Journal of Addiction Research & Therapy
ISSN: 2155-6105
Research Article Open Access

In-Vivo Anti-inflammatory Potential of *Malvastrum Tricuspidatum* in Addiction

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Abstract

Anti-inflammatory activity of plant *Malvastrum Tricuspidatum* was evaluated by in-vivo (carrageenan induced paw edema in rats) method. To evaluate anti-inflammatory activity; rats were divided into four groups each having six rats. Group I served as carrageenan control group and received carrageenan only. Group II received aqueous plant extract (200 mg/kg), Group III received aqueous plant extract (400 mg/kg) and Group IV received Indomethacin (10 mg/kg, p.o). Paw volume was measured using digital Plethysmometer at 1, 2, 3 and 4hr. Percentage inhibition of paw edema of each group were determined. The oral administration of 200 mg/kg, 400 mg/kg of the plant extract significantly ($p < 0.05$) inhibited inflammatory response induced by carrageenan in rats in a dose related manner. The most prominent inhibition was recorded for 400 mg/kg (45.45%) at 4hr of study while standard indomethacin showed 67.80% at dose of 10 mg/kg. The study shows that plant extract increase % inhibition in paw volume of rats. Plant extract have various phytoconstituents which may be responsible for anti-inflammatory activity but further investigation are required to isolate bioactive compounds to prove anti-inflammatory effect of plant.

Keywords: Addiction Research; Addiction Therapy; Addiction; Anti-inflammatory activity; *Malvastrum tricuspidatum*; Carrageenan; Indomethacin

Introduction

Inflammation is a localised defensive response of mammalian tissues towards allergic or chemical irritation, damage, and infection [1]. Inflammation is characterized by pain, redness, heat, and swelling [2]. Increased levels of endogenous biological substances such as nitric oxide, reactive oxygen species, prostaglandin E2, and cytokines are linked to the development of inflammation. [1] Increased vascular permeability, protein denaturation and membrane modification are all concerned with inflammation. [3] Cyclooxygenase (COX) is a key enzyme in the synthesis of prostacyclins, prostaglandins, and thromboxanes, all of which are involved in inflammation, pain, and platelet aggregation. Vasoactive compounds enhance the permeability of arterioles, allowing blood cells, chemical substances, blood proteins, and fluid to accumulate in that location. This fluid buildup causes swelling and can be painful because it compresses nerves in the region. Prostaglandins may also produce nerve irritation and contribute to pain [4]. Uncontrolled inflammatory reactions may have a role in the onset and development of inflammatory disorders such as atherosclerosis, rheumatoid arthritis, cardiovascular disease, and cancer [5]. In addition to these diseases prostatitis, anaphylaxis, diabetes, chronic renal disease, digestive disorders, alzheimer's disease, bacterial infections, and new corona virus infection are all linked with inflammation, particularly at the cellular level, which can lead to major health issues [6]. Inflammatory free radicals assault biomembranes and macromolecules, exacerbating inflammatory consequences. Furthermore, free radicals such as peroxyl and hydroxyl are extremely reactive molecules that include charged nitrogen and oxygen ions, causing oxidative stress if not well controlled. Antioxidants are utilized to prevent oxidative stress by scavenging free radicals and protecting tissues from oxidative damage. However, synthetic antioxidants such as propyl gallate, butylated hydroxytoluene, and butylated hydroxyanisole have been linked to carcinogenicity, hepatotoxicity, and other undesirable effects in patients, as well as being more labile and inaccessible that limiting their utility. As a result, antioxidant supplements derived from natural sources such as herbs and fruits provide a feasible alternative for combating oxidative stress in the body, particularly during inflammation[7]. Herbal medicines have been used for a long time to prevent and treat disorders, including inflammation. Many people are now use herbal treatments in their daily lives as Phytonutrients or nutraceuticals, and the usage of herbal pharmaceuticals and Phytonutrients or nutraceuticals is spreading quickly over the world. According to the World Health Organization (WHO), three-quarters of people get their daily healthcare from traditional and plant-based medicine. Herbal medications are becoming increasingly popular since they have fewer adverse effects than synthetic pharmaceuticals [8]. A wide range of plants have been employed for medicinal reasons throughout antiquity. Most plant components have been extracted and may have anti-inflammatory and antioxidant qualities. Use of plant extract can protect against chronic inflammation by down regulating inflammatory mediators. Plant bioactive principles can also modulate oxidative stress [9]. The active phytochemical elements of herbal formulations or medications, such as alkaloids, flavonoids, terpenoids, phenols, polyphenols, tannins, saponins, polysaccharides, proteins, lipids, and peptides, are responsible for their pharmacological activity [10]. *Malvastrum Tricuspidatum* (Malvaceae family) is also known as False mallow or kharenti [11]. Malvastrone is found in the leaves of *Malvastrum Tricuspidatum*. Phenylethylamine, dotriacontane, dotriacontanol, sitosterol, stigmasterol, campesterol, lutein, N-methyl-phenylethylamine, and an unidentified indole alkaloid are found in the aerial portions[12]. Palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, malvalic acid, and sterculic acid are also found in the plant. *Malvastrum Tricuspidatum* has several therapeutic benefits, including the application of the leaves to inflamed sores and

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Received: 07-Sep-2022, Manuscript No. jart-22-77394; **Editor assigned:** 09-Sep-2022, PreQC No. jart-22-77394(PQ); **Reviewed:** 23-Sep-2022, QC No. jart-22-77394; **Revised:** 24-Sep-2022, Manuscript No. jart-22-77394 (R); **Published:** 30-Sep-2022, DOI: 10.4172/2155-6105.100489

Citation: Gaurav S (2022) In-Vivo Anti-inflammatory Potential of *Malvastrum Tricuspidatum* in Addiction. J Addict Res Ther 13: 489.

wounds, the administration of a plant decoction in dysentery, and the use of the flowers in cough, chest, and lung disorders. *Malvastrum Tricuspidatum* is used as an anti-inflammatory, analgesic and in the treatment of jaundice and ulcers as mentioned in traditional Indian system of medicine [11]. Numerous tribal tribes throughout the world use various components of this plant. The crushed leaves of this plant, along with salt or alcohol, are used by Mexican Kickapoo Indians to treat ringworm infection. The Bhil tribes of Rajasthan use a decoction of this herb to treat jaundice. This plant's leaf infusion is used to treat diabetes in Mexico. Pharmacological testing revealed that this plant has antinociceptive, analgesic, and antibacterial properties [13, 14]. As mentioned in the Indian traditional system of medicines that plant possesses anti-inflammatory activity but not proved scientifically so in the present study we evaluate anti-inflammatory activity of plant *Malvastrum Tricuspidatum* by using invivo animal models to justify its traditional use in experimental animals (Figures 1 and 2).

Materials and Methods

Plant

Fresh leaves of plant *Malvastrum Tricuspidatum* were procured from Maharashtra and were authenticated. The voucher specimens were submitted and preserved as Institute Herbarium Nos: LORDS/2022/PCOG/08 Herbarium sample was also preserved.

Animals

The study used healthy Wistar albino male rats weighing between 150 and 200 gram. They were kept in temperature-controlled circumstances (22-30°C), with a relative humidity of at least 30% but no more than 70%. (Other than during room cleaning). According to OECD Guideline 423, lighting was artificial with 12 hour light and 12 hour dark cycles. All animals received a standard pellet diet and water. The present work was carried out with a prior permission by IAEC of Lords University with CSPSEA registration number 1386/PO/Re/S/10/CPCSEA.

Chemicals

All substances necessary to assess anti-inflammatory activity were acquired at Jaipur local market and are of standard grade.

Aqueous leaf extraction of Malvastrum Tricuspidatum

Malvastrum Tricuspidatum aqueous leaf extraction: We obtained aqueous extract of *Malvastrum Tricuspidatum* using the Soxhlet equipment with hot continuous extraction technique. The dry and powdered medication was packaged. The Soxhlet device is a continuous, automated process that does not require any more operation. This procedure is not time-consuming, as the extraction period for a standard-sized sample is 48 hours. The extract was refrigerated until further investigation [15].

Phytochemical Characterization

Aqueous extract were subjected to general phytochemical analysis for the presence of carbohydrates, proteins, amino acids, tannins, phenolic flavonoids, alkaloids, anthraquinone, glycosides, saponins, and steroidal nucleus using the standard methods [16-18].

In- vivo anti-inflammatory activity

Carrageenan was formed as 1 percent W/V solution in 0.9 percent saline no more than 24 hours before use. The lambda form does not gel at room temperature and is injectable to elicit an inflammatory reaction. The protocol was followed for performing an anti-inflammation experiment in inflamed rodents generated by carrageenan.

Procedure

Paw edema was produced by injecting 0.1 ml of 1% w/v carrageenan suspended in 1% CMC into the sub-plantar tissues of each rat's left hind paw. The rats were put into four groups of six animals each. Group I Carrageenan as the control, Group II Aqueous extract (200 mg/kg), Group III Aqueous extract (400 mg/kg), and Group IV Indomethacin (10 mg/kg) as the standard reference. The paw volume was determined by immersing the foot in the plethysmograph mercury bath before injecting the carrageenan, as well as after 1, 2, 3, and 4 hours. The anti-inflammatory activity was measured as a percentage reduction of oedema in animals administered with the extract under test versus a carrageenan control group. Food was withheld from 12 hours before medication delivery till the trial was completed. Following a 60-minute injection of 0.1 ml of 1 percent w/v carrageenan into the subplantar area of the left paw, the test and control drug were administered [19,20]. Immediately following carrageenan injection in both the control and other treatment groups (Vt). The percentage of inhibition for each group was calculated using the formula.

$$\% \text{ Inhibition of Paw edema} = \frac{Vc - Vt}{Vc} \times 100$$

Where Vc and Vt represent average Paw volume of control and treatment animal respectively [21].

Statistical analysis

After submitting the data gathered from numerous investigations, the results were interpreted. Graph pad Prism 9.0.2 was used for

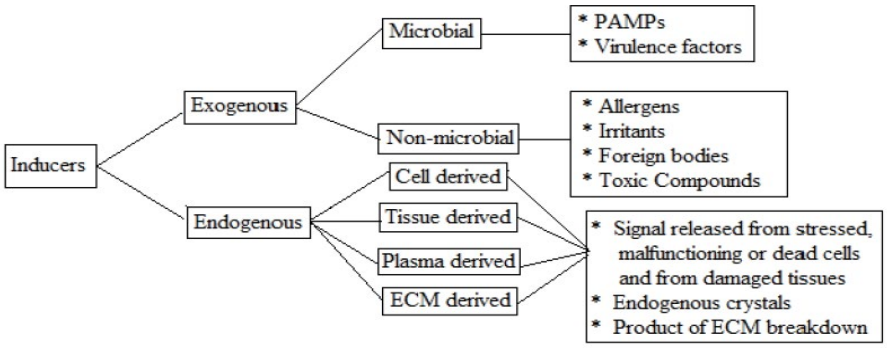

Figure 1: Various inducers of inflammation.

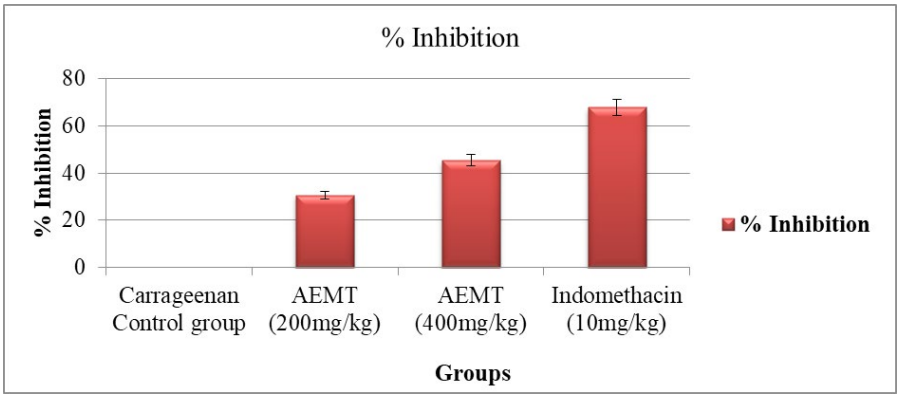

Figure 2: Effect of aqueous extract of plant on carrageenan induced paw edema in rats.

Source: AEMT - Aqueous extract of *Malvastrum Tricuspidatum.*

Table 1: Effect of aqueous extract of plant *Malvastrum Tricuspidatum* on carrageenan induced paw edema in rats.

Sr. No	Treatment	1hr	2hr	3hr	4hr	Average	% Inhibition
1.	Carrageenan Control Group	1.34±0.1	2.37±0.118	3.64±0.147	3.23±0.161	2.64	---------
2.	AEMT (200mg/kg)	1.14±0.103	1.74±0.217*	2.50±0.106*	1.97±0.115*	1.83	30.68
3.	AEMT (400mg/kg)	1.00±0.12	1.54±0.163*	1.81±0.099*	1.41±0.188*	1.44	45.45
4.	Indomethacin Control Group	0.59±0.118*	0.8±0.121*	1.14±0.125*	0.9±0.118*	0.85	67.80

Values are Mean ± Standard Deviation; where *P<.05, **P<.01, ***P<.001 when compared to control group
AEMT – Aqueous extract of *Malvastrum Tricuspidatum*

statistical analysis, which comprised one-way ANOVA followed by tests such as Dunnett and t-test. P<0.05 is deemed statistically significant.

Result

Phytochemical investigation shows that aqueous extract of plant *Malvastrum Tricuspidatum* known to possess Carbohydrate, steroids, flavonoids, tannins and glycosides. Paw volume of various treated group at 1, 2, 3, and 4hr and their % inhibition are tabulated in Table 1, result shows that aqueous extract at dose 200mg/kg and 400mg/kg shows 30.68 % and 45.45 % inhibition in paw volume respectively. Standard drug indomethacin (10mg/kg) shows 67.80 % inhibition in paw volume. Result obtained was statistically significant (p<0.05).

Discussion

Inflammation is a process of the organism's immunological defence in reaction to an external (burn, infection, allergy, trauma) or endogenous (infection, allergy, trauma) aggression (cancer cells or autoimmune pathologies). Inflammation is defined at the tissue level by redness, swelling, heat, discomfort, and loss of tissue function as a result of local immunological, vascular, and inflammatory cell responses to infection or damage. Vascular permeability alterations, leukocyte recruitment and accumulation, and inflammatory mediator release are all important microcirculatory processes that occur throughout the inflammatory phase [22]. The aim of the inflammatory response is the prevention and elimination of the infecting agents from the organism, reparation of damaged tissues and prevention of further disease development. Although many factors can trigger the inflammation, the mechanisms of the immune system response are generally common for all of them [23]. Chronic inflammation is the important cause of many chronic illnesses, including autoimmune diseases, allergies, metabolic syndrome, cardiovascular dysfunction, and cancer [24]. Acute inflammation progresses in two stages the first phase begins with the release of histamine, serotonin, and kinins. The second phase is associated with the production of prostaglandin-like compounds within 2-3 hours. The second phase is responsive to both steroidal and nonsteroidal anti-inflammatory agents that are therapeutically beneficial. Prostaglandins are the primary cause of acute inflammation. Carrageenan is a powerful molecule that promotes the production of inflammatory and proinflammatory mediators [25]. Many anti-inflammatory treatments, including as corticosteroids and nonsteroidal anti-inflammatory medicines, are available to treat this, however these synthetic agents have side effects such as GIT damage, gastric erosions, and, in extreme cases, serious bleeding and death [26]. In addition to anti-inflammatory effect, NSAIDs also have antipyretic and analgesic properties. These medications inhibit COXs enzymes, which are rate-determining enzymes for prostaglandins and other prostanoids synthesis [27]. Plants are the primary source of compounds used in the creation of new medications [28]. Several studies have been conducted in order to identify natural bioactive compounds with anti-inflammatory properties. Secondary metabolites of medicinal plants, such as polyphenols, flavonoids, terpenoids, and alkaloids, are important sources for anti-inflammatory drug development. According to recent research, these molecules give anti-inflammatory properties by suppressing inflammatory mediators involved in inflammatory processes, such as cytokines, chemokines, and pro and neo-inflammatory mediators [29,30]. According to the study, aqueous extract of the plant *Malvastrum Tricuspidatum* contains a variety of phytoconstituents such as steroids, flavonoids, tannins, and glycosides etc. Table 1 shows that drug extracts at 200 mg/kg and 400 mg/kg enhance the percent inhibition in paw volume by 30.68% and 45.45%, respectively. This might account for the anti-inflammatory action of aqueous extracts at the given dosage.

Conclusion

Our findings indicate that *Malvastrum Tricuspidatum* has significant antiinflammatory efficacy. More research into the separation of the bioactive chemical compound of the plant *Malvastrum Tricuspidatum*, as well as research into biochemical pathways, may result in the creation of an effective anti-inflammatory medicine with minimal toxicity.

Acknowledgement

Providing research facility from faculty of Pharmacy, Lords University, Alwar is gratefully acknowledged.

Conflict of Interest

Nil

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