RESEARCH ARTICLE

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Gibberellin A_3 Induced Histological and Histochemical Alterations in the Liver of Albino Rats

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ABSTRACT: Gibberellin A_3 (GA_3) is a plant growth regulator used in many countries, including Egypt, to accelerate the growth of fruits and vegetables. The present work was conducted to investigate the histopathological and histochemical effects of GA_3 on the liver of albino rats. Administration of GA_3 by gavage at a dose level of 24 p.p.m. in 0.2 ml saline, 3 times a week for 3 weeks induced many histopathological changes in the liver such as cytoplasmic vacuolization of the hepatocytes with pyknotic nuclei, blood vessel congestion and inflammatory leucocytic infiltrations. Histochemical observations revealed marked reduction in total carbohydrates and total protein contents in the hepatocytes. These changes proved to be time dependent. Moreover, the level of the enzymes GOT, GPT and alkaline phosphatase in serum were significantly decreased especially after the third week. In conclusion, the present study showed that GA_3 affected the structure and function of the rat liver.

KEYWORDS: gibberellic acid, liver, histopathology, histochemistry

INTRODUCTION

Gibberellic acids are a group of plant growth regulators that have been identified in different plants[1] and they are used in agriculture as plant regulators to stimulate both cell division and cell elongation that affect leaves as well as stems.[2] If gibberellic acid or one of its metabolites is applied to dwarf varieties of peas, broad beans or maize, growth is greatly accelerated.[3] In *Alstroemeria hybrida*, leaf senescence is retarded effectively by application of gibberellins.[4] Feeding toads *Bufo regularis* with gibberellin A_3 induced hepatocellular carcinomas in 16% of the animals.[5] Moreover, El-Mofty *et al*[6] showed that gibberellin A_3 induced breast and lung adenocarcinomas in mice. Gibberellic acid was found to induce chromosomal aberrations in human lymphocytes[7] and mice.[8] The World Health Organization[9] listed gibberellin-A_3 as a plant growth regulators related to pesticides. Gibberellic acid (gibberellin A_3) is used extensively in Egypt to increase the growth of some fruits (such as strawberries and grapes) and some vegetables (such as tomatoes, cabbages and cauliflower).[10] The present work was conducted to study the histopathological and histochemical effects of gibberellic acid (GA_3) on the liver of albino rats.

MATERIALS AND METHODS

Sexually mature male albino rats *Rattus norvigicus* weighing 150±5 g were used. Animals were kept in the laboratory under constant temperature (24±2°C) for at least one week before and throughout the experimental work. They were maintained on a standard diet and water were available *ad libitum*. Animals were divided into two groups. Twenty five rats in the first group were orally given gibberellin-A_3 (Berelex**,** BDH chemical, Pool, UK) at a dose level of 24 p.p.m in 0.2 sterile saline, 3 times per week for 3 weeks. Animals in the second group (15 rats) were served as controls.

The treated animals and their controls were sacrificed by decapitation after 1, 2 and 3 weeks of treatment. For enzyme determination, sera were obtained by centrifugation of the blood samples and stored at –20°C until assayed for the biochemical parameters. Transaminases (GOT, GPT) activities were determined on the basis of Reitman and Frankle[11] and alkaline phosphatase was measured using the method of Kind and King.[12] The results were analyzed statistically using Student's t-test.

For histological examination, liver was removed and fixed in Bouin's fluid; for histochemical study, they

were fixed in Carnoy's fluid. Fixed materials were embedded in paraffin wax and sections of 5 microns thickness were cut. Slides were stained with haematoxylin and eosin for histological examination. Total carbohydrates were demonstrated using periodic acid Schiff's technique (PAS).[13] Total proteins were detected using the mercury bromophenol blue method of Mazia *et al.*[14]

Results

Data in Table1 shows that there was an insignificant elevation in GOT in the sera of treated animals during the first and second week of treatment followed by a significant decrease ($P<0.05$) after the third week. Similarly, GPT exhibited a significant increase after the second week followed by a significant decrease after the third week. Determination of alkaline phosphatase activity revealed insignificant increase during the first two weeks and significant decrease after the third week.

Table 1. Effect of gibberellin–A_3 on serum transaminases (GOT, GPT) and alkaline phosphatase.

Weeks after treatment	GOT (u/ ml)	GPT (u/ ml)	Alkaline phosphatase (u/ 100 ml)
Control	122.5±5.2	58.9±1.1	43.8±2.6
1	129.0±6.6	69.6±3.5	41.2±0.45
2	138.0±2.3	78.0±2.2*	46.5±3.1
3	88.0±1.8*	32.0±3.7*	23.5±1.4*

Each value represent mean of 8 animals ± standard deviation
*significant at $P<0.05$.

Fig 1 shows the histological structure of the liver of a control rat. Examination of the liver of rats after one week of treatment with GA_3 showed no detectable changes. After two weeks, the liver sections revealed masses of inflammatory leucocytic infiltrations comprise mainly of lymphocytes and sparse eosinophils in several areas (Fig 2). The intrahepatic blood vessels, central and portal veins were congested and their lining epithelia were eroded. The sinusoidal spaces were somewhat dilated and infiltrated by lymphocytes (Fig 3). Injurious signs were observed in the individual hepatocytes and such injuries were more obvious in the peripheral lobular zones than the pericentrally located ones. The hepatocytes are swollen with granular cytoplasm and others appeared with cytoplasmic vacuolization with giant nuclei (Fig 4). Hyperplasia of bile ducts associated with peribiliary leucocytic cells aggregations were observed (Fig 5) . After three weeks of treatment with GA_3, the liver tissue lost its characteristic archetictural organization. The individual hepatocytes were deteriorated and appeared with severe cytoplasmic vacuolization and their nuclei ranged in their degenerative changes from karyolysis to severe karyorrhexis and complete pyknosis (Fig 6). The intrahepatic blood vessels were congested and the bile ducts were hyperplastic. There was massive cellular infiltrations (Fig 7).

Histochemical examination of the liver of control rats showed that a considerable amount of total carbohydrates was observed in the cytoplasm of the hepatic cells as indicated by PAS-positive reaction (Fig 8). Animals treated with GA_3 for one week showed a mild depletion of carbohydrate content. Liver tissues examined after two weeks of treatment with GA_3 showed that a large number of cells appeared with a reduced amount of total carbohydrates (Fig 9). A marked reduction of total carbohydrates was observed in the liver after three weeks of treatment (Fig 10). Total proteins were demonstrated in the hepatic cells of control rats as deeply stained blue granules diffused homogenously through both the cytoplasm and nuclei. The nuclear membrane and the nucleoli were intensely stained. Examination of liver sections after two weeks of treatment with GA_3 showed a noticeable decrease in the total protein contents. After three weeks of treatment with GA_3, many hepatocytes showed signs of degeneration and their cytoplasms were vacuolated. These cells showed a marked decrease in total proteins.

Discussion

It is well known that the liver is the first target organ in toxicological prospects regarding its role in detoxification, biotransformation and excretion of xenobiotic. After enteric uptake of injurious materials, it is the first organ to be exposed to such hazards via the portal circulation.[15] Results obtained in the present study showed that gibberellic acid induced many histopathological changes in the liver of rats. Similarly, Abdelhamid *et al*[16] reported that feeding chickens with gibberellic acid led to numerous histological lesions in different organs including liver and that two-week withdrawal period did not ameliorate the effect of GA_3. Among the pathological symptoms observed in the present work was the remarkable abundance of leucocytic infiltrations in the liver of GA_3-treated animals. These leucocytic infiltrations were considered as a prominent response of the body tissue facing any injurious impacts.[17] The hepatocytes showed severe cytoplasmic vacuolization, especially after the third week of treatment with GA_3. The interpretation of vacuolar formation has been subjected to wide speculations by many investigators. Robbins and Angell[18] demonstrated that cytoplasmic vacuolization is one of the important primary response to all forms

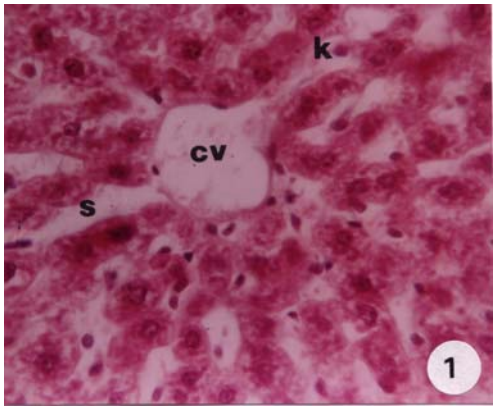

Fig 1. Paraffin section of the liver of a control rat showing a central vein (cv), Kupffer cell (k) and sinusoidal spaces (S). Hematoxylin-eosin X 550.

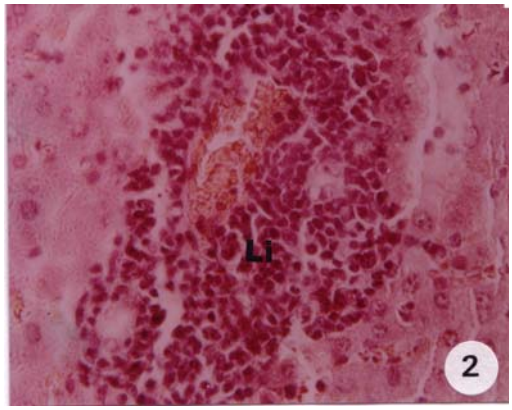

Fig 2. Section of the liver of a rat treated with GA_3 for 2 weeks showing a large mass of leucocytic inflammatory cells (Li). Hematoxylin-eosin X 550.

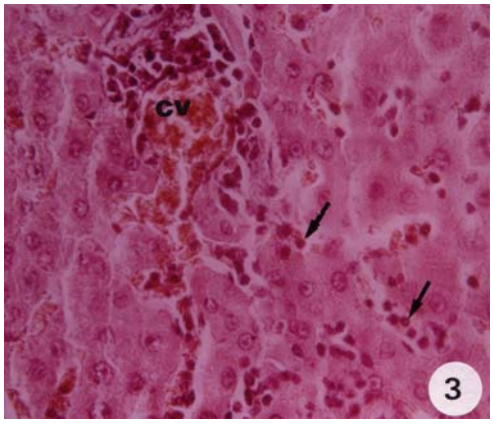

Fig 3. Section of the liver of a rat treated with GA_3 for 2 weeks showing congested central vein (cv) and infiltration of lymphocytes to sinusoidal spaces (arrows). Hematoxylin-eosin X 550.

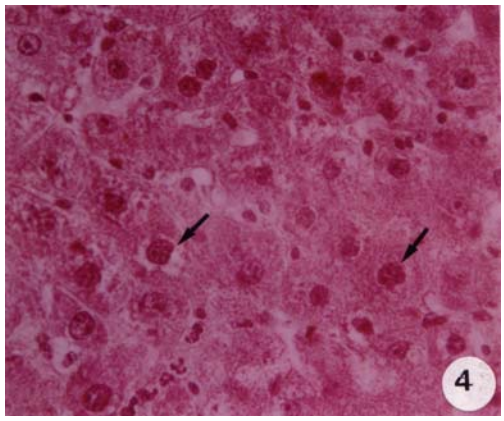

Fig 4. Section of the liver of a rat treated with GA_3 for 2 weeks showing hepatocytes with cytoplasmic vacuolization and giant nuclei (arrows). Hematoxylin-eosin X 550.

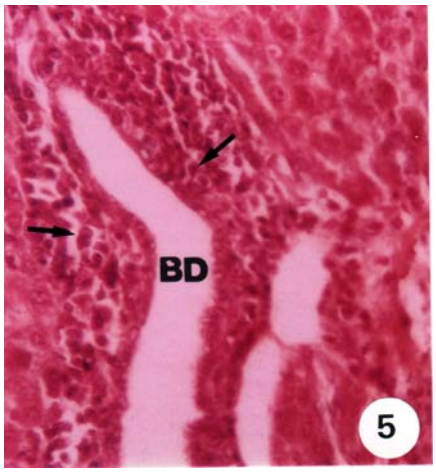

Fig 5. Section of the liver of a rat treated with GA_3 for 2 weeks showing enlarged bile duct (BD) and peribiliary cellular infiltrations (arrows). Hematoxylin-eosin X 550

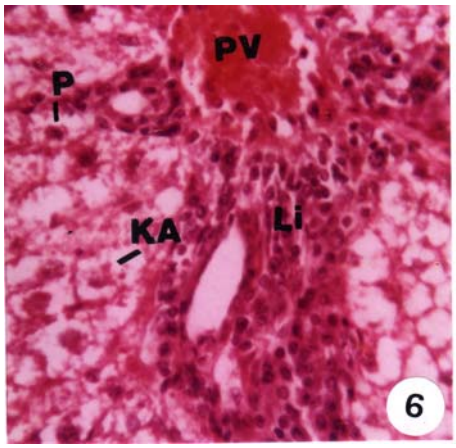

Fig 6. Section of the liver of a rat treated with GA_3 for 3 weeks showing hepatocytes with cytoplasmic vacoulization . Congested portal vein (PV), and Leucocytic infiltrations (Li), are seen. Hematoxylin-eosin X 550.

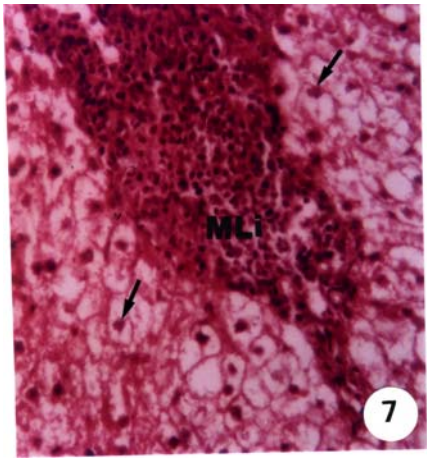

Fig 7. Section of the liver of a rat treated with GA_3 for 3 weeks showing a mass of inflammatory leucocytic infiltration (MLi) and cytoplasmic vacuolization of the hepatocytes with pyknotic nuclei (arrows). Hematoxylin-eosin X 550.

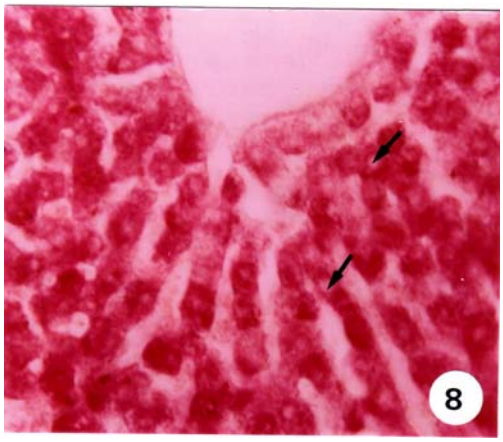

Fig 8. Section of the liver of a control rat showing PAS positive inclusions in the cytoplasm of the hepatocytes, the nuclei gave negative reaction (arrows). PAS stain X 450.

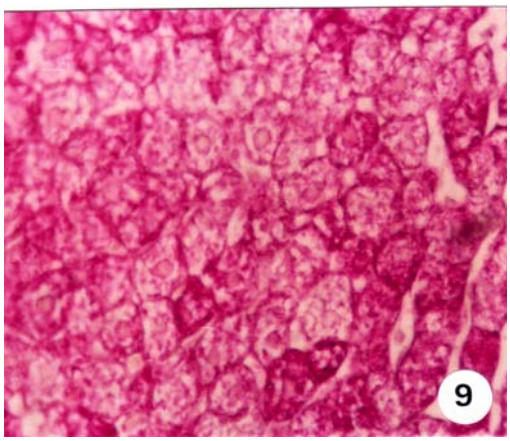

Fig 9. Section of the liver of a rat treated with GA_3 for 2 weeks showing a decrease in PAS reactivity in most of hepatocytes. X 550.

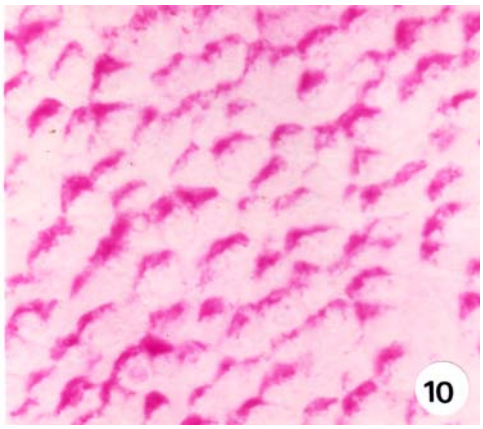

Fig 10. Section of the liver of a rat treated with GA_3 for 3 weeks showing a weak PAS staining in the cytoplasm of the hepatocytes. X 550.

of cell injury. It implies increased permeability of cell membranes leading to an increase of intracellular water. As water sufficiently accumulates within the cell, it produces cytoplasmic vacuolization. Zhang and Wang[19] correlated the vacuolar degenerative changes with the marked disturbances which take place in lipid inclusions as a result of injurious treatments.

GA_3 was found to affect serum transaminases (GOT, GPT) and alkaline phosphatase. The activity of these enzymes increased during the first two weeks of treatment followed by a marked decrease after the third week. It was reported that serum transaminases were considered to be a sensitive measure in evaluating hepatocellular damage and elevation of serum alkaline phosphatase activity provided a measure of extrahepatic or intrahepatic biliary obstruction.[20] The decrease in the enzyme activity recorded in this study after the third week of treatment may be due to the hepatotoxic potency of GA_3 which leads to severe destructive changes in the hepatic cells. More specifically, Jones and Berk[21] attributed this effect to the decreased synthesis of the proteinous moiety of the enzyme by the liver.

Treating rats with GA_3 caused a decrease of total carbohydrates in the hepatic cells. Carbohydrates depletion, especially glycogen was observed in kideny of rats treated with GA_3[22] and in a variety of animals under different pathological conditions.[23, 24] This glycogen loss was interpreted by some investigators. Orr *et al*[25] attributed this phenomenon to the lost capacity of such pathologically altered cells to metabolize glycogen properly and maintain its storage in normal pattern. Feuer *et al*[26] speculated that glycogen diminution may be attributed to the increased

activities of lysosomal enzymes brought about by deleterious impacts. However, one or more of such factors could be considered as the causal agent of total carbohydrate loss observed in the liver of GA_3-treated rats.

The present study also revealed that treatment with GA_3 caused marked decrease in liver total proteins. This result is in agreement with that of Abdelhamid *et al*[16] who indicated that GA_3 induced a reduction of muscular proteins in chickens. A reduction in total proteins was also observed in liver of some animals exposed to insecticides.[27, 28] These investigators attributed the reduction in protein content partially to the decreased level of protein synthesis in the hepatic cells suffering from pathological changes due to the hyperactivity of hydrolytic enzymes.

Thus, the present study collectively indicated that GA_3 affected the structure and function of rat liver. Since GA_3 is extensively used in some countries, its use must be under strict control.

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