



BioBacta



Journal of Medical and Life Science

www.jmals.journals.ekb.eg



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

\*Ehab Tousson, Afaf El-Atrsh, Mervat Mansour, Abdallah Assem

Department of Zoology, Faculty of Science, Tanta University, Tanta, Egypt

\*Corresponding author: [toussonehab@yahoo.com](mailto:toussonehab@yahoo.com)

DOI: [10.21608/jmals.2019.179684](https://doi.org/10.21608/jmals.2019.179684)

### Abstract

Ethephon is a plant growth regulator that can release ethylene which could affect the plant growth and development processes. The current study was conducted to examine the possible toxic effects of Ethephon on both liver and kidneys of male rats. A total of 20 adult male rats were divided randomly into 2 groups, the first group is the control and the second group received Ethephon at a 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. Intoxication of Ethephon to rats showed marked injury in the liver and kidney. Also, liver and kidney sections in rats intoxicated with Ethephon showed a strong positive affinity for PCNA expressions. Our results indicated that Ethephon could be harmful to the liver and kidney. Awareness must be raised for producers and consumers about the toxic effects of Ethephon.

**Keywords:** Ethephon, kidney, Liver, PCNA, Plant growth regulator, Toxicity.

### 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 (Hajam et al., 2017; Wani et al., 2017; Abd Eldaim et al., 2019). Ethephon (2- chloroethyl phosphonic acid; Ethrel®) is a plant growth regulator that can release ethylene which is a simple gas produced in small amounts by several plant tissues and it acts as a powerful regulator of growth and development (Zhang et al., 2012; Dhall et al., 2013; Hussain et al., 2015), 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 of the plants and indirect through the diet; as in crops which have been sprayed with this chemical (Pierik et al., 2006; Taheri et al., 2012;

Moustakime et al., 2018). Ethylene is found very prominently in physiologically matured fruits undergoing ripening. Dietary studies have been conducted on Ethephon toxicity in different experimental animals (Haux et al., 2000, 2002; Tuluze and Çelik, 2006; Anant and Avinash, 2012). Ethephon has been reported to cause toxic, mutagenic, and teratogenic effects, reduce DNA and RNA concentrations and inhibit cholinesterase enzyme activity (Al Twaty, 2006; El-Okazy, 2008; Abd El Raouf and Girgis 2011). Ethephon alone or combined with other herbicides could be harmful to the kidney and liver (Yazar and Baydan, 2008). Plant growth regulator Ethephon has been reported to induce reproductive toxicity in rats (Dutta, 2015; Abd Eldaim et al., 2019). Also, Ethephon induced immunotoxicity and oxidative stress in mice (Abou-Zeid et al., 2018). Ethephon has toxic and harmful effects on insects and can induce oxidative stress (Altuntaş et al., 2015). Therefore; the present study

was designed to study the possible toxic effects of Ethephon on the liver and kidneys of male albino rats.

## 2. Materials and methods

### 2.1. Animals

The experiment was performed on 20 male rats weighing  $150 \pm 10$ g and of 10-12 weeks of age. The rats were held in suitable plastic cages for one week before the experimental work for acclimation in an animal house at Zoology Department, Faculty of Science, Tanta University, Egypt, and maintained on a standard rodent diet and water available *ad libitum*. After one week of acclimation, rats were equally divided into two groups.

### 2.2. Experimental groups

The first group controlled included rats received no treatment and the second group was Ethephon group included rats received Ethephon at a dose (200 mg/Kg body weight/day) by oral gavages for four weeks (Bhadoria et al., 2015).

### 2.3. Histopathological investigation

Immediately after decapitation animals were dissected, liver and kidneys 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 a rotary microtome and mounted on clean slides. Sections were stained with Ehrlich's hematoxylin and counterstained with eosin as a routine method after Tousson (2016).

### 2.4. Proliferating cell nuclear antigen (PCNA) expression analysis

Proliferating cell nuclear antigen immunoreactivity (PCNA-ir) was performed according to Tousson et al. (2011, 2012). Distribution of PCNA stained nuclei was examined in deparaffinized sections (5  $\mu$ m) using an Avidin-Biotin-Peroxidase immunohistochemical method (Elite-ABC, Vector 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

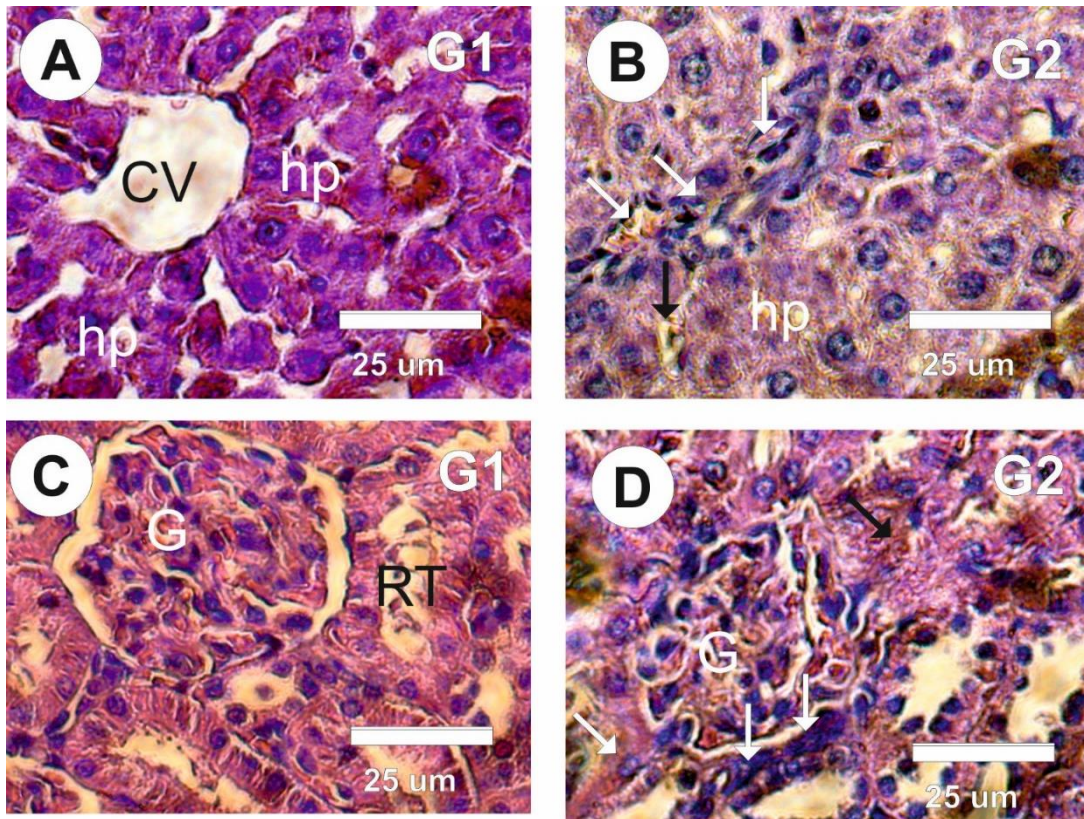
Liver sections in male rats in the control (G1) group showed the normal structure of hepatocytes where the hepatocytes are polygonal in shape with prominent round nuclei, eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged in between the hepatic cords with a fine arrangement of Kupffer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

Kidney sections of the control (G1) group showed normal histological structures of the glomeruli and renal tubules in the cortical and medullary portions. The glomerulus is surrounded by the Bowman's capsule, proximal and distal convoluted tubules without any inflammatory changes (Figure 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 the glomerulus and renal tubules (Figure 1D).

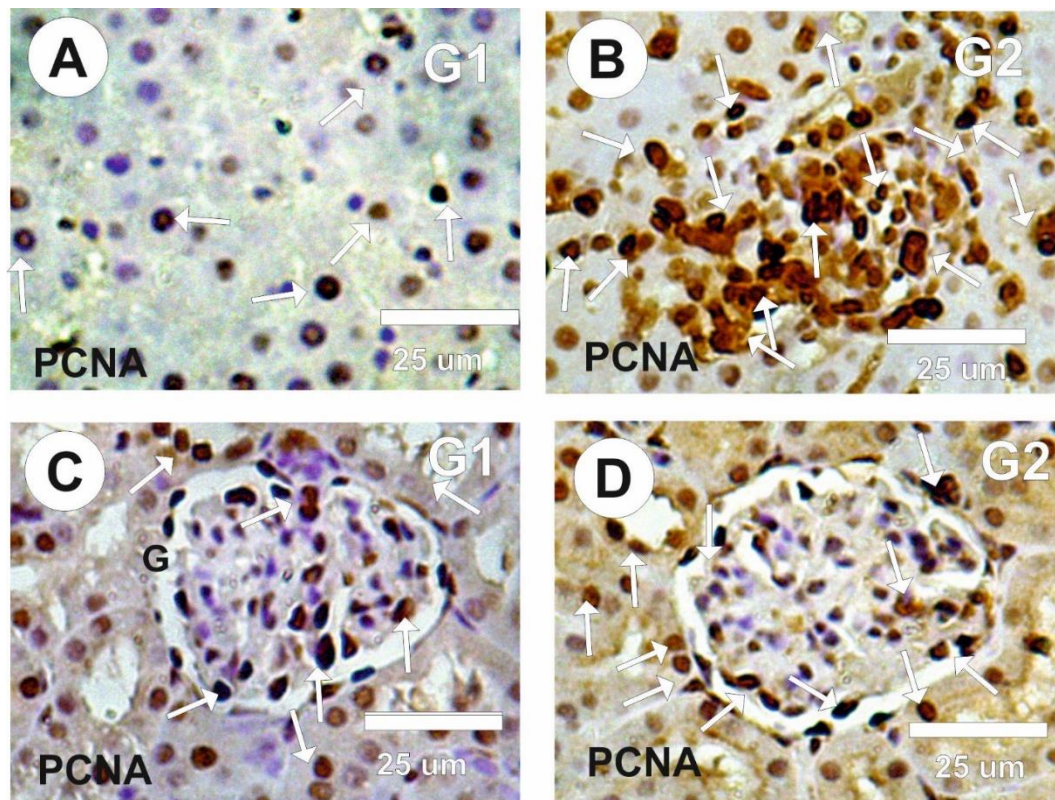
### 3.2. PCNA expressions in liver and kidney

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 the 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).



**Figure 1(A-D)** Photomicrographs of rat liver sections stained by HE. **A:** The liver section in the male rat in the control (G1) showed the 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:** The kidney section in the 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).



**Figures 2(A-D):** Photomicrographs of liver and kidney sections stained with PCNA-ir in the different groups. **A:** The 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:** The kidney section in control (G1) showed mild positive PCNA expression (arrows) in the glomerulus and renal tubules. **D:** The kidney section in Ethephon (G2) group revealed moderate to strong positive expressions for PCNA.

#### 4. Discussion

Plant growth regulators have become of vital importance in agriculture for increasing agricultural productivity. Commonly, the most used ones are ethylene, gibberellins, abscisic acid, and cytokinins. Ethephon is a plant growth regulator that decomposes and liberates active metabolite ethylene that can modify many developmental and physiological processes, for example, maturation and ripening and it remains in the fruits for a long time; thus, it may be dangerous to the health of consumers (Deka and Dutta, 2015). The liver is the main target of many toxic compounds as most of these elements undergo the first-pass metabolism there. Hence, it becomes an organ of vital importance for studying the effects of different chemicals distributed into the body (Saggu et al., 2014; Salama et al., 2015; Tousson et al., 2015; Al-Rasheed et al., 2018). 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 (Ferguson et al., 2008; Salama et al., 2013; El-

Moghazy et al., 2014). The current work is designed to study the possible toxic effects of Ethephon on the 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 the Ethephon group. Also, in the recent study; a significant increase in PCNA expressions in liver tissues after the intoxication of Ethephon when compared with control. These findings were consistent with those of Bhadoria et al. (2015) who reported that; Ethephon administration induced histological damages in rat liver tissues, also agree with Yazar and Baydan (2008); Abd El Raouf and Girgis (2011); Bhadoria et al. (2015) who reported that Ethephon induced damage to liver and kidney tissues of rats and mice. Our finding agrees with the studies by Hussein et al. (2011) who found mild piece-meal necrosis of hepatocytes on histopathological examination of liver in rats after the intoxication of plant growth regulator, gibberellic acid. Our result agrees with the results of Altin et al. (2011) who reported hepatocellular necrosis after administration of a high dose of 4-chlorophenoxy

acetic acid (4-CPA), a plant growth regulator, to rats. Our immunohistochemical results; revealed a significant increase in PCNA on the liver and kidney after Ethephon intoxication. So; the present results indicate that exposure to Ethephon has direct effects on rat's liver and kidney structures and functions. These results indicated that Ethephon could be harmful to the liver and kidney. Awareness must be raised for producers and consumers about the toxic effects of Ethephon.

## 5. References

- Abd Eldaim, M. A., Tousson, E., El Sayed, I. E. T., Awd, W. M. (2019). Ameliorative effects of *Saussurea lappa* root aqueous extract against Ethephon-induced reproductive toxicity in male rats. *Environmental Toxicology*, 34(2): 150-159.
- Abd El Raouf, A., Girgis, S.M. (2011). Mutagenic, teratogenic and biochemical effects of Ethephon on pregnant mice and their fetuses, *Global Veterinary*, 6(3): 251-257.
- Abou-Zeid, S. M., Allam, T.S., El-Bahrawy, A., Mohamed, M.A. (2018). Ameliorating effects of Green Tea on Ethephon-Induced Immunotoxicity and Oxidative Stress in Mice. *International Journal of Pharmaceutical Sciences and Research*, 4: 1-1.
- Al-Rasheed, N.M., El-Masry, T.A., Tousson, E., Hassan, H.M., Al-Ghadeer, A. (2018). Hepatic protective effect of grape seed proanthocyanidin extract against Gleevec-induced apoptosis, liver Injury and Ki67 alterations in rats. *Braz. J. Pharm Sci.*, 2018;54(2):e17391
- Altin, D.T., Ozer, C., Yesilkaya, E., Babül, A., Bideci, A., Cinaz, P. (2011). Effect of the plant growth regulator (4-Chlorophenoxy acetic acid) into the oxidative stress parameters in rat liver. *African Journal of Pharmacy and Pharmacology*, 5(22): 2498-2504.
- Altuntaş, H. (2015): Effects of Ethephon on the hemolymph metabolites of the greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Acta Physica Polonica A*, 128(2B).
- Al Twaty, N.H.A, 2006. Mutagenic effects of Ethephon on albino mice. *Journal of Biological Sciences*, 6(6): 1041-1046.
- Anant, J.D., Avinash, B.G. (2012). Modulation in serum biochemicals in European rabbit, *Oryctolagus cuniculus* (Linn.) exposed to Ethephon. *European Journal of Experimental Biology*, 2(3): 794-799.
- Bhadoria, P., Nagar, M., Bahrioke, V. Bhadoria, A.S. (2015). Effect of Ethephon on the Liver in Albino Rats: A histomorphometric study. *Biomedical Journal*, 38 (5): 421-427.
- Dhall, R. K. (2013): Ethylene in Post-harvest Quality Management of Horticultural Crops: A Review. *Journal of Crop Science and Technology*, 2(2).
- Dutta, U. (2015). Evaluation of Ethephon induced oxidative stress to gonadal disorder and its amelioration by ethanolic extract of shoot of *Bambusa balcooa* Roxb. in Albino rat. *Toxicology Letters*, 2(238): S269-S270.
- Dutta, U., Deka, M. (2015). Protective activity of *Bambusa balcooa* Roxb against repeated exposure to Ethephon induced haematological alteration in Albino rat. *Toxicology Letters*, 238(2): 84-85.
- El-Moghazy, M., Zedan, N.S., El-Atrsh, A.M., El-Gogary, M., Tousson, E. (2014). The possible effect of diets containing fish oil (omega-3) on hematological, biochemical and histopathological alterations of rabbit liver and kidney. *Biomedicine and Preventive Nutrition*, 4: 371-377.
- El-Okazy, A.M. (2008). The effects of combination of Gibberellic acid -3(GA3) and Ethephon (2 Chloroethyl Phosphonic Acid) (Plant Growth Regulators) on same physiological parameters in Mice. *The Journal of the Egyptian Public Health Association*, 83: 67-86.
- Ferguson, M. A., Vaidya, V. S., Bonventre, J. V. (2008). Biomarkers of nephrotoxic acute kidney injury. *Toxicology*, 245(3): 182-193.
- Hajam, M.A, Hassan, G.I., Bhat, T.A., Bhat, IA., Rather, A.M., Parray, E.A. (2017). Understanding plant growth regulators, their interplay: For nursery establishment in fruits. *International Journal of Chemical Studies*, 5(5): 905-910.
- Hussain, I., Ahmad, S., Amjad, M., Ahmed, R. (2015). Ethephon application at kimri stage accelerates the fruit maturation period and improves phytonutrients status (Hillawi and Khadrawi (cv)) of date palm fruit. *Pakistan Journal of Agricultural Sciences*, 52(2).
- Haux, J. E., Quistad, G. B., Casida, J. E. (2000). Phosphobutrylcholinesterase: Phosphorylation of the Esteratic Site of Butyrylcholinesterase by Ethephon [(2-Chloroethyl) phosphonic acid] Dianion.

- Chemical Research in Toxicology, 13: 646-651.
- Haux, J. E., Lockridge, O., Casida, J. E. (2002). Specificity of Ethephon as a butyrylcholinesterase inhibitor and phosphorylating agent. *Chemical Research in Toxicology*, 15: 1527-1533.
- Hussein, W.F., Farahat, F.Y., Abass, M.A., Shehata, A.S. (2011). Hepatotoxic potential of Gibberellic Acid (GA3) in adult male albino rats. *Life Science Journal*, 8(3): 373-383.
- Moustakime, Y., Zakaria, H., Joutei, K. (2018). Effect of Ethephon application on the cellular maturity of *Olea europaea* L. and on the extractability of phenolic compounds in virgin olive oil. *Chemical and Biological Technologies in Agriculture*, 5(2).
- Pierik, R., Tholen, D., Poorter, H., Visser, E.J.W., Voeselek, L.A.C.J. (2006). The janus face of ethylene: growth inhibition and stimulation. *Trends in Plant Science*, 11: 176-183.
- Saggu, S., Sakeran, M., Zidan, N., Tousson, E., Mohan, A., Rehman, H. (2014). Ameliorating effect of chicory (*Chichorium intybus* L.) fruit extract against 4-tert-octylphenol induced liver injury and oxidative stress in male rats. *Food and Chemical Toxicology*, 72: 138-146
- Salama, A.F., Tousson, E., Ibrahim, W., Hussein, M.W. (2013). Biochemical and histopathological studies in the PTU-induced hypothyroid rat kidney with reference to the ameliorating role of folic acid. *Toxicology and Industrial Health*, 29(7): 600-608.
- Salama, A.F., Tousson, E., Elfetoh, E.M., Elhaak, M.A., Elawni, M.A. (2015). Effect of Egyptian plant *Silybum marianum* on the kidney during the treatment of liver fibrosis in female Albino rats induced by alcohol in comparison to the medical silymarin from China. *International Journal of Current Microbiology and Applied Sciences*, 4(3): 557-570.
- Taheri, A., Cline, J.A., Jayasankar, S., Pauls, P.K. (2012). Ethephon-induced abscission of 'Redhaven' peach. *American Journal of Plant Sciences*, 3: 295-301.
- Tousson, E. (2016): Histopathological alterations after a growth promoter boldenone injection in rabbits. *Toxicology and Industrial Health*, 32(2) 299-305.
- Tousson, E., Ali, E. M., Ibrahim, W., Mansour, M. A. (2011). Proliferating cell nuclear antigen as a molecular biomarker for spermatogenesis in PTU-induced hypothyroidism of rats. *Reproductive sciences*, 18(7): 679-686.
- Tousson, E., El-Moghazy, M., Massoud, A., Akel, A. (2012). Histopathological and immunohistochemical Changes in the testes of rabbits after injection with the growth promoter boldenone. *Reproductive Sciences*, 19(3): 253-259.
- Tousson, E., Ibrahim, W., Barakat, L., Abd El-Hakeem, A. (2015). Role of Proplis administration in boldenone-induced oxidative stress, Ki-67 protein alterations and toxicity in rat liver and kidney. *International Journal of Scientific and Engineering Research*, 6 (8): 660-664.
- Tuluca, Y., Celik, I. (2006). Influence of subacute and subchronic treatment of abscisic acid and gibberellic acid on serum marker enzymes and erythrocyte and tissue antioxidant defense systems and lipid peroxidation in rats. *Pesticide Biochemistry and Physiology*, 86: 85-92.
- Yazar, S., Baydan, E. (2008). The subchronic toxic effects of plant growth promoters in mice. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 55: 17-21.
- Wani, A.W., Hassan, G.I., Dar, S.Q., Baba, T.A., Dar, M.I., Amir, S., Bisati, I.A., Bhat, I.G. Hassan, G.h. (2017). Influence of different phytohormones and nutrients on fruit set and chemometric attributes of apple. *Vegetos*, 30(2): 492-498.
- Zhang, L., Li, S., Liu, X., Song, C., Liu, X. (2012). Effects of Ethephon on physicochemical and quality properties of kiwifruit during ripening. *Postharvest biology and technology*, 65: 69-75.