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PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Study on Analgesic and Anti-inflammatory Properties of *Cordia myxa* Fruit Hydro-alcoholic Extract

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Abstract: *Cordia myxa* is a plant which is used in tropical regions of the world. Analgesic and anti-inflammatory effect of fruit of this medicinal plant was investigated in mice. Hydro-alcoholic extract of it was prepared by maceration method. Formalin test was conducted in six groups of mice (6 animals in each group) and acetic acid test in another six groups (6 mice). Groups one to six in each test were administered normal saline, oral indomethacin, intraperitoneal tramadol, 100 mg kg⁻¹ oral extract, 200 mg kg⁻¹ oral extract and 100 mg kg⁻¹ intraperitoneal extract, respectively. The duration of foot lickings were calculated in formalin- administered (1st) group within min 0 to 5 (acute phase) and 15 to 25 (chronic phase). Acetic acid-induced writhings were counted within 10 min in the 2nd group. The results showed that hydro-alcoholic extract of *Cordia myxa* fruit was considerably effective in formalin test. Also, analgesic and anti-inflammatory properties of this plant's fruit in both acute and chronic phase are somewhat similar to these properties in the study on animal model of experimental colitis.

Key words: *Cordia myxa*, fruit extract, analgesic, anti-inflammatory, mice

INTRODUCTION

The genus *Cordia*, with about 300 identified species and worldwide distribution, is one of the largest genera in the family Boraginaceae (Geller, 2010). *Cordia myxa*, a perennial generative and vegetative plant, is popularly used by the public for its efficacy in chest and urinary infections. Other known therapeutic properties of this plant such as anthelmintic, diuretic, demulcent, antidiarrheal, anti-gastric and anti-worm have been reported. It is also a liver tonic. Several preparations of *Cordia* species from its bark, leaf and fruit extracts have been used in traditional medicine for treatment of osteoarticular diseases, dysmenorrhea and as abortive (Afzal *et al.*, 2009). Analgesic, anti-inflammatory and anti-arthritis activities of them have been studied in rats (Ficarra *et al.*, 1995; Al-Awadi *et al.* 2001).

The fruit of *Cordia* is popularly used for the treatment of chest and urinary tract infections, diseases of the lung and spleen and as an astringent, anthelmintic, diuretic, demulcent and expectorant agent (Afzal *et al.*, 2007; Barroso and de Oliveira, 2009). The fruit

of *C. myxa*, locally known as “Sepestan” in some regions of Iran, has been used for the treatment of asthma and lung infections. It is utilized like as emollients, demulcents, antitussives and expectorants.

Therapeutic activities of different parts of certain species of *Cordia* such as *C. francisci*, *C. martinicensis*, *C. myxa*, *C. serratifolia* and *C. ulmifolia* have been studied in animal models. Analgesic and anti-inflammatory activity of leaf extracts of *C. myxa* has been ascertained by Ficarra *et al.* (1995). Also, Al-Awadi *et al.* (2001) in their study on experimental colitis, found *C. myxa* fruit preparation effective as anti-inflammatory agent.

Medicinal plants have been used as a source of remedies since ancient times. In Iran, medicinal plants have been widely used for the treatment of diseases in folk medicine. Nowadays, the herbal medicines have attracted interests of scientists, as the irrational use of therapies, such as inaccurate dosage, lack of proof of safety and efficacy and risk of interaction with other drugs may lead to health hazards (Rates, 2001; Schmeda-Hirschmann and Yesilada, 2005).

Additionally, search for new or alternative agents is always needed to replace harmful drugs. Researchers from different regions of the world have studied therapeutic utility of *Cordia* species' leaves, fruits and seeds. We realized a need to investigate anti-inflammatory and analgesic effects of *C. myxa* fruit extract in a mice model.

MATERIALS AND METHODS

Fruit extract preparation: This study was conducted in the veterinary medicine school, Shahid Chamran University of Ahvaz-Iran in (2006). Sepestan (*Corida myxa*) fruits were collected from the agriculture faculty educational garden. After careful cleaning of fruits and sufficient washing with tap water, its hydro-alcoholic extract was obtained by maceration method in 70% methanol. Then the *C. myxa* extract (CME) was stored in refrigerator till examination.

Animal model: Seventy two specified pathogen-free NMRI strain mice of either sex weighing 20 ± 3 g were used for this study. The animals were kept in lab animals' research center, Jundishapour University of Ahvaz, with free access to food and water, under 12 h light and 12 h dark conditions. Animal experimentation protocols conformed to the institutional animal ethics committee's guidelines. Mice were randomly allocated into two groups of 36 animals (6 mice in each group of 6). One part was examined for formalin-induced pain test and other for acetic acid-induces abdominal writhing test.

Test procedure

Formalin tests: For formalin test, it was subcutaneously injected in plantar surface of mice hind paw. The duration of licking of foot was calculated within 0-5 min (acute phase) and 15-25 min (chronic phase) after formalin injection. For acetic acid test, the number of writhing was counted within 10 min following intraperitoneal injection of a 1% solution of acetic acid.

Acetic acid test: Groups one to six in each test were administered oral normal saline (control), oral indomethacin (control; as standard anti-inflammatory drug), intraperitoneal tramadol (control; as standard analgesic drug), 100 mg k^{-1} oral CME, 200 mg k^{-1} oral CME and 100 mg k^{-1} intraperitoneal CME. Drugs were purchased from a registered pharmacist in Ahvaz city. All drug solutions and suspensions were freshly prepared.

Statistical analysis: The mean of obtained data was compared between groups by SPSS software

(version 16, USA), using ANOVA test. P value was considered significant when < 0.05 .

RESULTS AND DISCUSSION

Means of duration of foot licking in acute phase were 1.52, 0.67, 0.028, 0.81, 0.77 and 0.67 min (Fig. 1) and in chronic phase were 0.88, 0.77, 0, 0.013, 0.28 and 0.09 min, respectively (Fig. 2). Statistical analyses showed that *Cordia myxa* fruit extract was significantly effective in reducing perceived pain in the formalin test.

In acetic acid test, the mean numbers of writhing in groups one to six were 25.17, 34.67, 7.17, 25.13, 28.5 and 22.83, respectively in 10 min. Tramadol significantly inhibited writhing, but *Cordia myxa* fruit extract was not effective at a statistically significant level (Fig. 3).

The results of the present study showed that hydro-alcoholic extract of *Cordia myxa* fruit has analgesic and anti-inflammatory effect comparable to standard drugs such as indomethacin and tramadol, similar to findings by Ficarra *et al.* (1995) and Al-Awadi *et al.* (2001).

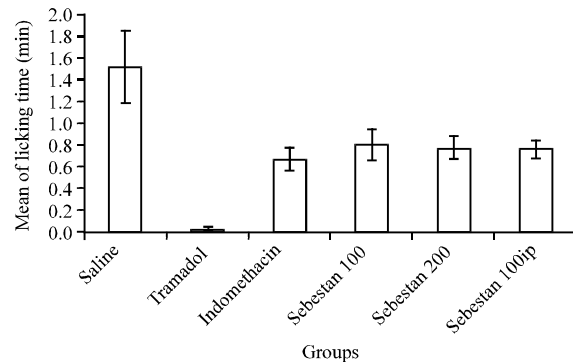


Fig. 1: Mean±SE duration of licking in acute phase of formalin test

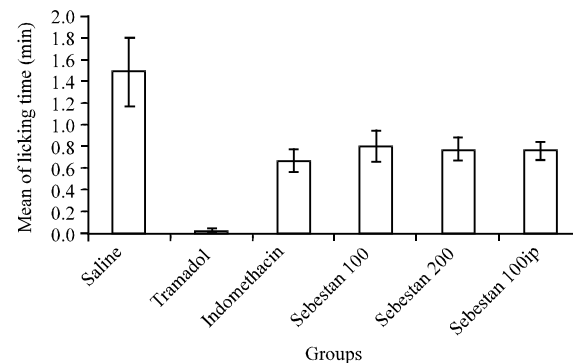


Fig. 2: Mean±SE duration of licking in chronic phase of formalin test

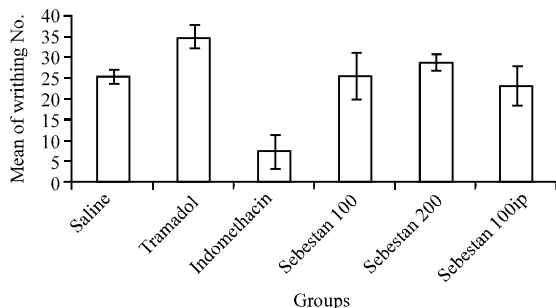


Fig. 3: Mean±SE number of writhing in acetic acid test

Hind paw formalin-induced inflammation is a biphasic process. The initial phase is associated with activation of sensory neurons, whereas the second accelerating phase is a reflection of the combined effects of afferent input pathways and central sensitization in the spinal dorsal horn (McNamara *et al.*, 2007). Acetic acid-induced inflammation is also a standard method in laboratory animals' researches, although it is not specific and about 10% of animals do not show writhings during experiments (Olapour and Najafzadeh, 2010). So we performed both of the methods in our study.

Polyphenols, alkaloids and flavonoids in plants are known to exert active anti-inflammatory effects (Luximon-Ramima *et al.*, 2002; Olukunle *et al.*, 2011). Afzal *et al.* (2009) reported high polyphenolic content of *C. myxa* fruit and suggested that protective effect on liver damage of rats could be due to this compound. *Cordia dichotoma* fruit extracts is reported to have anti-inflammatory activity against carrageenan- and dextran-induced paw edema in rats, which is said to be due to their content of tannins, flavonoids, alkaloids, glycosides, saponins and carbohydrates (Sharma *et al.*, 2010). *C. dichotoma* fruit is also shown to be effective in gastric ulcers in rat model, as well as wound healing (Kuppast and Nayak, 2006; Kuppast *et al.*, 2009). Ficarra *et al.* (1995) reported analgesic and anti-inflammatory activities of these medicinal plants in their research on five *Cordia* species' leaf extract on rat. They evidenced and determined four flavonoid glycosides, robinin, rutin, datiscoside and hesperidin, one flavonoid aglycone, dihydrorobinetin, two phenolic derivatives, chlorogenic and caffeic acid and concluded that therapeutic properties of these genus could be attributed to these compounds.

Al-Awadi *et al.* (2001) studied anti-inflammatory effect of *C. myxa* fruit in experimental colitis model and demonstrated that Sebestan preparation exerts an inhibitory effect on the oxidant stress factors that lead to progression of colitis, resulting in not only improvement

of total antioxidant status level, but also its restoration to normal levels. Furthermore, *C. myxa* fruit is a good source of several elements with respect to their bioavailability. They suggested that anti-inflammatory effect of the *Cordia myxa* fruit may be attributed partly to its antioxidant property and to restoration of the levels of trace elements in the inflamed colon, liver and plasma.

More recently rosmarinic acid and rosmarinic acid ethyl ester of ethanolic extract of *Cordia americana* are proved to exhibit the highest inhibitory effects on the pro-inflammatory mediators p38 and JNK3 (Geller, 2010). However further tests are needed to explore the exact mechanism of action at the molecular level and to know the actual constituents responsible for this activity.

CONCLUSION

Hydro-alcoholic extract of *C. myxa* fruit could serve as an alternative anti-inflammatory therapy in managing inflammatory conditions or as complementary therapy allowing patients to take smaller doses of conventional anti-inflammatory drugs, thereby minimizing the side effects of these standard drugs.

ACKNOWLEDGMENTS

The authors would like to thank Chamran University of Ahvaz for financial supports and Dr. Seyedhossein Hekmatimoghaddam for proofreading of the manuscript.

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