JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

"AN INVESTIGATION OF ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS EXTRACT OF *MALUS SYLVESTRIS* FRUITS IN EXPERIMENTAL ANIMALS"

Ramdas Bhat
Associate Professor, Department of Pharmacology, Karavali College of Pharmacy, Vamanjoor (post), Mangalore, Karnataka, India- 575028

Sujana V Mestha
UG Scholar, Department of Pharmacology, Karavali college of Pharmacy, Vamanjoor (Post), Mangalore, Karnataka, India- 575028

Sahana Nagesh
UG Scholar, Department of Pharmacology, Karavali college of Pharmacy, Vamanjoor (Post), Mangalore, Karnataka, India- 575028

Preeti Shanbhag
UG Scholar, Department of Pharmacology, Karavali college of Pharmacy, Vamanjoor (Post), Mangalore Karnataka, India- 575028

Grinton Josvi Veigas
Assistant Professor, Department of Pharmacology, Karavali College of Pharmacy, Vamanjoor (post), Mangalore, Karnataka, India- 575028

Dr. Ravikumar
Principal, Karavali college of Pharmacy, Vamanjoor (post), Mangalore, Karnataka, India- 575028

CORRESPONDING AUTHOR:
Ramdas Bhat*
Associate Professor, Department of Pharmacology, Karavali College of Pharmacy, Vamanjoor (post), Mangalore, Karnataka, India- 575028,
Email: ramdas21@gmail.com, Mobile: 7795772463

ABSTRACT:

The present study was conducted to examine the anti-inflammatory activity of aqueous fruits extract of *Malus sylvestris* using suitable experimental models. Wister rats were divided into 5 groups of n=6 each using Aspirin (300 mg/kg, p.o) as the standard drug. The Aqueous extract of *Malus sylvestris* was given at three doses of 200 mg/kg, 400 mg/kg and 800 mg/kg p.o. in distilled water respectively. Prepared extract was administered orally once daily for 7 consecutive days one hour prior to the administration of 0.1ml carrageenan injection in sub-plantar region. The volume of the rat paw was measured at the end of 7^{th} day. The present study reveals that the aqueous extract of *Malus sylvestris* at the doses of 400 mg/kg and 800 mg/kg significantly reduced the carrageenan induced paw edema ($P<0.001$) at 3hr and 6hr in a dose dependent manner as compared to the vehicle control. Whereas 200mg/kg does not show any significance with that of control. As a result of the foregoing findings, the aqueous extract of *Malus sylvestris* fruit was found to have considerable anti-inflammatory activity in the carrageenan induced paw edema but was unable to completely prevent the inflammation.

KEYWORD: Aqueous extract of *Malus sylvestris* fruit (AEMS), Paw edema, Carrageenan, Inflammation, Aspirin.

INTRODUCTION:

Nature has provided a complete store-house of remedies to cure all ailments of mankind. Historically, almost all medicines were derived from plants, with the plant serving as man's sole chemist for centuries[1]. A living tissue's response to injury is inflammation, which includes both systemic and local reactions[2]. The process of inflammation is more complicated than is currently thought. It ranges from an acute inflammatory response to an infection in the skin to a chronic inflammatory response that results in remodelling of the artery wall in atherosclerosis. It is diverse and is now recognised as the underlying pathologic process involved in the development of practically all diseases. Rubor, tumour, calor, and dolor were the first four indications of inflammation identified by the Roman author Celsus in the first century A.D. Later, Virchow added the fifth sign function, laesa (loss of function), to these. The symptoms and tissue damage that go along with an inflammatory response can be caused by a variety of factors. The main mediators in inflammatory disorders such rheumatoid arthritis, sepsis, and others are thought to be pro-inflammatory cytokines (TNF- and IL-1B) and nitric oxide (NO)[3]. A large percentage of the global population (80% of people) cannot afford the products of the pharmaceutical industry and must rely on the use of traditional medicines, which are primarily derived from plant material, despite our dependence on modern medicine and the immense advances in synthetic drugs [4].

Malus sylvestris, sometimes known as European Crab apple or common apple, is a member of the Rosaceae family. It is a tiny deciduous tree with a height of 4 to 10 meters. The fruit has a roughly spherical shape, is 2.5 x 2.8 cm, is flattened at each end, and is glossy, pale green with huge white dots that turn flushed or spotted crimson in the fall. The fruit is rich in carbohydrates, total dietary fats, sugars, protiens, fats and minerals. The investigations of the compounds isolated from crab apple includes Polyphenols (Tannins, Anthocyanins), Saponins, Alkaloid and steroid Flavonoids like Procyanidin, quercetin, phloretin, myricetin, and epicatechin are all flavonoids classified under the flavanol (catechin) are present[5]. The plants exert various pharmacological uses such as nerve sedative, relieves anxiety, lowers blood pressure, carminative, digestive, emollient, hypnotic, laxative, refrigerant, antioxidant and Antibacterial. In folklore they are used to treat cancer, malaria, warts, dysentery, fever, scurvy, spasms. Crushed fruits are used in the treatment of inflammation, light wounds, sore throat[6,7,8].

So, the present study was done to examine anti-inflammatory activity of *Malus sylvestris* as it has various active constituents to minimize the side effects caused by synthetic drugs and to promote the use of a novel natural medicinal plant.

MATERIALS AND METHODS:

The fruits of *Malus sylvestris* were obtained from the Mangalore local market and authenticated by botanist Pilikula Nisargadhama, Mangalore. The current study was carried out at Karavali College of Pharmacy's Department of Pharmacology.

ANIMALS:

The following study used Wistar rats of both sexes weighing around 150-200g. The animals were fed a standard pelleted diet (Lipton India Ltd., Mumbai) and distilled water ad libitum under a constant 12 hours light and dark cycle. Prior to the experiment, all animals were housed in laboratory conditions for 5 days. All experiments were carried out in accordance with the ethical standards for the investigation of experimental pain in animals and the guidelines for the investigation of inflammation in conscious animals.

PLANT EXTRACT:

The fruits were washed 2 or 3 times with tap water so that it was made free from all dust materials. They were cut into small pieces and made into a paste with the help of a blender. For the aqueous extract, 500 g of plant material was extracted by infusion boiled water (500 ml) for three days. The respective aqueous extracts were separated from its residues by gravity filtration. The final crude extract (15.2% d.w.) was produced as a brownish greasy powder in terms of dry weight. The extract was kept chilled at 4 – 8 ^{0} C[9].

EXPERIMENTAL DESIGN:

CARRAGEENAN INDUCED PAW-EDEMA TEST: After acclimatization, animals were randomly divided into 5 groups of six mice each (n = 6).

- Group I – Received Distilled water 25ml/kg orally.
- Group II – Received Aspirin 300mg/kg orally.
- Group III – Received 200 mg/kg AEMS fruit orally.
- Group IV – Received 400 mg/kg AEMS fruit orally.
- Group V – Received 800 mg/kg AEMS fruit orally.

Group 1 served as control (vehicle treated), Group II served as standard (received Aspirin 300mg/kg body weight); Group III, Group IV and Group V were treated with aqueous extract of *Malus sylvestris* fruit as 200mg/kg, 400 mg/kg, 800mg/kg body weight. The test extract was administered in distilled water and taken orally. The drugs were given orally 1hr prior to the administration of the 0.1 ml carrageenan (1% w/v in water) injected into the sub-plantar are of the right hind paw and treatment

were continued for 7 consecutive days. On the 7th day the paw edema was measured using plethysmometer during the treatment period. The anti-inflammatory activity is measured by comparing the mean hind paw swelling to the initial hind paw thickness.

RESULTS AND OBSERVATION:

In the test all the extracts of AEMS 200mg/Kg, 400mg/kg, 800mg/kg reduce the paw edema volume at the time of 6hrs to 0.35±0.004ml, 0.33±0.004ml and 0.25±0.004ml respectively but unable to prevent the inflammation completely. The AEMS at the dose of 400mg/kg and 800mg/kg significantly ($P < 0.001$) shows anti-inflammatory activity and reduced the carrageenan induced paw edema at the 3 hr and 6 hr when compared to the control.

Group	Treatment	Paw Edema Volume (ml)		
1.	Control	0.49±0.001	0.47±0.004	0.25±0.004
2.	Standard	0.42±0.001^{ns}	0.16±0.047	0.08±0.003***
3.	Test-1	0.47±0.001^{ns}	0.39±0.007^{ns}	0.35±0.004^{ns}
4.	Test-2	0.46±0.000^{ns}	0.33±0.015**	0.33±0.004**
5.	Test-3	7.53±7.093***	0.28±0.009***	0.25±0.004***

Table no.1; The effect of aqueous fruit extract of *Malus sylvestris* on Carrageenan-induced paw edema.
Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test. **P < 0.01, ***P < 0.001 vs. control

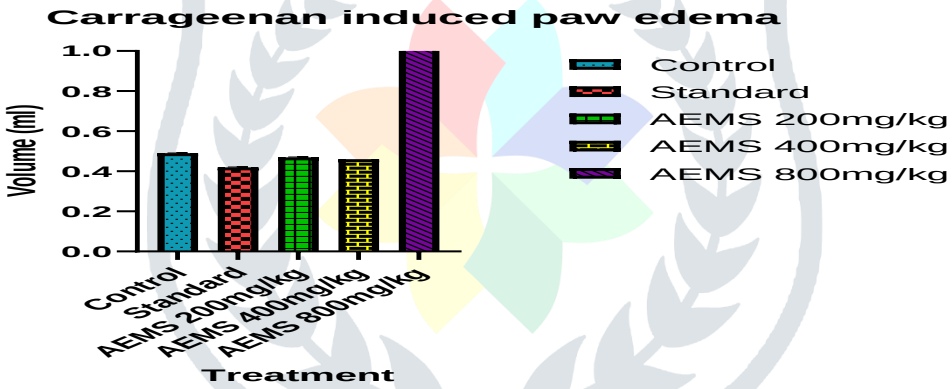

Figure 1. Comparative profile of volume of paw edema at 0 hr after oral administration 200, 400, 800mg/kg of AEMS.

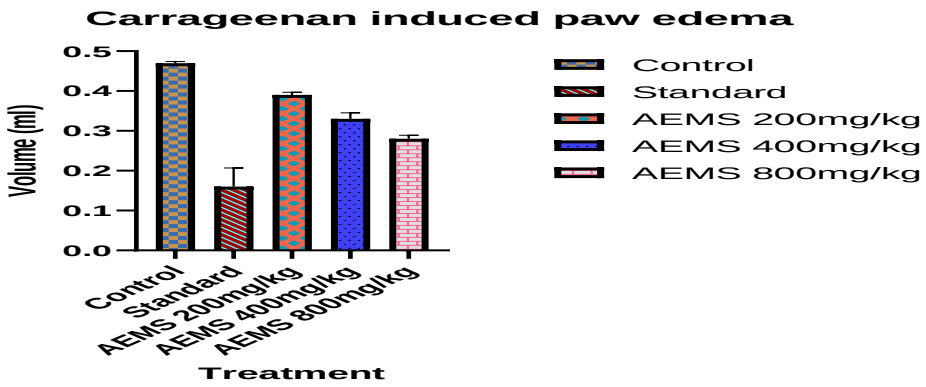

Figure 2. Comparative profile of volume of paw edema at 3 hr after oral administration 200, 400, 800mg/kg of AEMS.

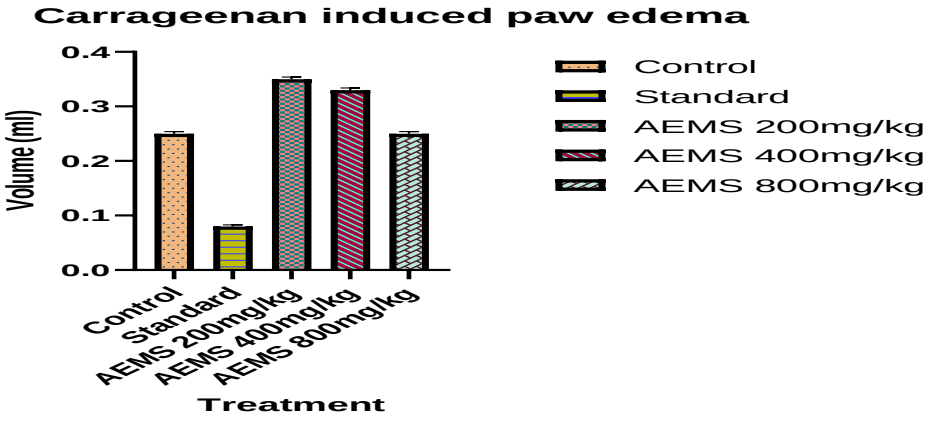

Figure 3. Comparative profile of volume of paw edema at 3 hr after oral administration 200, 400, 800mg/kg of AEMS.

DISCUSSION:

The goal of this research was to determine the anti-inflammatory activity of an aqueous extract of *Malus sylvestris* fruits. Because of its biphasic nature, the carrageenan-induced paw edema model is widely used for the screening of NSAIDs and inflammation, with the release of chemical mediators such as serotonin, bradykinin, and histamine at I-Phase followed by prostaglandin release at II-Phase.

Histamine is an important chemical mediator of inflammation in addition it is a potent vasodilator that causes increased vascular permeability. In both phases, the release of these chemical mediators causes pain and fever, and both extracts significantly reduced paw edema in the I-Phase of the inflammation, indicating their effect on prostaglandins[10].

The current study found that aqueous extract of *Malus sylvestris* fruits at doses of 400 mg/kg and 800 mg/kg significantly reduced carrageenan-induced paw edema ($P<0.001$) at 3hr and 6hr when compared to the vehicle. Whereas 200mg/kg has no significance when compared to the control. As a result of the preceding findings, the aqueous extract of *Malus sylvestris* fruits was found to have significant anti-inflammatory activity in the carrageenan-induced paw edema control but was unable to completely prevent the inflammation.

CONCLUSION:

We can conclude from the above study that the aqueous extract *Malus sylvestris* fruits demonstrated significant dose-dependent anti-inflammatory activity in carrageenan-induced paw edema, making it a novel promising medicinal plant with diverse anti-inflammatory effects that are free of the side effects of conventional anti-inflammatory drugs, but it was discovered that it cannot completely reduce the inflammation. However, more research is needed to determine the specific mechanism and active principles responsible for its anticonvulsant properties.

CONFLICT OF INTEREST:

No conflict of interest.

ACKNOWLEDGEMENTS:

Authors are thankful to Principal and Management Karavali college of Pharmacy, Vamanjoor, Mangalore for providing necessary facilities for research.

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