

Hepatotoxic Potential of Gibberellic Acid (GA₃) in Adult Male Albino Rats

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Abstract: Gibberellic acid (GA₃), a plant growth regulator, is widely used in agriculture of many countries including Egypt. However, its potential hazardous effects on human health were relatively unexplored. The purpose of this study was to investigate the effects of sub- chronic toxicity of GA₃ on hepatic function and structure in adult male albino rats and also to determine the effects of withdrawal of GA₃ on the affected parameters following 6 weeks of follow up. Forty adult male albino rats were equally divided into four groups; the first group was used as a negative control, while the second group (positive control group) received NaOH; the vehicle. Animals of the third group (GA₃ group) received 75 ppm of GA₃ daily in drinking water for six weeks. Animals of the last group (Recovery group) received the same treatment as the third group for six weeks then were left without any treatment for another 6 weeks. At the end of the experimental period, all rats were sacrificed for assessment of liver function tests; ALT, AST, GGT and ALP. Liver specimens were collected for histopathological examination and assessment of hepatic levels of SOD, CAT, GSHPx and MDA. The results revealed that GA₃ sub-chronic toxicity induced a significant increase in AST, ALT, GGT and ALP as compared to control group. There was also a significant increase in hepatic malondialdehyde level with a significant decrease in SOD, CAT, and GSHPx enzymes activity in comparison with control groups. Histopathological examination using light microscope showed; hepatocyte vacuolization and inflammatory cellular infiltration. Most of hepatocytes appeared shrunken with pyknotic nuclei. Moreover, Bcl-2 immunolocalization revealed over-expression of this protein in both hepatocytes and endothelial cells of hepatic sinusoids. Electron microscopic examination revealed most of hepatocytes were with shrinkage nuclei with condensation of its heterochromatin and cytoplasmic vacuolization. On the other hand, stoppage of GA₃ administration for 6 weeks has resulted in some sort of regression of the previously mentioned hepatotoxic effects. In conclusion: results of the current study suggested gibberellic acid was a potent pro-oxidant that induced a significant hepatotoxicity in adult male albino rats, while 6 weeks period of follow up was insufficient for complete recovery of these toxic effects.

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1. Introduction

Many chemicals are currently used in agriculture nowadays. One of these chemicals is the plant growth regulators where its use began in the 1930s (Fishel, 2006). Plant growth regulators (PGRs) are also known as plant hormones or phytohormones. They are chemicals that regulate plant growth (Osborne and McManus, 2005).

Gibberellins are one of the six major classes of plant growth regulators according to the American Society of Agricultural Science (Fishel, 2006). Gibberellic acid (GA₃) is one of the most active hormones of gibberellins. It affects many mechanisms of plant growth including stem elongation by stimulating cell division and elongation, flowering, fruit development and breaking dormancy (Neil and Reece, 2002).

Saber *et al.* (2003) mentioned that gibberellic acid (GA₃) is used extensively in Egypt

and other countries, to increase the growth of many fruits (such as strawberries and grapes) and vegetables (such as tomatoes, cabbages and cauliflower).

Gibberellic acid (GA₃) is highly persistent and bioactive in soil for months. The Environmental Protection Agency has determined its use to be only allowed in low doses (Schwechheimer and Willige, 2009).

People may be exposed to residues of GA₃ in diet derived from consumption of different types of fruits and vegetables treated with GA₃. Exposure to residues may also be through drinking water (Tomlin, 2004).

Occupational exposure of the agricultural workers to GA₃ may occur through inhalation of powder and dermal contact with this compound at work places where GA₃ is produced or used giving the picture of acute toxicity (Arteca, 1996).

GA3-treated cells lose their ability to scavenge reactive oxygen species and this loss ultimately results in oxidative damage and cell death (Fath *et al.*, 2001).

A growing amount of evidence indicates that GA3 alters the antioxidative systems in the rat's tissues. Antioxidant enzyme activities were significantly decreased in the erythrocyte, liver, and brain tissues of rats treated with GA3 (Tuluca and Celik, 2006).

Three groups of enzymes play significant roles in protecting cells from oxidant stress: superoxide dismutase (SOD), catalase (CAT) glutathione peroxidases (GPx). These enzymes have been shown to be sensitive indicators of increased oxidative stress (Stadtman and Levine, 2000).

Furthermore, the lipid peroxidation end product malondialdehyde (MDA) was significantly increased in the erythrocyte, liver, brain and muscle of rats treated with GA3. MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation (Celik *et al.*, 2007).

The liver is the main target for the toxicity of several compounds. This is because 75% of blood coming to the liver arrives directly from gastrointestinal organs and then spleen via portal veins which bring drugs and xenobiotics in concentrated form (Lee and Senior, 2005). The pathophysiological mechanisms of hepatotoxicity are still being explored and include both hepatocellular and extracellular mechanisms (Andrad *et al.*, 2005). There are reports in support of GA3 impairment effects on the hepatic function and structure of rats (Saber *et al.*, 2003).

Ozmen *et al.* (1995) found that, exposure to GA3 resulted in a significant reduction of the total protein amount of hepatic tissue when compared with the control mice. Moreover, Sakr *et al.* (2003) found that oral administration of GA3 induced different biochemical and histochemical changes in the liver of the treated rats. Biochemical changes were in the form of early increase followed by late decrease in liver enzymes ALT and AST. Histochemical observations revealed marked reduction in total carbohydrates and total protein contents in the hepatocytes.

The increasing use of this substance in agriculture making it as an interesting subject to investigate its possible adverse effects on the liver as one of the main target organs for different xenobiotics. So, the aim of this study was to evaluate hepatotoxic effects of GA3 in adult male albino rats for 6 weeks, and also to determine the effects of withdrawal of GA3 on the affected parameters following 6 weeks of follow up.

2. Material and Methods

Material:

1- Chemicals:

A- Gibberellic Acid (2,4a, 7-Trihydroxy-1-methyl-8-methylenegibb-3-ene-1, 10 - dicarboxylic acid 1,4a-lactone) in the form of white crystalline powder, was from Sigma -Aldrich chemical Co., Germany.

B- Sodium hydroxide: It was used to dissolve gibberellic acid. It was obtained from El- Nasr Co., Egypt.

2- Kits:

- Eli tech-diagnostic kits: for estimation of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST).
- Gamma – Glutamyl transferase (GGT) kit from Bio ADWIC, Egypt was used for this assay.
- Alkaline phosphatase (ALP) kit from Bio-Systems Co., Spain.
- Bio-diagnostic kits: for estimation of superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GSHpx) and malondialdehyde (MDA) levels. Kits were purchased from Diagnostic and Research Reagents (Giza, Egypt).

3- Experimental Animals:

In this experiment, 40 adult male albino rats weighting 150-200 g were obtained from Animal House in Zagazig Faculty of Veterinary Medicine. All animals were subjected to 14 days of passive preliminaries in order to adapt themselves to their new environment and to ascertain their physical wellbeing. They were housed in a separate well ventilated cages, under standard conditions, with free access to the standard diet and water *ad libitum*. The experiment was conducted at the Animal House of Faculty of Medicine Zagazig University. The experiment was performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, 1996).

Methods:

1-Experimental design:

Grouping of animals:

The rats were divided into four groups, each group consisted of ten rats.

Group 1 (Negative control group): kept without treatment till end of experiment.

Group 2 (Positive control group): Sham received only the vehicle (each rat received 1ml of 1N NaOH added to 1000 ml of tap water and given orally for 6 weeks).

Group 3 (GA₃ group): 75 mg of GA3 were dissolved in 1ml of 1N NaOH and then were diluted with tap water until 1000ml to obtain a 75 ppm dose

(Tuluca and Celik, 2006) to be added to drinking water of these animals for 6 weeks.

Since all rats have the same physiologic characters, daily water consumption of all rats was approximately 30 ± 3 ml during the tests. Consequently, the GA₃ intake amount of each rat was about 2.2 ± 0.3 mg per day (Tuluca and Celik, 2006).

Group 4 (Recovery Group): Animals of this group received the same treatment as group 3 for 6 weeks, then they were left without any treatment for 6 weeks (Saly, 1998).

At the end of the study, all rats were anaesthetized by ether and sacrificed for collection of blood samples for estimation of AST, ALT, GGT and ALP serum levels. Liver specimens were collected to be subjected for assessment of oxidative stress markers and histopathological examination using both light and electron microscopes.

2- Assessment of liver function tests (LFTs):

-Alanine aminotransferase (ALT) (IU/L): estimation of ALT has been carried out using kits of Eli Tech-diagnostic. It was done as described by **Reitman and Frankel (1957)** according to the pamphlet of Eli tech-diagnostic by method of enzymatic UV kinetic.

-Aspartate Aminotransferase (AST) (IU/L): estimation of AST has been carried out using kits of Eli Tech-diagnostic. It was done as described by **Reitman and Frankel (1957)** according to the pamphlet of Eli Tech-diagnostic by enzymatic UV kinetic.

-Gamma – Glutamyl Transferase (GGT) has been carried out using Biolabo- France kit. It was determining as described in **Tietz (1986)**.

-Alkaline Phosphatase (ALP) enzyme assessment was done using bio- systems Co., kit, Spain.

1- Assessment of tissue oxidative stress markers:

Each liver specimen was divided into 2 parts, one part was wrapped with aluminum foil and embedded in liquid nitrogen for 1 hour then kept frozen in -80°C till used to assess SOD, CAT & GSHpx enzymes activity and MDA level in tissues. Assessment of enzyme activity of GSHpx & SOD & CAT was done according to Paglia and Valentine (1967) & Durak *et al.* (1996) and Soliman *et al.* (2010) respectively, while assessment of MDA level was done as described by Satoh (1978) according to the pamphlet of Bio diagnostic kits using calorimetric method. The 2nd parts were preserved for histopathological examination.

4- Histologic Study:

For light microscopic study, liver specimens were fixed in 10% formalin saline for histopathological examination using H&E stain by following the method described by Wilson and Gamble (2002).

For electron microscopic study, liver specimens were fixed in 2% glutaraldehyde and then post fixed in 1% osmium tetroxide, dehydrated and embedded in epoxy resin. Ultrathin sections were cut and double stained with uranyl acetate and lead citrate and examined by transmission electron microscope at electron microscope laboratory in Histology Department, Faculty of Medicine, Zagazig University (**Glauret and Lewis, 1998**).

5- Immunohistochemical study:

Immunostaining was performed using the avidin-biotin peroxidase technique for localization of Bcl-2. Paraffin sections mounted on coated slides were deparaffinized and treated with 0.01 M citrate buffer for 10 minutes to unmask antigens. Then sections were incubated in H₂O₂ for 10 minutes to abolish endogenous peroxidase activity before blocking with 5% horse serum for 2 hrs at room temperature to inhibit the nonspecific immunoreactions. Primary monoclonal anti- Bcl-2 serum (Cell Marque Lot., 27068) were applied at 1:5000 dilutions. Sections were incubated with primary monoclonal antisera for 36 h at 4°C. after washing they were incubated with biotinylated secondary antibodies for 5 hrs, then followed by avidin-biotin peroxidase complex. Finally immune reaction was visualized with 0.05% diaminobenzidine. Then the slides were counter stained with Mayer's hematoxylin before mounting (Happerfield *et al.*, 1993).

6- Statistical analysis:

Data were represented as means \pm SD. The differences were compared for statistical significance by ANOVA, LSD tests and student's t-test. Difference was considered significant at $p < 0.05$. The statistical analysis was performed using Epi-Info version 6.1 (Dean *et al.*, 2000).

3. Results:

1- Biochemical results:

Statistical comparison between the negative and positive control groups regarding LFTs (AST, ALT, GGT and ALP) and hepatic oxidative stress markers (SOD, CAT, GSHpx and MDA) revealed no significant difference ($P > 0.05$), so the negative control was used for comparison with other groups of the study (**Table 1**).

Table 1: Statistical analysis by student t-test of means values of LFTs and oxidative stress markers of negative control rats (groups 1) and positive control rats (groups 2) .

Groups		Group 1 (Negative Control)	Group 2 (Positive Control)
Liver Function Tests	AST (IU/L)	43.6 ± 1.9	44.9±1.5#
	ALT (IU/L)	27±1.82	26±1.63#
	GGT (IU/L)	2.73±0.462	2.88±0.502#
	ALP (IU/L)	45.0±1.72	45.8±1.63#
Oxidative Stress Markers	SOD (U/gm)	70.57±3.82	69.88±3.94#
	CAT (nmol/min/ml)	18.84±2.12	18.75±2.59#
	GSHPx (U/gm)	166.92±4.10	168.40±3.94#
	MDA (nmol/gm)	21.33±3.30	21.88±3.29#

Data are expressed as means ± SD with non significance (F) ≠ > 0.05, n=10 in each group

1-1- Liver function tests (AST, ALT, GGT and ALP):

Processing the mean values of LFTs (AST, ALT, GGT and ALP) of the adult male albino rats of the negative control group, GA₃ treated group and the recovery group through (ANOVA) test revealed that there were statistically significant differences (p<0.001) between them (Table 2).

There was a statistically significant increase in the mean values of LFTs of GA₃ treated rats in comparison with those of the negative control group rats (p<0.001). By the end of the follow up period there was an improvement in the mean values of LFTs of the recovery group. The results showed a statistically significant decrease in the mean values when compared with the mean values of LFTs of GA₃ treated rats (p<0.001). This improvement was partial because these values still higher (p<0.001) than those of the negative control group (Table 2) .

Table 2: Statistical analysis by ANOVA test and LSD of means values of LFTs of negative control rats (Group 1), GA3 treated rats (Group 3) and Recovery group (group 4) .

Groups		Group 1 (Negative Control group)	Group 3 (GA3 group)	Group 4 (Recovery group)	P
Liver Function Tests	AST (IU/L)	43.6 ± 1.9	82± 2.9*	57.4± 2.5*#	<0.0001†
	ALT (IU/L)	27± 1.82	66± 1.84*	42± 1.92*#	<0.0001†
	GGT (IU/L)	2.73± 0.462	12.55±0.51*	7.42± 0.331	<0.0001†
	ALP (IU/L)	45.0± 1.72	79.82± 2.34*	51.55± 1.50*#	<0.0001†

†: significant difference (p<0.05) as compared by ANOVA test, *: Significant difference as compared to the negative control group (P < 0.05), #: significant difference as compared to GA₃ treated group n=10 in all groups

1-2- Hepatic oxidative stress markers (SOD,CAT, GSHPx and MDA):

The mean values of the antioxidant enzymes (SOD, CAT, and GSHPx) and the lipid peroxidation end product MDA of the negative control group, GA₃ treated group and the recovery group through (ANOVA) test showed a highly (p<0.001) statistically significant difference (Table 3) .

There was a statistically significant decrease in the mean values of the antioxidant enzymes activities of GA₃ treated rats in comparison with those of the negative control group rats (p<0.001). on the other hand GA₃ treatment induced a significant increase in the hepatic level of MDA (p<0.001). the six weeks period of recovery didn't reveal non significant improvement in the hepatic MDA level, as statistical comparison of the mean values of MDA of the recovery group showed non significant difference when compared to those of GA₃ treated group. In contrary there was a significant improvement in the mean values of antioxidant enzymes activities of the recovery group. The results showed a statistically significant increase in the mean values when compared with those of GA₃ treated rats (p<0.001). This improvement was partial because these values still higher (p<0.001) than those of the negative control group (Table 3).

Table 2: Statistical analysis by ANOVA test and LSD of means values of LFTs of negative control rats (Group 1), GA3 treated rats (Group 3) and Recovery group (group 4) .

Groups Parameters		Group 1 (Negative Control group)	Group 3 (GA3 group)	Group 4 (Recovery group)	P
Oxidative Stress Markers	SOD (U/gm)	70.57± 3.82	54.94± 3.31*	63.92± 4.05*#	<0.0001†
	CAT (nmol/ min/ml)	18.84± 2.12	15.61± 0.33*	17.04±1.5*#	<0.0001†
	GSHPx (U/gm)	166.92± 4.10	129.64±3 .86*	146.17±3.57 *#	<0.0001†
	MDA (nmol/ gm)	21.33± 3.30	144± 1.54*	142± 5.4*	<0.0001†

†: significant difference (p<0.05) as compared by ANOVA test, * : Significant difference as compared to the negative control group (P < 0.05), #: significant difference as compared to GA3 treated group
n=10 in all groups

2- Histopathological Results:

2-1 Light microscopic examination:

2-1-1 H&E stain:

Examination of H&E stained liver sections of control groups showed the normal hexagonal or pentagonal lobules with central veins and peripheral hepatic triads (portal areas) contained branches of the portal vein, hepatic artery, and bile duct. Hepatocytes are arranged in trabeculae running radiantly from the central vein. They had stippled appearance of the acidophilic cytoplasm and contained large vesicular nuclei. The trabeculae were separated by sinusoidal spaces (Fig. 1).

Examination of H&E stained liver sections of GA3 treated group showed, mild piecemeal necrosis of hepatocytes and inflammatory cellular infiltrates. Most of hepatocytes appeared apoptotic. They appeared shrunken with pyknotic nuclei. The hepatocytes also appeared vacuolated with few inflammatory cells in-between (Figs. 2,3).

Light microscopic examination of H&E stained liver sections of the recovery group showed partial recovery. Most of hepatocytes showed ground glass appearance. The cytoplasm appeared pale stained with apparently normal basophilic nuclei with appearance of some binucleated cells (Fig. 4).

2-1-2 Immunolocalization of Bcl-2:

Immunolocalization of Bcl-2 in all examined liver specimens of control groups revealed that hepatocytes were negative for Bcl-2 expression. On the other hand, 6 weeks of treatment with GA₃ resulted in increased overexpression of Bcl-2 in hepatocytes especially those surrounding the central vein and endothelial cells of blood sinusoids (Fig.5). Upon recovery both hepatocytes and endothelial cells regain its negative expression of Bcl-2 (Fig. 6).

2-2 Electron microscopic examination:

Electron microscopic study of the control group showed the ultra structure of the liver. The hepatocytes appeared with euchromatic nuclei containing prominent nucleoli. The cytoplasm contained numerous mitochondria, smooth endoplasmic reticulum and rough endoplasmic reticulum. The hepatocytes were adjacent to each other (Fig. 7).

On the other hand, the electron microscopic study of GA3 treated group showed apoptosis of some hepatocytes with shrinkage of their nuclei and condensation of their heterochromatin. The cytoplasm appeared empty of cytoplasmic organelles (cytoplasmic rarification). Some apoptotic cells showed swelling of mitochondria and vacuolations (Figs. 8,9).

The electron microscopic study of the recovery group showed partial improvement of hepatocytes. There was reappearance of cell organelles (mitochondria & cisternae of ER). The nuclei appeared euchromatic with prominent nucleoli. The rest of cytoplasm was occupied by hypertrophied SER. There was some cytoplasmic vacuolization (Fig. 10).

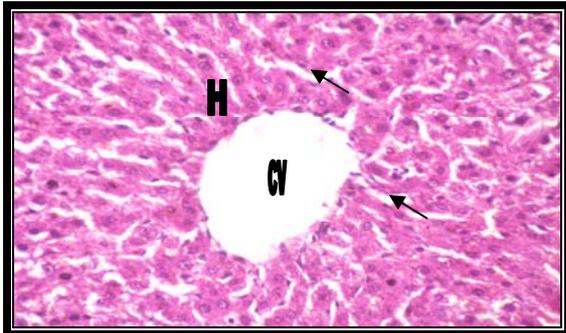


Fig. (1): A photomicrograph of a section in the liver of a negative control adult male albino rat showing part of hepatic lobule with central vein (CV) and sheets of hepatocytes (H) with sinusoidal spaces in between (arrows). (H&E X400)

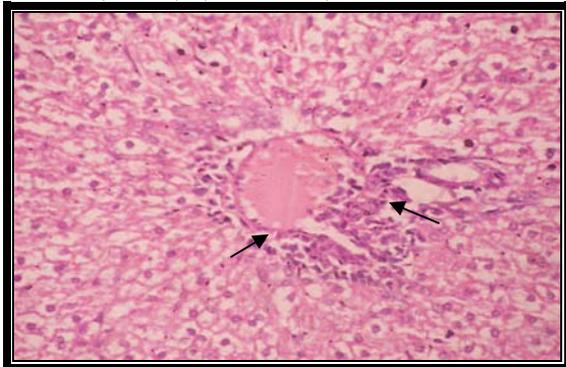


Fig. (2): A photomicrograph of a section of the liver obtained from an adult male albino rat from the 3rd group (GA₃ group) showing inflammatory cellular infiltrates (arrows). Most of hepatocytes showing cytoplasmic vacuolations. (H & E X400)

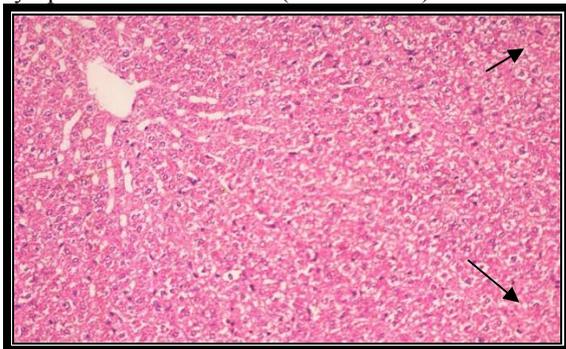


Fig. (3): A photomicrograph of a section of the liver obtained from an adult male albino rat from GA₃ group showing cytoplasmic vacuolations of hepatocytes and few inflammatory cells in between. Hepatocytes at the periphery of the lobules showed mild piecemeal necrosis (arrows)(H & E X 200)

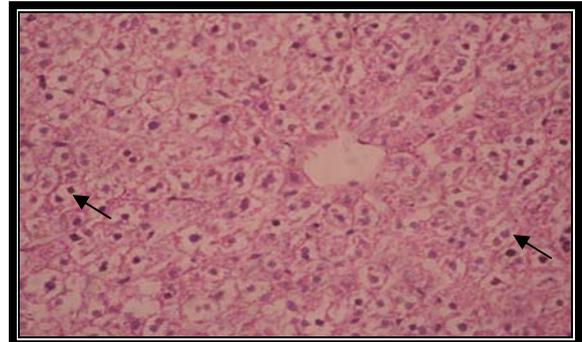


Fig. (4): A photomicrograph of a section of the liver obtained from an adult male albino rat from the 4th group (Recovery Group) showing ground glass appearance of hepatocytes. The cytoplasm appears pale stained. Apparently normal basophilic nuclei with appearance of some binucleated cells (arrows). (H&E X 200)

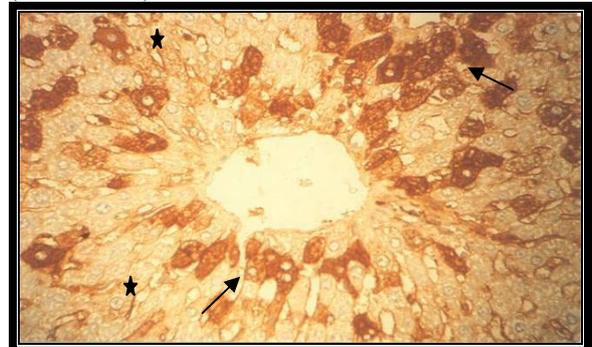


Fig. (5): A photomicrograph of a section of the liver obtained from an adult male albino rat from the 3rd group (GA₃ Group) showing strong expression of Bcl-2 in hepatocytes surrounding the central vein (arrows) and endothelial cells of blood sinusoids (*). (avidin- biotin immunoperoxidase for Bcl-2 x 400)

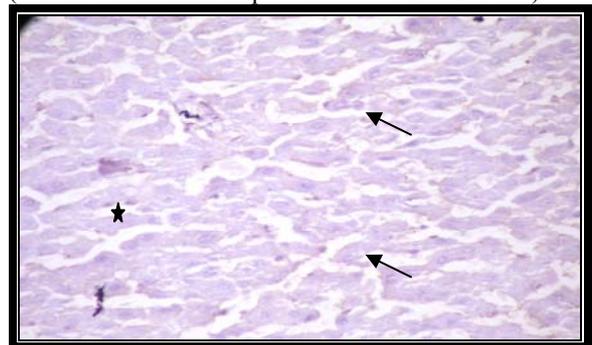


Fig. (6): A photomicrograph of a section of the liver obtained from an adult male albino rat from the recovery group showing negative expression of Bcl-2 in hepatocytes (arrows) and endothelial cells of the blood sinusoids (*). (avidin- biotin immunoperoxidase for Bcl-2 x 400)

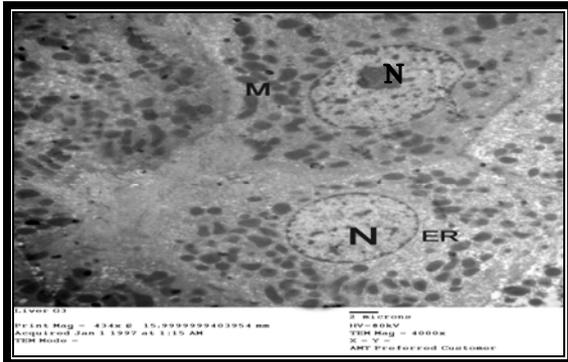


Fig. (7): An electron micrograph of a section in the liver of a negative control adult male albino rat showing adjacent hepatocytes with euchromatic nuclei (N) containing prominent nucleoli. The cytoplasm contains numerous mitochondria (M), smooth, and rough endoplasmic reticulum (ER). (X 4000)

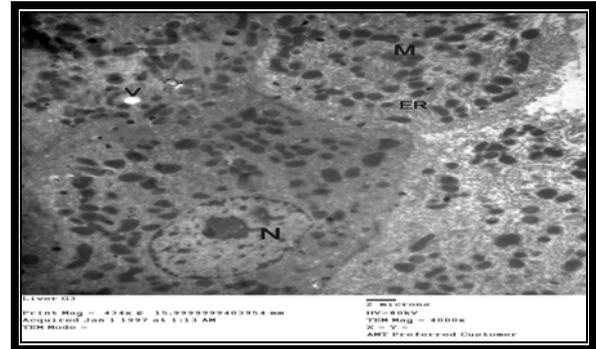


Fig. (10): An electron micrograph of a section of the liver obtained from an adult male albino rat from recovery group showing reappearance of cytoplasmic organelles (mitochondria (M) & cisternae of ER). The nucleus (N) appears euchromatic with prominent nucleolus. There is still some cytoplasmic vacuolization (V). (X4000)



Fig. (8): An electron micrograph of a section of the liver obtained from an adult male albino rat from GA₃ group showing cytoplasmic rarification (*) and vacuolization (V) of hepatocytes with shrinkage of the nucleus and condensation of its heterochromatin (arrow). (X 4000)

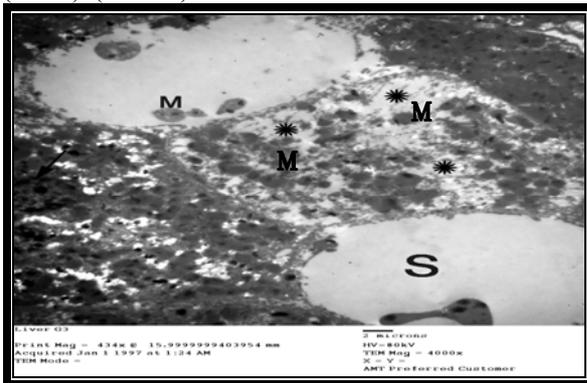


Fig. (9): An electron micrograph of a section of the liver obtained from an adult male albino rat from GA₃ group showing apoptotic nuclei (arrow), swelling of mitochondria (M) and cytoplasmic rarification (*). Notice part of sinusoid is seen (S)(X4000)

4. Discussion

Gibberellic acid (GA3) is produced by a naturally-occurring fungus in large vats (Schwechheimer and Willige, 2009).

GA3 is used to increase fruit size, increase cluster size (in grapes), delay ripening of citrus fruits, speed up flowering of strawberries, and stimulate starch break down in barley (for beer making). Also, it is used to promote growth of male flowers on female plants and allows production of female-only seeds and seedless fruits (Cambell and Jane, 2002 & Seiler, 2005).

Although GA3 is extensively used in Egypt and other countries, little is known about its toxic effects in mammals as well as its potential hazardous effects on human health (Saber *et al.*, 2003 & Erin *et al.*, 2008).

The increasing use of this substance in agriculture making it as an interesting subject to investigate its possible adverse effects on the liver as one of the main target organs for different xenobiotics. So, the aim of this study was to evaluate hepatotoxic effects of GA3 in adult male albino rats for 6 weeks, and also to determine the effects of withdrawal of GA3 on the affected parameters following 6 weeks of follow up.

The present study revealed that, both control groups showed no abnormal findings as regards liver enzyme biomarkers: AST, ALT, GGT and ALP and also the antioxidant enzymes activities (SOD, CAT, GSHPx) and MDA level in liver. There was no significant difference between the negative and the positive control group as regard all these parameters. Also, there were no abnormal histopathological changes in the liver specimens of the adult male

albino rats of these groups all over the period of the study.

GA₃ treatment for 6 weeks induced a significant increase in the mean values of serum AST, ALT, GGT & ALP when compared with the negative control group. Upon recovery, there was an improvement in the mean values of liver function tests of the treated group as the recovery group showed a statistically significant decrease in LFTs when compared with those of GA₃ treated group. This improvement was partial because these mean values still significantly higher than those of the negative control group.

These findings could be explained by Jaeschke *et al.* (2002) who found that leakage of the enzymes were produced within hepatocytes and small amounts constantly leak through the cell membrane which gave the normal serum enzymes level of these enzymes. Liver damage caused by liver cell injury (hepatocellular toxicity) made the membranes more permeable.

The results of the present work were parallel to Sakr *et al.* (2003). They observed a significant increase in serum ALT & AST after treatment with GA₃ which were considered to be a sensitive measure in evaluating hepatocellular damage.

In the present study the disturbance in LFTs was accompanied with disruption of the hepatic antioxidant enzymes activities with accumulation of MDA indicating GA₃ induced oxidative stress and lipid peroxidation in the treated animal livers. As there were statistically significant decreases in the mean values of SOD, CAT, GSHPx enzymes activities and significant increase in the mean values of MDA level in the hepatic tissues of the treated rats in comparison to that of the control group.

Upon recovery, the mean values of SOD, CAT and GSHPx enzymes showed a significant increase in comparison to GA₃ treated group. These mean values were still significantly lower than those of the negative control group. Also, the mean values of MDA level showed a non significant decrease in comparison to GA₃ treated group.

The result of the present study was in agreement with Orrenius *et al.* (2003) who found that the plant growth regulators compounds including GA₃ can accelerate lipid peroxidation up to 65-fold, in different tissues and this was attributed to the formation of OH radicals that may react with the lipids, possibly by hydrogen abstraction leading to oxidative damage within the cell.

Moreover, Muthuraman and Srikumar (2009) investigated the effect of GA₃ on the antioxidant defense status and lipid peroxidation level in rats. They stated that sub-chronic treatment of rats with GA₃ caused enhancement of lipid peroxidation

and reduction of antioxidant defense in treated animals when compared to the control rats.

Regoli and Principato (1995) mentioned that decreased CAT, GSHPx enzymes activities might have reflected a cellular oxidative stress due to GA₃ exposure. This decrease in antioxidant enzymes activities might be due to excessive consumption secondary to the flux of superoxide radicals or due to further decrease in the activities of these protective enzymes.

The flux of superoxide radicals can attack molecules in biological membranes, tissues, and mediate chain reactions which target lipids (Radi *et al.* 1991), polysaccharides, DNA and proteins, leading to various forms of cell injury (Stadtman and Levine, 2000).

Results of light and electron microscope examination of stained liver sections obtained in the present study have supported the above mentioned biochemical results.

Light-microscope examination of H&E stained liver sections of GA₃ treated rats after 6 weeks, showed mild piecemeal necrosis, inflammatory cellular infiltrates and appearance of many apoptotic cells. These apoptotic cells appeared shrunken with pyknotic nuclei. The hepatocytes also appeared vacuolated with inflammatory cells in-between. Furthermore, 6 weeks of treatment with GA₃ resulted in increased intensity of staining of Bcl-2 especially in hepatocytes surrounding the central vein and endothelial cells of sinusoids.

Electron microscope examination of liver sections had clarified the above mentioned results. 6 weeks GA₃ treatment, resulted in apoptosis of some hepatocytes with shrinkage of the nuclei and condensation of their heterochromatin. The cytoplasm appeared empty of cytoplasmic organelles (cytoplasmic rarification). Some apoptotic cells showed swelling of mitochondria and vacuolations.

The histopathological findings of this study were coincided with Sakr *et al.* (2003) and Troudi *et al.* (2009) who stated that the liver sections of GA₃ treated rats revealed that hepatocytes were swollen and appeared with severe cytoplasmic vacuolization with degeneration of their nuclei. They also stated that such injuries were more obvious in the peripheral lobular zones than the pericentrally located ones. The intrahepatic blood vessels were congested. They also noticed massive cellular infiltrations with inflammatory cells in several areas of the lobules.

These results were in accordance with Abd El Maksoud *et al.* (1996) who recorded that the oral administration of GA₃ revealed significant histological changes in the liver cells in the form of appearance of large areas of rarified cytoplasm with disappearance of cellular organelles.

The biochemical interpretation of the cytoplasmic swelling and vacuolations had been subjected to wide speculations by many investigators. Sherlock and Dooly (2002) demonstrated that cytoplasmic swelling and vacuolization are one of the most important primary responses to all forms of cell injury. They occurred due to increased permeability of cell membranes leading to an increase of intracellular water. As water sufficiently accumulates within the cell, it produces cytoplasmic vacuolization.

Many earlier studies disclosed that mitochondrial dysfunction contributed to apoptosis via the production of reactive oxygen species (Mignotte and Vayssiere, 1998).

For interpretation of the mechanism of mitochondrial swelling reported in this study, Jaeschke *et al.* (2002) mentioned that oxidative stress had contributed to the opening of the mitochondrial permeability transition pore (PTP) which led to the formation of a high-conductance channel, in the inner mitochondrial membrane, and led to mitochondrial swelling and subsequent release of cytochrome c from the intermembrane space.

PTP opening appears to be associated with apoptosis or necrosis according to the presence or deficiency of ATP (Lemasters, 2002).

The histopathological changes in the present study as regard swelling of mitochondria were in accordance with the results of Pessayre *et al.* (2001) and Krahenbuhl (2001) who reported that inhibition of mitochondrial function together with accumulation of reactive oxygen species and lipid peroxidation, all these factors led to cell death.

As regarding the follow up period in this study, examination of H&E stained liver sections showed partial improvement. Most of hepatocytes showed ground glass appearance with appearance of some binucleated cells. Immunolocalization of Bcl-2 revealed that most of hepatocytes and endothelial cells of sinusoids became negative for Bcl-2 expression.

The ground glass appearance of hepatocytes was clarified by electron microscopic picture which showed partial improvement in the form of reappearance of cell organelles (mitochondria & cisternae of RER). The nuclei appeared euchromatic with prominent nucleoli. The rest of cytoplasm was occupied by abundant SER. There still was some cytoplasmic vacuolization.

These results were coincided with Abd El Maksoud *et al.* (1996) who stated that following GA₃ withdrawal, a few cells became nearly similar to those of the control group, while the majority of cells remained affected.

The above mentioned results coincided with Saly (1998) who studied the chronic toxic effects of

GA₃ on liver, heart and kidney and stated that 4 weeks of recovery from the destructive effects of GA₃ was incomplete.

The ground glass appearance of hepatocytes detected in the recovery period of this study was coincided with Droge (2002) who mentioned that the glass appearance reflect adaptation of cells upon recovery.

These results were coincided with Kimball (2008) who stated that the ground glass appearance of liver cells was considered as adaptive mechanism and occurred as a result of increased synthesis of cellular organelles such as mitochondria and RER probably to increase function of individual hepatocytes.

Abundant SER in the recovery period could be also explained by Benedetti (2005) who stated that it takes part in the synthesis of phospholipids for building of cell membrane and membranes of cell organelles.

On the other hand the increased RER was explained by Kimball (2008) who mentioned that RER had a very important role in the synthesis and packaging of proteins. Some of these proteins might be used by the cell to synthesize membrane, other cell organelles, and the others were sent out. This was considered as a structural-functional response to enable cells to release the oxidative stress secondary to GA₃ toxicity.

Conclusion

From the previously mentioned results we can conclude that gibberellic acid was a potent pro-oxidant that induced a significant hepatotoxicity in adult male albino rats following 6 weeks of daily exposure. The hepatotoxic effects of GA₃ were manifested by impairment of the LFTs that associated with inhibition of the hepatic antioxidant enzymes with accumulation of MDA the end product for lipid peroxidation. GA₃ induced lipid peroxidation promote local apoptosis and upregulation of Bcl-2 that end in hepatocyte apoptosis and cell death. On the other hand, 6 weeks period of follow up was in sufficient for complete recovery of these toxic effects.

RECOMMENDATIONS

1. Gibberellic acid (GA₃) use should be under strict control.
2. Periodic monitoring of GA₃ concentration in the soil and plants.
3. More studies are needed to explore other hazardous effects of GA₃ on other body systems and organs.
4. Other studies with prolonged periods of administration of GA₃ are recommended to learn more about its toxic effects.

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