Global Veterinaria 9 (2): 184-191, 2012
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2012.9.2.63199

# Protective Effect of Dates (*Phoenix dactylifera L.*) And Licorice (*Glycyrrhiza glabra*) on Carbon Tetrachloride-Induced Hepatotoxicity in Dogs

*[1]H.A. Abdelrahman, [1]S.I. Fathalla, [2]A.A. Mohamed, [3]H.K. Jun and [3]D.H. Kim*

[1]Department of Physiology, Faculty of Veterinary Medicine, Menofia University (Sadat Branch), Egypt
[2]Department of Anatomy, Faculty of Medicine, El-Azhar University, Egypt
[3]College of Veterinary Medicine, Chungnam National University, Daejeon, Korea

**Abstract:** The ameliorative activity of aqueous extracts of the dates (*Phoenix dactylifera L.*) and Licorice (*Glycyrrhiza glabra*) on carbon tetrachloride ($CCl_4$)-induced hepatotoxicity was studied in dogs. Fifteen dogs were divided into 3 equal groups. Group 1 ($CCl_4$-positive control) animals were injected intra-peritoneal with a fresh mixture of equal volumes of $CCl_4$ and olive oil (1:1 – v: v) at doses of 0.6 ml/kg of body weight for 3 successive days and receiving distilled water orally (15ml/kg) for 14 consecutive days. Group 2 (prophylactic group) receiving both date and licorice extracts orally for 24 consecutive days and injected IP with $CCl_4$ on days 10, 11 and 12 of the experimental period. Group3 (curative group) were received aqueous extract of date and licorice extracts for 14 consecutive days and were injected IP with $CCl_4$ on days 1, 2 and 3 of the experimental period. Liver damage was assessed by liver morphology, histology and estimation of plasma concentration of enzyme activities of phospatase (ALP), gamma-glutamyltransferase (GGT), aspartate aminotransferases (AST) and alanine aminotransferase (ALT) and protein profile (total proteins, albumin, globulin and A/G ratio). Treatment with aqueous extract of date flesh and licorice significantly ($P<0.05$) reduced $CCl_4$-induced elevation in plasma liver marker enzymes concentrations and ameliorated histopathological liver damage and stop fibrosis and edema of hepatic parenchyma in dogs. Hence these synergistic effects of date and licorice extract can be used alone and/or in polyherbal formulations of hepatoprotective drugs, thereby preventing the process of initiation and progress of hepatocellular diseases.

**Key words:** Dates Palm · Licorice · Hepatoprotective · $CCl_4$ · Dogs

## INTRODUCTION

The role of traditional medicines in the solution of health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine [1]. With the associated side effects of the modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions [2]. Research work on medicinal plants has intensified and information on these plants has been exchanged. This research will go a long way in the scientific exploration of medicinal plants for the benefit of man and is likely to decrease the dependence on synthetic drugs [3]. Moreover, plant medicines are more often used in combination rather than in a single in order to get maximum benefit from their combined strength [4].

Hepatotoxicity from drugs and chemicals is the commonest form of liver disease. Some of the inorganic compounds producing hepatotoxicity are arsenic, phosphorus, copper and iron. The organic agents include certain naturally-occurring plant toxins such as pyrrolizidine alkaloids, mycotoxins and bacterial toxins. The synthetic group of organic compounds is a large number of medicinal agents. In addition, exposure to hepatotoxic compounds may be occupational, environmental or domestic that could be accidental, homicidal or suicidal ingestion [5].

Hepatoprotective agents are those compounds, which alleviate the liver injury caused by hepatotoxic agents. Hepatoprotective effects of plant drugs and herbal formulations are studied against chemicals (alcohol, $CCl_4$, alcohol, beta galactosamine, thioacetamide) and drugs (paracetamol, nimusalide,

**Corresponding Author:** Hassan A. Abdelrahman, Department of Physiology, Faculty of Veterinary Medicine, Menofia University (Sadat branch), Egypt.

antitubercular drugs like isoniazid, etc.) induced hepatotoxicity in rats and mice as they virtually mimic any form of naturally-occurring liver disease [6].

Licorice (*Glycyrrhiza glabra*) has many traditional uses include treating peptic ulcers, asthma, pharyngitis, malaria, abdominal pain, insomnia and infections [7]. Decoction of the dried root is taken orally as a diuretic, depurative and emollient [8]. It is also used in the treatment of kidney stones and stomach ache [9]. Licorice has been reported to have antioxidant, antiulcer, hepatoprotective [10- 12], anti-asthmatic [13], anti-inflammatory [14], antiviral, anti-diabetic [15] and anticancer activities [16, 17].

Date palms (*Phoenix dactylifera*) have been cultivated in the Middle East since at least 6000 BC [18]. Date palm fruits have been an important component of the diet in most of the arid and semiarid regions of the world [19]. The various parts of this plant are widely used in traditional medicine for the treatment of various disorders which include memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders [20]. The fruits of *Phoenix dactylifera* are used as a detersive and astringent in intestinal troubles, treatment for sore throat, colds, bronchial asthma, to relieve fever, cystitis, gonorrhea, edema, liver and abdominal troubles and to counteract alcohol intoxication [21]. It is also scientifically proved to possess a variety of pharmacological activities which indicate its usefulness in various kinds of diseases and disorders.

Many researchers were used only licorice [10-12] or date palm [22] in different liver affection as a hepatoprotective. So, these results urged us to evaluate the hepatoprotective activity of the co-supplementation (synergistic effects) of date palms (*Phoenix dactylifera L.*) and licorice (*Glycyrrhiza glabra*) against carbon tetrachloride-induced hepatotoxicity in dog.

## MATERIALS AND METHODS

**Plant Material:** Fresh fruits of dates (*P. dactylifera L.*) and dried roots of licorice (*Glycyrrhiza glabra*) were obtained from the native market and authenticated by a taxonomist of Botany Department, Faculty of Science, Alazhar University, Cairo Egypt. Samples of these plants were kept frozen for future reference.

**Plant Preparation and Administration:** The date fruits were manually separated from the pits. The water extract of the date fruit was made by adding distilled water to the fruit (1:3 w/v) and leaving for 48 h in a refrigerator (4°C) with continuous stirring. The daily dose of date fruits per a dog was 1g/kg of body weight according to studies of Al-Qarawi *et al*. [22] and dose translation from animal to human studies [23]. The aqueous extract (1 g/kg) was then used daily through experimental period. The daily dose of dried roots of licorice per a dog was 0.4g/kg of body weight according to studies of Al-Qarawi *et al*. [12] and dose translation from animal to human studies [23]. The roots were soaked in distilled water (1:2 w/v) at temperature of about 25°C for 4 h. The obtained extract was filtered then stores it at -20°C until use. The aqueous extract (0.4 g/kg) was then used daily through experimental period.

**Animals:** Fifteen apparently healthy dogs of native breeds (3 females, 12 males) were used in the current study. Their body ranged from 7 to 10 kg. The dogs were acclimatized to the cages for 15 days prior to experiment. They were housed in well-ventilated room at 25°C (±2) and photoperiod of 12-h light/dark cycle. The dogs were fed twice daily, in the morning and evening, with commercially prepared foods (55% rice, 25% meat, 10% milk, 5% oil and 5% fiber, with the addition of purified calcium carbonate (1400 mg/kg dry matter), potassium iodide (250 mg/kg dry matter) and vitamins). Water was provided ad libitum. The dogs were bathed with a pyrethrin-based insecticide, dewarmed one week before experimental period. Animals and their care were conducted in conformity with international laws and policies.

**Experimental Design:** Fifteen dogs were randomly assigned equally into three equal groups of five each, Group 1 (G.1) ($CCl_4$-treated group): The dogs were injected with a fresh mixture of equal volumes of $CCl_4$ and olive oil (1:1 v/v) by three intra-peritoneal (IP) injections at doses of 0.6 ml/kg body weight/day on days 1, 2 and 3 of the experimental period and receiving distilled water orally (15 ml/dog) for 14 consecutive day.

**Group 2 (G. 2) (Prophylactic Group):** The animals were received both date (1g/kg) and licorice (0.4g/kg) extracts orally (dissolved in 15 ml distilled water/dog) for 24 consecutive days and injected IP with $CCl_4$ on days 10, 11 and 12 of the experimental period.

**Group 3(G. 3) (Curative Group):** The animals were received aqueous extract of date and licorice extracts for 14 consecutive days and were injected IP with $CCl_4$ on days 1, 2 and 3 of the experimental period.

**Blood Sampling:** Venous blood was sampled for each dog by jugular vein puncture 10 days pre-experiment (pre- $CCl_4$ injection) and on 6 and 15 day of the experimental period. The clear serum was separated by centrifugation at 900 g for 10 min and stored at -20°C until biochemical investigations were carried out to assess liver function tests.

**Blood Parameters:** The concentrations of alkaline phospatase (ALP), gamma-glutamyltransferase (GGT), aspartate aminotransferases (AST) and alanine aminotransferase (ALT), total proteins and albumin were determined using commercial kits according to the instructions of the manufactures (Bio-Merieux Laboratory Reagent and Products, France).

**Histopathological Studies:** On day 15 (25 for group 2), all the animals were euthanized by overdose of anesthetic agent (90 mg/kg i.v. pentobarbital sodium injection, Hospira, Inc. USA) to obtain liver autopsy specimens. Liver was washed with normal saline (0.9%) and fixed in 10% formol saline. Paraffin sections of 5ì thickness were prepared and microscopically examined after staining with hematoxylin and eosin [24, 25].

**Statistical Analysis:** Results are expressed as mean ± standard error (SE). Differences between means in different groups were tested for significance using a one-way analysis of variance (ANOVA) followed by Duncan's test and P value of 0.05 or less was considered significant using the statistical analysis system, [26].

## RESULTS

In dogs, hepatic damage induced by $CCl_4$ (group 1) caused a significant rise ($P < 0.05$) in liver marker enzymes (except GGT– Table 1) relative to prophylactic (G.2) and curative (G.3) groups on 6 and 14 days post $CCl_4$ injection and the levels at pre-experiment. Oral treatment with co-supplement of date and licorice extracts significantly ($P < 0.05$) decrease the levels of ALP, AST and ALT at 6 and 14 days post $Ccl_4$ injection (Table 1). The concentrations of protein profile showed no significant change between groups either at pre-experiment samples or post-$CCl_4$ injection (Table 2).

Histopathological examination of the liver sections of dogs treated with $CCl_4$ showed mononuclear leucocytes inflammatory cells infiltration surrounding the dilated central vein associated with fatty change in the most of

Table 1: Effect of date palm and licorice aqueous extracts on liver markers enzymes of $CCl_4$-induced liver damage in dogs

| Time | Group | ALP U/L | GGT U/L | AST units/ml | ALT units/ml |
|---|---|---|---|---|---|
| Pre-Experiment | 1 | 87.20±9.86$^{a}$ | 6.20±0.37$^{a}$ | 24.60±4.27$^{a}$ | 26.80±1.50$^{a}$ |
| | 2 | 97.60±6.91$^{a}$ | 6.00±0.77$^{a}$ | 29.00±7.87$^{a}$ | 27.40±3.85$^{a}$ |
| | 3 | 80.80±5.08$^{a}$ | 6.80±0.58$^{a}$ | 37.60±7.44$^{a}$ | 29.00±3.35$^{a}$ |
| 6-days post $CCL_4$ injection | 1 | 880.80±37.53$^{b}$ | 6.80±0.86$^{a}$ | 294.00±26.27$^{c}$ | 2687.20±420.43$^{b}$ |
| | 2 | 368.60±28.36$^{a}$ | 5.20±0.58$^{a}$ | 112.60±13.35$^{a}$ | 228.20±23.34$^{a}$ |
| | 3 | 335.80±21.09$^{a}$ | 6.40±0.81$^{a}$ | 215.20±31.16$^{b}$ | 251.60±29.05$^{a}$ |
| 15-days post $CCL_4$ injection | 1 | 242.80±10.44$^{b}$ | 12.00±1.92$^{a}$ | 265.60±63.83$^{b}$ | 453.80±50.22$^{c}$ |
| | 2 | 173.80±11.22$^{a}$ | 8.40±1.29$^{a}$ | 98.40±19.63$^{a}$ | 96.80±10.66$^{a}$ |
| | 3 | 261.80±28.99$^{ab}$ | 10.40±1.21$^{a}$ | 125.40±12.63$^{a}$ | 267.20±57.52$^{b}$ |

Mean ± SE (n=5) $^{a,b,c}$ Means having different superscripts are significantly different at $p<0.05$.

Table 2: Effect of date palm and licorice aqueous extracts on serum protein profile of $CCl_4$-induced liver damage in dogs

| Time | Group | Total protein g/dl | Albumin g/dl | Globulin g/dl | A/G Ratio |
|---|---|---|---|---|---|
| Pre-Experiment | 1 | 7.48±0.15$^{a}$ | 3.00±0.14$^{a}$ | 4.48±0.28$^{a}$ | 0.69±0.07$^{a}$ |
| | 2 | 7.30±0.19$^{a}$ | 2.84±0.01$^{a}$ | 4.46±0.19$^{a}$ | 0.64±0.03$^{a}$ |
| | 3 | 7.30±0.18$^{a}$ | 2.92±0.19$^{a}$ | 4.38±0.34$^{a}$ | 0.69±0.08$^{a}$ |
| 6-days post $CCL_4$ injection | 1 | 7.28±0.60$^{a}$ | 2.40±0.08$^{a}$ | 4.88±0.61$^{a}$ | 0.52±0.05$^{a}$ |
| | 2 | 6.88±0.32$^{a}$ | 2.42±0.18$^{a}$ | 4.46±0.20$^{a}$ | 0.54±0.03$^{a}$ |
| | 3 | 6.80±0.60$^{a}$ | 2.20±0.09$^{a}$ | 4.60±0.67$^{a}$ | 0.52±0.07$^{a}$ |
| 15-days post $CCL_4$ injection | 1 | 7.02±0.50$^{a}$ | 2.54±0.21$^{a}$ | 4.48±0.44$^{a}$ | 0.58±0.06$^{a}$ |
| | 2 | 6.62±0.34$^{a}$ | 2.46±0.17$^{a}$ | 4.16±0.24$^{a}$ | 0.60±0.04$^{a}$ |
| | 3 | 6.56±0.24$^{a}$ | 2.38±0.27$^{a}$ | 4.18±0.29$^{a}$ | 0.59±0.11$^{a}$ |

Mean ± SE (n=5) $^{a,b,c}$ Means having different superscripts are significantly different at $P<0.05$

**Plate 1: (Group1- $CCL_4$)**

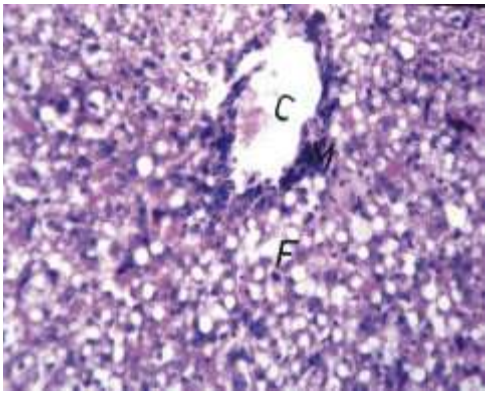



**Fig. 1**

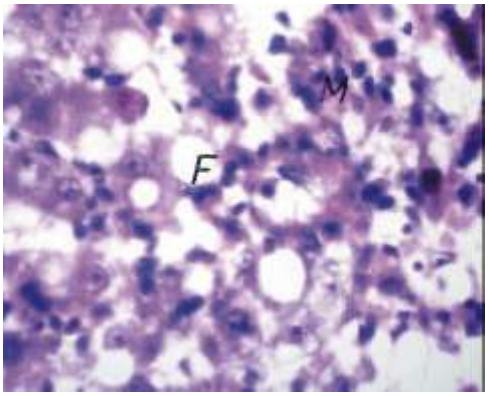



**Fig. 2**

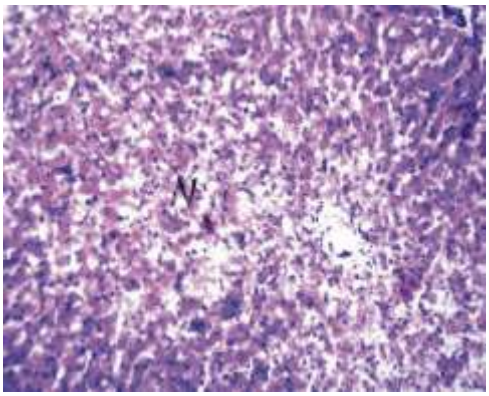



**Fig.3**

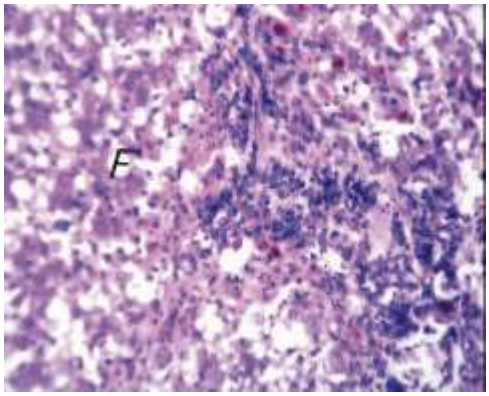



**Fig. 4**

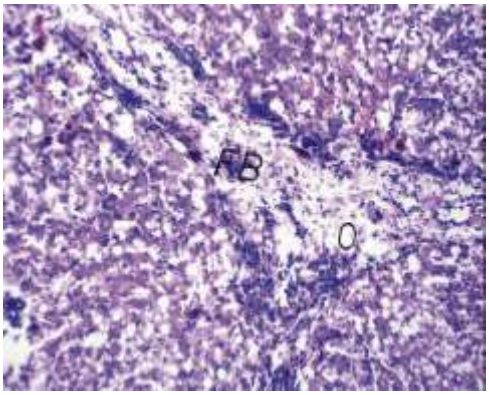



**Fig. 5**

Fig. 1: Dog liver in group 1 showing mononuclear leucocytes inflammatory cells infiltration (M) surrounding the dilated central vein (C) with fatty change in most of the surrounding hepatocytes (F). (H&E x64).

Fig. 2: Dog liver in group 1 showing mononuclear leucocytes inflammatory cells infiltration (M) in between the fatty changed hepatocytes (F). (H&E x160)

Fig. 3: Dog liver in group 1 showing focal coagulative necrosis in hepatocytes (N). x40

Fig. 4: Dog liver in group 1 showing focal necrosis infiltrated by mononuclear leucocytes inflammatory cells (M) in between the fatty changed hepatocytes (F). (H&E x64)

Fig. 5: Dog liver in group 1 showing fibrosis (FB) with oedema (O) replaced the hepatic parenchyma. (H&E x40).

**Plate 2: (Control, 2 & 3 Groups)**

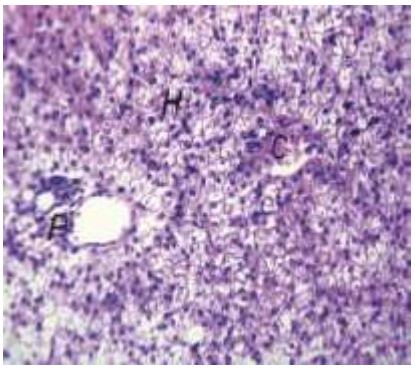
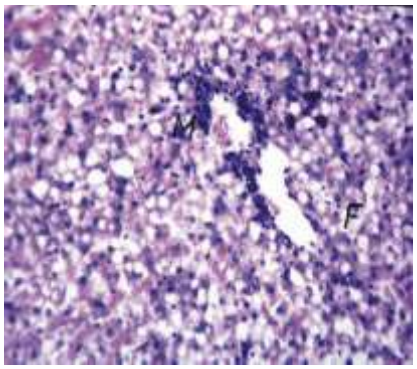
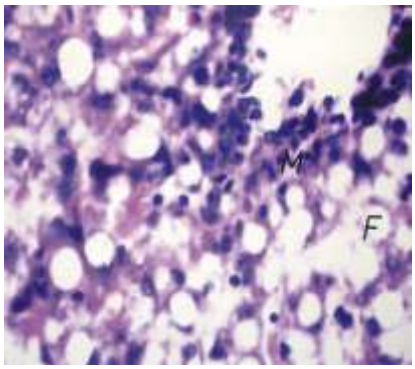
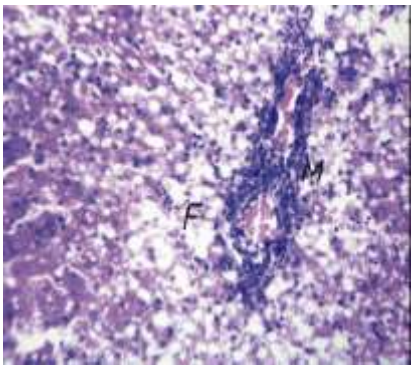
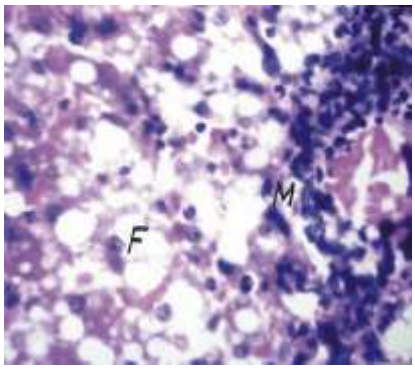
| Group | H&E- x64 | H&E- x160 |
|---|---|---|
| Control |  | |
| | **Fig.6** | |
| Prophlactic **(Gp-2)** |  |  |
| | **Fig. 7** | **Fig.8** |
| Curative **(Gp-3)** |  |  |
| | **Fig. 9** | **Fig. 10** |

Fig. 6: Dog liver in control group showing hydropic degeneration with normal structure of the central vein (C), portal area (P) and surrounding hepatocytes (H). H&E x64.

Fig. 7: Dog liver in group 2 showing mononuclear leucocytes inflammatory cells (M) surrounding the central vein with fatty change in adjacent hepatocytes (F). x64.

Fig. 8: Magnification of fig. 7 to identify in flammatory cells infiltration surrounding the central vein (M) and fatty change in adjacent hepatocytes. H&E x160.

Fig. 9: Dog liver in group 3 showing massive number of mononuclear leucocytes inflammatory cells (M) surrounding the dilated central vein (M) with fatty change in adjacent hepatocytes (F). H&E x64.

Fig. 10: Dog liver in group 3 showing the magnification of fig. 9 to identify flammatory cells infiltration surrounding the central vein (M) with fatty change in adjacent hepatocytes (F). H&E x160.

Table 3: Histopathological assessment of protective effect of dates and licorice extracts on carbon tetrachloride- induced hepatotoxicity in dogs

| $CCl_4$ (GP1) | Prophylactic (Gp2) | Curative (Gp3) |
|---|---|---|
| | *Fatty change in hepatocytes* | |
| ++++ | ++ | ++ |
| | Inflammatory cells infiltration surrounding central vein | |
| +++ | + | ++ |
| | Focal necrosis | |
| +++ | - | - |
| | Focal necrosis with inflammatory cells | |
| +++ | - | - |
| | Diffuse Inflammatory cells infiltration between the hepatocytes | |
| +++ | - | - |
| | Focal necrosis with edema | |
| ++ | - | - |

++++=very sever, +++= sever, ++=moderate, += mild, - = nil

the hepatocytes (Fig.1) and inflammatory cells infiltration in parenchyma (Fig.2). Focal coagulative necrosis was detected in the hepatic parenchyma with inflammatory cells infiltration (Fig. 3& 4). There was focal fibrosis with edema replacing the hepatic parenchyma (Fig.5- Plate 1). There were inflammatory cells infiltrations surrounding the central vein (Fig. 7), with fatty change in the surrounding hepatocytes in dogs of prophylactic group 2 (G. 2) (Fig. 8 - Plate 2). Massive number of inflammatory cells infiltration was detected surrounding the dilated central vein associated with fatty change in the adjacent hepatocytes in curative group (G.3) (Fig. 9,10- Plate-2). The summary of the histopathological assessment of protective effect of dates and licorice on carbon tetrachloride–induced hepatotoxicity in dogs was shown in Table 3. However, the protection offered by prophylactic administration of date and licorice extracts seemed relatively greater than curative one, which was reflected on blood marker enzymes and stoppage of focal fibrosis with edema replacing the hepatic parenchyma induces by $Ccl_4$.

## DISCUSSION

Administration of $CCl_4$ to normal dogs increased serum levels of ALP, AST and ALT. The enzymes leaking out from damaged liver cells into circulating blood. The hepatotoxic effects of $CCl_4$ are attributed to its metabolism by $P_{450}$ to yield toxic trichloromethyl radicals ($CCl_3$) or trichloroperoxyl radical ($CCl_3O_3$) that can act as free radical initiators [27]. These radicals are believed to induce injury either by interacting with the unsaturated fatty acids of cell membranes, there by causing lipid peroxidation, or by binding covalently to important macromolecules such as proteins, lipids, or DNA [28,29].

In our study, the synergistic effect of date and licorice extracts caused significant decline of the liver marker enzymes in both prophylactic (G. 2) and curative (G. 3) groups against $CCl_4$ toxicity owing to the following facts.

The protective effect of many plant extracts against $CCl_4$ may be attributed to the presence of flavonoids, tannins and steroids [30, 31]. Flavonoids are known to be antioxidants, free radical scavengers and anti lipoperoxidants leading to hepatoprotection [22].

Phytochemical studies on date palm recorded that the fruits contain carbohydrates, alkaloids, steroids, flavonoids, vitamins and tannins [32, 19]. HONG *et al*. [33] were identified 13 flavonoid glycosides in the date palm (*Phoenix dactylifera*) fruits.

Licorice contains flavonoids and pentacyclic triterpene saponins (liquiritigenin, liquiritin and glycyrrhizin). Glycyrrhizin is the major constituent and comprises 4% to 13% of the dried root weight [34]. Glycyrrhizin one of hepatoprotective compound against CCl4-induced liver injury in rats [35]. 18ß-Glycyrrhetinic acid (found in glycyrrhizin) is also a potent hepatoprotective compound [36].

These findings can be further confirmed with histopathological studies. The groups of dogs (G.2 and G.3) treated with date and licorice extracts showed no necrosis or edema and less inflammatory reaction relative to untreated one (G.1). This suggests the reparative quality and maintenance of structural integrity of hepatocytic cell membrane of damaged liver cells by the extracts. Furthermore, the synergistic effects of date and licorice have the power to stop the adverse and unfavorable effects induce by $CCl_4$. Identical histopathological findings were reported by Al-Qarawi *et al*. [12] and [22] in rat liver injury models.

In conclusion, the daily oral consumption of an aqueous date palm and licorice extract possess hepatoprotective activity which may be due to its antioxidant effect. This article tries to explore the safety, efficacy and inexpensive co-supplement herbal extract in the treatment of liver disorders which require prolonged therapy. Hence these synergistic effects of date and licorice extract can be used alone and/or in polyherbal formulations of hepatoprotective drugs, thereby preventing the process of initiation and progress of hepatocellular diseases.

## REFERENCES


1. Krentz, A.J. and C.J. Bailey, 2005. Oral antidiabetic agents: current role in type 2 diabetes mellitus. Drugs, 65: 385-411.
2. Gupta, Y.K. and S. Briyal, 2004. Animal models of cerebral ischemia for evaluation of drugs. Indian J. Physiol Pharmacol., 48: 379-394.
3. Amadou, C.K., 1998. Promoting alternative medicine. Africa Health J., 2: 20-25.
4. Nadeem, M., P.C. Dandiya, K.V. Pasha, M. Imran, D.K. Balani and S.B. Vohora, 1996. Hepatoprotective activity of some herbal formulations available in India. Indian Drugs, 33: 390-396.
5. Harsh, M., 2002. The liver, biliary tract and exocrine pancreas. In: Text book of pathology, 4th Ed, Jaypee Brothers Medical Publishers (P) Ltd. New Delhi. 22-24 and 569-630.
6. Lee, M.K., H. Yeo, J. Kim and Y.C.J. Kim, 2000. Protection of rat hepatocytes exposed to $CCL_4$ *in vitro* by cynandione A, a biacetophenone from Cynanchum wilfordii. J. Pharm. Pharmacol., 52(3): 341-345.
7. Joseph, E., J.R. Pizzorno and T.M. Michael, 1999. Textbook of Natural Medicine. 2nd Ed. Harcourt Publishers Limited. pp: 768.
8. Novaretti, R. and D. Lemordant, 1990. Plants in the traditional medicine of the Ubaye Valley. J. Ethanopharmacol., 30(1): 1-34.
9. Dafni, A., Z. Yaniv and D. Palevitch, 1984. Ethnobotanical survey of medicinal plants in Northern Israel. J. Ethanopharmaco., 10(3): 295-310.
10. Acharya, S.K., S. Dasarathy, A.Tandon, Y.K. Joshi and B.N. Tandon, 1993. A preliminary open trial on interferon stimulator (SNMC) derived from Glycyrrhiza glabra in the treatment of subacute hepatic failure. Indian J. Med. Res., 98: 69-74.
11. Luper, S., 1999. A review of plants used in the treatment of liver disease: part two. Altern Med. Rev., 4(3): 178-188.
12. Al-Qarawi, A.A., H.A. Abdel-Rahman and S.A. El-Mougy, 2001. Hepatoprotective activity of licorice in rat liver injury models. Journal of Herbs, Spices & Medicinal Plants, 8(1): 7-14.
13. Taniguchi, C., M. Homma, O. Takano, T. Hirano, K. Oka, Y. Aoyagi, T. Niitsuma and T. Hayashi, 2000. Pharmacological effects of urinary products obtained after treatment with saiboku-to, an herbal medicine for bronchial asthma, on type IV allergic reaction. Planta Med., 66(7): 607-611.
14. Azimov, M.M, U.B. Zakirov and Sh.D. Radzhapova, 1988. Pharmacological study of the anti-inflammatory agent glyderinine]" Farmakol Toksikol., 51(4): 90-93.
15. Nakagawa, K., H. Kishida, N. Arai, T. Nishiyama and T. Mae, 2004. Licorice flavonoids suppress abdominal fat accumulation and increase in blood glucose level in obese diabetic KK-A(y) mice. Biol. Pharm. Bull., 27(11): 1775-1778.
16. Craig, W. and L. Beck, 1999. Phytochemicals: Health Protective Effects. Can J Diet Pract Res., 60(2): 78-84.
17. Agarwal, R., Z.Y. Wang and H. Mukhtar, 1991. Inhibition of mouse skin tumor-initiating activity of DMBA by chronic oral feeding of glycyrrhizin in drinking water. Nutr Cancer., 15(3-4): 187-93.
18. Copley, M.S., P.J. Rose, A. Clampham, D.N. Edwards, M.C. Horton and R.P. Evershed, 2001. Detection of palm fruit lipids in archaeological pottery from Qasr Ibrim, Egyptian Nubia. Proceedings of the Royal Society, London, 268: 593-597.
19. Biglari, F., A.F.M. AlKarkhi and M.E. Azhar, 2008. Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. Food Chemistry, 107: 1636-1641.
20. Nadkarni, K.M., 1976. Indian Materia Medica, 1st ed. Vol 1. Mumbai: Bombay popular prakashan Pvt. Ltd.
21. Barh, D. and B.C. Mazumdar, 2008. Comparative nutritive values of palm saps before and after their partial fermentation and effective use of wild date (*Phoenix sylvestris* Roxb.) sap in treatment of anemia. Research Journal of Medicine and Medical Sciences, 3: 173-176.
22. Al-Qarawi, A.A., H.A. Abdel-Rahman, B.H. Ali, H.M. Mousa and S.A. El-Mougy, 2004. Protective effect of extracts from dates (*Phoenix dactylifera* L.) on carbon tetrachloride-induced hepatotoxicity in rats. Intern J. Appl. Res. Vet. Med., 2(3): 176-180.

23. Reagan-Shaw, S., M. Nihal and A. Ahmad, 2007. Dose translation from animal to human studies. The FASEB Journal, (2): 659-661.
24. Banchroft, J.D., A. Stevens and D.R. Turner, 1996. Theory and Practice of Histological Techniques. 4thEd. Churchil Livingstone, New York, London, San Francisco, Tokyo.
25. Valeer, J.D., 2003. Liver tissue examination, Journal of Hepatology, 39: S43-S49.
26. SPSS® for WindowsTM 1996. Version 7.5, Copyright SPSS Inc.
27. Johnston, D.E. and C. Kroening, 1998. Mechanism of early carbon tetrachloride toxicity in cultured rat hepatocytes. Pharmacol Toxicol., 83: 231-239.
28. Yasuda, H., N. Izumi, O. Shimada, T. Kobayakawa and M. Nakanishi, 1980. The protective effect of tinoridine against carbon tetrachloride hepatotoxicity. Toxicol Appl Pharmacol., 52: 407-413.
29. Slater, T.F., 1984. Free-radical mechanisms in tissue injury. Biochem J., 222: 1-15.
30. Gilani, A.H. and K.H. Janbaz, 1995. Preventive and curative effects of Artemisia absinthium on acetaminophen and $CCl_4$-induced hepatotoxicity. Gen. Pharmacol., 26: 309-315.
31. Giulia, D.C., N. Nicola, A.I. Angelo and C. Fracesco, 1999. Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life sciences, 65(4): 337-353.
32. Dowson, V.H.W., 1982. Date production and protection. FAO Plant Production and Protection Paper No. 35. Food and Agriculture Organization of the United Nations.
33. Hong, Y.J., F.A. Tomas-Barberan, A.A. Kader and A.E. Mitchell, 2006. The Flavonoid Glycosides And Procyanidin Composition of Deglet Noor Dates (*Phoenix dactylifera*). J. Agric. Food Chem., 54: 2405-2411.
34. Wang, Z.Y. and D.W. Nixon, 2001. Licorice and cancer. Nutr. Cancer, 39: 1-11.
35. Gumpricht, E., R. Dahl, M.W. Devereaux and R.J. Sokol, 2005. Licorice compounds glycyrrhizin and 18beta-glycyrrhetinic acid are potent modulators of bile acid-induced cytotoxicity in rat hepatocytes. J. Biol Chem., 280: 10556-10563.
36. Nose M.M. Ito, K. Kamimira, M. Shimizu and Y.A. Ogihara, 1994. Comparison of the antihepatotoxic activity between glycyrrhizin and glycyrrhetinic acid. Planta Med., 60: 136-139.