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First *in vitro* lipid peroxidation and *in vivo* anti-inflammatory activity of the Omani frankincense of *Boswellia sacra* Flueck.

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Abstract

The use of frankincense for the medicinal and religious purposes dates back to the early Egyptians, the Greeks, and the Chinese and today, it is commonly used as an anti-inflammatory, antiseptic, anti-tumor, sedative and diuretic agent. To prove the scientific basis of the anti-inflammatory properties and the *in vitro* lipid peroxidation of the Omani Frakincense on pharmacological grounds, the present study was conducted. The crude extract, the essential oils and various sub-fractions of the crude methanolic extract (300 mg/kg of the body weight of the animals) obtained from the resin of *Boswellia sacra* Flueck. were evaluated for their *in vivo* anti-inflammatory activities using carrageenan-induced hind paw edema in rats and *in vitro* lipid peroxidation by using the thiobarbituric acid reactive substance (TBARS) assay. The tremendous folk use of frankincense as an anti-inflammatory agent was rationalized by our present study of the *in vitro* TBARS assay and the *in vivo* anti-inflammatory activities. To the best of our knowledge, this is the first report of the anti-inflammatory activity studies which has been conducted thoroughly on the essential oils, the crude extract and the various sub-fractions obtained by using various polarity organic solvents on the resin of the plant *Boswellia sacra* Flueck.

Keywords: Frankincense, Essential oil, *Boswellia sacra*, Anti-inflammatory activity, Lipid peroxidation

Introduction

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli [1, 2]. It has long been recognized as a localized protective reaction of tissue to irritation, injury, or infection that is characterized by pain, redness and swelling [3]. During inflammation, high levels of reactive oxygen species (ROS) are also produced to exert a defense against pathogens [4]. Among them, abnormal excess nitric oxide produced by an inducible nitric oxide synthase (iNOS) is believed to act as a toxic radical that can damage cellular macromolecules such as proteins, DNA, and lipids, triggering several unfavorable cellular responses [3, 5]. Therefore, inhibition of the cellular reaction is one of the strategies for treatment of inflammatory diseases. For *in vivo* tests, inflammation can be induced in animals by many substances. Mice paw edema is the most commonly used model for acute inflammation while subcutaneous implantation of biomaterial is usually used for inflammatory model [3]. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can easily be induced [6]. The side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses [7]. Therefore, the development of newer and more potent anti-inflammatory drugs with lesser side effects is necessary. Furthermore, the link between inflammation and TBARS measurement, antioxidant deficiency or the synthesis of inflammatory cytokines has been reported in literature [8]. Our present study includes the TBARS assay for the lipid peroxidation.

Frankincense is the gum resin obtained by the incisions in the trunk of the trees of *Boswellia* genus of the family Burseraceae. *Boswellia sacra* Flueck. is native to the southern part of Oman; a closely related specie (*B. carteri*) growing also in Yemen and in Northern Somalia [9]. The pharmacological activities of frankincense, as crude extracts, the distilled essential oil [10, 11] and the isolated compounds have been investigated [12-16]. According to several published reports the frankincense essential oil exhibits *in vitro* antibacterial, antifungal and immunomodulatory activity [17]. Despite the extensive phytochemical and pharmacological investigations of frankincense obtained from other

spices, the Omani frankincense obtained from *B. sacra* is the least explored. To the best of our knowledge, this is the first study which invistigates the anti-inflammatory activity of the Omani frankincense from *B. sacra* Flueck. which aimed to evaluate the anti-inflammatory potential of the methanol extract, various sub-fractions and the crude oils of frankincense in both *in vitro* and *in vivo*.

Materials and Methods

Plant Material

The aerial parts of *B. sacra* Flueck. and the various grades of resin were collected in April and May 2010 from different locations in Dhofar (Southern part of Oman) and were supplied by a trustful Dhofari partner (Dr. Saleh Al-Amri). SHG (Super Hougari Green), HR (Hougari Regular), RHW (Royal Hougari White), and HY (Hougari Yellow) grade resins were collected from Wadi Hougar, while SF (Shabi Frankincense) was collected from Wadi Magsyl. All these samples were authenticated by Dr. Mustafa Mansi (botanist), Department of Biological Sciences and Chemistry, University of Nizwa, the Sultanate of Oman.

Extraction and Isolation

The methanol extract (100 g) of the Hougari Regular (HR) grade resin was subjected to vacume liquid chromatography (VLC) over a silica gel column (1000 g, 70-230 mesh, Merck) for the sub-fractionation which yielded various sub-fractions. The polarity of the eluting solvent was increased with 5% chloroform gradient in polarity up to 100%, then with 1% gradient of methanol up to 10%, followed by the 5% gradient of methanol up to 40% and then finally washed with methanol. The extraction of oils was carried out by Soxhlet apparatus. *n*-Hexane was used as a mobile phase and the extraction was carried out by the standard method reported in literature [18]. Prior to the oil extraction, all the resin samples were shade dried, and the dried resins thus obtained were ground to make powder using the grinder (IKA, MF 10 basic).

TABLE-1: Effect of test samples (300 mg/kg) on the rat paw edema induced by 2% carrageenan

Samples	Paw volume measurement at different time intervals (% inhibition)							
	0.0 min	15 min	30 min	1 hr	2 hr	3 hr	4 hr	12 hr
Control	0.90 ± 0.13 (-)	1.30 ± 0.08 (-)	1.45 ± 0.07 (-)	1.55 ± 0.08 (-)	1.60 ± 0.02 (-)	1.70 ± 0.05 (-)	1.82 ± 0.08 (-)	1.30 ± 0.67 (-)
SHG oil	0.92 ± 0.05 (2.2)	1.30 ± 0.22 (0.0)	1.40 ± 0.22 (3.5)	1.45 ± 0.17 (6.6)	1.45 ± 0.14 (9.4)	1.45 ± 0.16 (14.7) *	1.40 ± 0.21 (23.1) **	1.30 ± 0.17 (0.0)
HR oil	0.89 ± 1.05 (1.1)	1.30 ± 1.08 (0.0)	1.50 ± 1.16 (3.4)	1.52 ± 0.08 (1.9)	1.45 ± 0.16 (9.4)	1.35 ± 0.17 (20.6) **	1.35 ± 0.16 (25.8) **	1.25 ± 0.19 (3.9)
RHW oil	0.94 ± 0.06 (4.4)	1.40 ± 0.21 (7.7)	1.35 ± 0.26 (6.9)	1.54 ± 0.32 (0.7)	1.55 ± 0.28 (3.1)	1.60 ± 0.22 (5.9)	1.65 ± 0.24 (9.4)	1.42 ± 0.21 (9.2)
SF oil	0.95 ± 0.05 (5.6)	1.00 ± 0.08 (23.1) **	1.20 ± 0.07 (17.3)	1.22 ± 0.06 (21.3) **	1.30 ± 0.09 (18.8) **	1.41 ± 0.08 (17.1) *	1.35 ± 0.98 (25.8) **	1.22 ± 0.22 (6.2)
HY oil	0.90 ± 0.08 (0.0)	0.95 ± 0.13 (26.9) **	1.25 ± 0.06 (13.8)	1.32 ± 0.05 (14.8) *	1.43 ± 0.12 (10.6) *	1.51 ± 1.22 (11.2) *	1.45 ± 1.02 (20.3) **	1.32 ± 1.21 (1.5)
Crude Extract (HR)	0.87 ± 0.05 (3.3)	1.20 ± 0.15 (7.7)	1.32 ± 0.65 (8.9)	1.32 ± 0.45 (14.8) *	1.35 ± 0.87 (15.6) *	1.42 ± 0.18 (16.5) *	1.36 ± 0.15 (25.3) **	1.30 ± 0.16 (0.0)
36% $CHCl_3$/*n*-hexane	1.10 ± 0.33 (22.2)	1.10 ± 0.15 (15.4) *	1.24 ± 0.12 (14.5) **	1.32 ± 0.14 (14.8) *	1.44 ± 0.32 (10.0) *	1.50 ± 0.31 (11.8) *	1.45 ± 0.18 (20.3) **	1.35 ± 0.17 (3.8)
40% $CHCl_3$/*n*-hexane	1.00 ± 0.15 (11.1)	1.10 ± 0.21 (15.4) *	1.10 ± 0.31 (24.1) **	1.30 ± 0.65 (16.1)*	1.41 ± 0.77 (11.9) *	1.51 ± 0.32 (11.2) *	1.45 ± 0.12 (20.3) **	1.32 ± 0.13 (1.5)
60% $CHCl_3$/*n*-hexane	0.93 ± 0.15 (3.3)	1.20 ± 1.14 (7.7)	1.30 ± 1.23 (10.4) *	1.41 ± 1.15 (9.1)	1.40 ± 1.12 (12.5) *	1.42 ± 1.14 (16.5) *	1.38 ± 0.15 (24.2) **	1.28 ± 0.07 (1.5)
70% $CHCl_3$/*n*-hexane	0.95 ± 0.25 (5.6)	1.22 ± 0.15 (6.2)	1.31 ± 1.27 (10.4) *	1.39 ± 0.13 (10.3)*	1.39 ± 0.13 (13.1) *	1.38 ± 0.12 (18.9) **	1.35 ± 0.13 (25.8) **	1.25 ± 0.13 (3.9)
2% MeOH/$CHCl_3$	0.90 ± 0.05 (0.0)	1.20 ± 0.41 (7.7)	1.35 ± 0.76 (6.9)	1.41 ± 0.45 (9.1)	1.35 ± 0.14 (15.6) *	1.32 ± 0.16 (22.4) **	1.32 ± 0.21 (27.5) **	1.30 ± 0.31 (0.0)
4% MeOH/$CHCl_3$	0.92 ± 0.12 (2.2)	1.20 ± 0.14 (7.7)	1.36 ± 0.25 (6.2)	1.41 ± 0.18 (9.1)	1.37 ± 1.22 (14.4) *	1.35 ± 0.23 (20.6) **	1.32 ± 0.16 (27.5) **	1.29 ± 0.12 (0.8)
6% MeOH/$CHCl_3$	0.96 ± 0.08 (6.7)	1.30 ± 0.22 (0.0)	1.32 ± 0.16 (9.0)	1.42 ± 0.16 (8.4)	1.36 ± 0.15 (15.0) *	1.39 ± 0.12 (18.2) **	1.35 ± 0.22 (25.8) **	1.30 ± 0.14 (0.0)
Aspirin (300 mg/kg)	0.85 ± 0.25 (5.6)	1.10 ± 0.18 (15.4) *	1.32 ± 0.17 (9.0)	1.35 ± 0.05 (12.9) *	1.37 ± 0.12 (14.4) *	1.45 ± 0.15 (14.7) *	1.42 ± 0.09 (22.0) **	0.90 ± 0.75 (30.8) **

Animals

Male Wistar rats weighing 180-200 g were used as test animals. The animals were housed in standard cages at room temperature under controlled light and temperature with free access to food and water. Prior to the experiments (*ca*. 24 h), the animals were maintained at the laboratory conditions with free access to water only. The animals submitted to oral administration of the test samples were fasted for 12 h.

Anti-inflammatory Activity Studies

The determination of the anti-inflammatory activity was carried out using the carrageenan-induced hind paw edema model [19]. Animals were treated with the test samples (each 300 mg/kg of the body weight) by oral administration. The rat paw edema was induced by injecting 2% carrageenan (0.1 mL) into the right hind paw plantar surface of the selected animals. This test was performed according to the method already reported in literature [20]. Saline solution (0.9%, 0.1 mL) was injected into the left paw as the negative control reference. The standard drug aspirin (300 mg/kg) was used as the positive standard. The foot volume measurements were carried out following the plethysmometer method described in the literature [21]. The rat paw volume was measured before the drug injection and then consecutively after 15 and 30 min. It was then measured at 1 h intervals for 4 h and finally after 12 h. The results of these studies are presented as mean ± SEM in Table 1.

Thiobarbituric Acid Reactive Substance (TBARS) Assay

Lipid peroxidation or the oxidative degradation of lipids is assayed by the TBARS method reported in the literature [19]. MDA (malondialdehyde), a possible end product of the lipid peroxidation, is reacted with thiobarbituric acid in the acidic high temperature and formed a red complex TBARS. The amount of tissue TBARS is measured by the thiobarbituric acid assay (TBA) as previously described by Buege and Aust [22]. Typically, 20 µL of substrate (Phosphotidyl choline), 5 µL of Tris-HCL buffer (pH 7.1), 5 µL of ferrous sulphate (1 mM), and 20 µL (0.5 mM) of sample inhibitor were added in 96 well plate. Then 30 µL of double distilled water was added. It was then incubated at 37 C for 15 min. Then 50 µL of TCA (50%) and 120 µL of TBA (0.35g) were added to the reaction mixture. It was then incubated for 15 minutes at boiling water bath and pink color chromogen appeared. Readings were taken at 532 nm (spectra Max-340). Percent radical scavenging activity by samples was determined in comparison with a DMSO treated control group. Following formula was used to calculate % lipid peroxidation inhibition activity. Quercetine and BHA (0.5 mM) were used as standard inhibitors in anti Lipid per oxidation assay which showed 85.025% and 90.321% inhibition, respectively.

Statistical Analysis

The statistical analyses were performed according to the method described by Alcaraz and Jimenez [23]. The values are expressed as mean ± SEM. The significance of difference between means was determined by Student's *t*-test at value of $p<0.05$.

Table 2 Results of the *in vitro* TBARS assay (% inhibition)

Samples	% inhibition TBARS assay
HR oil	14.2
HRA oil	18.3
HRB oil	25.6
SF oil	33.1
Crude extract	16.4
Pure hexane	6.5
36% $CHCl_3$/*n*-hexane	3.3
40% $CHCl_3$/*n*-hexane	5.8
60% $CHCl_3$/*n*-hexane	3.3
80% $CHCl_3$/*n*-hexane	17.9
Pure $CHCl_3$	26.6
2% MeOH/$CHCl_3$	8.1
8% MeOH/$CHCl_3$	8.1
40% MeOH/$CHCl_3$	5.5
Quercetin	85.0

Result and discussion

The intraplatar injection of carrageenan induces rat paw edema which is a model of acute inflammation being used for the investigation of anti-inflammatory drugs. According to this model, a large variety of mediators are released in two distinctive phases; histamine and serotonin in the first phase and prostaglandins in the second phase [24].

The crude methanol extract and the various sub-fractions (36, 40, 60, 70% chloroform/*n*-hexane and 2, 4, 6% MeOH/chloroform) were obtained through VLC of the HR grade resin of *B. sacra* Flueck. The crude fats were obtained through Soxhlet extraction of all available grades (SHG, HR, RHW, SF, and HY) of the Omani frankincense. All these samples were investigated for the *in vitro* lipid peroxidation and the *in vivo* anti-inflammatory activities. The *in vivo* studies were carried out using the reported procedure of carrageenan-induced paw edema model in rats [20].

The results obtained through paw edema model in rats induced by carrageenan (Table 1) showed a significant reduction in the inflammatory growth in the hind paw of the rats. The SF oil inhibited the formation of edema by 21.3, 18.8, 17.1, and 25.8% after 1, 2, 3, and 4 h, respectively. This is higher than aspirin (standard drug), which showed the respective inhibition of 12.9, 14.4, 14.7, and 22.0% after 1h, 2h, 3h, and 4h, respectively. Similarly, the HR oil, the crude extract, and the more polar fractions (60% chloroform/*n*-hexane or above) showed the inhibition of edema formation after 3h and 4h of the injection of the inflammatory stimulus (Table 1). It is reported that the highest volume of carrageenan-induced edema is characterized by the presence of prostaglandins and other autacoids [25, 26]. The mechanism of action of our active samples (SF oil, HR oil, crude extract, and the polar fractions obtained at 60% chloroform/*n*-

hexane or above), is likely to follow that of the standard drug aspirin used in this study.

The carrageenan-induced inflammatory response has been demonstrated to be associated with the neutrophil infiltration and the production of neutrophil-derived mediators and free radicals [27]. On the other hand the MDA production is due to free radical attack on the plasma membrane. Thus the carrageenan-induced inflammatory effect is associated with the accumulation of MDA. Hence the results obtained from the *in vitro* TBARS assay (Table 2) are in agreement with the results of our anti-inflammatory studies. The same sequence of relative potency was observed in this assay; however the sample of the highest polarity showed lower inhibition when compared to those obtained at lower polarity (80% and 100% chloroform/*n*-hexane) (Table 2, Figure 1).

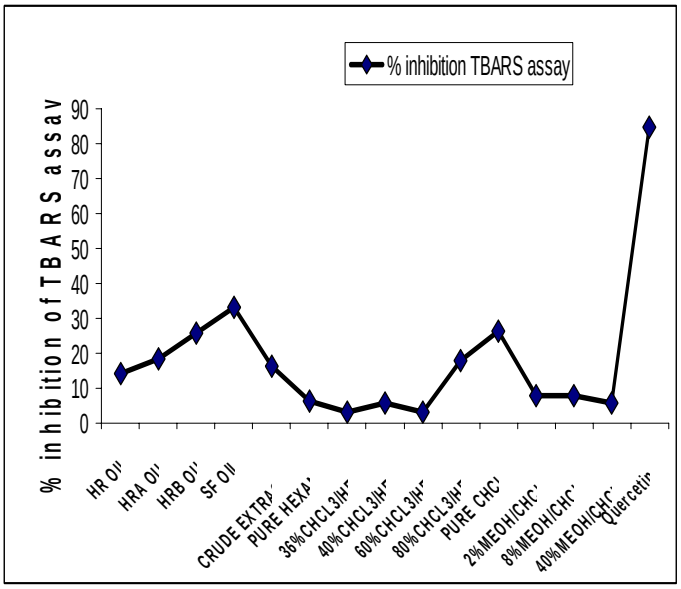

Figure 1: % Inhibition of TBARS of essential oils and extracts

Conclusions

The results of this study indicated that the various samples possessed significant anti-inflammatory activities. Some of our tested samples showed higher activity than the standard drug (aspirin) used in this study. The present study supports the traditional use of the resin and its preparations which are being used by the local communities for various inflammatory problems. It also suggests the presence of active ingredients in these sub-fractions, which can be developed into anti-inflammatory agents. Thus, there is a dire need for further phytochemical studies to investigate the pharmacological and toxicological profiles for the complete understanding of the medicinal potential for this valuable resin.

Authors' contributions

Ahmed Al-Harrasi, Ahmed Al-Rawahi and Javid Hussain designed and administrated the experiment and gave technical support and conceptual advice. They edited the manuscript too. Liaqat Ali, Hidayat Hussain, and Najeeb ur Rehman performed the experiments, analyzed the experiment results and compiled the manuscript. Ghulam Abbas, Mahjabeen and Rashid Al-Harrasi performed the pharmacological and biological tests.

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