ORIGINAL RESEARCH

Effect of *Citrullus colocynthis* extract on reduction in pain and inflamation

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ABSTRACT

The use of medicinal plants in treatment has been very common nowdays. Colocynth, Scientifically kwon as *Citrullus colocynthis* is used to reduce pain and inflammation in traditional medicine. *C. colocynthis* are already reported as rich source of different phytochemical constituents and responsible for many pharmacological applications. The aim of present study is to evaluate the effects of acetone extract of Colocynth fruit (AECC) on pain and inflammatory mediators at doses of 100 mg/kg, 200mg/kg, 400mg/kg by using tail flick and formaline induced pain methods. Our observations indicated that *C. colocynthis* fruit acetone extract can reduce pain and inflammation and we can use this plant as analgesic and anti inflammatory agent in the future.

Key words- *C. colocynthis*, pain, inflammation, acetone extract, analgesic.

INTRODUCTION

Citrullus colocynthis (L.) Schrad is important cucurbit plant, widely distributed throughout the desert areas of the world. (1). Plant species *C. colocynthis* (L.) Schrad is belongs to (family *Cucurbitaceae*) is common in India and the southern islands (2). Colocynth/Bitter Apple is the English name for the fruit *C. colocynthis*, also known as Indrayan in Hindi, Rakhal in Bengali, Anedri in Sanskrit, Kattu, and Pcitummatti in Tamil (3,4).

C. colocynthis grows from its perennial roots; this plant features angular, harsh, rough, and vine-like stalks that can ascend nearby vegetation. A solitary yellow flower blooms in the leaf axils. They have lengthy peduncles, a tuberous base, and trailing or ascending stems, and are monoecious (5).

C. colocynthis is a very old remedy in the Indian medicine. The fruit has been described as cathartic and useful in biliousness, fever, constipation and intestinal parasites. The root is used in janndice, ascites, rheumatism and urinary diseases. In severe cases of ascites, jaundice, and a number of uterine disorders, including amenorrhea, this medicine is often used as a purgative by doctors (6).

Worldwide Leprosy, jaundice, constipation, diabetes, asthma, cancer, bronchitis, joint pain, and mastitis are a few of the conditions for which *C. colocynthis* has been used medicinally (7-10).

Human and animal cases of bacterial infections, intestinal diseases, diabetes, and cancer have all been successfully treated with the fruits in India and Pakistan (9, 11-13).

C. colocynthis has a long history of traditional usage as a diabetic therapy in tropical and subtropical regions (14-17).
There for the present study was carried out to evaluate the anti-arthritc activity of *C. colocynthis* acetone extract by using tail flick and formalin induced pain in rats.

MATERIAL AND METHODS

COLLECTION OF PLANT MATERIALS

The fruits of *C. colocynthis* were collected from Tosham area of Bhiwani district, Haryana in November 2019 and was identified and verified by Dr. S.S. Yadav, Assistant Professor, Botany Department, M.D.U, Rohtak, Haryana.

PROCESSING OF PLANT MATERIALS

The *C. colocynthis* fruits were washed and rinsed with fresh water. After that, they were dried in the shade until all of the moisture had disappeared. Plants material were dried, then chopped up into pieces and processed into a coarse powder.

PREPARATION OF ACETONE EXTRACT BY MACERATION TECHNIQUE

The maceration technique was used to extract the acetone extract of both plant materials. Extracts of acetone were obtained by soaking a measured amount (300gm) of dry powder from both plants in 900 mL acetone for seventy-two hours. The extracts were filtered via Whatman No. 40 filter paper and evaporated at 45ºC with 200 revolutions per minute in a rotary evaporator, and the prepared extract was used in further analysis, and the remaining was kept in the fridge at 4ºC for storage (18).

ANIMALS

Wistar male rats were purchased from the Jaipur-based animal facility, Bilwal Medchem, and Research Laboratory Pvt. Ltd (BMRL). All animals were acclimated to the lab atmosphere for 7 days before the experimental procedure started. Animals were maintained in a light/dark cycle (twelve hours) at room temperature. The experimental protocol and all procedures utilized for this experimental study were approved by the Institutional Animal Ethics Committee of BMRL Pvt. Ltd, Jaipur, with protocol approval number BMRL/IAEC/2020-64.

TAIL FLICK TEST

An analgesiometer and the procedures outlined by D'Amour and Smith (19) were used to establish the significance of the tail-flick test. In this case, latency was defined as the amount of time it takes for an animal to react to brief exposure to high temperatures (such as flicking or retracting its inflicted tail).
A sensitivity test was done to select the rats for this experimental work and the rats that were unable to retract their tails within four seconds were eliminated from this study.

EXPERIMENTAL DESIGN FOR TAIL FLICK METHOD

There were total five groups, each including six albino Wistar rats
Group I (Control) - Only distilled water was given to the Rats.
Group II (Standard) - The standard drug Pentazocine(30 mg/kg) diluted with distilled water was given to rats (p.o.)
Group III, IV and V - AECC (100, 200 and 400 mg/kg, p.o.), respectively
Ten seconds was set as the cut–off time. After thirteen minutes of administering the test medications and fifteen minutes of administering pentazocine (standard drug), the latency time was recorded at fifteen minute intervals for one hour.

FORMALIN TEST

A slightly modified version of the procedure given by Tjølsen *et al.* (20) was utilized to analyze this investigation. Subcutaneously 0.05 mL of formalin (2.5%) was given to animals in their right hind feet (subplantar region) to induce pain. After thirty minutes of injecting formalin, all extract doses were administered orally to all test animals.

Rats were separated into different cages for observation. The duration of the animal's licking of the injected paw was taken as an indication of its level of pain. In the early (5 minutes) and late (15–30 minutes) phases after injecting formalin, the nociceptive responses were recorded.

EXPERIMENTAL DESIGN FOR FORMALIN TEST

There were total five groups, each including six albino Wistar rats

Group I (Control) - Only distilled water was given to the Rats.

Group II (Standard) - The standard drug Aspirin (300 mg/kg), diluted with distilled water was given to rats (p.o.)

Group III, IV and V - AECC (100, 200 and 400 mg/kg, p.o.), respectively

STATISTICAL ANALYSIS

One-way ANOVA (analysis of variance) followed by Dunnett's multiple comparison tests were used for statistical analysis in SigmaStat® version 3.5 Software. To illustrate differences between groups, we reported data as mean S.E.M. and evaluated differences to be statistically significant when when $p<0.05$.

RESULTS

TAIL FLICK TEST

In tail flick method, Pentazocine (30 mg/kg) significantly ($p<0.01$) increased the reaction time at 30, 60 and 90 minutes. Onset of action was observed at 30 minutes of administration of Pentazocine. Moreover, AECC significantly ($p<0.01$) inhibit pain produced by thermal means at the doses of 400 mg/kg at 60 minutes (Table1) as compared to control. AECC at the dose of 100 mg/kg and 200mg/kg didn't produce any significant reduction in pain.

Table -1 Effect of AECC in tail flick test

Group	Reaction time (sec)			
	0 min	30 min	60 min	90 min
Control	4.11±0.32	4.52±0.59	4.51±0.22	4.41±0.78
Pentazocine (30 mg/kg)	4.46±0.62	6.32±0.13**	7.99±0.09**	7.89±0.29**
AECC 100mg/kg	4.07±0.55	4.4±0.32	4.56±0.42	4.42±0.72
AECC 200mg/kg	4.17±0.42	4.51±0.48	5.22±0.10	4.98±0.31
AECC 400mg/kg	4.76±0.36	5.12±0.62	5.84±0.22**	5.42±0.38

Data presented as Mean ± SEM (n=6), **p <0.01, *p<0.05, are considered significant (one-way analysis of variance followed by the Dunnett's post hoc test) as compared to control.

FORMALIN TEST

AECC (400 mg/kg) showed a significant ($p<0.05$) reduction in paw licking when compared to vehicle control group. While AECC (100 and 200 mg/kg) showed non-significant reduction in paw licking. Paw licking in the formalin vehicle control group was found to be 81±0.12. Aspirin (300 mg/kg) appears to be more effective in reducing the paw licking, it significantly ($p<0.01$) reduced the paw licking (26±0.51). (Table1)

Table 1. Effect AECC in formalin induced pain test

Group	Paw licking Early phase (0-5 min)	Paw licking Late phase (15-30 min)
Control (Formalin-0.05 ml)	66±0.37	81±0.12
AECC 100mg/kg	61±0.51	63±0.58
AECC 200mg/kg	55±0.34	52±0.67
AECC 400mg/kg	51±0.29	41±0.36*
Standard	48±0.48	26±0.51**

Data presented as Mean ± SEM (n=6), **p <0.01, *p<0.05, are considered significant (one-way analysis of variance followed by the Dunnett's post hoc test) as compared to control.

DISCUSSION AND CONCLUSION

Nowadays, synthetic medicines are widely used to treat number of illnesses, but they come with a range of negative effects and have the potential to cause serious health problems. The formulation of herbal medicines which are manufactured by using plants, rich in bioactive compounds has been practiced in the traditional medical systems (Unani and Ayurveda) for a very long time[21].

In the present study, acetone extracts of both plants was assessed using analgesic animal models such as the tail flick method and the formalin-induced pain method.

Maximum reaction time in tail flick assay was observed at 60 minutes for the standard drugs (pentazocine), for AECC showed significant analgesic effect in the tail flick test.

Maximum response time was recorded at 60 minutes for the standard drugs (pentazocine) and the AECC and also AECC showed significant analgesic effects in the tail flick test, when compared to control rats.

In formalin induced pain test, AECC at 400 mg/kg decrease the paw licking in both, early phase (neurogenic pain) and the late phase (inflammatory pain) compared to control animals at 400mg/kg , which demonstrated that this plant has ability to block pain inducing neurotransmitters, thus prove its significant analgesic property.

The outcomes of present study revealed that *C. colosynthis* acetone extract exhibits both central as well as peripheral antinociceptive activities which make it a dual pain inhibitor. The therapeutic role of Flavonoids' role in management of, neuropathic pain, colitis, arthritis, cancer pain, osteoarthritis, and in cardiovascular illnesses etc. have already documented in different researches [22] but exact mechanisms of action for flavonoids are unknown; however researches have shown that they can be used as analgesics, antioxidants, and anti-inflammatory agents. According to these studies, flavonoids can reduce cellular inflammatory responses and pain by inhibiting the activation and synthesis of a range of cellular regulatory proteins such as cytokines and transcription factors. In consideration of the above findings, it is concluded that the flavonoids found in *C. colosynthis* are responsible for the plant's analgesic and anti inflammatory activity [23].

CONFLICT OF INTEREST

Authors declare no conflict of interest

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