Evaluation of antioxidant, anti-inflammatory and analgesic potential of Citrullus lanatus seed extract in rodent model

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Citation

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Abstract

The present study was carried out to evaluate the antioxidant, anti-inflammatory and analgesic potential of the Citrullus lanatus seeds. Extraction of the seeds were carried out using solvents of increasing polarity. The free radical scavenging activity of all extracts were measured by DPPH and H_2O_2 methods. The methanolic extract of the Citrullus lanatus seeds was further evaluated for its in vivo anti-inflammatory activity using carrageenan induced rat paw edema and analgesic activity by tail flick and tail immersion methods. The methanolic extract of the seeds (MECL) had shown highest antioxidant activity. The results were obtained in a dose dependent manner. 200mg/kg of MECL had shown significant ($p<0.05$) anti-inflammatory and analgesic activity as compared to diclofenac sodium and morphine respectively. It may be concluded that the methanolic extract of seeds of Citrullus lanatus has good antioxidant, anti-inflammatory and analgesic potential and may be used as a future food medicine.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Higher plants, containing medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times (1). Before the availability of synthetic drugs man was completely dependent on natural medicinal plants for curing diseases (2). Natural plant products such as herbs, fruits and vegetables became more popular in recent years due to public awareness and increasing interest among consumers and scientific community (3). For a variety of reasons more individuals nowaday prefer to control their health with the help of natural plant medicines (4). Epidemiological evidence has revealed that constituents in natural products show many biological and pharmalogical activities, including antioxidative, anti- inflammatory and antiviral effects (5). The main reason of all these diseases is supposed to be the free radicals. The free radicals are created as a consequence of ATP (adenosine triphosphate) production by the mitochondria. These by-products are generally called reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) that result from the cellular redox process (6, 7). If free radicals are not inactivated, their chemical reactivity can damage all cellular macromolecules including proteins, carbohydrates, lipids and nucleic acids. Their destructive effect on protein may play a role in the causation of cataracts, damage to DNA which may cause cancer, effect on LDL cholesterol which is very much responsible for heart disease and free radicals are also involved in the inflammation processes like gout and asthma(8, 9). The common link between free oxidant radicals and inflammatory reactions has been well established (9). Natural medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. Investigations into the chemical and biological activities of plant during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents (10). Natural products play an important role in drug development programs of the pharmaceutical industry (11). In developing countries, especially in rural context people usually turn to traditional healers when in diseased conditions and plants of ethnobotanical origin are often presented for use. Natural products which contain antioxidant properties such as phenolics, include flavonoids and phenolic acids, carotenoids and vitamins etc. They have less side effects,

easily available and are cost effective (12). Considering these aspects the aim of the present study is to investigate the Citrullus lanatus seeds for their antioxidants, anti-inflammatory and analgesic potential.

MATERIALS AND METHODS

CHEMICALS

DPPH (1,1-diphenyl-2-picrylhydrazyl) was obtained from Hi-media, carrageenan, ascorbic acid and diclofenac sodium from Jackson Laboratories, Amritsar. Punjab), Physician sample of morphine was procured from Government Medical College and Hospital, Patiala (Punjab) and the solvents like hexane, chloroform, ethyl acetate and methanol were of analytical grade and purchased from SD fine chemical.

PLANT MATERIAL

Citrullus lanatus seeds were purchased from the local grain market, sector 26 in Chandigarh. The seeds were authenticated and the voucher specimen no: 0394 has been deposited in the Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar. For examination only healthy looking seeds were chosen (without mechanical damages and bacterial infection). The seeds were cleaned, washed, shade dried and carefully powdered in the grinder by maintaining the temperature below 20°C. All samples were kept in air tight light-protected containers.

EXTRACTION

Extraction was carried out using solvents of increasing polarity such as hexane, chloroform, ethyl acetate and methanol by simple maceration process for 24 hrs. The solvents were completely removed by rotary evaporator and crude extracts were obtained and stored in the refrigerator. These crude extracts were further used for their antioxidant anti-inflammatory and analgesic potential.

ANIMALS

Wister albino rats (160-180 g) of either sex, Swiss albino mice (20- 30 g) were purchased from Sanjay Biologicals, Amritsar for experimental study. They were acclimated to standard animal house conditions such as temperature (24.0±1.0 C), relative humidity

(55-65%) and 12hrs light/12hrs dark cycle. They were fed with commercial pelleted rat feed and had free access to water. The experimental protocol was approved by the IAEC (Institutional Animal Ethical Committee) of CPCSEA, (Committee for the Purpose of Control and Supervision of Experiments on Animal) registration no. 874/ac/05/CPCSEA.

ANTIOXIDANT ASSAYS

QUALITATIVE EVALUATION OF THE DPPH SCAVENGING ACTIVITY

The qualitative assay was performed according to the reported method (9, 13). Two milligrams of the extract was diluted with 1 ml of the appropriate solvent, then small quantity of each dilution of the hexane, chloroform, ethyl acetate and methanol was carefully loaded individually into the baseline of the TLC plates (20 cm x 10 cm) and the sample was allowed to dry. Hexane-ethyl acetate (7:3) was used as mobile phase. Once dried, the plates were sprayed with a 0.2% solution of DPPH (1,1-diphenyl-2-picrylhydrazyl) in ethanol. Extracts containing antioxidant component showed a yellow-on-purple spot due to the discoloration of DPPH.

QUANTITATIVE EVALUATION OF THE DPPH SCAVENGING ACTIVITY

The antioxidant activity of different extracts (chloroform, ethyl acetate, methanol and acetone extract) was carried out. Extracts were dissolved in methanol. 1 ml of this solution was mixed with 1 ml of 0.05mM DPPH in methanol and adjusted up to 5 ml with methanol. Final concentrations (50-300 µg/ml) were taken. Mixtures were vigorously shaken and left for 30 min in dark and analyzed at 517 nm on (Shimadzu UV-1700 Pharma Spec Japan) using methanol as a blank.1 ml of 0.05mM DPPH diluted with 4 ml of methanol was used as control. All the readings were taken in triplicate and their mean value was taken into consideration. Ascorbic acid was used as a reference standard. Inhibition of DPPH radical was calculated using the equation:

$$I\ (\%) = 100 \times (A - A_s)/A$$

Where A is the absorbance of the control and A_s is the absorbance of the tested sample. The IC_{50} value represented the concentration of the extract that caused 50% inhibition (13, 14).

QUANTITATIVE EVALUATION OF THE HYDROGEN PEROXIDE SCAVENGING ACTIVITY

All the different solvent extracts of Citrullus lanatus seeds were evaluated for their hydrogen peroxide radical

scavenging potential by reported method (15, 16). An aliquot of 0.6 mL of hydrogen peroxide (43 mM) and 1.0 ml of different concentrations of extracts ranging from (50-300 µg/ml) was mixed, then 2.4 ml of 0.1M phosphate buffer (pH 7.4) was added. The resulting solution was kept for 10 min and the absorbance was recorded at 230 nm using uv spectrophotometer(Shimadzu UV-1700 Pharma Spec Japan) against a blank (without hydrogen peroxide). All the readings were taken in triplicate and their mean value was taken into consideration. Ascorbic acid was used as a reference standard. The percentage scavenging of hydrogen peroxide was calculated using the following formula:

Scavenging activity (%) = $[(V_c - V_t)/V_c] \times 100$

Where V_c is absorbance of control and V_t is absorbance of test sample.

ANTI-INFLAMMATORY ACTIVITY

The animals of either sex were divided into five groups each composed of six animals.

Group I (Control): Carrageenan (1%, p.o.)

Group II (Standard): Diclofenac sodium (12.5 mg/kg, p.o.)

Group III: Methanolic extract of Citrullus lanatus (MECL), (50 mg/kg, p.o.)

Group IV: MECL (100 mg/kg, p.o.)

Group V: MECL (200 mg/kg, p.o.)

Percentage inhibition of edema = $(V_c - V_t / V_c) \times 100$

Where, V_c is the inflammatory increase in paw volume in control group of animals and V_t is the inflammatory increase in paw volume in drug-treated animals. Paw edema was induced by injecting 0.1 ml of 1% carrageenan in physiological saline into the sub plantar tissue of the left hind paw of each rat (17-18). The different doses of the extract were administered orally 30 min prior to carrageenan administration. The paw volume was measured at intervals of 60, 120, 180 and 240 min by the mercury displacement method using a plethysmometer (Labco,India). The percentage inhibition of paw volume in treatment group was compared with the carrageenan control group (Group- I). Diclofenac sodium (12.5 mg / kg p.o.) was used as reference drug.

ANALGESIC ACTIVITY

The albino mice were divided into five groups of six animals each.

Group I (Control): Carboxy methyl cellulose suspension (0.3 ml, 1% w/v, p.o.)

Group II (Standard): Morphine (10 mg/kg, p.o.)

Group III: Methanolic extract of Citrullus lanatus (MECL), (50 mg/kg, p.o.)

Group IV: MECL (100 mg/kg, p.o.)

Group V: MECL (200 mg/kg, p.o.)

All analgesic tests were performed in different time intervals i.e. 0, 30, 60, 120 and 180 minutes.

ANALGESIC ACTIVITY BY TAIL FLICK TEST

In the present study analgesia was assessed according to the reported method (19). The terminal part of the tail (about 1cm) of the mice was placed on analgesiometer, at uniform distance from the nichrome wire. Temperature of heating element of the instrument was maintained at 52±0.5°C. Cut-off time 20 seconds was maintained.

ANALGESIC ACTIVITY BY TAIL IMMERSION TEST

In present study analgesia was assessed according to the reported method (20). 3-4 cm area of the tail was marked and immersed in the water bath thermo-statistically maintained at 51°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. The maximum cutoff time for immersion was 20 seconds to avoid the injury of the tissues of tail.

STATISTICS

Descriptive statistics and comparisons of differences between each data set were calculated by the use of Sigma Stat 3.5 trial version software. The data were expressed as Mean ± SEM, and analyzed by one way ANOVA in each experiment. Statistical significance was accepted at the level of $p<0.05$. In the case of significant variation ($p<0.05$), the values were compared by Dunnet test.

RESULTS

QUALITATIVE INHIBITION OF DPPH RADICAL

The TLC of methanolic extract sprayed with 0.2% DPPH solution showed good antioxidant activity. The spots showed

good yellow coloration over the violet coloration of the DPPH.

QUANTITATIVE ANTIOXIDANT ACTIVITY

Scavenging of the DPPH radical: The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517 nm induced by antioxidants. Due to rapid hydrogen donating ability of DPPH, it reacts with antioxidants and gets converted into 1, 1-diphenyl-2-picryl hydrazine and hence shows decrease in absorbance. All the extracts showed some concentration dependent antioxidant activity but the maximum activity was found in methanolic extract (300 µg/ml) (Fig.1).

Scavenging of Hydrogen Peroxide: The measurement of H_2O_2 scavenging activity is one of the useful methods of determining the ability of antioxidants to decrease the level of pro-oxidants such as H_2O_2 (15). Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because of rise in the hydroxyl radicals in the cells (21). The hydrogen peroxide scavenging activity of various extracts is shown in Fig.2. The maximum activity was found in methanolic extract followed by ethyl acetate and chloroform. Concentration dependent antioxidant activity was observed for methanol extract.

Figure 1

Figure 1. Percentage inhibition of DPPH free radical by seed extracts at different concentrations in vitro

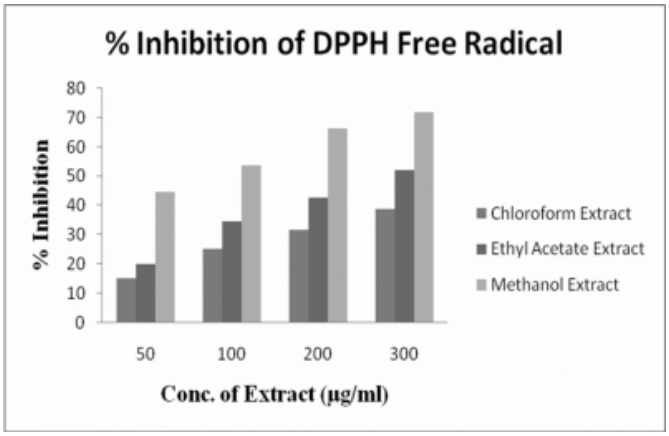
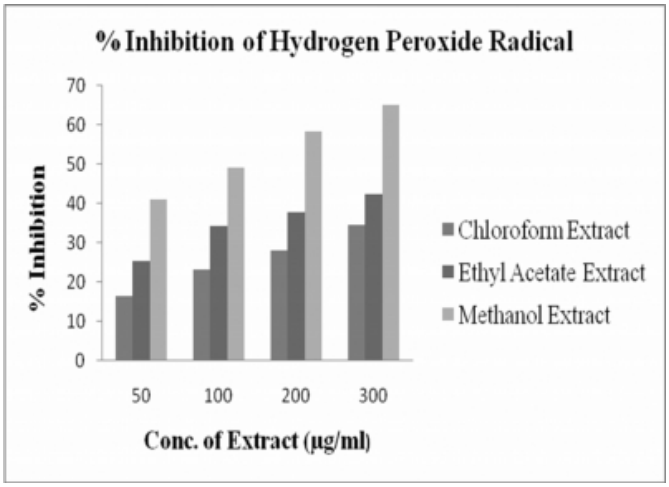


Figure 2

Figure 2. Percentage inhibition of hydrogen peroxide free radical by seed extracts at different concentrations in vitro



PHARMACOLOGICAL EVALUATION

ACUTE INFLAMMATION STUDY IN RATS

Effect of MECL on acute inflammation in rats: The methanolic extract of Citrullus lanatus seeds significantly attenuated carrageenan induced paw volume in rats. However, the maximum reduction of paw volume was observed at higher dose (200mg/kg, p.o.) which was comparable to the effect of diclofenac sodium (12.5mg/kg, p.o.) as shown in Table 1.

Figure 3

Table 1. Anti inflammatory activity by carrageenan induced paw edema in rat

Groups (mg/kg)	60 min	120 min	180 min	240 min	% inhibition of paw edema
Carrageenan (1%)	0.34 ± 0.05	0.55 ± 0.04	0.65 ± 0.04	0.70 ± 0.04	0.00
Diclofenac (12.5)	0.25 ± 0.04 [a]	0.45 ± 0.05 [a]	0.26 ± 0.05 [a]	0.19 ± 0.05 [a]	72.85 [a]
MECL (50)	0.43 ± 0.03 [b]	0.54 ± 0.05 [b]	0.63 ± 0.01 [b]	0.67 ± 0.09 [b]	4.28 [b]
MECL (100)	0.35 ± 0.04 [b]	0.49 ± 0.05 [b]	0.59 ± 0.04 [b]	0.50 ± 0.03 [b]	28.57 [b]
MECL (200)	0.30 ± 0.04 [a]	0.49 ± 0.05 [a]	0.35 ± 0.03 [a]	0.29 ± 0.04 [a]	64.28 [a]

Table 1. Data were expressed as mean ± S.E.M., n = 6 rat in each group. a = $p<0.05$ vs Carrageenan control groups, b = $p<0.05$ vs diclofenac control group. Results were expressed as paw volume in ml.

ANALGESIC ACTIVITY IN MICE

Effect of MECL on Tail flick test: The methanolic extract of Citrullus lanatus significantly attenuated the spinal pain sensation against conduction heat in mice. Moreover, this ameliorative effect of tail withdrawal response was observed in a dose dependent manner. The maximum analgesic effect was shown at higher dose (200mg/kg, p.o.) which was comparable to that of morphine (10mg/kg, p.o.) as shown in Table 2.

Figure 4

Table 2. Tail flick test

Groups (mg/kg)	0 min	30 min	60 min	90 min	120 min
Control (1% CMC)	1.97 ± 0.03	2.44 ± 0.02	2.43 ± 0.09	2.37 ± 0.07	2.35 ± 0.06
Morphine (10)	2.09 ± 0.02[a]	6.76 ± 0.01[a]	12.13 ± 0.04[a]	13.16 ± 0.07[a]	13.2 ± 0.02[a]
MECL (50)	2.04 ± 0.01[b]	3.43 ± 0.01[b]	5.55 ± 0.02[b]	5.9 ± 0.01[b]	5.23 ± 0.05[b]
MECL (100)	2.08 ± 0.08[b]	3.63 ± 0.01[b]	6.86 ± 0.04[b]	7.19 ± 0.02[b]	6.15 ±0.08[b]
MECL (200)	2.15 ± 0.02[a]	5.86 ± 0.01[a]	10.13 ± 0.01[a]	11.3 ± 0.08[a]	11.9 ± 0.09[a]

Table 2. Data were expressed as mean ± S.E.M., n=6 mice in each group. a = p<0.05 vs CMC control groups, b = p<0.05 vs morphine control group. Results were expressed as tail withdrawal latency in seconds.

Effect of MECL on Tail immersion test: The methanolic extract of Citrullus lanatus significantly attenuated the spinal pain sensation against hot water in mice. Moreover, this ameliorative effect of tail withdrawal response was observed in a dose dependent manner. The maximum analgesic effect was shown at higher dose (200mg/kg, p.o.) which was comparable to that of morphine (10mg/kg, p.o) as shown in Table 3.

Figure 5

Table 3. Tail immersion test

Groups (mg/kg)	0 min	30 min	60 min	90 min	120 min
Control (1% CMC)	2.17 ± 0.07	2.34±0.02	2.42±0.01	2.36±0.06	2.36±0.04
Morphine (10)	2.19 ± 0.03[a]	5.65 ± 0.07[a]	9.18±0.02[a]	11.24±0.04[a]	10.24±0.06[a]
MECL (50)	2.15 ±0.02[b]	3.27 ± 0.02[b]	4.34 ± 0.06[b]	5.62 ± 0.03[b]	5.23 ± 0.02[b]
MECL (100)	2.17 ± 0.06[b]	3.42 ± 0.03[b]	6.64 ± 0.06[b]	6.94 ± 0.03[b]	6.26 ± 0.04[b]
MECL (200)	2.12 ± 0.05[a]	4.88 ± 0.09[a]	8.87 ± 0.03[a]	10.66 ± 0.04[a]	8.26 ± 0.01[a]

Table 3. Data were expressed as mean ± S.E.M., n=6 mice in each group. a = p<0.05 vs CMC control groups, b = p<0.05 vs morphine control group. Results were expressed as tail withdrawal latency in seconds.

DISCUSSION

In the present study, the seed extract of Citrullus lanatus in different solvents were evaluated for their in vitro antioxidant activity. Results have revealed that methanolic extract possessed highest in vitro antioxidant activity as compared to others. Hence, MECL was further evaluated for its in vivo anti-inflammatory and analgesic potential. Carrageenan induced rat paw edema test has frequently been used to assess the anti-edematous effect of natural products. Carrageenan is a strong chemical for the release of inflammatory and pro inflammatory mediators. It has also been reported that several ROS are released during such inflammation (22). Therefore, antioxidant with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated (24). Moreover, recent studies suggest that the inflammatory tissue damage is due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites (25). Again, as MECL demonstrated good antioxidant action in vitro so, it may be assumed that it reduces inflammation due to its antioxidant potential. Tail immersion and tail flick tests were carried out using morphine as standard drug, which is known to act by centrally mediated mechanism. As MECL also showed decrease in the tail withdrawl latency which indicated that it may possess analgesic property. This analgesic activity may be via central pain pathway due to its free radical scavenging activity. According to these findings of the present experiment, oral MECL was found to have anti-

inflammatory and analgesic actions and these pharmacological actions are may be due to the free radical scavenging activity. However, more elaborative studies are required to identify the active components and precise mechanism of analgesic and anti-inflammatory actions.

CONCLUSION

Based on the results of the present study, it can be concluded that the methanolic extract of Citrullus lanatus seeds possess remarkable antioxidant, anti-inflammatory and analgesic potential. However, further studies are needed to understand the exact mechanisms of action and to isolate the compound (s) responsible for such activities.

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