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Histological Evaluation of Kidney Toxicity induced by Hydrochlorothiazide and its Ameliorative Effect via Royal Jelly Treatment in Albino Rats

¹Rasha Hussain Kuba, ²Lina A.Salih, ³Yasser A. H. Al-Issa

^{1,2}Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

³Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq
Rasha.hussain@sc.uobaghdad.edu.iq

ABSTRACT: Royal jelly (RJ), it is a nutrient material which has a milky-white color that secreted by worker bees to feed developing larvae and to provide exclusive nourishment to the queen bee throughout her lifespan, this protein-rich material enables the queen to grow larger, extend her lifespan, and maintain her fertility. RJ rich with nutrients which have very therapeutic effects

The purpose of the current study was to assess the effect of this substance (royal jelly) on histological features of kidney in adult male albino rats following a long-term use of popular diuretic medication; Hydrochlorothiazide (HCTZ).

Thirty albino rats and also they are healthy, their weights ranged between 150-200g were employed in this investigation as a model. These rats under study were divided at random into three groups (each group consist of 10 subjects). Group 1: Control group, rats received 0.5 ml isotonic saline solution via gavage.

Group 2: rats were given hydrochlorothiazide (3 mg/kg/day) via gavage group. Group 3: rats treated with hydrochlorothiazide (3 mg/kg/day and RJ (100 mg/kg) for 4 weeks.

The histopathological findings of the animal's kidney showed marked tubular injury with congestion of intertubular blood vessels and hemorrhage. The lesions were appeared in the corticomedullary junction showed glomerulus mild congestion, and focal necrosis of collecting tubules with cast formation within others. While in the drug-treated animals group administrated later with RJ the histological section showed moderate degeneration and necrosis of proximal convoluted tubules without glomerular deterioration. Normal appearance of Bowman space and blood capillaries mild necrosis was seen in the collecting tubules. Thus, based on the ongoing study can concluded *in vivo* the Royal jelly serves as a powerful nephroprotective substance through diminishing the renal toxicity caused by agents such as HCTZ is mainly facilitated by diminishing oxidative stress and inflammation, thereby averting damage to both tubular and glomerular structures.

INTRODUCTION

Royal jelly (RJ) is a substance consist of a group of protein called gluten. The worker bees of genus *Apis mellifera* release this material from two glands: hypopharyngeal and submandibular [1]. It serves as nutrients-rich substance for juvenile larvae bees and is crucial to the queen's diet. Additionally, it has been demonstrated that royal jelly a pivotal role in different biological activities in animal models, cells and tissues [2].

This substance consists of different biological molecules (Protein, lipids, sugars, and a lot of nucleic acids, such as DNA and RNA), Mineral salts (K, Ca, Na, Zn, Fe, Cu, and Mn.) in descending order, in addition to large amount of enzymes antibiotic components [3,4]. According to several studies, Royal jelly offer a variety of pharmacological properties, including: anti-inflammatory and antitumor effects. It can also blood pressure reducing, blood vessel dilation, growth enhancement, and give rise the resistance to infections, and for these reasons, why Royal jelly has been utilized commercially in foods, cosmetics, and pharmaceuticals for over 30 years. products [5].

The kidney is a sensitive vital organ that the body needs to carry out various critical functions, including maintaining homeostasis, regulating the extracellular environment, detoxifying substances, and excreting harmful metabolites and chemicals [6]. One of the most important function of kidney is the removing of many medications and their metabolites by tubular secretion, glomerular filtration, or, in certain cases, a combination of the two [7,8]. The majority of treatments cause nephrotoxicity via one or more common pathogenic pathways [9]. Treatment-induced nephrotoxicity is frequently more common in specific subjects and in specific therapeutic situations [10].

Many reports indicate that effective prevention requires knowledge of the pathogenic mechanisms with renal injury, patient-related risk factors, drug-related risk factors, proactive interventions, vigilant monitoring, and timely intervention [11]. Hydrochlorothiazide is a thiazide diuretic used to treat edema related to a number of conditions, especially blood hypertension through the blocking of sodium chloride transporter, which situated on the apical membrane of the kidneys distal convoluted tubules [12]. Sodium reabsorbing in kidney via distal convoluted tubule, which is responsible for re-absorption of sodium about 5% to 10% [13]. Therefore this study tried to focus on the toxic effect of the chronic dose of hydrochlorothiazide on histological features of kidney and assessment the beneficial effects of royal jelly by using adult male rats as a paradigm.

MATERIALS AND METHODS

Animals and treatment

Twenty four adult male Wistar albino rats (200 ± 50 gm) were acquired for this investigation from animal house of national center for drug control and research, and permitted unlimited access to food and water under regulated conditions of 12/12 hr of light / dark, ($22 \pm 2^\circ\text{C}$), and ($55 \pm 5\%$) humidity. Three groups of eight rats were randomly selected. Group 1: the control group, rats received 0.5 ml isotonic saline solution intra peritoneally (negative control). Group 2: treated with hydrochlorothiazide drug (3 mg/kg/day), Group 3: treated with hydrochlorothiazide (3 mg/kg/day) and RJ (100 mg/kg) for 2 weeks [14].

Histological examination:

After two weeks of treatment, the animals were killed under diethyl ether anesthesia, and the kidneys were cleaned with normal saline and they were cut into short slices and fixed 10 % formalin before being dehydrated succession of ethanol grades, embedded in paraffin wax, and sectioned at a thickness 5-6 μm in order to make slides. The slides were stained for histological analysis using hematoxylin and eosin (H&E) [15].

RESULTS

Kidney tissue sections in the control group (G1) exhibited a typical structure, normal glomerulus with normal convoluted tubules (Fig.1)

The histopathological sections of the kidney of rats treated with chronic dose of hydrochlorothiazide (G2), marked tubular injury showed congestion of intertubular blood vessels and hemorrhage. The lesions were appeared in the corticomedullary junction showed glomerulus mild congestion, and focal necrosis of collecting tubules with cast formation within others (Fig.2).

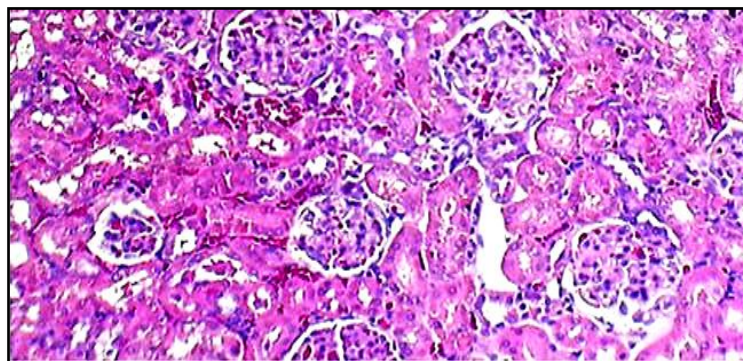


Fig. 1 Histological sections of kidney in control group (H&E stain, 40X).

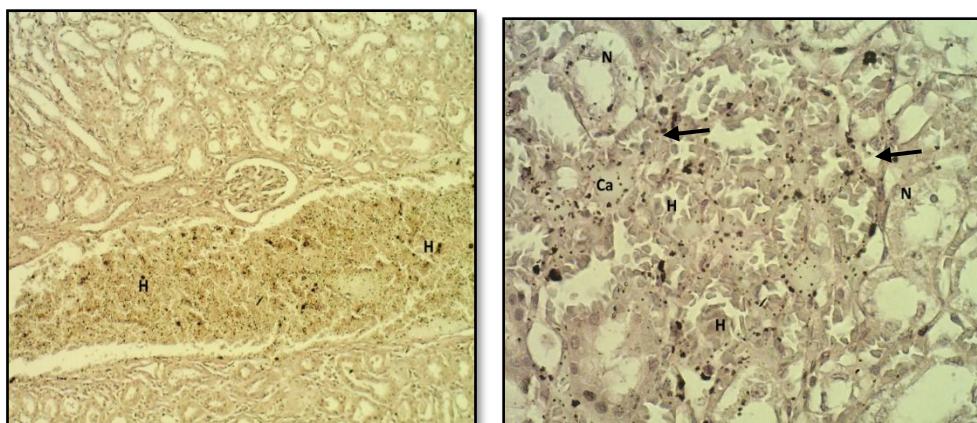


Fig. 2: Histological sections of the renal medulla in the treated group of rats with (drug) H&E 40X showed: focal hemorrhage within cortical medullary zone (H), necrosis (N), focal areas of mononuclear cells infiltrations (arrow), and cast formation (Ca). H&E 100X.

While in the treated animals group administrated later with RJ the histological section showed moderate degeneration and necrosis of proximal convoluted tubules without glomerular deterioration. Normal appearance of Bowman space and blood capillaries mild necrosis was seen in the collecting tubules (Fig.3).

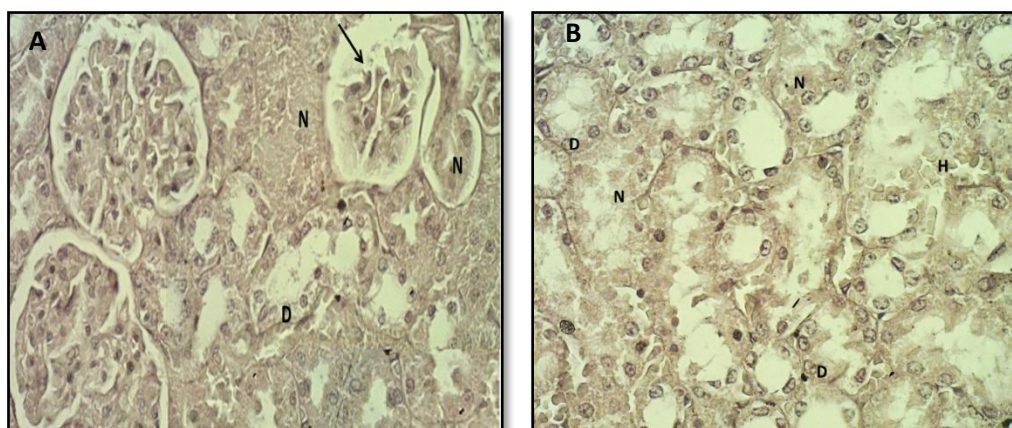


Fig.3: magnified section renal cortex (A) and medulla (B) (Royal & Drug) shows moderate degeneration (D) & mild necrosis of the proximal tubules and collecting tubules (N) with no glomerular deterioration (arrow). H&E 400X

DISCUSSION

In this study the 3 mg/kg/day HCTZ induced nephrotoxicity in rats lead to histopathological changes in the kidney compared to control, these changes include hemorrhage within cortical medullary zone, necrosis in the convoluted tubules, infiltrations of mononuclear cells, and cast formation in the medulla. The hemorrhagic area indicate vascular damage due to increase the permeability, the inflammatory infiltration reflects an ongoing immune response because accumulation of structural disruption.

The presence of tubular casts suggests impaired tubular function and may obstruction which leads to weak renal filtration, administration of 100mg/kg of Royal jelly reduce the histological nephrotoxicity in rats, the histological investigation of rat kidney showed moderate degeneration and necrosis of proximal convoluted tubules without glomerular deterioration. Normal appearance of Bowman space and blood capillaries mild necrosis was seen in the collecting tubules. The prognosis may be negatively impacted by the side effects of diuretics, such as electrolyte imbalances and loss of renal function. Chronic doses of HCTZ can lead to significant hypokalemia, this occur as a result of the damage of renal tubular [16]. By bolstering the antioxidant system, Royal jelly raises the kidneys thiol level and catalase enzyme activity [17], therefore, Royal jelly considered as free radical scavenger and act as anti-oxidant substance [18].

By decreasing ROS generation and NADPH oxidase2 activity, Royal jelly seems to lessen oxidative damage [19]. Lipid peroxidation reduction and stop its formation, and GSH capacity increases and upstream anti-oxidants in the kidney, these results agree with [20]. The inflammatory properties of Royal jelly as reported in earlier research may be attributed to the protective role against nephrotoxicity in rats [21].

From the histological sections, it can be observed that the control renal medulla displayed a normal architecture characterized by prominent basophilic nuclei and well-defined eosinophilic cytoplasm, which indicates intact cellular integrity and appropriate Hematoxylin and Eosin (H&E) staining affinity

In contrast, the sections obtained from the Hydrochlorothiazide-treated groups exhibited a pale yellowish appearance, which can be ascribed to drug-induced histopathological changes. These alterations encompass cellular degeneration and necrosis, resulting in nuclear loss (karyolysis) and a subsequent reduction in hematoxylin binding and basophilia.

Moreover, the occurrence of hemorrhage and tubular cast formation disrupts the normal organization of tissue and contributes to the modified staining pattern. Overall, these observations imply that Hydrochlorothiazide may cause structural damage to the renal medulla, as evidenced by the decreased nuclear staining and the overall faded coloration of the tissue [22]. These results may because the toxicity of drug sometimes alter the chemical compositions and density of tissue especially in H&E stain resulting in weak, pale staining [23].

CONCLUSION:

In vivo Royal jelly serves as a powerful nephroprotective substance. Its capacity to mitigate renal toxicity caused by agents such as HCTZ is mainly facilitated by diminishing oxidative stress and inflammation, thereby averting damage to both tubular and glomerular structures.

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