

HEPATOPROTECTIVE PROPERTIES OF *COMMIPHORA OPOBALSAMUM* ("BALESSAN"), A TRADITIONAL MEDICINAL PLANT OF SAUDI ARABIA

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Summary: *The hepatoprotective activity of an ethanolic extract of Commiphora opobalsamum ("Balessan") was investigated in rats by inducing hepatotoxicity with carbon tetrachloride:liquid paraffin (1:1). This extract has been shown to possess significant protective effect by lowering serum transaminase levels (serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase), alkaline phosphatase and bilirubin. Pretreatment with an extract of Balessan prevented the prolongation of the barbiturate sleeping time associated with carbon tetrachloride induced liver damage in mice. On the other hand, CCl₄-induced low-level nonprotein sulphydryl concentration in the liver was replenished by the Balessan extract. These data suggest that the plant C. opobalsamum may act as an antioxidant agent and may have a hepatoprotective effect.*

Introduction

Balessan is the Arabic or local name of *Commiphora opobalsamum* (L.) Engl., family *Burseraceae*. This plant is one of the most ancient plants, with a mag-

nificent history of healing, and was a valuable medicinal agent in ancient Arabia. It grows wild in countries on both sides of the Red Sea (1). It has been used in diseases of liver, stomach and urinary tract. A decoction or tincture is used by local traditional healers for the treatment of chest, stomach and kidney complaints; to promote digestion; and to relieve rheumatism, scurvy and jaundice (2). Abdul-Ghani and Amin (3) have reported an antihypertensive activity of an aqueous extract of this plant in rats. However, there is

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a dearth of scientific data on this plant and therefore the present investigation was undertaken to evaluate the antihepatotoxic potential of an ethanolic extract in laboratory animals.

Materials and methods

Plant collection and extraction. The aerial parts of the plant were collected from the Farasan Island of the Red Sea (Saudi Arabia) in March 2002 and were identified by our taxonomist Dr. Atiqur Rahman (College of Pharmacy, King Saud University). A voucher specimen (#14312) was deposited at the herbarium of the College of Pharmacy for future reference. Powdered shade-dried aerial parts of the plants were macerated in 96% ethanol for 36 h. Solvent elimination was carried out under reduced pressure which yielded a brownish semisolid compound. A solution of the extract was made in distilled water for administration to animals.

Animals. Wistar albino rats, of either sex and approximately the same age (8-10 weeks), weighing 180-200 g, obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, were used. Swiss albino mice were used for studies of sleeping time. The animals were kept in constant temperature (22 ± 2 °C), humidity (55%) and light-dark conditions (12/12 h light/dark ratio). The animals were provided with Purina chow and free access to drinking water *ad libitum*.

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 and cyanogenic glycosides, glucosinolates, coumarins, sterol and/or triterpenes (4).

Induction of acute hepatotoxicity by carbon tetrachloride (CCl₄). Male Wistar rats were divided into four groups containing six animals in each group. Group I was kept as a control group. Groups II, III and IV received 0.25 ml of CCl₄ in liquid paraffin (1:1) per 100 g body weight intraperitoneally (5). Group II received only CCl₄ treatment. Groups III and IV were treated with 250 and 500 mg/kg of ethanolic extract of Balessan, respectively. Drug treatment was started 5 days prior to CCl₄ administration and continued until the end of the experiment. After 48 h, following CCl₄ administration, animals were sacrificed using ether anesthesia. Blood was collected by heart puncture and the serum was separated. The liver was immediately removed and a small piece was fixed in 10% formalin for histopathological assessment.

Assay of serum glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvate transaminase (GPT), alkaline phosphatase (ALP) and total bilirubin activities. The collected blood was centrifuged at 3,000 rpm for 10 min to separate the plasma. The plasma was analyzed for the biochemical parameters including GOT, GPT, alkaline phosphatase and total bilirubin (6, 7).

Estimation of nonprotein sulfhydryl groups (NP-SH). The activity of NP-SH was measured according to the method of Sedlak and Lindsay (8). The liver tissue was homogenized in ice-cold 0.02 M ethylenediaminetetraacetic acid (EDTA). Aliquots of 5 ml of the homogenates were mixed in 15 ml test tubes with 4 ml of distilled water and 1 ml of 50% trichloroacetic acid. The tubes were shaken intermittently for 10-15 min and centrifuged at 3,000 g. Two milliliters of supernatant were mixed with 4 ml of 0.4 M Tris buffer, pH 8.9, and 0.1 ml of 0.4% (5,5-dithio-bis [2-nitrobenzoic acid]) (DTNB) was added and the sample was shaken. The absorbance was read within 5 min of addition of DTNB at 412 nm against a reagent blank with no homogenate.

Measurement of phenobarbital-induced sleeping time. Mice were divided into four groups of 10 animals each. Group I received the vehicle (0.3 ml of saline); group II received CCl₄ only. Groups III and IV received Balessan extract (250 and 500 mg/kg orally). Thirty minutes after the administration of the extract, animals of groups II, III and IV were treated with sodium phenobarbital (50 mg/kg, intraperitoneally). The time interval between the onset and the regaining of the righting reflex was measured as sleeping time (9).

Histopathological studies. The liver tissue was fixed in 10% ethanol buffered formalin and processed through graded ethanol, xylene and impregnated with paraffin wax, sections were made by microtome. After staining with hematoxylin-eosin stain, the sections were examined under a research microscope by a person who was not aware of the experimental protocols. The different histopathological indices were scored (10).

Statistical analysis. The data were statistically analyzed using Student's *t*-test.

Results

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

The effects of ethanolic extract on CCl₄-induced hepatotoxicity in rats are shown in Table I. Rats subjected to the CCl₄ regimen alone developed significant hepatocellular damage as evidenced by a significant elevation in serum activities of GOT, GPT, ALP and bilirubin concentrations compared with normal values, which have been used as reliable markers of hepatotoxicity. Oral administration of an ethanol extract of Balessan (250 and 500 mg/kg) exhibited a significant reduction in CCl₄-induced increased levels of SGOT, SGPT, ALP and serum bilirubin concentrations.

NP-SH contents in liver were significantly decreased following the administration of CCl₄. Treatment with Balessan extract (either dose) significantly reversed the NP-SH level (Table II).

There was a significant lowering of phenobarbital-induced sleeping time following the administration of the Balessan extract (500 mg/kg) in the CCl₄-induced acute liver injury model (Table III). In contrast, the lower dose (250 mg/kg) showed an insignificant reduction in sleeping time.

Histological observations supported the results obtained from liver enzyme assays. Confluent hepatic cell necrosis and karyorrhexis and karyolysis of hepatocytes were also noted in the control CCl₄ treated rat livers. Extensive hepatic cell steatosis was seen. No confluent necrosis was observed in either of the groups treated with Balessan extract (Figs. 1-4).

Table I. Effect of an ethanol extract of Balessan on some enzymes and bilirubin in rats with CCl₄-induced liver damage

Treatment (n = 6)	Dose mg/kg	GOT mg/kg	GPT mg/dl	ALP mg/dl	Bilirubin mg%
Control	N. saline	151.00 ± 9.76	91.4 ± 3.94	399.16 ± 46.60	0.72 ± 0.026
CCl ₄	1.25 ml/kg	592.25*** ± 48.35	474.41*** ± 35.67	729.85*** ± 33.65	1.69*** ± 0.12
Balessan + CCl ₄	250	256.41*** ± 13.74	123.66*** ± 6.60	508.33*** ± 23.06	1.45 ± 0.003
Balessan + CCl ₄	500	200.08*** ± 30.80	115.56*** ± 9.99	489.00*** ± 24.56	1.08*** ± 0.03

***p < 0.001 Student's *t*-test; *as compared with the control (normal saline) group; †as compared with the CCl₄ group. GOT = glutamic-oxaloacetic transaminase; GPT = glutamic-pyruvate transaminase; ALP = alkaline phosphatase.

Table II Effect of an ethanolic extract of Balessan on the level of nonprotein sulfhydryl (NP-SH) groups in the liver of rat treated with CCl₄

Treatment (n = 6)	Dose mg/kg orally	NP-SH (mean ± SE) μmol/g of tissue
Control normal saline	—	1.62 ± 0.13
Control CCl ₄	—	0.95 ± 0.04***
Balessan extract + CCl ₄	250	1.28 ± 0.006**
Balessan extract + CCl ₄	500	1.50 ± 0.07***

*As compared with the control (normal saline) group; **as compared with the control (CCl₄); ***p < 0.01; ****p < 0.001 Student's t-test.

Discussion

The efficacy of any hepatoprotective drug is essentially dependent on its capability to either reduce harmful effects or to maintain the normal hepatic physiological mechanisms that have been unbalanced by the hepatotoxin (5). The results of the present study reveal that the ethanolic extract of Balessan possesses significant hepatoprotective and antioxidant activities against CCl₄-induced liver damage in rats. It has been observed that CCl₄ is bio-transformed by the cytochrome P-450 system to the trichloromethyl free radical. This free radical may react again with oxygen to form a trichloromethyl peroxy radical, which may attack lipids on the membrane of endoplasmic reticulum. The trichloromethyl peroxy free radical leads to lipid peroxidation, the disruption of Ca⁺⁺ homeostasis and, finally, results in cell death (11, 12). Therefore, leakage of large quantities of enzymes into the blood stream are often associated with massive necrosis of the liver (13).

Administration of CCl₄ results in a rapid increase of serum GOT, GPT and ALP levels (14). Serum GOT can be found in the liver, cardiac muscle, kidney, brain, pancreas, lungs, skeletal muscle, leukocytes and erythrocytes (in decreasing concentrations) (15), whereas the highest concentration of Serum GPT is found in the liver. In tissues, Serum GPT occurs in two locations, the cytosol and mitochondria (16). Serum GPT appears to be a more sensitive and specific test of acute hepatocellular damage than Serum GOT (14). Therefore, the possible hepatoprotective mechanism of Balessan extract on CCl₄-induced liver injuries may be due to the following factors: (i) inhibition of cytochrome P-450 activity; (ii) prevention of lipid peroxidation; (iii) stabilization of the hepatocellular membrane; and (iv) enhancement of protein synthesis (17).

Furthermore, alkaline phosphatase (ALP) is the prototype of these enzymes that reflects the pathological alteration in biliary flow (18). CCl₄-induced elevation of this enzymatic activity in serum is in line with

Table III Effect of the ethanolic extract of Balessan on duration of phenobarbital sleeping time of mice treated with CCl₄

Treatment (n = 6)	Dose mg/kg	Sleeping time (min)	Reduction in sleeping time
Only phenobarbital	50	33.2 ± 2.53	—
CCl ₄ + phenobarbital	—	132.2 ± 6.15***	—
CCl ₄ + Balessan extract + phenobarbital	250 + 50	127.8 ± 4.77*	3%
CCl ₄ + Balessan extract + phenobarbital	500 + 50	109.00 ± 5.12**	18%

*p < 0.05, **p < 0.001 Student's t-test. *As compared to the phenobarbital group; **as compared to the CCl₄ + phenobarbital group.

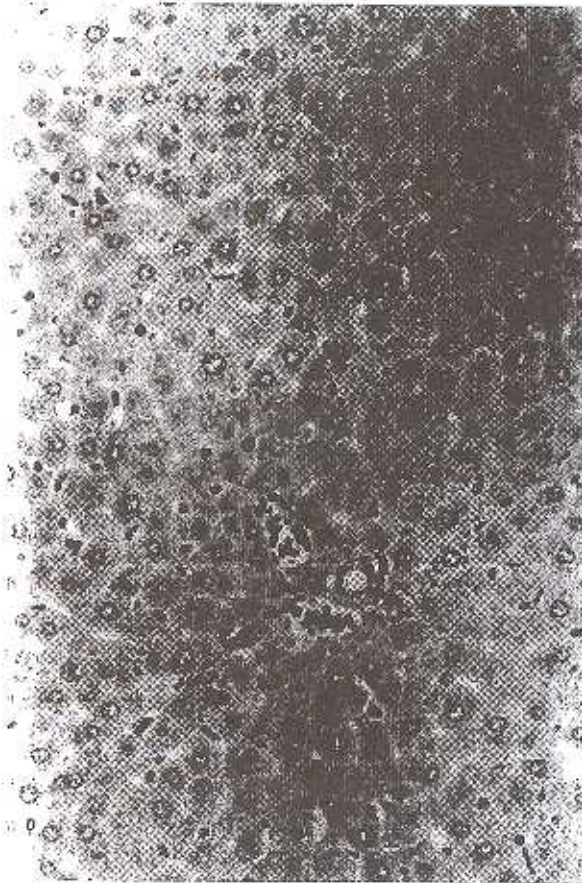


Fig. 1 Control animal (no treatment) portal space (in the center) surrounded by normal liver parenchymal cells. Hematoxylin eosin x 200.

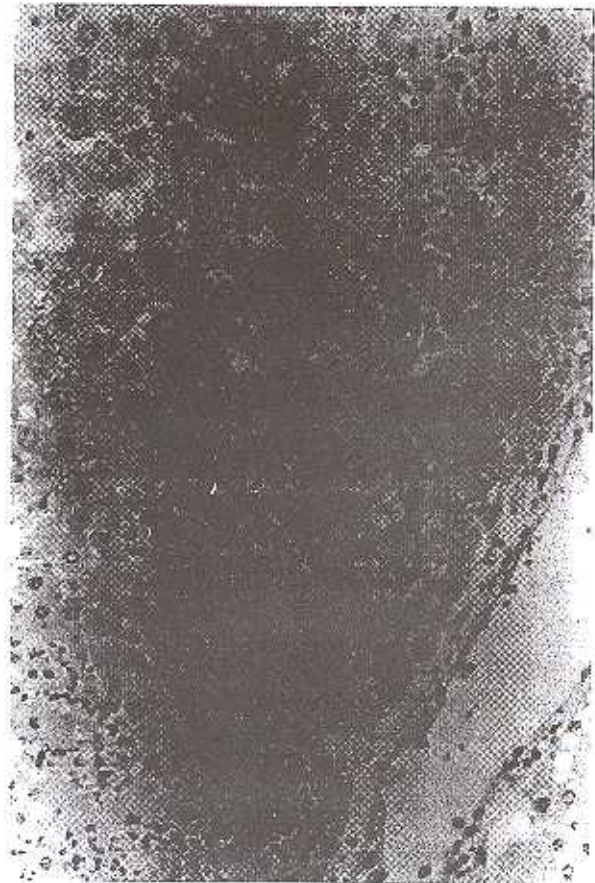


Fig. 2 Liver parenchyma after CCl₄ treatment (exposure), central venous lobule surrounded by confluent hepatic cell necrosis. Note karyorrhexis and karyolysis of hepatocytes. Hematoxylin eosin x 200.

the high level of serum bilirubin content (17). The extract mediated suppression of the increased ALP activity with the concurrent depletion of raised bilirubin level suggests the possibilities of the extract being able to stabilize biliary dysfunction in the rat liver, thereby indicating its effectiveness in maintaining the normal functional status of the liver (20). Our observations in the present study also indicate that treatment with CCl₄ caused a significant reduction in

NP-SH concentration in the rat liver. An ethanolic extract of Balessan, however, offered a significant replenishing of the NP-SH level. Thus, sulphydryl seems to have a role hepatoprotection through its antioxidant potential (21, 22). Additionally, phenobarbitone-induced sleeping time is significantly prolonged in liver damage and this parameter may be employed as a measure of functional status of the hepatic drug metabolizing system (23).

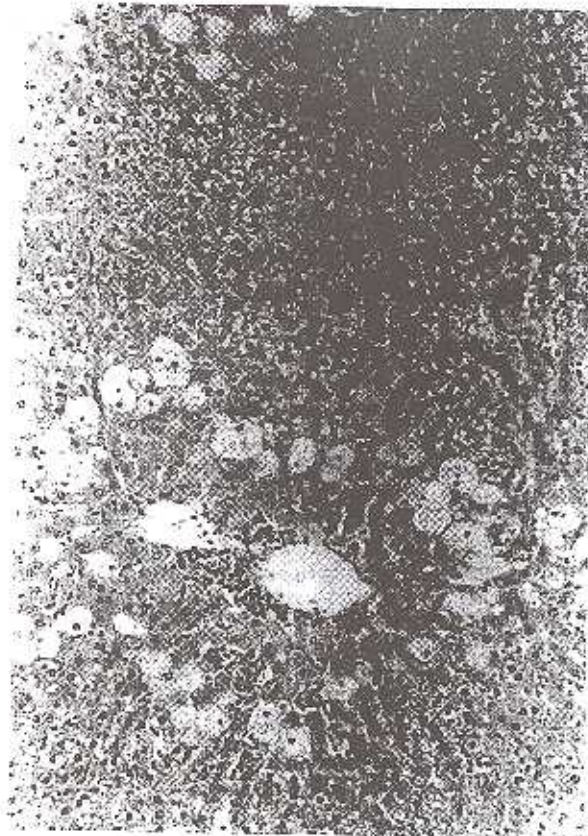


Fig. 3A Liver parenchyma after CCl_4 exposure and Balessan 250 mg/kg treatment. No confluent necrosis is evident. Instead, the pericentral area displays extensive hepatic cell steatosis as well as inflammatory infiltrate. Hematoxylin-eosin x 200.

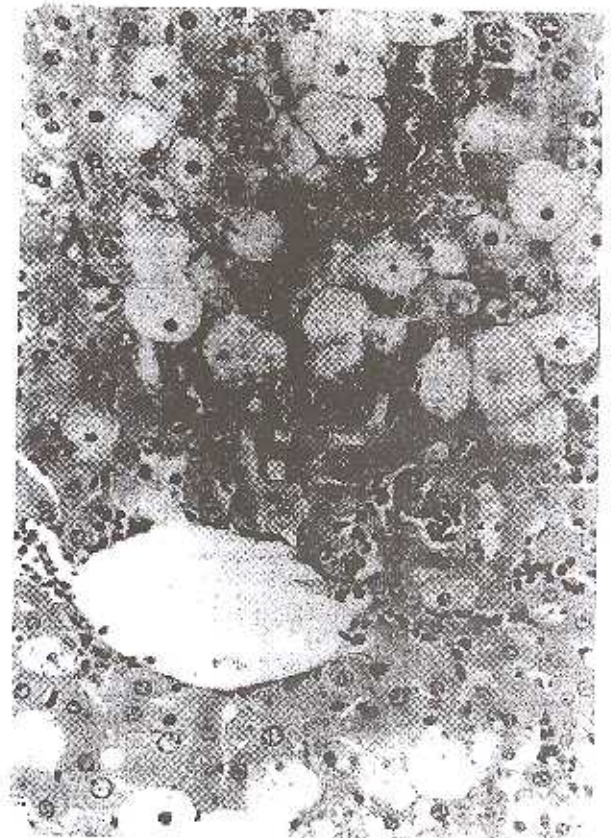


Fig. 3B Liver parenchyma after CCl_4 exposure and Balessan 250 mg/kg treatment. No confluent necrosis is evident. Instead, the pericentral area is localized around central vein. Hematoxylin-eosin x 200.

Regarding the effect of CCl_4 on liver cells and the protective effect of Balessan, no confluent necrosis was observed in either Balessan extract-treated groups, which supports our biochemical findings.

The chemical constituents of Balessan, responsible for its hepatoprotective activity against chemical injury, is not known. However, Balessan contains a number of phytochemical constituents, including flavonoids, saponin, volatile oils, sterol and/or triterpenes. All of these constituents are known to

exhibit antioxidant activity, offer protection against cell damage and possess free radical scavenging effects (24, 25). Interestingly, some *Commiphora* species have been shown to possess diversified activities through various mechanisms. These include *Commiphora molmol* and *Commiphora mukul*, which showed pharmacological effects that included anti-inflammatory, antihepatotoxic, antiholesterolemic, antiulcer and cytotoxic actions (26-29).

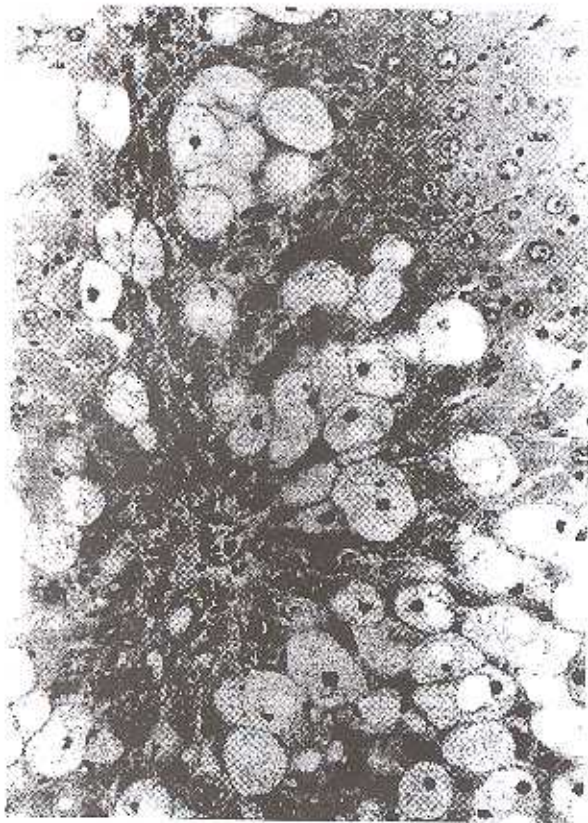


Fig. 4 Liver parenchyma after CCl_4 exposure and treatment with 500 mg/kg of Balessan extract. There is no evidence of necrosis or bile retention. The rim of steatotic hepatocytes appeared and surrounds the perportal space. Hematoxylin-eosin $\times 200$.

In conclusion, this study demonstrates that Balessan possesses significant hepatoprotective and antioxidant effects in rats. Further studies are necessary to isolate the active chemical component(s) and to elucidate its exact mechanism(s) of action.

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