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Therapeutic and preventive effects of *Commiphora gileadensis* against diethylnitrosamine-induced hepatic injury in albino rats

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Commiphora gileadensis **is a tree in the burseraceae family, cultivated widely in Hadhramaut governorate (Yemen) and known locally as "Besham" or Balsam" used traditionally for many ailments. The aim of this study was to evaluate the therapeutic and preventive effects of *C. gileadensis* against diethylnitrosamine (DEN) -induced hepatic injury in albino rats. 40 albino rats were divided randomly into five groups (each contains 8 animals). Group I (Negative control) was given normal saline i.p and distilled water, group II (Positive control) toxicity-initiated with single dose of DEN 200 mg/kg i.p and promoted after 2 weeks with 0.05% of phenobarbitone in drinking water to complete 10 weeks. Group III was given 500 mg/kg extract of *C. gileadensis* bark for 10 weeks. Groups IV (preventive group) pretreated with 500 mg/kg extract of *C. gileadensis* bark and injected with DEN 200 mg/kg i.p for 10 weeks. Groups V (Treatment group) was given single dose of DEN same like group II but for 6 weeks, then treated with 500 mg/kg *C. gileadensis* bark extracts orally for an additional 4 weeks. All doses were used according to the effective dose fixation. Liver function enzymes, complete blood count (CBC) and lipid profile were measured. In addition, fasting blood sugar (FBS) and total body weight were taken weekly. At the end of experiment relative weight of liver was calculated. *C. gileadensis* showed significant hepatoprotective effect as it reduced the liver function enzyme's level, this effect was supported by hepatic histopathological improvement against DEN-induced hepatic injury. In addition, it demonstrated potent anti-platelets activity. The outcomes of this study suggested that *C. gileadensis* has novel hepatoprotective and remarkable anti- platelets effect.**

Key words: *Commiphora gileadensis*, diethylnitrosamine, phenobarbitone, hepatic injury.

INTRODUCTION

Commiphora gileadensis (opobalsamum L.) or the Arabian balsam tree is one of a famous plant spread in the Arabian Peninsula especially Yemen, Saudi Arabia and Oman. The ancient product that is secreted from this

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tree like resins or even the uses of woods cutting from plants were used for making perfumes, incense and different medical products (Jones, 1924). It is a member of the highly studied and commercially used resinous plant family burseraceae, comprising, among others, the biblical frankincense and myrrh (Zohary, 1973). It contains flavonoids, saponins, volatile oils, sterols and triterpenes (Zohary, 1982).

In Yemen, especially in Hadhramaut governorate this plant is used for thousands of years for many different ailments like skin disorders (wounds and burns), respiratory disease, gynecological purposes as contraceptive, labor pain, laxative and diuretic effect (Pliny, 1989). Nitrosamines are chemical compounds with the chemical structure R1N (- R2)-N=O. They are used in the manufacturing of some cosmetics, pesticides, and most rubber products. Nitrosamine occurs in latex products such as balloons (Altkofer et al., 2005) and in many foods, and other consumables. Diethylnitrosamine chemically belonging to the N-Nitrosamine family is proved to be one of the potent carcinogens that is primarily metabolized by the cytochrome P-450 enzymes to reactive electrophiles (O6 alkyl-guanine and N7 alkyl-guanine) which are proved to be cytotoxic (Archer, 1989), carcinogenic (Swann and Magee, 1971) and mutagenic (Magee and Barnes, 1967). Moreover, phenobarbitone may promote the toxic effects induced by diethylnitrosamine (DEN) (Mohammed et al., 2014).

The aim of the present study is to evaluate the therapeutic and preventive effects of *C. gileadensis* against DEN-induced hepatic injury in albino rats.

MATERIALS AND METHODS

Drugs and natural product

N-Nitrosodiethylamine (DEN) and phenobarbitone were purchased from Sigma-adrich Co. USA. *C. gileadenesis* bark was freshly collected in December, 2014 from Hadhramaut governorate. The plant was identified and authenticated at Botany Department, College of Science-, Sana'a University.

Animals

Experimental animals being Wister albino rats (Rattus norvegicus albinus), with average weight of 260±20 g and age of 3 to 4 months were obtained from the animal house of Biology Department, Sana'a University. They were allowed for one week to acclimatize and maintained in 12 h dark/light cycle. They were kept under free water and normal rat chow.

Preparation of extract

1500 g of air-dried bark of *C. gileadensis* was powdered and macerated in 4 L of 99.9% of methanol for one week. The macerated barks then were put in Orbital shaker (0S10B-IKA®-Werke- Germany) for further 48 h to mix all the contents of macerated preparation. Then filtered and the filtrate evaporated under reduced pressure using rotary evaporator (RE3022C-Stuart) at 40°C. Repeated steps were done till the extraction of bark was completed. This process yielded about 24.39 g dark brownish color semi solid extract that was dissolved freshly in distilled water and given to animals (Al Howiriny et al., 2004).

Animal study design

Forty albino rats were divided randomly into five groups (each contains 8 animals). Group I (**Negative control**) was given normal saline i.p and distilled water. In group II (**Positive control**) toxicity was initiated with single dose of DEN 200 mg/kg i.p and promoted after 2 weeks with 0.05% of phenobarbitone in drinking water to complete 10 weeks. Group III was given 500 mg/kg extracts of *C. gileadensis* bark for 10 weeks. Groups IV (**Preventive group**) was pretreated with 500 mg/kg extract of *C. gileadensis* bark and injected with DEN 200 mg/kg i.p for 10 weeks. Groups V (**Treatment group**) was given single dose of DEN same like group II but for 6 weeks, then treated with 500 mg/kg *C. gileadensis* bark extracts orally for further 4 weeks. All doses were used according to the effective dose fixation according to method of Kalaiselvan et al. (2013). Aspartate aminotransferase (AST) (Schumann et al., 2002) and alanine aminotransferase (ALT) (Sonntag and Scholer, 2001), Alkaline phosphatase (ALP) (Abicht et al., 2001), lipid profile (Stein and Myers, 1995; Pisani et al., 1995; Bachoric, 2000) and complete blood count (CBC) were measured. In addition, fasting blood sugar (FBS) (Knudson and Weinstock, 2001) and total body weight were taken regularly. At the end of experiment relative weight of liver was calculated.

Dose fixation study

Different doses of *C. gileadenesis* extract (50, 100, 250, 500 and 750 mg/kg body weight) were used for 4 weeks in albino rats. The effective dose was based on the biochemical studies including liver and kidney function tests. The doses of 500 and 750 were found to be effective, but the minimum effective dose (500 mg/kg) was chosen and fixed throughout this study.

Acute toxicity study

Another twelve albino rats (330±10 g) were randomly divided into 3 groups, each contained 4 animals (the least no. was used for toxicological study). First group served as control group were only drunk distilled water, second and third groups were given extract of *C. giladenesis* bark 2 g/kg and 3 g/kg dissolved in distilled water. All animals were given the tested extract through oral gavage for 72 h according to the method of Jaykaran (2008). The following parameters were observed and measured:

1. Observational parameters: Mortality of animals, motor activity, tremors, convulsion, posture, spasticity, ataxia, writhing, skin color, diarrhea, salivation, lacrimation and respiration. Additionally, daily body weight and food intake were also noted. These observational parameters were monitored immediately at 0, 2, 4, 8, 24, 48 and 72 h of given tested extract.

2. Biochemical parameters: After 72 h of closed observation, animals were sacrificed by decapitation under anesthesia. Blood samples were collected for biochemical studies AST, ALT, CBC and FBS. Liver and heart were dissected out for histopathological examination. All the study procedures were in accordance with the guidelines for the care and use of laboratory animals, and approval was received prior to the experiments from the Institutional Research and Ethics Committee, UST.

Table 1. Effect of *C. giladenesis* on the (mean± SE) liver function enzymes (AST, ALT and ALP) for 10 weeks in albino rats (n=.8).

Treatment	Mean± SEM		
	AST (U/l)	ALT(U/l)	ALP(U/l)
Control	153.2±3.94	37.6±3.86	101.4±10.33
DEN	281.0±23.87*	45.6±8.22	162.0±4.93*
Plant	158.2±6.47**	35.2±1.06	95.8±7.72**
Preventive	208.6±3.59**	42.7±4.85	106.2±19.88**
Treatment	197.4±20.38**	44.2±5.75	103.4±18.12**

*Significant as compared with control at (P< 0.05), ** significant as compared with DEN- induced liver damage at (P< 0.05), AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase.

Table 2. Effect of *C. giladenesis* on the (mean± SE) body weight (g) and relative weight of liver (g) for 10 weeks in albino rats (n=8).

Parameter	Mean± SEM				
	Control	DEN	Plant	Preventive	Treatment
Initial body weight (g)	272.7±12.1	274.2±14.0	273.0±15.5	277.8±14.2	275.5±21.4
Final body weight (g)	320±12.7	252±11.3	290±17.4	280±6.68	285±19.6
Weight of liver (g)	8.1±1.68	13.0±1.00*	8.8±0.75**	9.50±1.5**	10.3±1.12**
Relative weight of liver	2.5	5.1*	3.03**	3.39**	3.61**

*Significant as compared with control at (P< 0.05), ** significant as compared with DEN-induced liver damage at (P< 0.05).

Table 3. Effect of *C. giladenesis* on the (mean± SE) lipid profile for 10 weeks in albino rats (n=8).

Treatment	Mean± SEM			
	Cholest (mg/dl)	LDLc(mg/dl)	TG(mg/dl)	HDL(mg/dl)
Control	74.2±2.75	18.2±0.80	53.4±3.05	62.0±3.57
DEN	114.6±14.1*	26.0±3.0	64.6±12.1	36.8±10.7*
Plant	63.8±9.29**	19.2±1.25**	42.0±5.05	64.4±7.32**
Preventive	70.4±4.05**	20.3±1.33**	49.0±6.82	63.8±4.38**
Treatment	80.7±6.98**	20.8±0.91**	46.6±9.45	60.8±4.96**

*Significant as compared with control at (P< 0.05), ** significant as compared with DEN- induced liver damage at (P< 0.05), Cholest.: cholesterol, LDL-c: low density lipoprotein lipids, TG: Triglyceride, HDL: High density lipoprotein lipids

Statistical analysis

Data were summarized as means ± SEM. One way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test was used to conduct the significance of association using statistical package for the social sciences (SPSS) program version 21. Differences were considered significant at P values of less than 0.05.

RESULTS

Hepatoprotective effect

Methanolic extract of *C. gileadenesis* showed significant amelioration of DEN-induced liver injury as it reduced the level of liver function enzymes AST, ALT and ALP (Table 1). This effect was supported by reduction in the relative weight of liver and lipid profile (Tables 2 and 3) as well as tissue and hepatocytes improvements. In addition, *C. gileadenesis* showed significant reduction in platelets aggregation and lymphocyte levels as seen in Table 4 and 5. Moreover, continuous administration of *C. gileadenesis* in albino rats for 10 weeks counteracted the weight loss caused by DEN, and improved the health state of animals without harmful effect on blood glucose level as shown in Figures 1 and 2.

Toxicological study

There were no acute mortality and biochemical toxicity observed after oral administration of *C. gileadenesis* even

Table 4. Effect of *C. giladenesis* on the (mean± SE) complete blood count (CBC) for 10 weeks in albino rats (n=8).

Treatment	Mean± SEM					
	Hb (g/dl)	RBC (X10^12/L)	MCV (Femtoliters)	MCH (pg)	MCHC (g/dl)	Platelets (X10^9/L)
Control	16.0±0.45	8.77±0.488	52.0±0.23	17.1±0.07	31.6±0.65	494.0±64.4
DEN	17.0±0.17	9.87±0.298	54.5±1.28	17.3±0.29	32.2±0.58	837.0±126*
Plant	16.1±0.39	8.63±0.750	53.7±2.07	16.7±0.21	31.9±0.56	553.0±11.3**
Preventive	15.9±0.98	8.35±9.428	54.4±0.81	17.8±0.57	31.7±0.41	593.064.2**
Treatment	16.8±0.47	9.19±0.257	53.6±1.26	17.2±0.14	32.6±0.41	616.0±30.6**

*Significant as compared with control at (P< 0.05), ** significant as compared with DEN-induced liver damage at (P< 0.05), Hb: hemoglobin, RBC: Red blood cell, MCV: mean cell volume, MCH: mean corpuscular hemoglobin. MCHC: mean corpuscular hemoglobin concentration.

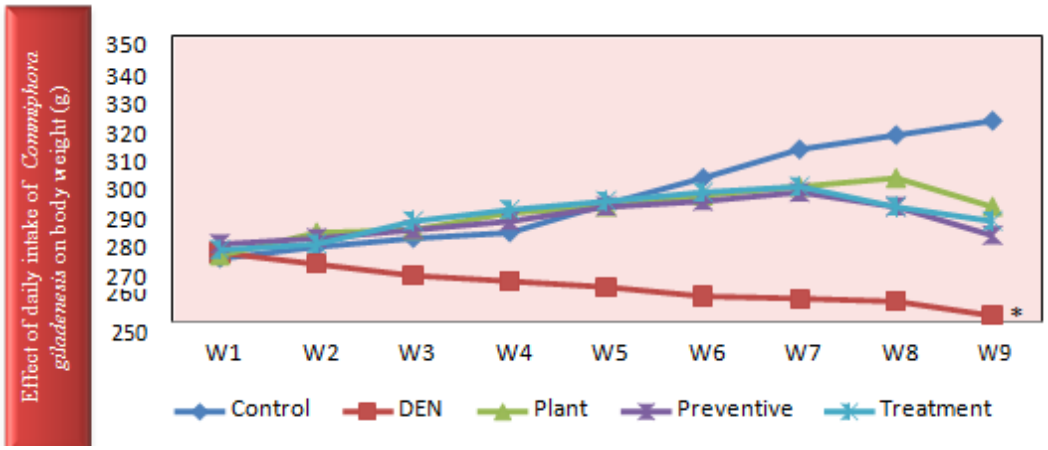

Figure 1. *C. giladenesis* on the (mean± SE) body weight (g) for 10 weeks in albino rats (n=8),*Significant as compared with control at (P< 0.05), W: week's number.

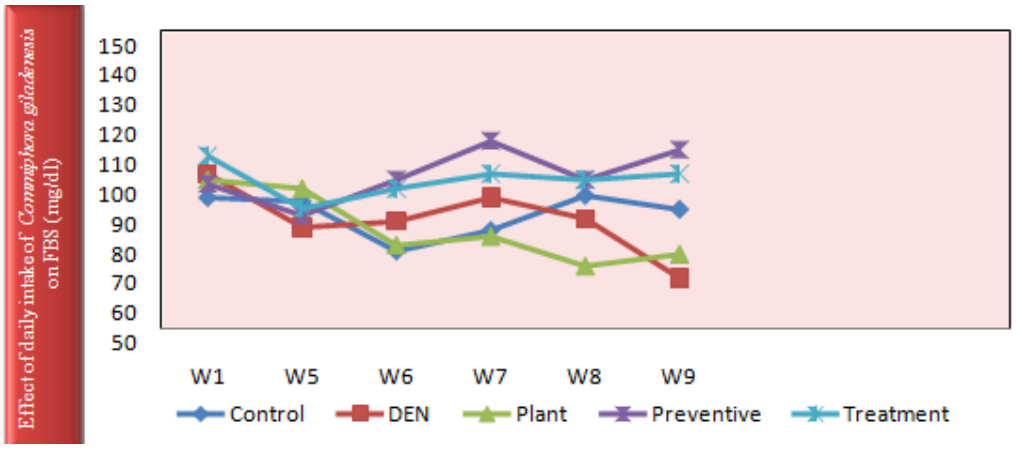

Figure 2. Effect of daily intake of *C. giladenesis* on the (mean± SE) fasting blood sugar (mg/dl) for 10 weeks in albino rats.

at a dose of 3 g/kg. All animals were found to be normal at the end of 72 h with no significant differences between control and treated groups in the measured parameters (Tables 5 and 6).

Table 5. Effect of *C. giladenesis* on DEN-induced alteration in CBC in male albino rats.

Treatment	Mean ±SE				
	T.WBC (×10^9/L)	Lymphocyte (%)	Monocyte (%)	Neutrophil (%)	Eosinophil (%)
Control	9.1±0.63	64.3±5.16	0.7±0.16	20.5±2.91	1.7±0.29
DEN	11.4±0.84*	80.6±5.08*	0.9±0.23	22.2±1.19	1.9±0.23
Plant	8.78±0.55**	60.8±2.39**	0.7±0.16	20.7±1.01	1.5±0.33
Preventive	9.52±0.92	68.3±2.40**	0.8±0.18	19.5±1.42	1.1±0.30
Treatment	10.3±0.399	67.6±2.91**	0.6±0.20	20.8±1.89	1.3±0.14

*Significant as compared with control at (P< 0.05), ** significant as compared with DEN-induced liver damage at (P< 0.05), TWBC: total white blood cell.

Table 6. Acute toxicity study of extract of *C. gileadenesis.*

Test	Mean ±SE		
Animal groups	**Control**	**Extract 2 g/kg**	**Extract 3 g/kg**
Clinical observation	No effect	No effect	No effect
Body weight (g) Pretreatment	323.0±26.5	314.0±15.0	330.0±14.9
After 24 hours	323.7±27.3	314.8±15.1	330.5±15.3
After 48hours	325.1±28.1	316.2±14.9	332.0±14.8
After 72 hours	325.9±28.3	316.7±14.6	334.5±15.3
Food intake (g)			
Pretreatment	79.0±3.23	76.1±3.48	77.5±3.84
After 24 hours	78.9±3.20	77.8±2.19	81.1±2.57
After 48hours	80.2±3.32	78.6±2.70	82.7±2.40
After 72 hours	81.0±3.46	80.0±2.65	89.6±3.17
Biochemical studies			
Hematology	12.4±0.63	11.9±1.07	14.1±0.54
Hb (g/dl)	10.2±0.41	10.5±0.29	9.33±1.01
Total W.B.C liver function enzymes			
AST	62.3±19.4	55.0±20.4	37.3±11.3
ALT	56.0±19.5	51.3±21.6	39.7±8.66
Fasting blood sugar			
Pretreatment	119.3±4.66	126.0±12.0	113.7±9.56
Post -treatment	117.5±13.9	120.7±3.17	91.0±6.51
Histophathology study	Normal	No change	No change
Liver Heart	Normal	No change	No change

DISCUSSION

In the present study, diethylnitrosamine was used as a model of hepatic injury. It is an N-nitroso alkyl compound, categorized as a potent hepatotoxin and hepato-carcinogen in experimental animals (Figure 3a-j) (Jose et al., 1998). This compound is metabolized in liver by P450 enzymes to form reactive electrophiles which cause oxidative stress leading to cytotoxicity, mutagenicity and carcinogenicity (Archer, 1989). It is considered as a pollutant found in environment, foods, alcoholic beverages and pharmaceutical agents (Sivaramkrishnan et al., 2008; Gupta et al., 2010).

The result of this study showed that DEN elevated significantly the liver function enzymes as well as it caused severe histopathological changes in the liver tissues. DEN led marked elevation of serum enzyme levels of AST, ALT and ALP is good indication of hepatocellular damage. However, elevation of these enzymes may lead to liver necrosis due to leakage of these enzymes to blood stream (Ala-Kokko et al., 1987; Al-Rejaie et al., 2009). Furthermore, an accumulation of connective tissue

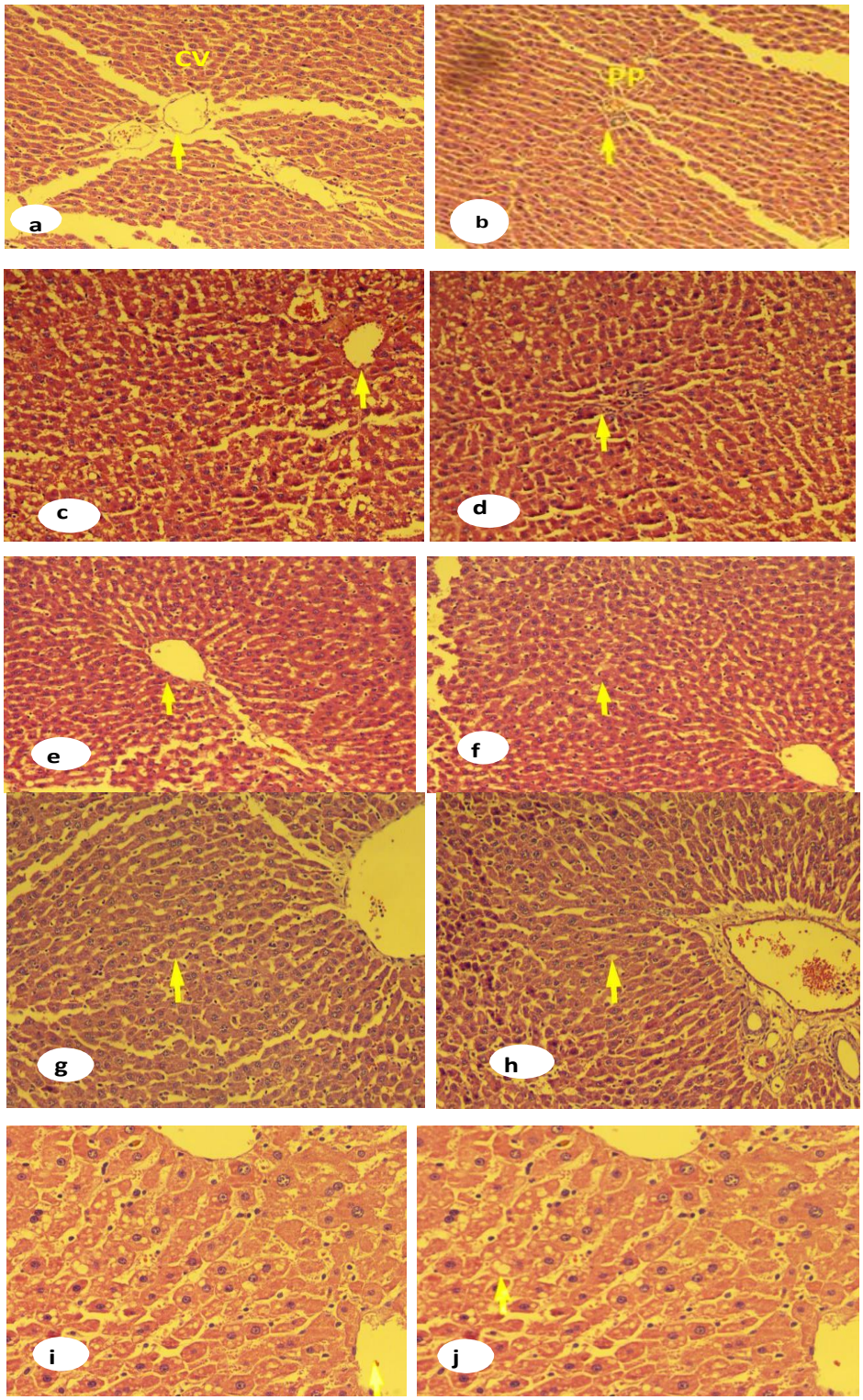

Figure 3. a and b: Control group section showing liver with preserved architecture with normal preportal, central vein and trabeculae of hepatocytes separated by blood sinusoids (H&E; original magnification: X 200); c and d: DEN treated group, showing inflammatory cells, the hepatocytes show macro and micro steatosis involving zone 3 in few acinus (H&E; original magnification: X 200); e and f: Plant treated group, showing normal liver architecture of central vein and mid zone (H&E; original magnification: X 200); g and h: Preventive group, showing marked improvement of liver with no evidence of steatosis in mid zone and preportal area (H&E; original magnification: X 200); i and j: Treatment group showing marked improvement with mild steatosis in zone3 (H&E; original magnification: X 200).

protein especially collagen, have been reported in DEN induced liver injury (George and Chandrakasan, 1996). Moreover, decreased synthesis of collagenolytic in the impaired hepatocytes may cause an accumulation of collagen (George et al., 2001). All these effects were ameliorated by the using of methanolic extract of *C. gileadenesis* bark. This plant showed significant preventive and therapeutic effects against DEN-induced hepatic injury through reduction of the liver function enzymes like AST, ALT and ALP as well as lipid profile especially cholesterol, LDL-c supported by the histopathological tissue improving in commiphora-treated groups.

However, the mechanism underlying the improvement of hepatic functions after continuous using of this plant may be referred to prevent the formation of reactive metabolite of DEN through direct inhibition of the hepatic cytochrome P450, additionally, it inhibits lipid peroxidation processes, stabilizes the hepatocyte membrane and enhances protein synthesis (Al-Howiriny et al., 2003). The presence of phytochemical antioxidant constituents like flavonoids, volatile oils, saponins, triterpenes and sterols are considered as key role for the protective effects and free radical scavengers in this plant (Vogel, 1977; Kikuzaki et al., 2000).

Regarding the effect of *C. gileadenesis* on complete blood count, it was found that this plant has significant reduction of platelets levels caused by DEN-induced toxicity. The precise reason for diminished platelets aggregation is still unknown. This worthwhile and remarkable anti-platelets activity may be due to either anti-inflammatory effect (Al-Howiriny et al., 2003) through the action on inflammatory mediators like thromboxane A2, 5HT and ADP or direct action on GPIIb/IIa receptors of platelets.

Conversely, the outcome of the present study was that, this plant counteracted the weight loss in DEN- treated group when it was administered for preventive or therapeutic purposes, this effect was not accompanied with any change in fasting blood sugar level. It suggested that this plant is characterized by appetite modulating effect without harmful metabolic changes as it reduced lipid profile especially cholesterol and low density lipoprotein (bad lipids). In contrary, it raised high density lipoprotein level which is known as a good lipid (Al-Amoudi, 2009). All these magic effects make this plant as a novel complementary medicine for many ailments. Additionally, beside its worthwhile health benefits, it is also considered as a safe plant assessed by preliminary toxicological study. It saves up to 3 g/kg/d (2.5 fold of the dose used) without any observational, biochemical side effects and/or histopathological changes.

Conclusion

From the outcomes of the present study, it is suggested that *C. gileadenesis* possesses protective effect against DEN-induced liver injury and anti -platelets activity. The presence of phytochemical antioxidant constituents in this plant like flavonoids, volatile oils, saponins, triterpenes and sterols may be responsible for its protective effects as they working as free radical scavengers. Additionally, the toxicological study showed that this plant can be used safely without any harmful effects. Further studies using more technical methods to elucidate the exact constituent (s) responsible for these benefits without side effects are required in order to approve and expand these findings.

Conflict of interests

The authors have not declared any conflict of interests.

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