Original Article

Ethephon, an organophosphorous, a Fruit and Vegetable Ripener: Has potential hepatotoxic effects?

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Abstract

Introduction: In the recent years, ethephon, 2-chloroethylphosphonic acid, is one of the most commonly used plant growth regulators. At present, it is being used on fruits, vegetables, and cereals for promoting pre- and post-harvest ripening. The effect of artificial ripening has become questionable because of various health-related issues. This study was conducted to note the morphology of liver after ethephon administration as it is the site where chemicals undergo first pass metabolism and probably will be affected by ethephon. **Materials and Methods:** Adult Wistar albino rats were divided into experimental and control groups (10 each). Ethephon was administered at a dose of 200 mg/kg/day by a gavage tube in the experimental rats for 14 days. The animals were sacrificed within 24 h of the last dose; liver was dissected and processed for light microscopy. Hematoxylin and eosin-stained sections were studied using an image-pro express analyzer. The data obtained from control and experimental groups were statistically analyzed. **Results:** In the experimental rats, the body weight was found to be significantly decreased. The orderly arrangement of hepatocytes was disrupted and was replaced by blood-filled sinusoids. At sites, hepatocytes appeared to be degenerated. Councilman bodies with pyknotic nuclei and inflammatory infiltrations were seen. The population per unit area of the hepatocytes and Kupffer cells was 29.53 ± 10.65 versus 44.18 ± 10.31 and 25.12 ± 4.41versus 13.05 ± 6.5 in experimental and control groups, respectively. The decrease of hepatocytes and increase of Kupffer cells were found to be statistically significant. **Conclusions:** The observations in the liver are probably indicative of degenerative changes associated with ethephon. Hence, we can conclude that this plant growth regulator, Fruit and Vegetable Ripener, has hepatotoxic potential. General awareness and regarding the use of such plant growth regulators is must to reduce the intake.

Keywords: Ethephon, Fruit Ripeners, hepatotoxiciy

Introduction

Nutritionists advise to include fruits and vegetables in our daily diet to improve our immune system. However, we do not know that these fruits, vegetables, and food products available in the market today, which we are consuming daily to boost our immune system, are really safe. We are aware about the artificial ripening of fruits and vegetables to meet consumer's demand, increasing their shelf lives, and other economic factors.

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In the recent years, ethephon, 2-chloroethylphosphonic acid, is the most widely used plant growth regulator as it promotes fruit coloration, leaf, flower, or fruit abscission, fruit ripening, fruit yield, germination, and flower induction.[1] The color of oranges, lemons, and grapefruits often remains green when they are ripe, but consumers do not buy them because of their external green appearance. The application of ethylene to these green citrus fruit results in the development of desirable citrus color.

The use of ethephon varies with plant species, chemical concentration, and time of application as it regulates the phases of plant growth and development by application to various growth sites.[2] It is applied to plants in the form of a mist or spray. After

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application, ethephon penetrates through stomata and cuticles to the apoplast where, at pH of 5 and above, it decomposes to form ethylene, chloride, and phosphate.[3] Ethylene is a naturally occurring plant hormone that is produced by many fruits and vegetables. It affects the physiological processes in plants and initiates the ripening process when internal concentrations increase from 0.1 to 1.0 ppm (parts per million). Externally applied ethylene can also initiate the ripening process.[4] According to the Ministry of Agriculture, ethylene is considered safe in the concentration varying from 0.001% to 0.01% depending on the crop, variety, and maturity.[5]

Ethephon is widely being used throughout the world as an insecticide.[6] It is an organophosphorus compound, and in experimental animals, it has been reported to get rapidly absorbed in the gut.[7] Some of it is expected to convert to ethylene oxide, then to ethanediol and hydroxyethyl-glutathione and mercapturic acid.[8] Ethephon is one of the few synthetic compounds, and perhaps, the only agrochemical that spontaneously reacts to generate phosphoproteins, and it is a much better inhibitor of butrylcholinestrase than acetylcholinestrase.[6,9] The involvement of 2-chloroethylphosphonic acid in biogenesis of some antibiotics has been determined. It affects the growth of streptomyces and antibiotic production.[10]

Scientists have reported that regular consumption of artificial-ripened fruits may cause dizziness, weakness, skin ulcer, and heart- and liver-related diseases.[11,12] Olson and Hinsdill have reported adverse health effects on mice after subchronic treatment with plant growth regulators.[13] In recent studies, dose-related inhibition of brain, red blood cell, and plasma cholinesterase has been reported in rats and mice.[14] A significant increase of ^{14}C-acetate incorporation into cholesterol and lipids of serum, liver, heart, and brain is reported.[15] A significant decrease in body weight, food consumption, vocalizations, and motor activity in birds, rabbits, and dogs has been observed.[16] Toxic effects of ethephon reported in human adults till date are salivation, lacrimation, diarrhea, urgency of bowel movement, stomach cramps, increased urgency, and frequency of urination with decreased appetite with inhibition of plasma cholinesterase activity.[17] Despite the known toxicities, there are only few studies documenting the effect of ethephon on of liver, where most of the chemicals undergo first pass metabolism.

Materials and Methods

The study was approved by the Institutional Animal Ethics Committee (IAEC), University College of Medical Sciences (UCMS), New Delhi, on March 10, 2011. The IAEC approval number was IAEC/2011/46.

Inbred adult Wistar albino rats weighing 150–200 g were used for the study. The rats were procured from the animal house of UCMS and Guru Tegh Bahadur Hospital, Delhi. The animals were divided into two groups, experimental and control groups, containing 10 animals each. The animals were group housed (12 h light/dark cycle) with *ad libitum* access to food and water. The body weights were recorded before the onset of the experiment and on day 7 and day 14 of the experiment. The animals of Group I was given ethephon at a dose of 200 mg/kg body weight/day for 14 days by oral gavage during the morning hours. In Group II, the gavage tube was introduced for 14 days at the same time and these rats served as controls. The animals of both groups were sacrificed within 24 h of the last dose by perfusion with formal saline under anesthesia.

Perfusion of animals

The animals were anesthetized by keeping them in an inverted glass jar containing a large piece of cotton soaked in anesthetic ether. Anesthesia was achieved in 5–10 min. The anesthetized rats were pinned up on the dissection board, and a midline incision was made on the skin extending from the xiphoid process to the jugular notch. Sternum was lifted by cutting the ribs along its sides. The heart and the ascending aorta were exposed by removing the overlying fat and thymus. A ligature was passed under the ascending aorta, and a small nick was made in the left ventricle through which a cannula was inserted into the aorta and tied with the help of a ligature. A small nick was also made in the right auricle, and blood was allowed to clear out of the system by injecting normal saline through the cannula. Following this, about 200 ml of 10% formal saline was injected under low pressure with the help of a syringe till a clear solution started flowing out and the animal became pale and stiff. The perfused rats were kept in formalin for 3–4 days.

Tissue preparation for microscopy

The liver was dissected out and cut into smaller pieces (5 mm). It was then washed in running tap water to remove surplus fixation. The tissue was dehydrated through changes in ascending grades of ethyl alcohol. Clearing of tissue was done in cedarwood oil followed by xylene (15 min) and then a mixture of xylene and paraffin wax in the ratio of 1:1 for half an hour. Tissue was further given three changes in paraffin wax (melting point 60°C) for 1½ h each and then embedded in wax. Blocks were prepared with the help of Leukart's "L"shaped bars. The blocks were trimmed, labeled, and mounted on a block holder. Eightmicronthick sections were cut using a rotatory microtome. The sections were picked up by flotation method on a glass slide smeared with egg albumin, glycerin, and thymol mixture. The slides were allowed to stand upright to drain and allow the sections to dry completely. They were then kept in the incubator at 37°C overnight to prevent displacement of sections during the staining process. The sections were later stained with hematoxylin and eosin. Observations were made on randomly selected sections of the liver stained with hematoxylin and eosin on a Zeiss light microscope for both groups.

Observations and Results

Among control group, all the rats survived well during the period of the experiment, i.e., 14 days. No appreciable change was

observed in their behavior, appetite, and motor activity during the experimental period. During the experimental period of 14 days, it was observed that the rats became hypoactive after receiving ethephon for few days. The decrease in activity was accompanied by decrease in food intake. On subsequent days, the animals appeared weak before administration of the drug but became very aggressive and showed resistance while introducing the gavage tube for dosing.

The mean body weight of the rats at the beginning of the experiment was181.3 ± 12.2 g and 181.5 ± 13.1 g; after 7 days of treatment, the weight was 201 ± 10.7 and 185.5 ± 12.4 g while just before the sacrifice, it was 217 ± 9.2 and 188.9 ± 12.6 g in control and experimental group, respectively [Table 1]. Before the start of experiment, there was no significant difference in weight among control and experimental groups. On day seven, higher weight gains were observed among control group, but the difference was statistically insignificant ($P = 0.06$). Whereas just before the sacrifice statistically significant, higher weight gain was observed among the control group ($P < 0.0001$).

In hematoxylin and eosin-stained sections of the control group, hepatocytes (parenchymal cells) appeared to be polyhedral in shape with a centrally placed, rounded nucleus. The cytoplasm of the hepatocytes appeared vacuolated and stained pink with eosin. Vacuolated appearance was probably due to the glycogen granules and the lipids, which were washed off during processing. The sinusoids of the liver were seen between the plates of hepatocytes [Figure 1].

In the experimental group, the one cell thick orderly arrangement of the hepatocytic plates appeared to be disrupted. The hepatocytes varied in size and were not so appropriately fitted together. In most places, the hepatocytes appeared shrunken with a small hetrochromatic nucleus. The cytoplasm stained dark pink with eosin and an increased cytoplasmic eosinophilia was seen. At sites, few of the hepatocytes appeared to be binucleated. This is probably suggestive of regenerative attempts. The Kupffer cells which were present along the sinusoidal endothelium appeared swollen and distended [Figure 2].

These were foci of inflammatory infiltrate, i.e., macrophages and lymphocytes. The bile canaliculi between the hepatocytes throughout the liver appeared distended. The portal triads showed proliferation of bile ductules and infiltration of macrophages, eosinophils, lymphocytes, and neutrophils [Figure 3].

The mean number of hepatocytes in a unit area (57,600 μ^2) was 44.2 ± 10.3 and 29.5 ± 10.6 in control and experimental group, respectively. There was statistically significant decrease in the number of hepatocytes in a unit area when compared with the control group by Student's *t*-test ($P < 0.0001$) [Table 1].

The Kupffer (macrophage) cell count was found to be increased with the mean population per unit area (57600 μ^2) to be 13.1 ± 6.5 and 25.1 ± 4.4 in control and experimental group, respectively [Table 1]. The mean number of Kupffer cells per unit area of the experimental rats was found to be statistically significantly increased as compared to that of the control rats analyzed by Student *t*-test ($P < 0.0001$) [Table 1].

Discussion

In the present study, a statistically significant decrease in the body weight gain ($P < 0.001$) was observed in the ethephon-treated rats as compared to control group. The decrease in body weight gain in experimental group was in accordance with the findings of Henwood and El-Okazy.[18,19] The body weight gain in contrast to our findings was reported by Xian-Hui *et al.*, who found a significant increase in body weight gain after treating adolescent female rats with ethephon for a short period of 20 days.[20]

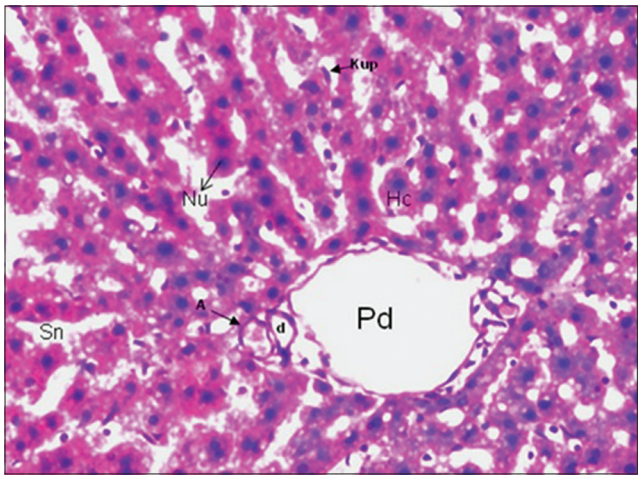

Figure 1: Photomicrograph of a transverse section of the liver in the control rat showing the portal triad consisting of the tributary of the portal vein (pd) lined by endothelium, a branch of hepatic artery (A) and a bile ductule (d). The hepatocytes (Hc) are polyhedral in shape with centrally placed euchromatic nucleus (nu). The Kupffer cells (kup) are attached to the endothelium of sinusoids (Sn) (H and E, ×200)

Table 1: Comparison of body weight and other histological features in control and experimental rats

Parameters	Experimental group*	Control group*	*P*#
Body weight before the experiment (g)	181.5±13.1	181.3±12.2	0.97
Body weight after 7 days (g)	185±12.4	201±10.7	0.06
Body weight prior to sacrifice (g)	188.9±12.6	217±9.2	<0.001
Hepatocytes count per unit area	29.5±10.6	44.2±10.3	<0.001
Kupffer cells count per unit area	25.1±4.4	13.1±6.5	<0.001

*Mean±SD, #Un-paired *t*-test. SD: Standard deviation

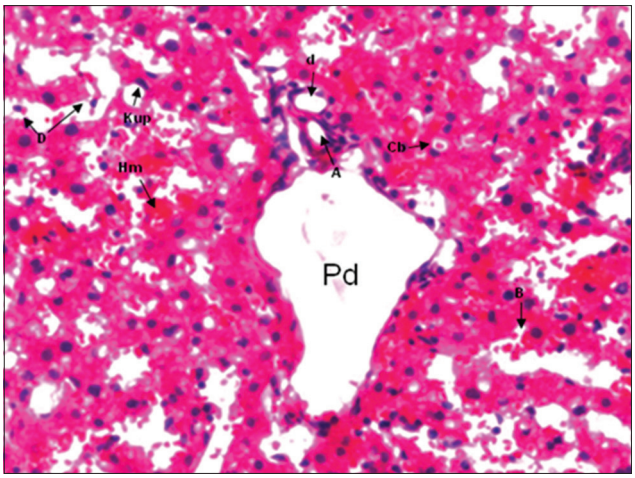

Figure 2: Photomicrograph of a transverse section in the liver of an experimental rat showing the portal triad, and the hemorrhage (Hm) disrupting the radiating pattern of hepatocytes. The hypertrophied Kupffer cells (kup) and councilman bodies (Cb) are visible. The portal triad shows dilatation of the tributary of portal vein (pd), many bile ductules (d), and a branch of hepatic artery (A). The bile canaliculi (B) and space of disse (D) appear to be dilated. At sites, degenerating hepatocytes with pyknotic nucleus are also seen (H and E, ×200)

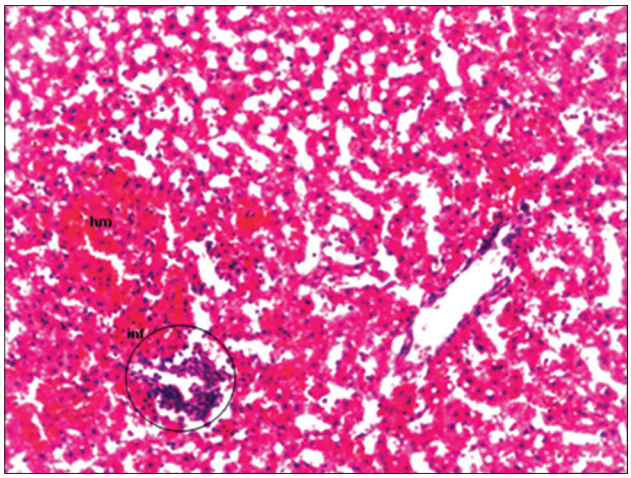

Figure 3: Photomicrograph of a transverse section in the liver of an experimental rat showing an area of parenchyma with inflammatory infiltration (inf) consisting of lymphocytes, macrophages, neutrophils, and eosinophils. A massive area of hemorrhage (hm) is also visible (H and E, ×100)

The mean number of hepatocytes and Kupffer cells of control group in a unit area [Table 1] was in accordance with the findings of Gershbein and Elias and Hase and Brim.[21,22] No study has commented on effect of ethephon on liver cells.

Areas around the portal triads and central vein showed shrunken hepatocytes with pyknotic nucleus and pale staining cytoplasm. At places, dying hepatocytes appeared to be what is known as the acidophilic bodies or the Councilman bodies. These observations are in consistent with the findings of Hussein *et al*.[23]

At places, areas of hemorrhage were seen disrupting the normal parenchyma which was replaced by large blood-filled spaces. This is in consistent with findings of Yazar and Baydan who administered oral ethephon to mice for 45 days and demonstrated microscopic changes in terms of hyperemic areas.[24]

According to Miller and Van and Troup, the only sign of toxicity in mice treated with ethephon was the inhibition in plasma and red blood cell cholinesterase.[25] Similar cholinergic effects were also noted by Berouty *et al*. along with other findings.[17]

El-Okazy demonstrated that ethephon caused several signs of toxicity other than affecting cholinesterase.[19] These signs included an increase in the weights of liver, kidney, and spleen in groups treated with ethephon together with gibberellic acid. Decreased hemoglobin and total erythrocyte count were recorded in addition to an increased total leukocyte count and blood urea. They postulated that a combination of ethephon and gibberellic acid exhibited an additive dose-dependent effect on these parameters.

According to Nada and Al-Twaty, ethephon reduce the DNA and RNA concentrations in liver and testis.[26] He reported similar results with protein content and cholinesterase enzyme activity in blood plasma. The results showed that ethephon could be mutagenic in mice.

Conclusions

The observed statistically significant decrease in the hepatocyte count per unit area may be attributed and correlated with the fact that ethephon caused a decrease in the metabolic activity of the hepatocytes thereby causing their atrophy, resulting in the decrease of cells in a unit area. The statistically significant increase in Kupffer cells, inflammatory infiltrations, and areas of hemorrhage suggest the ongoing damage. These observations after administration of the ethephon are suggestive of the alarming possibility of toxic poisoning that may result in human either by occupational exposure, lack of knowledge, unsafe attitudes, faulty sprayers, lack of protective equipment, or consumption of this plant growth promoter in our daily diet. The lack of information at all levels can be one of the most important causative factors of insecticides intoxications.

Recommendations

- Health education to increase public awareness about the ill effects of the commonly used artificial fruit and vegetable ripeners on most of the body systems (central nervous system, pulmonary hepatic, and cardiac). Primary care physicians should be sensitized to spread this knowledge
- It is important to perform qualitative and quantitative analysis of the presence of ripening agents within the fruit skin and flesh to understand the relevant health hazard
- Need to promote healthy practices such as the fruits and vegetables with visible spots or necrosis (lesions) or any other abnormality should not be consumed. Wash fruits and vegetables thoroughly with water before consuming. Peeling of fruits before consumption and vegetables before cooking should be promoted

- Ensure the quality of fruits and vegetables by sending them to voluntary testing laboratories
- Efforts of policy makers are required to reduce the use of artificial ripeners. Residue data sheets of United States have summarized the residues found on a variety of commodities and their by-products in the USA. They have given the preharvest intervals, concentration factors, and application rates.[27] We should work on those fact sheets to limit the use of ethephon and other artificial ripening agents. Safe limit of residue data should be worked on according to the Indian environment and the use can be limited accordingly
- Organic farming is the best solution, so it should be adopted and promoted so that our immune boosters remain boosters not toxicants.

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Conflicts of interest

There are no conflicts of interest.

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