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The Protective Effect of different Feeding Methods of Ethanolic Extract of *Rosmarinus officinalis* on Overdose Toxicity of Hepatorenal induced by Paracetomol

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Abstract

The present work was achieved to investigate the protect effect of ethanolic extract from the leaves and stems of *Rosmarinus officinalis* in reducing the toxicity of paracetamol in liver and kidney. Ethanolic extract was obtained from maceration of powdered leaves and stems of *R. officinalis* with ethanol (90%) at room temperature. The Histopathological experiments were done on 24 male albino rats in Karbala University /animal house of Pharmacy College for two months, and the research was done with the agreement of the animal ethical committe in the college, the rats randomly divided to four groups: Group1: control group feeder on the standard diet.Group2: drenched 1 ml/Kg 1000 mg of paracetamol Solves with water (normal saline) 85% every day for six weeks. Group 3: drenched by gavage needle 220 mg/Kg alcoholic extract of rosemary, and 1 ml/Kg 1000 mg paracetamol every day for six weeks. Group 4: drenched 1ml /Kg 1000 mg of paracetamol for two weeks followed by oral administration of rosemary extract, every day for six weeks. As results, group 3 that were treated with combination of *Rosmarinus officinals* extract and toxic dose of paracetamol, showed low significant protective effect to reduce the toxicity in the liver and kidney. The Group 4 that was treated with *Rosmarinus officinals* extract alone exhibited high significant protective activity against paracetamol-induced liver and Kidney damages.

Key words: *Rosmarinus officinalis*, Protective Effect, Hepatorenal, Paracetomol, Overdose Toxicity

1. INTRODUCTION

Today, many medicinal drugs are used and consumed, without knowing the side effects and health risks of the frequent use. One among them is paracetamol as an over-the-counter product, which often used to get relief from headache, fever and certain pains such as muscle aches, arthritis, backache, toothache and cold [Manchanda *et al.*, 2013]. However, recent reports indicate that big overdoses of paracetamol can produce toxicity. In 2005, the most commonly toxicity reports in the United States were from toxic ingestion of paracetamol, there were 67,000 reports and 37% of these were occurred in children. The toxicity overdose of paraecetamol was reported to cause hepatic necrosis and acute renal failure. [Mazer and Perrone, 2008]

Although Modern medicine is progressing, there is no synthetic medication available to treat hepatic disorders. Even though, there are a several herbal formulations claimed to hold beneficial activity in remediation hepatic disorder [Ramachandra Setty et al., 2007]. In one of our field surveys we found that a widely distributed in the Mediterranean region plant, *Rosmarinus officinalis* is a medicinal and aromatic plant, which belongs to the genus Rosmarinus of Lamiaceae family. Rosemary is recommended plant, to be widely employed in folk medicine and pharmaceutical products as a digestive, astringent, diuretic, tonic, and diaphoretic, in addition it can be used for urinary ailments treatments. Recently, interested bioactive phytochemicals have been isolated from rosemary, including a diversity of phenolic components like carnosol, carnosic acid, rosmanol, 7-methyl-epirosmanol, isorosmanol, rosmadial and caffeic acid. The components were used as anti-oxidant effective. [Cui *et al*., 2012, Machado *et al*., 2013]

The present work aimed to show the effect of different feeding of alcoholic extract from *Rosmarinus officinalis* in treatment and protective effect of liver and kidney damage caused by acetaminophen over dose.

2. EXPERIMENTAL

2.1 Plant Materials

The leaves and stems of Rosemary officinalis were collected in August 2016 from garden of medicinal plants of Pharmacognosy department, Pharmacy College, Karbala University in Iraq. The species was identified by Dr. Ibraheim Saleh Abbas of the Pharmacognosy department, pharmacy faculty, Al-Mustansiriya University.

2.2 Extraction of Leaves of Rosemary officinalis

Leaves and stems (450.0 g) of *R. officinalis* were dried, powdered and, then soaked with ethanol (90%, 3.0 L) for 7 days at room temperature. The crude extract was filtered using filter paper. The plant residues were again soaked in 3.0 L of absolute ethanol for 5 days. The filtrates of ethanol 90% and absolute ethanol were combined, and evaporated to dryness using water bath at 60°C, to obtain 100 g of *R. officinalis* (R.O.).

2.3 Experimental of Histopathology

2.3.1 Animals and treatments

Albinos mature male rat's Rattus norvegicus were used in this study. These animals weighing 250-350 g ±5 gm and (3-4) months old*, were kept in the animal house of pharmacy collage university of Karbala, housing conditions were maintained at 28 ± 2°C for 7days.

The litters of cages were changed every second days. They were given a rodent diet supplement with a Vit.C and supplied with water ad-libitum. The experimental animal randomly divided into four groups: Group1: control group. Group2: drenched 1ml /Kg 1000 mg paracetamol Dissolves with water (normal saline) 85% every day for six weeks.

Group3: drenched 220mg/Kg alcoholic extract of Rosmarinus, and 1ml / Kg 1000 mg paracetamol every day for six weeks. Group4: drenched 1ml /Kg 1000mg of paracetamol for two weeks

next that given oral drenched of rosemary extract every day for 42 days.

2.3.2. Preparation of Histopathological Study

Sample was taken from liver and kidney after killed the animal by high dose of anesthesia, histological sections were prepared according to Luna [Luna, 1992] for histological study and as follow:

In the histological methods we take the samples (1 cm) from the fixative organs in formalin 10 % to washing for 5 minutes in tap water to remove the fixative effect , then making the steps of routine histological technique which include the dehydration by serial of progressive concentrations of ethanol (50% - 100%) , the clearing by using the xylene or chloroform , infiltration by using paraffin wax path , embedding by paraffin wax blocks , sectioning by using Rotary Microtome to thin 6-7 micrometer plates which placed in water path and then placed in glass slides to become ready to staining by using the (Hematoxline - Eosine stain) After that the histological sections examined by light microscope to study the histological changes may be occurred in these sections prepared from the animals of control and three treated groups.

3. RESULTS

The results of histopathological investigation for treatment and protective effect of *R. officinalis* on the liver and kidney were displayed as follow:

Group 1 The histological examination of the liver and kidney in native group (control). The liver appears properly and naturally in the central vein and liver cell, the kidney sections exhibit normal histological structure of the glomeruli and tubules at the cortex (Fig 1-2 a,b).

Group 2 Histological sections taken from the liver of rats, which were given with paracetomol only through the gastrointestinal tube, were degenerated into hepatic degeneration of hepatic cells (Fig.3-a), Accompanied by acute congestion and enlargement of the hepatic portal vein in addition to fibrosis in peri-ductal tissue around the bile ducts dilatation, and there is a vacoulation in the hepatocytes lead to (Fig.3-b). Entire separation with further necrotic cell, and pyknotic nuclei.

The microscopic structure of kidney tissue shows suffering from atrophied and endothelial cells contain gaps in it (Fig.4-a). In addition, there is bleeding in the interstitial tissue and strong congestion in the blood vessels), (Fig.4-b). The cells lining the proximal and distal convoluted tubules in the kidney suffer from necrosis.

Fig 2(a) The precise histological structure of the kidney in the control group shows a normal and proper structure of both cortex, glomeruli **g** and tubules **t** (H&E, 100).

Group 3 In liver, reduction in hydropic degeneration in the hepatocytes with mild infiltration of inflammatory cells and there is moderate dilatation in portal area with few congestion. (Fig.5)

Kidney revealed normal intact kidney tissue morphology with tubules (Fig.6)

Administration rosemarry extract lead to dramatically decrease the toxic effect of paracetamol in liver and prevent occurs any little thing in the kidney.

Group 4: showed full protection against paracetamol induced damage and show normal liver and kidney without pathological altration (Fig.5-6).

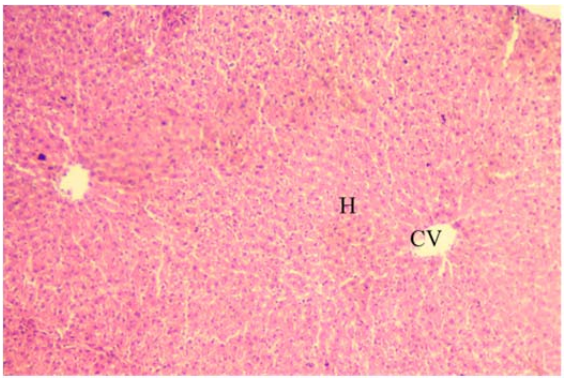

Fig 1 Microscopic appearance of liver in native group (control) demonstration the histological structure of both hepatocytes **H** and central vein **CV** appears (H & E 100).

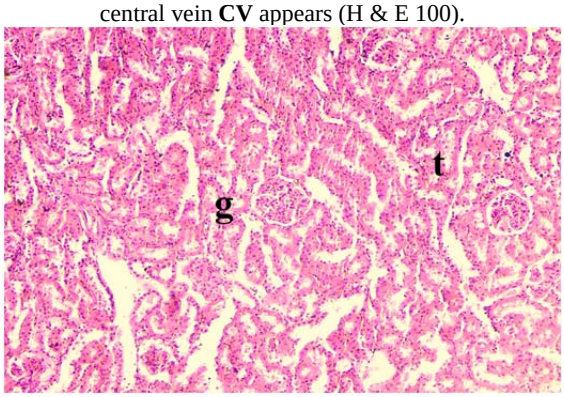

Fig 2(a) The precise histological structure of the kidney in the control group shows a normal and proper structure of both cortex, glomeruli **g** and tubules **t** (H&E, 100).

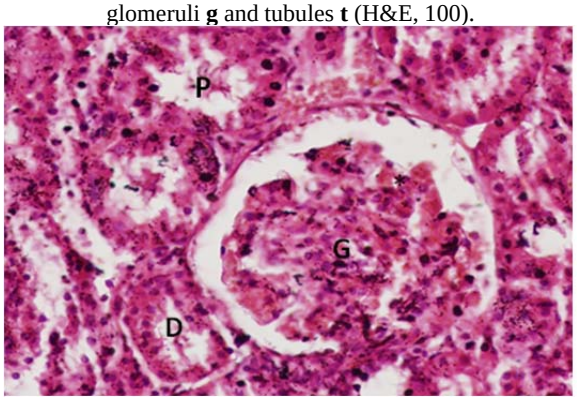

Fig 2(b) Kidney of the control group (group 1), sections demonstrating glomerular capillaries g, Proximal **P** and distal tubules **d**, ((H&E, X100).

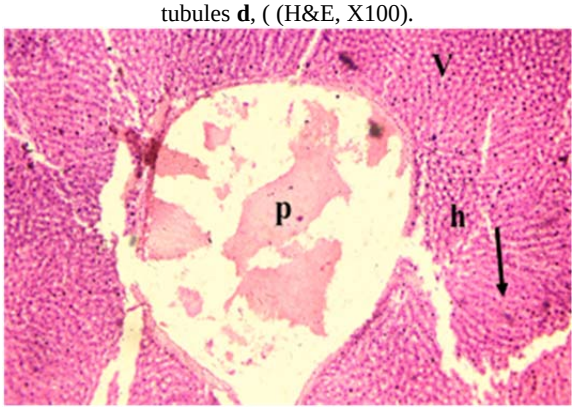

Fig 3-A Microscopic appearance of liver (group 2), demonstrating enlargement in portal area (p) hepatocyte suffering hydropic degeneration (h) (H&E, 640).

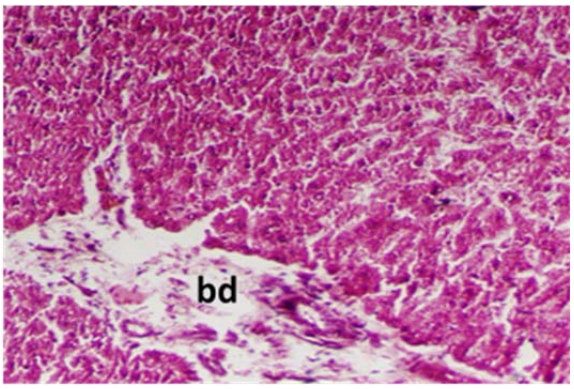

Fig 3-b Histopathological structure of liver in (group 2), demonstrating distention in the portal vein with acute congestion, peri-ductal have fibrosis around the bile duct dilatation **bd** (H&E, X400).

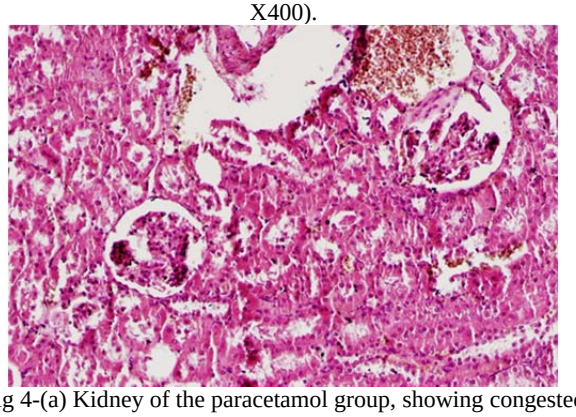

Fig 4-(a) Kidney of the paracetamol group, showing congested peritubular blood vessels (bv) and interstitial hemorrhage (H&E, X400).

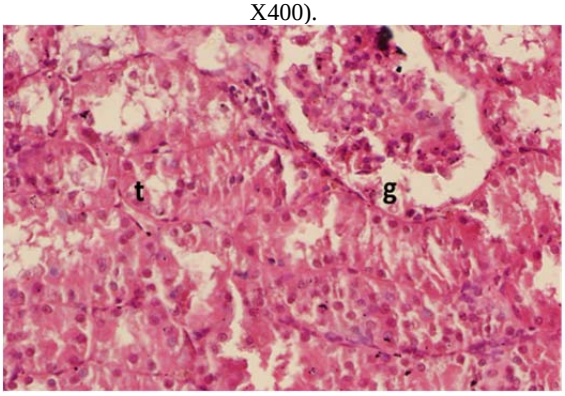

Fig 4-B) Kidney microscopic appearance (group 2), reveled gaps in the lining endothelium of the glomerular tuft **g**, and tubule **t** (H&E, X1000).

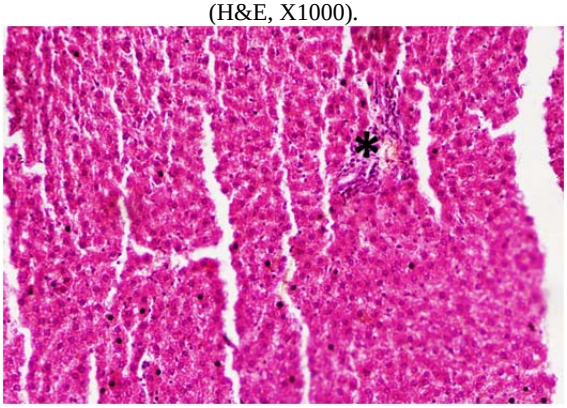

Fig. 5 liver show mild infiltration of inflammatory cells (*) in portal tract in Administration of the combination between Rosmarinus extract and paracetamol (H&E, 640).

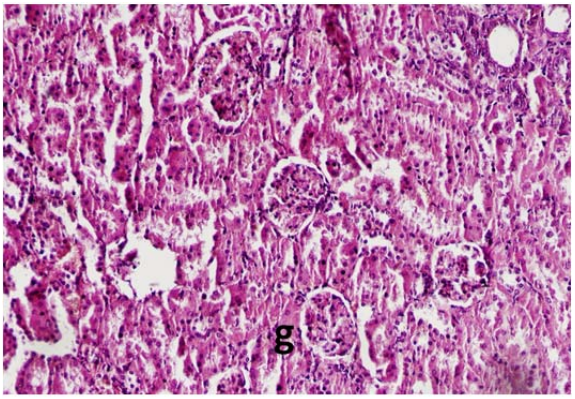

Fig 6 Kidney of the paracetamol+rosemary group and rosemary group, showing intact normal structure of the glomeruli **g** and tubules **t** (H&E, 640).

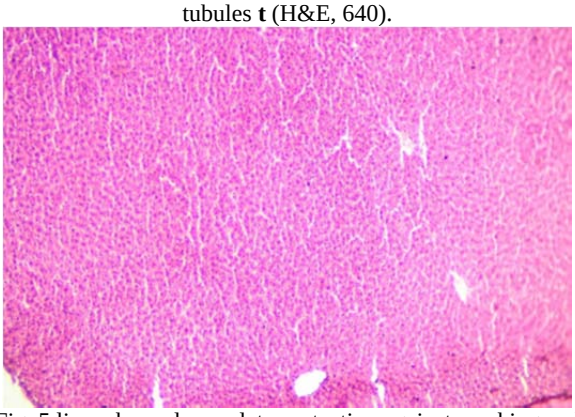

Fig. 5 liver showed complete protection against cracking and decomposition of paracetamol and show normal liver (H&E, 640).

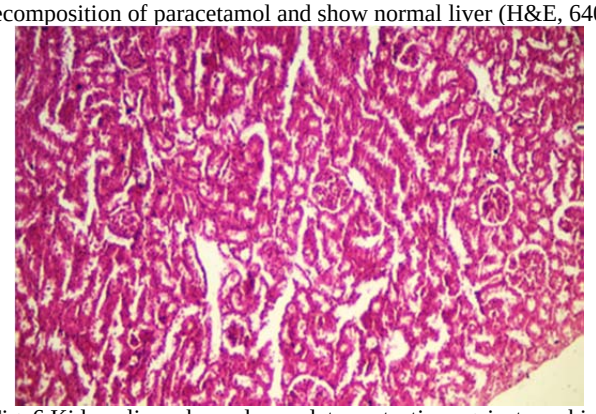

Fig. 6 Kidney liver showed complete protection against cracking and decomposition of paracetamol and show normal kidney (H&E, 640).

4. DISCUSSION

Paracetamol is well known as common drug to treat fever and analgesic over the world. High overdose associated to the commonly using and over the counter that cause acute necrosis. In kidney atrophy of glomerular tuft and showed gaps in the endothelial cells lining tubule (fig. 4-a). In addition, there is bleeding in the interstitial tissue and strong congestion in the blood vessels (Fig.4-b). Necrotic cells present in tubular epithelial in both convoluted tubules proximal and distal, renal failure, and liver dysfunction [Eman et al., 2015]. Liver and kidney disease is one of the most causes of death effectiveness in nation in all ages, around the world. The paracetamol that are now available to therapeutic this condition has strong difficult, which justifies searching for new hepato-protective agents [Horvathova et al., 2010].

In the present study, it is found the administration of animals with *R. officinalis* extracts lead to protect and treated the liver and kidney that induced toxicity by high dose of Paracetamol.
The histopathological examinations of liver tissues and kidney showed the infiltration of of cells inflammation, congestion, with distention in the liver stroma near the portal area and in kidney there is vacuolization in the lining endothelium of glomeruli and interstitial hemorrhage that due to degeneration in the interstitial tissue and around glomeruli was observed and reported in the group two which administrated with high dose of paracetamol. Our finding was agreed with similar researches [Soyal et al., 2010] .

This perception may be attributed to Nitric Oxide cytotoxicity and peroxy nitrite which produced from the restrained reaction between .nitric oxide and superoxide anion which stimulate response of the cell ranging from exquisite modfication of cell warn to astounding oxidative injury, committing cells to apoptosis or necrosis. [Rababah et al., 2004]

In group 3, the administration of *R. officinalis* extract in combination with the high dose of paracetamol, then the histopathological observation for liver tissue showed a little effect in hepatocytes, portal area and bile duct, and the stroma of liver defiance the toxicity effect of paracetamol. The kidney histopathology exhibits resistance to toxicity by paracetamol by reduces the hemorrhage, necrosis, and vacuolation due to anti-inflammation properties by reducing the release of pro-inflammatory cytokines during inflammatory injury, that finding compliance with [Sintayehu et al., 2012] who revealed the same result.

The combination of *R. officinalis* with overdose of paracetamol displayed a little protect effect, and that may be attributed to the interaction between bioactive components of the extract with paracetamol

The histopathology finding in group 4, of the liver and kidney that were given Rosemary extract after high dose administration of Paracetamol, displayed disappearing of the degeneration and necrosis that showed normal intact tissue indicating high protect effect. Our finding was agreement with other study, confirming the anti-hepatotoxic action of rosemary extract [Jung, et al., 2009,] and with that showed *Rosmarinus officinalis* leaves extract provides a full perfects protective effect against kidney injury due to CCl4 [Saber et al., 2012]. others reports stated the crude extract also shows antioxidant and anti-inflammatory activities in the inhibition of NO production, and reduction of pro-inflammatory IL-1ß cytokine and COX-2 mRNA expression in LPS-activated RAW 264.7 cells which substantiates its chemopreventive potential [Soyal et al., 2010].

That Therapeutic action of *R. officinalis* extract due to the presence of many bio-actively secondary metabolites in ethanolic extract of rosemary leaves and stems. The major families found in rosemary are flavonoids such as Kaempferol, luteolin, genkwanin and ladanein; phenolic diterpenes contain, carnosic acid, carnosol or rosmanol; and triterpenes like the ursolic acid. Flavonoids and phenolic diterpenes are strong antioxidants and behave as scavenger compounds for free radicals, $RO^{\bullet}$ and $NO^{\bullet}$. In practice, the acidity ofcarnosic acid and carnosol acid is considered to be one of the most important antioxidants component found in this herb, which have many reported to have broad anticancer properties in several cell line models like the prostate, breast, leukemia and others [Cui et al., 2012, Kontogianni et al., 2013, Habtemariam, 2016, Fernández-Ochoa et al., 2017].

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