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Analgesic Activity of Aqueous Extract of Citrullus Lanatus Peels

Amrita Kumari, Juhi Rao, Jyoti Kumari, Neha Sharma , Pankaj Jain, Vivek Dave, Swapnil Sharma*

Department of Pharmacy, Banasthali University, Tonk, Rajasthan
*Corresponding Author: skspharmacology@gmail.com

Abstract The present study was undertaken to evaluate the analgesic activity of aqueous extract of *Citrullus lanatus* peels (AECL) using Eddy's hot plate method. The AECL produced a significant analgesic activity in a dose dependent manner. All the doses of AECL (250, 500 and 1000mg/kg) had shown analgesic activity. The reaction time obtained for these three doses after 90 minutes of drug administration was found out to be 5.15 mins, 8.92 mins and 10.82 mins respectively which was comparable to Diclofenac sodium (5 mg/kg) that showed the reaction time of 12.36 mins. It was concluded that the AECL has good analgesic potential and may be adopted as alternative to conventional NSAIDS.

Keywords Analgesic, *Citrullus Lanatus,* Peels, Diclofenac Sodium, Eddy's Hot Plate

1. Introduction

Pain has been defined by International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [1]. Clinically, pain can be labeled "nociceptive" if it is inferred that the pain is due to ongoing activation of the nociceptive system by tissue injury. Although neuroplastic changes (such as those underlying tissue sensitization) are clearly involved, nociceptive pain is presumed to occur as a result of the normal activation of the sensory system by noxious stimuli, a process that involves transduction, transmission, modulation and perception.

Tissue injury activates primary afferent neurons called nociceptors, which are small diameter afferent neurons (with A-delta and C-fibers) that respond to noxious stimuli and are found in skin, muscle, joints, and some visceral tissues.[5] These fibers have specific receptors that may be responsible for noxious mechanical, chemical or thermal stimuli. One class, called transient receptor potential (TRP) receptors, has been undergoing intensive investigation in the hope of ultimately yielding new therapies for pain [2]. Opioids or nonopioids are the drugs that are currently used for the management of pain. All these drugs carry potential toxic effects. It has been observed that the risk of gastrointestinal bleeding are significantly associated with acute use of non-steroidal anti-inflammatory drugs (NSAIDs) like regular-dose aspirin, diclofenac, ketorolac, naproxen or nimesulide. Piroxicam increased the risk of bleeding in both acute and chronic therapy [3]. Opioids are the commonly used drugs for the management of acute postoperative pain [4]. On the contrary many medicines of plant origin have been used since ages without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop more effective and cheaper drugs. Plants represent a large natural source of useful compounds that might serve as lead for the development of novel drugs.

In the present study, *Citrullus lanatus* was selected as it is one of those medicinal plants that are commonly used in ayurvedic system of medicine. *Citrullus lanatus*, commonly known as watermelon, belongs to family Cucurbitaceae. Gill et al. evaluated the antioxidant, anti-inflammatory and analgesic potential of the *Citrullus lanatus* seeds [5]. Bartalis et al. reported various cucurbitacin analogues from *Cucurbita texana* and *Citrullus lanatus* [6]. Tirupur et al. investigated that the watermelon seeds are good source of linoleic acid (18:2 ω-6) as a major fatty acid [7]. Sevcan Altaş et al. evaluated that the watermelon juice protects the liver, kidney and brain tissues from experimental CCL_4 toxicity in rats and the protective effect of watermelon juice may be due to its antioxidant activity and inhibition of lipid peroxide formation [8]. The seed is demulcent, diuretic, pectoral, vermifuge, hypotensive and tonic [9]. The aim of this study was to investigate analgesic potential of aqueous extract of *Citrullus lanatus* peels.

2. Material and Methods

2.1. Plant Material

The fruit of *Citrullus lanatus* was purchased from the local commercial market of Jaipur in February month 2012. The peels were collected from the fruit and shade dried. The collected peels were identified and authenticated by Dr. K.C.

Sharma Department of Botany, University of Rajasthan. A voucher specimen number RUBL20685 was prepared and preserved along with the crude drug sample at the herbarium of Department of Botany, University of Rajasthan (Raj.), India.

2.2. Plant Extraction

The peels were fully shade dried and cleaned from extraneous matter. They were mechanically grinded and converted into coarse powder. Then extract was prepared by simple maceration process. The coarse peel powder was weighed and adequate distill water was added. They were kept for seven days with occasional stirring. After the completion of duration of maceration they were filtered using whatmann filter no.1 and filtrate(s) were collected. The filtrate so obtained, was termed as aqueous extract of Citrullus lanatus peels (AECL) and used for carrying out the further studies.

2.3. Animals

Swiss albino mice weighing 25-30 gm of either sex were obtained from Banasthali University Rajasthan, (India). The animals were housed in Animal house, Pharmacy Department, Banasthali university,(Rajasthan) in polycarbonate cages, in a room maintained under controlled room temperature 22 ± 20 C, relative humidity 60 -70% and provided with food and water. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (Register Number: 1283/C/09/CPCSEA and were in accordance with the guidelines of the CPCSEA. The animals were deprived of food for 24 h before experimentation but allowed free access to water ad libitum throughout.

2.4. Phytochemical Analysis

The AECL was then subjected to qualitative phytochemical screening for the identification of different phytoconstituents [10,11].

2.5. Analgesic Activity Test

The analgesic activity was evaluated using Eddy's hot plate method. For analgesic activity the animals were divided into five groups consisting of six mice in each.
Group I (control) received 1 ml of distill water (i/p).
Group II (standard) received Diclofenac sodium 5mg/kg (i/p)
Group II received AECL 250mg/kg (i/p).
Group IV received AECL 500mg/kg (i/p).
Group V received AECL 1000mg/kg (i/p).

2.5.1. Eddy's Hot Plate Method

The hot-plate was used to measure response latencies according to the method described by Eddy and Leimbach, with minor modifications. The paws of mice are very sensitive to heat at temperature, which are not damaging the skin. The response is in the form of jumping, withdrawal of the paws or the licking of the paws. The animals were placed on Eddy's hot plate kept at a temperature of 55±0.5°C. A cut off period of 15 sec, was observed to avoid damage of the paw. Reaction time and the type of response were noted using a stopwatch. The latency was recorded before and after 30, 60 and 90 min of both test and standard [12].

2.5.2. Statistical Analysis

All data were represented as mean ± SEM and as percentage. Results were statistically evaluated using Dunnett's t- test. $P<0.01$ was considered significant.

3. Result

The preliminary phytochemical studies revealed the presence of carbohydrates, phytosterol, tannins, saponins, glycoside and flavonoids in the extract. Data of the phytochemical analysis are listed in Table-1. Acute toxicity studies did not reveal any toxic symptoms or death in any of the animals up to the doses of 3000 mg/kg body weight, with AECL. The result of the present study indicates that the AECL possess analgesic activity in mice. The reaction time obtained for 250mg/kg, 500mg/kg and 1000 mg/kg after 90 minutes of drug administration was found out to be 5.15 mins, 8.92 mins and 10.82 mins respectively which was comparable to Diclofenac sodium (5 mg/kg) that showed the reaction time of 12.36 mins. The result were significant at p <0.001. The analgesic activity is presented in Table 2 and in figure 1.

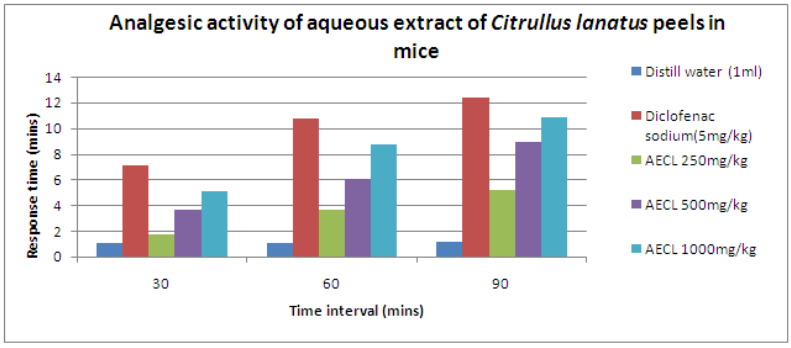

Figure 1. Effect of different doses of aqueous extract of Citrullus lanatus peels (AECL) on reaction time in mice.

Table1. Phytochemical analysis of AECL

Tests	AECL
1.Tests for sterols	
a. Test solution+Conc.H_2SO_4	+ve
b. Libermann Buchard's Test	+ve
c. Test solution + sulphur	+ve
d. Salkowski test	+ve
2.Tests for Glycosides	
a. Keller Killiani's Test	+ve
b. Balget's Test	+ve
c. Bromine water Test	+ve
d. Legal's Test	+ve
e. Raymonds Test	+ve
3. Test for Saponins	
a. Haemolytic Test	+ve
b. Foam Test	+ve
4. Test for Tannins	
a. Gelatin Test	+ve
b. Ferric Test	+ve
5. Test for Alkaloids	
a. Dragendroff's Test	-ve
b. Mayer's Test	-ve
c. Hanger's Test	-ve
d. Wagner's Test	-ve
6.Test for Carbohydrates	
a. Barfoed's Test	
b. Benedict's Test	+ve
c. Molisch's Test	+ve
	+ve
7. Test for Flavonoids	
a. Shinoda Test	+ve
b. Alkaline Reagent Test	+ve
c. Ferric Chloride Test	+ve
d. Lead Acetate Test	+ve
e. Zn-HCl reduction Test	+ve

Table 2. Effect of different doses of aqueous extract of *Citrullus lanatus peels (*AECL) on reaction time in mice.

Groups (mg/kg)	Reaction Time in mins		
	30 min	60 min	90 min
Control(distill water, 1 ml)	1.08±0.20	1.07±0.12	1.15±0.28
Diclofenac sodium (5mg/kg)	7.08±1.10	10.73±1.30	12.36±2.52
AECL(250mg/kg)	1.69±0.33	3.60±0.95	5.15±0.51
AECL(500mg/kg)	3.69±0.31	6.02±0.54	8.92±0.19
AECL(1000mg/kg)	5.12±0.57	8.74±0.58	10.82±0.47

4. Discussion

Pain and inflammation are associated with pathology of various clinical conditions like arthritis, cancer, and vascular diseases [13]. In various traditional medicinal systems a number of natural products are used to relieve the symptoms of pain. The AECL exhibited a significant analgesic activity using Eddy's hot plate model of pain. The hot plate method has been found to be suitable for evaluation of centrally acting analgesics. The nociceptors seem to be sensitized by sensory nerves. The involvement of endogenous substances such as PGs may be minimized by this model. NSAID such as diclofenac sodium used in this study are known to inhibit cyclooxygenase enzymes I and II which are implicated in the production of inflammation mediating agent prostaglandin (PGE2) from arachidonic acid [14-16]. The pattern of analgesic activity exhibited by the extract was similar to that of diclofenac sodium which suggests that the plant's activity

may be mediated by cyclooxygenase I and II inhibition. Sharma et al. reported the presence of phytoconstituent such as carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, tannins and polyphenols in crude fruit pulp extract. Similarly Oseni et al. worked on seed and whole fruit and reported Saponins, flavonoids, terpenoids, alkaloids, cardiac glycosides

In centrally acting analgesic methods, the 250mg/kg, 500mg/kg and 1000 mg/kg doses of AECL was found to be significantly effective.

5. Conclusion

From the above results, it can be concluded that the AECL showed dose dependent significant analgesic activity in Eddy's hot plate method. As the phytochemical screening has confirmed the presence of flavonoids, tannins, glycosides, saponnin and phytosterol in the aqueous extract, its pharmacological activity may be attributed due to the presence of these phytoconstituents. More detailed phytochemical studies are, however, necessary to identify the active principle(s) and exact mechanism of action.

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