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PHARMACOLOGICAL INVESTIGATION OF *AESCULUS INDICA* AQUEOUS-ETHANOL EXTRACT FOR ITS ANTI-NOCICEPTIVE ACTION

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ABSTRACT

Aesculus indica is used in traditional medicine for various health problems, including rheumatic pain. The present research project was aimed to investigate the analgesic effect of *Aesculus indica*, in order to substantiate its therapeutic use in rheumatism. *Aesculus indica* crude extract (Ai.Cr) was investigated, using chemical and thermally induced nociceptive models in mice. Ai.Cr showed the presence of carbohydrates, flavonoids, proteins, saponins, steroids, tannins and terpenes. In chemical-induced nociception, Ai.Cr dose dependently (10, 30 and 60 mg/Kg) inhibits acetic acid evoked writhes as exhibited by diclofenac sodium. In thermally-induced nociceptive models Ai.Cr at 100, 300 and 600 mg/Kg prolonged the latency period, measured at different time intervals of 30, 60, 90 and 120 min. both in hot plate and tail immersion test, similar to tramadol. These results proposed that *Aesculus indica* possesses anti-nociceptive property, occurred possibly through dual inhibition of peripheral (chemically) and central (thermally) nociception mechanisms, which provides scientific background in support of its folk use in pain management.

Keywords: *Aesculus indica,* analgesic activity, mice.

INTRODUCTION

Aesculus indica (Wall. ex Cambess.) Hook. (family: Sapindaceae) commonly called "Indian Horse Chestnut" and locally known as Bankhor. It is a large herbaceous tree found at 2000-3000 m altitude in Himalayas lowland and Kashmir to West Nepal (Kaur, 2011). It is generally used traditionally for the treatment of abdominal colic, thrombosis, phlebitis, haemorrhoids (Singh *et al.,* 1986), diabetes (Chakraborthy, 2009), rheumatism and skin diseases (Bibi *et al.,* 2012). The plant contains aescin, astragalin, β-sitosterol, quercitrin and rutin (Srijayanta *et al.,*1999).

*Aesculus indic*a is pharmacologically reported to possess cytotoxic (Bibi *et al.,* 2012), anti-bacterial (Bibi *et al.,* 2011), anti-oxidant (Chakraborthy, 2009), immune-modulatory (Chakraborthy *et al.,* 2009), anti-viral (Singh *et al.,* 2003), neurodepressive (Bhatt, 1992), spasmolytic (Qayum *et al.,* 1988) and anti-inflammatory (Ikram *et al.,* 1986) properties. In the present investigation, we evaluated the anti-nociceptive activity of *Aesculus indica*, mediated via dual peripheral and central analgesic pathways, justifying its ethnomedicinal application in rheumatic pain.

MATERIALS AND METHODS

Plant material and extraction: *Aesculus indic*a leaves (950 g) were collected from Muzaffarabad, Azad Kashmir in May 2015 and were authenticated by Dr. Mushtaq Ahmad (a taxonomist at Department of Plant Sciences, Quaid-i-Azam University, Islamabad).The voucher specimen (AI-113) was submitted to the herbarium at same Department. The plant was washed, dried in shade and ground to a coarse powder. The powdered material was extracted with 70% aqueous-ethanol with occasional shaking at room temperature. The material was strained by using a muslin cloth and then Whatman filter paper grade 1 (Williamson *et al.,* 1998). The filtrate was evaporated with a rotary evaporator under reduced pressure to obtain a thick semi-solid paste i.e. *Aesculus indica* crude extract (Ai.Cr), was solubilised in saline.

Chemicals: Acetic acid was purchased from Daejung Chemicals & Metals Co., Ltd. Korea. Diclofenac sodium was purchased from Olive Laboratories National Industrial Zone Rawat, Islamabad. Ethanol was purchased from BDH Laboratory Supplies, poole, England and tramadol obtained from Searle Company, Karachi respectively. All the chemicals used in this study, were of analytical quality.

Animals: Both male and female Balb-C mice (25-35 g) were used for this study, divided in to five different groups, each having 5 mice. Animals were housed (23-25°C) at the Animal House of Riphah Institute of Pharmaceutical Sciences. Mice were given standard diet and tap water *ad libitum.* Experiments were carried out according to the rules of Institute of Laboratory Animal Resources, Commission on Life Sciences University, National Research Council (1996) and were approved by

Ethical Committee of Riphah Institute of Pharmaceutical Sciences (Ref. No: REC/RIPS/2015/005).

Phytochemical Analysis: Phytochemical studies were performed for the detection of various phytochemical classes, according to standard procedures as described by (Evans, 1996; Harborne, 1998), with some modifications.

Test for carbohydrates: Benedict's, Fehling's and Molisch's test was performed for the detection of carbohydrates.

Test for flavonoids: Alkaline reagent test was used; yellow colour appears when test solution reacts with sodium hydroxide.

Test for proteins: Crude extract reacts with ninhydrin reagent; appearances of blue colour indicated the presence of amino acid and proteins (Yadav and Agarwala, 2011).

Test for saponins: Presence of saponins was detected upon vigorous shaking of diluted samples marked the formation of froth (Harborne, 1998; Yadav and Agarwala, 2011).

Test for steroids: Steroids were considered positive, if plant material treated with chloroform and sulfuric acid subsequently produced red coloration (Evans, 1996; Yadav and Agarwala, 2011).

Test for tannins: Crude extract was treated with few drops 0f 1% ferric chloride solution dark blue precipitate or green precipitate indicated the presence of tanins.

Test for terpenes: Copper acetate and Salkowai's test was used for the detection of terpenes (Evans, 1996; Harborne, 1998).

Acetic acid-induced writhings: Plant extract (10, 30, 60 mg/Kg; i.p), standard drug (diclofenac sodium 10 mg/Kg, i.p.) and normal saline (10 mL/Kg, i.p.) was injected into five different groups (n=5) of animals. The pain was induced in mice after 30 min. by intraperitoneal injection of 0.7% acetic acid (0.1ml/kg). The mice were placed individually in transparent cage. After 5 min elapse, the number of acid-induced writhes (abdominal stretching and/or simultaneous stretching of one hind limb) was counted for 20 min. (Bashir and Gilani, 2008; Khan and Gilani, 2010).

Hot plate assay: Mice were individually positioned on hot plate (temperature of 55°C) and latency to a pain reaction (licking of paw or jumping) was recorded in seconds (sec.), assessing their response to thermal stimulus. Cut off time was 20 sec. to avoid thermal paw injury. Previously selected animals were divided into five groups (I, II, III, IV and V) having five mice in each group. The group I, II and III received different doses of Ai.Cr (100, 300 and 600 mg/Kg, i.p.), group IV received tramadol (30 mg/Kg, i.p) and group V (control animal) received normal saline (10 mL/Kg, i.p.), 30 min. before their placement on hot plate. The reaction time was taken at 0, 30, 60, 90 and 120 min. after administration of the treatment. The latency time in Sec. of control animals was compared to that of test drug treated animals (Bukhari *et al.,* 2013).

Tail immersion test: After dividing mice into five different groups (I, II, III, IV and V). Group I, II and III served as a test drug (Ai.Cr 100, 300 and 600 mg/Kg, i.p), group IV received tramadol (30 mg/Kg, i.p) and group V received normal saline (10 mL/Kg, i.p.). After 30 minutes, the treated mice were individually placed in a restrained device (positioned vertically, extending out the animal tail). The marked area (5cm) of tail was dipped in hot water (55°C). Within few seconds, each mouse reacted to removes its tail out of hot water. Withdrawal time (sec.) was noted at 0, 30, 60, 90 and 120 min. The cut-off time was 15 sec. to avoid the injury (Toma *et al.,* 2003).

Statistical analysis: The data indicated are mean ± standard error of mean (SEM, n= number of experiment). The statistical parameter applied is ANOVA (one-way analysis of variance) with Tukey post-hoc test $P \leq 0.05$ was noted as significantly different. The bar-graphs were scrutinized using the GraphPad prism version 6.01 for windows, GraphPad software, San Diego CA, USA (Khan and Gilani, 2010).

RESULTS

Phytochemical analysis: Phytochemical screening of Ai.Cr showed the presence of carbohydrates, flavonoids, proteins, saponins, steroids, tannins and terpenes.

Effect on acetic acid mediated writhings: Ai.Cr dose-dependently (10, 30 and 60 mg/Kg) decreased number of writhes triggered by acetic acid (Figure 1). The number of writhes in control saline group was 75 ± 1.4. Ai.Cr at the doses of 10, 30 and 60 mg/Kg reduced number of writhes to 43 ± 3.9, 25 ± 0.7and 17 ± 0.9 ($P \leq 0.001$ vs. saline group) respectively. Diclofenac sodium (20 mg/Kg) diminished number of writhes to 12 ± 1.46 ($P \leq 0.001$ vs. saline group).

Hot plate test: Ai.Cr at doses of 100, 300 and 600 mg/Kg prolonged the latency time against thermal pain generation. The latency times of control saline group animals at 0, 30, 60, 90 and 120 min. were 7.89 ± 0.48, 8.21 ± 0.68, 8.25 ± 0.27, 8.58 ± 0.53 and 8.61 ± 0.55 respectively. The latency time of Ai.Cr (100 mg/Kg) treated group at 0, 30, 60, 90 and 120 min. were 8.43 ± 1.24, 9.72 ± 0.39, 10.22 ± 0.42, 10.76 ± 1.28 and 12.45 ± 0.82 ($P \leq 0.05$ vs. saline group) respectively. The latency time of Ai.Cr (300 mg/Kg) treated group at 0, 30, 60, 90 and 120 min. were 8.64 ± 0.57, 11.11 ± 0.40, 11.58 ± 1.0

($P \leq 0.05$ vs. saline group), 11.88 ± 0.53 and 13.78 ± 0.48 ($P \leq 0.01$ vs. saline group) respectively. The latency time of Ai.Cr (600 mg/Kg) treated group at 0, 30, 60, 90 and 120 min. were 8.43 ± 0.51, 11.82 ± 1.13 ($P \leq 0.05$ vs. control group), 14.68 ± 0.87 ($P \leq 0.01$ vs. saline group), 15.54 ± 1.41 ($P \leq 0.01$ vs. saline group) and 17.64 ± 0.85 ($P \leq 0.001$ vs. saline group) respectively. The latency time of tramadol (30 mg/Kg) treated group at 0, 30, 60, 90 and 120 min. were 7.74 ± 0.87, 13.39 ± 0.70 ($P \leq 0.001$ vs. saline group), 14.60 ± 0.99 ($P \leq 0.001$ vs. saline group), 15.38 ± 1.57 ($P \leq 0.01$ vs. saline group) and 16.32 ± 1.0 ($P \leq 0.001$ vs. saline group) respectively, as shown in Figure 2.

Tail immersion: Ai.Cr at the doses of 100, 300 and 600 mg/Kg prolonged the reaction time against thermal stimuli. The withdrawal time of control saline group animals at 0, 30, 60, 90 and 120 min. were 1.34 ± 0.07, 1.32 ± 0.06, 1.27 ± 0.04, 1.41 ± 0.14 and 1.34 ± 0.05 respectively. The withdrawal time of Ai.Cr (100 mg/Kg) treated group at 0, 30, 60, 90 and 120 min. were 1.33 ± 0.09, 1.54 ± 0.13, 1.81 ± 0.06, 2.02 ± 0.17 and 2.36 ± 0.15 ($P \leq 0.01$ vs. saline group) respectively. The withdrawal time of Ai.Cr (300 mg/Kg) treated group at 0, 30, 60, 90 and 120 min. were1.29 ± 0.13, 2.01 ± 0.08 ($P \leq 0.01$ vs. saline group), 2.28 ± 0.16 ($P \leq 0.001$ vs. control group), 2.46 ± 0.13 ($P \leq 0.001$ vs. saline group) and 2.87 ± 0.16 ($P \leq 0.001$ vs. control group) respectively. The withdrawal time of Ai.Cr (600 mg/Kg) treated group at 0, 30, 60, 90 and 120 min. were 1.22 ± 0.13, 2.38 ± 0.93 ($P \leq 0.001$ vs. saline group), 2.71 ± 1.17 ($P \leq 0.001$ vs. saline group), 2.98 ± 0.09 ($P \leq 0.001$ vs. saline group) and 3.25 ± 0.17 ($P \leq 0.001$ vs. control group) respectively. The withdrawal time of tramadol (30 mg/Kg) treated group at 0, 30, 60, 90 and 120 min. were 1.02 ± 0.15, 2.60 ± 0.16 ($P \leq 0.001$ vs. saline group), 2.98 ± 0.17 ($P \leq 0.001$ vs. saline group), 3.26 ± 0.18 ($P \leq 0.001$ vs. saline group) and 3.39 ± 1.18 ($P \leq 0.001$ vs. saline group) respectively, as shown in Figure 3.

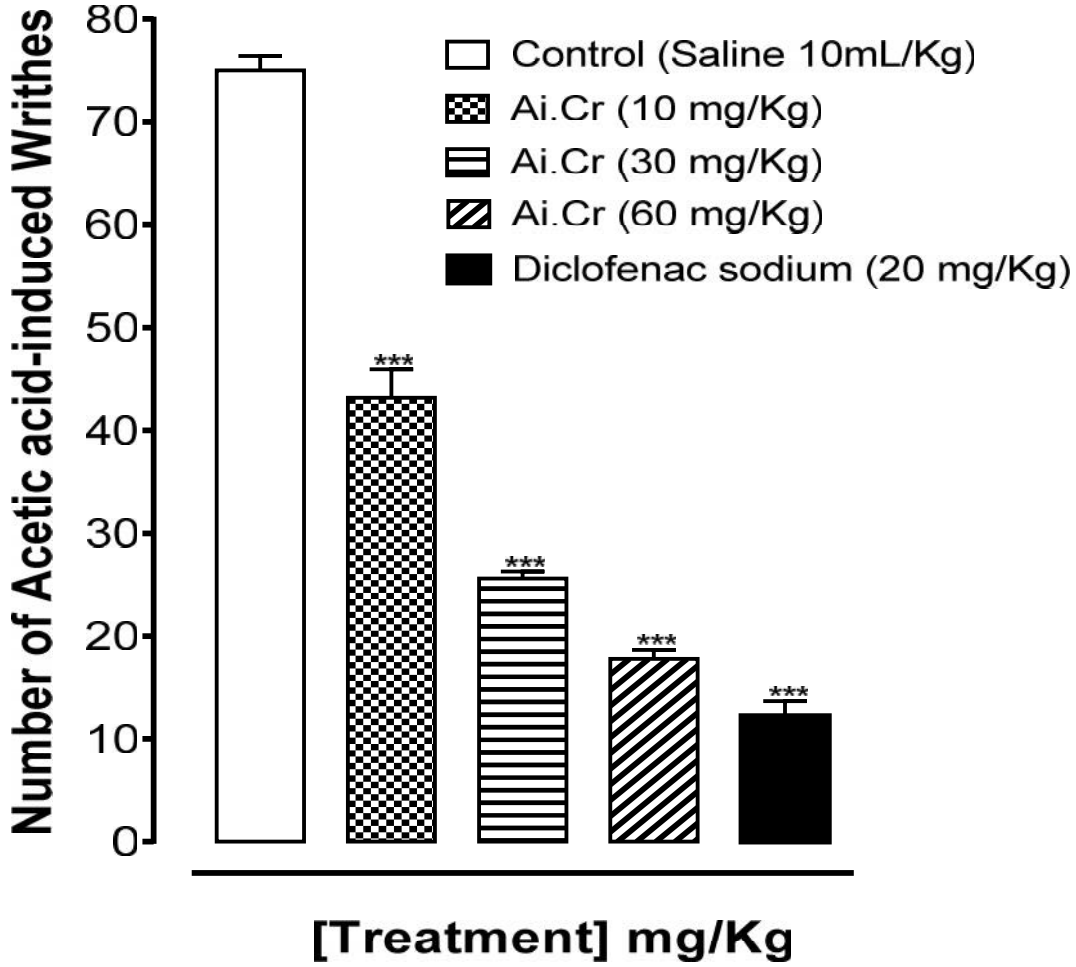


Figure 1. Bar chart showing inhibitory effect of the *Aesculus indica* crude extracts (Ai.Cr) and diclofenac sodium on the acetic acid-induced writhes in mice. Values shown are mean ± SEM, n=5. $^{***}P \leq 0.001$ vs. saline group, one-way analysis of variance with post-hoc Tukey test.

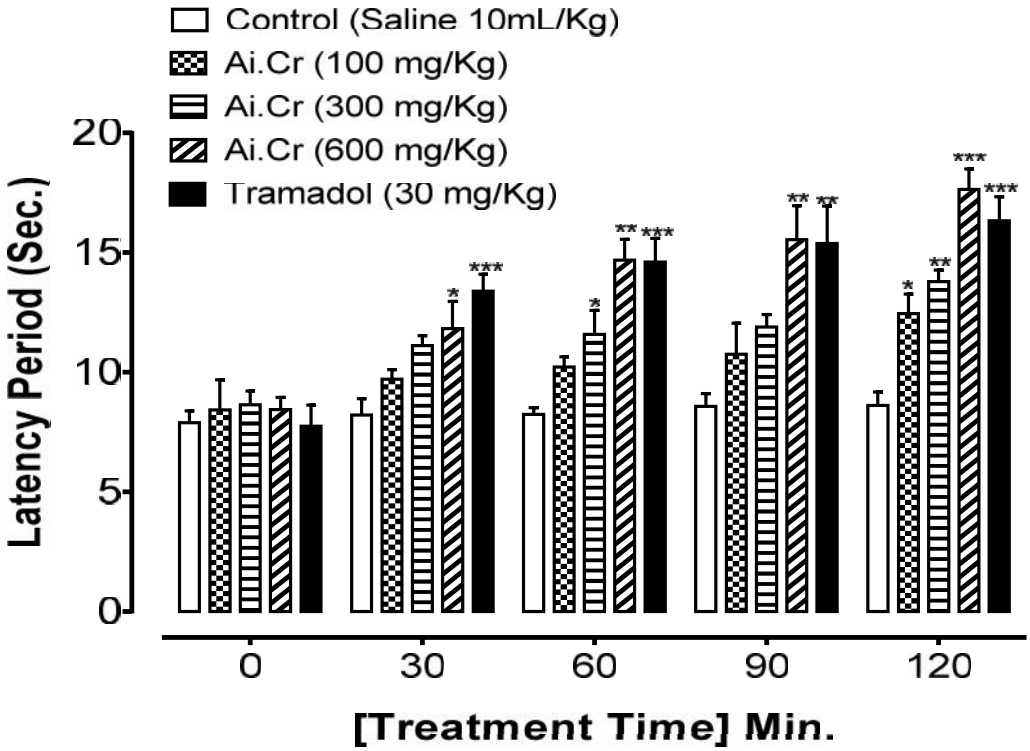


Figure 2: Bar chart showing effect of the *Aesculus indica* crude extracts (Ai.Cr) and tramadol on the latency time of mice in hot plate test model. Values shown are mean ± SEM, n=5. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ vs. saline group, one-way analysis of variance with post-hoc Tukey test.

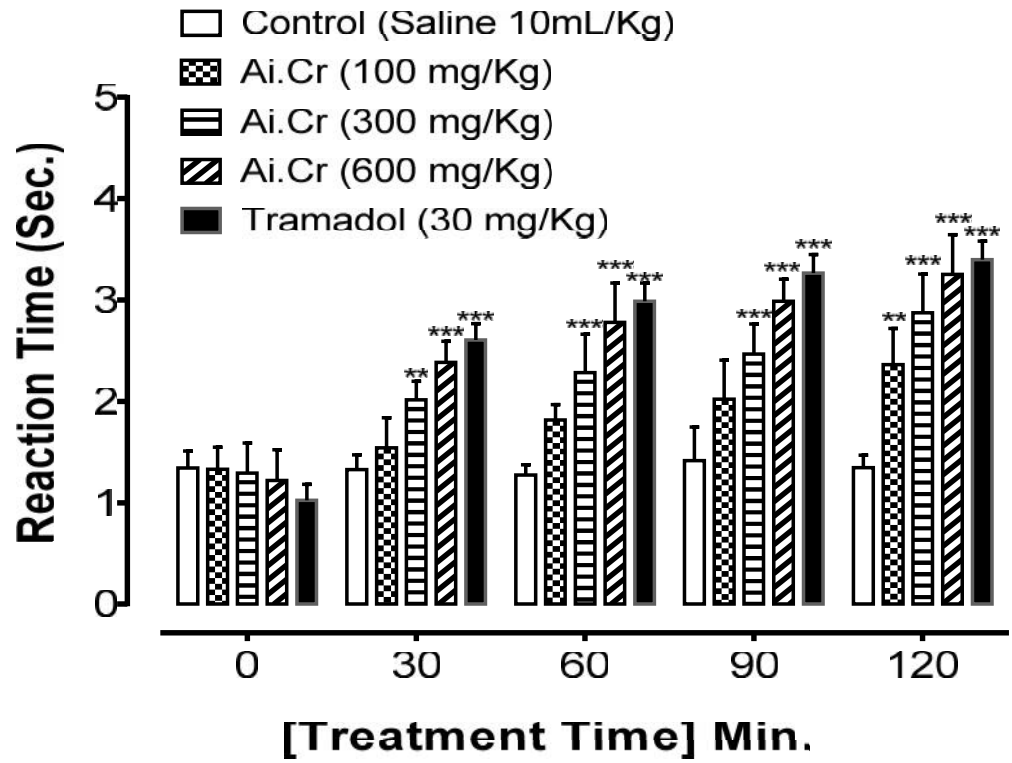


Figure 3: Bar chart showing effect of the *Aesculus indica* crude extracts (Ai.Cr) and tramadol on the reaction time of mice in Tail immersion model. Values shown are mean ± SEM, n=5. $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ vs. saline group, one-way analysis of variance with post-hoc Tukey test.

DISCUSSION

In present study, *Aesculus indica* was investigated against chemical (acetic acid evoked writhings) and thermally (tail immersion and hot plate) induced pain models to evaluate its peripheral and central mediated anti-nociceptive effect. Assay of acetic acid-induced writhings, is an established protocol for screening of peripherally acting analgesics (De Souza *et al.,* 2009). The i.p. injection of acetic acid causes release of different chemical mediators (bradykinin, histamine, serotonin, substance P and prostaglandins). These mediators stimulate chemosensitive nociception, developing abdominal constrictions (Bukhari *et al.,* 2013; Ferreira *et al.,* 2013). Such pain sensations are inhibited by non-steroidal anti-inflammatory drugs (NSAIDs), which exhibit antinociceptic effect by inhibition of prostaglandin synthesis (Derardt *et al.,* 1980; Santa-Cecília *et al.,* 2011). Ai.Cr at 10-60 mg/Kg reduced the acetic acid-induced writhings, like that caused by diclofenac sodium, revealing the antinociceptic effect of *Aesculus indica*, possibly through the inhibition of peripheral pain mediated pathways.

The supraspinal reflex is elicited by hot plate method while the tail immersion test demonstrates the spinal reflex by stimulating various types of opiods receptors (Chapman *et al.,* 1985).To evaluate central analgesic effect of the plant, hot plate and tail immersion assays were used. The agents, which increase reaction time against heat stimulus, act centrally to subside pain (Ibironke and Ajiboye, 2007; Gul *et al.,* 2014). Ai.Cr in dose-dependent manner (100, 300 and 600 mg/Kg) prolonged the latency period and latency for the tail withdrawal response in both aforementioned used models, as that caused by tramadol, a standard analgesic drug indicating the centrally mediated anti-nociceptive activity of *Aesculus indica.* The plant extract was more potent for its peripheral analgesic activity, compared to the central effects.

The presence of flavonoids and tannins may account for the observed anti-nociceptive effect of *Aesculus indica,* as the phytochemicals of such classes are known for their analgesic effect (Zulfiker *et al.,* 2010), though the role of other constituents present in the plant cannot be neglected.

Conclusion: These results reveal that *Aesculus indica* possesses analgesic activity, mediated through dual peripheral and central analgesic pathways. Further in-detail, advance molecular studies are warranted to explore nature of chemical constituents and to confirm pharmacodynamics base of the pharmacological action.

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