Hepatoprotective Activity of Date Palm (*Phoenix dactylifera*) Pollen Grains in Rats

By

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بسم الله الرحمن الرحيم

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صدق الله العظيم

مريم (الاية *25*)

Dedication

I dedicate this

work.....

to my beloved....

Mother...

who suffered..

a lot with me.

Itimad

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Firstly, my heartfelt thanks to Almightily Allah for giving me strength and patience to perform this study.

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ABSTRACT

Hepatoprotective Activity of Dates Palm (*Phoenix dactylifera*) Pollen Grains in Rats

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The present study was carried out to investigate hepatoprotective effect of Sudanese dates palm (*Phoenix dactylifera Linn*) pollen grains powder (produced from Elgolid Locality, Northern State of Sudan) on experimental albino rats.

No systemic studies on its hepatoprotective activity have been reported before. The study was carried for (five) days on albino rats in which hepatptoxicty was achieved by injecting carbon tetrachloride (CCl_4) subcutaneously (3ml/kg body weight) which dissolved in equal preparation with olive oil 1:1 on the third day for all groups except group1(the control).

Date palm pollen grains powder dissolved in distilled water and orally administered to rats at dose rates of 250 and 500 mg/kg body weight for five days, to groups 3 and 4 respectively, group 2 served as CCl_4 control, Silymarin was given orally to group 5 at dose rate of 50 mg/kg body weight as reference drug for 5 days.

Date palm pollen grains powder showed significant ($P< 0.05$) hepatoprotective activity which indicated by decreased of alanine amino transferase(ALT), aspartate amino tranferase (AST) and alkaline phosphatase (ALP) of treated groups when compared with CCl_4

control which showed higher levels of these enzymes, these results correlated with histopathological changes.

The toxicity study of date palm pollen grains powder was also performed on rats at doses of 500,1000 and 2000 mg/kg body weight orally for seven days which revealed that date palm pollen grains powder had no adverse effect, but mild changes in liver cells when examined microscopically in the groups that received 1000 and 2000 mg/kg body weight.

ملخص الأطروحة

الأثر الواقى لحبوب لقاح شجرة النخيل على الكبد فى فئران التجارب

بواسطة

إعتماد أبو عوف أحمد أبو عوف

في هذه الأطروحة، تمت دراسة الأثر الواقي علي الكبد لبدرة حبوب لقاح شجرة النخيل التي تم جلبها من محلية القولد الولاية الشمالية، لم تتم دراسة الأثر الواقي للكبد لهذا الجزء من النبات من قبل.

أجريت الدراسة لمدة خمسة أيام في الجرذان البيضاء معطوبة الكبد بواسطة حقنها تحت الجلد بمادة رابع كلوريد الكربون المذاب في زيت الزيتون بنسبة 1:1 في اليوم الثالث لكل المجموعات ما عدا المجموعة الأولي، بجرعة 3 ملجم/ كجم وزن حي.

عند إعطاء بدرة حبوب لقاح النخيل المذابة في الماء المقطر بواسطة التجريع ألفموي بجرعتي 250 و500 ملجم/ كلحم وزن حي لمدة خمسة أيام، تم ملاحظة انخفاض ($P<0.05$) نشاط إنزيمات ALT,ASTوALP وتركيز البروتين مقارنة مع المجموعة التي أعطيت رابع كلوريد الكربون فقط مع تغيير واضح في خلايا الكبد يشمل موت الخلايا والتغير الدهني في مجموعة رابع كلوريد الكربون.

أثبتت هذه الدراسة أن بدرة حبوب لقاح النخيل المذابة في الماء المقطر لها أثر واقي للكبد لأنها أحدثت انخفاضا ($P<0.05$) في إنزيمات الكبد عند تجريعها للجرذان معطوبة الكبد.

تمت دراسة الأثر السمي لبدرة حبوب شجرة نبات النخيل في الجرذان البيضاء بجرعات 1000,500 و2000ملجم/ كلحم وزن حي لمدة سبعة أيام، وأثبتت الدراسة أن بدرة حبوب لقاح شجرة النخيل ليس لها أثر سمي واضح، بل هنالك تغيير طفيف في خلايا الكبد عند فحصها مجهريا، في المجموعات التي أعطيت جرعتي 1000و2000 ملجم/ كلحم وزن حي.

INTRODUCTION

Knowledge about herbs that have curative or preventive effect of disease conditions has been transmitted from one generation to another by experienced elders and by tribal herbal specialists.

The disorders associated with the liver are numerous and varied because it is the key organ for metabolism. Many Sudanese plants are used by traditional healers for treatment of jaundice and liver disorders. *Phoenix dactylifera Linn* has been used in Middle East as staple food. The palm family is a symbol of prosperity and love to Muslims and its legend dates back to Judeo- Christian mythology (Al-Qarawi *et al.,* 2004). In local practices dates are considered a tonic, some consider it to be aphrodisiac, the flower of the plant is used as purgative (Zohget and Elsheikh, 2000).

Many Middle Easterners believe that consumption of dates particularly in the morning on empty stomach can reverse the action of any toxic material that the subject may have been exposed to. Therefore, studies done to assess the ability of date flesh and pits to treat or prevent some of the toxic actions of carbon tetrachloride (CCl_4) on the liver of rats, and their hepatoprotection was proved to CCl_4-induced toxicity in rats (Al-Qarawi *et al.,* 2004).

Experimentally, date extract had been shown to increase sperm and increase stimulating concentration of testosterone count in Guinea pigs and to enhance spermatogenesis, follicle hormone (FSH) and Lueteinizing hormone (LH) in rats. Date pits were known to enhance growth, an action that has been ascribed to increase in the plasma level of estrogen (Elgasim *et al*., 1995) or testosterone (Ali *et al*., 1999).

The early Egyptians and ancient Chinese used Date palm pollen grains as a rejuvenating medicinal agent, it was called “Fountain of Youth”. Pollen preparations distributed wide world for dietary purposes and used as dietary supplement by increasing the total dietary intake (Kroyer and Hegedus, 2001).They are good sources of protein, amino acids, vitamins, dietary fiber, fatty acids, enzymes, hormones and minerals (Alferez and Campos, 2000).

The objectives of this study were: to evaluate the hepatoprotective activity of date palm (*Phoenix dactylifera L.*) pollen grains on rats and to evaluate the toxicity of date palm (*Phoenix dactylifera L.*) pollen grains on rats.

CHAPTER ONE
LITERATURE REVIEW

1.1 The liver

Liver is the largest gland in the body that performs functions that essential for life and it has enormous functional reserve. The main sign of liver damage is jaundice, which is a clinical sign and often arises in diseases of liver and billiary system, but also in diseases in which there are no lesion of these organs. It is a result of accumulation of billirubin. The staining is more pronounced with direct billirubin than with indirect billirubin.

For the importance of the liver and its disease susceptibility, many plants were used in folk medicine to protect the liver. There are about 600 commercial therapeutic preparations against hepatitis alone (Ortomans, 1979).

1.2 Plants with hepatoprotective activity

Several species of plants have been reported to have hepatoprotective effect. For example, aqueous extract of roots of *Cochospermum tincorium* used in treatment of liver complains in man in Senegal and West Africa (Sere *et al*., 1984). In rats two traditional medicinal plants namely *Crepst ruppelli* and *Anisote trisulcus* recorded for their hepatoprotective action against CCl_4-induced hepatitis. The leaf extract of equal parts of each in mixture given at 200 mg/kg body weight showed significance hepatoprotective activity and used for liver disorders and gall stones (Fleurentin *et al*., 1986).

Alcoholic extract of whole plant of *Boerhavia diffusa* exhibited hepatoprotective activity when given to rats and mice orally after induction of hepatotoxicity by CCl_4 and the extract increase in normal bile flow in rats (Chandan *et al*., 1991).

Hepatoprotective activity of Hepatogard tablets were studied in rats (Rao *et al*., 1993). Hepatogard (650 mg/kg) is composed of crude powder of plants *Picrorhiza kurra*, *Andrographis paniculata*, *Phyllanthus amanus, Boerharvia indica, Azadirachta indica*, *Ectipta alba*, *Zingiber officinalis* and *Piper longum*. The tablets exhibited hepatoprotective activity and reversed the biochemical and histological changes induced by CCl_4.

In Turkey ethanolic extract of seven plants were used as hepatoprotective plants, these plants are *Carduns acanthoides*, *C. nutans*, *Cichorium intybus*, *Fumaria aseplae*, *F. vailantii*, *Gentiana olivieri* and *Platago lanceolatl* (Aktay *et al*., 2000).

Ahmed *et al*. (2002) tested different extracts of *Apium graveolens* and *Croton oblongifolius* for their hepatoprotective activity against CCl_4-induced hepatotoxicity in albino rats, The methanolic extract showed the most significant hepatoprotective activity compared with the standard drug silymarin and other extracts of petroleum ether and acetone also exhibited a potent activity.

In Jordian, folk medicine hepatoprotective effect of boiled and non-boiled aqueous extracts of *Pistacia lentiscus*, *Phillyrea latifolia* and *Nioctiana glauca* showed their effectiveness in the treatment of jaundice which was evaluated in vivo using CCl_4-intoxicated rats as experimental model. Plants extracts administered orally at dose rate of 4 ml/kg body

weight. The effect of boiled extract was more pronounced than that of the non-boiled extract (Janakat and Al- Merie, 2002). Also significant hepatoprotective effect was obtained against CCl_4, exhibited potent activity.

1.3 Use of Date palm (*Phoenix dactylifera Linn*) as Hepatoprotective and its parts

Phoenix dactylifera L. belonging to family *Arecaceae*, called "Nakhla" genus *Phoenix* that consist of 12 species native to tropical Asia and Africa.

1.3.1 Taxonomy

According to Wealth of India 1985:

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Liliopsida*

Order: *Arecales*

Family: *Arecaceae*

Genus: *Phoenix*

Species: *Phoenix dactylifera*

Binomial name: *Phoenix dactylifera Linn*

1.3.2 Distribution

Arab countries lay within the date palm latitude 16-27˚N, producing 80% of total world date production. Sudan is one of the Arab countries that cultivate date palm trees and rank fifth in production among Arab date producing countries and third in Africa (AOAD, 1998)

and date cultivation has been confined to Northern and River Nile States of Sudan.

1.3.3 Description

The date palm (*Phoenix dactylifera L.*) is medium-sized tree with pinnate leaves, containing about 150 leaflets having spines on the petiole. There are male and female date palm trees; hermaphrodism is rare (Mason, 1915). The female inflorescence carries about 800-1000 tiny whitish flowers and more flowers are usually carried by the male inflorescence (Candler, 1958).

Female flowers are fertilized by the pollen grains of the male producing a fruit with one seed called date.

1.3.4 Phytochemistry

Phytochemically, the whole plant (namely shoot tip, pollen grains, leaves, fruits, callus, embryogenesis and invitro leaf tissue) contains carbohydrates, alkaloids, steroids, flavonoids, vitamins and tannins. Four phenolic acids and nine bound phenolic acids were tentatively identified (Ziouti *et al*., 1996; Eong *et al*., 2006).

Dates contain at least six vitamins including small amount of vitamin C, vitamin B_1 (thiamine), B_2 (riboflavin), nicotinic acid (niacin) and vitamin A (Al-Shahib and Marshall,1993). Enzymes such as phytase, invertase and peroxidase have been isolated in dates (Nadkarni, 1976). Presence of estrone has been reported in the dried date seeds and pollens (Heftmann and Bennet, 1965).

The date palm pollen grains showed the presence of alpha-amirin, triterpenoids, saponins and a crude gonadotrphic substance (Mahran *et al*., 1976).

1.4 Medicinal uses of *Phoenix dactylifera L.* fruits

1.4.1 Effect on the gastrointestinal tract

Pretreatment with date fruit ethanolic and aqueous extracts at a dose rate of 4 mg/kg body weight for 14 days markedly ameliorated the ulcer index, histopathological indices such as necrosis, haemorrhage, congestion and oedema in stomach sections and biochemical levels of some enzymes such as gastrin in plasma, mucin and histamine in gastric mucosae of ethanol-induced gastric ulceration in rats (Al-Qarawi *et al*., 2005).

Phoenix dactylifera L. spathe aqueous extract at dose rates of 3, 6 and 12 mg/kg body weight produced a satisfactory significant reduction in both caster oil-induced intestinal transit and frequency of diarrhea in rats (Abdalla and Al- Taher, 2008).

Water and ethanolic extracts from date flesh and date pits at doses of 0.01, 0.02, 0.04 ml/kg body weight showed a dose dependant increase in the gastrointestinal transit time, while water extract from dialyzed date flesh extract induced a dose-dependant decrease in the gastrointestinal transit time, the possible reason for this may be the method based on extraction of particular component which could be valuable towards respective clinical conditions (Al-Qarawi *et al*., 2003).

1.4.2 Anti-inflammatory activity

Oral administration of the methanolic and aqueous extracts of edible portion of date fruits suppressed the swelling in the foot significantly by 67.8 and 61.3% respectively, while the methanolic extract of date seeds showed significant reduction by 35.5% in adjuvant arthritis in rats by mechanistically reducing plasma fibrinogen and normalizing the plasma level of antioxidants. Adminstration of the extracts also produced significant increase in body weight gain and food efficiency ratio (Mohammed and Okbi, 2004).

1.4.3 Nephroprotective activity

Extracts of date flesh and pits significantly reduced the increase in plasma creatinine and urea concentrations induced by gentamycin nephrotoxicity and ameliorated the proximal tubular damage. Antioxidant components in the date (e.g. melatonin, vitamin E and ascorbic acid) were suggested to be the basis of the nephroprotection (Al-Qarawi *et al*., 2008).

1.4.4 Anti-oxidant activity

Phytochemicals from fruits have been shown to possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of degenerative disease in human (Javanmardi *et al*., 2003). Studies conducted on antioxidant activity and phenolic content of various fruits of *Phoenix dactylifera L.* demonstrated a linear relationship between antioxidant activity and the total phenolic content of date fruit extract (Alliath and Abdalla, 2005).

1.4.5 Invitro antiviral activity

The crude acetone extract of date palm pits at doses of 100 and 1000 mg/kg body weight showed a rapid, strong and dose dependant ability to inhibit the infectivity of *Pseudomonas* phage to *Pseudomonas aeruginosa* by binding to the phage and completely prevented bacterial lyses, as evidenced by the presence of higher numbers of *Pseudomonas aeruginosa* cells surviving. These results suggested that the pit extract of *Phoenix dactylifera L*. fruit could play an important role in controlling the replication of the Human Immuno Deficiency Virus (HIV-1) by a novel mechanism of interaction with binding of the phage to the host bacterium and injection of its genome which demonstrate its use in the treatment of Acquired Immune Deficiency Syndrome (AIDS) (Burgoyne and Tan, 2008).

1.4.6 Effect on hemolytic activity of *Streptococcus pyogenes*

Phoenix dactylifera L. fruit extract at 5, 10 and 20% dilution when incubated with *Streptococcus pyogenes* for 24 hours effectively slowed the growth of *Streptococcus pyogenes* to 30.8, 64.7 and 88.5% respectively. Date extract neutralized the hemolytic activity of the streptococcal exotoxin, streptolysin O and 96% inhibition was obtained at a very low concentration (1:262144 date extract dilution) (Sabah *et al*., 2007).

1.4.7 Anti-hyperlipidemic activity

Phoenix dactylifera L. fruit pulp was evaluated for its effect on the lipid metabolism in high cholesterol diet induced hypercholesterolemic in hamsters where it significantly reduced the

elevated levels of plasma lipids including cholesterol, triglycerides and low density lipoprotein (LDL) in treated animals as compared to high cholesterol-diet supplemented animals including its possible beneficial effects in atherosclerosis development in human. It also significantly reduced the total body, liver and kidney weights of hamsters, which were increased by the high cholesterol diet suggesting its possible use in the treatment of obesity (Salah and Al-Maiman, 2005). In another report, the *Phoenix dactylifera L*. seed fibers (2.5%) supplemented with the basal diet significantly reduced the plasma triglyceride, LDL and total cholesterol levels and increased the high density lipoprotein (HDL) levels in the rats (Al-Mougy *et al*., 1991).This may be of particular importance in occurrence of coronary heart disease which is strongly related to decreased HDL, cholesterol concentration and increased LDL, cholesterol concentrations.

1.5 Hepatoprotective activity

Pre and post treatment with the aqueous extract of date flesh or pits significantly reduced CCl_4-induced elevation in plasma activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) enzymes and billirubin concentration and ameliorated morphological and histological liver damage in rats. This data suggests that CCl_4-induced liver damage in rats can be reversed by treatment of extracts from date flesh or pits and moreover it can also be used prophylactically as a dynamic liver support (Al-Qarawi *et al*., 2004)

1.6 Effect on reproductive system

Oral administration of date palm suspension at doses of 120 and 240 mg/kg body weight improved the sperm count, morphology and DNA quality with a concomitant increase in the weights of testis and epididymis (Bahmanpours *et al*., 2006), moreover date extracts have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in rats (Elgasim *et al*., 1995). This study suggested its usefulness in solving infertility problems in males.

1.7 Medicinal uses of date palm pollen grains

Pollen grains applications and uses in traditional and herbal medicine have been recorded throughout history. They have been used in the treatment of sexual incapacity and weakness in the Arab world (El-Desoky *et al*., 1995). The date palm pollen grains have been reported to be used directly for human consumption. A variety of pollen containing food products such as candy and chocolate bars are commercially available in health food stores in the Western world (Stanly and Linkens, 1974).

Hassan and Abou-Elwafa (1947) detected a non crystalline estrogenic substance in the extract of date palm pollen grains. El-Ridi and Abou-Elwafa (1950) and El-Ridi *et al*., (1953) studied rutin content in date pollen grains and isolated as crude gonadotrophical active substance. Bennett and Heftman, (1966) detected and isolated esterone and cholesterol from date palm pollen grains.

In an other study, Wojcicki *et al*. (1987) recorde the antioxidant and hypolipedemic effects of pollen extracts in male rabbits and Wister rats. They demonstrated the reduction of total cholesterol and triglyceride count under the influence of cernitins, indicating their antioxidant properties.

Wojcicki *et al*. (1986) investigated the influence of pollen extract on experimental atherosclerosis in rabbits for over a period of 12 weeks. Significant reduction in lipid metabolism profile in the degree of the plaque formation occurred after the pollen extract was administered. The total cholesterol content in serum and liver homogenate was significantly decreased by 67% and 45% respectively, while serum high lipoprotein cholesterol and alpha-lipoprotein levels were increased by 19% and 14% respectively. Atherosclerotic plaque intensity at 12 weeks, measured planomerically, averaged 85% in high fat diet fed animals versus 33.7% in pollen extracts treated rabbits. Their findings suggested that pollen extracts not only significantly lowered the serum lipid content of the experimental animals, but also modified deposition in major arteries. Earlier in 1985 the same investigators recorded the protective effects of pollen extracts against the allyl alcohol damage of liver. They indicated that pollen extracts significantly reduced the serum glutamate oxaloacetate (GOT) and glutamate pyruvate tranminase (GPT) activities and the allyl alcohol induced alkaline phosphatase (ALP) elevation. Zhao *et al*. (1990) isolated several components from typhae pollen grains and their structures and biochemical effects.

El-Desokey *et al*. (1995) reported the effect of date palm (*Phoenix dactylifera L*.) pollen grains on sexual hormonal balance, cholesterol, total lipids, albumin, globulin and liver functions in control male and female and in castrated and ovariectomized rats. Their findings showed a decline in serum testosterone levels in control male rats, but a slight increase was detected in the ovariectomized and castrated rats. Similarly, serum estradiol content was elevated in both control and ovariectomized rats. Progesterone level, however, decreased in control female rats, with slight increase of serum follicle stimulating hormone (FSH) and luteinizing hormone and (LH) in both normal and ovariectomized female rats. Pollen grains significantly increased serum globulin, total protein and total lipids in ovariectomized rats. Also serum alkaline phosphatase (ALP) activity was increased in normal male rats. There was an increase in serum plasma glutamate pyruvate (GPT) activity in normal male, ovariectomized female and castrated male rats and similarly, glutamate oxaloacetate transaminase (GOT) activity was also increased in ovariectomized female and normal male rats. All these GPT and GOT values were still within the normal ranges in rats.

Reshod and Shagrawi (1998) studied the effects of pollen grains on lipid fraction, total lipid, triglyceride, total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol of plasma, liver and brain of rats as well as fatty acid composition and the activities of liver function enzymes (plasma glutamate pyruvate, glutamate oxaloacetate transaminase, lactate dehydrogenase, alkaline phosphatase and γ-glutamyl transferase) tested in male rats, fed synthetic diet which were supplemented with different concentrations of date palm pollen

grains (0.0, 2.0 and 4.0%) for 35 days. There were significant reduction in plasma total lipid by 39.1% and 39.86%, triglyceride by 6.9% and 41.8% and low density lipoprotein cholesterol by 54.7% and 21.8% in rats consumed modified diet containing 2.0% and 4.0% date pollen grains respectively. However there was significant elevation in plasma high density lipoprotein cholesterol of treated rats compared to the control. Lipid fractions of the liver and brain in treated rats were also significantly lowered compared to the control. The liver function enzymes activities were significantly reduced in treated rats. The percentage of stearic acid, arachidic acid and lignoceric acid showed significant elevation in rats which consumed 2% and 4% pollen grains in the diets. The study showed that date palm pollen grains play a role in lowering lipid fractions and protect the liver by maintainace of liver function enzyme activities.

1.7.1 Effect on cisplatin- induced genotoxicity

Aqueous extract of pollen grains administration by oral route to mice at doses of 250 and 500 mg/kg body weight significantly inhibited the cisplatin-induced genotoxicity. At histological level, significant recovery of the testis histology was observed in animals administered with pollen grains prior to cisplatin treatment.

Furthermore, administration of the pollen grains extract caused a decrease in epididymal sperm with tail abnormalities that would interfer with sperm motility and the highest dose retained normal epididymal sperm number. These findings suggested the preventive role of pollen

grains against the chemotherapeutic-induced infertility in males (Al-Kharage and Rokaya, 1982).

1.7.2 The gonadotrophic activity of date palm pollen grains

It is commonly claimed in Egypt that pollen grains of date palm (*Phoenix dactylifera L.*) are effective in the treatment of both male and female infertility. Previous investigations by Hassan and Abu El-Waffa (1947) indicated the presence of estrogenic activity in this type of pollen grains. Other investigations were able to isolate substance of plant origin with a gonad stimulating capacity (Friedman and Friedman, 1939).

The estrogenic and androgenic effects are function of LH (Frevold and Simpsox, 1957). The gonadotrophic activity of pollen grains seemed to be mainly of a follicle stimulating nature. When an extract obtained from 100 g pollen was injected intravenously into immature female rabbits, it resulted in an increase in the number of Graffian follicles which were at different stages of development. It caused the maturation of the follicles and some of them were atretic, but ovulation did not taked place.

1.8 Clinical studies on date (*Phoenix dactylifera L.*)

Partysmart is a herbal formulation containing date fruit powder as one of the ingredients. It was evaluated for its effect on blood and urinary levels of alcohol and acetaldehyde after alcohol ingestion. It is safety as well as efficacy values in the prevention of the alcohol-induced hangover symptoms in human volunteers in a prospective, randomized, double-blind, comparative, crossover clinical trial. In this study, the formulation significantly reduced the mean hangover score along with

the blood levels of alcohol and acetaldehyde without producing any clinically significant adverse effects providing a safe and effective novel herbal composition for the prevention of hangover syndromes and liver disorders in acute and chronic alcoholics (Thornfeldt *et al*., 2006).

A cosmeceutical composition containing alcoholic extract of *Phoenix dactylifera L*. fruit and seeds showed a significant wrinkles, toughness, fine lines, mottled hyper pigmentation, inflammatory papules and pustules, lesions, in the skin of various groups of patients suffering from different types of mucocutaneous skin disorders such as visible cheek in nonhypertrophic actinic keratoses, sebaceous hyperplasia, premalignant keratoses, acne vulgaris, extrinsic aging in controlled clinical studies indicating its ability to compensate the great need for new and effective treatment products for age and environmental insult related skin changes, disorders and diseases. Furthermore, kernel extract of *Phoenix dactylifera L*. fruit with clinically proven anti-wrinkle efficacy and free radical scavenging properties is being induced as an ingredient of D'Orientine formulation which is used to protect the skin from environmental sources of aging and wrinkling (WIPO 2006).

A placebo-controlled clinical trial with 5% date pulp versus placebo in 10 patients was applied to the eyelid area twice daily for 5 weeks. A statistically significant reduction in wrinkled surface (27.6%) and wrinkle depth was achieved. Six of the participants said that visual improvement occurred. Moreover, *Phoenix dactylifera L*. is one of the ingredients of Epionce Renewal Eye Cream and Epionce Renewal Facial Cream which is applied to protect against signs of aging by the virtue of its antioxidant and soothing properties (HHP 2007).

1.9 Medicinal applications of *Phoenix dactylifera L.*

The water in which fresh dates are soaked for a while is a drink given to relieve alcohol intoxication. The milk in which clean and fresh dates are infused is a very nourishing and restorative drink to the children as well as adults especially during convalescence from fevers and smallpox. The sweet pulp of the date fruits is useful in dysentery. Date fruits are used as an ingredient in various aphrodisiac and tonic confections; they are also useful in asthma. Paste made from ground seeds is said to be applied for opacity of cornea and to the head to relieve headache. The smoke produced from burning of the date seeds in powder form is a useful fumigatory for piles. Some doctor's advice dates as consumptives as they promote expectoration, soothe the chest and prevent constipation (WOI 1985). Seeds roasted and ground into powder make a beverage like coffee called "date coffee". A gum or juice obtained from the stem and named "laghi" is used as a demulcent, diuretic and refrigerant in genitourinary affections.

The liquid distillate obtained from spathe of *Phoenix dactylifera L.* known as Maa Al-liqah or Maa Al-Tilal or water of the spathe, is believed to have certain medical uses as it relieves abdominal gases and pain especially after heavy meal and claimed to have anti-spasmodic activity (Nadkarni, 1976). The flower of the plant is used as a purgative (Zoghet and Alsheikh 2000). The pollen grains of date palm have been used in Egyptian local practice to improve fertility in women (Amin *et al*., 1969).

CHAPTER TWO
MATERIALS AND METHODS

2.1 Materials and experimental designs

Two experiments on rats were performed. The first experiment was run to determine the hepatoprotective activity of date palm pollen grains and the second experiment was done to study the toxicity of the same plant.

2.1.1 Hepatoprotective activity of date Palm (*Phoenix dactylifera L.*) pollen grains in rats:

2.1.1.1 Animals, housing and management

Twenty five Wister albino rats of either sex weighting from 85-105 gms were obtained from the Animal House, Faculty of Pharmacy, University of Khartoum. The rats were kept within the premises of the Institute of Medicinal and Aromatic Plants, National Centre for Research, Khartoum. The rats were allowed one week adaptation period. They were housed in cages and maintained in a room under standard environmental conditions and controlled temperature (22 ± 2˚C), relative humidity (60%) with free access to water and fed at 2.5 MCal and 20% crude protein rat formula feed. Rats were apparently healthy and they were identified by color tail marks.

2.1.1.2 Collection and preparation of pollen grains of *Phoenix dactylifera L.*

About 30 pods were freshly obtained from Elgolid Locality, Northern State, Sudan, during March 2009, when male pollen grains

were ripe. The pods were allowed to dry at room temperature and then powdered dissolved in distilled water for oral administration.

2.1.1.3 Administration of date palm pollen grains powder

At the end of the adaptation period, rats were divided randomly to 5 groups of 5 rats each. Group 1, the control, received only normal saline for 5 days. Group 2 the carbon tetrachloride (CCl_4) control, received CCl_4 by subcutaneous injection (3ml/kg body weight) as single dose on the third day. Group 3 received orally 250 mg/kg body weight of pollen grains watery solution for 5 days and liver damage was produced by injecting 3ml/kg body weight CCl_4 subcutaneous injection in equal volume of olive oil (1:1) on the third day. Group 4 received orally 500 mg/kg body weight pollen grains watery solution for 5 days and also liver damage was produced as with group 3 on the third day. Group 5 received standard drug Silymarin orally 50 mg/kg body weight for 5 days and liver damage was produced likewise to group 3on the third day.

2.1.1.4 Blood samples and parameters

Blood samples were obtained from the orbital plexus on the first day and on the last day for serum analysis and haematological examinations. Sera were analyzed for the activities of the alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST) and total protein and albumin concentrations. Haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cells (RBCs), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were estimated. The specimens of

livers were collected immediately after slaughter of animals and were fixed in 10% formal saline.

2.1.1.5 Toxicity of date palm (*Phoenix dactylifera*) pollen grains powder in rats

Twenty Wister albino rats of either sex weighting 80-115gm were brought from the Animal House Faculty of Pharmacy, University of Khartoum. The rats were kept in cages within the premises of the Institute of Medicinal and Aromatic Plants, National Centre for Research, Khartoum. They were given one week adaptation period. Rats maintained in a room under standard environmental conditions and controlled temperature (22 ± 2˚C) and relative humidity (60%) with free access to water and rat formula feed (2.5 MCal and 20% crude protein). Rats were apparently healthy and were identified by color tail mark.

2.1.1.5.1 Administration of the date palm pollen grains powder

The rats were weighted after the adaptation period and then divided randomly into 4 groups of 5 rats each. Group 1 (control), received orally only normal saline for 7 days. Group 2 received 500 mg/kg body weight pollen grains powder dissolved in distilled water orally for 7 days. Group 3 received 1000 mg/kg body weight pollen grains watery solution orally for 7 days. Group 4 received 2000 mg/kg body weight pollen grains watery solution orally for 7 days.

2.1.1.5.2 Blood samples and parameters

Blood samples were obtained from the orbital plexus on the first day and on the last day for serum analysis and haematological

examination. Sera were analyzed for the activities of alkaline phosphatase (ALP), alanine amino transferee (ALT). aspartate amino transferase (AST) and total proteins, albumin and creatinine. Haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cells (RBCs), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were estimated. Clinical signs and mortality rate were recorded; specimens of liver and kidney were collected immediately after slaughter of rats and were fixed in 10% formal saline for histopathology.

2.2 Methods

2.2.1Chemical methods used for determination of serum constituent

Blood samples were obtained from the orbital plexus or at slaughter of rats. The sera were separated by centrifugation at 3000 r.p.m. for 5 minutes in a Hettich EBA 35 centrifuge and stored at -20˚C until analyzed.

2.2.1.1 Alkaline phosphatase (ALP)

It is an optimized method according to the recommendation of Chemie (1972).

Principle

In alkaline medium serum alkaline phosphatase (ALP), splits p-nitro-phenyl phosphatase in the presence of Mg^{+2} ions, into p-nitro-phenol and phosphate. At the pH of the reaction p-nitro phenyl was colored yellow, the optical density measured in a Spectrophotometer (Jenway 6305 UV/VIS) at the wave length 405nm.

Reaction

P-nitro phenyl phosphate + H_2O → phosphate + p- nitro phenol

Calculation

U/I-2760 x A 405 nm/min. (A= the mean of sample absorbance reading).

2.2.1.2 Alanine amino transferase (ALT)

It is an enzymatic method, which measure alanine amino transferase (ALT) by monitoring the concentration of pyruvate hydrozone formed with 2-4 dinitrophenyl hydrazine.

Principle

The absorbance of the samples was read against the reagent blank, after 5 minutes at wave length 546 nm in Jenway 6305 UV/ VIS Spectrophotometer.

2.2.1.3 Aspartate amino transferase (AST)

It is an enzymatic method that measure aspartate amino transferase (AST) by monitoring the concentration of oxaloacetate hydrozone formed with 2-4 dinitrophenyl hydrazine.

Principle

The absorbance of the samples was read against the reagent blank after 5 minutes at wave length 546 nm in Jenway 6305 UV/ VIS Spectrophotometer and cuvette of 1 cm light path.

2.2.1.4 Albumin

Serum albumin was measured by a colorimetric method using a commercial kit (Randox Laboratories Ltd., U.K.).

Principle

The measurement of serum albumin is based on its quantitative binding to the indicator 5, 5-di-purple, BCP.

Serum was mixed with a buffered BCP reagent and the mixture was incubated for 2 minutes at room temperature. The absorbance of the sample (A sample) and of the standard (A standard) was measured against blank at a wave length of 600 nm albumin concentration (C) was calculated as follows:

$$C\ (g/dl) = \frac{\text{A sample x concentration of standard}}{\text{A standard}}$$

2.2.1.5 Total protein

Total serum protein was measured by a colorimetric method using a commercial kit (Randox Laboratories Ltd., U.K.).

Principle

Colorimetric determination of total protein in serum is based on the Biuret reaction. The serum protein reacts with copper sulphate in the presence of sodium hydroxide. The Rochelle salt (K-Na-tartarate) contained in the Biuret reagent is utilized to keep the formed cupric hydroxide in solution which gives the blue color. The intensity of the color produced is proportional to the amount of protein in the sample.

The absorbance of the sample (A sample) and of the standard (A standard) were read against the reagent blank in the Spectrophotometer at a wave length of 545 nm. The total serum protein concentration (C) was calculated as follows:

$$C\ (mg/dl) = \frac{A\ sample \times concentration\ of\ the\ standard}{A\ standard}$$

2.2.1.6 Creatinine

Serum creatinine concentration was measured by a colorimetric method using commercial kit (Randox Laboratories Ltd., U.K.).

Principle

Creatinine forms a colored complex with picric acid in alkaline medium. The color intensity is proportional to the concentration of the reaction mixture. Serum was mixed with a mixture of picric acid and sodium hydroxide. Absorbance change/minute (A_{492}/min.) of the sample and standard was recorded at a wave length of 492 nm and change in calculation as follows:

$$C\ (mg/dl) = \frac{A\ sample \times concentration\ of\ standard}{A\ sample}$$

2.2.2 Haematological methods

These were described by Schalm, (1965). Blood samples from rats were collected into dry clean bottles with anticoagulant ethylene diamine tetra acetic acid (EDTA) added.

2.2.2.1 Haemoglobin (Hb) concentration

The concentration of haemoglobin (Hb) was measured by the cyanmethaemoglobin technique using Colorimeter (Ciba Corning, model 252).

Avolume of 0.02 ml of blood was added to the 4 ml of Drabkins solutions 0.2 gm potassium cyanide, 0.2 potassium ferri cyanide and 1 gm bicarbonate per liter of distilled water.

After 10 mins. The haemoglobin (Hb) concentration measured in g/dl of blood. The method is based on the conversion of haemoglobin by Drabkin solution to cyanmethaeglobin.

2.2.2.2 Packed cell volume (PCV)

Blood samples were drawn into microhaematocrit capillary tubes and sealed at one end with cristaseal (Hawksley). The capillary tubes were centrifuged for 5 minutes in microhaematocrit centrifuged (Hawksley and Sons, Ltd. England) and the (PCV) percentage was determined from the scale on the Hawksley microhaematocit reader.

2.2.2.3 Red blood cells count (RBCs)

Erythrocytes were counted by using Neudauer haemocytometer (Hawksly and Sons Ltd, England) and Hayem's solution as a diluent consisting of 0.5 gm sodium sulphate, 0.5 mercuric chloride and 1 gm sodium chloride made up to 200 ml with distilled water.

2.2.2.4 Mean corpuscular volume (MCV)

The mean volume of the red corpuscle was calculated from erythrocyte count and the volume of packed red cells as described by Maxweil and Wintrobe, (1967) as follows:

$$\text{MCV} = \frac{\text{Vol. packed red cells per ml per 1000ml(fl)}}{\text{Red cells count per millions per cm}}$$

2.2.2.5 Mean corpuscular haemoglobin concentration (MCHC)

MCHC was calculated when the Hb concentration and the volume of packed red cells are known (Maxweil and Wintrobe, 1967).

Calculation

$$\text{MCHC} = \frac{\text{Haemoglobin per gm per 100 ml}}{\text{Volume packed cell per 100 ml}}$$

2.2.3 Histopathological methods

The specimens of the liver and kidney were collected immediately after slaughter of animals and fixed in 10% formal saline, embedded in paraffin wax sectioned at 5µm and stained routinely with Haemotoxylin & Eosin (H &E).

2.4 Statistical analysis

Mean values in serum parameters were compared using ANOVA. Changes in individual serum parameters against time were figured out as histograms (Mendelhall, 1971).

CHAPTER THREE

RESULS

3.1 Effect of date palm (*Phoenix dactylifera L.*) pollen grains powder against CCl_4-induced liver damage in rats

3.1.1 Clinical findings

There were no clinical signs in group 1 (the control group) and the groups treated with dates pollen grains (3and4) and also in group 5 (Silymarin group). In group 2 (CCl_4 group), depression and nervous signs were observed.

3.1.2 Post-mortem findings

In CCl_4 group (2), the liver showed fatty changes, congestion and adhesion in the lobes. In group 3(CCl_4+250 mg/kg pollen grains powder), no post-mortem changes were observed in the vital organs. In group 4 (CCl_4 + 500 mg/kg pollen grains powder) there was fatty change in the liver. In group 5 (CCl_4+ 50 mg/kg Silymarin) no post-mortem changes were observed in the vital organs.

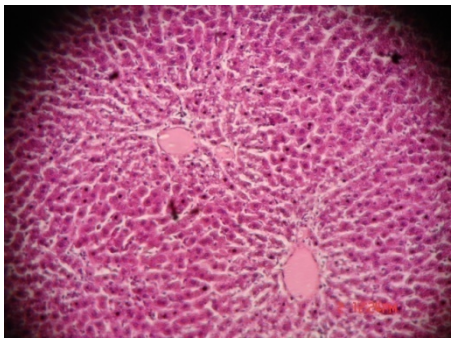
3.1.3 Histopathological changes

Histopathological sections of the experimental groups were presented in Figure 1 (a-d). In group1 (the control), no pathological changes were seen. In group 2 (CCl_4 group), there was centrilobular necrosis, congestion and haemorrhage in the central vein. In group 3(CCl_4 + 250 mg/kg body weight date pollen powder), the liver showed centrilobular necrosis with slight congestion. In group 4 (CCl_4+ 500 mg/kg body weight date pollen powder), the liver showed scattered fatty

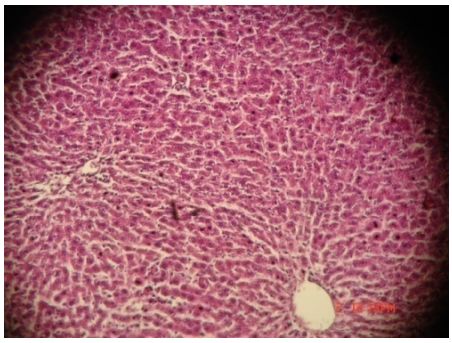
fatty changes and slight congestion. In group 5(CCl_4+ Silymarin 50mg/ kg body weight), no pathological changes were seen.

Figure (1):- Histopatological changes in livers of rats given pollen grains powder of *Phoenix dactylifera L.* and CCl_4 (H & E x 10).

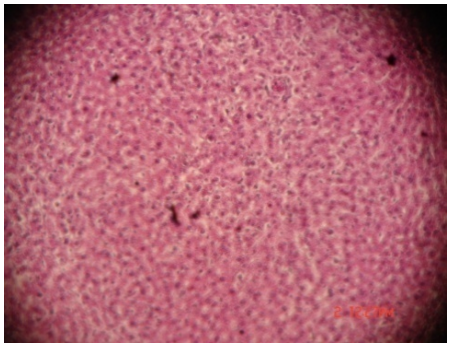
a



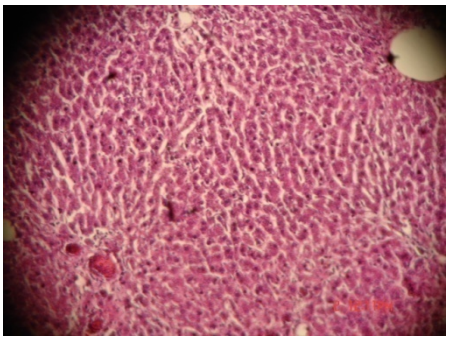
b



c



d



a:- Section of liver cells of CCl_4 group showing centrilobular necrosis, congestion and haemorrhge in the central vein.

b:- Section of liver cells treated with 250 mg/kg body weight *Phoenix dactylifera L.* pollen grains and CCl_4 showing centrilobular necrosis with slight congestion.

c:- Section of liver cells treated with 500 mg/kg body weight *Phoenix dactylifera L.* pollen grains and CCl_4 showing fatty change (scattered) and slight congestion.

d:- Section of liver cells treated with 50 mg/kg body weight Silymarin and CCl_4 showing no pathological change.

3.1.4 Changes in serum constituents

The effect of the date palm pollen grains powder and CCl_4 on the activities of enzymes ALT, AST and ALP and in the concentrations of total protein and albumin in serum of rats given in table 1 and Figures 2-6.

In rats treated with CCl_4 only (group 2), the activities of serum ALT, AST, ALP and the concentrations of total protein and albumin were significantly ($p< 0.05$) increased when compared with the untreated control group.

Treatment with date palm pollen grains powder to rats in groups 3 and 4 (250 mg/kg and 500 mg/kg each with CCl_4 respectively) resulted in significant ($p < 0.05$) fall in the levels of the enzymes ALT, AST and ALP and in the concentrations of total protein and albumin compared with CCl_4 control group.

3.1.5 Haematological changes

The mean values of Hb, PCV, RBC, MCV and MCHC were presented in Table 2. There were no significant ($p < 0.05$) differences between the test groups and the control group.

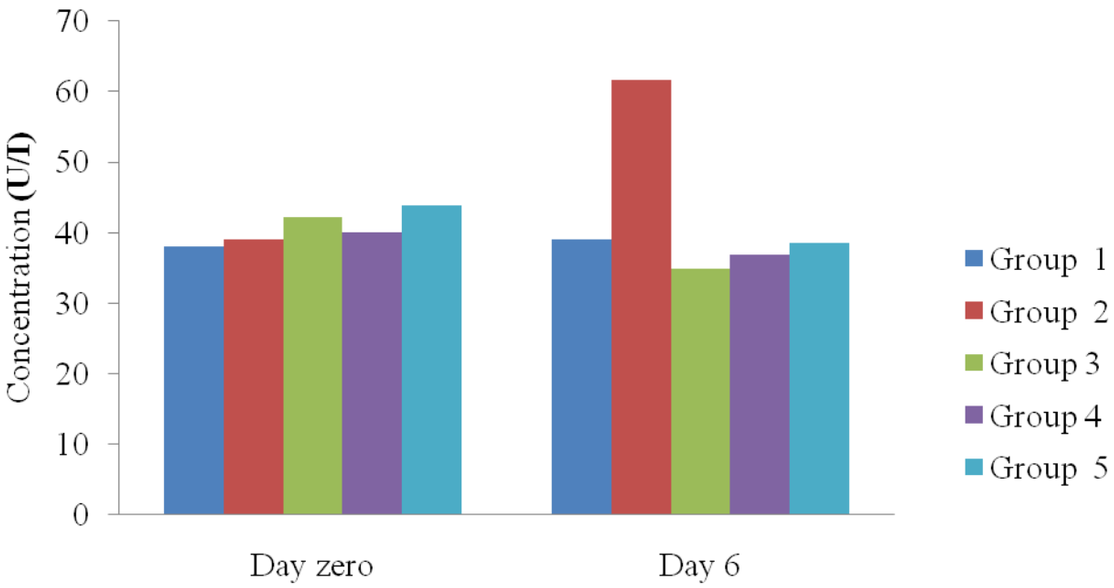
Table (1) Changes in serum constituents in rats treated with *Phoenix dactylifera L.* pollen grains powder andCCl_4

Groups	ALT U/I (Means ± S.E.)		AST U/I (Means ± S.E.)	
	Day zero	Day 6	Day zero	Day 6
Group 1	38 ± 1.41 a	39 ± 1.94 b	177.6 ± 3.61 a	178.7 ± 3.52 b
Group 2	39 ± 2.26 a	61.6 ± 6.94 a	164.6 ± 9.93 a	254.0 ± 20.8 a
Group 3	42.2 ± 1.71 a	34.8 ± 1.66 b	181.0 ± 1.79 a	178.0 ± 2.35 b
Group 4	40.0 ± 1.64 a	36.8 ± 1.56 b	180.2 ± 1.56 a	178.0 ± 2.17 b
Group 5	43.8 ± 2.01 a	38.6 ± 1.63 b	181.4 ± 3.3 a	162.0 ± 3.27 b

Groups	ALP U/I (Means ± S.E.)		Total protein g/dl (Means S.E)		Albumin g/dl (Means ± S.E)	
	Day zero	Day 6	Day zero	Day 6	Day zero	Day 6
Group 1	190.4 ± 3.1 a	193.4 ± 2.2b	6.68 ± 0.3a	6.76 ± 0.31b	4.74 ± 0.17a	4.84 ± 0.2b
Group 2	189.6 ± 3.43 a	255.0 ±23.4 a	5.95 ± 0.2a	7.09 ± 0.19 a	4.84 ± 0.19a	7.2 ± 0.21 a
Group 3	191.2 ± 2.3a	164.8 ± 11.b	6.18 ± 0.3a	5.94 ± 0.29b	5.1 ± 0.31 a	4.08 ± 0.2b
Group 4	195.8 ± 1.88 a	191.8 ± 2.4 b	6.54 ± 0.1a	5.89 ± 0.02b	5.08 ± 0.19a	4.38 ± 0.1b
Group 5	192.6 ± 2.1a	170.6 ± 4.03b	6.88 ± 0.26a	5.86 ± 0.15 b	4.8 ± 0.17 a	4.32 ± 0.1b

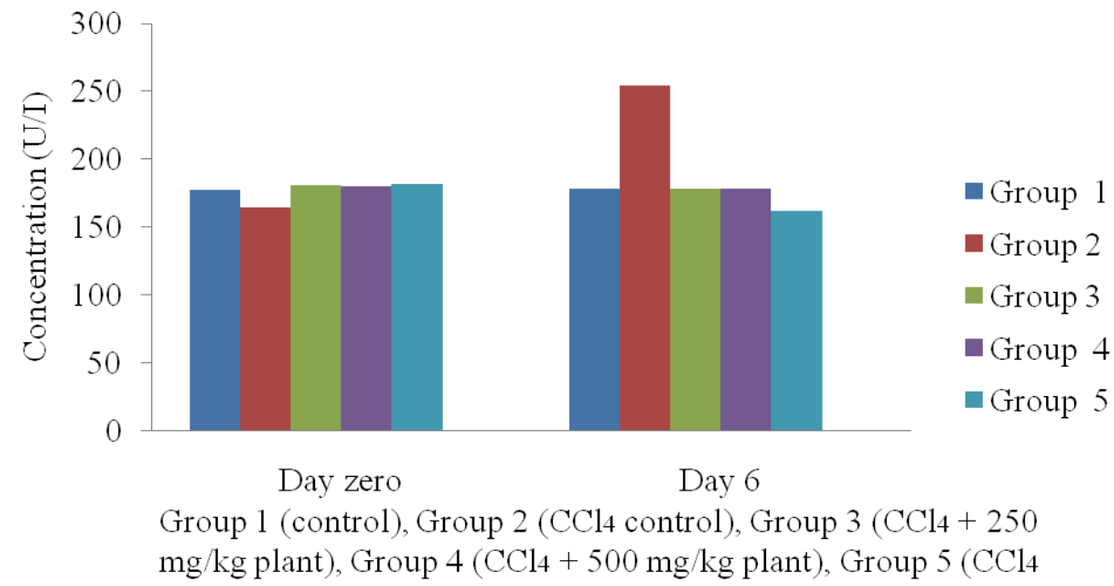
Group 1 (control group), Group2 (CCL_4 control), Group 3 (CCl_4 + 250 mg/kg date pollen grains powder),
Group 4 (CCl4 + 500 mg/kg date pollen grains powder), Group 5 (CCl_4 + Silymarin drug).
Means in the same column with the same letter are not significantly ($p < 0.05$) different.

Figure 2. Changes in serum ALT in rats treated with *Phoenix dactylifera L.* pollen grains powder and CCl_4.



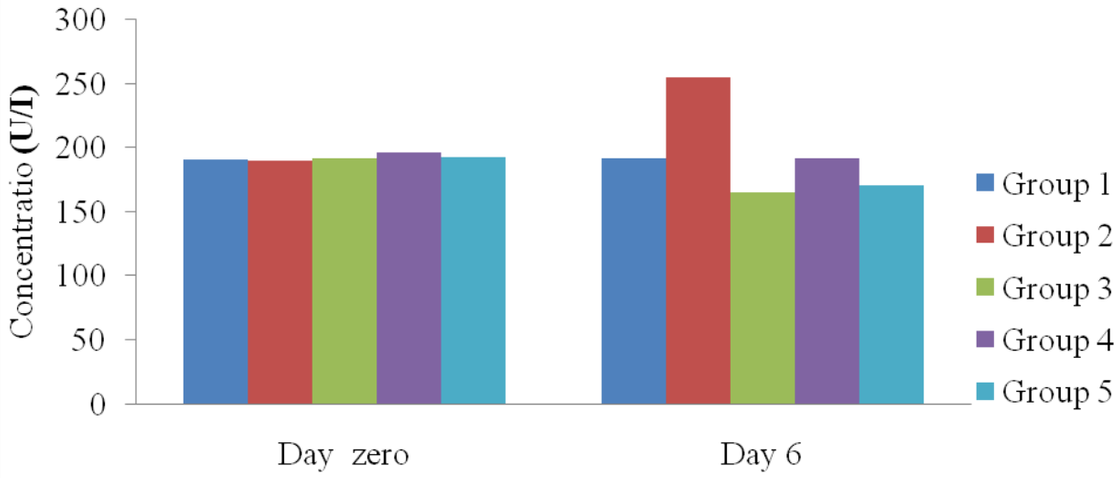
Group 1 (control), Group 2 (CCl_4 control), Group 3 (CCl_4 + 250 mg/kg plant), Group 4 (CCl_4 + 500 mg/kg plant), Group 5 (CCl_4 + Silymarin)

Figure 3. Changes in serum AST in rats treated with *Phoenix dactylifera L.* pollen grains powder and CCl_4.



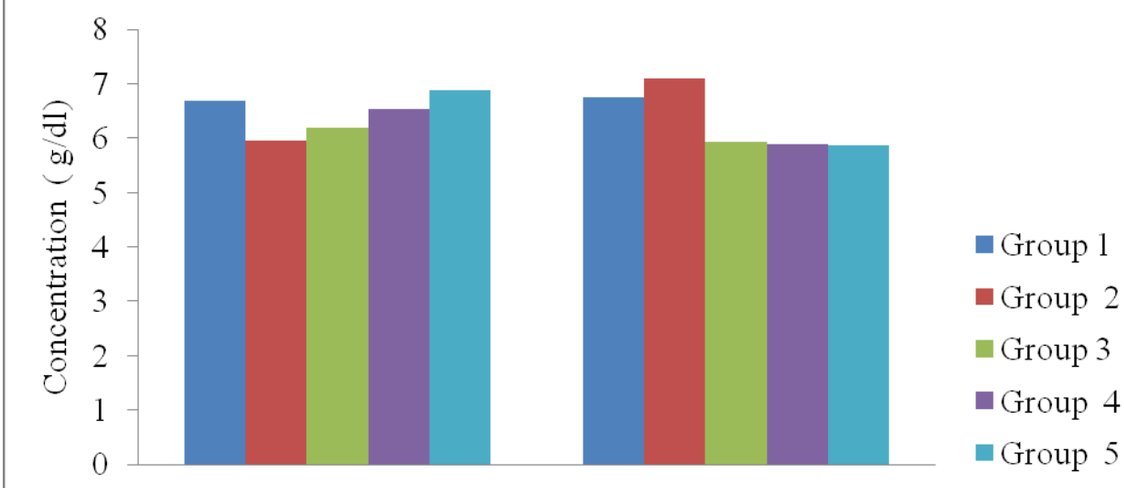
Group 1 (control), Group 2 (CCl_4 control), Group 3 (CCl_4 + 250 mg/kg plant), Group 4 (CCl_4 + 500 mg/kg plant), Group 5 (CCl_4 + Silymarin)

Figure 4. Changes in serum ALP in rats treated with *Phoenix dactylifera L.* pollen grains powder and CCl_4.



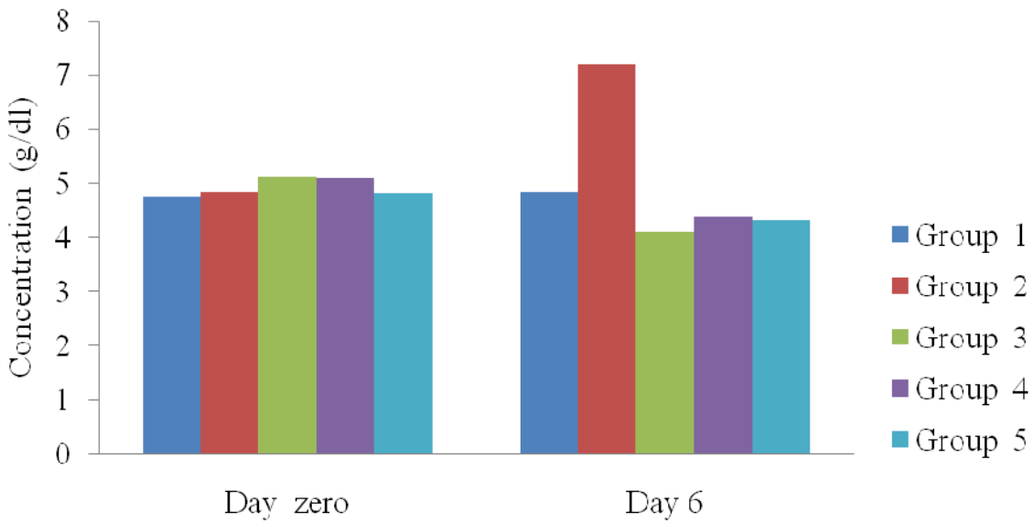
Group 1 (control), Group 2 (CCl_4 control), Group 3 (CCl_4 + 250 mg/kg plant), Group 4 (CCl_4 + 500 mg/kg plant), Group 5 (CCl_4 + Silymarin)

Figure 5. Changes in serum Total Protein in rats treated with *Phoenix dactylifera L.* pollen grains powder and CCl_4.



Group 1(control), Group 2 (CCl_4 control), Group 3 (CCl_4 + 250 mg/kg plant), Group 4 (CCl_4 + 500 mg/kg plant), Group 5 (CCl_4 + Silymarin)

Figure 6. Changes in serum Albumins in rats treated with *Phoenix dactylifera L.* pollen grains powder and CCl_4.



Group 1 (control), Group 2 (CCl_4 control), Group 3(CCl_4 +250mg/kg/plant), Group 4 (CCl_4 + 500 mg/kg plant), Group 5 (CCl_4– Silymarin)

Table (2) Changes in haematological values in rats treated with *Phoenix dactylifera L.* pollen grains powder and CCl_4

Groups	Hb g/dl (Means ± S.E.)		PCV % (Means ± S.E.)	
	Day zero	Day 6	Day zero	Day 6
Group 1	16.14 ± 0.25 ab	15.58 ± 1.13 a	61.74 ± 4.34 a	61 ± 4.39 a
Group 2	17.42 ± 0.22 a	15.18 ± 0.35 a	63.36 ± 2.44 a	59.32 ± 4.39 a
Group 3	16.10 ± 0.24 ab	14.66 ± 0.36 a	62.88 ± 0.66 a	56.82 ± 1.29 a
Group 4	15.88 ± 0.37 ab	14.50 ± 0.39 a	61.72 ± 1.73 a	54.74 ± 1.57 a
Group 5	16.38 ± 0.49 ab	15.1 ± 0.29 a	67.84 ± 2.45 a	62.66 ± 1.54 a

Groups	RBC $x10^6$ (Means ± S.E.)		MCV fl (Means ± S.E.)		MCHC % (Means± S.E.)	
	Day zero	Day 6	Day zero	Day 6	Day zero	Day 6
Group 1	9.81 ± 0.25 a	9.21 ± 0.69 a	64 ± 2.02 a	69.6 ± 2.25 a	262.4 ± 6.62 a	249.0 ± 6.43 a
Group 2	8.36 ± 0.44 a	8.95 ± 0.20 a	62.8 ± 0.37 a	66.2 ± 0.66 ab	230.4± 1.94 bc	255.4±1.96 a
Group 3	7.70 ± 0.54 a	8.62 ± 0.21 a	63.0 ± 0.89 a	65.8 ± 0.37 a	239.6 ± 3.83 a	256.0 ± 1.70 a
Group 4	7.28 ± 0.29 a	8.22 ± 0.24 a	63.2 ± 0.49 a	66.2 ± 0.97 a	247.8 ± 3.02 a	262.0 ±1.76 a
Group 5	7.94 ± 0.49 a	8.93 ± 0.53 a	64.8 ± 0.73 a	70.4 ± 0.75 a	225.6 ±4.07 a	244.2±9.37 a

Group 1 (control group), Group2 (CCL_4 control), Group 3 (CCl_4 + 250 mg/kg date pollen grains powder),
Group 4 (CCl4 + 500 mg/kg date pollen grains powder), Group 5 (CCl_4 + Silymarin drug).
Means in the same column with the same letter are not significantly ($p < 0.05$) different.

3.2Toxicity experiment of *Phoenix dactylifera* pollen grains powder in rats

3.2.1 Clinical signs

There were no obvious clinical signs in group1 (control) and in group 2 (500 mg/kg body weight pollen grains powder) throughout the period of the experiment, but in group 3 (1000 mg/kg body weight pollen grains powder) and group 4 (2000 mg/kg body weight pollen grains powder), rats showed depression and general unthriftness.

3.2.2 Post-mortem changes

At necropsy, in groups 1 (control) and in group 2 (500mg/kg body weight pollen grains powder), there were no changes in all vital organs. In group3 (1000mg/kg body weight pollen grains powder) and in group 4 (2000 mg/kg body weight pollen grains powder), the livers and spleens of the rats showed slight congestion .

3.2.3 Histopathological changes

In histopathological sections of the specimens, no changes were observed in all vital organs of group 2 (500 mg/kg body weight pollen grains powder) and in group 3 (1000 mg/kg body weight pollen grains powder). In group 4(2000 mg/kg body weight pollen grains powder), there were generalized fatty change in the liver (Figure 7) and congestion and shrinkage of the kidney glomeruli (Figure 8).

3.2.4 Serum constituent changes

The activities of ALT, AST, and ALP and concentrations of total protein, albumin and creatinine are presented in Table (3). There were no significant ($p< 0.05$) changes in all parameters except there was significant ($p < 0.05$) increase in ALP in groups 3 and 4 and in creatinine in group 4 at day 8. are presented in Table (3) and there

3.2.5Haematological changes

The mean values of RBCs, Hb, PCV, MCV and MCHC are presented in Table 4. The mean values of these parameters fluctuate within the normal range and remained similar ($p < 0.05$) during the period of investigation in all treatment groups.

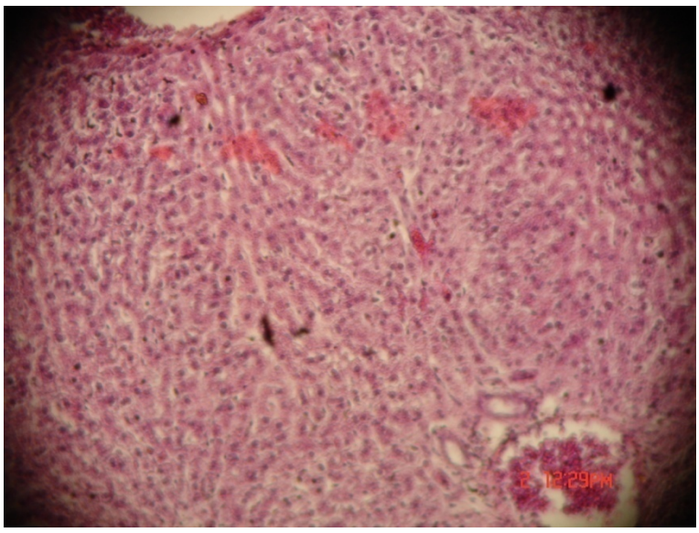


Figure 7. Generalized fatty change and slight congestion in a liver of a rat treated with 2000 mg/kg body weight *Phoenix dactylifera L.* pollen grains powder (H & E x 10).

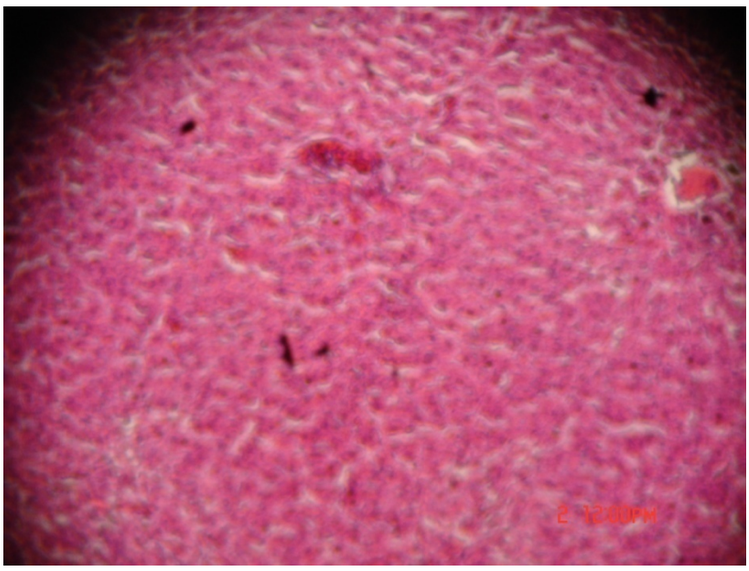


Figure 8.Congestion and shrinkage of the glomeruli in a kidney of a rat treated with 2000 mg/kg body weight *Phoenix dactylifera L.* pollen grains powder (H & E x 10).

Table (3) Changes in serum constituents in rats treated with different doses of *Phoenix dactylifera L.*pollen grains powder.

Groups	ALT U/I (Means ± S.E.)		AST U/I (Means ± S.E.)		ALP U/I (Means ± S.E.)	
	Day zero	Day 8	Day zero	Day 8	Day zero	Day 8
Group 1	34± 0.707 a	34 ± 0.707 a	187.98 ± 0.43 a	187.98 ± 0.41a	190.32± 0.43 c	190.4 ±0.4 c
Group 2	33.4 ± 0.51 a	34.8 ± 0.8 a	187.4 ± 0.41 a	188.02± 0.33 a	190.58 ± 0.37 c	191.76± 0.5 bc
Group 3	34 ± 1.0 a	34.2 ± 1.07 a	188.6 ± 0.38 a	188.74±0.35 a	194.1 ± 0.37 a	194.4 ± 0.37 a
Group 4	34 ± 0.707 a	33.8 ± 0.58 a	188.34 ± 0.38 a	188.62 ± 0.34a	192.04± 0.44 b	193.14± 0.41ab

Groups	Total protein g/dl (Means± S.E.)		Albumin g/dl (Means± S.E.)		Creatinine mg/dl (Means± S. E.)	
	Day zero	Day 8	Day zero	Day 8	Day zero	Day 8
Group 1	6.45 ± 0.11 a	6.53 ± 0.1 a	4.54 ± 0.17 a	4.58 ± 0.18 ab	0.61 ± 0.006 b	0.61 ± 0.005 b
Group 2	6.32 ± 0.17 a	6.54 ± 0.1 a	4.59 ± 0.11 a	4.61 ± 0.21 a	0.56 ± 0.031 bc	0.6 ± 0.031 b
Group 3	6.29 ± 0.16 a	6.61 ± 0.15 a	4.43 ± 0.13 a	4.48 ± 0.15 a	0.5 ± 0.032 c	0.54 ± 0.024 b
Group 4	6.53 ± 0.12 a	6.55± 0.13 a	4.77 ± 0.08 a	4.78 ± 0.07 a	0.66 ± 0.02 a	0.72 ± 0.02 a

Group 1(control), Group 2(500 mg/kg date pollen grains powder), Group 3 (1000 mg/kg date pollen grains powder), Group 4(2000 mg/kg date pollen grains powder).
Means in the same column in the letter are not significantly ($p < 0.05$) different.

Table (4) Changes in haematological values in rats treated with different doses of*Phoenix dactylifera L.* pollen grains powder.

Groups	RBC 10^6 (Means ± S.E.)		Hb g/dl (Means ± S.E.)	
	Day zero	Day 8	Day zero	Day 8
Group 1	9.7 ± 0.11 a	9.3 ± 0.75 a	16.1 ±0.26 a	15.6 ±1.14 a
Group 2	9.5 ± 0.20 a	8.6 ± 0.39 a	15.7 ±0.14 a	14.8 ±0.31 a
Group 3	9.3 ± 0.30 a	8.8 ± 0.14 a	16.1 ±0.58 a	15.2±0.32 a
Group 4	9.3 ± 0.29 a	9.6 ± 0.33 a	15.9±0.29 a	15.2±0.48 a

Groups	PCV % (Means ± S.E.)		MCV fl (Means ± S.E.)		MCHC %(Means ± S.E.)	
	Day zero	Day 8	Day zero	Day 8	Day zero	Day 8
Group 1	60 ± 1.37 a	62.7 ± 4.5 a	62.2 ± 1.0 b	70.8 ± 1.77 a	268 ± 3.89 a	243.3 ± 4.08 a
Group 2	59.6 ± 0.63 a	62.1 ± 2.1 a	62.8 ± 1.0 b	72 ± 1.09 a	264 ± 3.7 a	238.2 ± 3.8 a
Group 3	61.9 ± 0.2 a	64.1 ± 0.4 a	66.6 ± 0.87 a	72.8 ± 2.44 a	259.8 ± 3.96 a	229.2 ± 8.3 a
Group 4	62.3 ± 2.25 a	73 ± 2.46 a	66.8 ± 0.7 a	77 ± 1.05 a	256.6 ± 7.2 a	205 ± 4.39 a

Group 1 (control), Group 2 (500 mg/kg plant powder), Group 3 (1000 mg/kg plant powder)

Group 4 (2000 mg/kg plant powder).

Means in the same column in the same letter are not significantly ($p < 0.05$) different.

CHAPTER FOUR

DISCUSSION

The present study has been an attempt to elucidate, hepatoprotective properties and toxicity of date palm (*Phoenix dactylifera L.*) pollen grains powder in rats.

Liver damage induced by CCl_4 is perhaps the best- studied model of hepatotoxicity (Cornelius, 1993). Several mechanisms underlying this toxicity have been suggested (Recknagel *et al*., 1989).The mechanism of CCl_4 - induced elevated plasma activities of AST, ALT and ALP and the concentrations of total protein and albumin levels in animals pre and post- treated with date pollen grains powder respectively showed their ability to restore the normal functional status of the poisoned liver and also to protect against subsequent CCl_4 hepatotoxicity.

The mechanism by which the date palm fruit extract induced its hepatoprotective activity is not certain. However, it is possible that β-sitosterol, a constituent of *Phoenix dactylifera L.* (EL- Mougy *et al*., 1991), is at least partly responsible for the protective activity against hepatotoxicity (Nan-Lin and Pin-Tome, 1988).

The inactive metabolites of carbon tetrachloride, are transformed to free radicals through the microsomal cytochrome P-450 dependent enzyme, resulting in activation of CCl_4 toxicity.

The efficacy of a hepatoprotective drug, are essentially dependant on its capability of either reducing the harmful effect or

maintaining the normal hepatic physiological mechanisms which have been unbalanced by the hepatotoxin (Basu *et al*., 1992).

An additional important factor in the hepatoprotective activity of any drug is the ability of its constituents to inhibit the aramatose activity of cytochrome P-450, by there favoring liver regeneneration. On that basis, it is suggested that flavonoids in *Phoenix dactylifera L.* could be contributing as a factor to its ability for hepatoprotection through inhibition of cytochrome P-450 aromatase (Kowalska *et al*., 1990). In addition, the recorded content of vitamin C in the date palm pollen grains may also play a role in hepatoprotection. Previous invivo studies indicated that hepatic microsomal drug metabolic rate decreases in ascorbic acid deficiency and is augmented when high supplements of the vitamin are given to guinea pig (Sato and Zannoni, 1976 and Burtis and Ashwood, 2001). Liver cytochrome P-450 activity is significantly reduced in ascorbic acid - deficient guinea pigs (Rikans *et al*., 1978).

The results revealed that *Phoenix* dactylifera *L.* pollen grains possess significant hepatoprotective activity against CCl_4 at the dose 500 mg/kg body weight pollen grains powder by an oral route and was able to prevent liver damage as indicated by low levels of ALT,AST and ALP, at the same time, these enzymes showed higher levels in CCl_4 group and these results were correlated with histopathology. The increase levels of ALT and AST have been reported in CCl_4-induced necrosis of the liver (Gadgoli and Mishra1995). Also increased liver enzymes is a feature of the toxicity reported by El-Badwi *et al.* (1995 and 1998) in chicks intoxicated with *Rinus*

communis and goats intoxicated with of *Calotropis procera.*

In this study the levels of enzymes were reduced significantly on treatment with pollen grains after CCl_4 -intoxication and to same extent better than the reference drug (Silymarin) especially at the higher dose (500 mg/kg body weight).

The findings revealed that various biochemical alterations produced by CCl_4 were prevented by the therapy with the plan pollens.

Phoenix dactylifera L. pollen grains powder protected the liver against CCl_4 toxicity, although some alterations in the liver cells, due to CCl_4, was present and the overall damage to cells and blood vessels was less when compared to CCl_4 control group.

For the importance of the medicinal uses of *Phoenix dactylifera L.*, pollen grains, especially the traditional use of it in Northern State for the treatment of infertility in women, the toxicological effects of this plant in rats were tested.

The results of toxicity experiment showed no significant changes in serum enzymes, no significant changes in haematological parameters and in histopathological findings at the doses 500 and 1000 mg/kg body weight. This indicated that *Phoenix* dactylifera *L.* pollen grains powder had no toxic effects on rats at the doses mentioned throughout the period of the experiment, except for slight toxic effects at the higher dose (2000 mg/kg).

Conclusion and recommendations

1.This study has investigated the toxicity and hepatoprotective activity of date palm (*Phoenix dactylifera L.*) pollen grains powder against CCl_4- induced liver damage in rats.

2.The date pollens in this study had hepatoprotective effect against CCl_4 at the dose rate of 250 mg/kg body weight and 500 mg/kg body weight which is dose dependant and this effect may be related to the antioxidant properties of this plant.

3.The date palm (*Phoenix dactylifera L.*) pollen grains powder possess no toxicological effect at the doses tested (500 mg/kg and 1000 mg/kg body weight) with lesser toxic effects at the higher dose (2000 mg/kg body weight).

Based on the above mentioned findings, we suggest the following recommendations:-

1. Further biologically-guided fractionation of the extract to identify exactly the chemical nature of the active ingredients.

2. Detailed pharmacological and toxicological investigations of the active components of the extract.

3. Standardzation and formulation of the acceptable dosage form.

REFFERENCES

Abdalla, Y. and Al-Taher, E. (2008). Possible anti- diarrhoeal effect of the date palm (*Phoenix dactylifera*) spathe aqueous extract in rats. Scientific Journal of King Faisal University vol.-**9**No.1 1429-1435.

Ahmed, B. Alam, T. Varshney, M. and Khan, S. A.(2002). Hepatoprotective activity of two plants belonging to the Apiaceae and the Euphorbiaceae family.Ethnopharmacology, **79 (3):**313-316.

Aktay, G. Deliorman, D. Ergun, F. Yesilada, E. and Gevik, C. (2000). Hepatoprotective effects of Turkish folk remedies on experimental liver injury. J. Ethnopharmacol., **73:121**-129.

Alferez, M.J.M. and Campos, M.S. (2000). Beneficial effect of pollen calcium and magnesium in rats with nutritional feropenic anemia. J. Agric. Food Chem., **48:**5715-5722.

Ali, B. H. Bashir, A. K. and Al-Hadrami, G. (1999). Reproductive **66:** 437-441.

Alliath, A. and Abdalla, A. A. (2005). In vitro evaluation of antioxidant activity of different extracts of *Phoenix dactylifera* fruits as functional foods. Deutsche Lebensmittel Rundschan **101:** 305-308.

Al-Kharage, E. and Rokaya, A.(1982). Protective effects of *Phoenix dactylifera* (date palm) against Cisplatin- induced genotoxicity. Food Science and Technology Abstracts, vol.**1** 148-331.

Al-Mougy, S.A. Abdel-Aziz, S.A. Al-Shanawany, M. Omer, A (1991). The gonadotrophic activity of *Palamae* in mature male rats. Alexandria J. Phamac. Sci., **5:**156-159 .

Al-Qarawi, A.A. Ali, B.H. Al-Mougy, S.A. Mousa, H.M.(2003). Gastrointestinal transit in mice treated with various exracts of date palm (*Phoenix dactylifera Linn*). Food and Chemical Toxicology, **41**: 37-39.

Al-Qarawi, A.A. Mousa, H.M. Ali, B.E.H. Abdel- Rahman, H. and El-Mougy, S.A. (2004). Protective effect of extracts from date (*Phoenix dactylifera L.*) on the carbon tetrachloride –induced hepatotoxicity in rats. Intern. J . Appl. Res. Vet. Med., **2:** 176 -180.

Al-Qarawi, A.A. Abdel-Rahman, H. Ali, B.H. Mousa, H.A. and El-Mougy, S. A. (2005). The ameliorative effect of dates (*Phoenix dactylifera L.*) on ethanol-induced gastric ulcer in rats. J. Ethnopharmacol., **98 :** 313-317.

Al- Qarawi, A.A. Abdel-Rahman, H. Mousa, H.M. Ali,B.H. and El-Mougy, S. A. (2008). Nephroprotective action of date *Phoenix dactylifera L.* in gentamicin-induced nephrotoxicity. Pharmaceutical Biology, **4:** 227-230.

Al-Shahib, W. and Marshal, R.J. (1993). The fruit of the date palm: its possible use as the best food for the future? Int. J. Food Sci. Nut., **54:** 247-259.

Amin, E.S. Awad, O. Abdel Samad, M. and Iskander, M.N. (1969). Isolation of estrone from Moghat roots and from pollen grains of Egyptian date palm. Phytochem.,vol. **9** Issue 1 295-297.

AOAD. (1998). Arab Agric. Statistics year book vol. **18:** p. 107.

Bahmanpour, S. Talaei, T. Vojdani, Z. Panjehshahin, M.R. Poostpasand, L,A. Zareei, S.and Ghaemini, M. (2006). Effect of *Phoenix dactylifera Linn* pollen grains on sperm parameters and reproductive system of adult male rats. Ind.J.Med.Sci. **31**: 208-212.

Basu, A. Sen, N. Ray, R. N. and Chaudhuri, A. K. N. (1992). Hepatoprotective effects of *Calotropis procera* root extract on experimental liver damage in animals. Fitoterapia, **36 (6):** 507-513.

Bennett, R.D. and Heftmann, E. (1966). Isolation of Estrone and Cholesterol from the date palm *Phoenix dactylifera L.* Phytochemistry, **5:** 231-235.

Broadhurt, C.L. (1999). Bee products: medicine from the live. Nutr. Sci. News, **4:** 366-368.

Burgoyne, R.W. and Tan, D.H. (2008). Prolongation and quality of life HIV-infected adults treated with highly active anti-retroviral therapy (HAART): a balancing act. J.Antimicrob. Chemother, **61:** 469-73.

Burtis, C. A. and Ashwood, E. R. (2001). Tietz's Fundamentals of Clinical Chemistry. Philadelphia W.B. Saunder, Vol. **2:** 565.

Chandan, B.K. Sharma, A.K. and Anand, K.K. (1991). *Boerhavia diffusa*: A study of its hepatoprotective activity. Journal of Ethnopharmacology, **31 (3):** 299-307.

Chandler,W.H. (1958). Ever Green Orchards. Lex and Fabiger, Philadelphia, USA.

Chemie, D . G. K. (1972). Alkaline phosphatase. Journal of clinical chemistry and clin. Biochemistry, **10:** 182-192.

Cornelius., C.E. (1993). Animal Models in Liver Research. Academic Press, San Diego,**37**:341.

El-Badwi, S.M.A. Adam, S.E.I. and Hapke,H.J.(1995). Comparative Toxicity of *Ricinus communis* and *Jatropha curcas* seed on brown Hisex chicks. Vet. Hum. Toxicology, 34:112.

El-Badwi, S.M.A. Adam, S.E.I. Shigidi, M.T. and Hapke H.J. (1998). Studies on *Laticiferous* plant. Toxic effect on goats of *Calotropis procera* given by different routes of administration, Deutsche Tierarztliche Wochenschidt, 105-425.

El-Desoky, G.E. Ragab, A.A. Ismail, S.A. and Kamal, A.E. (1995). Effect of palm pollen grains (*Phoenix dactylifera*) on sex hormones, proteins, lipids and liver functions. J.Agri. Sci. Mansoura Univ., **20:** 4249-4268.

Elgasim, E.A. Al-Yosif, Y.A. and Homeida, A.M. (1995). Possible hormonal activity of date pits and flesh fed to meat animals. Food Chem., **52:** 149-150.

El-Mougy, S.A. Abdel-Aziz, S.A. Al-Shanawany, M. andOmer, A. (1991). The gonadotrophic activity of Palmae in mature male rats. Alexandria J. Pharmac. Sci., **5:** 156-159.

El-Ridi, M.S. and Abou-Elwafa, M. (1950). "General composition and vitamin content of the pollen grain of the date palm *Dactylifera palma* J. Roy. Med. Assoc. Egypt., **33:**168.

El-Ridi. M. S. Strait, L. A. and Abou-Elwafa, M. H. (1953). Isolation of Rutin from the pollen grain of the date palm *Dactylifera palma.* J. Roy. Med. Assoc. Egypt., **3:** 317-322.

Eong, Y.J. Hong, F.A. Tomas-Barberan, Adel, A, Kader, S. and Alyson, E. (2006). The flavonoid glycosides and procyannidin composition of Deglet Noor dates (*Phoenix dactylifera*). J. Agric. Food Chem., **54:** 2405-24011.

Fleurentin, J., Hoefler, C. Lexa, A. Mortier, F. and Pelt, J.M. (1986). Traditional medicinal plants of Yemen. Journal **Ethnopharmacol**., **16:** 105-111.

Frevold, H.L. and Simpsox, (1957). gonadotrophic activity of date palm pollen grains. Endocrinology, vol. **28:** 28-33.

Friedman, M. H. and Friedman, G.S. (1939). The gonadotrophic extracts from green leaves. American J. Physiology,**6:**133-486.

Gadgoli, C. and Mishra, S. H. (1995). Preliminary screening of *Achillea millefolium*, *Cichorium intybus* and *Capparis spinosa* for anti-hepatotoxic activity. Fitoterapia, **56 (4):** 323 - 329.

Hassan A.I.Y. and Abou-Elwafa, M.H. (1947). "An estrogenic substance in pollen grains of date palm tree *Phoenix dactylifera L*. Nature, **159**:409.

HHP. (2007). Lotion and Potion Havard University.USA.

Heftmann, E. and Bennett, R.D. (1965). Identification of estone in date seeds by thin layer chromatography. Naturwissenschaften, **2:**431-438.

Janakat, S. and AL-Merie, H.(2002). Evaluation of hepatoprotective effect of *Pistacia lentiscus*. J.Ethnanopharmacol., **83:**135-138.

Javanmardi, I. J. Stushno, C. Locke, E.and Vivanco, J.M.(2003). Antioxidant activity and total phenolic content of Iranian Ocimum accession. Food Chemistry, **83:** 547-550.

Kowalska, M.T. Brandt, M.E. and Puett, D. (1990). Inhibition of cytochrome (P-450) aramatase activity by plant extracts. Planta Med. **56 :** 675-677.

Kroyer, G. and Hegedus, J.(2001). Evaluation of bioactive properties of pollen extracts as functional dietary food supplement. Innovative Food Sci. Emerging Technol, **2 (3):** 171-174.

Mahran, G.H. Abdul-Wahab, S.M. and Attia, A. (1976). A phytochemical study of date palm pollen Planta Medica, **29:** 171-175 .

Mason, S.C. (1915). Botanical characters of the leaves of the date palms used in distingwishing cultivated varieties. USDA. Bull. **223**.

Maxweil, M. and Wintrobe, M.D.(1967). Clinical Haematology. 6th ed, Lean and Fibiger, Philadelphia, USA.

Mendenhall, W. (1971). Introduction to Probability and Statistics. 3rd ed, Wadsworth Publishing Company In., Belmont, California, USA.

Mohamed, D. A. and Al-Okbi, S.Y. (2004). In vivo evaluation of antioxidant and anti-inflammatory activity of different extracts of date Fruits in adjuvant arthritis. Polish Journal of Food and Nutrition Sciences, **13:** 397-402.

Nadkarni, K.M. (1976). Indian Materia Medica Mumbai: Bombay Popular Prakashan Pvt.Ltd. Vol. **1**.

Nan-Lin, C. and Pin -Tome, W. (1988). Antihepatotoxic principles of *Sambucus formosana*. Planta Med. **54:**223-224.

Nizar, M.A. El-Sukhon, S.Msameh Y.and Abdul-Karim, J.S. (1999). Effect of date fruits (*Phoenix dactylifera L.*) onthe Hemolytic activity of Streptolysin O.Pharmaceutical Biology,37**:** 335-339.

Ortomans, H., (1979). Med. Pharm., **2:** 129.

Rao, P.G.M. Rao, S.G. Kumar, V. Ramnarayan, K. Nayak, S.S. Seth, S. and Srinivasan, K. K. (1993). Effects of the epatogard against carbon tetrachloride-induced liver damage. Fitoterapia, **64 (2):** 108-113.

Recknagel, R.O. Glende, E.A. J.R. Dolak, J.A. and Waller, R.L. (1989). Mechanisms of carbon tetrachloride toxicity. Pharmather, **43:** 139-154.

Reshod, A. and Al-Shagrawi.M.A. (1998). Enzyme activities, lipid fractions and fatty acid composition in male rats fed palm pollen grains (*Phoenix dactylifera*). Res. Bult., **79:** 5-18.

Rikans, L.E., Smith, C.R., and Zannoni , V.G.,(1978). Ascorbic acid and cytochrome P-450 .J. Pharmacol Exp. Ther., **204:**702-705.

Sabah, A.A.J. and Mazen, A.N. (2007). Invitro Evaluation of the antiviral activity of an extract of date palm (*Phoenix dactylifera*) pits on a *Pseudomonas* Phage. CAM., **15:** 1-6.

Salah, A. and Al-Maiman, M. (2005). Effect of date palm (*Phoenxi dactylifera*) seed fibers on plasma lipids in rats J. King Saud. Univ., **17:** 117-123.

Sato, P.H. and Zannoni, V.G. (1976). Ascorbic acid and hepatic drug metabolim. J. Pharmacol Exp. Ther., **198:** 295-307.

Schalm, O.W. (1965). Veterinary Haematology, 4th edition., Lea and Febiger., Philadelphia, USA.

Sere, A. Batalha, M.V. Diaw, M.M. Agba, K.C. Ba, A.C. and Gay, O. (1984). Pharmacodynamic study of the pato-protective properties of *Cochlospermum tinctorium* a rich Cochlospermuaceae. Revue de Medicine Veterinaire, **135:** 199-209.

Stanley, R.G. and Linkens, H.G. (1974). Pollen Biology,Biochemistry Management. Spriger- Verleg Berlin Heidelberg, New-York, P. **44.**

Thornfeldt, C.R. Pastor, E.K. Inventors, L. R. Dykas S. Nipper, L.L.P. and Assignee, U.S. (2006). Treatment of muco - cutaneous disorders through reversing chronic inflammation and barrier disruption. US Patent J.,**2:**33-39.

Vayalil, P.K. (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit (*Phoenix dactylifera L. Arecaceae*). J. Agric. and Food Chem., **50:** 610-17.

WOI. (1985). A Dictionary of Indian raw Materials Products. Ist. Edition, Vol. **3** Council of Scientific and Industerial Research. Wealth of India New Dalhi.

Wojcicki, J. Samochowice, L. Bartlomowicz, B. Hinek, AJaworska, M. and Gawronska, S. (1986). Effect of pollen extract on the development of experimental atherosclerosis in rabbits. Atherosclerosis, **62:** 39-45.

Wojcicki, J. Samochowiec, L. Kadlubowska, O. and Kownacka, A.B. (1987). Study on the antioxidant properties of pollen extracts. Arechimmunol. **35:** 725-729.

WIPO. (2006). Herbal formulation capable of preventing alcohol-induced hangover, methods of preparing the use. World Intellectual property organization. **1:** 31-32.

Zhao, J. Zhang, C.Y. D.M. Huang, G.Q. X.U. Y.L. Wang, Z.Y., Fang, S.D. Chen, Y. and Gu, Y.L. (1990). The antiatherogenic effects of components isolated from pollen typhae. Thromb- Res.Mar., **57:** 966-975.

Ziouti, A.C. Modafar, E.L. Fleuriet, A.S. Boustani, E.L. and Macheix J.J. (1996). Phenolic compounds in date palm cultivars sensitive and resistant to *Fusarium.* Biologia Plantarum, **38:** 451-457.

Zoghet, M. and Al Alsheikh, A. (2000). Wild Plants in the Region of Riyadh. King Saud University Press. pp.185-186.