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

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ABSTRACT

*Aesculusindica*is used in traditional medicine for various health problems, including rheumatic pain. The present research project was aimed to investigate the analgesic effect of *Aesculusindica*, in order to substantiate its therapeutic use in rheumatism. *Aesculusindica* crude extract (Ai.Cr) was investigated, using chemical and thermally induced nociceptive models in mice. Ai.Crshowed the presence of carbohydrates, flavonoids,proteins, saponins, steroids,tannins and terpenes. In chemical-inducednociception,Ai.Cr dose dependently(10, 30 and 60 mg/Kg) inhibitsacetic acid evoked writhes as exhibited by diclofenac sodium. In thermally-induced nociceptiveAi.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 *Aesculusindica*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: *Aesculusindica,*analgesic activity, mice.

INTRODUCTION

Aesculusindica(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 usedtraditionallyfor the treatment of abdominal colic, thrombosis, phlebitis, haemorrhoids (Singh *et al.,* 1986), diabetes(Chakraborthy, 2009a),rheumatism andskin diseases(Bibi*et al.,* 2012).Theplant containsaescin, astragalin, β-sitosterol, quercitrinand rutin(Srijayanta*et al.,*1999).

*Aesculusindic*ais pharmacologically reported to possesscytotoxic (Bibi*et al.,* 2012), anti-bacterial (Bibi*et al.,*2011), anti-oxidant (Chakraborthy, 2009a), immune-modulatory (Chakraborthy*et al.,* 2009b), anti-viral (Singh *et al.,* 2003), neurodepressive (Bhatt, 1992), spasmolytic (Qayum*et al.,* 1988) and anti-inflammatory (Ikram*et al.,*1986)properties.Inthe present investigation, we evaluated the anti-nociceptiveactivity of *Aesculusindica*, mediated via dual peripheral and central analgesic pathways, justifying its ethnomedicinalapplication inrheumatic pain.

MATERIALS AND METHODS

Plant material and extraction:*Aesculusindic*aleaves (950 g)were collected fromMuzaffarabad, Azad Kashmir inMay2015 and were authenticated byDr.Mushtaq Ahmad(ataxonomistat Department of Plant Sciences,Quaid-a-Azam University, Islamabad).The voucher specimen (AI-113)was submitted to the herbarium at same Department. Theplant was washed, dried in shadeand ground to a coarse powder. The powdered material was extracted with 70% aqueous-ethanol with occasional shakingat room temperature. The material was strainedby using a muslin cloth and then Whatman filter papergrade 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. *Aesculusindica*crudeextract (Ai.Cr), was solubilisedin 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 fivedifferent 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 outaccording to the rules of Institute of Laboratory Animal Resources, Commission on Life Sciences University, National Research Council(1996) and were

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

Phytochemical Analysis: Phytochemical studies wereperformed for thedetection of various phytochemicalclasses,according to standard proceduresas described by (Evans, 1996; Harborne, 1998),with some modifications.

Test for carbohydrates: Benedict's,Fehling's andMolisch'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 ninhydrinreagent;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 withfew drops 0f 1% ferric chloride solutiondark 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)wascounted for 20 min.(Bashir andGilani, 2008; Khan andGilani, 2010).

Hot plate assay: Mice were individually positioned on hot plate(temperature of 55°C)and latency to a pain reaction (licking of paw orjumping) was recorded in seconds (sec.), assessing their response to thermal stimulus. Cut off time was 20 sec. to avoid thermalpaw injury.Previously selected animals were divided into five groups (I, II, III, IV and V) having five mice in each group. Thegroup I, II and III received different doses of Ai.Cr(100, 300 and 600mg/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 placementon hot plate. The reaction time was taken at 0, 30, 60, 90 and 120 min. after administrationof the treatment.The latency time in Sec. of control animals was compared to that of test drug treatedanimals (Bukhari, 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 wasdippedin hot water (55°C). Within few seconds,each mouse reacted to removes its tail out of hot water. Withdrawal time (sec.) was notedat 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 $P< 0.05$ was noted as significantly different. The bar-graphs were scrutinizedusing 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.Crshowed 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/Kgreduced number of writhes to 43 ± 3.9, 25 ± 0.7and 17 ± 0.9($P< 0.001$ vs. saline group)respectively.Diclofenacsodium (20 mg/Kg)diminishednumber ofwrithes to12 ± 1.46 ($P< 0.001$ vs. saline group).

Hot plate test: Ai.Crat doses of 100, 300 and 600 mg/Kgprolonged the latency time against thermal pain generation.The latency timesof control saline group animalsat 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($P< 0.05$ 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< 0.05 vs. saline group), 11.88±0.53 and 13.78±0.48(P< 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.were8.43 ± 0.51, 11.82 ± 1.13 (P< 0.05 vs. control group), 14.68±0.87 (P< 0.01 vs. saline group), 15.54± 1.41 (P< 0.01 vs. saline group)and 17.64±0.85(P< 0.001 vs. saline group) respectively. The latency timeof tramadol (30 mg/Kg) treated group at 0, 30, 60, 90 and 120 min. were 7.74±0.87, 13.39±0.70 (P< 0.001 vs. saline group), 14.60 ± 0.99 (P< 0.001 vs. saline group), 15.38± 1.57 (P< 0.01 vs. saline group)and 16.32±1.0(P< 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.Thewithdrawal 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< 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< 0.01 vs. saline group), 2.28±0.16 (P< 0.001 vs. control group), 2.46± 0.13(P< 0.001 vs. saline group) and 2.87± 0.16(P< 0.001 vs. control group) respectively. The withdrawaltime 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< 0.001 vs. saline group),2.71± 1.17 (P< 0.001 vs. saline group),2.98± 0.09 (P< 0.001 vs. saline group) and 3.25± 0.17(P< 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< 0.001 vs. saline group),2.98± 0.17 (P< 0.001 vs. saline group),3.26± 0.18 (P< 0.001 vs. saline group) and 3.39± 1.18(P< 0.001 vs. saline group) respectively, as shown in Figure 3.

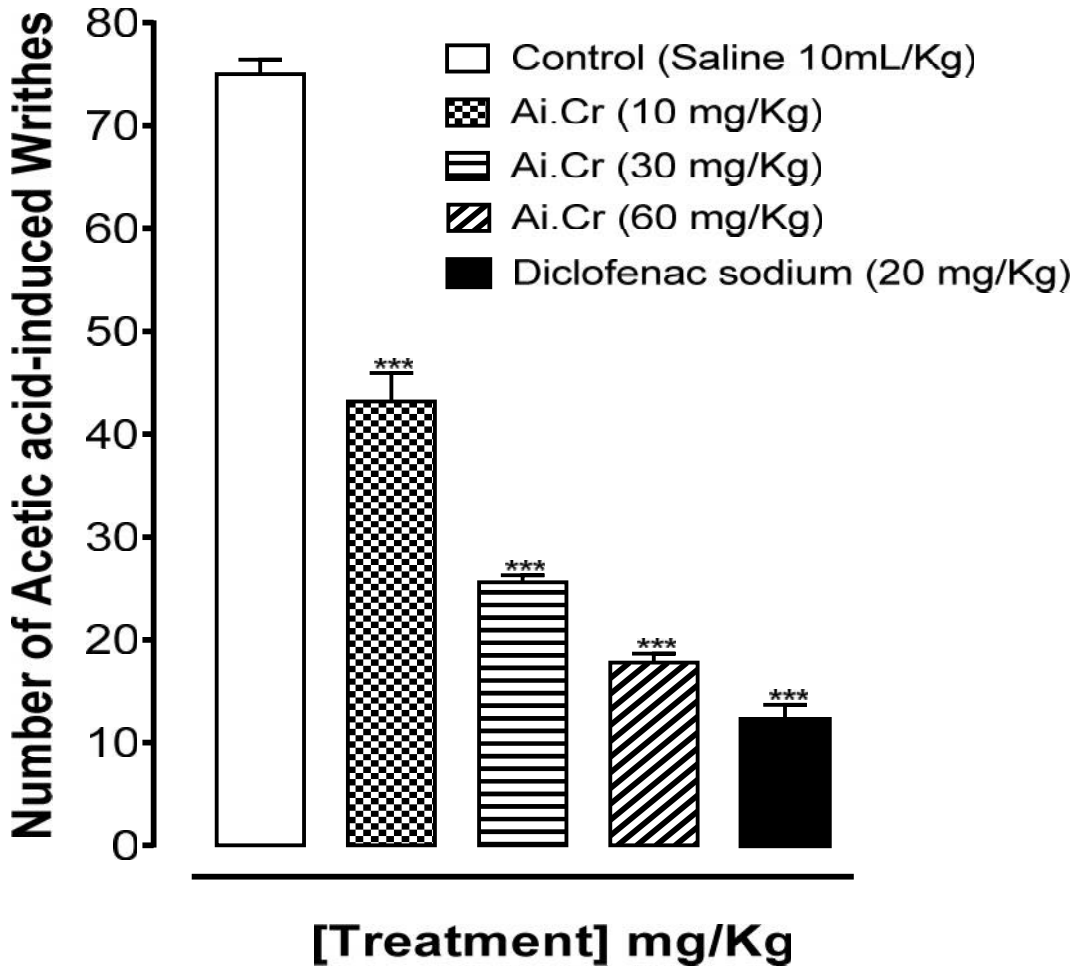

Figure 1. Bar chart showing inhibitory effect of the *Aesculusindica* crude extract (Ai.Cr) and diclofenac sodium on the acetic acid-induced writhes in mice. Values shown are mean ± SEM, n=5. $^{***}P$ < 0.001 vs. saline group, one-way analysis of variance with post-hoc Tukey test.

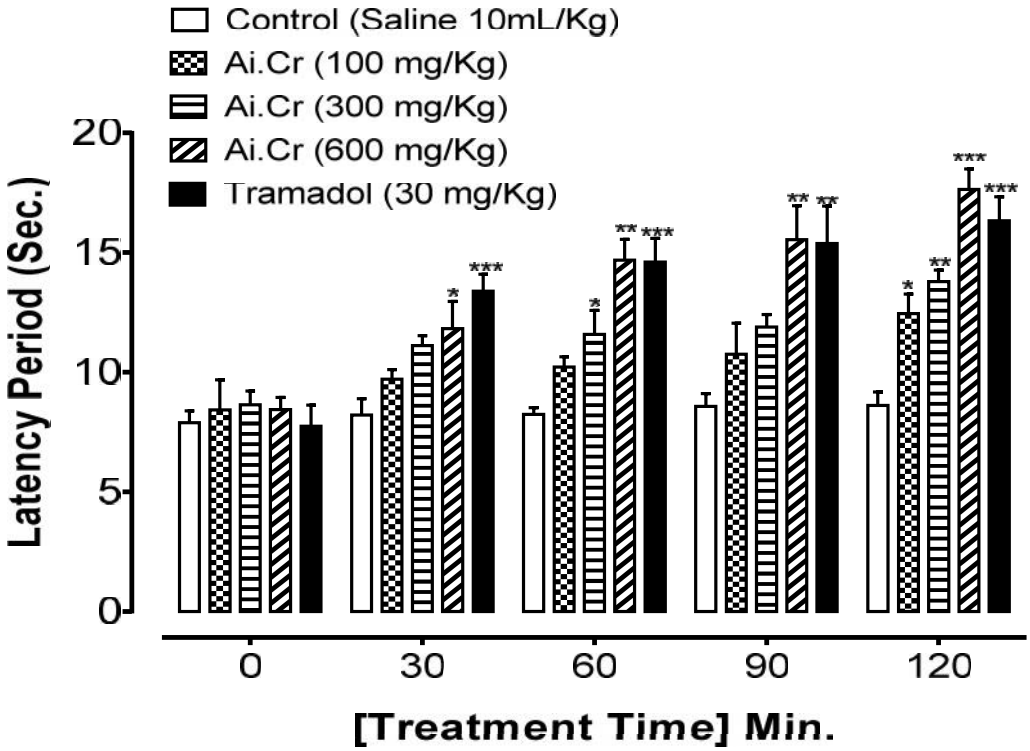

Figure 2: Bar chart showing effect of the *Aesculusindica* crude extracts (Ai.Cr) and tramadol on the latency time of mice in hot plate test model. Values shown are mean ± SEM, n=5. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. saline group, one-way analysis of variance with post-hoc Tukey test.

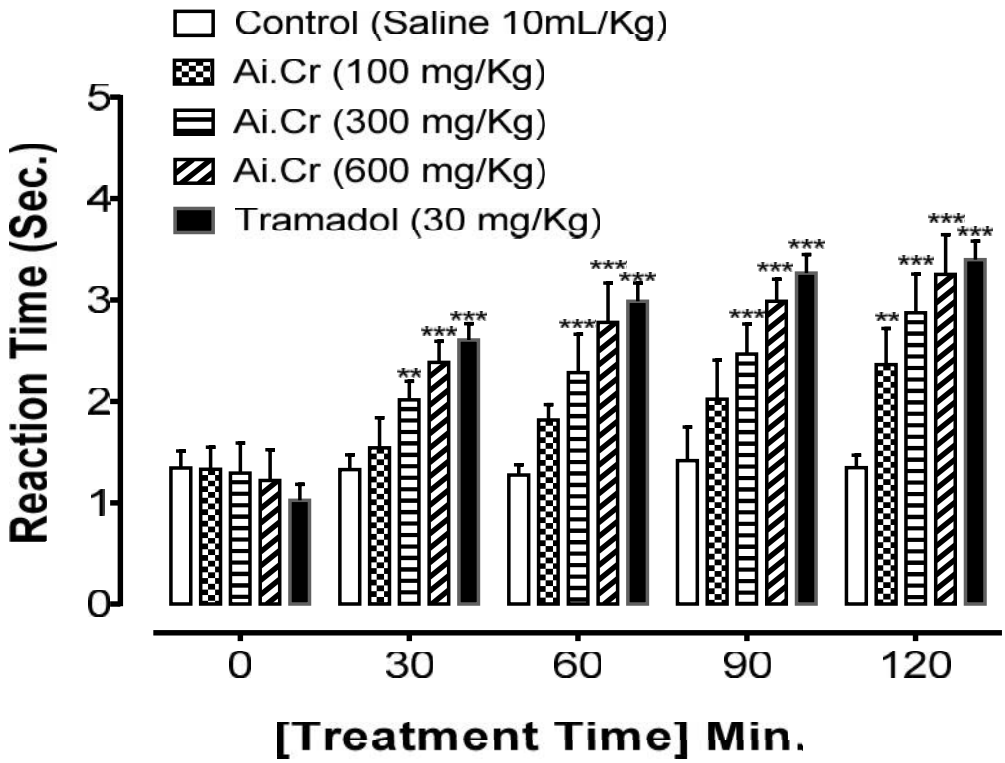

Figure 3: Bar chart showing effect of the *Aesculusindica* crude extracts (Ai.Cr) and tramadol on the reaction time of mice in Tail immersion model. Values shown are mean ± SEM, n=5. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. saline group, one-way analysis of variance with post-hoc Tukey test.

DISCUSSION

In present study, *Aesculusindica* was investigated against chemical (acetic acid evoked writhings) and thermally (tail immersionandhot plate) induced pain models to evaluate its peripheral and central mediated anti-nociceptive effect. Assay of acetic acid-induced writhings, is anestablished protocol for screening of peripherally acting analgesics(De Souza *et al.,* 2009).The i.p. injection of acetic acid causesrelease of different chemical mediators (bradykinin, histamine, serotonin, substance P and prostaglandins). These mediators stimulate chemosensitivenociception, developing abdominal constrictions(Bukhari*et al.,* 2013; Ferreira *et al.,* 2013).Such pain sensations are inhibited by non-steroidal anti-inflammatory drugs (NSAIDs), which exhibitantinociceptic effectby inhibition of prostaglandin synthesis(Derardt*et al.,* 1980;Santa-Cecília*et al.,* 2011).Ai.Crat 10-60 mg/Kg reduced the acetic acid-induced writhings, like that caused by diclofenac sodium, revealing the antinociceptic effect of *Aesculusindica*, possibly through theinhibition 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, hotplate and tail immersion assays were used.The agents,whichincreasereaction time against heat stimulus, act centrally to subside pain (IbironkeandAjiboye, 2007; Gul*et al.,* 2014). Ai.Crin dose-dependent manner (100, 300 and 600 mg/Kg) prolonged the latency period and latency for the tail withdrawal responsein both aforementioned used models, as that caused by tramadol, a standard analgesic drugindicatingthe centrally mediated anti-nociceptive activity of *Aesculusindica.* 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-nociceptiveeffect of*Aesculusindica,* asthe 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 *Aesculusindica*possesses analgesic activity, mediated through dual peripheral and central analgesic pathways. Further in-detail, advance molecular studies are warranted toexplore nature of chemical constituents and to confirm pharmacodynamics base of the pharmacological action.

REFERENCES

Bashir, S. and A.H. Gilani(2008). Studies on the antioxidant and analgesic activities of Aztec marigold (*Tageteserecta*) flowers.Phytother. Res. 22: 1692-1694.

Bhatt, J.P. (1992). Neurodepressive action of a piscicidal glycoside of plant, *Aesculusindica* (Colebr.) in fish.Indian J. Exp. Biol.30: 437-439.

Bibi, Y., S. Nisa, F. Chaudhary, and M.Zia (2011). Antibacterial activity of some selected medicinal plants of Pakistan. BMC Complement. Altern. Med. 11: 52.

Bibi, Y., S. Nisa, M. Zia, A.Waheed, S. Ahmed,and M.F. Chaudhary(2012). In vitro cytotoxic activity of *Aesculusindica* against breast adenocarcinoma cell line (MCF-7) and phytochemical analysis. Pakistan J Pharm Sci. 25: 183-187.

Bukhari, I.A., M.S. Alhumayyd, A.L.Mahesar, and A.H. Gilani (2013). The analgesic and anticonvulsant effects of piperine in mice. J. Physiol. Pharmacol. 64: 789.

Chapman, C.R., K.L. Casey, R.Dubner, K.M. Foley, R.H.Gracely, and A.E. Reading (1985). Pain measurement: an overview. Pain. 22: 1-31.

Chakraborthy, G.S. (2009a). Antioxidant Activity of the Successive Extracts of *Aesculusindica* Leaves. Int. J. Pharm. Sci. Drug Res. 1: 121-123.

Chakraborthy, G.S., V. Patil, and K.N. Kaushik(2009b). Evaluation of immunomodulatory activity of *Aesculusindica*. Int J PharmTech Res. 1: 132-134.

Deraedt, R., S.Jouquey, F. Delevallée, and M. Flahaut(1980). Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur. J. Pharmacol. 61: 17-24.

De Souza, M., M. Pereira, J.Ardenghi, T. Mora, L.Bresciani, R.Yunes, M. DelleMonache, and V.Cechinel-Filho (2009). Filicene obtained from *Adiantumcuneatum* interacts with the cholinergic, dopaminergic, glutamatergic, GABAnergic and tachykinergic systems to exert antinociceptive effect in mice. Pharmacol. Biochem. Behavior. 93: 40-46.

Evans, W.C. (1996). Trease and Evan's Pharmacognosy. 4th Ed. WB Saunders; Edinburgh(London). 161-408 p

Ferreira, L., A.Grabe-Guimarães, C. de Paula, M. Michel, R.Guimarães, S.Rezende, J. de souza,and A.Saude-Guimarães, (2013). Anti-inflammatory and antinociceptive activities of *Campomanesiaadamantium*. J. Ethnopharmacol. 145: 100-108.

Gul, S., S. Ahmed, N.Kifli, Q.T.Uddin, N.B.Tahir, A.Hussain, H.Z.Jaafar, M. Moga,and M. Zia-Ul-Haq (2014). Multiple pathways are responsible

for Anti-inflammatory and Cardiovascular activities of *Hordeumvulgare* L. J. Transl. Med. 12: 316.

Harborne, J.B., (1998). Phytochemical Methods A guide to modern techniques of plant analysis. 3rd Ed.Chapman and Hall; New York. 1-302 p

Ikram, M., and S.N. Gilani(1986). Anti-inflammatory activity of *Aesculusindica*fruit oil. Fitoterapia. 57: 455-456.

Ibironke, G.F., and K.I. Ajiboye(2007). Studies on the anti-inflammatory and analgesic properties of *Chenopodiumambrosioides* leaf extract in rats. Int. J. Pharmacol. 3: 111-115.

Kaur, L.A.K.H.V.I.R., L.I.N.C.Y. Joseph,andM.A.T.H.E.W. George (2011). Phytochemical analysis of leaf extract of *Aesculusindica*.Int. J. Pharm. Pharma Sci. 3: 232-234.

Khan, A.U., and A.H. Gilani(2010). Antisecretory and analgesic activities of *Terminaliabellerica.* Afr. J. Biotechnol. 9: 2717-2719.

National Research Council (1996). Guide for the Care and Use of Laboratory Animals. National Academy Press; Washington. 1-7 p

Qayum, A., N. Ahmed, K.D. Ahmad, and N. Gilani(1988). Pharmacological Screening of Indigenous Medicinal Plants. Pakistan J. Pharm. Sci. 37: 39.

Santa-Cecília, F., L.Freitas, F.Vilela, C.Veloso, C. da Rocha, M. Moreira, D. Dias, A. Giusti-paiva, and M. dos Santos (2011). Antinociceptive and anti-inflammatory properties of 7-epiclusianone, a prenylatedbenzophenone from *Garciniabrasiliensis*. Eur. J. Pharmacol. 670: 280-285.

Singh, B., P. Agrawal, and R. Thakur (1986). Aesculuside-A, A New Triterpene Glycoside from *Aesculusindica*. Planta Med. 52: 409-410.

Singh, B., M.Katoch, R. Ram, and Z. Aijaz(2003). A new antiviral agent from Indian Horse Chestnut (*Aesculusindica*). European Patent Specification. International publication No.WO, 79795.

Srijayanta, S., A.Raman, and B. Goodwin (1999). A Comparative Study of the Constituents of *Aesculushippocastanum* and *Aesculusindica*. J. Med. Food. 2: 45-50.

Toma, W., J.D.S.Gracioso, C.A.Hiruma-Lima, F.D. Andrade, W. Vilegas, and A.S. Brito(2003). Evaluation of the analgesic and antiedematogenic activities of *Quassiaamara* bark extract. J. Ethnopharmacol.85: 19-23.

Williamson, E.M., D.T. Okpako, andF.J. Evans (1998). Selection, Preparation and Pharmacological Evaluation of Plant Material, John Wiley and Sons, Chichester. 1: 15-23.

Yadav, R. N. S., and M. Agarwala, (2011). Phytochemical analysis of some medicinal plants. J.phytol. 3(12).

Zulfiker, A.H.M., M.M.Rahman, M.K.Hossain, K. Hamid, M.E.H. Mazumder, and M.S. Rana(2010). In vivo analgesic activity of ethanolic extracts of two medicinal plants *Scopariadulcis* L. and *Ficusracemosa*Linn. Biol. Med. 2: 42-48.