Phytochemical Analysis And Anti-Nociceptive Activity Of Ethanolic Leaves Extract Of Crataeva Adansonii DC In Rodents

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Abstract
Objective: The aim of this study was to screen the phytochemical constituents and evaluate the analgesic activity of crude ethanolic leaves extract of Crataeva adansonii at 50,100 and 200 mg/kg doses in experimental animal models.
Methodology: The ethanolic extract was prepared by maceration method followed by use of rotary evaporator. Phytochemical screening was done by using various tests. Analgesic activity was examined by acetic acid induced writhing for the evaluation of peripheral pain and tail flick and hot plate test were used for central pain evaluation.
Result: Ethanolic leaves extract and diclofenac sodium showed significant activities in both central and peripheral analgesic models as compared to control ($p<0.001$). Although leaves extract at dose of 50 and 200 mg/kg showed extremely significant result than 100 mg/kg of Crataeva adansonii. It was noted that diclofenac sodium was more potent among all with significantly greater activities as compared to all doses ($p<0.05$).
Conclusion: Crataeva adansonii can be used as an effective drug in the treatment of peripheral as well as in central pain due to its promising activity in animal models.

Keywords: Crataeva adansonii, Analgesic, maceration, tail flick, hot plate.

1. INTRODUCTION:

An unpleasant feeling and sensitive experience in everyday life is pain. It is an indication of tissue injury to prevent tissue damage (Silva et al., 2017). Acute pain is a useful biologic response and self-limiting in nature that arises in result to a specific injury. Chronic pain, in contrast, may be considered as a disease state. It may outlast the usual duration of recovery, if accompanied with a disease or injury. It may be classified as central or peripheral, depending on the site of the lesion (Shah and Shah, 2015).

The usage of natural products, principally herbal medicines is one of the ancient therapies used by humanity. During the recent years, people are eager to use herbal medicines due to their lower complications and fewer side effects than synthetic drugs. Regarding to the increasing demand for medicinal plants and related compounds the phytopharmaceutical remedies for the management of pain have been growing throughout the world (Borges et al., 2013).

Natural products in the form of herbal medicine have been used since ancient times. Crataeva adansonii DC. belongs to the small genus Crataeva L. is the member of family, Capparidaceae. It is well known for its different names i.e. barna, varuna, sacred garlic pear or temple plant. It is a small or medium sized tree which is unarmed, glabrous attains a height approximately 6-15m tall. C.adansonii sub species adansonii having elliptic or elliptic shaped lanceolate leaflets including acuminate apex. Oleanolic acid and 4-epi hederagenin are two bioactive compounds which are obtained from C.adansonii. From the leaves of plants some phytochemicals have been isolated which are aurantiamide acetate, Ethylpyropheophorbide A, purpurin -18 ethyl ester and pyropheophorbide. Leaves are attributed to antitrypanosomal activity against African trypanosome. Owing to antimicrobial properties young leaves are applied for otitis media. The overall plant used to cure blood pressure regulation restricts growth of intestinal worms which ease female delivery. However, it also helps to cure hepatic problems, indigestion and urinary troubles. The yellow color found in leaves used to dye clothes. In dry season people utilized foliage as forage. Its durable wood is used to make agricultural tools, joinery, toys, drums, utensils, writing boards and models. The seeds also used as condiment in culinary uses. Wood material has been recycled in the production of charcoal. Large and colorful petals attracts honey bee for nectar (Maduka et al., 2016;

Zingue et al., 2016; Udeh and Onoja, 2015; Abdullahi et al., 2015). The aim of this study was to evaluate the analgesic activity of crude ethanolic leaves extract of Crataeva adansonii at 50,100 and 200 mg/kg doses in rodents.

2. MATERIALS AND METHODS:

Chemicals

Absolute Ethanol (EtOH), Dimethyl sulfoxide, acetic acid were obtained from Merck, Germany. While diclofenac sodium (Voren) was purchased from the local market.

Preparation of plant extracts

The aerial part of the plant were cleaned with distilled water, dried under shade for 5 days. 10 kg of leaves were soaked in Ethanol (90%) at room temperature for 8-12 days. The percolate was filtered through Whatmann no.1 filter paper. Then remaining solvent was evaporated by rotary evaporator (Eyela, Japan) at 40^{o}C under reduce pressure. This process was repeated thrice and then combine all three filtrates to obtain crude Ethanolic extract (550g).

Qualitative Phytochemical Determinations

Preliminary phytochemical testing were performed to identify the various primary and secondary metabolites such as: (Flavonoids, Phytosterols, Glycoside, Alkaloid, Carbohydrate, Saponin, Gums, Proteins and amino acids, Tannins, Tartaric acid, Ascorbic acid (vitamin c), Fixed oil and Anthocyanins) in the leaves of C.adansonii DC.

Experimental animals

Adult Wistar Albino mice (20-25g) of either sex were bought from animal house of ICCBS, University of Karachi. Animals were retained in well aerated laboratory cages at a temperature of 27±2^{o}C, standard laboratory diet and water ad libitum were given to the mice. All the animals were kept on dark and light cycles of 12h.

Acetic acid induced writhing test

Ethanolic extracts of different doses were screened for acetic acid induced writhing. The animals were divided into five different groups (n=6). Group I or control group received normal saline at a dose of 10 ml/kg body weight while diclofenac sodium at a dose of 50 mg/kg body weight was administered to Group II. The plant extract were administered orally to the remaining groups III, IV and V at a dose of 50, 100 and 200 mg/kg body weight one by one. After 30 min of the above mentioned protocol, 0.2ml, 0.6% acetic acid was injected to all groups through intra-peritoneal route. Then after 5 min of acetic acid injection abdominal constrictions were started to count for next 10 min. The analgesic effects of different doses were calculated according to the formula mentioned below (Chowdury et al., 2015).

$$\% \text{ Protection} = \frac{\text{Mean control group} - \text{Mean treated group}}{\text{Mean control group}} \times 100$$

Tail Flick test

The analgesia was evaluated according to tail flick method. Mice were divided into five groups having six animals in each group. The mouse was held in the water bath (50 ± 2 ^{o}C) with the whole tail extending out. The extracts of C.adansonii were given orally with help of feeding cannula at a dose of 50, 100, 200 mg/kg body weight individually. The initial readings were taken immediately before administration of different doses at 60 minutes after administration. Tail flick apparatus use to measure analgesia. To avoid injury in mice cut off time was set as 10 seconds. Normal saline 10 ml/kg used as the negative control while standard diclofenac sodium as (50mg/kg, positive control) were subjected to assess analgesic activity (Owoyele et al., 2004). Tail flick antinociceptive index (TFAI) of all tested doses were determined by the following expression.

TFAI= reaction time-baseline/cutoff time x 100

Hot Plate test

Five groups of six mice each were used in this study. The analgesia measured by placing the animals on a hot plate (50±2^{o}C). The response of animals to heat was observed when the animal licked and jumped and then they were displaced from the hot plate quickly. Normal saline (10 ml) and standard drug diclofenac sodium (50 mg/kg) were given to group I and II respectively. Ethanolic extracts at dose of 50, 100 and 200 mg/kg were administered to group III, IV and V correspondingly. Stainless steel feeding tube was used to administer drug. After 30 minutes of treatment, the observations were recorded. The time period of no reaction was assumed at 30 seconds (Dharmasiri et al., 2003). Calculation of percent analgesia were done by using the following formula:

$$\%\text{Analgesia} = \frac{\text{Test latency - Control latency}}{\text{Cutoff time - control latency}} \times 100$$

3. RESULT:

Qualitative Phytochemical Determinations

From this plant various metabolites were identified including carbohydrate, Ascorbic acid, amino acid, proteins, phytosterols, gums, tartaric acid, tannins, flavonoids, fixed oil, anthocyanin and glycosides (Table 1).

Table-1: Phytochemical screening of ethanolic leaves extract of Crataeva adansonii DC

Plant Constituents	Test Name	CA-1
		E t O
Flavonoids	Alkali reagent test	+
	Zn-HCl test	+
Phytosterols	Liberman's burchard test	+
	Salkowaski test	+
Glycoside	Keller killiani test	+
	Borntrager's test	-
Alkaloid	Wagner test	-
	Dragendroff test	-
	Hagers test	-
Carbohydrate	Molish test	+
Saponins	Foam test	-
Gums	Benedict test	-
Proteins and amino acids	Millions test	+
	Biuret test	+
	Ninhydrin test	+
Tannins	$Fecl_3$ test	+
	Potassium di chromate test	+
	Lead acetate test	+
Tartaric acid		+
Ascorbic acid (vitamin c)		+
Fixed oil	Spot test	+
Anthocyanins		+

Acetic acid induced writhing test:

In this test the ethanolic extract at 200 mg/kg exhibited most prominent and significant effect ($p < 0.001$) i.e. 68% as compared to diclofenac sodium which shows drastically decrease writhes significantly ($p < 0.001$) i.e. 67% at 50 mg/kg p.o dose (Table-2)

Table-2: Analgesic effect of extracts of C.adansonii by Acetic acid induced constriction in mice.

Treatments	Given Dose mg/kg	Mean no. of writhes ± S.E.M	%inhibition
Control	-	49.50±0.83	-
Diclofenac Sodium	50	16±0.63***	67
CA-1 (EtOH)	50	21.83±1.94***	56
	100	19±3.03***	62
	200	15.83±2.63***	68

Mean ± S.E.M (n=6). Statistical significance were calculated by ANOVA followed by post hoc test when compare to the control group *P < 0.05, ** P< 0.01, ***p < 0.001.

Table-3: Analgesic effects of various extracts by tail flick test

Treatment Group	Dose (mg/kg)	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Control	–	0.91 ±0.11	1.03 ±0.12	0.90 ±0.18	0.99 ±0.11	0.90 ±0.07	0.91 ±0.12	0.92 ±0.05
Diclofenac sodium	50	1.23 ±0.09	3.38 ±0.09***	5.50 ±0.08***	6.45 ±0.14***	6.16 ±0.13***	5.31 ±0.25***	4.36 ±0.36***
CA-1 (EtOH)	50	0.96 ±0.02	3.21 ±0.63***	4.39 ±0.71***	4.88 ±1.02***	4.80 ±1.21***	2.55 ±0.60***	1.38 ±0.17
	100	1.00 ±0.04	2.99 ±0.42***	3.89 ±0.95***	4.27 ±0.69***	3.37 ±0.54***	1.96 ±0.24*	1.46 ±0.14
	200	1.00 ±0.02	2.40 ±0.53***	2.38 ±0.48**	3.38 ±0.43**	3.15 ±0.72***	1.58 ±0.51	1.08 ±0.14

Tail Flick test

The Standard drug diclofenac sodium shows significant (P<0.001) difference as compared to the control. Most prominent analgesia (4.39 seconds) was shown in tail flick test at 50 mg/kg dose while diclofenac sodium decreases pain sensation in 5.5 seconds at same dose.

Mean ± S.E.M (n=6). Statistical significance were calculated by ANOVA followed by post hoc test when compare to the control group *P < 0.05, ** P< 0.01, ***p < 0.001.

Hot Plate Test

In hot plate analgesic model mice at 50 mg/kg dose showed latency to thermal stimuli at 16.00 seconds whereas, the pain delay response of standard drug was 17 .33 seconds at same dose

Table-4: Analgesic effect of extracts of Plant B by hot plate Analgesiometer in mice.

Treatment Group	Dose (mg/kg)	0 min	30 min	60min	90min	120 min	150min	180 min
Control	–	6.00 ±0.89	6.50 ±0.54	5.83 ±0.98	6.00 ±0.89	6.33 ±0.89	6.16 ±0.89	6.33 ±0.51
Diclofenac Sodium	50	6.66 ±0.51	12.83 ±0.75***	17.33 ±2.33***	15.50 ±2.34***	11.50 ±1.64***	11.00 ±1.54***	10.83 ±1.16***
CA-1 (EtOH)	50	8.33 ±0.81***	15.83 ±0.40***	16.0 ±0.63***	17.36 ±0.40***	16.33 ±0.89***	12.58 ±0.54***	9.42 ±0.54***
	100	6.33 ±0.51	9.66 ±1.21	14.33 ±2.36***	14.66 ±2.73***	16.5 ±3.92***	11.0 ±2.1***	8.66 ±1.03***
	200	7.33 ±1.21	12.33 ±2.87***	13.66 ±1.86***	15.66 ±2.50***	16.5 ±1.51***	15.33 ±2.94***	10.45 ±0.54***

Mean ± S.E (n=6). Statistical significance were calculated by ANOVA followed by post hoc test when compare to the control *p < 0.05; **p < 0.01; ***p < 0.001.

4. DISCUSSION:

Pain sensations is associated with complex cascade in relation to central and peripheral nervous system. Evidently it hinders physical, physiological and quality attributes of life. Pain or inflammation considered as a defensive modality to reduce harmful stimuli as well as initiates healing process and diagnostic tool of various pathological issues (Fazal-ur-Rehman, 2014). Although any injury or damage in tissues related with pain and inflammation therefore, persistent inflammation results permanent tissue damage or may be failure of organ (Shikha et al., 2015). Natural medicine with promising pharmacological effects proved as a potential resource for generation of newer molecules with high therapeutic value and lesser side effects (Zhu et al., 2017).

Phytochemical screening confirms the presence of various metabolites carbohydrate, Ascorbic acid, amino acid, proteins, phytosterols, gums, tartaric acid, tannins, flavonoids, fixed oil, anthocyanin and glycosides (Table-1). According to literature search, this plant has an excellent property of antimicrobial, antitrypanosomal, antigout, analgesic, antioxidant, anti-itching, counter irritant and vermifuge. Leaves also used to treat hepatic problems, indigestion, constipation, urinary troubles, blood pressure regulation, asthma, and snake bites, post-menopausal complaints and ease in female delivery etc (Gidwani et al., 2009; Maduka et al., 2016; Zingue et al., 2016; Udeh and Onoja, 2015; Abdullahi et al., 2012). It is also used as routine vegetable for salads and soups and fulfill nutritional needs in many regions of Africa (Agbankpé et al., 2015). Our studies suggested that due to its content of proteins, carbohydrates and Vitamin C it may be a potential source of growth supplement as complemented its use in diseases occurred due to malnutrition problems. The high level of flavonoids in it indicative of anti-inflammatory, antidiarrheal, anti-oxidative, antiallergy, antimicrobial and anticancer properties (Okafor and Ezejindu, 2014). Therefore, it has great remedial actions in asthma, cancer, gout, jaundice etc. In support of our research findings, significant value of steroids in C.adansonii may contributed in hormonal imbalances and reproductive complaints (Oyedeji and Bolarinwa, 2013). The presence of tannins in plants suggested there use as antibacterial, antiviral and anti parasitic (Akiyama et ak., 2001; Kolodziej and Kiderlen, 2005; Lu et al., 2004) Hence plant used to treat snake bites, trypanosomiasis, hepatic and urinary infections etc.

Assay of acetic acid induced writhing measures peripheral analgesia produced by crude extract suggested that secretion of notorious agents from mast cells and macrophages like cytokines, tumor necrosis factor and interleukins were responsible for generation of abdominal constriction (Ribeiro et al., 2000). It was evident that all doses were decreases abdominal constriction significantly in animals due to restriction of arachidonic acid metabolism.

The ethanolic extract at 200 mg/kg exhibited most prominent and significant effect ($p < 0.001$) i.e. 68% as compared to diclofenac sodium which shows drastically decrease writhes significantly ($p < 0.001$) i.e. 67% at 50 mg/kg p.o dose (Table-2). It was reported that Hydromethanolic extract of stem bark of C.adansonii at 200mg/kg decreases 57% writhing reaction whereas aspirin used as positive control exhibited 50% efficacy at 100 mg/kg (Prempeh, 2008).

Most prominent analgesia (4.39 seconds) was shown in tail flick test at 50 mg/kg dose while diclofenac sodium decreases pain sensation in 5.5 seconds at same dose. (Table-3). In hot plate analgesic model mice at 50 mg/kg dose showed latency to thermal stimuli at 16.00 seconds whereas, the pain delay response of standard drug was 17 .33 seconds at same dose (Table-4).

It means crude leaves extracts and diclofenac sodium possess comparable pain relieving effects. Table 35 It was suggested that activity of crude ethanolic extract in hot plate test is similar as activity of non-steroidal and anti-inflammatory drugs by interrupting in pain sensations mechanism in animals might be proved as good source in various painful inflammatory conditions as non-steroidal agents produce analgesia by blocking endogenous prostaglandins, bradykinins and other noxious stimuli (Guay et al., 2004).

5. CONCLUSION:

In this study, Crataeva adansonii showed extremely significant results at 50 mg/kg and 200 mg/kg when compared to the control group i.e. diclofenac sodium. It is therefore concluded that Crataeva adansonii can be used as an effective drug in the treatment of peripheral as well as in central pain due to its promising activity in animal models.

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