Cardioprotective and antioxidant effects of oleogum resin "Olibanum" from *Boswellia carteri* Birdw. (Burseraceae)

Ahmed A. Zaki *, Nadia E. Hashish, Mohamed A. Amer, Mohamed-Farid Lahloub

Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, 35516, Egypt

Available online 20 May 2014

[ABSTRACT] One of the leading causes of death worldwide is cardiovascular disease, hence searching for a cure is an important endeavor. The totally safe, edible, and inexpensive *Boswellia* plant exudate, known as olibanum or frankincense, is considered to possess diverse medicinal values in traditional medicine and from recent biological studies. Investigating the cardioprotective and antioxidant activities of olibanum from a *Boswellia* species, family Burseraceae, namely *Boswellia carteri* Birdw. was the aim of this study. Cardioprotective activity was evaluated using a model of myocardial infarction induced by isoproterenol (ISO), while antioxidant activity was tested adopting nitric oxide scavenging (NOS) and azino-bis-3-ethyl benzthiazoline-6-sulfonic acid (ABTS) assays. The results revealed a mild cardioprotective effect and weak antioxidant activity.

[KEY WORDS] *Boswellia carteri*; Olibanum; Frankincense, Cardiovascular diseases; Antioxidant;


Introduction

According to the World Health Organization (WHO), cardiovascular diseases are the world’s largest killer, claiming the lives of 17.1 million persons each year [1]. Myocardial infarction results from any interruption in the blood supply to any part of the heart, and leads to the death of the cardiac tissue (myocardial necrosis). The consequences of myocardial infarction include hyperlipidemia, peroxidation of membrane lipids, and loss of plasma membrane integrity [2].

High levels of catecholamines induce myocardial infarction through depleting the reserved energy of cardiac muscle cells, leading to complex biochemical and structural changes that cause irreversible cellular damage and ultimately necrosis. Iso-proterenol (ISO), a synthetic catecholamine, produces myocardial infarction through the generation of highly cytotoxic free radicals resulting from its auto-oxidation [3]. The biochemical and histological changes occurring after administration of ISO in animals, including ISO-induced myocardial necrosis were first reported by Rona et al. in 1959 [3].

The pharmacological effects of ISO are also associated with its β-adrenergic effects, such as an increase in heart rate, decrease in blood pressure, and diminished oxygen supply to the myocardium. ISO-induced myocardial necrosis is a well-known standard model to study the beneficial effects of drugs on cardiac dysfunction. Within six minutes of i.p., intraperitoneal, injection of ISO, histological changes such as myofilament fragmentation occur [4].

Known since antiquity, Frankincense oleo gum resin is produced by either the natural exudation or by incision of the bark of several species of *Boswellia* (Burseraceae). Frankincense is also known as Olibanum, Luban Dakar, Bakhor, or Kendar (in Arabic), and Salai Guggal (in Ayurvedic medicine) [5-6]. The plant is native to India, the Arabian Peninsula, and the North-East of Africa [7-10]. Olibanum played an essential role in mummification as embalming liquid, and was also used as incense. In traditional medicine, olibanum is used in the treatment of cough and asthma, as a diuretic, and as an emmenagogue, while the essential oil and the absolute are used as fixatives in perfumes, soaps, creams, and lotions. Frankincense oleogum resin is a complex mixture containing a series of mono-, sesqui-, di-, and triterpenoids [11-12]. Compounds isolated from *Boswellia* resin, have shown various biological activities including anti-inflammatory activity [13], leukotriene biosynthesis-inhibitory activity [14-15], antitumor activity [16], and immunomodulatory effects [17].

[Received on] 01-Feb.-2013

[Corresponding author] Ahmed A. Zaki: E-mail: ahmedawadzaki@yahoo.co.uk

These authors have no conflict of interest to declare.

Published by Elsevier B.V. All rights reserved.
Medicinal plants with antioxidant properties are also able to impart cardio-protection [31], thus *Boswellia* species are candidates to possess the same activity since, for example, an extract of *B. serrata* showed free radical scavenging activity. The essential oil of many *Boswellia* species reportedly demonstrated weak antioxidant activities [19-20, 32].

WHO recommends the use of plant-based medicines as an alternative medicine, especially in developing countries [1]. Therefore, screening plants as potential sources for new drugs is a rational approach. In this study, the oleogum resin of *Boswellia carteri* Birdw. (Burseraceae), known as olibanum, was evaluated as a prophylactic and as a therapy for cardiovascular diseases.

**Materials and Methods**

**Chemicals**

Isoprenaline hydrochloride (ISO), malondialdehyde (MDA), creatine kinase (CK), glutamic oxaloacetic transaminase (GOT), and lactate dehydrogenase (LDH), and all other chemicals are of analytical grades were purchased from Bio-diagnostic Co., Dokki, Giza, Egypt and Sigma Chemical Co., St. Louis, MO, USA. Marker enzymes were assayed in serum using standard kits supplied from SweMed diagnostics, Bangalore, India.

**Olibanum oleogum resin of *Boswellia carteri* Birdw. (Burseraceae) was purchased from a local herbal store in May 2012, and compared with a genuine sample kept in the Drug Museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University. Reviewing the phytochemical differentiation between *Boswellia* species exudates in the literature further confirmed the sample as olibanum of *B. carteri* [19, 21]. A voucher specimen of the sample was deposited in the herbarium of the department numbered as: olibanum/B. carteri/OGR/105.**

**Preparation of extract**

The oleogum resin (100 g) was finely powdered and then extracted with methanol (400 mL). The extract was filtered and concentrated under reduced pressure to yield the alcohol-free extract (20 g).

**Phytochemical analysis**

Phytochemical screening was performed according to standard procedures for the presence of different groups, including alkaloids, glycosides, resins, tannins, saponins, terpenoids, and carbohydrates. The results of phytochemical screening are cited in Table 1.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Response</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystalline sublimate</td>
<td>—</td>
<td>Claus &amp; Tyler, 1968 [24]</td>
</tr>
<tr>
<td>Volatile constituents</td>
<td>++</td>
<td>Egyptian Pharmacopoeia, 1984 [15]</td>
</tr>
<tr>
<td>Carbohydrates and/or glycosides</td>
<td>++</td>
<td>Balbaa et al., 1981 [30]</td>
</tr>
<tr>
<td>Tannins</td>
<td>—</td>
<td>Wall et al., 1954 [31]; Trease &amp; Evans, 1996 [4]</td>
</tr>
<tr>
<td>Saponins</td>
<td>—</td>
<td>Wall et al., 1954 [37]</td>
</tr>
<tr>
<td>Oxidase Enzyme</td>
<td>—</td>
<td>Wall et al., 1954 [37]</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>—</td>
<td>Shellard, 1957 [40]</td>
</tr>
<tr>
<td>Unsaturated sterols and/or triterpenes</td>
<td>++</td>
<td>Burke et al., 1974 [41]; Wall et al., 1954 [37]</td>
</tr>
<tr>
<td>Cardenolides</td>
<td>—</td>
<td>Neher, 1969 [42]; Kedde, 1981 [43]</td>
</tr>
</tbody>
</table>

**Assessment of the antioxidant activity of olibanum extract**

**Nitric oxide scavenging assay**

Nitric oxide (NO) generated by sodium nitroprusside in aqueous solution at physiological pH is measured by the Griess reaction according to Naskar et al. [21], as follows: 1 mL of 10 mmol L⁻¹ sodium nitroprusside is mixed with 1 mL of test solution at different concentrations (100, 250, 500, 1000 μmol L⁻¹) in phosphate buffer saline (pH 7.4) and the mixture is incubated at 25–30 °C for 150 min. One mL from the incubated mixture is mixed with 1 mL of Griess’ reagent (1% sulphanilamide, 2% ortho-phosphoric acid 0.1% naphthyl ethylene diamine dihydrochloride). This leads to diazotization of nitrite with sulphanilamide and coupling with naphthyl ethylene diamine dihydrochloride. The absorbance of the chromophore formed is read at 546 nm. The percentage inhibition of the absorbance is calculated by comparing the results of the test with those of the control using the given equation. The mean of three readings is cited in Table 2.

Percentage inhibition = (1– absorbance of test/absorbance of control) × 100%

**ABTS assay**

The assay employs a radical cation derived from ABTS (azino-bis-3-ethyl benzthiazoline-6-sulfonic acid) as a stable free radical to assess the antioxidant activities of different extracts [23]. The advantages of this assay are the stability of the free radical and that the reaction is stoichiometric. The mean of three readings is cited in Table 2.

**Experimental animals**

Wistar male albino rats weighing 150–200 g were obtained from the Experimental Animal House of the Faculty of Pharmacy, Mansoura University. The study protocol was reviewed and approved by the faculty Ethics Committee for the
Table 2  Results of radical scavenging activity of olibanum, where NO = Nitric Oxide, and ABTS = azino-bis-3-ethyl benzthiazoline-6-sulfonic acid

<table>
<thead>
<tr>
<th>Assay</th>
<th>ABTS</th>
<th>% Inhibition</th>
<th>NO Scavenging</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µg·kg⁻¹</td>
<td>0.123</td>
<td>15.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250 µg·kg⁻¹</td>
<td>0.119</td>
<td>18.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 µg·kg⁻¹</td>
<td>0.113</td>
<td>22.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 000 µg·kg⁻¹</td>
<td>0.106</td>
<td>33.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.041</td>
<td>87.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As: Absorbance of sample, Ac: Absorbance of control

Use and Cdelare of Experimental Animals. The animals were acclimatized to the departmental animal house and housed under standard laboratory conditions of temperature at (27 ± 2) °C, and a light: dark cycle of 12 hours. They were group housed in polypropylene cages with no more than four animals per cage, and had free access to food pellets and tap water.

Experimental protocol

A total of 30 rats were used and randomly divided into five main groups, each of six animals.

Group 1: negative control (distilled water p.o.)

Group 2: positive control (ISO-treated; 85 mg·kg⁻¹, s.c.) at an interval of 24 hours for two days [24].

Groups 3, 4, and 5 were administered Boswellia carteri alcohol extract at 100, 200, and 400 mg·kg⁻¹ body weight/day, p.o., respectively for 30 days, followed by s.c. administration of ISO (85 mg·kg⁻¹) at an interval of 24 h for two days. Twelve hours after the second injection of ISO, an aliquot (5 mL) of the blood was collected through eye vein and centrifuged for 10 min. The serum was then withdrawn into Eppendorf tubes for the biochemical analyses. The rats were sacrificed, the hearts were excised, and left ventricles were isolated and then stored in formalin solution for histopathological assessment. The hearts were washed in cold phosphate buffer then kept frozen.

Biochemical assay

The marker enzymes AST, CK-MB, and LDH were assayed in serum using standard kits. The heart tissue was excised immediately, washed with chilled isotonic saline, tissue homogenates were prepared in ice cold 0.1 mol·L⁻¹ tris-HCl buffer (pH 7.2), and used for the assay of lipid peroxidation (MDA).

Table 3  Results of biochemical analyses in serum, where ISO = Isoprenaline (X ± s, n = 6)

<table>
<thead>
<tr>
<th>Parameter/groups</th>
<th>Normal control</th>
<th>ISO only</th>
<th>ISO + 100 mg·kg⁻¹ olibanum</th>
<th>ISO + 200 mg·kg⁻¹ olibanum</th>
<th>ISO + 400 mg·kg⁻¹ olibanum</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (µmol·L⁻¹)</td>
<td>95.02 ± 1.73</td>
<td>270.30 ± 3.75</td>
<td>212.28 ± 6.17</td>
<td>210.06 ± 4.51</td>
<td>184.31 ± 4.04</td>
</tr>
<tr>
<td>LDH (µmol·L⁻¹)</td>
<td>64.67 ± 2.08</td>
<td>248.05 ± 3.78</td>
<td>192.03 ± 3.44</td>
<td>143.30 ± 4.93</td>
<td>132.01 ± 5.13</td>
</tr>
<tr>
<td>CK-MB (µmol·L⁻¹)</td>
<td>39.22 ± 4.03</td>
<td>230.23 ± 5.27</td>
<td>113.33 ± 2.64</td>
<td>105.01 ± 5.02</td>
<td>101.44 ± 6.07</td>
</tr>
</tbody>
</table>

Differences in means were estimated by ANOVA followed by Dunnett’s multiple comparison test. The values of serum AST, LDH and CK-MB values of Groups treated with 100, 200, and 400 mg·kg⁻¹ olibanum vs Group ISO only and Group ISO vs Group Normal. * P < 0.05 significant difference

Table 4  Results of the biochemical analyses in heart tissue homogenate, where ISO = Isoprenaline (X ± s, n = 6)

<table>
<thead>
<tr>
<th>Parameter/groups</th>
<th>Normal control</th>
<th>ISO only</th>
<th>ISO + 100 mg·kg⁻¹ olibanum</th>
<th>ISO + 200 mg·kg⁻¹ olibanum</th>
<th>ISO + 400 mg·kg⁻¹ olibanum</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol·g⁻¹ tissue)</td>
<td>41.99 ± 1.84</td>
<td>394.10 ± 6.25</td>
<td>258.00 ± 2.46</td>
<td>222.01 ± 6.56</td>
<td>172.60 ± 6.01</td>
</tr>
</tbody>
</table>

Differences in means were estimated by ANOVA followed by Dunnett’s multiple comparison test. The values of MDA level of Groups treated with 100, 200 and 400 mg·kg⁻¹ olibanum vs Group ISO only and Group ISO vs Group Normal. * P < 0.05 significant difference

Fig. 1  Results of biochemical analyses in serum, where ISO = Isoprenaline

**Fig. 2** Results of biochemical analyses in heart tissue homogenate, where ISO = Isoprenaline

**Preparation of heart homogenate**

The homogenates were prepared according to Ojha et al. [25], as follows. The frozen hearts were thawed then weighed a 10% homogenate was prepared from the minced heart in phosphate buffer (50 mmol·L\(^{-1}\), pH 7.4), and the supernatant was used for biochemical estimations.

**Histopathological studies of myocardium**

The myocardial tissue was fixed in 10% buffered formalin solution. Cross sections of 5 µm thickness of myocardial tissues were cut and stained with hematoxylin and eosin followed by microscopic examination of the myocardial histoarchitecture. Representative area images were captured in an image analysis system (Fig. 3). The slides were evaluated for myonecrosis, inflammatory cell infiltration, and edema. About 5-10 fields for each slide were examined and the severity of changes of the above-mentioned criteria were graded (Table 5).

**Statistical analysis**

The results were expressed as \( \bar{X} \pm s \). The individual data of each treated and the control group were submitted to one-way ANOVA with the level of significance set at \( P < 0.05 \), using program GraphPad Prism (version 5).

**Fig. 3** Histopathological changes of rats myocardium, where ISO = Isoprenaline

<table>
<thead>
<tr>
<th>Groups</th>
<th>Myonecrosis</th>
<th>Inflammation</th>
<th>Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>A ISO + 100 mg·kg(^{-1}) olibanum</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>B ISO + 200 mg·kg(^{-1}) olibanum</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C ISO + 400 mg·kg(^{-1}) olibanum</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D ISO treated</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>E Normal control</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

**Table 5** Histopathological changes of rat myocardium, where ISO = Isoprenaline

**Results**

Nitric oxide (NO) is considered a free radical which is involved in physiological regulation. However, excess production of NO is associated with several diseases [26] since it reacts with oxygen to produce stable nitrates and nitrite. At a concentration of 1,000 µg·mL\(^{-1}\), the alcohol extract of olibanum showed moderate concentration dependence of NO-scavenging action, along with weak antioxidant activity.

The subcutaneous administration of ISO induces severe stress in the cardiac muscle leading to development of myocardial infarction. This resulted in significant increase in the serum levels of heart marker enzymes, namely, LDH, AST, and CK-MB (Table 3, Fig. 1), as well as the lipid peroxidation marker MDA (Table 4, Fig. 2). Pretreatment with the alcohol extract of olibanum reduced the activities of these enzymes to near normal levels. ISO-induced myocardial infarction showed membrane permeability alterations leading to the loss of function and integrity of myocardial membrane.

Enzymes are the best markers of tissue damage because of their tissue specificity and catalytic activity. Increased ac-
tivities of these marker enzymes in the serum are indicative of cellular damage, severity of necrotic damage, and loss of functional integrity of cell membrane. AST, LDH, and CK-MB enzymes are present in cardiac muscle, and their levels in plasma are directly proportional to the number of necrotic cells present in the cardiac tissue, hence, they are diagnostic markers of myocardial infarction. These results established that pretreatment of experimental animals with olibanum decreased the leakage of the marker enzymes from the myocardium into the systemic circulation, suggesting a myocyte-protective action.

Lipid peroxidation is an important pathogenic event in myocardial necrosis, and the accumulation of lipid hydroperoxides reflects cardiac damage. Hence, increased lipid peroxides in ISO-induced myocardial necrosis might be indicative of free radical mediated membrane damage accompanied by an increase of MDA level. In this study, pretreatment with olibanum to ISO-administered rats reduced MDA level. This effect probably due to the slight antioxidant effect of the total extract mentioned earlier.

The results of the histopathological study of the effect of olibanum on ISO-induced necrosis, inflammation, and edema on the heart tissues are displayed in Table 5 and Fig. 3. The data revealed that the hearts of the normal group rats showed normal cardiac muscle fiber arrangement without edema and inflammation, the ISO-treated rats showed myo-necrosis with edema, inflammatory cells, and separation of myofibers, while the olibanum-pretreated groups exhibited the architecture of myofibrillar and continuity with the adjacent myofibrils near to the normal striações.

**Discussion**

The olibanum methanol extract exhibited a cardioprotective effect, particularly on administering high doses, determined biochemically and histopathologically. These promising results led to an ongoing attempt to isolate the compounds responsible for this cardioprotective effect. The extract also showed antioxidant activity, however, the precise mechanism of protection is unclear.

**Acknowledgement**

The sincere efforts of Mr. Ahmed Abbas, technician in the biology laboratory, are highly appreciated.

**References**


[23] Lissi E, Modak B, Torres R, et al. Total antioxidant potential of


