

Comparative histological study of protective effect of oil and alcoholic extracts of dry palm dates and leaves (Phoenix dactylifera L) against CCL4 induced oxidative stress in rats

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Abstract

A study was performed to investigate the protective effect of different extracts of alcoholic and oil of leave and dry date of (Phoenix dactylifera) against oxidative stress induced by CCL4 at a dose of 100 mg/kg given orally daily to five treated groups (CCL4 control (+ve) , alcoholic extract of leave (150 mg/kg) , alcoholic extract of dry date (100 mg/kg) , oil extract of leave and date (250 mg/kg), also two control dosed with vehicles (distal water and corn oil) were used . At the end of two months experiment the animal scarified and histopathological sections of selected organs were examined for all rat groups including (liver, kidney and spleen). The result showed in CCL4 group (shrinkage of glomerular renal tubular , necrosis of epithelial cell lining renal tubule, congested blood vessel, interstitial fibrosis in kidney while liver cell showed necrosis of hepatocyte, vacuolar degeneration, sever central fatty change , congested of blood vessel and fibrosis. No lesion absorbed in spleen of all groups while less kidney and liver cell lesion were observed in groups treated by by oil of dry date and leave extracts indicating partial protection by such extract against oxidative stress induce by CCL4. No histological lesion were observed in kidney and liver of groups treated with methanolic dry date and leave extract that indicate complete protection of these extract against oxidative stress induced by CCL4

Aim of study: To study the histopathological effects and compare the antioxidant of different palm dry date and leave extracts against CCL4 induce oxidative stress in rats

key: leave, dry date, oxidative stress, antioxidant , histopathology lesion.

دراسة نسيجية مقارنة للتأثير المضاد للاكسدة المستحثة ب CCL4 للمستخلصات الكحولية والدهنية للتمر الجاف والسعف (phoenix dactylifera) في الجرذان

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الخلاصة

تم اجرا دراسة حول تأثير المستخلصات الكحولية والدهنية للتمر الجاف والسعف للنخيل العراقي من صنف فيونكس دكتي لايفيرا phoenix dactylifera ضد الاكسدة المستحثة عن طريق تجريع CCL4. اعطيت يوميا عن طريق الفم خمسة مجاميع معالجة تمثل مجموعة سيطرة ايجابية CCL4, مجاميع علاج بالمستخلص الكحولي للاوراق النخلة (150 ملغم / كغم) والتمر الجاف (100 ملغم / كغم) , مجاميع علاج بالمستخلص الدهني لاوراق والتمر الجاف بجرعة 250 ملغم / كغم مع مجموعتي سيطرة سلبية اعطيت ماء مقطر او دهن ذرة ولمدة شهرين. بعد مرور شهرين قتل الحيوانات وتم اجراء فحص النسيجي المرضي لكل من الاعضاء المختارة (الكبد والكلى والطحال) . اظهرت النتيجة في مجموعة CCL4 انكماش انبوبي الكلوي الكببي, التليف الخلالي , التنخر الخلايا المبطنة للنبيبات واحتقان الاوعية الدموية في الكلى بينما اظهرت تنخر خلايا

الكبد وانحطاط فجوي والارتشاح الدهني المركزي , التليف واحتقان الاوعية الدموية في الكبد بينما مجاميع المستخلص الدهني اظهرت نتائج اقل شدة بالتاثير وذلك يشير الى الحماية الجزيئية ضد الاكسدة اما مجاميع المستخلص الكحولي للاورق وثمره النخيل فلم تظهر تغيرات نسيجية وهذا يشير الى الحماية الكاملة ضد الاكسدة.

Introduction

Phoenix dactylifera

The date palm (*Phoenix dactylifera* L.) is one of oldest cultivated plants of human kind and used as food for 6000 years . There are more than two hundred varieties of dates available worldwide especially in Iraq. It is the main crop in Egypt, Saudi Arabia, and Middle Eastern countries. (1)

The fruits are a rich source of carbohydrates, dietary fibers, certain essential vitamins, contain at least six vitamins including a small amount of vitamin C, and vitamins B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin) and vitamin A, and minerals , lipids and protein. Date palm fruits have been an important component of the diet in most of the arid and semiarid regions of the world . (Umar, et al. 2015). Dates are widely used in traditional medicine for the treatment of various disorders e.g., memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders.(2) and as a deterrent and astringent in intestinal troubles. It is also used in the treatment for sore throat, colds, bronchial asthma, to relieve fever, cystitis, gonorrhea, edema, liver and abdominal troubles and to counteract alcohol intoxication. (3)

Materials and Methods

Phytochemicals detection of the active components

Phytochemical tests were carried out on the leave and date alcoholic extracts by using standard procedures to identify the constituents as follows

- Detection of Phenolic according to Harbone,1973 (4)
- Detection of Tannins, Saponin, Resins Components, and Glycosides Components according to Harbone,1984 . (5)
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- Detection of Coumarins Components according to Geissman, 1962 .(6)

- Detection of Flavonoids Components according to Jaffer et al., 1983 (7)

- Detection of Steroids and Terpenoids Components according to Al-Bid, 1985(8)

- Test for alkaloids according to Sofowora, 1993 (9)

Experimental Design

Collection of plant material and preparation of Extract

Phoenix dactylifera fresh leaves, dry fruits collected were washed thoroughly in running tap water, then the leaves was separated, cut into smaller pieces and dried for four week in room temperature then blended to fine powder using a mechanical blender. The dry date was dried at room temperature before grinding it with a meat grinder to produce date paste, that used for alcoholic and oil extraction

Alcoholic Extraction

According to Harbone,J.B. and Mabray ,H.1975 (10) method was done by using methanol 70% and magnetic stirrer for 24 hours then concentrated under reduced pressure at 40°C, 90 rpm using a rotary evaporator.

Leave oil extraction

Extraction of leave oil was done by using hexane and soxhlet apparatus heated at 45°C for period 5- 6 hours under reflex (10– 12cycle/h) after that the extraction solvent was evaporated using a Rotary vacuum evaporator at a temperature 40 °C with 90 rpm according to Charef M et all, 2008 (11) method.

Dry Fruit oil extraction

According soxhlet extraction method by Luque, de.et all, 2010 (12) was done by hexane to which added methanol as modified method achieve by the researcher

this increase the yield of oil extraction 2 fold. Extraction was done under electric thermal at temperature 45 C for 6 hrs during 10-12 cycles. The extract is concentrated by Rotary vacuum evaporator for removing of solvent at temperature 40 C with 90 rpm.

Preparation and dosing of fruit and leave extracts

The doses of extracts were chosen after pilot study for the antioxidant effects of different doses in rats.

The chosen doses were dissolved in distilled water for alcoholic fruit and leave extracts and corn oil for both leave and fruit oil extracts and suitable concentration were prepared and adjusted to be given at a dose volume of 0.1 ml/100 g B.W orally daily for two months using gavages for intubation in rats

Animals

Forty nine adult males of Sprague dawley albino rats weighing (200 - 225 g) and aged between 3-5 months were used in the present study. They have free access to standard laboratory feed and water, with a 8-16 hrs dark / light cycles. The animals were left in optimum conditions for two week for acclimatization in animal house of al kufa veterinary medicine college.

Experiment

Forty nine male adult rats were randomly divided to seven groups treated daily orally for two months according to the following design :

Group I: Control: (-ve) standard diet and distal water orally administered

Group II: control: (-ve) standard diet and corn oil orally administered at 0.1ml/100g

Group III: control (+ve) CCl₄ 100 mg/kg B.W.

Group IV(fruit alcoholic extract group) : CCl₄ (100 mg/kg) + fruit alcoholic extract (100 mg/kg B.W).

Group V(leave alcoholic extract group):CCl₄ (100 mg/kg) + leave alcoholic extract (150 mg/kg B.W).

Group VI (fruit oil extract group) : CCL₄(100 mg/kg) +fruit oil (250mg/kg BW).

Group VII (leave oil extract group) : CCl₄(100 mg/kg) + leave oil (250 mg/kg B.W.)

At the end of two month treatment, all animals were sacrificed after anaesthetization with chloroform by opening abdominal area and the samples of liver, kidney and spleen were removed then putted in 10% formalin, fixed and embedded in paraffin, from which sections were made and stained using haematoxylin-eosin (H & E). (13)..Histopathological changes in liver, kidney and spleen section for each groups were detected.

Results

The results of the phytochemical screening of different leave and date alcoholic and oil extracts are presented in (Table 1) Phytochemical analysis of crude powder of leaf and past dry date of methanol extracts, and crude oil of leaf and dry date palm revealed the presence of tannins, Steroid, phenolic , flavonoid , saponin, coumarin in methanolic and oil extract of leaf and dry date but terpenoid was absent in oil extract of date, the coumarin was more detected in methanol extracts of dry date and more in oil extract of leave and dry date while flavonoid and saponinolic were more present in alcoholic date and leave extracts. Results indicated the presence of glycoside and carbohydrate, in methanol extracts of leave and dry date only while alkaloid and Resin was presented only in oil extract of leave and dry date aslisted in table

Table - active components phytochemicals of the of different leave and fruit alcoholic and oil extracts.

Phytochemical test	Alcoholic Leave extract	Alcoholic date extract	Leave oil extract	Dry date oil extract
Tannins	+	+	+	+
Phenolic	+	+	+	+
Flavonoid	++	++	+	+
Saponin	++	++	+	+
Cumarin	+	++	+++	+++
Glycoside	+	+	-	-
Carbohydrate	+	+	-	-
Alkolioid	-	-	+	+
Resine	-	-	+	+
Steroid	+	+	+	+
Terpenoid	+	+	+	-

(-)negative result (+) obvious result (++) more obvious (+++) very clear result

Histopathology liver

CCl₄ considers a hepatotoxicant agent therefore in the present study CCl₄ group showed histopathological changes in the liver represented necrotic hepatocyte, at the periphery of central vein, vacuolar degeneration of hepatocytes, severe central fatty change, congested blood vessel and severe fibrosis in liver. (Figure 2). Tissue damages and necrosis were not seen in groups treated by alcoholic extracts of dry date and leave + CCl₄ (figure -3,4-) nearly similar to control group (figure -1-). leave oil extract showed less changes include central fatty change (figure-6-), while the group treated with oil extract of leave group were showed hemorrhage, central fatty change, necrosis of hepatocyte and vacuolar degeneration in liver. (figure -5-)

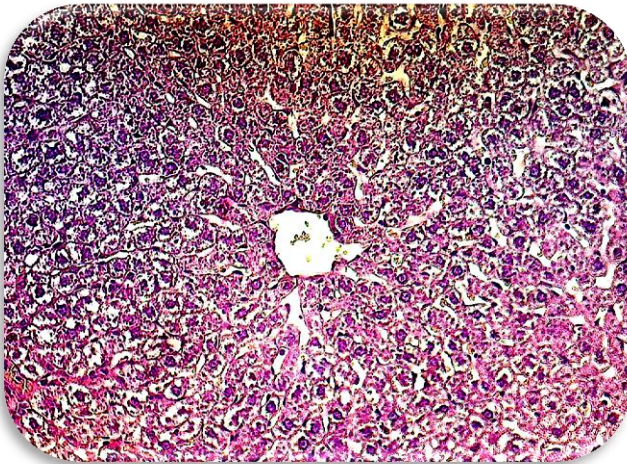


Figure -1- histological liver section of control group distal water showed normal peripheral fatty change liver. H&E stain 40X

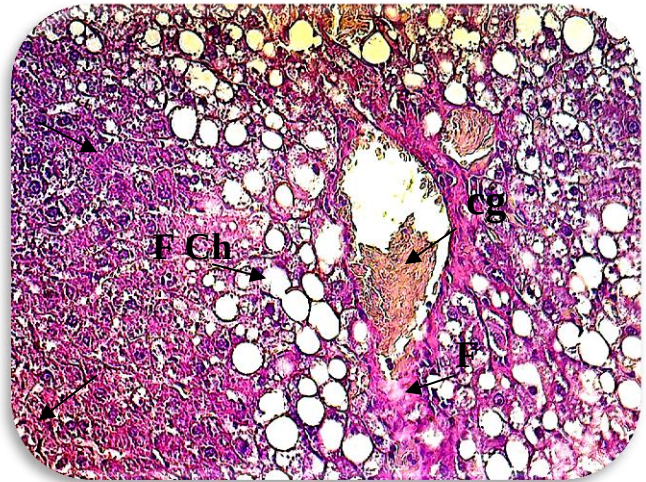


Figure -2- histological liver section of ccl4 treated rats group showed (N) necrosis in hepatocyte (V) vacuolar degeneration (FCh) more central fatty change (cg) congested blood vessel (e) fibrosis in liver. H&E stain 40X

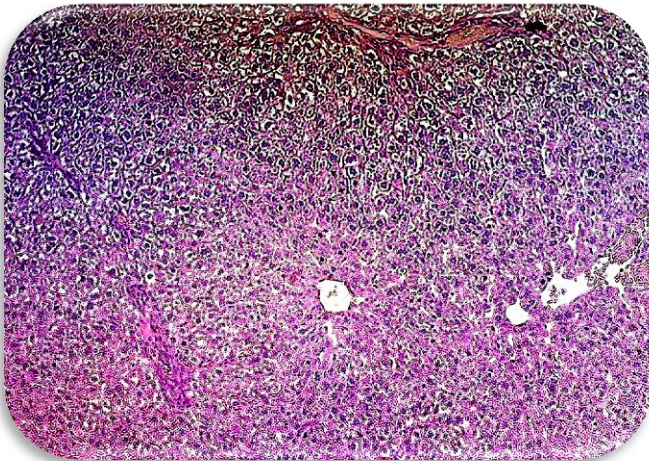


Figure-3- histological liver section of methanolic extract of leave group showed normal peripheral fatty change liver. H&E stain 10X

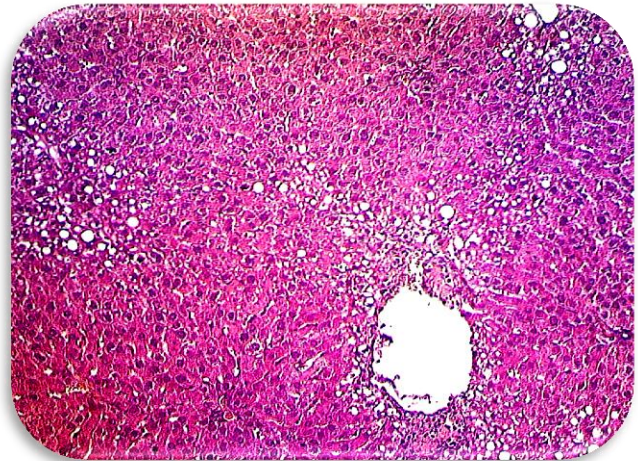


Figure -4- histological liver section of methanolic extract of date group showed normal peripheral fatty change liver. H&E stain 10X

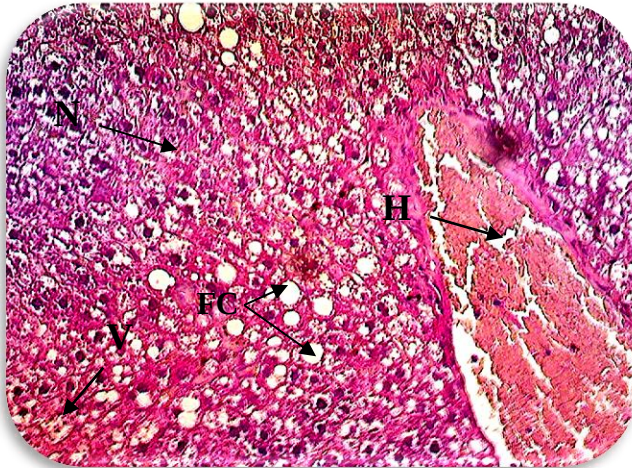


Figure -5- histological liver section of leave oil extract group showed (H) hemorrhage (FCh) central fatty change (N) necrosis of hepatocyte (V) vacuolar degeneration . H&E stain 40X

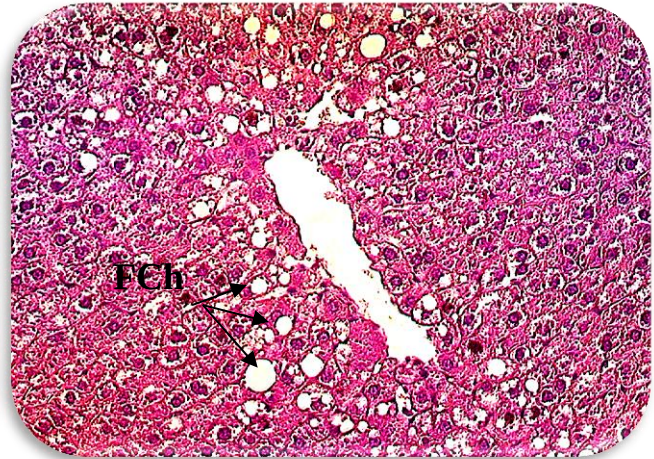


Figure -6 histological liver section of date oil extract group showed (FCh) central fatty change in liver. H&E stain 40X.

Kideny

CCL4 treated group showed histopathological lesion in kidney represented by, shrinkage of glomerular renal tubule , necrosis of renal tubule epithelial cell lining, congested blood vessel, and interstitial fibrosis (figure -8-). Tissue damage and necrosis were not observed in groups treated by alcoholic extracts of dry date and leave with CCL4 similar to control groups (figure -7,9,10-). The group treated with oil extract of dry date showed hemorrhage in interstitial tissue and congested blood vessel (figure -11-). Oil extract of leave groups showed necrosis of renal tubule epithelial cell lining , and interstitial fibrosis (figure -12-)

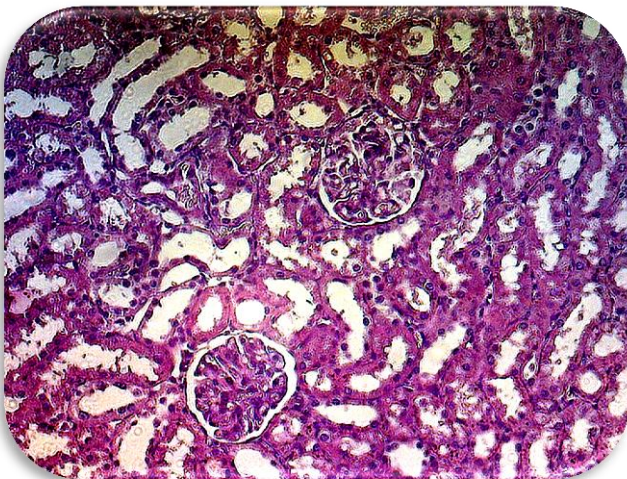


Figure -7- histological kidney section of control (-v) group with distal water showed normal kidney. H&E stain 40 X

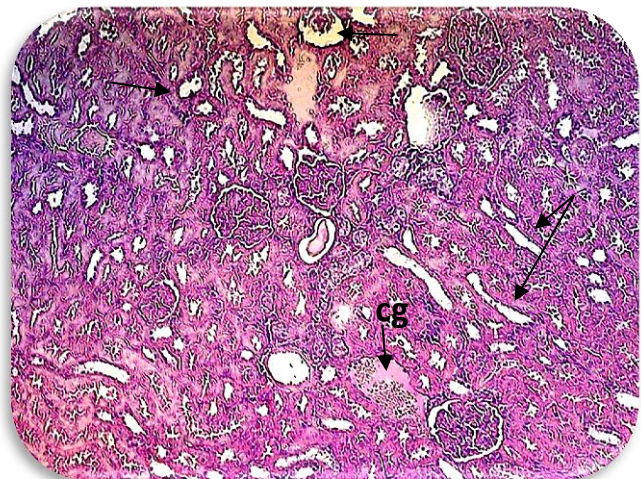


Figure -8- histological kidney section of CCL4 treated rats group showed (Sh) shrinkage of glomerular renal tubule (N) necrosis of epithelial cell lining renal tubule (cg) congested blood vessel (d) interstitial fibrosi. H&E stain 40X

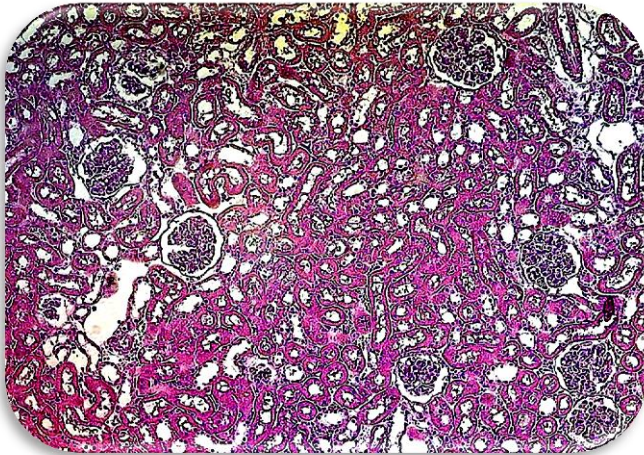


Figure -9- histological kidney section of methanolic extract of leaf groups showed normal kidney. H&E stain 40X

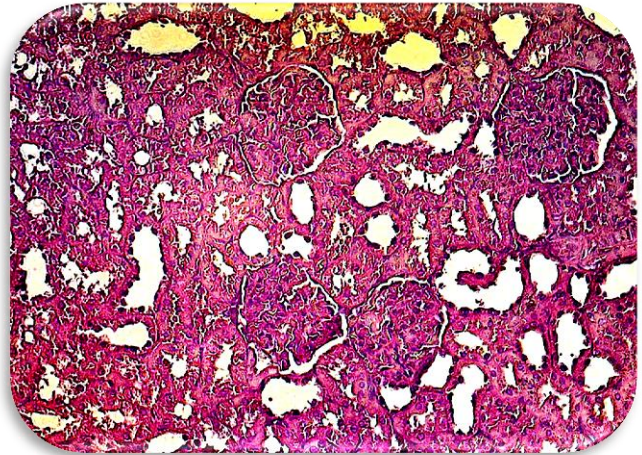


Figure -10- histological kidney section of methanolic extract of dry date group showed normal kidney. H&E stain 40X

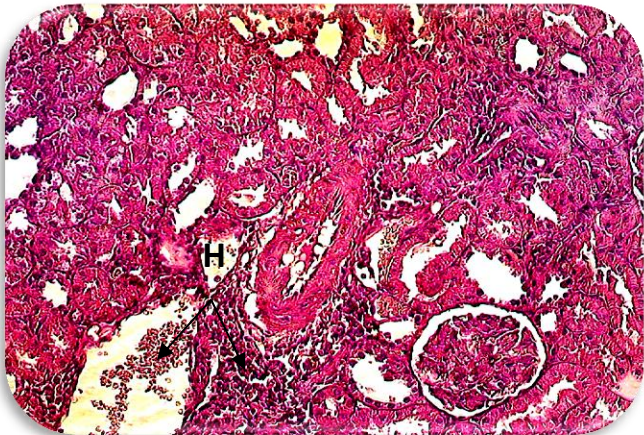


Figure -11- histological kidney section of date oil extract group showed (H) hemorrhage in interstitial tissue congested blood vessel . H&E stain 40X.

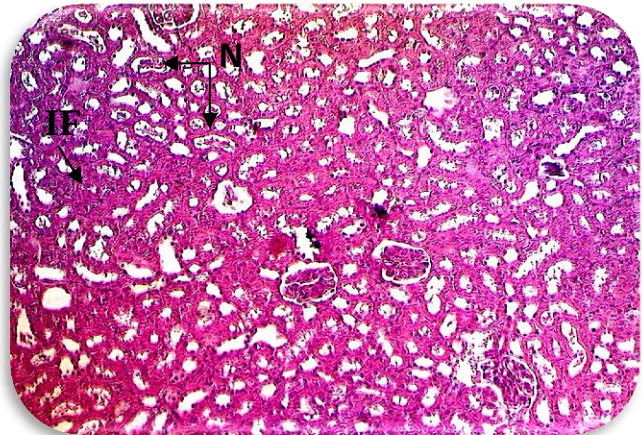


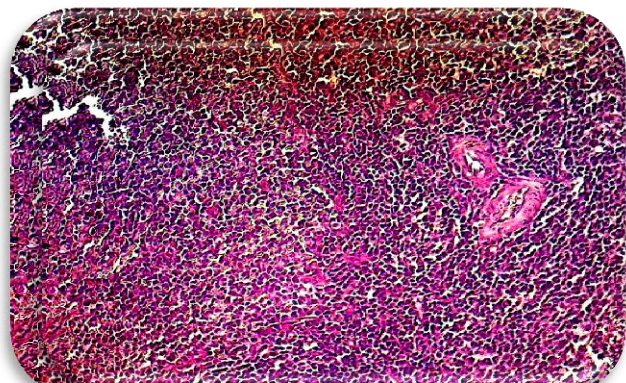
Figure -12 - histological kidney section of leave oil extract group showed (N) necrosis of renal tubule epithelial cell lining (IF) interstitial fibrosis . H&E stain 40X

Spleen

Histopathological section of spleen showed normal no lesion cell with in all groups treated rats as seen figure (13,14,15, 16)



Figure -13 - histological spleen section of methanolic date extract group with normal red and white pulp. H&E stain 40X



85 Figure -14- histological spleen section of control group with normal red and white pulp . H&E stain 40X

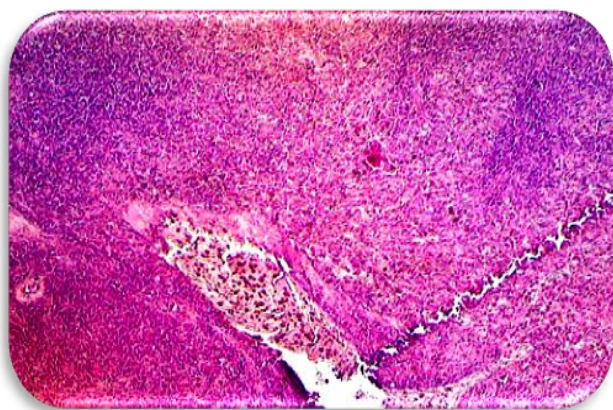


Figure- 15 - histological spleen section of spleen of leave oil extract group with normal cell. H&E stain 10X

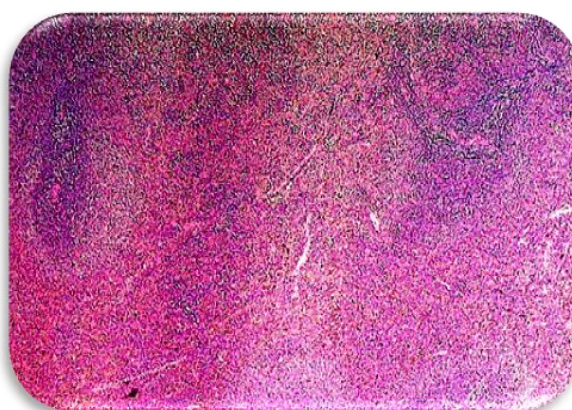


Figure- 16 - histological spleen section of spleen of CCL4 group with normal cell. H&E stain 10X

Discussion

Carbon tetrachloride, having its toxic effect on liver, also reportedly gets distributed at higher concentrations in the kidney than in the liver. The mechanism of CCl₄ renal toxicity is almost the same as that of liver, but CCl₄ shows a high affinity to the kidney cortex which contains cytochrome P-450 predominantly. Due to CCl₄ hepatorenal injury, the transport function of hepatocytes and nephrotic cells gets disturbed resulting in the leakage of plasma membrane. (14)

Following administration, CCL4 is activated by cytochrome P450 system to form trichloromethyl (CCL3) radical. This radical binds to cellular molecules (nucleic acids, proteins and lipids) thereby impairing crucial cellular processes such as lipid metabolism, with the potential outcome of fatty degeneration, while the reaction between trichloromethyl (CCL3) radical and DNA is thought to function as initiator of hepatic cancer. This radical can also react with oxygen to form the trichloromethylperoxy (CCL3OO) radical, a highly reactive species. This compound initiates the chain reaction of lipid peroxidation, culminating in destruction of polyunsaturated fatty acids, especially those associated with

phospholipids. This leads to alteration of permeabilities of mitochondrial, endoplasmic reticulum and plasma membranes, resulting in the loss of cellular calcium sequestration and disruption of calcium, homeostasis with subsequent cell damage. (15)

Antioxidants are substances capable of counteracting the damaging effects of oxidation in body tissues. Antioxidants are divided into two classes based on mechanism of action: chain-breaking antioxidants, such as Vitamin E and beta-carotene, “break the chain” of free radical formation by donating an electron to stabilize an existing free radical and preventive antioxidants are enzymes that scavenge initiating radicals before they start an oxidation chain. (16).

The results recorded no lesions in spleen of all groups while lesions in kidney and liver cell were less observed in groups treated by dry date and leave oil extracts indicating partial protection by such extract against oxidative stress induced by CCL4. Nearly no histological lesion were observed in kidney and liver of groups treated with methanolic dry date and leave extract that indicate complete protection of these extract against oxidative stress induced by CCL4 that caused more histopathological

lesions in kidney and liver cells of CCL4 treated group. Such lesions in liver and kidney were also recorded by many studies (17, 18 & 19) which attributed the cause of such effects to the formation of highly reactive ROS metabolite inducing oxidative stress or lipid peroxidation in liver or kidney cell membrane. (20), (21) and (22)

The groups treated by CCL4 + oil extract of fruit and leave were showed lessens histological changes in liver and kidney possibly due to the partial protective effect against the CCL4 liver and kidney cell damage induced in rat attributed to extracts antioxidant components including flavonoid, saponine phenol, cumarin, alkaloid and resin these play role to reduce lesion in liver and kidney. (23), (24) & (25)

Phytoconstituents like the flavonoids, triterpenoids, saponins and alkaloids are known to possess hepatoprotective activity. The presence of flavanoids in our extract may be responsible for its antioxidant and thus hepatoprotective activity. Numerous studies have suggested that flavonoids commonly function as antioxidants and may protect plants against oxidative stress caused by suboptimal environmental conditions. (26) and (20)

Current studies on flavonoids from various plant extracts disclosed their antioxidant properties and showed their stimulatory action on cellular enzymatic antioxidants. Moreover it is reported that some flavonoids put forth a stimulatory act on the gene expression of certain cellular enzymatic antioxidants. (27) and (28)

The histological changes in the methanolic extract of dry date and leave - treated groups were quite similar to that of the control group indicating that the alcoholic extract given complete protection against CCL4 induce cellular oxidative damage in rats possibly because alcoholic extract have more antioxidant compounds than that from

the oil extracts. The results of phytochemical indicate the presences of more polyphenol flavonoid, cumarin and saponin components in alcoholic extracts more than that oil extracts with only presence of carbohydrate and glycoside in these extracts may give them more potential antioxidant effect than the date and leave oil extract. This explain the nearly complete antioxidant protection of such extract over the oily one that give partial effect.

The results of effect of CCL4 and palm extracts reveal normal spleen sections that may be attributed to either the dose of CCL4 was not enough to cause histopathological effect or spleen have its own antioxidant effect even against the powerful CCL4 oxidant effect possibly because spleen may get benefit from the antioxidant component of the decomposed RBC giving it more oxidative resistant ability.

Conclusion

The present study has shown that alcoholic and oil extracts of fruit and leave had protective effect against CCL4-induced oxidative changes in the liver and kidney. This effect may be due to the rich vitamins and antioxidants factors for example, phenolic and flavonoidal compounds in the extract. This study highlights the interest to change toward the use of natural medicinal plants with antioxidant activity for protection against diseases.

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