

## Anti-inflammatory and antioxidant activity of Joshanda partially mediated through inhibition of lipoxygenase

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### Abstract

Joshanda, a polyherbal product is commonly used to treat inflammation of the mucous membranes of nose and air passages. The current study was planned to evaluate the antiinflammatory and antioxidant activity of Decoction of Joshanda (DJ). DJ was challenged in carrageenan induced paw edema and cotton pellets induced granuloma at 100, 200 and 300 mg/kg i.p. for its antiinflammatory profile. However, for its antioxidant activity, DDPH free radical scavenging test was used. DJ manifested marked attenuation of edema (56%) induced by carrageenan, at 300 mg/kg after 4<sup>th</sup> hour of administration. In cotton pellet induced granuloma, DJ exhibited profound reduction in granuloma formation which was 52.74% at 300 mg/kg i.p. respectively. When tested against lipoxygenase, DJ illustrated profound inhibition (55% at 100 µg/ml) in a concentration dependent manner. Against DDPH free radical, DJ elicited prominent scavenging activity (58%) and thus strongly harmonized the antiinflammatory activity. Our findings suggest that DJ strongly ameliorated the induced inflammation at least partially mediated through lipoxygenase inhibition and thus the results were consistent with the traditional uses of Joshanda as an antiinflammatory agent.

**Keywords:** Joshanda; antiinflammatory; carrageenan; cotton pellets; antioxidant

### Introduction

Joshanda is a polyherbal formulation of Unani origin (Greco-Arab). It is largely used against the inflammation of the mucous membranes of nose and air passages (Azmi et al.,

2010). Joshanda (DJ) is one of the leading house hold remedy against upper respiratory infections, catarrh, cold and flu in Pakistan. Such practices are even more common in paediatric age group (Ashraf et al., 2010). This polyherbal formulation consists of expectorant, respiratory demulcent and anticatarrhal herbs which assist in relieving the enervating cough. It is also recommended for the treatment of premenstrual syndrome (Akram et al., 2011). The effect of the drug on the bronchial smooth muscles in isolated tissues is already explored (Kheterpal et al., 1989).

To the best of our knowledge, the antiinflammatory activity of Joshanda has not been evaluated yet. For that account, we are presenting in this article, the anti-inflammatory activity of the decoction of Joshanda both in acute and chronic phases of inflammation induced in animal models; *in vitro* lipoxygenase inhibition assay followed by its antioxidant potential.

## Material and Methods

### Sample Collection

Joshanda (Hamdard Laboratories Waqf Pakistan) in commercial pack was purchased from herbal medical store in Peshawar. Each commercial packet contained the same composition of plants. 100 g of each Joshanda packet contains 3 g of *Althaea officinalis*, 9 g of *Cordia latifolia*, 5 g of *Glycyrrhiza glabra*, 3 g of *Malva sylvestris*, 5 g of *Onosma bracteatum* 5 g of *Viola odorata* and 5 g of *Zizyphus Sativa*.

### Sample preparation

All the materials from the commercial packets were taken out and ground to powder form using grinding machine. The powdered materials were weighed. The sample was taken in hot water at 70 °C for 24 h to make a decoction. Then it was filtered while hot on Buckner funnel using vacuum pump. The filtrate was centrifuged for 40 min at 5000 rpm to separate out solid particles. The liquid mixture was concentrated using vacuum rotary at 70 °C. Then it was dried in oven and finally ground to powder using mortar and pestle. The %yield was calculated as 19.25%.

### Experimental animals

Healthy wistar rats (180-270 g) of either sex were used in different tests. They were kept under the standard laboratory conditions at  $25 \pm 2$  °C; the light cycle was maintained as 12 h dark: 12 h light. Animals were fed with laboratory diet *ad libitum* and allowed free access to drinking water. The rulings of the institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council were maintained during all the experiments performed (Khan et al., 2010).

### Drugs and reagents used

Carrageenan, ascorbic acid, linoleic acid (Sigma Chemicals Co., St. Louis, MO, USA), Aspirin (Reckit & Colman, Pakistan). DPPH (Waka Ltd. Japan). Carrageenan was used as suspension in acacia. Best quality chemicals/solvents were used during various tests.

### ***Carrageenan-induced edema***

Anti-inflammatory profile of decoction of Joshanda (DJ) was evaluated using carrageenan induced hind paw edema test (Khan et al., 2011a; Khan et al., 2011b). The test animals were grouped, each containing six animals. Group I received normal saline (10 ml/kg) as control. Animals of group II, III and IV received crude extract (100,200 or 300 mg/kg i.p.). Group V received standard drug, aspirin (100 mg/kg i.p.) as a reference drug. Inflammation was induced by sub-plantar injection (0.1 ml of 1% suspension of carrageenan with 2% gum acacia in normal saline) in the right hind paw of the rats after 30 min of the drug administration in all groups. The paw volume was measured using plethysmometer (Ugo Basile, Italy) at 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> h after the carrageenan injection. Statistics was applied on the raw data for the calculation of reduction in rat paw volume (ml) for each group against saline, followed by the calculation of percent reduction in the rat paw using the following formula:

$$\text{Inhibition (\%)} = 1 - (\text{dt/dc}) \times 100$$

Where “dt” showing the difference in paw volume of the treated group “dc” against the difference in paw volume in the control group.

### ***Cotton pellet granuloma***

Wistar rats of either sex were divided into groups (n= 6). Briefly, cotton pellets (30±1 mg) were autoclaved and implanted subcutaneously into the groin region of each rat (Khan et al., 2009a). For seven consecutive days, DJ was administered at a dose of 100, 200 and 300 mg/kg i.p. Similarly, standard drug, aspirin (100 mg/kg i.p.) was treated in the same way. On day seven, the rats were sacrificed and the pellets inserted in the granuloma tissues were carefully removed. Subsequently, it was dried in an oven at 60 °C and weighed (Wang et al., 2012). The resulting weight was then compared with the weight of control for the determination of weight of granuloma followed by the calculation of percent control.

### ***In vitro Lipoxygenase inhibition assay***

DJ was subjected to the challenge of lipoxygenase (LOX) inhibitory assay *in vitro* (Khan et al., 2011d; Razziq et al., 2011) using Soybean lipoxygenase and linoleic acid. Equal volume (10 mL) of the sample (DJ) and standard drug along with 20 mL of solvent lipoxygenase solution were mixed simultaneously followed by incubation for 5 min at 25 °C. The biochemical reaction was initiated by the addition of linoleic acid solution (10 µL) as substrate and the absorption change with the formation of (9Z, 11E)-13S)-13-hydroperoxyoctadeca-9, 11-dienoate was followed for 10 min at 234 nm. The test sample and the control were dissolved in 50% ethanol. All the reactions were performed in triplicate. Baicalein was used as positive control.

### ***Antioxidant activity***

To study the free radical scavenging activity, DJ was tested against the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Khan et al., 2011c, Raziq et al., 2011). The solution of DPPH (0.1 mM) was prepared by dissolving it in the ethanol and kept in the dark on of 10, 50 and 100 µg/ml. 1 ml of DPPH solution was added to 3 ml of various concentrati-

Table 1. Anti-inflammatory effect of the DJ in carrageenan induced in hind paw edema in rats.

Group	Dose	Increase in paw volume (mean $\pm$ SEM) in ml			
		1 h	3 h	4 h	5 h
Saline	10 ml/kg	0.70 $\pm$ 0.06	0.69 $\pm$ 0.09	0.75 $\pm$ 0.07	0.70 $\pm$ 0.08
DJ	100 mg/kg	0.60.07 (14.28)	0.58 $\pm$ 0.09 (15.94)	0.58 $\pm$ 0.04 (22.67)	0.60 $\pm$ 0.09 (14.28)
	200 mg/kg	0.55 $\pm$ 0.07 (21.42)	0.51 $\pm$ 0.05* (32)	0.45 $\pm$ 0.06* (40)	0.52 $\pm$ 0.05* (25.71)
	300 mg/kg	0.44 $\pm$ 0.07* (37.14)	0.33 $\pm$ 0.04* (56)	0.29 $\pm$ 0.04** (61.33)	0.36 $\pm$ 0.05** (48.57)
Aspirin	100	0.25 $\pm$ 0.07** 64.28	0.19 $\pm$ 0.06** 72.46	0.18 $\pm$ 0.06** 76	0.19 $\pm$ 0.02** 72.85

Experimental data are shown as mean  $\pm$  S.E.M. (n=6). One-way ANOVA was utilized as judgment test of significant differences among groups followed by Dunnett's multiple comparison post test. A probability of \* $P < 0.05$ , \*\* $P < 0.01$  was considered significant from control.

on of drug. The absorbance was taken at 517 nm in terms of colour change. The antioxidant activity of the test materials was calculated by comparison of the results with the ethanol. Ethanol was used as negative control while butylated hydroxyl toluene (BHT) as a standard antioxidant drug. All the analyses were performed in triplicate. The % free radical scavenging activity (RSA) was calculated using the following formula

$$\text{RSA (\%)} = (\text{control absorbance} - \text{sample absorbance}) / \text{control absorbance} \times 100$$

### Statistical analysis

Results of various tests are illustrated as the mean  $\pm$  S.E.M. of six independent animals. Statistical significance was determined by using one-way ANOVA followed by a post-hoc Dunnett's test for comparisons against vehicle.  $P > 0.5$  was considered as significant.

## Results

### Effect of DJ in carrageenan induced paw edema

DJ elicited intense antiinflammatory activity against inflammation caused by carrageenan as presented in Table 1. The effect was dose dependent (100, 200 and 300 mg/kg i.p.) while maximum attenuation was observed at 300 mg/kg after 4<sup>th</sup> h of drug administration

Table 2. Effect of DJ in cotton pellet induced granuloma at 100, 200 and 300 mg/kg i.p.

Groups	Dose	Weight of the cotton pellet (mg)
Saline	10 ml/kg i.p.	67.27 $\pm$ 1.25
	100 mg/kg	57.45 $\pm$ 0.9
Crude extract	200 mg/kg	48.77 $\pm$ 1.45*
	300 mg/kg	31.79 $\pm$ 1.35**
Aspirin	100 mg/kg	21.90 $\pm$ 1.10**

Experimental data are shown as mean  $\pm$  S.E.M. (n=6). One-way ANOVA was utilized as judgment test of significant differences among groups followed by Dunnett's multiple comparison post test. A probability of \* $P < 0.05$ , \*\* $P < 0.01$  was considered significant from control.

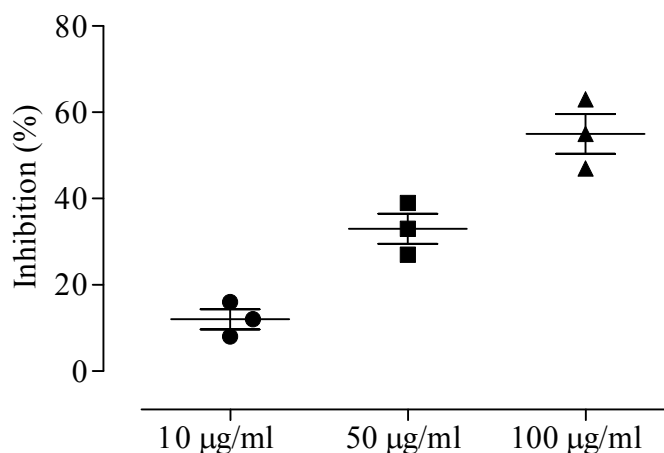


Figure 1. Effect of *in vitro* lipoxigenase inhibition of DJ. Experimental data are shown as mean  $\pm$  S.E.M. (n=6). One-way ANOVA was utilized as judgment test of significant differences among groups followed by Dunnett's multiple comparison post test. A probability of \* $P < 0.05$ , \*\* $P < 0.01$  was considered significant from control.

similar to standard drug, aspirin. However, the effect was remained significant during various assessment times (1–5 h) at 300 mg/kg.

#### ***Effect of DJ in cotton pellet induced granuloma***

The effect of DJ on cotton pellet induced granuloma is presented in Table 2. It showed in the dose dependent manner marked protection by reducing granuloma. Maximum protection (52%) was observed at 300 mg/kg i.p. after treatment of day seven (Figure 1).

#### ***Effect of DJ in lipoxigenase inhibitory assay***

In lipoxigenase inhibitory challenge, DJ provoked profound inhibition against soybean lipoxigenase (Figure 1). The inhibitory profile was based on DJ concentration. Of the various concentrations tested in the assay, maximum inhibition (55%) was observed at 100 µg/ml.

#### ***Effect of DJ against DPPH free radical***

Decoction of Joshanda (DJ) showed prominent free radical scavenging activity against DPPH free radical Figure 2. The effect was in a concentration dependent mode. Maximum (58%) scavenging was note at the maximum tested concentration (300 µg/ml) of DJ. The free radical scavenging activity showed strong correlation with the carrageenan induced paw edema (Figure 3) and cotton pellet induced granuloma (Figure 4).

### **Discussion**

The current study revealed profound antiinflammatory activity of the decoction of the polyherbal product, Joshanda (DJ) in both acute and chronic *in vivo* antiinflammatory mode ls; probably mediated at least in parts through lipoxigenase inhibition. Additionally the acti-

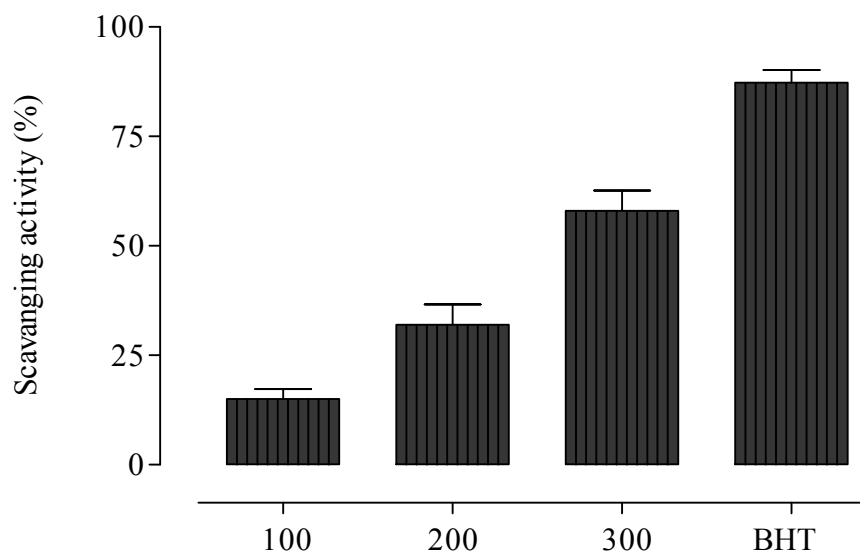


Figure 2. Antioxidant activity (%) of DJ on DPPH at 100, 200 and 300 µg/ml. Data are expressed as mean  $\pm$  SEM of three different findings.

vity was strongly augmented by potent antioxidant activity in *in-vitro* assay.

Carrageenan induced paw edema test is a significant tool for the assessment of antiinflammatory profile of natural products (Roome et al., 2008; Alqasoumi et al., 2012). It is believed that the local oedema is induced by the subplantar injection of carrageenan that increased progressively. Oedema formation due to carrageenan in the rat paw is the biphasic event during 1–5 h; the initial phase (1h or 1.5h) is predominately a nonphagocytic edema followed by a second phase with increased edema formation that remained up to 5 h (Meckes et al., 2004; Chouhan et al., 2012; Geroushi et al., 2011). Researchers have been described the involvement of different mediators in various stages of carrageenan induced oedema. The initial phase (up to 1.5 h) is attributed to the release of histamine, 5-hydroxytryptamine, platelet activating factor and serotonin. Kinin was released from 1.5 to 2.5 h and at the last st-

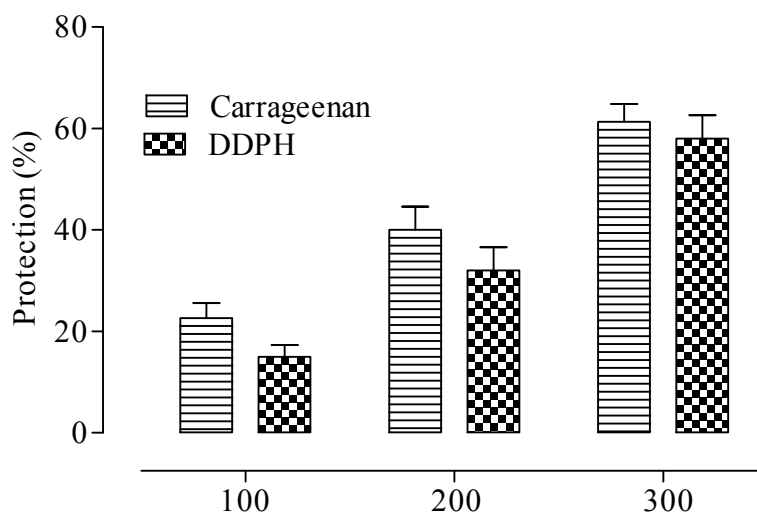


Figure 3. Correlation of carrageenan induced paw edema test (100, 200 and 300 mg/kg) with DDPH free radical scavenging activity (100, 200 and 300 µg/ml).

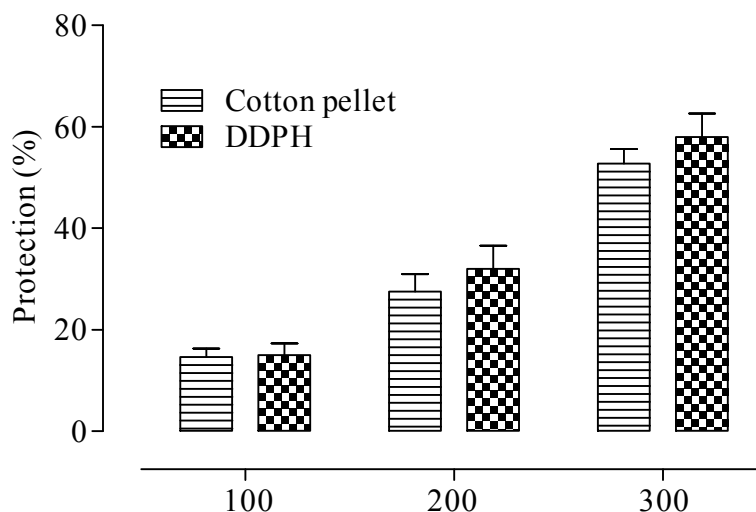


Figure 4. Correlation of cotton induced granuloma test (100, 200 and 300 mg/kg) with DDPH free radical scavenging activity (100, 200 and 300 µg/ml).

ep inflammation was continued until 5 h by the lipid derived eicosanoids (prostaglandins, leukotrienes, HPETEs, etc.). Our result showed marked anti-inflammatory activity of DJ in carrageenan induced paw edema model at all test doses in various assessment times. It could be assumed that the active principles of Joshanda inferred with the release of various mediators of inflammation.

Cotton pellet granuloma is a well established test for the assessment of chronic anti-inflammatory process. Chronic inflammation is generated when the body fails to respond against inflammatory agents (Marina et al., 2010; Saha et al., 2011). The chronic condition is predominantly consists of a transudative phase and a proliferative phase. The dry weight of the implanted cotton pellet correlates well with the amount of granulomatous tissue formation (Guang-Qin et al., 2008). DJ demonstrated comprehensive reduction in the dry weights of implanted cotton pellets. Based on our results, it may be suggested that the secondary mentalities of Joshanda has diverse activity profile; even firmly prevent granuloma formation.

Leukotrienes (LTs) are the downstream products of arachidonic acid that exert pivotal biological functions as well as pathogenic effects in wide range of inflammatory process. Polymorphonuclear leukocytes (PMNL) and monocytes/macrophages are the major cells capable of synthesizing LTs due to a high LOXs expression and activity and represent crucial components in chronic inflammatory diseases (Thorsten et al., 2008; Khan et al., 2009b). The role of lipoxygenase is also documented in carrageenan provoked oedema (Viji and Helen, 2008). Inhibition of lipoxygenase activity results in down regulation of the pro-inflammatory activity of leukocytes and platelets (Claria and Romano, 2005; Kuhn and O'Donnell, 2006) which may cause a diminished or delayed outcome of the inflammatory reaction. DJ elicited marked inhibitory activity on lipoxygenase. Keeping this in view, it can be suggested that the mechanism underlying the anti-inflammatory activity of DJ is at least in parts due to lipoxygenase inhibition.

In the course of inflammation, free radicals generation play a crucial role in its pathophysiology (McDowell et al., 2011; Albano et al., 2012). Researchers have been suggested that the over expression of the inducible forms of the cyclooxygenase (COX) and the lipoxygenase (LO) enzymes are responsible for the generation of the lipid mediators and damaging free radicals are intricately involved during the inflammatory process (Tanas et al., 2010; Guimaraes et al., 2011). As the experimental finding showed that DJ possesses strong antioxidant activity, therefore this effect further substantiate the overall anti-inflammatory potential of DJ.

It is worth mentioning that the antiinflammatory and antioxidant activity of most of the individual plant species in Joshanda are already reported in literature (Arif et al., 1989; Akamatsu et al., 1991; Koochek et al., 2003; Elmastas et al., 2004; Patel et al., 2008; Benammar et al., 2010; Hage-Sleiman et al., 2011). Our findings on Joshanda are therefore consistent with the activities of various plant species in its composition.

In short, Joshanda showed in-depth antiinflammatory activity not only in acute form of inflammation but also in chronic condition. The antiinflammatory activity was strongly complemented by its free radical scavenging activity and thus validated its traditional use as an antiinflammatory agent. Additionally, the pharmacologically active constituents in Joshanda sustained their structural integrity even at excessive heat treatment. The isolation of such heat stable antiinflammatory agents could lead to ideal therapeutic modality in the treatment of various inflammatory conditions.

### Conflict of Interest

There is no conflict of interest associated with the authors of this paper.

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