were observed carefully for first 30 min. and periodically for 24 h with special attention during first four hours. Animals were further observed daily for subsequent 14 days. Effects such as changes in skin fur, eyes and mucous membranes were observed daily. Also the circulatory, autonomic, respiratory, and central nervous systems, behaviour pattern and somatomotor activities were observed during study. Animals were further observed for salivation, diarrhea, tremors, lethargy, convulsions, sleep, and coma. The parameters like body weight, food, and water intake were checked periodically every two days.

Anti-nociceptive activity:

The anti-nociceptive activity of ethanol extract was tested using different animal models namely hot plate, acetic acid induced writhing and tail immersion test. Doses of extract were selected based on results of acute toxicity study.

Healthy Swiss albino mice (18–22 g) were used for the study. Animals were divided into five groups of six in each. Group I was control and received 0.1% CMC, group II was received standard drug, group III, IV and V were received ethanol extract of *Aesculus indica* seeds with low, medium and high dose by oral route.

Evaluation parameters

Tests were performed on same animals after 14 days washing period.

Hot plate test

Analgesic activity in mice was executed according to the method described previously.[15,16] The hot plate analgesiometer (IITC, USA) was used to determine the analgesic activity of ethanol extract of *Aesculus indica* seeds. Animals were divided into five groups of six in each. Group I received vehicle i.e 0.1% CMC by oral route, group II received standard drug pentazocine 17 mg/kg by intraperitoneal route, group III, IV and V were received ethanol extract of *Aesculus indica* seeds 100mg/kg, 200mg/kg and 300mg/kg respectively in 0.1% CMC solution by oral route.

Animals were placed on hot plate at different time points (0, 15, 30, 45, 60, 90 and 120 min) after administration of standard drug pentazocin, extract and vehicle, time require for first response (flickering or licking of hind paw or jumping) has been measured. Hot plate was maintained at 55 ± 0.5 °C and cutoff time fixed for 15 sec. to avoid tissue damage.

Acetic acid induced writhing test

The acetic acid induced writhing test was performed as described previously using 0.1 ml of 0.6% v/v acetic acid solution in normal saline.[17,18] Swiss albino mice (18–22 g) of either sex were divided into five groups of six in each.

Group I received vehicle i.e 0.1% CMC by oral route, group II received standard drug Aspirin 30 mg/kg by oral route, group III, IV and V received ethanol extract of *Aesculus indica* seeds 100mg/kg, 200mg/kg and 300mg/kg respectively in 0.1% CMC solution by oral route. Thirty minutes after administration of standard drug Aspirin and test extracts, 0.1 ml of 0.6% acetic acid were administer via intra-peritoneal route. The number of writhing will be counted for 20 minutes after administration of acetic acid. The percentage inhibition of writhing has been calculated.

percentage inhibition of writhing = (C – T / T) x 100

Where C- Average number of writhes in control group.

T- Average number of writhes in test group.

Tail immersion test

The tail immersion test was carried out in Swiss albino mice (18–22 g) according to method described by previous researchers.[19,20]Animals were divided into five groups of six in each. Group I received vehicle i.e 0.1% CMC by oral route, group II received standard drug pentazocine 17 mg/kg by intraperitoneal route, group III, IV and V were received ethanol extract of *Aesculus indica* seeds 100mg/kg, 200mg/kg and 300mg/kg respectively in 0.1% CMC solution by oral route. Animals were adapted for restrainer 30 min. before study leaving the tail hanging out freely. After administration of standard drug pentazocine, extract and vehicle, the tail immersed in hot water (Temperature 55 ± 0.5° C) and the reaction time require for removal of tail has been recorded as response. The cutoff time for tail exposure to hot water fixed to 15 s. The response was recorded at 0, 15,30,60,90,120 and 180 minutes after administration of dose.

Statistical Analysis

The data presented as a mean ± SD (Standard Deviation). Two way ANalysis Of VAriance (ANOVA) was used to make comparisons between the treated groups. The level of statistical significance was set at $P < 0.001$.

RESULTS

Preliminary phytochemical analysis:

Table 1.Shows the findings of qualitative analysis of *Aesculus indica* seeds extract. According to the obtained resultscarbohydrates, saponins, tannins, flavonoids were found to be present in extract.Alkaloids, glycosides, amino acids, steroids and terpenoids were found to be absent.

Table 1. Qualitative analysis of the phytochemicals inseeds extracts of *Aesculus indica*.

Sr. No.	Test for Phytoconstituents	Present/Absent
1.	**Alkaloids**	
	Mayer's Test	Absent
	Dragendroff's Test	Absent
	Wagner's Test	Absent
	Hager's Test	Absent
2.	**Glycosides**	
	Keller Killiani's test (Cardiac Glycosides)	Absent
	Borntrager's test (Anthraquinone Glycosides)	Absent
3.	**Carbohydrates**	
	Molish Test	Present
	Fehling test (reducing sugar)	Present
4.	**Steroids**	
	Salkowski's Test	Absent
5.	**Flavonoids**	
	Lead Acetate Test	Present
	Sodium Hydroxide Test	Present
6.	**Saponins**	
	Foam Test	Present
7.	**Tannins and phenolic compounds**	
	Ferric Chloride Test	Present
	Lead Acetate Test	Present
	Dilute Nitric Acid Test	Present
	Dilute Iodine Solution Test	Present
	Acetic Acid Solution test	Present
8.	**Proteins**	
	Biuret test	Absent
9.	**Amino acids**	
	Ninhydrin Test	Absent

Acute toxicity study

The acute toxicity study began with a 300mg/kg starting dose. During a 14-day observation period, oral administration of a 300 mg/kg dosage of ethanol extract of *Aesculus indica* seeds caused no significant toxicity. From above results it is clear that given dose was safe and hence further study was performed by administering 2000mg/kg dose of extract to next group of animals. There were no indicators of toxicity and mortality [Table 2.], as well as the animals' morphological characteristics and general appearance did not change. There was no salivation, diarrhoea, tremors, convulsions, lethargy or unusual behavior observed during study in treatment group. When compared to control group mice, extract-treated animals did not demonstrate any significant changes in body weight, food and water intake [Table 4.]. For further confirmation of results effect was checked by giving same dose (2000mg/kg) to another group of three animals and results

were repeatedly same. Table 3, shows the parameters measured before and after the test extract of *Aesculus indica* seeds. According to results even at the highest dosage of 2000mg/kg body weight of the test animal, all parameters were normal. The oral LD_{50}could be over 2000mg/kg body weight. As a result, greater dose testing of the extracts may not be necessary, and the extracts were practically non-toxic.

Table 2. Effect of *Aesculus indica* seeds extract for sign of toxicity and mortality (n = 3).

Group	Treatment	Sign of toxicity (ST/NB)	Mortality (D/S)
Normal Control	Vehicle	0/3	0/3
Aqueous extract	2000 mg/kg	0/3	0/3
Alcoholic extract	2000 mg/kg	0/3	0/3

ST = Sign of toxicity, NB = Normal behaviour, D = Died, S = Survived.

Table 3. Effects of A.i. seeds extractat dose 2000mg/kg on morphological characteristics and general appearance in mice (n=3).

Sr. No.	Response	Before	After
1.	Alertness	Normal	Normal
2.	Touch response	Normal	Normal
3.	Torch response	Normal	Normal
4.	salivation,	Normal	Normal
5.	Diarrhoea	Absent	Absent
6.	Tremors	Absent	Absent
7.	Convulsions	Absent	Absent
8.	Lethargy	Absent	Absent
9.	Skin fur	Normal	Normal
10.	Pinna reflux	Normal	Normal
11.	Corneal reflux	Present	Present
12.	Pupils	Normal	Normal
13.	Lacrimation	Normal	Normal
14.	Gripping strength	Normal	Normal
15.	Urination	Normal	Normal
16.	Hyper activity	Absent	Absent

Table 4. Effect of extract of *Aesculus indica* seeds extract 2000mg/kg on body weight, food intake and water intake of mice (n = 3).

Day	Normal control			Test group		
	Body weight (g)	Food intake (g)	Water Intake (ml)	Body weight (g)	Food intake (g)	Water intake (ml)
0	19.20 ± 1.15	6.16 ± 0.51	6.86 ± 0.25	19.60 ± 0.85	6.06 ± 0.23	6.20 ± 0.17
2	19.23 ± 1.05	6.20 ± 0.36	6.93 ± 0.73	19.73 ± 0.90	6.20 ± 0.20	6.66 ± 0.40
4	19.56 ± 0.95	6.06 ± 0.61	7.13 ± 0.72	19.80 ± 0.80	5.90 ± 0.17	6.90 ± 0.10
6	19.76 ± 0.76	6.20 ± 0.36	7.20 ± 0.65	20.03 ± 0.85	5.83 ± 0.05	7.03 ± 0.56
8	19.90 ± 0.75	6.26 ± 0.25	6.93 ± 0.51	20.20 ± 0.90	6.16 ± 0.15	7.03 ±0.11
10	20.20 ± 0.65	6.46 ± 0.41	7.30 ± 0.45	20.50 ± 0.75	6.33 ± 0.15	7.03 ±0.23
12	20.46 ± 0.65	6.46 ± 0.57	7.03 ± 0.11	20.56 ± 0.86	6.03 ± 0.20	6.76 ±0.25
14	20.66 ± 0.60	6.46 ± 0.41	7.00 ± 0.17	20.93 ± 0.75	6.13 ± 0.30	6.80 ±0.30

All data is expressed as Mean ± SD (n = 3).

Antinociceptive Activity:

Hot plate test:

The effect of ethanol extract of *Aesculus indica*seeds is represented in Table 5. Extract significantly delayed the response dose dependently at doses 100, 200 and 300 mg/kg between 15 and 120 min after administration of extract when compared to control group. At dose 300 mg/kg showed maximum response at 45 min with response time 8.80 ± 0.14 ($p < 0.001$) when compared with control group (3.05 ± 0.18). The standard drug pentazocine showed maximum response at 30 min with reaction time 10.03 ± 0.17 ($p < 0.001$) when compared with normal control animals (3.2 ± 0.30).

Table 5: Effect of ethanol extract of *Aesculus indica* seeds in hot plate method.

Groups	Reaction time in seconds						
	Basal	**15 min.**	**30 min.**	**45 min.**	**60 min.**	**90 min.**	**120 min.**
Normal control	2.88 ± 0.14	3.06 ± 0.19	3.2 ± 0.25	3.05 ± 0.18	2.96 ± 0.08	3.03 ± 0.10	3.00 ± 0.15
Pentazocine 17 mg/kg i.p.	2.93 ± 0.26***	8.70 ± 0.08***	10.03 ± 0.16***	9.93 ± 0.17***	7.80 ± 0.14***	5.78 ± 0.07***	4.76 ± 0.12***
Ai extract 100mg/kg p.o.	2.86 ± 0.10***	4.01 ± 0.14***	6.18 ± 0.17***	6.58 ± 0.17***	4.83 ± 0.17***	3.88 ± 0.14***	3.25 ± 0.15
Ai extract 200mg/kg p.o.	2.68 ± 0.14***	5.31 ± 0.14***	6.68 ± 0.19***	7.21 ± 0.19***	5.03 ± 0.12***	4.38 ± 0.28***	3.53 ± 0.32***
Ai extract 300mg/kg p.o.	2.98 ± 0.14***	6.11 ± 0.25***	7.36 ± 0.16***	8.80 ± 0.14***	7.66 ± 0.20***	5.08 ± 0.22***	4.63 ± 0.08***

All data is expressed as Mean ± SD (n = 6).

*** $p < 0.001$when compared with control.

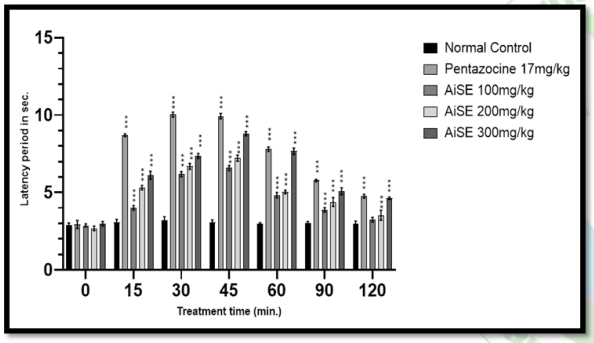

Figure 1: Effect of the *Aesculus indica* seeds extract and pentazocine on the latency time of mice in hot plate model. Values shown are mean ± SD, *** $p < 0.001$when compared with control, n=6.

Tail immersion test

The table 6. Shows effect of ethanol extract of *Aesculus indica* on time required for tail withdrawal response in mice. Extract at all doses significantly increased response time in comparison with control group. The highest dose (300 mg/kg) of extracts (9.63 ± 0.23; $p < 0.001$) showed maximum response at 1.5 h which was considerable response in comparison with pentazocine (17 mg/kg) (12.55 ± 0.25).

Table 6: Effect of ethanol extract of *Aesculus indica* seeds in tail immersion test.

Groups	Time for tail withdrawal response (Seconds)						
	Basal	**15 min.**	**30 min.**	**60 min.**	**90 min.**	**120 min.**	**180 min.**
Normal control	2.11 ± 0.23	2.06 ± 0.10	2.25 ± 0.13	2.18 ± 0.17	2.21 ± 0.18***	2.30 ± 0.16	2.25 ± 0.19
Pentazocine 17 mg/kg i.p.	2.15 ± 0.17***	4.08 ± 0.16***	7.05 ± 0.16***	10.10 ± 0.19***	12.55 ± 0.16***	10.08 ± 0.16***	8.21 ± 0.13***
Ai extract 100mg/kg p.o.	2.18 ± 0.11***	3.05 ± 0.12***	4.60 ± 0.26***	6.13 ± 0.13***	7.30 ± 0.14***	4.26 ± 0.18***	3.25 ± 0.13***
Ai extract 200mg/kg p.o.	2.21 ± 0.11***	3.26 ± 0.19***	5.25 ± 0.22***	7.35 ± 0.13***	8.36 ± 0.11***	6.16 ± 0.08***	4.16 ± 0.17***
Ai extract 300mg/kg p.o.	2.11 ± 0.13***	3.53 ± 0.12***	6.21 ± 0.11***	8.26 ± 0.10***	9.63 ± 0.16***	7.53 ± 0.17***	5.25 ± 0.10***

All data is expressed as Mean ± SD (n = 6).

*** $p < 0.001$when compared with control.

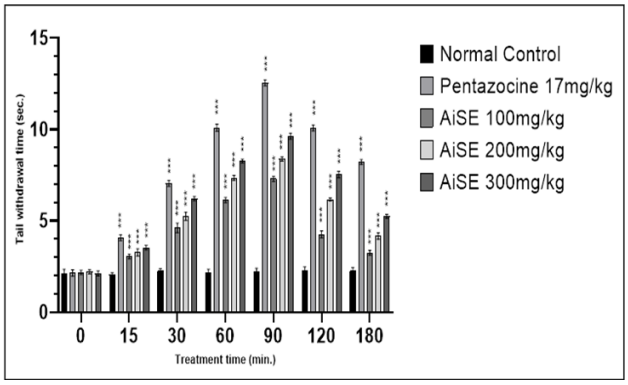

Figure 2: Effect of the *Aesculus indica* seeds extract and pentazocine on the reaction time of mice in Tail immersion model. Values shown are mean ± SD,*** p < 0.001when compared with control, n = 6.

Acetic acid induced writhing test

Table 7.Shows the effect ofethanol extract of *Aesculus indica* seeds on the number of writhing in mice. When compared to the control group, extract significantly reduced the number of writhes at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg. In comparison with control group (44.66 ± 1.44) the extract at 300 mg/kg showed the greatest suppression of writhes (27.16 ± 1.16, p < 0.001). When compared to the normal control, the aspirin (30 mg/kg) inhibited writhing by 87.41 percent, whereas the extract at 300 mg/kg inhibited writhing by 64.43 percent.

Table 7: Effect of ethanol extract of *Aesculus indica* seeds in writhing test.

Groups	No. of writhing	% Inhibition
Normal control	44.66 ± 0.81	-
Aspirin 30 mg/kg p.o.	23.83 ± 0.75***	87.41
AiSE 100mg/kg p.o.	37.60 ± 0.81***	18.77
AiSE 200mg/kg p.o.	32.5 ± 0.54***	37.41
AiSE 300mg/kg p.o.	27.16 ± 0.75***	64.43

All data is expressed as Mean ± SD (n = 6).

*** p < 0.001when compared with control.

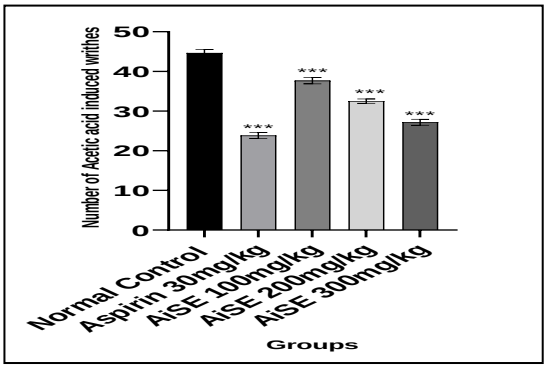

Figure 3. Inhibitory efect of the *Aesculus indica* seeds extract and aspirin on the acetic acid-induced writhes in mice, Values shown are mean ± SD, *** p < 0.001when compared with control, n = 6.

DISCUSSION

For centuries, medicinal plants have been utilised to treat human illnesses. The active compounds are mostly responsible for the crude drug's biological activity. However, evidence-based scientific research on the biological activity and toxicity of medicinal herbs are limited. The findings of this study revealed that the *Aesculus indica* seeds extract contained carbohydrates, saponins, tannins, and flavonoids [Tab.1]. These secondary metabolites produce a definite physiological action on the human body.[21-27] Toxicity data aids in determining the maximum dose of a substance that can be safely utilised in animals and humans. There were no reports on the toxicity of *Aesculus indica* seeds extract. As a result, the current investigation began with acute toxicity of the extract at a dose of 300 mg/kg and then progressed to a dose of 2000 mg/kg after obtaining satisfactory results. During 14 days of treatment with a single dose of *Aesculus indica*, there was no death. There was no substantial change in body weight, food, or drink intake, safety of extract.No symptoms of toxicity were noticed at the limit dose throughout the investigation, indicating that it was well tolerated. The findings indicated that the extract is safe to use.

The central mechanism of analgesic action of the extract was evaluated using hot plate and tail immersion techniques.To measure central anti-nociceptive activity, the tail immersion and hot plate tests are standard and very sensitive assays. With this nociception models, the extract showed substantial action. It is well known that centrally acting analgesics raise the pain threshold of mice when they are exposed to heat.[28] These tests are important in determining whether or not a heat induced nociception is present and whether or not narcotics are involved.[29] The hot plate test is very sensitive test to determine central anti-nociceptive activity. It involve neuronal signaling pathways to respond thermal stimuli.Supraspinal reflex is elicited by hot plate method. The substances, which increases the reaction time against heat stimulus, act centraly to mimic pain.[30]*Aesculus indica* (100, 200 and 300 mg/Kg) prolonged the latency period in hot plate model and tail withdrawing time as that produced by pentazocine, a standard analgesic drug indicating the centraly mediated anti-nociceptive activity.

Both tests use neural signalling pathways to respond to heat stimuli and are linked to central activity.[31] As a result, these tests were used to distinguish between central and peripheral analgesics. The hot plate involves higher brain functions and indicates a supra-spinally structured reaction to thermal pain stimuli, whereas the tail immersion test involves spinal motor reflexes to thermal nociceptive stimuli. The effect of central analgesics like opioids is mediated via regulation of spinal (μ2, κ1, δ2) and supraspinal (μ1, κ3, δ1, σ2) receptors.[32]

To test peripheral nociception acetic acid-induced writhing paradigm was used. Acetic acid causes the hind limbs to extend, the back to arch, and the abdominal muscles to contract in response to peripheral nociception.[33,34] TNF-, interleukins, and other inflammatory mediators such as histamine, bradykinin, serotonin, substance P and prostaglandins are released when acetic acid is given intraperitoneally.[35,36]The nociceptors in the dorsal horn of the central nervous system are activated by cytokines and inflammatory mediators, which then activate inflammatory

pathways, resulting in pain feeling.37,38 These mediators stimulate chemosensitve nociception, developing abdominal constrictions. Such pain sensations are inhibited by non-steroidal anti-inflammatory drugs (NSAIDs), which exhibit antinociceptive effect by inhibiton of prostaglandin synthesis.[39,40]Ethanol extract*of Aesculus indica* seeds reduced the acetic acid-induced writhing, similar to caused that of aspirin revealing the antinociceptic effect of *Aesculus indica*, possibly through the inhibition of peripheral pain mediated pathways.

The presence of saponins, flavonoids and tanins in *Aesculus indica* may account for the anti-nociceptive effect found, as these phytochemicals are renowned for their analgesic effect, while the involvement of additional constituents present in the plant cannot be overlooked.

CONCLUSION

The current study found that at the dose levels tested as per the acute toxicity studies, the ethanol extract of *Aesculus indica* seeds has considerable dose dependent antinociceptive effects in laboratory animals. The findings show that antinociceptive activity of *Aesculus indica* is mediated by two analgesic pathways: peripheral and central.

The tail immersion and hot plate tests are linked with central activity and involve neuronal signaling pathways to respond thermal stimuli. Tail immersion test involves spinal motor reflexes to thermal nociceptive stimuli, whereas the hot plate involves higher brain functions and represents supra-spinally organized response to thermal pain stimuli. The peripheral nociception induced by acetic acid was inhibited by extract. Possible mechanism peripheral analgesic effect may likely due to cytokine inhibition and other inflammatory mediators.

The presence of saponins, flavonoids and tannins in *Aesculus indica* seeds, along with the existence of other phytochemicals, may be responsible for antinociceptive effects. The findings appear to back up the plant's historic use in the treatment of several painful illnesses and also point to the presence of biologically active compounds. However more research is needed to isolate and characterize the bioactive components responsible for the anti-nociceptive effect.

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