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Evaluation of the Hepatoprotective Effect of *Aloe vera*, *Clematis hirsute*, *Cucumis prophetarum* and Bee Propolis Against Experimentally Induced Liver Injury in Rats

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Abstract: In a project to search for new natural hepatoprotective agents 3 plant extracts; *Aloe vera*, *Clematis hirsute* and *Cucumis prophetarum*, in addition to Bee Propolis were studied. The ethanol extracts of the 3 plants and propolis were subjected to hepatoprotective assay using Wistar albino rats. Liver injury induced in rats using carbon tetrachloride. The biochemical parameters; serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin were estimated as reflection of the liver condition. Based on the good results of the biochemical parameters measurements, histopathological study was performed on the liver of rats treated with Propolis and *Aloe vera*. The livers of rats treated with Propolis showed good protection against the toxic effect of carbon tetrachloride. On the other hand treatment with *Aloe vera* extract failed to restore the normal appearance of hepatocytes. All the results were compared with silymarin, as a reference hepatoprotective drug.

Key words: *Aloe vera*, *Clematis hirsute*, *Cucumis prophetarum*, propolis, carbon tetrachloride, hepatoprotection, silymarin, rats

INTRODUCTION

The liver is an organ of prime importance and plays a significant role not only in metabolism and detoxification of exogenous toxins and therapeutic agents, but also in the bioregulation of fats, carbohydrates, amino acids and proteins. A number of pharmacological and chemical agents act as hepatotoxins and produce a variety of liver ailments (Ram, 2001). Numerous herbal extracts are used for liver problems, however, considerable number of them lack the scientific prove for these claims. In our search for hepatoprotective natural remedies three plant extracts; *Aloe vera*, *Clematis hirsute* and *Cucumis prophetarum* in addition to Bee Propolis were tested against liver injury induced in rats using carbon tetrachloride. In Saudi folk medicine, the leaves of *Aloe vera* are used as hepatic stimulant and in liver enlargement (Mossa *et al.*, 1987). The aerial parts of *Cucumis prophetarum*, known locally as Shari-al-deeb is used by traditional medicine practitioners as remedy for liver disorders (Al-Yahya *et al.*, 1990). *Clematis hirsute* known locally as Ghashoua and Shajarat Al-Riyah (Chaudhary, 1998) is used as other *Clematis* species members as anti-inflammatory (Morgan, 1981; Xu *et al.*, 1996). Natural medicine practitioners often use propolis for the relief of

various conditions, including inflammations and viral diseases. Propolis also exhibits immunomodulatory effects (Brätter *et al.*, 1999; Ansorge *et al.*, 2003).

MATERIALS AND METHODS

Test material: The fruits of *Cucumis prophetarum* L. (Cucurbitaceae) were collected from the Northern part of Saudi Arabia in March 2003. The aerial parts of *Clematis hirsuta* Guillemin and Perr (Ranunculaceae) were collected from Tanuma region in March 2000. The plants were identified by Dr. Mohammad Atiqur Rahman, taxonomist of the Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Voucher specimens (#12465 and 11411) were deposited at the herbarium of the center.

The extract of *Aloe vera* (L.) Burm. f. (Asphodelaceae) was obtained from E. Merck. Extracted Bees Propolis (98% purity, Flavonoids: 14%) was obtained from Guangzhou Longstone Import and Export Trading Co., Ltd., China.

Extraction: Fifty grams of the dried ground aerial parts of *Clematis hirsute* and the dried fruits of *Cucumis*

prophetarum were extracted to exhaustion by percolation at room temperature with 90% ethanol, the extracts were evaporated *in vacuo* to leave 4.25 and 2.41 g extracts, respectively.

Animals: Wistar albino rats (150-200 g) of either sex roughly the same age (8-10 weeks), obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh were used. The animals were housed under constant temperature ($22\pm 2^\circ\text{C}$), humidity (55%) and light/dark conditions (12/12 h). They were provided with Purina chow and free access to drinking water *ad libitum* (Abdel-Kader and Alqasoumi, 2008). The experiments and procedures used in this study were approved by the Ethical Committee of the College of Pharmacy, King Saud University.

Chemicals: Silymarin (Sigma Chemical Company, USA).

Hepatoprotective activity: Male Wistar rats were divided into 5 groups 6 animals each. Group 1 was kept as a control group. Groups 2, 3, 4 and 5 received 0.125 mL of CCl_4 in liquid paraffin (1:1) per 100 g body weight intraperitoneally. Group 2 received only CCl_4 treatment. Group 3 was administered silymarin at a dose of 10 mg kg^{-1} p.o. Groups 4 and 5 were treated with 250 and 500 mg kg^{-1} of extracts respectively. Drug treatment was started 5 days prior to CCl_4 administration and continued till the end of the experiment. After 48 h, following CCl_4 administration the animals were sacrificed using ether anesthesia. Blood samples were collected by heart puncture and the serum was separated for evaluating the biochemical parameters. The liver was immediately removed and a small piece was fixed in 10% formalin for histopathological assessment.

Determination of the enzyme levels: The biochemical parameters such as Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP) and total bilirubin were estimated by reported methods (Boon *et al.*, 2006). The enzyme activities were measured using diagnostic strips (Reflotron®, ROCHE) and were read on a Reflotron® Plus instrument (ROCHE).

Statistical analysis: For each set of experiments where two or more than two groups were compared, an analysis of variance (ANOVA) test was used to determine the significance of the differences. Differences between the control and CCl_4 -treated group were compared for significance using student's t-test for non paired samples (Woolson and Clarke, 2002). All the values shown are the mean $\pm$ SE.

Histopathology: The livers of treated animals were immediately removed and a small piece was fixed in 10% formalin for histopathological assessment. All specimens were placed in cassettes and loaded into tissue baskets. The specimens were subjected to dehydration, clearing and inflation by immersion in different conc of ethanol (70-100%), xylene (3 times, 1 h each) and finally paraffin wax (4 times, 1 h each). The tissues were then transferred into moulds filled with paraffin wax. After orienting the tissues by hot forceps the moulds were chilled on cold plates and excess wax were trimmed off using a knife. The rotary microtome (Leitz, 1512) was used for making thin sections ($3\text{ }\mu\text{m}$). The sections were placed onto clean slides that were drained vertically for several min. before placing them onto a warming table at $37\text{--}40^\circ\text{C}$ (Prophet *et al.*, 1994). The slides were then deparaffinized, hydrated and to stained in Mayer's hematoxylin solution for 15 min. The slides were then washed in lukewarm running tap water for 15 min, placed in distilled water, 80% ethyl alcohol for 1 to 2 min then counterstained in eosin-phloxine solution for 2 min. The slides were then dehydrated and cleared through 2 changes each of 95% ethyl alcohol, absolute ethyl alcohol and xylene, 2 min each and finally mounting with resinous medium.

RESULTS AND DISCUSSION

Treatment of animals with the hepatotoxic agent carbon tetrachloride resulted in Significant Increase of Transaminases (SGOT and SGPT) and Alkaline Phosphate Levels (ALP) due to hepatocytes damage (Zafar and Ali, 1998). Sever jaundice was reflected by increase level of serum bilirubin (Lin *et al.*, 1997) (Table 1).

Treatment of animals with the known hepatoprotective agents silymarin resulted in significant decrease in the elevated levels of SGOT, SGPT, ALP and bilirubin ($p<0.001$) (Table 1). Silymarin act as an antioxidant by scavenging prooxidant free radicals and by increasing the intracellular concentration of GSH. It also exhibits a regulatory action of cellular membrane permeability and increase in its stability against xenobiotics injury; increasing the synthesis of ribosomal RNA by stimulating DNA polymerase-I, exerting a steroid like regulatory action on DNA transcription and stimulation of protein synthesis and regeneration of liver cells (Dehmlow *et al.*, 1996a, b; Gakova *et al.*, 1992; Saller *et al.*, 2007). Silymarin efficacy is not limited to the treatment of toxic and metabolic liver damage; it is also effective in acute, chronic hepatitis and in inhibiting fibrotic activity (Saller *et al.*, 2007).

Table 1: Effects of ethanolic extracts of *Clematis hirsuta*, propolis powder, *Aloe vera* and *Cucumis prophetarum* on serum biochemical parameters

Treatments (n = 6)	Dose mg kg ⁻¹ (Orally)	Biochemical parameters							
		SGOT (units L ⁻¹)		SGPT (units L ⁻¹)		ALP (units L ⁻¹)		Bilirubin (mg dL ⁻¹)	
		Mean±SE	Decrease (%)	Mean±SE	Decrease (%)	Mean±SE	Decrease (%)	Mean±SE	Decrease (%)
Normal (control)	Normal saline	106.35±17.11		47.53±12.15		512.16±29.74		0.61±0.07	
CCl ₄ only (toxicity control)	1.25 mL kg ⁻¹	432.00±32.46****		384.50±36.24****		1003.66±46.26****		2.75±0.26****	
Silymarin + CCl ₄	10	201.16±18.57****	53.4	153.00±22.66****	60.20	566.66±39.40****	43.5	1.18±0.14****	57.0
<i>Clematis hirsuta</i> + CCl ₄	250	407.83±14.19 ^b	5.5	350.83±33.29 ^b	8.70	969.83±25.94 ^b	-	2.79±0.23 ^b	-
<i>Clematis hirsuta</i> + CCl ₄	500	386.00±15.32 ^b	10.6	313.33±19.39 ^b	18.50	876.00±25.47 ^{b*}	12.7	2.30±0.22 ^b	16.3
Propolis powder + CCl ₄	250	342.16±22.22 ^b	20.7	301.50±22.94 ^b	21.50	809.16±22.87***	19.3	1.98±0.16 ^{b*}	28.0
Propolis powder + CCl ₄	500	305.00±16.13***	29.4	241.00±25.89***	37.30	747.16±31.28****	25.5	1.92±0.12 ^{b*}	30.1
Normal (control)	Normal saline	75.00±9.95		40.80±7.010		475.83±41.38		0.59±0.050	
CCl ₄ only (toxicity control)	1.25 mL kg ⁻¹	531.33±29.47****		381.00±29.07****		770.83±34.26****		2.27±0.29****	
Silymarin + CCl ₄	10	205.33±22.91****	61.3	135.50±18.97****	64.40	507.33±30.09****	34.1	1.04±0.15****	54.1
<i>Aloe vera</i> + CCl ₄	250	473.16±32.10 ^b	10.9	297.16±16.26 ^{b*}	22.00	778.50±44.26 ^b	-	1.76±0.19 ^b	22.4
<i>Aloe vera</i> + CCl ₄	500	428.82±17.94 ^{b*}	19.2	242.33±28.82 ^{b**}	36.30	607.66±27.94***	21.1	1.28±0.10 ^{b**}	43.6
<i>Cucumis prophetarum</i> + CCl ₄	250	571.00±34.66 ^b	-	327.00±24.72 ^b	14.10	784.16±35.46 ^b	-	1.81±0.16 ^b	20.2
<i>Cucumis prophetarum</i> + CCl ₄	500	494.66±36.63 ^b	6.9	279.66±26.08 ^{b*}	26.50	663.33±35.08 ^b	13.9	1.43±0.10 ^{b*}	37.0

*p<0.05; **p<0.01; ***p<0.001; ^aAs compared with the normal saline (control) group; ^bAs compared with the CCl₄ only group

Treatment of animals with crude extract of *Clematis hirsuta* (Aerial parts) at doses (250 and 500 mg kg⁻¹) showed little decreases in all the parameters estimated (Table 1). In addition, the decreases were not statistically significant.

The SGOT, SGPT, ALP and bilirubin levels in Propolis treated rats (500 mg kg⁻¹) were significantly decreased when compared with CCl₄ treated group (p<0.05-p<0.001) (Table 1). The reduction in the biomarkers levels with the 250 mg kg⁻¹ doses were significant (p<0.05 and p<0.01) except for that of SGPT which resulted in a 21.5% statistically insignificant reduction. These results revealed a good degree of protection against the toxic substance CCl₄.

The group of animals treatment of *Aloe vera* extract exhibited a significant reduction only in SGPT level (22%, p<0.05) at the lower dose (250 mg kg⁻¹). Where as the higher dose (500 mg kg⁻¹) significantly lowered the levels of SGOT, SGPT, ALP and bilirubin (p<0.05, p<0.01, p<0.01 and p<0.01), respectively, indicating a good level of protection against the toxicity of CCl₄.

The crude extracts of *Cucumis prophetarum* at the lower (250 mg kg⁻¹ body weight) dose failed to protect against elevated levels of all biomarkers. Whereas, only

SGPT and bilirubin levels were found to be reduced significantly (26.5 and 37.0% reduction; p<0.05 and p<0.05, respectively) at higher dose in *C. prophetarum* crude extract treated animals (Table 1).

Based on the above results the livers of rats treated with Propolis and *Aloe vera* were subjected to histopathological study to evaluate the extent of protection of hepatocytes. The histological appearance of the hepatocyte reflects their conditions (Prophet *et al.*, 1994). Exposure of hepatocytes to toxic agents such as CCl₄ leads to histopathological changes from the normal histological appearance (Fig. 1A). The hepatocytes of rat livers treated with a single dose of 1.25 mL CCl₄ kg⁻¹, showed centrilobular hepatocyte necrosis and extensive fatty change were observed on the midzonal or entire lobe at 24 h (Fig. 1B). Liver tissue of rats treated with CCl₄ and silymarin showed good recovery with absence of necrosis and fatty depositions (Fig. 1C).

Histological appearance of rat liver treated with *Aloe vera* (500 mg kg⁻¹) and CCl₄. Effects showed extensive inflammation around central veins, less apoptosis, microvesicular fatty changes and ballooning degeneration. This appearance indicates poor protection of the hepatocyte against the hepatotoxic agent.

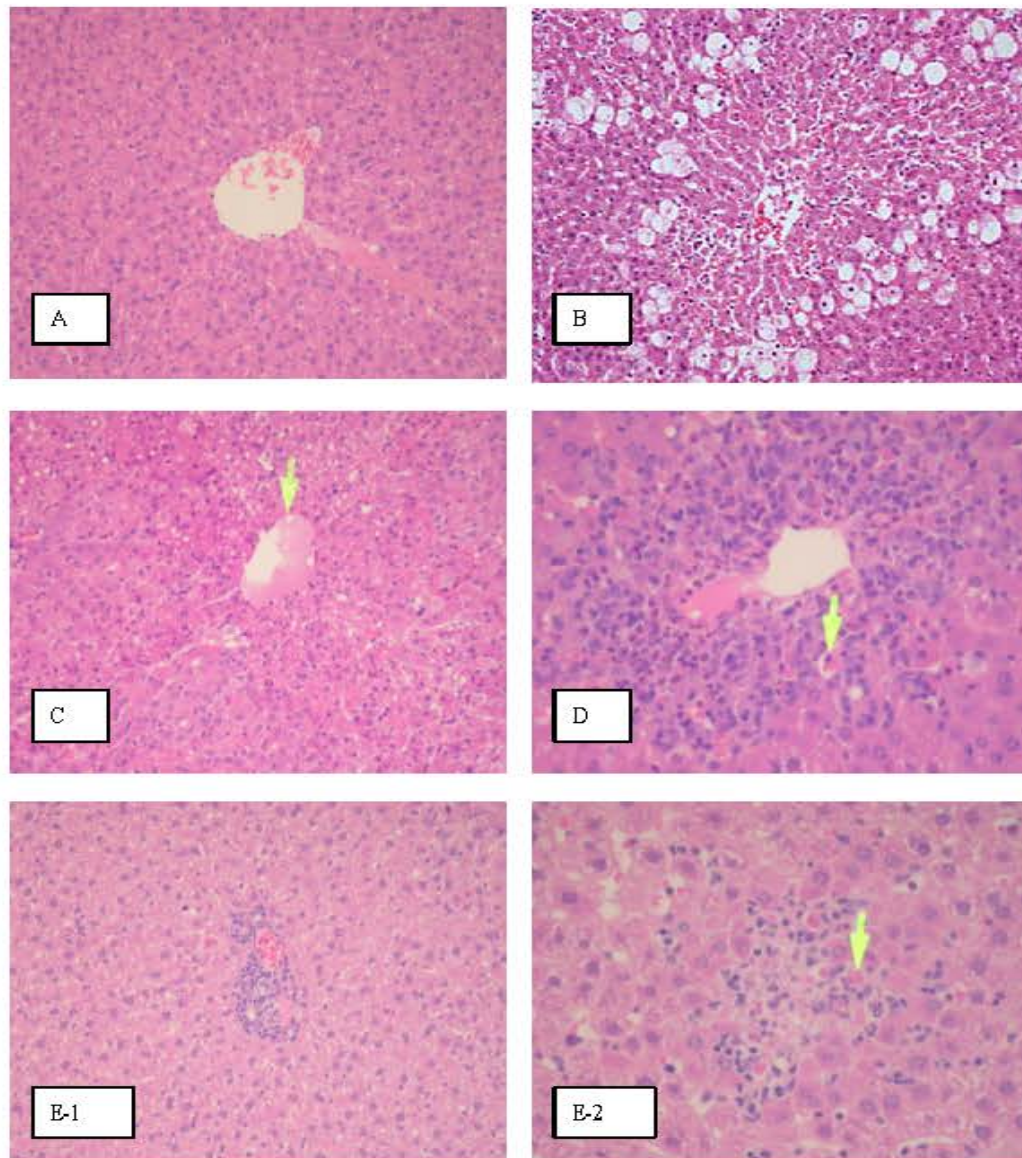


Fig. 1: Histopathological appearance of liver cells; (A): Normal cells; (B): Liver cells of rats treated with CCl_4 ; (C): Liver cells of rats treated with CCl_4 and silymarin; (D): Liver cells of rats treated with CCl_4 and *Aloe vera*; (E): Liver cells of rats treated with CCl_4 and Propolis powder

Liver tissue of rats treated with CCl_4 and Propolis powder (500 mg kg^{-1}) showed mild to moderate portal inflammation (Fig. 1E). It displayed a good progress with disappearance of fatty changes and necrosis. This appearance of the hepatocytes indicates the effectiveness of Propolis powder to express significant protective effect of the liver cells against CCl_4 .

The present study proves that Propolis has some protective effect against the hepatotoxic agent CCl_4 . Although there are some claims for the benefits of *Cucumis prophetarum* and *Aloe vera* for liver problems

(Mossa *et al.*, 1987; Al-Yahya *et al.*, 1990), the obtained results could not prove their effectiveness as hepatoprotective agents. No significant results were obtained from the use of *Clematis hirsute* indicating the lack of any protective effect for the liver.

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