Short Communication

Preliminary investigations on *Lens culinaris* Med. Seeds for anti-inflammatory and antioxidant properties

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Abstract

Objective: The objective of present study was to evaluate anti-inflammatory and antioxidant activities of *Lens culinaris* Med. seeds. **Materials and methods**: Methanolic extract of *Lens culinaris* Med. seeds was screened for presence of chemical constituents. Extract was evaluated for anti-inflammatory activity using carrageenan induced paw edema method on rats. Two different dose 100 and 200 mg/kg of methanol extract were selected for anti-inflammatory and antioxidant studies. Effect was measured by rat paw edema and measurement of antioxidant (SOD, CAT and GSH) level in rats. **Results and conclusion**: The methanolic extract contains flavonoids, tannins, proteins and glycosides. Extract was reduces rats inflammation in dose dependent manner and 200 mg/kg showed significant anti-inflammatory activity. The natural antioxidants level also significantly increases after extract administration in the rats. The anti-inflammatory and antioxidant effects of *Lens culinaris* Med. Seeds may be due to presence of flavonoids and tannins.

Keywords: Anti-inflammatory, *Lens culinaris*, carrageenan, edema, antioxidant

Introduction

Inflammation constitutes body's response to injury and is characterized by a series of events that mainly occur in three distinct phases. The first phase is caused by an increase in vascular permeability resulting in exudation of fluids from the blood into the interstitial space; the second phase involves the infiltration of leukocytes from the blood into the tissue and third phase is characterized by granuloma formation and tissue repair (Jain et al., 2016). Inflammation is a complex pathophysiological process mediated by a variety of molecules produced by leukocytes, macrophages and mast cells and mediator release resulted edema formation by extravasation of fluid and proteins and accumulation of leukocytes at the inflammatory site (White, 1999). Many inflammatory diseases are associated with the synthesis of prostaglandins, which are responsible for a sensation of pain. The primary enzyme responsible for prostaglandins synthesis is the membrane-associated cyclooxygenase, which occurs in two isoforms, COX-1 and COX-2. COX-1 is constitutively expressed while COX-2 is induced in the inflamed tissue. Modulation of the activity of the enzyme implies that the inflammation process can be modified (Janadri and Gowda, 2016).

The genus Lens is the name that indicative lens-like shape means the seeds of lentil is similar to the lens shape. *Lens culinaris* Med. (family Fabaceae) is commonly known as 'masoor' in hindi and “lentils” in English (Rastogi and Mehrotra, 1991). It is distributed and cultivated throughout the North region of India. Seeds are rich source of minerals such as calcium, iron, vitamin B and an important component of variety of food in many countries. They also reported to have high protein content, carbohydrates and fibers. Paste of lentil seeds is used to get rid of skin marks and to treat kidney and gastric disorders (Kirtikar and Basu, 2003; Vohra and Gupta, 2012). The seeds are also used as antifungal medicine and antioxidant medicine (Ahmad et al., 2010; Amarowicz et al., 2010).

Materials and methods

The seeds of *Lens culinaris* was identified and authenticated

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in JNKVV, Jabalpur (M.P.). The seeds were collected, dried and extracted with Petroleum ether for defatting and methanol by using soxhlet apparatus. Methanol extract was filtered and concentrated by rotary vacuum evaporator at 50-60° C. The yield of ethanol extract was calculated. Phytochemical analysis was carried out by using different chemical test to detect different chemical constituents (Kokate, 1999).

Acute toxicity study of methanol extract was done according to the OECD 425 guidelines on rats. The rats were kept in the animal house in a controlled room temperature at 24±10C temperature in a 12h/12h day/night cycle with free access to food and water ad libitum. All the experiments were carried out according to standard CPCSEA approved protocol. Albino rats (150-180g) were acclimatized to the laboratory conditions for at least seven days prior to the experiments. Methanol extract was administered orally in doses of 2000, 3000, 4000, 5000 mg/kg to the group of rats (n=5) and the percentage mortality was recorded for a period of 24 hours. Administration of 5000 mg/kg maximum dose did not produce mortality or general signs of toxicity for 24 hours. 1/10th of safe dose was taken as therapeutic dose for animals.

Anti-inflammatory activity of methanol extract was investigated using carrageenan induced edema in the hind paw of rats produced by the subplantar injection of 0.1 ml of a 1% carrageenan suspension in normal saline (Maswadeh et al., 2006). Test extracts (100 and 200 mg/kg) and vehicle (10 ml/kg) were given orally 1 h prior to carrageenan injection. Paw volume was measured immediately after the carrageenan injection, at 30 min interval for 3 h later and the difference from initial volume was taken as the edema volume, which was compared to standard.

The inhibition of inflammation was calculated using the following formula:

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

Where, V_t is the paw volume of test group and V_c is the paw volume of control group.

The blood sample from each group of animal was collected after 5 h of treatment for determination of antioxidants level. Level of Superoxide dismutase (SOD) was assayed (Misra and Fridovich, 1972) based on the inhibition of epinephrine autoxidation by the enzyme. Reduced glutathione (GSH) level was determined by the method of Moron (Moron et al., 1979). Catalase was estimated following the breakdown of hydrogen peroxide (Beers and Sizer, 1952).

Results and discussion

The paw edema of different groups of rats was observed. Results observed that paw edema was increased progressively and reached its maximum after 120 min of carrageenan injection. Methanol extract was reduced carrageenan-induced inflammation significantly (P<0.001) at dose of 200 mg/kg as compared to the control group (Table 1). The antioxidants level i.e. SOD, CAT and GSH were also found significantly increased after extract treatment (Table 2). It means methanol extract also showed antioxidant effect during inflammation process.

The results can be concluded that the significant activity may be due to presence of flavanoids and in methanol extract. It can be suggested from the study that the effectiveness for suppression of oedema is due to the ability of extract to either inhibit the synthesis, release or action of carrageenan involved in the inflammation. Carrageenan-induced paw edema in mice has been accepted as a useful phlogistic tool for investigating anti-inflammatory agents. Serious signs of inflammation develop immediately following subcutaneous injection, resulting from action of pro-inflammatory agents. The development of edema is a biphasic event: the early phase (0–2.5 h after carrageenan injection) involves the release of inflammatory mediators like histamine, serotonin and bradykinins; the late phase (3–6 h post-injection) is associated with the release of prostaglandins (Wang et al., 2014). In the present study, extracts and standard significantly inhibited the paw edema

Table 1. Effects of methanol extract of *Lens culinaris* seeds on carrageeninin induced paw edema in rats

Animal groups	Carrageenan induced paw edema Mean ± SEM (% inhibition of paw volume)					
	30 min	60 min	90 min	120 min	150 min	180 min
Control	0.57±0.12	0.72±0.08	0.87±0.15	1.08±0.13	0.97±0.17	0.86±0.08
Methanol extract	0.49±0.21	0.61± 0.11	0.70±0.24	0.79±0.20	0.71± 0.31	0.65±0.28
(100 mg/kg, p.o.)	(14.03)	(15.27)	(19.54)	(26.85)	(26.80)	(24.41)
Methanol extract	0.40±0.27 (29.82)	0.47± 0.34	0.55±0.37	0.50±0.51	0.46±0.31	0.44±0.51 (48.83)
(200 mg/kg, p.o.)		(34.27)	(36.78)	(53.70)*	(52.57) *	
Diclofenac sodium (25 mg/kg, i.p.)	0.32±0.43 (43.85)	0.39± 0.27	0.45±0.42	0.48±0.24	0.44± 0.61	0.41± 0.36 (52.32)
		(45.83)	(48.27)	(55.55) *	(54.63) *	

Value represents mean ± S.E.M. (n = 5), *P < 0.01 compared with control and standard

during the early phase of inflammation, indicating that the extract and standard may blocks histamine and serotonin release within the early phase. In future, detail pharmacological and chemical studies are needed in order to characterize the mechanism responsible for the anti-inflammatory action and also to identify other active constituents present in *Lens culinaris* seeds.

Table 2. Effect of methanol extract of *Lens culinaris* seeds on various antioxidants level in rats

Animal groups	Enzymatic study		
	SOD(U/mL)	CAT(U/mL)	GSH(U/mL)
Control	17.52±1.20	11.64±1.30	20.64±1.43
Methanol extract (100 mg/kg, p.o.)	22.24±1.63	18.64±1.52	28±1.47
Methanol extract (200 mg/kg, p.o.)	29.53±1.72*	26.67±1.82*	30.28±2.10*
Diclofenac sodium (25 mg/kg, i.p.)	30.52±1.24*	27.72±1.67*	32.54±2.08*

In conclusions, methanol extract of *Lens culinaris* seeds showed anti-inflammatory activity significantly with reducing oxidative stress. In addition anti-inflammatory effect can be connected with the reduction of release of inflammatory mediators.

Conflict of Interest

The authors have declared that there is no conflict of interest.

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