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Abstract

Background: The water extract of oleo-gum-resin of *Boswellia sacra* Fleuckiger (Burseraceae) is used in the treatment of liver problems in the Middle East and Arab-African countries. The present study was carried out to evaluate its effect on liver injury induced by carbon tetrachloride in acute and chronic liver injury models.

Materials and Methods: The *Boswellia sacra* extract was administered at doses of 2 ml/kg and 5 ml/kg. Silymarin, a known hepato-protective agent was used as standard. The extract was subjected to GC-MS analysis to determine the presence of various phyto-constituents.

Results: The *Boswellia sacra* extract at tested doses of 2 ml/kg and 5 ml/kg significantly reduced the elevated serum levels of biomarkers in liver damage. The hepatoprotective effect was supported by changes in histopathology. The GC-MS analysis of the extract revealed the presence of several phyto-constituents that included menthol, 3-cyclohexen-1-ol and octanoic acid.

Conclusion: The water extract oleo-gum-resin of *Boswellia sacra* possesses hepatoprotective activity, as claimed by traditional healers within the Middle East and Arab-African countries.

Key words: *Boswellia sacra*, hepatoprotective, carbon tetrachloride, frankincense, liver damage.

Introduction

The treatment of liver diseases with herbal drugs is a common practice across the globe, more so in developing countries. Several herbs, crude and standardized herbal extracts and isolated active components are used for the treatment of liver damage. It is estimated that around 700 herbal formulations from 100 plants are in clinical use (Roy et al., 2012).

Most of these herbal extracts/formulations have been tested scientifically to verify claimed benefits on liver function. We had earlier reported hepatoprotective effects of *Psidium guajava* (Roy et al., 2006), *Boswellia serrata* (Jyothi et al., 2006) and *Tectona grandis* (Majumdar et al., 2007) that are used widely for treatment of liver disorders. However, there are other herbal products that are marketed specifically for the treatment of 'liver problems', but their effectiveness has not ascertained through either experimental or clinical studies. One of such product is the water extract of *Boswellia sacra* Fleuckiger (Burseraceae) oleo gum resin (frankincense), known in Arabic as *Luban*. It is widely used in the Middle East and Arab-African countries for the treatment of cough, stomach ache and liver problems. Furthermore, the *Boswellia* oleo gum resin (frankincense) is also chewed as chewing gum for its essence. The *Boswellia sacra* oleo gum resin and its products are marketed by several companies throughout the world (www.alibaba.com, 2014; frankincense, 2014; *Boswellia sacra*, 2014).

The present study was undertaken to determine the effect of administration of water extract of *Boswellia sacra* on carbon tetrachloride induced liver damage in rats.

Materials and Methods

Materials

The water extract of *Boswellia sacra* oleo gum resin was purchased from the market. The most widely used brand in Saudi Arabia (*Luban-e-hakim*) was used in the present study. The extract was a clear solution with a very strong essence similar to that of dried *Boswellia sacra* oleo gum resin. Preparation of water extract by traditional method in our laboratory, which involves boiling the oleo gum resin with water in a closed jar or soaking the *Boswellia sacra* oleo gum resin in water overnight did not produce an extract with such strong odorous extract. Hence, the marketed extract was used in the present study. All other chemicals used were of analytical grade from different companies.

Animals

Wistar albino rats of either sex weighing between 180-210 g were used. The animals were maintained under standard conditions of 12:12 hr light dark cycle at a temperature of 25 ± 2 °C. The experimental protocol was approved by the research committee of the institute for its ethical and scientific content.

Preliminary Phytochemical Analysis

The extract was subjected to preliminary phytochemical analysis to detect the presence of phyto-constituents such as carbohydrates, proteins, amino acids, saponins, tannins and phenolic compounds, flavonoids and glycosides.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis of Extract

For performing the GC-MS analysis, the water extract of *Boswellia sacra* was extracted using dichloromethane and this extract was injected into GC-MS system (Shimadzu-QP2010S). For GC-MS detection, an electron impact ionization system was used. Helium gas (99.95%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1 µl was employed (a split ratio of 10:1). The injector

temperature was maintained at 250 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 60 °C (isothermal for 2 min), with gradual increase to 325°C at 15 min. Mass spectra were taken at 1.4 kV. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Interpretation of mass-spectrum GC-MS was conducted using NIST, WILEY and SZTRIP libraries. The spectrum of the unknown components was compared with the spectrum of known components.

Evaluation of Hepatoprotective Activity

Carbon Tetrachloride (CCl₄) Induced Acute Hepatic Damage (Roy et al., 2006)

The animals were divided into five groups consisting of six animals and treated as follows;

Group 1: Vehicle (1 ml/250 g, *p.o.*)

Group 2: Distilled water for 9 days + CCl₄ (1 ml/kg, *p.o.*) on ninth day

Group 3: Silymarin (100 mg/kg, *p.o.*) for 9 days + CCl₄ (1 ml/kg, *p.o.*) on ninth day

Group 4: *Boswellia sacra* extract (2 ml/kg/day, *p.o.*) for 9 days + CCl₄ (1 ml/kg, *p.o.*) on ninth day

Group 5: *Boswellia sacra* extract (5 ml/kg/day, *p.o.*) for 9 days + CCl₄ (1 ml/kg *p.o.*) on ninth day

The CCl₄ was administered after dilution with liquid paraffin the ratio of 1:1. Food was withdrawn 12 hr before carbon tetrachloride administration to enhance liver damage in animals of groups 2, 3, 4 and 5. Blood samples were collected 24 hr after the administration of CCl₄. and serum was used for assay of marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum bilirubin. All the biomarkers were determined using commercially available kits. The animals were then sacrificed; liver was isolated, washed with normal saline, dried and weighed immediately. The liver was then subjected to histo-pathological examination.

Carbon Tetrachloride (CCl₄) Induced Chronic Hepatic Damage (Roy et al., 2006)

The animals were divided into five groups of 6 rats each and treated as follows

Group 1: Vehicle (1 ml/250 g, *p.o.*) for 6 weeks

Group 2: Distilled water for 6 weeks + CCl₄ (1 ml/kg, *p.o.*) weekly twice for 6 weeks

Group 3: Silymarin (100 mg/kg, *p.o.*) for 6 weeks + CCl₄ (1 ml/kg, *p.o.*) weekly twice for 6 weeks

Group 4: *Boswellia sacra* extract (2 ml/kg/day, *p.o.*) for 6 weeks + CCl₄ (1 ml/kg, *p.o.*) weekly twice for 6 weeks

Group 5: *Boswellia sacra* extract (5 ml/kg/day, *p.o.*) for 6 weeks + CCl₄ (1 ml/kg, *p.o.*) weekly twice for 6 weeks.

Blood samples were collected 24 hr after the administration of CCl₄. and serum levels of biomarkers were determined as mentioned above. The animals were then sacrificed and liver was subjected to histo-pathological examination.

Statistical Analysis

Values are expressed as mean ± standard error of mean (SEM). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's test for comparison of all parameters. The statistical analysis was done using computer software (Graphpad Instat DATASET 1, ISD, software version 3.0 for Windows). Values of $p < 0.05$ were considered to indicate statistical significance.

Results

The water extract of *Boswellia sacra* oleo gum resin did not shown presence of any of the tested phyto-constituents indicating that it contains only essential oils.

GC-MS chromatogram analysis of the water extract of *Boswellia sacra* shows many peaks indicating presence of several volatile constituents (Figure 1). On comparison of the mass spectra of the constituents with the NIST, WILEY 7 and SZTERP library, several phyto-compounds were characterized and identified (Table 1). Some of the phytochemicals that are reported to influence the development of ulcers and their mass spectra of these phytochemicals are presented in Table 2. Of all the compounds identified, the most abundant compounds were 1,8-cineole (4.16%), 1-octanol (6.91%), (-) menthol (10.81%), 3-cyclohexen-1-ol (21.33%), octanoic acid (18.91%), thymol (6.14%) and carvacrol (5.67%).

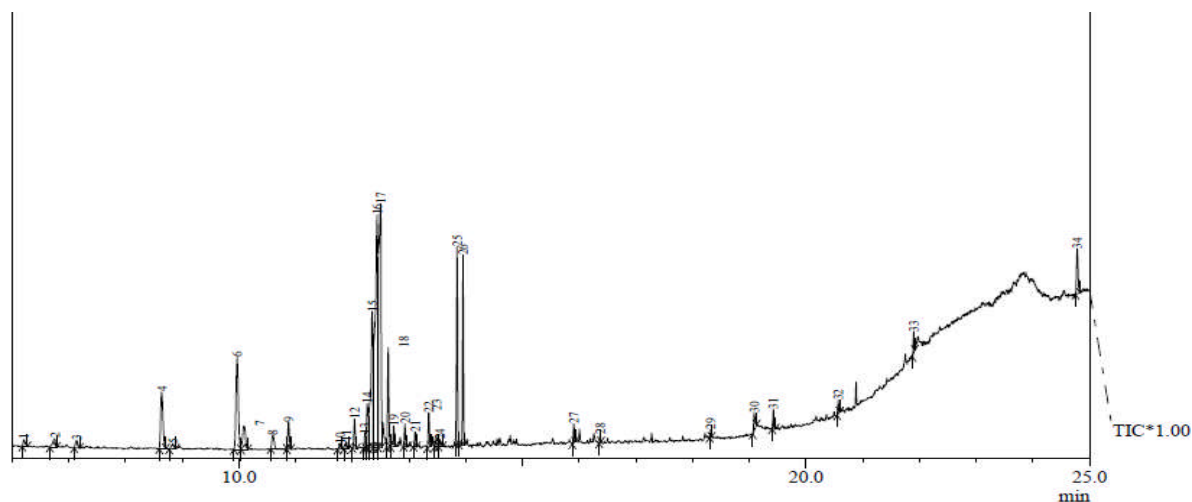


Figure 1: GC-MS chromatogram of water extract of *Boswellia sacra*

Table 1: List of major chemical constituents present in the water extract of *Boswellia sacra*

Number	Retention time	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	8.634	1,8-Cineole	C ₁₀ H ₁₈ O	154	4.16
2	9.964	1-Octanol	C ₈ H ₁₈ O	130	6.91
3	12.346	(-)-Menthol	C ₁₀ H ₂₀ O	156	10.81
4	12.433	3-Cyclohexen-1-ol	C ₁₀ H ₂₀ O	156	21.33
5	12.496	Octanoic acid	C ₈ H ₁₆ O ₂	144	18.91
6	13.843	Thymol	C ₁₀ H ₁₄ O	150	6.14
7	13.949	Carvacrol	C ₁₀ H ₁₄ O	150	5.67

Table 2: Mass spectra of major constituents in *Boswellia sacra* water extract

Compound name	Mass spectra
1,8-Cineole	<p>Hit# 1 Entry: 43993 Library: WILEY7.LIB SI: 95 Formula: C₁₀H₁₈O CAS: 470-82-6 MolWeight: 154 RetIndex: 0</p>
1-Octanol	<p>Hit# 2 Entry: 21969 Library: WILEY7.LIB SI: 97 Formula: C₈H₁₈O CAS: 111-87-5 MolWeight: 130 RetIndex: 0</p>
Menthol	<p>Hit# 1 Entry: 45541 Library: WILEY7.LIB SI: 95 Formula: C₁₀H₂₀O CAS: 89-78-1 MolWeight: 156 RetIndex: 0</p>
3-cyclohexen-1-ol	<p>Hit# 2 Entry: 43760 Library: WILEY7.LIB SI: 91 Formula: C₁₀H₁₈O CAS: 562-74-3 MolWeight: 154 RetIndex: 0</p>
Octanoic acid	<p>Hit# 2 Entry: 4091 Library: NIST12.LIB SI: 95 Formula: C₈H₁₆O₂ CAS: 124-7-2 MolWeight: 144 RetIndex: 0</p>
Thymol	<p>Hit# 4 Entry: 38608 Library: WILEY7.LIB SI: 95 Formula: C₁₀H₁₄O CAS: 89-83-8 MolWeight: 150 RetIndex: 0</p>
Carvacrol	<p>Hit# 2 Entry: 38622 Library: WILEY7.LIB SI: 95 Formula: C₁₀H₁₄O CAS: 499-75-2 MolWeight: 150 RetIndex: 0</p>

**Evaluation of Hepatoprotective Activity
Carbon Tetrachloride (ccl₄) Induced Acute Hepatic Damage**

Administration of CCl₄ produced a significant increase in all the biomarkers of liver damage in serum when compared to vehicle control. Both doses of *Boswellia sacra* (2 ml/kg and 5 ml/kg) and silymarin (100 mg/kg *p.o.*) significantly reduced the levels of ALT, AST, ALP and bilirubin when compared to CCl₄ treated control (Table 3). Liver sections from CCl₄ treated animals showed severe liver damage indicated by hydropic degeneration, inflammation and steatosis in periportal region. In animals treated with extract or silymarin, inflammation, steatosis and congestion were reduced though it was difficult to differentiate the effect produced by silymarin with that produced by both doses of the extract.

Table 3: Effect on serum enzyme levels in CCl₄ induced acute liver damage

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilirubin (mg/dl)
Vehicle control	86.23± 6.35	142.31± 12.56	416.54± 27.53	0.36± 0.025
CCl ₄ control	365.21± 12.54 ^{***}	445.45± 11.02 ^{***}	825.23± 26.15 ^{***}	0.89± 0.066 ^{***}
CCl ₄ + Silymarin	145.78± 18.59 ^{###}	218.69± 14.33 ^{###}	687.99± 33.48 [#]	0.58± 0.045 ^{###}
CCl ₄ + <i>Boswellia sacra</i> (2 ml/kg)	258.11± 12.03 ^{###}	321.45± 21.96 ^{###}	703.76± 28.35 [#]	0.71± 0.056 ^{###}
CCl ₄ + <i>Boswellia sacra</i> (5 ml/kg)	199.87± 11.25 ^{###}	284.21± 12.66 ^{###}	700.29± 22.57 [#]	0.66± 0.027 ^{###}

All values are mean ± SEM, n=6, ^{***}P<0.001 compared to control, [#]P<0.05, ^{###}P<0.001 compared to CCl₄ treated control.

Carbon Tetrachloride (CCl₄) Induced Chronic Hepatic Damage

A significant difference in biochemical markers, AST, ALT, ALP and bilirubin was observed between normal and CCl₄ treated animals (Table 4). Comparison of *Boswellia sacra* and silymarin treated groups with CCl₄ control revealed that both doses of *Boswellia sacra* extract (2 ml/kg and 5 ml/kg) and silymarin significantly reduced the hepatic damage as indicated by reduction in biochemical parameters and histological studies similar to that mentioned above.

Table 4: Effect on serum enzyme levels in CCl₄ induced chronic liver damage

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilirubin (mg/dl)
Vehicle control	77.54± 5.29	125.63± 23.65	468.21± 35.94	0.29± 0.018
CCl ₄ control	297.33± 9.28 ^{***}	395.28± 10.25 ^{***}	816.25± 63.27 ^{***}	1.27± 0.054 ^{***}
CCl ₄ + Silymarin	119.94± 10.97 ^{###}	248.21± 16.84 ^{###}	506.49± 22.53 ^{###}	0.76± 0.081 ^{###}
CCl ₄ + <i>Boswellia sacra</i> (2 ml/kg)	158.33± 17.52 ^{###}	305.21± 13.25 [#]	614.55± 33.89 [#]	0.99± 0.040 [#]
CCl ₄ + <i>Boswellia sacra</i> (5 ml/kg)	144.19± 14.65 ^{###}	282.24± 11.26 ^{###}	574.66± 21.88 [#]	0.86± 0.019 ^{###}

All values are mean±SEM, n=6, ^{***}P<0.001 compared to control, [#]P<0.05, ^{###}P<0.01, ^{###}P<0.001 compared to CCl₄ treated control.

Discussion

The water extract of *Boswellia sacra* oleo gum resin reduced the serum enzyme levels indicating good hepatoprotective activity at both tested doses. However, the effect was less compared to that of the standard drug; silymarin.

As mentioned earlier, the *Boswellia sacra* oleo gum resin, commonly known as frankincense or incense is widely used in different parts of the world. In the Middle East and Arab-African countries, it is chewed as chewing gum because of its flavor, burnt and used as incense for its smell apart from its used for treatment of diseases. The water extract of *Boswellia sacra* oleo gum resin is a house hold medicine used for the treatment of cough, stomach problems and liver problems. The word 'liver problems' is confusing and discussion with traditional healers revealed that liver problems include; jaundice, liver infection and other liver diseases.

There are several methods for the preparation of the water extract of frankincense. The frankincense covered in a muslin cloth or a wire mesh is boiled with water in closed vessels for 15-30 min and the water extract is used after cooling. Another method used is soaking the frankincense in water overnight followed by filtering. Some people add few drops of frankincense oil to water and use it (Nancy Healing, 2014;

Enfluerage, 2014; Paul et al., 2012). However, most of the people now prefer to buy the readymade water extract available in the market. We tried the first two methods in our laboratory but could not get the strong flavor of frankincense present in the readymade water extract. Hence, the prepared water extract from the market was used in the present study.

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of CCl₄ are largely due to its active metabolite, trichloro methyl radical (Shenoy et al., 2002).

Boswellia serrata, a member of boswellia species is reported to reduce hepatic damage in both experimental animals and human subjects. Earlier reports indicate that supplementation of *Boswellia serrata* is reported to reduce liver damage in type II diabetic patients (Ahangarpour et al., 2014). We had earlier reported the hepatoprotective effect of hexane extract of *Boswellia serrata* in rats (Jyothi et al., 2006). The beneficial effects of *Boswellia serrata* have been attributed mainly to the presence of boswellic acids (Liu et al., 2013). The boswellic acids are insoluble in water (Shah et al., 2009) and in the present study, the extract contained only volatile oils and it did not show presence of boswellic acids.

Review of literature on the chemical constituents present in the extract shows that carvacol, cineole, linalool and furanmethanol are reported to have or presumed to have hepatoprotective effect (Baser, 2008; Alipour et al., 2014; Chen et al., 2011). The effect of other chemical constituents on liver is not known. Hence, the hepatoprotective action may be because of carvacol, cineole, linalool and furanmethanol. Though the effect of other constituents is not known, their contribution to the hepatoprotective cannot be overruled and further studies on their antioxidant and/or hepatoprotective effect may indicate their effect on liver.

Conclusion

The water extract of *Boswellia sacra* oleo gum resin reduced the carbon tetrachloride induced acute and chronic liver damage in rats. The GC-MS analysis of the extract revealed presence of several constituents, some of which are reported to possess hepatoprotective effects.

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