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Histopathological Study of *Ricnius Communis* Methanolic Extract on Different Albino Male Mice Organs

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Abstract

The objective of this study was to considered as an explorer for In vivo studies on the production of some secondary metabolites from local medical plants named Ricinus communis to study their effect on mice organs (In vivo). Preparation of methanol extract for seeds primary indicators for chemical secondary metabolites groups were used to identify the chemical compounds exist in seed obtained. Three doses were used in this study (0,005, 0,01 , and 0,05 mg\kg) which represented 10,20 and 30 % of LD50 of plant which were given intra peritoneal for seven days. These doses tested on liver function enzymes (aspartate aminotransferase{AST}, alanine aminotransferase{ALT} and alkaline phosphotase {ALP}) and kidney function test (Albumin , Creatinin and Urea Concentrations) and in sera of albino male mice and on histology of mice organs (liver, kidney and spleen). The Results revealed a very low cytotoxic activity of the crude extracts in the all liver function enzymes (L.F.E.) and kidney function test (K.F.T) and liver tissue and spleen tissue and no cytotoxic activity in kidney tissue.

Key words: castor oil, Ricin, liver function enzymes, kidney function test.

INTRODUCTION

The species Ricinus communis be in the right place to family Euphorbiaceae, monotypic genus, Ricinus, and subtribe, Ricininae. It is commonly famous as 'castor plant (1). It is originating all over the country and commonly cultivated in the tropics and warm regions (2). The plant can grow up to a height of 6 to 15 feet and be able to leave for many years. The large, palatably lobed leaves can be over 20 inches. The seeds hold approximately 46- 60% oil besides are the only commercial source of ricinoleic acid that is used as industrial emollients, dyes, coatings, and plastics (3). R. communis has very high traditional medicinal values. The oil extracted as of the seeds of this plant has been used by native people since about 2000 BC. (4). The use of diverse parts of this plant for the treatment of numerous diseases in traditional or folk remedies all over the world has been reviewed (5). *R. communis* has antioxidant activity because has significant radicals scavenging abilities on 2,2dipehyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) (6). Also *R. communis* has antidiabetic activity according to World Health Organization (WHO) has mentioned the evaluation of traditional plant treatments for diabetes as they are active, non-toxic, with less or no side effects (7). Main component of castor oil also showed remarkable analgesic and anti-inflammatory effects (8). Anti-hepatotoxicity: The hepatoprotective activity is the capability to protect liver damage. Liver cirrhosis and drug induced liver damage are major health problem in western and developing countries . Ricin is a heterodimeric protein isolated from the seeds. It holds cytotoxic activity by virtue of its aptitude to fatally disrupt protein synthesis. Therapeutically, it be able to be used to specifically target and put an end to cancer cells (9).

METHODS

Plant Collection

The seeds of Plant were collected from the local market in Baghdad during the period between sep., till Oc., 2017. The plant was previously identified by Iraqi center of herbs then it homogenized to fine powder by using electric mill and then stored in airtight bottles.

Plant extraction

The seeds of plant (20 g) were extracted with 250 ml of methanol (70%) by soxhlet extraction
apparatus for 6 hours at 55C (10). After that the extract filtered and evaporated by using rotary evaporator to dry extract and stored at 4c until used.

Detection of active compounds

Detection of Tannins

The plant powder (10 gm) dissolved in about (50 ml) distilled water by using a magnetic stirrer, the mixture was left in a boiling water bath for few minutes, at that time few drops of 1% lead acetate solution were added, the development of Gelated precipitate (greenish-blue) was an indication of the existence of tannins (11).

Detection of Saponins

A) Foam tests for aqueous: Thick foam existence as an indication.

B) Aqueous mercury chloride 1%: (5 ml) of plant extract was added to (1-4 ml) of indicator the existence of white precipitate was an indication of saponin (12).

Detection of Flavonoids

Methanolic extract of plant was separated with petrolium ether; the aqueous layer was mixed with the ammonia solution. The appearance of dark color is an indication for the presence of flavonoids (13).

Detection of Terpenes

This indicator used to identify for the terpenes usually about (1 ml) of chloride antimon chloroform 20% was added to (5 ml) of plant extract, the appearance of white precipitate indicated the existence of terpenes (13).

Determination of toxicity of methanol extract of *R.communis* on albino male mice:

Plant Tested Doses:

The selection of these doses which used in this research were (10, 20 and 30%) of LD50 (20 mg /kg) which determined in according to EFSA journal (2008).

Experimental animals: -

Sixteen adult males' albino mice were used. Their weights at the beginning of experiment were (22- 28g). These mice

which were obtained from General Company of veterinary medicine, maintained in well-conditioned room, on special pellets regime and water. The animals were separated into four groups, three group received one of the graded doses of the methanol extract, and fourth group was control negative.

Mice were randomized into four groups; each groups contained four mice as explain below:

-Group 1: mice were administrated with D.W (control group 4 animals)

-Group 2: mice were administrated with first dose 10% of *R.communis* methanolic extract (4 animals)

-Group3: mice were administrated with the second dose 20% of *R.communis* methanolic extact(4 animals)

-Group4: mice were administrated with the third dose 30% of *R.communis* methanolic extract (4 animals)

In order to repair these doses, the plant extract dissolved at few drops of DMSO and then completed by distilled water to reach the required doses which injected intraperitoneally (0.1 ml) for seven days as single dose\day. Finally mice were sacrificed at day 8 for laboratory assessments.

Determinations of Hepatoprotective and Kidney protective effects

For Hepatoprotective determinations, the parameters of assessment were ALT, AST and ALP enzymes in serum, as well as histopathological evaluation of liver tissue.

For Kidney protective determinations, the parameters of assessment were albumin, creatinin and blood urea.

For both assessments a commercials kits were used (Randox Company).

Blood was collected via heart puncture, collected in Eppendorf tube and allowed to clot at room temperature for 15 minutes; the serum was separated via centrifugation at 3000 rpm for 10 minutes (14). Later on, it was used for the evaluation of liver function enzymes (aspartate aminotransferase; GPT, alanine aminotransferase; GOT), in addition to alkaline phosphatase (ALP) and kidney function test as well as mice organs were obtained and stored in formalin (10%) for histological examinations (15).

Alanine Amino-Transferase (GOT)

The activity of enzyme of ALT was determined in mouse serum according to (16), as in GOT determination using a commercial kit (Randox Company). GPT activity (Unit/L) was calculated from the kit standard curve.

Aspartate Amino-Transferase (GPT)

The enzyme activity of GPT was calculated in mouse serum according to evaluation method of (17). For this purpose, a commercial kit (Randox Company) was used. The activity of GPT (Unit/L) was calculated from the kit standard curve.

Alkaline Phosphatase (ALP)

The ALP enzyme was measured in mouse serum using a specific kit manufactured by Bio Merieux Company and the greatest traditional way used is that of (18) in which di-sodium phenyl phosphate is hydrolyzed with liberation of phenol and formation of sodium phosphate. The quantity of phenol formed is determined calorimetrically.

C. Calculations

The following equation was employed to assess the activity of ALP.

$$\text{ALP Activity (Unit/ml)} = \left(\frac{\text{Sample Absorbance - Control Absorbance}}{\text{Standard Absorbance - Blank Absorbance}}\right)$$

Histopathological Study

Samples were obtained & cut into small pieces (2×2×2 mm.) then pre-fixed in 2.5% gluteraldehyde diluted in phosphate buffer PH (7.4).

After that specimen were rinsed in the same buffer for several times and left in PBS for 12 hrs, and the procedure of (19) was followed to prepare histopathological sections. The procedure is outlined as follows:

• Washing: specimen was rinsed in the same buffer for several times and left in PBS for 12 hrs, and then specimens were post- fixed in 1% osmium tetra oxide for 1 hr.

• Dehydration: The sample was dehydrated with ascending concentrations (50, 70, 90 and 99%) of ethanol. There was (2 hours) for each concentration.

• Clearing: The sample was located in xylene for two hours.

• Infiltration: The sample was first located in paraffin-xylene (1:1) for 30 minutes at 57-58ºC, and then in paraffin alone for (2 hours) at 6070ºC. • Embedding: The sample was embedded in pure paraffin wax (melting temperature: 57-58ºC) and left to solidified at room temperature.

• Sectioning: The paraffin block was sectioned (rotary microtome) at a thickness of 5 microns, and then the sections were transported to a slide covered with Mayer's albumin. The slide was located in a hot plate (35-40ºC) for 1-2 hours.

• Staining: The slide was first placed in xylene for 15-20 minutes, descending concentrations (90, 80 and 70%) of ethanol (two minutes for each concentration) and finally distilled water. After that, the slide was stained with heamatoxylin for 1020 minutes and then washed with distilled water for 5 minutes. Then, the slide was placed in acidic alcohol for one minutes and washed with distilled water. After washing, the slide was placed in eosin stain for two minutes, then in ascending concentrations (70, 80, 90 and 99%) of ethanol (two minutes for each concentration). As a final point, the slide was cleared with xylene for 10 minute.

• Mounting: Mounted the slide was with a Canada balsam and covered with a cover slip. Then, the slide was examined microscopically to inspect the histopathological change

RESULTS

Chemical tests for the active components in *R. commuins*

The detection of active components in plant seeds demonstrated the following (Table 1) seed extract with methanol (70%) and distilled water containing the following:

Table (1) Detection of some active compounds in *R.communis* seed extracts

Test	Reagents	Indicator	*Ricinus communs* seeds
Tannins	Aqueous lead acetate	Gelated precipitate Blue – green color precipitate	positive
Flavonoids	Petrolium ether + ammonia	Yellow color	positive
Terpens	Chloride antimony chloroform	White precipitate	negative
Saponins	Foam test for aqueous mercury chloride	Thick foaming White precipitate	negative

Table (2): Effect of *Ricinus* on (L.F.E.) (aspartate aminotransferase{AST}, alanine aminotransferase{ALT} and alkaline phosphotase {ALP}) in sera of albino male mice.

Different letters: Significant difference ($P \leq 0.05$) between means.

Groups	Dose (mg\kg)	AST (U\L) Mean± S.E.	ALT (U\L) Mean± S.E.	ALP (U\L) Mean± S.E.
Control negative(I)	--------	45.02 ± 0.81^{A}	40.05 ± 0.57^{A}	71.67±2.60 ^{A}
Ricinus (II)	0.005	15.33±1.45 ^{D}	28.34±1.20 ^{C}	40.02±1.14 ^{B}
Ricinus(III)	0.01	20.31±0.88 ^{C}	33.01±1.53 ^{B}	41.67±2.60 ^{B}
Ricinus(IV)	0.05	24.67±0.89 ^{B}	40.11±1.15 ^{A}	72.66±3.92 ^{A}

Table (3): Effect of *Ricinus* on kidney function test (K.F.T.) (Albumin, Creatinin and Urea concentrations) in sera of albino male mice

Groups	Dose (mg\kg)	Albumin concentration(g\dl) Mean± S.E.	Creatinin concentrations (mg\dl) Mean± S.E.	Urea Concentrations (mg\dl) Mean± S.E.
Control negative(I)	-------	5.13±0.24 ^{A}	1.46±0.12 ^{AB}	44.83±2.01 ^{A}
Ricinus (II)	0.005	2.80±0.05 ^{B}	1.67±0.24 ^{A}	42.73±1.88 ^{AB}
Ricinus(III)	0.01	2.40±0.23 ^{B}	1.03±0.88 ^{B}	37.73±1.18 ^{B}
Ricinus(IV)	0.05	2.36±0.12 ^{B}	1.30±0.20 ^{AB}	38.91±0.57^{B}

Different letters: Significant difference ($P \leq 0.05$) between means.

Determination of Liver function enzymes (L.F.E.)

The AST activity in untreated mice (control I) was 45.02 ± 0.81 Unit/L, but these activity was significantly ($P \leq 0.05$) decreased to (15.33±1.45, 20.31±0.88 and 24.67±0.89 Unit/L) after mice treated with ricinus for three doses tested (0.005, 0.01 , and 0.05 mg\kg) respectively. Also, The same results were obtained for ALT in which significant reduced in concentration of that enzyme when mice treated with the first and second doses (28.34±1.20, 33.01±1.53) in comparison with negative control (40.05 ± 0.57 U\L). In addition to that, ALP concentrations were significantly reduced when mice treated with first and second doses respectively (40.02±1.14 and 41.67±2.60 U\L) as compared to negative control (71.67±2.60 U\L) (Table:

Determination of Kidney function test (K.F.T.)

The concentration of albumin was significantly ($P \leq 0.05$) reduced in mice after treatment with all doses of ricinus as compared to control negative (2.80±0.05, 2.40±0.23and 2.36±0.12 *vs*. 5.13±0.24 g/dL), and the results of creatinin indicated that the second and third doses of ricinus were able to reduces creatinin concentration in comparison to negative control (1.03±0.88, 1.30±0.20 *vs* 1.46±0.12 g\dl). The results of urea concentration explained that all doses of Ricinus had the ability to decrease urea in sera of mice in comparison to negative control **(**42.73±1.88**,** 37.73±1.18 and 38.91±0.57 *vs* 44.83±2.01mg\dl).

Histological Effects of *R. communis* on different mice organs

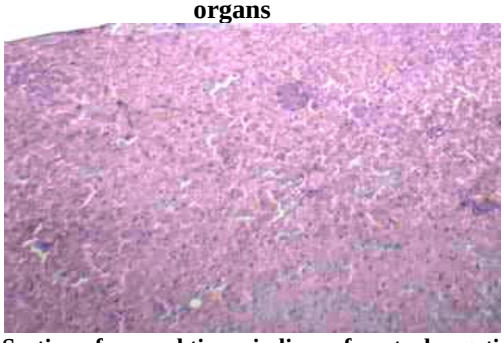

Figure1.Section of normal tissue in liver of control negative of mice (100 X).

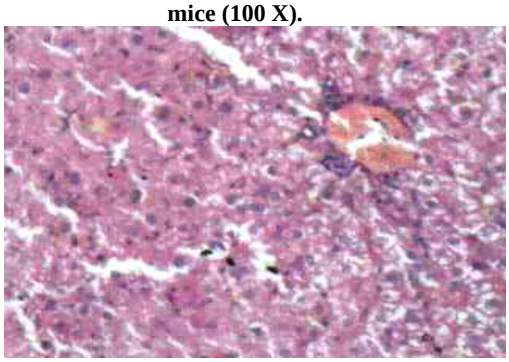

Figure 2. Liver tissue exposure to 10% (0.005 mg\kg) of crude extracts (200 X) showing mild degeneration changes on it.

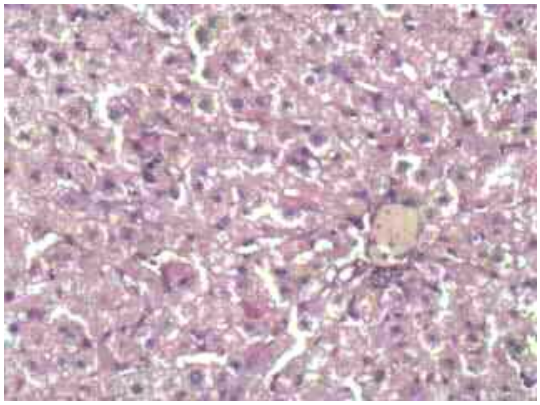

Figure 3. Liver tissue expose to 0,01mg/kg (20%) concentration of crude extracts (x200) showing mild to moderate degenerative changes.

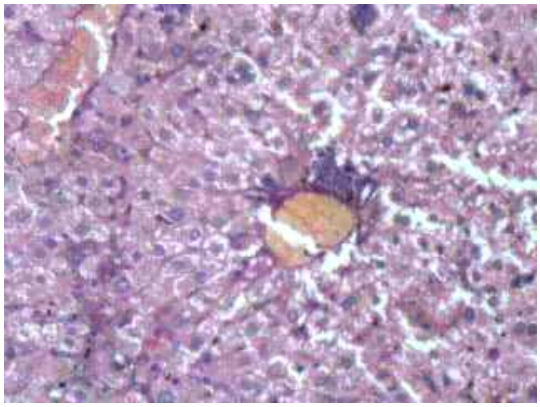

Figure 4. Liver tissue exposure to 30% concentration of crude extracts (x200) with moderate degenerative changes.

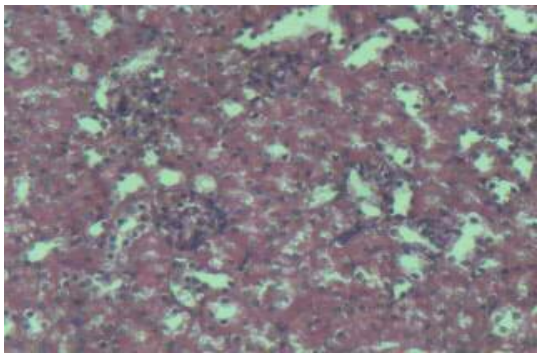

Figure 5. Section of control kidney tissue of mice (normal) (x100)

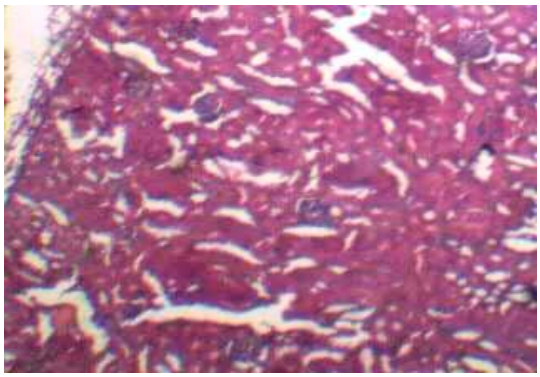

Figure 6. Kidney tissue exposure to 10% concentration of crude extracts (x100) showing no changes.

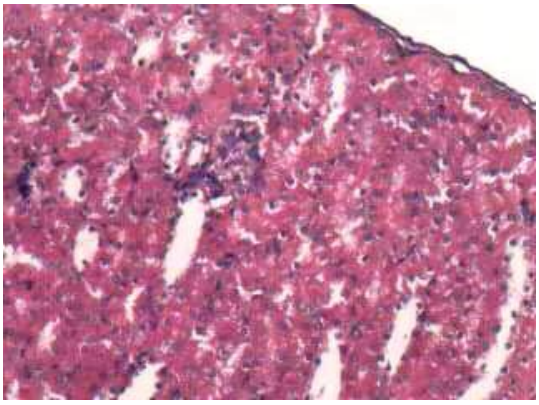

Figure 7. Kidney tissue exposure to 20% concentration of crude extracts (x200) showing mild tubules changes.

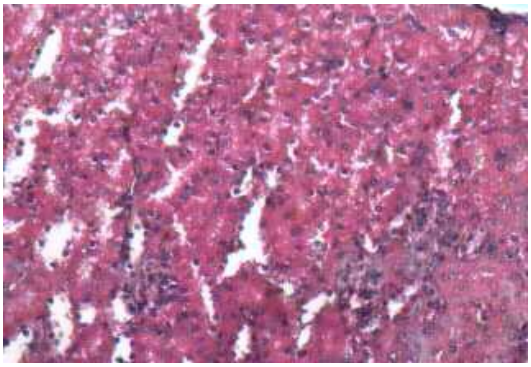

Figure 8. Kidney tissue exposure to 30% concentration of crude (x200) extracts showing mild to moderate degenerative changes.

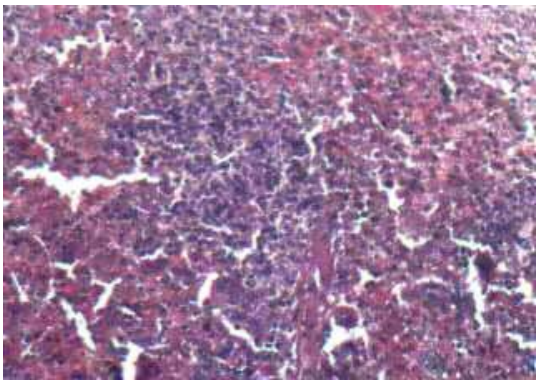

Figure 9. Control spleen tissue of mice (normal) (x100)

Figure 10. Spleen tissue exposure to10% concentration of crude extracts (x200) showing light widening of white pulp.

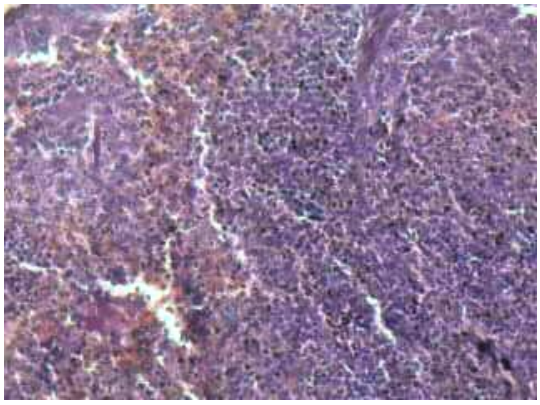

Figure 11. Spleen tissue exposure to 20% concentration of crude extracts (x100) showing mild degenerative changes with hemorrhage.

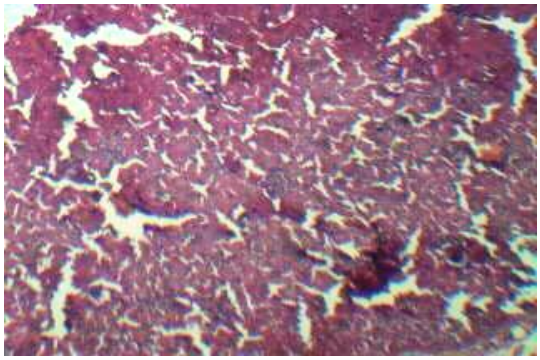

Figure 12. Spleen tissue exposure to 30% concentration of crude extracts (x200) showing degenerative necrosis of pulp tissue with hemorrhage.

DISCUSSION:

The state of imbalance between the level of antioxidant defense system and production of ROS called (Oxidative stress), such as superoxide radical (O^{2-}), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2). Further than the techniques with artificial pharmacology, the search as well chases alternative techniques that depend on natural products. In particular, it targets those plants with famous medical history or confirmed prospective of positive results against the sicknesses of the liver or other body parts (20). Approximately herbal extracts are known to avoid the oxidative damages in different organs by changing the levels of cytochrome P-450 through their antioxidant properties (21). *Ricinus* methanol extract caused a significant decrease in serum ALT, AST and ALP activities in comparison to negative control. This supported the ability of methanol extract anti-oxidants mechanism in down-regulating ROS, DNA damage inhibiting, and attenuating protein and lipid oxidation (22). Also, the significantly reduced the level of these marker enzymes by the plant extract can be a consequence of stabilization of plasma membrane, in addition to repair of damaged hepatic tissue (23). This protective effect has remained attributed in part to flavonoids, through modulation of several enzymes of the P450 family complicated in pre-carcinogen metabolism. Therefore, the suggestion is that this effect is caused by flavonoids, although a possible synergistic or antagonistic role of flavonoids with additional compounds in the extracts cannot be excluded. Flavonoids possess potent hepatoprotective activities and were conjectured as the main active contents (24). Phenolic compounds can also contribute directly to antioxidant action, because of their redox properties, which let them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (25). Also, the essential oils showing that the oil could scavenge the reactive free radicals that finally reduced the oxidative damage to the tissues and subsequently enhanced the level of this anti-oxidant (26). Also, sesequterpene has the ability to reduce the oxidative stress and prevented any damage via inhibiting the critical control points of apoptosis (27).In addition to all that, the essential oil administration reduced the oxidative hepatic injuries such as necrotic aggregations (28). The literature also revealed that the plants containing flavonoids and triterpenoids can control liver diseases. Therefore, the hepatoprotective activity of *Ricinus* may be attributed to aforesaid classes of compounds present in it (29).

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