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Studies on the Pharmacological Activities of an Ethanol Extract of Balessan (*Commiphora opobalsamum)*

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Abstract: The pharmacological activities of an ethanolic extract of Balessan (*Commiphora opobalsamum)* on inflammation induced by carrageenan and cotton pellets implant in rats; the nociceptive writhing response and the tail flick tests and the yeast induced pyretic activity and urine flow were examined in rats and mice. Oral administration of Balessan extract at doses of 250 and 500 mg kg^{-1} body weight showed a significant anti-inflammatory activity in rats. The extract significantly decreased the number of contractions and stretching induced by acetic acid and heat-induced pain in mice. Moreover, a significant increase in urine volume was noted in rats. A preliminary phytochemical screening of Balessan revealed the presence of a volatile oil, flavonoids, saponin, sterols and/or triterpenes. The observations suggest that Balessan extract possesses an anti-inflammatory, analgesic and diuretic activities in laboratory animals

Key words: Balessan, *Commiphora opobalsamum*, phytochemical tests, pharmacological activities, Arabian tradition medicine

INTRODUCTION

Commiphora opobalsamum (L). Engl., family Burseraceae, locally known as Balessan or Ood-e-Balsan, is an ancient herb used in Arabian folk medicine for the treatment of various diseases including sore throat, cough, laryngitis, chronic bronchitis and inflammations due to rheumatism and arthritis[1]. A tincture has been beneficially used in treatment of diseases of the chest, stomach, kidney, rheumatic pains and scurvy[2]. With oil they form a useful external application in bruises, swellings, wounds and rheumatic pains. Moreover, in Arabian traditional medicine it is used to remove kidney stone and as a diuretic (Personal communication, 2002). However, there is no scientific study to substantiate these claims of traditional healers. Therefore the present study was undertaken to evaluate the anti-inflammatory, analgesic, antipyretic and diuretic potential of this plant in laboratory animals.

MATERIALS AND METHODS

Plant material and extraction: Aerial parts of the plant were collected from Farasan Island of Red Sea (Saudi Arabia) in the month of March, 2002 and identified by our taxonomist (Dr. M. A. Rahman, College of Pharmacy, King Saud University, Riyadh, KSA). Powdered shade dried aerial parts of the plant were macerated in 96% ethanol for 36 hours; the solvent was then evaporated by a rotavap in vaccuo. The extract was kept in refrigerator for biological studies.

Preliminary phytochemical screening: A phytochemical analysis of the aerial parts of Balessan was conducted for the detection of alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oil, cyanogenic glycosides, glucosinolates, coumarins, sterols and/or triterpenes[3].

Biological tests: Wistar albino rats roughly the same age (8-10 weeks), weighing 180-200 g and Swiss albino mice (25-30 g body weight) obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh were used in these studies. The animals were kept in constant temperature (22±2°C), humidity (55%) and light-dark conditions (12/12 h light/dark). They were provided with Purina chow and free access to drinking water *ad libitum*.

Carrageenan-induced paw edema in rats: Experimental inflammation was induced according to the method

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described by Winter *et al.*[4]. 0.05 mL of 1% carrageenan sodium salt (BDH) were injected into the right hind foot of each rat under the plantar aponeurosis. The test groups of rats were treated orally with ethanolic extract of Balessan 250 and 500 mg kg^{-1} body weight 1 h before the carrageenan injection. At the same time, the control group was administered normal saline 5 mL kg^{-1} and the reference group was administered an aqueous solution of Phenylbutazone 100 mg kg^{-1} orally. The measurements of paw volumes were done by the displacement technique using a plethysmometer (Apelex, France) immediately and +3 h after the injection of carrageenan. The inhibitory activity was calculated according to the following formula:
Percent inhibition= 100 [1- (a-x/b-y)]
where, 'b' is the mean paw volume of control rats after carrageenan injection and 'y' before the injection; whereas 'x' is the mean paw volume of treated rats before injection and `a' is the mean paw volume after carrageenan injection.

Cotton pellet granuloma in rats: The method of Goldstein *et al.*[5] was used with a few modifications. Sterilized cotton pellets weighing 30 mg each were introduced s.c. in the groin region of rats. The rats were treated orally with 250 and 500 mg Balessan extract per kg once daily for four consecutive days. Animals in the control group received normal saline. Phenylbutazone 100 mg kg^{-1} (used as a standard drug) was given in other test group. On the fifth day, the animals were sacrificed with ether, the pellets were removed, freed from extraneous tissues, dried overnight at 60°C and weighed.

Analgesic activity

Inhibition of acetic acid-induced writhing in mice: The test was carried out using the technique of Siegmund *et al.*[6] as modified by Koster *et al.*[6]. The extract was administered orally, to 16 h fasted mice, divided into groups of six animals each. One hour after treatment, they were injected intraperitoneally with 0.2 mL of 3% acetic acid solution to induce the characteristic writhings. The number of writhings occurring between 5 and 15 min after the acetic acid injection was recorded. The responses of extract treated groups were compared with those of animals receiving indomethacin (as standard drug), as well as with the controls.

Tail flick test: Acute nociception was assessed using a tail flick apparatus (Tail Flick model DS 20 Socrel Apelex, France) following the method of D' Amour and Smith[7]. Briefly, each animal was placed in a restrainer, 2 min before treatment and baseline reaction time was measured by focusing an intensity controlled beam of light on the distal one-third portion of the animals tail. The extract was orally administered immediately after this step and, the post drug reaction time was measured at 15 min intervals after the administration of the drug for 3 h. A 10 seconds cut off time was used in order to prevent tissue damage.

Antipyretic activity: An increase in body temperature was induced in mice by subcutaneous injection of 20 mL kg^{-1} of a 20% aqueous suspension of brewer's yeast according to[8]. The mean rectal temperature was measured at different intervals after i.p. administration of the extract (250 and 500 mg kg^{-1} orally or indomethacin (4 mg kg^{-1}).

Diuretic activity: The animals in three groups (one group served as control) were put in the metabolic cages and after the initial adjustment period of 3 days, the test substance (Balessan extract) was orally administered to the rats of the experimental group at doses of 250 and 500 mg kg^{-1} body weight. The effect of extract at different doses was recorded by measurement of urine volumes of each group[9]. The urines were analysed for their content of Na^+, K^+, Ca^{++}.

RESULTS

The preliminary qualitative phytochemical screening of the aerial parts of the Balessan revealed the presence of flavonoids, saponins, volatile oil, sterols and/or triterpenes.

The ethanolic extract was found to suppress carrageenan-induced rat paw edema significantly. The extract also exhibited the ability to significantly reduce the granulation mass formation at the higher dose of 500 mg kg^{-1}. Although, the inhibition of granuloma formation was recorded in the group of animals receiving 250 mg kg^{-1} of ethanol extract, but this inhibition was found to be statistically non-significant (Table 1).

Pretreatment of mice with ethanol extract (250 and 500 mg kg^{-1}) produced a significant reduction in writhes induced by acetic acid (Table 2). Treatment of mice with an ethanolic extract of Balessan significantly increased the tail flick latency to the nociceptive stimuli (Table 3). However, no variation of the reaction time was observed on this test when a lower dose (250 mg kg^{-1}) was administered. The extract produced a dose-and-time-dependent decrease in yeast-induced hyperthermia in mice (Table 4).

Table 1: Effect of an ethanolic extract of Balessan on Carrageenan-induced paw edema and cotton pellet granuloma in albino rats.

		Carrageenan-induced edema of right hind paw		Cotton pellets granuloma	
Treatments(n=6)	Dose orally mg kg^{-1}	Mean increase in paw volume±SEM after (3 h)	% Inhibition	Mean increase	% Inhibition
Normal control only carrageenan	-	0.41± 0.01	-	53.00±3.29	-
Balessan ext.+ Carrageenan	250	0.33±0.01***	19	46.5 ±2.33	12
Balessan ext.+ Carrageenan	500	0.26±0.01***	36	42.00±2.17*	21
Phenylbutazone+ Carrageenan	100	0.20±0.01***	50	22.00±2.93***	58

*p<0.01, ***p<0.001 Student's t-test

Table 2: Effect of an Ethanolic extract of Balessan on Acetic acid-induced writhing in mice

Groups n=6	Dose orally mg kg^{-1}	Number of writhings/10 min	% Inhibition
Control	-	39.00±2.44	
Balessan extract	250	32.00± 1.66*	18
Balessan extract	500	27.00±1.46**	31
Indomethacin	4	18.33±1.89***	53

*p<0.05, **p< 0.01, ***p<0.001 Student's t-test

Table 3: Effect of an Ethanolic extract of Balessan on yeast induced hyperpyrexia in mice

			Rectal temperature (°C) Post drug				
Groups n=6	Dose orally mg kg^{-1}	Pre drug	15 Min	30 Min	60 Min	90 Min	120 Min
Balessan extract	250	36.60±0.22	36.35±0.26	36.16±0.26	35.96±0.10*	35.93±0.27	35.80±0.32
Balessan extract	500	37.06±0.32	36.65±0.17	35.91±0.19*	35.88±0.24*	35.66±3.29**	35.76±0.29*
Indomethacin	4	36.55±0.19	36.35±0.16	35.50±0.16**	34.63±0.17***	34.55±0.32***	34.53±0.32***

*p<0.05, **p<0.01, ***p<0.001 Student's t-test

Table 4: Effect of an ethanolic extract of Balessan on tail flick test in mice

			Reaction time (sec) Post drug				
Group n=6	Dose orally mg kg^{-1}	Pre drug	15 Min	30 Min	60 Min	90 Min	120 Min
Balessan extract	250	3.43±0.25	3.70±0.08	3.81±0.15	4.01±0.13	3.83±0.15	3.83±0.15
Balessan extract	500	3.58±0.23	3.80±0.11	4.50±0.24*	4.50±0.36**	5.15±0.31**	4.50±0.23*
Indomethacin	4	3.56±0.28	4.61±0.28**	5.83±0.26***	6.33±0.22***	6.06±0.10***	5.36±0.22***

*p<0.05, ** p<0.01, *** p<0.001 Student's t-test

Table 5: Effect of an ethanolic extract of balessan on diuretic studies

		Urine out put		pH of urine	
Groups n=6	Dose orally mg kg^{-1}	Volume of urine (mL)	% Increase of volume	pH of urine	% Decrease of pH
Control	-	5.41±0.71		10.21±0.162	
Balessan extract	250	6.61±0.72	22	9.26±0.180**	9
Balessan extract	500	8.30±0.74*	31	8.85±0.130***	13

*p<0.05, **p<0.01, ***p<0.001 Student's t-test

No significant changes in Na^+, K^+, Ca^{++} and creatinine excretion. Only significant increase in urine volume and decrease in urine pH was recorded (Table 5).

DISCUSSION

Among several traditional claims, the usefulness of Balessan *C. opobalsamum* in swellings, pain and fever have been emphasized more by the traditional healers (Personal Communication, 2002). The results of the present investigation revealed that the oral administration of an ethanolic extract of Balessan possesses a potential anti-inflammatory effect which was evidenced by the significant reduction in paw edema and cotton-pellet granuloma methods. However, the effect was less when compared with Phenylbutazone. Inflammation, a dynamic process considered as a protective mechanism, leads to a chronic inflammatory state when deregulated[9]. During the condition of inflammation associated with pain and fever, arachidonic acid is liberated from the phospholipids fraction of cell membranes and then enzymatically transformed to prostaglandins which sensitize blood vessels to the effect of mediators such as bradykinin, 5-HT and histamines that increase permeability[10,11]. It was

also observed that the extract significantly reduced the granuloma formation in rats. Multiplication of small blood vessels as well as proliferation of fibroblasts are the characteristic features at the repair phase of inflammation. Such proliferating cells penetrate the exudates, producing a highly vascularized reddened mass known as granulation tissue[12]. The extract of Balessan extract effectively reduced the cotton-pellet-induced granuloma suggesting its activity in the proliferative phase of the inflammation. The extract proved to possess analgesic activity. This may be due to the volatile oil, saponins, triterpenes and flavonoids which are present in Balessan. These substances are reported to possess analgesic, anti-inflammatory, fever reducing and antioxidant properties[13,14].

As the result of the Hippocratic screening test indicated that Balessan extract caused marked urination volume. Balessan extract was tested on kidney function and appeared to cause marked diuresis without any effect on Na^+, K^+ and Ca^{++} ions excretion. Thus, its apparent diuretic effect seemed to be due to inhibition of release of ADH (vasopressin) or blockade of its renal receptors. However, this diuretic potential may contribute, at least partly in reducing inflammation[15].

In conclusion, *C. opobalsamum* extract has an anti-inflammatory, analgesic, antipyretic and diuretic effects. These findings substantiate the claim of traditional herbal practitioners; the use of Balessan in joint swelling and pain. It deserves further studies to identify its active components and investigate their mechanism(s) of action.

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