ORIGINAL ARTICLE

EFFECT OF HEXANE EXTRACT OF *BOSWELLIA SERRATA* OLEO-GUM RESIN ON CHEMICALLY INDUCED LIVER DAMAGE

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ABSTRACT

The hexane extract of oleo-gum-resin of *Boswellia serrata* (BSHE) was evaluated for its effect on liver injury induced by carbon tetrachloride, paracetamol or thioacetamide. The BSHE was given in two different doses (87.5 mg/kg *p.o.* and 175mg/kg *p.o.*). Silymarin, a known hepatoprotective agent was used as standard. The lower dose of BSHE (87.5mg/kg *p.o.*) significantly reduced the elevated levels of serum marker enzymes and prevented the increase in liver weight in all three models of liver injury, while the higher dose showed mild hepatoprotective activity. The hepatoprotective effect of lower dose of BSHE was supported by changes in histopathology. It was concluded that hexane extract of oleo-gum-resin of *Boswellia serrata* plant in lower doses possess hepatoprotective activity.

Keywords: *Boswellia serrata*, hepatoprotective, carbon tetrachloride, paracetamol, thioacetamide.

INTRODUCTION

Liver diseases such as jaundice, cirrhosis and fatty liver are very common worldwide. There are many factors for the development of these diseases, one of the important factors being the use of drugs. *Boswellia serrata* (Burseraceae), occurs in tropical parts of Asia and Africa (Kokate, 1998). The oleo-gum-resin of the plant is known to possess a variety of activities such as antiarthritic, antiinflammatory, antitumour and anticarcinogenic effect (Singh and Atal, 1986; Huang *et al.,* 2000). This plant is widely used for its antiinflammatory activity, which is mediated by its active constituents, boswellic acids. Studies have also been carried out to determine the toxicity of this plant. It is reported that *Boswellia serrata* applied topically can produce allergic contact dermatitis (Acebo *et al.,* 2004). The hexane extract of the oleo-gum-resin of this plant, which is rich in boswellic acids, is reported to produce hepatotoxicity in large doses (1% in the diet) and it is also suggested that the hexane extract of the plant should not be administered to patients or volunteers participating in the clinical studies without informing them about hepatotoxic nature of the plant (Kiela *et al.,* 2005). On the contrary, Pandey *et al* (2005) reported that the alcoholic extract of oleo-gum-resin of *Boswellia serrata* possess hepatoprotective activity that may be due to presence of acetyl-α-boswellic acid. The present study was undertaken to evaluate the effect of hexane extract of oleo-gum-resin of *Boswellia serrata*, widely used for its antiinflammatory activity on experimental liver injury in rats.

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MATERIALS AND METHODS

Experimental animals

Albino Wistar rats weighing 175-250 g of either sex were used. Institutional Animal Ethics Committee approved the

Table 1: Effect of silymarin and BSHE on serum ALT, AST, ALP, bilirubin level and liver weight in CCl_4 induced liver injury in rats

Treatment	Dose (*p.o.*)	ALT (U/L)	AST (U/L)	ALP (U/L)	Serum Bilirubin (mg/dl)	Liver weight (g/100 g b.w.)
Vehicle control	-	49.73 ± 2.03	119.91 ± 4.73	424.86 ± 13.36	0.351 ± 0.020	2.83 ± 0.103
CCl_4 control	-	320.40 ± 17.04^{a}	617.83 ± 15.42^{a}	755.26 ± 22.78^{a}	0.868 ± 0.038^{a}	3.93 ± 0.197^{a}
CCl_4 + Silymarin	100 mg/kg	137.15 ± 6.59***	436.63± 18.90***	575.46± 18.55***	0.530 ± 0.014***	2.94 ± 0.276*
CCl_4 + BSHE	87.5 mg/kg	238.65± 15.25***	422.23± 15.22***	613.16± 13.51***	0.681 ± 0.022***	2.87 ± 0.277**
CCl_4 + BSHE	175 mg/kg	329.96 ± 8.96^{ns}	504.61 ± 26.07^{ns}	651.90 ± 25.58*	0.951 ± 0.030^{ns}	3.45 ± 0.075^{ns}

Values are mean ± S.E.M, n = 6, ^{a} $p < 0.001$ *vs* vehicle control. ^{ns} $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* CCl_4 control

Table 2: Effect of silymarin, BSHE on serum ALT, AST, ALP, bilirubin level and liver weight in paracetamol (PCM) induced liver damage in rats.

Treatment	Dose (*p.o.*)	ALT (U/L)	AST (U/L)	ALP (U/L)	Serum Bilirubin (mg/dl)	Liver weight (g/100 g b.w.)
Vehicle control	-	49.73 ± 2.03	119.91 ± 4.73	424.86 ± 13.36	0.351 ± 0.028	2.95 ± 0.075
PCM control	-	284.43 ± 7.27^{a}	399.28 ± 19.73^{a}	946.21 ± 20.60^{a}	1.060 ± 0.121^{a}	4.30 ± 0.240^{a}
PCM + Silymarin	100 mg/kg	136.15 ± 8.48***	268.53 ± 10.90***	580.51 ± 23.96***	0.585 ± 0.061***	2.88 ± 0.212***
PCM + BSHE	87.5 mg/kg	203.10 ± 7.77***	286.35 ± 15.84***	561.11 ± 24.88***	0.683 ± 0.060***	3.39 ± 0.153*
PCM + BSHE	175 mg/kg	306.86 ± 18.97^{ns}	319.26 ± 16.61*	878.63 ± 15.48^{ns}	0.951 ± 0.038^{ns}	4.00 ± 0.078^{ns}

Values are mean ± S.E.M, n = 6 ^{a} $p < 0.001$ *vs* vehicle control. ^{ns} $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* PCM control

Table 3: Effect of silymarin and BSHE on serum ALT, AST, ALP, bilirubin level and liver weight in thioacetamide (TAA) induced liver damage in rats.

Treatment	Dose (*p.o.*)	ALT (U/L)	AST (U/L)	ALP (U/L)	Serum Bilirubin (mg/dl)	Liver weight (g/100 g b.w.)
Vehicle control	-	49.73 ± 2.03	119.91 ± 4.73	424.86 ± 13.36	0.351 ± 0.028	2.83 ± 0.103
TAA control	-	393.33 ± 14.80^{a}	440.30 ± 8.24^{a}	785.93 ± 16.16^{a}	0.866 ± 0.041^{a}	4.01 ± 0.127^{a}
TAA + Silymarin	100 mg/kg	169.66 ± 8.29***	321.95 ± 7.58***	625.81 ± 10.59***	0.363 ± 0.021***	3.07 ± 0.081***
TAA + BSHE	87.5 mg/kg	321.01 ± 9.08***	320.25 ± 14.03***	664.84 ± 16.44***	0.506 ± 0.022***	3.11 ± 0.263***
TAA + BSHE	175 mg/kg	382.50 ± 11.31^{ns}	392.50 ± 12.20*	762.65 ± 12.72^{ns}	0.923 ± 0.066^{ns}	4.01 ± 0.124^{ns}

Values are mean ± S.E.M, n = 6, ^{a} $p < 0.001$ *vs* vehicle control. ^{ns} $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* TAA control

experimental protocol and animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Acute toxicity study

The acute oral toxicity study was performed according to the OPPTS (Office of prevention, pesticide and toxic substance) Up and Down procedure (Health effect test guidelines, 2004).

Evaluation of hepatoprotective activity

Carbon tetrachloride (CCl_4) induced liver injury (Matsuda *et al.,* 1991): The animals were divided into five groups consisting of six animals. The animals were then subjected to either one of the following treatments for 9 days.

Group 1: Vehicle (1 ml/250 g, *p.o.*)
Group 2: Distilled water for 9 days + CCl_4 (0.5 ml/kg, *p.o.*) on ninth day
Group 3: Silymarin (100 mg/kg/day, *p.o.*) for 9 days + CCl_4 (0.5 ml/kg, *p.o.*) on ninth day
Group 4: BSHE (87.5 mg/kg/day, *p.o.*) for 9 days + CCl_4 (0.5 ml/kg, *p.o.*) on ninth day
Group 5: BSHE (175 mg/kg/day, *p.o.*) for 9 days + CCl_4 (0.5 ml/kg *p.o.*) on ninth day

The CCl_4 was administered after dilution with liquid paraffin the ratio of 1:1. Food was withdrawn 12 h before carbon tetrachloride administration to enhance liver damage in animals of groups 2, 3, 4 and 5. The animals were sacrificed 24 h after the administration of CCl_4. Blood samples were collected and serum was used for assay of

marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum bilirubin. The liver was isolated and washed with normal saline, dried using filter paper and weighed immediately. The liver was then subjected to histopathological examination.

Paracetamol (PCM) induced liver injury (Asha and Pushpangadan, 1998): The same procedure as mentioned above was followed except that the liver injury was produced using PCM (2 g/kg, *p.o.*) diluted with sucrose solution (40% w/v). PCM was administered in 3 divided doses on day 9 and animals were sacrificed 48 hr after administration of PCM.

Thioacetamide (TAA) induced liver injury (Ahmad *et al.,* 1999): The same procedure was followed. Damage was induced by using TAA (100 mg/kg *s.c*), which was prepared in distilled water (2% solution).

STATISTICAL ANALYSIS

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The values are expressed as mean ± SEM and $p<0.05$ was considered significant.

RESULTS

Preliminary phytochemical investigation

The preliminary phytochemical investigation of the aqueous extracts of the *Boswellia serrata* showed that it contains volatile oils and terpenoids.

Acute oral toxicity study

Boswellia serrata hexane extract (BSHE) up to a dose of 1750 mg/kg *p.o.* body weight did not produce any mortality. Hence $1/10^{th}$ and $1/20^{th}$ of this dose i.e. 175 mg/kg and 87.5 mg/kg *p.o.* body weight were used.

Carbon tetrachloride induced acute hepatitis

Lower dose of BSHE (87.5 mg/kg *p.o.*) and silymarin (100 mg/kg *p.o.*) significantly reduced the levels of ALT, AST ALP and bilirubin ($p < 0.001$). Higher dose of BSHE (175 mg/kg *p.o.*) did not show any significant effect when compared to CCl_4 treated group. Administration of CCl_4 produced a significant increase in liver weight ($p < 0.001$). Silymarin and low dose of BSHE (87.5 mg/kg *p.o.*) showed a significant reduction in the liver weight ($p < 0.05$) when compared with CCl_4 treated group whereas high dose of BSHE (175 mg/kg *p.o.*) did not affect the liver weight (table 1). Liver sections from CCl_4 treated animals showed hydropic degeneration, inflammation and steatosis in periportal region. The inflammation was more in the sinusoids with congestion. In animals treated with either silymarin (100 mg/kg *p.o.*) or BSHE (87.5 mg/kg *p.o.*), the inflammation, steatosis and congestion were reduced. Liver sections from animals treated with higher dose (175 mg/kg *p.o.*) did not demonstrate any difference when compared to CCl_4 control (fig 1)

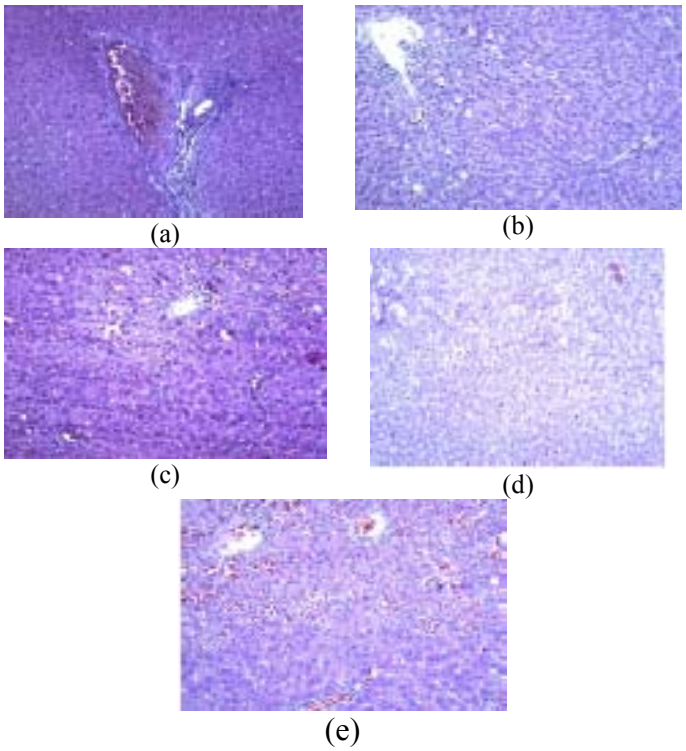

Fig. 1: Effect of silymarin and BSHE on CCl_4 induced liver damage.

(a) normal liver, (b) CCl_4 treated, (c) CCl_4 + silymarin (100 mg/kg *p.o.*), (d) CCl_4 + BSHE (87.5 mg/kg *p.o.*), (e) CCl_4 + BSHE (175 mg/kg *p.o.*) [H & E staining X 200]

Paracetamol induced liver toxicity

Forty eight hours after administration of PCM, the serum levels of ALT, AST, ALP and bilirubin were markedly increased ($p < 0.001$). Pretreatment with BSHE (87.5 mg/kg *p.o.*) and silymarin reduced the levels of biochemical markers levels significantly when compared to PCM treated control ($p < 0.001$). The liver weight of animals treated with either silymarin or lower dose of BSHE was significantly less when compared to paracetamol control. Pretreatment with BSHE (175mg/kg *p.o.*) did not show significant effect when compared with the PCM control (table 2). Histological examination revealed that paracetamol had produced severe congestion of blood vessels, mild hydropic degeneration, pyknosis of nucleus and occasional necrosis. Liver sections from silymarin treated animals showed mild pyknosis of the hepatocytes while those from BSHE lower dose showed mild hydropic degeneration with no pyknosis and no congestion. Sections obtained from animals treated with higher dose of BSHE were similar to paracetamol treated control animals (fig. 2)

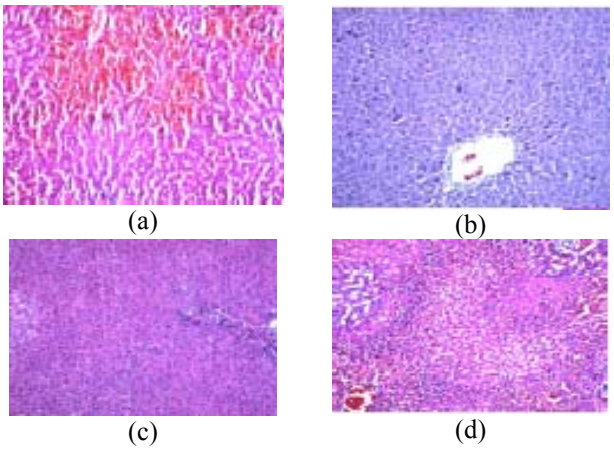

Fig. 2: Effect of silymarin and BSHE on PCM induced liver damage.
(a) PCM treated, (b) PCM + silymarin (100 mg/kg *p.o.*), (c) PCM + BSHE (87.5 mg/kg *p.o.*), (d) PCM + BSHE (175 mg/kg *p.o.*) [H & E staining X 200]

Thioacetamide induced liver necrosis

Pretreatment of animals with BSHE (87.5 mg/kg) and silymarin significantly reduced the levels of AST, ALT, ALP and bilirubin ($p < 0.001$). TAA induced acute toxicity had increased the weight of liver significantly ($p < 0.01$). When compared with TAA treated group, only low dose of BSHE (87.5 mg/kg *p.o.*) and silymarin had prevented the increase in liver weight, while the high dose of BSHE (175 mg/kg *p.o.*) did not produce significant change in liver weight (table 3). Thioacetamide administration produced perilobular necrosis, inflammation and congestion with cytoplasmic vacuolations in liver cells. Silymarin treatment reduced the inflammation and necrosis and BSHE lower dose also reduced inflammation and there was no sign of necrosis. Higher dose of BSHE did not produce any striking difference in histopathology when compared to thioacetamide treated control (fig. 3).

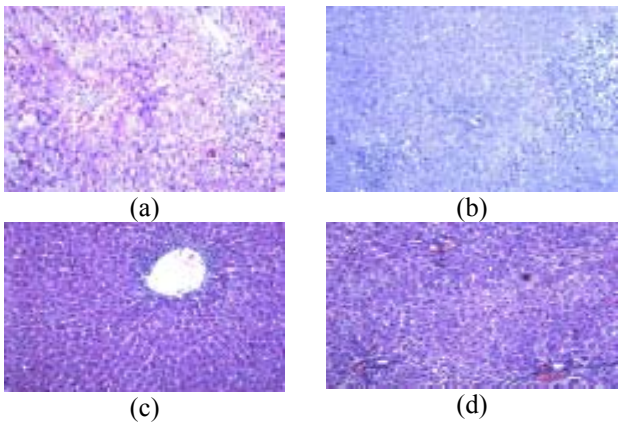

Fig. 3: Effect of silymarin and BSHE on TAA induced liver damage.
(a) TAA treated, (b) TAA + silymarin (100 mg/kg *p.o.*), (c) TAA + BSHE (87.5 mg/kg *p.o.*), (d) TAA + BSHE (175 mg/kg *p.o.*) [H & E staining X 200]

DISCUSSION

The hexane extract of *Boswellia serrata* oleo gum resin showed good hepatoprotective activity in lower doses (87.5 mg/kg *p.o.*). The effect produced was comparable to that produced by silymarin (100 mg/Kg *p.o.*), a well-known hepatoprotective agent.

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloro methyl radical (Shenoy *et al.,* 2001). Paracetamol induces liver damage by depletion of glutathione (GSH) (Udem *et al.,* 1997) and thioacetamide interferes with the movement of RNA from the nucleus to the cytoplasm, which causes membrane injury (Kumar *et al.,* 2004). The lower dose of BSHE (87.5 mg/kg *p.o.*) and silymarin (100 mg/kg *p.o.*) showed good hepatoprotective effect as evidenced by reduction in the levels of serum marker enzymes. The hepatoprotective effect was supported by histological changes produced by these drugs compared to hepatotoxin treated control. Antioxidants are known to reduce the hepatic injury induced by above-mentioned hepatotoxicants due to scavenging of free radicals (Feher *et al.,* 1984). There are reports to indicate that *Boswellia serrata* does not possess any antioxidant effect (Ammon *et al.,* 1993). However, the alcoholic extract of *Boswellia serrata* oleo gum reduces the production of nitric oxide in isolated peritoneal macrophages (Pandey *et al.,* 2005). It is reported that agents that modulate the production of nitric oxide in the liver have hepatoprotective effects (Majano *et al.,* 2004). It is difficult to explain with the present data, the precise mechanism by which BSHE is showing hepatoprotective action. Since both alcoholic extract of *Boswellia* and BSHE are rich in boswellic acids, the main active constituents of the plant, it is speculated that the BSHE may reduce the production of nitric oxide in the hepatocytes and this may be responsible for its hepatoprotective action.

The BSHE did not show dose dependent hepatoprotective action. The higher dose of the extract was less effective than the lower dose. As mentioned earlier, large doses of hexane extract of *Boswellia* (1% in the diet) is reported to be hepatotoxic. The hepatotoxic effect of the plant was reported to be due to dysregulation of a number of genes including a large number of lipid metabolism related genes and detoxifying enzymes (Kiela *et al.,* 2005). In our study, we did not observe any aggravation of the chemically induced hepatic damage when BSHE was administered in higher dose (175 mg/kg *p.o.*). On the contrary, a partial hepatoprotective action was observed in all three models as evidenced by a reduction in ALP in CCl_4 induced liver damage and a reduction in AST in paracetamol induced and thioacetamide induced liver damage. However, the histological examination of the liver tissues did not support

the hepatoprotective effect of higher dose of BSHE. From the above results, it can be suggested that BSHE at a dose of 175 mg/kg orally possess partial hepatoprotective effect. The results of the present study contradicts reports by other authors that BSHE is hepatotoxic (Kiela *et al.,* 2005).

To conclude, hexane extract of *Boswellia serrata* oleo-gum resin possess good hepatoprotective effect in lower doses and shows mild hepatoprotection in higher doses.

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