Lebda Medical Journal
Vol. 7, Jan. 2021, p.273-278

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Received 13 Nov. 2020; accepted in revised form 29Dec. 2020

Anti-inflammatory activity of Ethanolic extract of Cnicus Benedictus

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ABSTRACT

Introduction: Cnicus benedictus has been used as treatment for liver disorders and menstrual problems. Different studies have been undertaken for various activities on extracts of the plant but, so far and to the best knowledge of the author(s), no one has reported anti-inflammatory activity of ethanolic leaves extract of Cnicus benedictus .

Aim: The aim of this study is to investigate the anti-inflammatory activity of Ethanolic leaf extract of Cnicus benedictus in albino wistar rats upon oral administration (100 and 200 mg/kg .(

Method: Thirty albino wistar rats were taken and divided into five groups to study the anti-inflammatory activity of Ethanolic extract of Cnicus benedictus (CB). To induce oedema, 0.1 ml of 1% w/v carrageenan suspended in 1% CMC were injected into sub-plantar tissues of the left hind paw of each rat of Group 1, III, IV and V. Animals of group I were kept as the normal control group. Indomethacin (10 mg/kg) was given to group III. Ethanolic extract of CB in a dose of 100 and 200 mg/kg were given to group IV and V, respectively. The anti-inflammatory activity of CB was compared with standard indomethacin treated animals, using plethysmometer.

Result: The ethanolic extract of CB showed % inhibition of oedema 28.92 and 35.29 with dose of 100 mg/kg and 200 mg/kg, respectively .

Conclusion: Ethanolic extract of CB at higher dose possesses a significant anti-inflammatory activity ($P< 0.05$) compared to a lower dose.

Key words : Anti-inflammatory activity, Cniscus benedictus, Carrageenan

INTRODUCTION

Cnicus benedictus, belongs to family Asteraceae, and is widely spread to many Mediterranean countries (1). It is also cultivated in wide range of geographical areas of South Africa and South America. Earlier it was called as Carduus benedictus or "blessed thistle" because of its virtues. It was considered to possess cure-all property. For centuries, blessed thistle was used traditionally in treatment of various diseases, including plague and smallpox. However, these uses lack the evidence support.

Blessed thistle has been used as treatment for liver disorders and menstrual problems. It seems to detoxify the liver. In many European countries, blessed thistle tablets are prescribed along with acetaminophen or aspirin to counterbalance the potential liver damage by these drugs (2). Women consume blessed thistle in order to regulate their periods. It seems to stimulate the appetite and many herbalists prescribe it to their anorexic patients (3). These bioactivities properties of the plant are related to the phenolic composition such as phenolic acids, flavonoids, procyanins, anthocyanins, tannins and lignins (4). The plant also contains secondary metabolites such as glycosides, bioactive azo compounds, silymarin and rosmarinic acid (5, 6). These metabolites are important pharmaceutical agent for the treatment of hepatitis, lung and colon cancers (5, 8&9).

Inflammation is marked by acute and chronic phases. Acute inflammation which occurs few minutes after injury to tissue, is primarily due to an increase in permeability of blood resulting in extravasation of fluid and accumulation of white blood cells. The mediators involved in inflammation are histamine, serotonin, and cyclooxygenase-2 (C0X-2) (10). In chronic inflammation, prostaglandins (PGE2), nitric oxide and lipoxygenases are prominent inflammatory mediators.

Conventional medication such as the non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin, diclofenac, and ketoprofen are the most commonly used as the first line treatment of inflammation (11). Different studies have been undertaken for various activities on extracts of the plant but so far no one has reported anti-inflammatory activity of ethanolic leaves extract of Cnicus benedictus. Hence in the present study, we investigated the in- vivo anti-inflammatory activity of ethanolic leaves extract of Cnicus benedictus by carrageenan induced rat paw oedema method..

MATERIALS AND METHODS

Collection of plant material

Leaves of Cnicus benedictus were collected in December 2014 from Misurata, Libya and authenticated by Dr. Sarfaraz Hussain, Assistant Professor, Department of Pharmacognosy, Misurata University, Libya. The leaves were washed with water; shade dried, coarsely powdered, and kept in airtight containers until use.

Preparation of extract and preliminary phytochemical screening

Ethanolic extract was prepared by cold maceration technique. Extract was filtered and concentrated in rotary evaporator. The extract was dried in a vacuum desiccator to obtain a constant weight. The phytochemical screening was carried out as described by Norman (12).

Animals

A total of 30 albino wistar rats of either sex, weighing 150−200 g, were obtained from animal house, college of pharmacy, Misurata University, Libya. All animals were divided randomly into five groups and housed in polypropylene cages (6 in each cage), at an ambient temperature of 25 ± 2°C and relative humidity of 55−65%. Animal house was subjected to 12 h light/dark cycle and the animals were fed with standard diet and water ad libitum. Prior to the experiment they were deprived of food.

Drugs and chemicals

Carrageenan was purchased from Hi Media, Mumbai, India, Indocin Capsule 50 mg (indomethacin), Mylan Pharmaceuticals Private Limited, Italy Digital plethysmometer LE 7500 was used from Pan Lab, Harvard Madrein Spain. Standard chow diet was obtained from Misurata Agriculture food, Misurata, Libya.

Statistical analysis

For statistical analysis, data were presented by the one-way ANOVA followed by Newman – Keul's studentized range comparisons test using GraphPad Instat software (GraphPad Software Inc., CA, USA).

Acute toxicity study

To assess the acute toxicity of CB extract LD50 value was determined using the up-and-down method as described by Bruce (13).

Drug administration

The indomethacin was administered by suspending in 1% Carboxy methyl cellulose (CMC) solution.

In carrageenan model, ethanolic extract of Cnicus benedictus leaves at doses of 100 and 200 mg/kg were administered to the rats orally before carrageenan injection. Indomethacin at dose of 10 mg/kg was administered orally using gastric cannula 30 minutes prior to carrageenan injection in sub plantar region of rat left paw as shown in Figure 1A & B.

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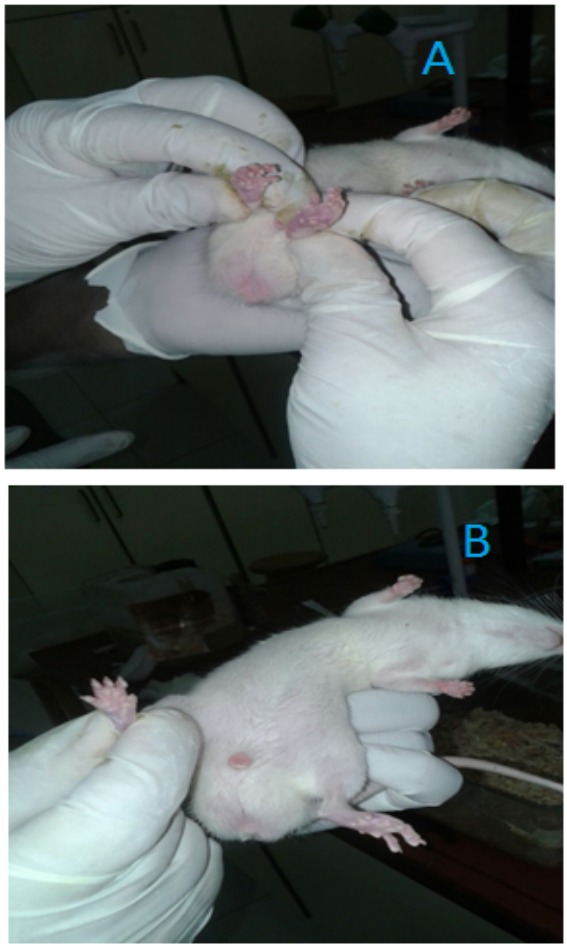

Figure 1: A) carrageenan induced paw oedema B) left paw showing oedema.

Evaluation of in-vivo anti-inflammatory activity

Paw oedema was induced by injecting 0.1 ml of 1% w/v carrageenan suspended in 1% CMC into sub-plantar tissues of the left hind paw of each rat (14). Rats were divided into five groups; each group consisting of six animals as per the scheme given in Table 1.

Table 1 Scheme for dose administration

Groups	No. of animals	Treatment	Dose	Scheme of evaluation
Group I	6	Normal Control	-	Paw thickness measurement at 1,2,3,4,5 hours
Group II	6	Carrageenan treated	1% w/v	
Group III	6	Indomethacin as standard reference	10 mg/kg	
Group IV	6	Ethanolic extract	100 mg/kg	
Group V	6	Ethanolic extract	200 mg/kg	

Paw thickness measurement

The inflammation was produced as as per the scheme given in Table 1 and the paw volume was measured using plethysmometer. The anti-inflammatory activity was calculated as the percentage of inhibition of oedema in the animals treated with the extract under test in comparison to the carrageenan control groupIndomethacin and individually according to the dose used.

The percentage (%) of oedema inhibition of oedema is was calculated using the formula

$$\% \quad \text{Inhibition} = \frac{T_0 - T_t}{T_0} \times 100$$

Where Tt is the thickness of left paw of rats given test extract at corresponding time and To is the paw thickness of rats of Carrageenan control group at the same time (15).

RESULTS:

Table 2 showed the effect of Ethanolic extract of CB and standard drug (indomethacin) as compared to carrageenan control at different hours in carrageenan-induced paw oedema model using plethysmometer. The Ethanolic extract administered at a dose of 100 mg/kg p.o prevented carrageenan-induced paw oedema with a percentage inhibition of 2.37 %, 6.39 %, 10.28 %, 24.94 and 28.92 at 1, 2, 3, 4 and 5 hours, respectively, while 5.14%, 7.67%, 13.36 %, 28.64% and 35.29 % at a dose of 200 mg/kg p.o. at 1, 2, 3, 4 and 5 hours, respectively. Indomethacin at a dose of 10 mg/kg p.o. prevented carrageenan induced paw oedema with a percentage inhibition of 11.07 %, 11.50 %, 15.94 %, 36.03% and 43.38 % at 1, 2, 3, 4 and 5 hours, respectively.

The result showed that the Ethanolic extract of CB at dose of 100 mg/kg and 200 mg/kg has a significant reduction in the carrageenan induced paw edema ($P < 0.05$) in a dose dependent manner when compared to control (Table 2 & Figure 2). The standard drug, indomethacin (10 mg/kg, i.p.) was more potent than the extract. The high dose of the extract (200 mg/kg) exhibited better anti-inflammatory activity than the low dose of the extract (100 mg/kg) as shown in Figure 2.

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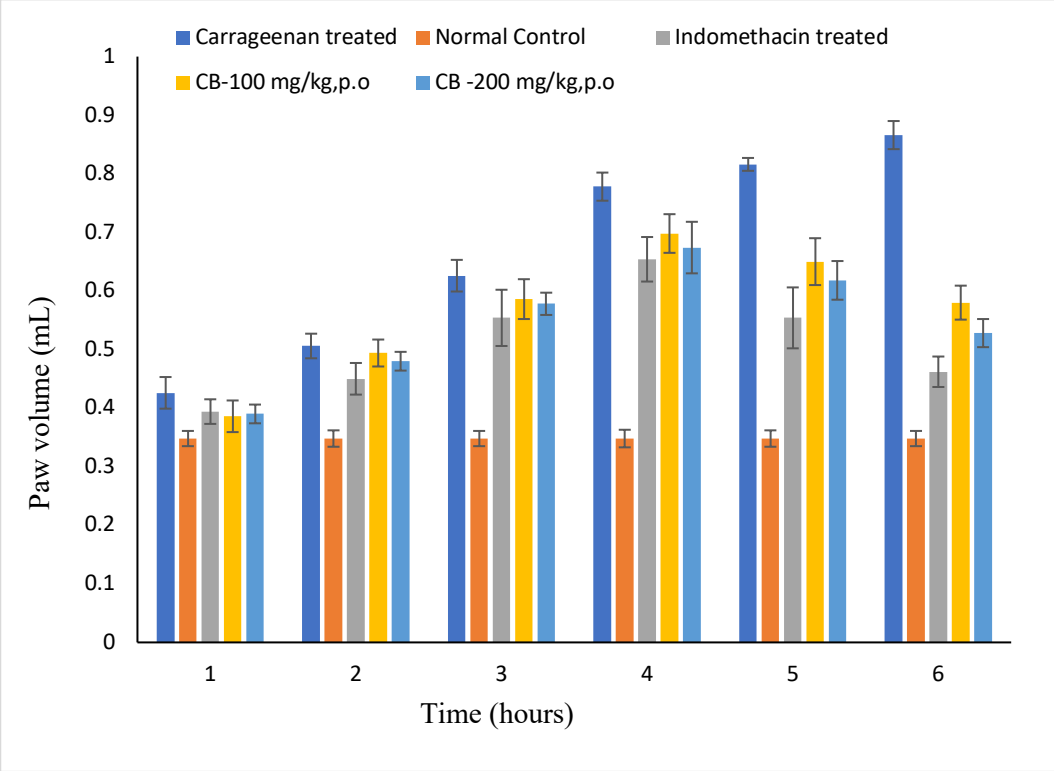

Figure 2: Anti-inflammatory activity of Ethanolic extract of Cnicus benedictus on carrageenan induced rat paw oedema method

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Table 2: % Inhibition of oedema in the carrageenan induced rat paw

Group		I	II	III	IV	V
Treatment		Normal control	Carrageenan treated	Indomethacin treated	CB- 100 mg/kg.p.o	CB- 200 mg/kg.p.o
% Inhibition	1 hour	31.22 ± 4.68	-	11.07 ± 1.49	2.37 ± 0.31	5.14 ± 0.62
	2 hours	44.41 ± 6.66	-	11.50 ± 1.55	6.39 ± 0.83	7.67± 0.92
	3 hours	55.27 ± 8.29	-	15.94 ± 2.15	10.28 ± 1.34	13.36 ± 1.60
	4 hours	57.35 ± 8.60*	-	36.03 ± 4.86	24.94 ± 3.24*	28.64± 3.44*
	5 hours	59.81 ± 8.97*	-	43.38 ± 5.86	28.92 ± 3.76*	35.29 ± 4.23*

*P < 0.05 when compared Normal control with CB 100 mg/kg.p.o and CB 200 mg/kg.p.o at 4 hours and 5 hours

DISCUSSION

The plant, Blessed thistle used in various diseases of liver and for menstrual problems. It is hepatoprotective and appetizer as proved by various studies. In this work we investigated the anti-inflammatory activity of the ethanolic extract. The evaluation of anti-inflammatory activity of ethanolic leaf extracts of Cnicus benedictus showed that there was no significant inhibition of paw oedema, 2.37 % and 5.14 % in the early hours of study by aqueous extract at 100 and 200 mg/kg, respectively (Table 2). Carrageenan-induced paw oedema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis (16). Carrageenan induced acute inflammatory model is the most suitable test procedure to ascertain the anti-inflammatory activity of medicinal agents in experimental animals (17).

Carrageenan plays a major role in the development of the second phase of inflammatory reaction, which is measured at the 4th hour (18). As shown in the Table 2, there was a significant (P < 0.05) percentage inhibition of paw oedema, 24.94 and 28.64% at doses of 100 and 200mg/kg, respectively, at the 4th hour by Ethanolic extract when compared with normal control. The inhibitory activity of indomethacin was much higher than Ethanolic extract of high dose CB at dose of 200mg. The Ethanolic extract showed lower activity than indomethacin. The obtained result is also supported the findings of Mascolo et al., 1987 (19). This result may suggest that the ethanolic extract of Cnicus Benedictus possessed mild anti-inflammatory activity.

CONCLUSION:

Ethanolic extract of CB at higher dose possess a mild anti-inflammatory potential, which is lower than the standard indomethacin treated group. However, when compared with the control (no treatment), the ethanolic extract of CB had significant anti-inflammatory activity (P< 0.05. Although more studies are needed, particularly on toxicity of the extract, these findings may support the use of the extract in traditional medicine for the management of mild inflammatory conditions.

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