INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR)
ISSN: 2231-6078
11(4): Oct.-Dec.: (2021), 1047-1052

Research Article

FORMULATION AND DEVELOPMENT OF HERBAL TOPICAL DRUG DELIVERY SYSTEM FOR ANTI-INFLAMMATION

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Abstract

Herbal formulations have growing demand in the world market. The present study was designed as formulation and evaluation of *salix alba* herbal tablet prepared. Tablet was prepared by direct compression method. The present communication also deals with the evaluation of formulated tablets (weight variation, friability, hardness and disintegration time. *Salix alba* contains a substance (salicine) that is converted by the body into a salicylate similar to the blood-thinner aspirin. *Salix alba* have anti-thrombotic action which can be use in Post Covid complication related to blood clot in place of aspirin.

Keyword : Herbal Topical Drug Delivery System, Anti-Inflammation, Herbal drug, Anti-Inflammation drug.

Introduction

Inflammation is implicated in the pathology of several debilitating diseases and pain is the most common symptom that occurs individually or often in association with inflammation. Acute inflammation, if remain unchecked, can develop into chronic state and leads to the initiation and progression of diseases like cancer, rheumatoid arthritis, diabetes and inflammatory bowel diseases. Pain of various types and origin, represents the condition that need therapeutic attention. The use of currently available anti-inflammatory and analgesic therapies is associated with various adversities. Therefore, the search for novel anti-inflammatory and analgesic drugs from natural sources is rational and productive strategy towards the cure of inflammatory and painful conditions (Sofidiya et al., 2014, Uddin et al., 2014).

Inflammation And Pain

Majority of human population has been affected by several inflammation related disorders and painful conditions. Inflammatory diseases and pain are important and global healthcare issues that demands for careful therapeutic attention. Chronic inflammatory diseases and related pain of diverse origin has been characterized as clinical, social and economic burden to the society and still its pharmacotherapy is the unsettled issue. There is an exceptional and vital need to develop anti-inflammatory and analgesic agents with novel mechanisms of action, which is a very challenging job. Translational gap between preclinical experimental data and clinical results, limitations of investigational techniques and species differences are major hurdles for the development of novel anti-inflammatory and analgesic therapies (Botz et al., 2017).

Triterpenoids And Natural Products A The Anti-Inflammatory And Analgesic Drugs

Discovery of novel biologically active phytoconstituents obtained from natural sources appears to be the important subject of many scientific investigations and pharmaceutical companies. Search of anti-inflammatory and analgesic drugs from natural sources is promising with respect to the cure of inflammatory diseases and painful conditions. Natural products containing pentacyclic triterpenoids are biocompatible and cost effective alternatives for the treatment of inflammatory diseases and management of pain. Historically, medicinal plants have established their worth as a source of therapeutically active molecules. Medicinal plants offer vast pool of phytoconstituents with diverse chemical scaffolds for the exploration of novel drug leads. In the last few decades, focus of major blue chief pharmaceutical industries for the search of new drug molecule was mainly centered towards the screening of synthetic compound libraries. Synthetic compounds are compatible with high throughput screening platforms and are easy to produce and resupply. Despite these advantages, this discovery approach has resulted in the decreased number of new drug approvals. Therefore, the interest of scientific community towards the discovery of drugs from natural sources has been renewed, instead of its recognized challenges (Atanasov et al., 2015).

Plant Profile

The Cucurbitaceous are mostlyy prostrate or climbing herbaceous annuals comprising about 125 genera and 960 species that are further characterized by commonly having 5-angled stems and coiled tendrils (Bates *et al*.,1990).

Figure 1: *Praecitrullus fistulosus* plant

.1 **Botanical Name:** ***Praecitrullus fistulosus (Stocks)*** **Pangalo**

Kingdom:	Plantae – Plants
Subkingdom:	Tracheobionta – Vascular plants
Super division:	Spermatopyta – Seed plants
Division:	Magnoliophyta – Flowering plants
Class:	Magnoliopsida – Dicotyledons
Subclass:	Dilleniidae
Order:	Cucurbitales
Family:	Cucurbitaceae
Sub Family:	Cucurbitoideae
Tribe:	Benincaseae
Sub Tribe:	Benincasinae
Genus:	Praecitrullus
Species:	*P. fistulosus*

Research Methodology

The fresh leaves of *Praecitrullus fistulosus*, were collected during March and April 2021 from Bhopal.The collected fresh leaves (18 g) were washed, shade-dried and powdered. For the preparation of aqueous, methanol extracts, 5 g of dried powder of the leaves were extracted using a maceration method for 5 days. The extract was filtered through membrane filters (0.45 µ), with the aid of suction pump. The yield based on dry weight was: aqueous extract 25.98% (w/w), methanol extract 2.74% (w/w). The filtrate was concentrated under reduced pressure, reconstituted in a minimal volume (less than 2%) of dimethyl sulphoxide (DMSO) and diluted in sterile water to a final concentration of 50 mg/ml. The DMSO solubilized forms of each of the extracts were stored at room temperature and used for the following bioassays.

Method

Maceration

The process consist of keeping the crude drug in intimate contact with whole of the menstrum in a closed vessel with occasionally shaking for 7 days, straining, pressing the marc. Mixing the liquid, & finally clarifying by subsidence or filtration. The drug should be properly communicated. The cellular structure get penetrated & the soluble portion are softening & dissolved. Occasionally shaking bring about a rapid equilibrium between the intra and extracellular fluid. A closed vessel is recommended so as to prevent loss of menstrum. As the degree of pressing the marc may very the final product in not adjusted to any complete extraction. The drug menstum ratio is 1:10. a sediment may form on standing for a few day before use. Maceration process is very simple & does not require a skilled operator.

Collection of plant

The plant material was collected from local area of Bhopal in february 2021 and was authenticated at the Department of Botany, Barkatullah University Bhopal. A voucher specimen number or the herbarium number is Bot/Her/Bl763 has been deposited.

Authenticated by

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Vehicle

Distilled water is the only vehicle was used for the whole experiment

Preparation of the extract

The preparation of extract was carried out according to the method. Briefly, the stem bark of Praecitrullus fistulosus was shade dried after collection for 5 days and was powdered. Approximately 0.95 kg of powdered drug material was extracted using 99% pure ethanol in the ratio of 1:2 (w/v) in a Soxhlet apparatus. The extract obtained (EBFR) was dried in a steam bath and the dried mass was weighed and recorded. The percentage of yield was calculated. The weight of dried crude extract obtained was approximately 0.16 g which commemorated with the percentage yield of 17.16%.

Experimental animals

Wistar rats weighing 130-165g were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) under 12 h light/dark cycle and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the commencement of experiment. The experimental protocol was approved by the Institutional Animal Ethical Committee,RKDF College of Pharmacy, Bhopal Madhya Pradesh, India.

LABORATORY MODELS

(A) ANTI-INFLAMMATORY SCREENING

The present study involved anti-inflammatory evaluation of fraction, isolated from the ethanol extract of Praecitrullus fistulosus isolated from the stem bark at the oral doses of 200mg/kg in experimental models of acute, chronic and immune inflammation. Acute anti-inflammatory activity was studied using carrageenan,

induced paw edema models. The cotton pellet assay and oxazolone induced contact dermatitis model were executed to study the effects of Praecitrullus fistulosus in chronic inflammation and delayed type hypersensitivity, respectively.

Chronic Inflammation

Cotton Pellet Induced Granuloma In Mice

The effect of BREAF and CA on chronic inflammation was evaluated through cotton pellet induced granuloma test in male C57BL/6 mice (Samy et al., 2006). Mice were divided into different groups, each containing six animals. Under anesthesia, sterile cotton pellets weighing 10 ± 1 mg were implanted subcutaneously and bilaterally in the groin region of mice through a single surgical blade incision (Hosseinzadeh et al., 2003, Boonyarikpunchai et al., 2014). Prior to implantation, the cotton pellets were soaked in 0.2 mL of water for injection containing 0.13 mg streptomycin and 0.1 mg penicillin. The control group animals were treated with the vehicle. The standard drug treated group received dexamethasone (1 mg/kg/day, p.o.) for 7 days. Other groups received either BREAF (5, 10 & 20 mg/kg/day, p.o.) or CA (5, 10 & 20 mg/kg/day, p.o.) for 7 days starting from the day of pellet implantation (Park et al., 2007). On 8th day mice were sacrificed by the overdose of anesthetics. The granulomas were carefully dissected out, weighed and dried at 60 °C to constant weight. The increase in wet and dry weight of pellets were recorded as a measure of granuloma formation. Percentage protection from granuloma development was calculated using following formula:

% Protection = [(Mean granuloma weight in control group − Mean granuloma weight in test group) / (Mean granuloma weight in control group)] × 100

(B) ANALGESIC SCREENING

Praecitrullus fistulosus were screened for the analgesic activity in various peripheral and central models of pain in experimental animals. Peripheral analgesic action was evaluated using acetic acid-induced writhing test in mice. Whereas, animal models like hot plate test, tail immersion test, formalin induced pain and capsaicin induced pain were used to evaluate the efficacy of Praecitrullus fistulosus in controlling the pain involving central mechanisms. Furthermore, mechanism based animal models are used and opioid involvement was studied using hot plate and tail immersion tests to delineate the likely mechanisms of action of Praecitrullus fistulosus involved in their analgesic activities.

Central Pain Models

Hot Plate Test In Mice

Hot plate test was performed as described previously by (Eddy and Leimbach, 1953) with brief modifications. Swiss albino mice were randomly divided into different groups consisting of 6 mice in each. Animals were placed on a hot plate maintained at 55 ± 1 °C and their pain responses (i.e. hind-paw licking and jumping) were observed. The time that elapsed between the placement of animal on the platform of apparatus and its pain reaction was recorded as the response latency (Rejón-Orantes et al., 2013). Basal latency was measured, and only animals that presented a basal latency between 7-15 sec were used. The cut-off time was fixed at 30 sec to avoid skin damage. After 30 min the mice were orally treated with the Praecitrullus fistulosus (200 mg/kg, p.o.) or vehicle or with fentanyl (0.1 mg/kg, i.p.) (Rejón-Orantes et al., 2013). Hot plate latency was recorded at 30 and 60 min after treatment with the Praecitrullus fistulosus or vehicle or fentanyl. Hot-plate latencies were then converted to percentage (%) of the maximal possible effect (% MPE) (Pietrovski et al., 2006, Souza et al., 2015).

% MPE = (postdrug latency — basal latency) / (cut-off time — basal latency) × 100

ANTI-INFLAMMATORY SCREENING

The present study involved anti-inflammatory evaluation of fraction, isolated from the ethanol extract of Praecitrullus fistulosus isolated from the stem bark at the oral doses of 200mg/kg in experimental models of acute, chronic and immune inflammation. Acute anti-inflammatory activity was studied using carrageenan, induced paw edema models. The cotton pellet assay and oxazolone induced contact dermatitis model were executed to study the effects of Praecitrullus fistulosus in chronic inflammation and delayed type hypersensitivity, respectively.

Chronic Inflammation

Praecitrullus fistulosus inhibited granuloma formation in cotton pellet model

Inflammation and granuloma develops in cotton pellet granuloma model during the course of several days. Chronic anti-inflammatory effect of Praecitrullus fistulosus was studied at the doses of 200 mg/kg, p.o. by using cotton pellet granuloma test in C57BL/6 mice and results are presented in **Table 1.** The formation of edematous and proliferative deposits around implants were reduced in Praecitrullus fistulosus (200 mg/kg, p.o.) and dexamethasone (1 mg/kg, p.o.) treated groups. Praecitrullus fistulosus treatment significantly reduced both wet ($P = 0.0003$) and dry ($P < 0.0001$) weights of the granuloma. Statistically significant reduction in granuloma formation by Praecitrullus fistulosus was recorded at all the tested doses of 200 mg/kg, p.o. ($P < 0.01$). Praecitrullus fistulosus demonstrated dose dependent decrease in granuloma weights with highest activity observed at the dose of 200 mg/kg, p.o. which was still less than the effect of standard drug dexamethasone **(Table 1).**

Table 1: Chronic anti-inflammatory activity of Praecitrullus fistulosus (PF) in cotton pellet test

Group	Wet weight (mg)	Dry weight (mg)
Control	157.0 ± 10.0	74.0 ± 4.6
PFAQ. (200 mg/kg/day, p.o.)	123.0 ± 3.5** (22.2)	58.0 ± 3.3** (23.3)
PFEE (200 mg/kg/day, p.o.)	114.0 ± 5.4** (28.0)	47.0 ± 3.1** (34.7)
Dexamethasone (1 mg/kg/day, p.o.)	106.0 ± 5.2** (32.4)	42.0 ± 3.4** (42.8)

Values represent mean ± S.E.M. The values in parenthesis represents percentage protection from granuloma formation, n=6 animals per group. One way ANOVA (***P = 0.0003 for wet granuloma weight and ***P < 0.0001 for dry granuloma weight). *P < 0.05, **P < 0.01 as compared with control group (One way ANOVA followed by Dunnett's multiple comparison post hoc test).

NOTE

PFAQ- Praecitrullus fistulosus Aquous Extract
PFEE- Praecitrullus fistulosus Ethanolic Extract

ANALGESIC SCREENING

Praecitrullus fistulosus were screened for the analgesic activity in various peripheral and central models of pain in experimental animals. Peripheral analgesic action was evaluated using acetic acid-induced writhing test in mice. Whereas, animal models like hot plate test, tail immersion test, formalin induced pain and capsaicin induced pain were used to evaluate the efficacy of Praecitrullus fistulosus in controlling the pain involving central mechanisms. Furthermore, mechanism based animal models are used and opioid involvement was studied using hot plate and tail immersion tests to delineate the likely mechanisms of action of Praecitrullus fistulosus involved in their analgesic activities.

Central Pain Models

Praecitrullus fistulosus demonstrated analgesic activity in hot plate test

Hot plate test was used to study the effect of centrally acting analgesics in increasing the reaction time of mice in response to thermal stimuli. The results of analgesic activity of Praecitrullus fistulosus in hot plate test are shown as **Table 2.** No significant difference in response to thermal stimulus was observed in the mice treated with vehicle throughout the entire time of observation. Praecitrullus fistulosus exhibited dose dependent effect against thermally induced pain in the mice. This inhibition was statistically significant (P < 0.01) in the mice treated with Praecitrullus fistulosus at the dosage of 200 mg/kg, p.o. at 30 and 60 min after the oral treatments as compared with vehicle treated group. Praecitrullus fistulosus at the lowest dose of 5 mg/kg, p.o. also showed significant (P < 0.01) analgesic activity at 60 min following drug treatment. Percentage maximal possible effect (% MPE) of Praecitrullus fistulosus (200 mg/p.o.) and fentanyl (0.1 mg/p.o.) treated mice after 30 min of drug administration was 7.6%, 14%, 23% and 85%. Whereas, % MPE at 60 min of drug administration was 13%, 24%, 40% and 48%, respectively. Due to transient action of Fentanyl (0.1 mg/kg, i.p) % MPE was decreased after 60 min when compared to % MPE at 30 min. However, time dependent increase in % MPE was observed in Praecitrullus fistulosus treated mice at all time intervals in dose dependent manner **(Table 2).**

Table 2: Maximal possible effect of Praecitrullus fistulosus in hot plate test in mice

Group	% Maximal Possible Effect (MPE)	
	30 min	60 min
Control	2.9 ± 0.6	3.23 ± 0.5
PFAQ (200 mg/kg/day, p.o.)	16.0 ± 1.8**	26.0 ± 1.4**
PFEE (200 mg/kg/day, p.o.)	24.0 ± 2.8**	42.0 ± 2.2**
Fentanyl (0.1 mg/kg, i.p)	86.0 ± 3.9**	48.0 ± 2.2**

Values represent mean ± S.E.M. n=6 animals per group. One way ANOVA (***P < 0.0001). **P < 0.01 as compared with control group (One way ANOVA followed by Dunnett's multiple comparison post hoc test)..

NOTE

PFAQ- Praecitrullus fistulosus Aquous Extract
PFEE- Praecitrullus fistulosus Ethanolic Extract

Discussion

Fruits of Praecitrullus fistulosus and leaves of Praecitrullus fistulosus have been traditionally used in Indian system of medicine for the treatment of pain and inflammatory conditions. In this study, a fraction of aquous and ethanolic extract isolated from the leaves of Praecitrullus fistulosus were methodically investigated for anti-inflammatory and analgesic activities using experimental models of inflammation and pain. Praecitrullus fistulosus A exhibited dose dependent anti-inflammatory activity in cotton pellet models of inflammation at the oral doses of 200 mg/kg. Praecitrullus fistulosus inhibited the phases of cotton pellet granuloma model inflammation. Likewise, reduction in paw inflammation by Praecitrullus fistulosus was also evident in exogenous histamine and serotonin-induced inflammation in rats. Results of cotton pellet granuloma model are in close agreement and suggested

the ability of Praecitrullus fistulosus to inhibit acute phase mediators of inflammation.

Reduction in formation of cotton pellet granuloma either by Praecitrullus fistulosus treatment demonstrated inhibition of proliferative changes associated with chronic inflammation.

Analgesic activity of Praecitrullus fistulosus against thermally induced pain was studied by using hot plate tests. Praecitrullus fistulosus increased the pain threshold and maximal possible effect (% MPE) in these models, which suggests the efficacy of these pentacyclic triterpenoids (PTs) in controlling pain of central origin. As, the concurrent administration of opioid receptor antagonist naloxone with Praecitrullus fistulosus resulted in reversal of their analgesic effects, it endorse the involvement of opioid mechanisms in analgesic activities of Praecitrullus fistulosus.

The peripheral analgesic property of Praecitrullus fistulosus was evaluated by using acetic acid-induced writhing test in mice. Acetic acid-induced abdominal constrictions is a useful experimental model for the exploration of peripheral analgesic activity of new drugs. Acetic acid causes stimulation of local peritoneal receptors, release of variety of endogenous pain mediators and stimulation of the neurons that are involved in pain sensation. These neurons are sensitive to the actions of opioids and non-steroidal anti-inflammatory drugs (NSAIDs). Endogenous substances that are released by acetic acid includes prostaglandins, histamine, 5-HT, leukotrienes and kinins. Pain sensation is also appears to be mediated by prostaglandin pathways, peritoneal mast cells and acid sensing ion channels. (Gupta et al., 2015)

Hot plate model test were used to assess the central mechanism of Praecitrullus fistulosus in making analgesia. In both these models, sensitization of nociceptors by sensory nerves and participation of endogenous substances like prostaglandins are abridged. In hot plate test, paw of animal is very sensitive to the temperatures above 50 °C, is founded on the observation that opioid drugs like morphine causes selective extension of reaction time of typical tail withdrawal response in experimental animals. Increase in reaction time in response to thermal stimuli in both these models suggest the analgesic activity of test drugs (Bacchi et al., 2000).

Summary Conclusion

Results of *in-vivo* experiments obtained through the present study can assist the design of virtual screening protocols for the natural products that inhibit the IKKβ mediated NF-κB signaling pathway and offer insights into the discovery of novel anti-inflammatory and analgesic leads. The bioactivity guided fractionation of Praecitrullus fistulosus leaves resulted in isolation of ethanolic Fraction. Anti-inflammatory activity of Praecitrullus fistulosus ethanolic extract (PFEE) was found to be mediated through the down regulation of NF-κB inflammatory pathway and subsequent inhibition of release and/or actions of various pro-inflammatory mediators. The analgesic activity shows the involvement of opioid system and transient receptor potential vanilloid 1 and ankyrin 1 channels. Isobolographic analysis of herbal compositions comprising of Praecitrullus fistulosus revealed that these compounds, exhibit synergism, when used in combination. It advocates the combined use of pentacyclic triterpenoids (PTs) as a potential anti-inflammatory and analgesic remedies. Correlation studies involving the pharmacokinetics and pharmacodynamics on the synthetic or semi-synthetic derivatives of these pentacyclic triterpenoids could further yield the compounds with better clinical potential. In conclusion, the Praecitrullus fistulosus possess the significant and potent anti-inflammatory and analgesic activities. Praecitrullus fistulosus and their semi-synthetic or synthetic analogues could be further developed as a novel anti-inflammatory and analgesic compounds.

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