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Original Article

Histopathological and immunohistochemical studies on the effects of Ethephon on liver and kidney in male rats

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ABSTRACT

Ethephon can release ethylene which could affect the plant growth process. The current study was designed to study the possible toxic effects of ethephon on both liver and kidneys of male rats. 20 adult male rats were divided into 2 groups; the first group is control and the second group received Ethephon at dose (200 mg/kg body weight/day) for four weeks. At the end of the study liver and kidney tissues were taken and examined histopathologically. Ethephon administration to rats showed marked injury in liver and kidney. Also, liver and kidney sections in treated rats with Ethephon showed strong positive affinity for PCNA expressions. These results indicated that Ethephon could be harmful on liver and kidney. Producers and consumers should become more aware about the toxic effects of Ethephon.

1. INTRODUCTION

Plant growth regulators or phytohormones are chemical compounds which are registered for the use in agriculture, it can be produced naturally in higher plants, active in minute amounts, it can modify different physiological processes of plants and are commonly active at very low concentrations [1,2]. Ethephon (2-chloroethylphosphonic acid; Ethrel®) is a plant growth regulator that can release ethylene which could affect different plant growth processes, it can stimulate the production of endogenous ethylene and it has both direct and indirect effects on humans, direct as through inhalation during the spraying process the plants and indirect through the diet (Fruits and vegetables) which has been sprayed with it [3-5]. Ethylene is a simple gas that is produced in small amounts by several plant tissues and they act as a powerful regulator of growth and development. They are found very conspicuously in physiologically matured fruits persisting ripening. Dietary studies have been performed on toxicity of ethephon to different experimental animals [6-8]. Ethephon alone or combined with other herbicides has been reported to be harmful to liver and kidney [9].

2. MATERIALS AND METHODS

2.1 Histopathological investigation

Immediately after decapitation animals were dissected, liver and kidney from different groups were quickly removed, washed in 0.9 saline solutions and fixed in 10 % neutral buffered formalin. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin (mp. 50– 58°C). Sections of 7 microns thickness were cut using rotary microtome and mounted on clean slides. Sections were stained with Ehrlich's haematoxylin and counterstained with eosin as a routine method after Bancroft and Cook [10].

2.2 Proliferating cell nuclear antigen expression

Proliferating cell nuclear antigen immunoreactivity (PCNA-ir) in liver and kidney was performed according to Tousson et al. [11,12] Distribution of PCNA stained nuclei were examined in deparaffinized sections (5 µm) using an Avidin–Biotin–Peroxidase immunohistochemical method (Elite–ABC, Vector

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Laboratories, CA, USA) with PCNA monoclonal antibody (dilution 1:100; DAKO Japan Co, Tokyo, Japan).

3. RESULTS

3.1 Effect of Ethephon on liver and kidney histopathology

Liver sections in male rats in the control (G1) group showed normal structure of hepatocytes where the hepatocytes are polygonal in shape with prominent round nuclei, eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged in between the hepatic cords with fine arrangement of Kupffer cells (Figure 1A). However, liver sections in Ethephon (G2) group showed hepatotoxicity manifested by marked inflammatory cells, degeneration in hepatic cords in addition to karyomegally and pyknotic nuclei indicating apoptosis, moderate fibrosis, and marked diffuse necrosis of hepatic tissue and congested blood sinusoids (Figure 1B).

Kidney sections of control (G1) group showed normal histological structures of the glomeruli and renal tubules in the cortical and medullary portions. The glomerulus surrounded by the Bowman's capsule, proximal and distal convoluted tubules without any inflammatory changes (Figures 1C). Kidney sections in Ethephon (G2) group showed variable pathological changes in glomeruli and some parts of the urinary tubules as moderate cellular infiltration degeneration and necrosis in glomerulus and renal tubules (Figure 1D).

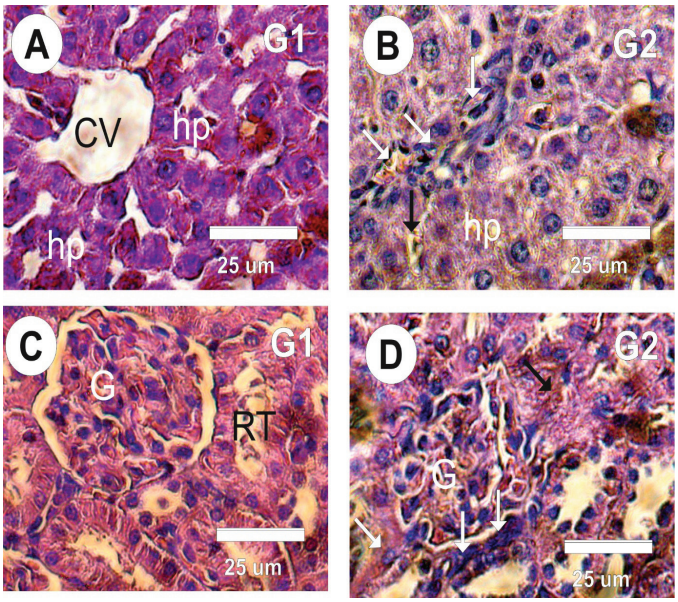

Fig. 1(A-D): Photomicrographs of rat liver sections stained by HE.

A: Liver section in male rat in the control (G1) showed normal structure of hepatocytes (hp).

B: Liver section in Ethephon (G2) showed marked inflammatory cells (White arrows), degeneration in hepatic cords, and marked diffuse necrosis (Black arrows) of hepatic tissue and congested blood sinusoids.

C: Kidney section in male rat in the control (G1) showed normal histological structures of the glomeruli (G) and renal tubules (RT).

D: Kidney sections in Ethephon (G2) group showed moderate cellular infiltration (White arrows), degeneration and necrosis in renal tubules (Black arrows).

3.2 PCNA expressions in liver and kidney

The detection of PCNA expressions in liver sections in the different groups was revealed in Figure (2). Liver sections in the control (G1) group showed mild positive PCNA expression (Grade 1) in hepatocytes nuclei (Figure 2A). In contrast; strong positive expressions for PCNA (Grade 4) in the liver sections Ethephon (G2) group were detected as compared with control (Figure 2B).

Kidney sections in the control (G1) group showed mild to moderate positive PCNA expression (Grade 2) in glomerulus and renal tubules (Figure 2C). In contrast; moderate to strong positive expressions for PCNA (Grade 3) in the kidney sections Ethephon (G2) group were detected as compared with control (Figure 2D)

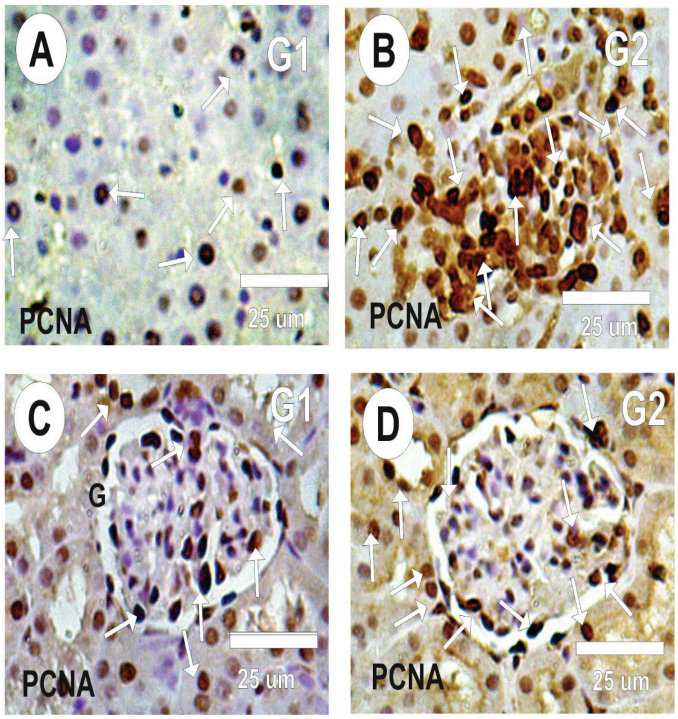

Fig. 2(A-D): Photomicrographs of liver and kidney sections stained with PCNA-ir in the different groups.

A: Liver section in control (G1) showed mild positive PCNA expression (arrows) in hepatocytes nuclei.

B: Strong positive affinity for PCNA arrows in the liver section in treated rats with Ethephon (G2).

C: Kidney section in control (G1) showed mild positive PCNA expression (arrows) in glomerulus and renal tubules.

D: Kidney section in Ethephon (G2) group revealed moderate to strong positive expressions for PCNA.

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4. DISCUSSION

Today, plant growth regulators have become of a vital importance in agriculture. Commonly, the most used ones are gibberellins, abscisic acid, cytokinins and ethylene. Ethephon is a plant growth regulator that decomposes and liberates an active metabolite ethylene that modifies many developmental and physiological processes, for example maturation and ripening and it remains in the fruits for a longer time; thus, it may be dangerous to human health [13]. Liver is the main target of many toxic compounds as most of these elements undergo first pass metabolism there. Hence, it becomes an organ of vital importance for studying the effects of different chemicals distributed into the body. The kidney is an essential organ responsible for performing many vital functions including the maintenance of homeostasis and regulation of the extracellular environment such as detoxification and excretion of toxic metabolites and drugs [14]. The current work was designed to study the possible toxic effects of Ethephon on liver and kidneys of male albino rats. In the recent study; distinct inflammatory cells, degeneration, mild fibrosis and distinct dispersed necrosis of hepatic tissue and congested blood sinusoids were determined in liver sections in Ethephon group. Also, in the recent study; a significant increase in PCNA expressions in liver tissues after oral gavage of Ethephon when compared with control. These findings were consistent with those of Bhadoria et al. [15] who reported that; Ethephon administration induce histological damages in rat liver tissue. Yazar and Baydan[9]; Abd El Raouf and Girgis [7]; Bhadoria et al. [15] who reported that; Ethephon induced hepato and nephrotoxicity. This finding agrees with the studies by Hussein et al. [16] who found mild piece-meal necrosis of hepatocytes on histopathological examination of liver in rats after administeration a plant growth regulator, gibberellic acid. Our result agrees with the results of Altin et al. [17] who reported hepatocellular necrosis after administration of a high dose 4-chlorophenoxy acetic acid (4-CPA), a plant growth regulator, to rats. Also; our results agree with Abd Eldaim et al. [18] who find that; Ethephon-induced reproductive toxicity, PCNA alteratins in rats. Our immunohistochemical results; revealed a significant increase in PCNA on the liver and kidney after Ethephon administration. So; the present results indicate that exposure to Ethephon has direct effects on rat's liver and kidney structure and functions.

5. CONCLUSION

These results indicated that Ethephon could be harmful on liver and kidney. Producers and consumers should become more aware about the toxic effects of Ethephon.

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