Biochemical And Molecular Profiles Of Gibberellic Acid Exposed Albino Rats

Hanan A.E.Soliman[1]; Mona M. Mantawy[2] and Hany M. Hassan[3]
[1]Chemistry Departement,Biochemistry Branch,Faculty of Science,Beni suef University,Egypt. [2] ; Department of Medicinal chemistry , National Research Center , Dokki , Egypt and [3] Immunobiology and immunopharmacology unit , Animal Reproduction Research Inst. , Giza , Egypt
dr_mona_zaki@yahoo.co.uk

ABSTRACT: The present study casts the light on the influence of the plant growth regulator ,Gibberellic acid (GA3), on antioxidant defense systems [glutathione peroxidase, superoxide dismutase (SOD), and catalase (CAT)] , lipid peroxidation level (malondialdehyde = MDA), AST, ALT , alkaline phosphatase , creatinine, total protein , albumin globulin , total lipids , total cholesterol, calcium and glucose . Moreover, histopathological examination of kidney and liver was done . On the molecular level the DNA damage was determined . The rats were received 75 ppm of GA3 in drinking water ad libitum for 50 days. Gibberellic acid (GA3) treatments caused different effects on the estimated parameters compared to control . Gibberellic acid exposure induced significant elevations of plasma AST, ALT , alkaline phosphatase , creatinine and malondialdehyde . However , Gibberellic acid produced non significant alterations in plasma total protein , albumin globulin , total lipids , total cholesterol, calcium and glucose . On the other hand, exposure elucidate significant reductions of catalase , superoxide dismutase and glutathione peroxidase in comparison to control group. The histopathological findings revealed that Kidney sections of Gibberellic acid treated rats suffered from areas of interstitial fibrosis which appear as segmental and global glomerular sclerosis tubulointerstitial injury . On the similar ground, liver section of Gibberellic acid treated rats , revealed that Gibberellic acid induced liver fibrosis; fatty metamorphosis and necrosis. The total genomic DNA electrophoretic pattern of lymphocytes deprived from Gibberellic acid treated rats revealed strong and obvious DNA damage as represented by a lot of fragments migrated from the wells. As a conclusion, Gibberellic acid(75 ppm) produce hepatonephrotoxicity , subsequently has oxidative stress role and DNA damage in albino rats 50 days post treatment. [Journal of American Science 2010;6(8):224_229]. (ISSN: 1545-1003).

Keywords: Biochemical; Molecular Profile; Gibberellic Acid; Rats

INTRODUCTION

Gibberellic acids are a group of plant growth regulators that have been identified in
different plants(MacMillian et al. ,1961)and they are used in agriculture as plant regulators
to stimulate both cell division and cell elongation that affect leaves as well as stems(Taiz and Zeige, 1991). Gibberellic acid (actually a group of related substances called Gibberellic acids) was discovered as a metabolic by product of the fungus *Gibberella fujikuroi* (Riley, 1987). If gibberellic acid or one of its metabolites is applied to dwarf varieties of peas, broad beans or maize, growth is greatly accelerated(Jones , 1973).

In *Alstroemeria hybrida*, leaf senescence is retarded effectively by application of Gibberellic acids(Kappers et al., 1997).Feeding toads, *Bufo regularis*, with Gibberellic acid A3 induced hepatocellular carcinomas in 16% of the animals.Moreover, it was showed that Gibberellic acid A3 induced breast and lung adenocarcinomas in mice(El-Mofty and Sakr ,1988). Gibberellic acid was found to induce chromosomal aberrations in human lymphocytes(Zalinian et al., 1990)and mice(Bakr et al., 1999). The World Health Organization(1990) classified Gibberellic acid-A3 as a plant growth regulators related to pesticides.

Gibberellic acid (Gibberellic acid A3) 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)(Weaver et al., 1961). Recently ,Kamel et al.(2009) recorded that Male rabbits treated with Gibberellic acid at all studied doses caused a significant increase in semen ejaculate volume, sperm concentration, total sperm out-put and sperm motility (%) and has direct androgenic-like action on testes compared to the control group.

The present investigation aimed to cast the light on the possible effects of Gibberellic acid on biochemical profile, histopathological image and DNA integrity of exposed rats.

MATERIALS AND METHODS

Twenty sexually mature male albino rats weighing 170±10 g were used. Animals were kept in the laboratory under constant temperature (24±2 ^{o}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. Ten rats in the first group were orally given Gibberellic acid-A3 (Berelex®, BDH chemical, Pool, UK) at 75 ppm in drinking water were continuously administered orally to rats ad libitum for 50 days (Celik et al.,2007). Animals in the second group (10 rats) were served as controls. The treated animals and their controls were sacrificed by decapitation after the end of treatment.

For enzyme determination, Plasma were obtained by centrifugation of the blood samples and stored at –20^{o}C until assayed for the biochemical parameters. Transaminases (ALT, AST) and alkaline phosphatase activities were determined on the basis of King(1965).

Plasma creatinine levels were measured using the photometric determination according to the Jaffe method (Ecoline Mega, DiaSys Diagnostic Systems GmbH, Holzheim, Germany) described earlier(van Dokkum et al ., 2004) .

Plasma total protein concentration as (g/dl) was measured by the Biuret method as described by Armstrong and Carr (1964). Albumin (A) concentration as (g/dl) was determined by the method of Doumas *et.al*. (1971). Globulin (G) concentration as (g/dl) was calculated as the difference between total protein and albumin. Plasma total lipids (PTL) concentration as (g/dl) were estimated according to Frings *et.al*. (1972). Total cholesterol (TCh) concentration as (mg/dl) was determined according to Richmond (1973). Plasma glucose (PG) concentration as (mg/dl) was estimated according to the method of Trinder (1969). Serum calcium (SCa) concentration as (mg/dl) was measured according to the method of Sarkar and Chauhan (1967) using commercial kits (Stanbio kits).

Catalase activity was estimated by the method of Cakmak and Horst (1991). The reaction mixture contained 100 crude enzyme extract, 500 µL 10 mM H_2O_2 and 1400 µL 25 mM sodium phosphate buffer. CAT activity of the extract was expressed as CAT units. Superoxide dismutase activity was determined with the reaction mixture contained 100 µL 1 µM riboflavin, 100 µL 12 mM L-methionine, 100 µL 0.1 mM EDTA (pH 7.8), 100 µL 50 mM Na_2CO_3 (pH 10.2) and 100 µL 75 µM Nitroblue Tetrazolium (NBT) in 2300 µL 25 mM sodium phosphate buffer (pH 6.8), 200 µL crude enzyme extract in a final volume of 3 mL. SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT glass test tubes containing the mixture were illuminated with a fluorescent lamp (120 W); identical tubes that were not illuminated served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm(Hernandez *et al*., 2000). Glutathione peroxidase (GSH-Px) was measured by spectrophotometric method developed by Paglia and Valentine (1967). One unit of GSH-Px activity was defined as the amount of the enzyme that converted 1 µM NADPH, substrate to NADP+ per minute. All the assays were carried out in triplicate using a spectrophotometer (Hitachi U-2000, Hitachi Ltd., Tokyo, Japan).

Malondialdehyde (MDA) was measured by colorimetric method (Stewart and Bewley, 1980).

Histological Examination

Rat kidneys were removed and portions fixed in 4% paraformaldehyde (PFA). Other kidney portions were frozen in optimum cutting temperature (OCT) compound (Miles, Elkhart, IN, U.S.A.) and stored at -80 °C. Fixed renal tissues were embedded in paraffin and cut into 4-µm-thick sections. The sections were stained with periodic acid-Schiff (PAS) stain and Masson trichrome stain to reveal histological changes and areas of interstitial fibrosis.

Liver tissue sections were fixed in 100 ml/L formalin saline in phosphate buffer and processed in paraffin wax. Sections from blocks were stained with hematoxylin-eosin , histological analyses were performed blindly using light microscope **.**

Total genomic damage of DNA analysis

DNA was extracted from lymphocytes of blood samples using DNA extraction Kit (Fermentas life Siences, Lithuania -Cat. #K0513).

Gel was prepared with 1.5% electrophoretic grade agarose (BRL) and 0.2% polyvinyl pyrolidine (PVP, Sigma). The agarose and PVP were boiled with Tris Borate EDTA buffer (TBE buffer; 89 mM Tris, 89 mM boric acid, 2mM EDTA, pH 8.3). 0.5 mg ethidium bromide /ml distilled water (Sigma) was added to the gel at 40^{o}C. Gel was poured and allowed to solidify at room temperature for 1h before samples were loaded (15 µl of extracted DNA/well). Electrophoresis was performed for 2 hrs at 50 volt using lX TBE buffer as a running buffer. Gel was photographed using a polaroid camera while DNA was visualized using a 312 nm UV light under a transilluminator (Herolab, Germany) . The photographes were analyzed using Phortex software version 3.0, UK to determine the degree of DNA damage (Cressman et al. ,1999) .

Statistical analysis

All values were expressed as mean ± standard error (SE). All statistical analyses were performed using SAS (version 8.02). Statistical differences among the experimental groups were assessed by one-way ANOVA. Tukey's test was used as a follow-up test and significance was defined at $p<0.05$.

RESULTS

Table (1) revealed that Gibberellic acid-A3 exposure induced significant elevations of plasma AST, ALT , alkaline phosphatase , creatinine and malondialdehyde . However , Gibberellic acid produced non significant alterations in plasma total protein , albumin globulin , total lipids , total cholesterol, calcium and glucose . On the other hand, Gibberellic acid exposure elucidate significant reductions of catalase , superoxide dismutase and glutathione peroxidase in comparison with control group.

Table(1): Effect of Gibberellic acid-A3 exposure on some biochemical parameters of rats.

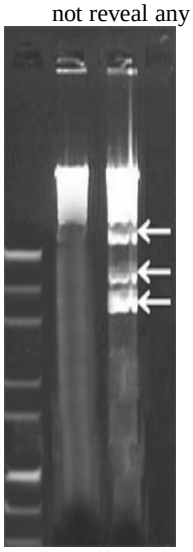
Items	**Control group**	**Exposed group**
AST (iu/l)	52.28 ± 4.28	66.85 ±4.51*
ALT(iu/l)	33.27 ± 4.33	44.31±3.07*
Alkaline phosphatase (iu/l)	103.85 ± 12.36	137.08±5.88*
Creatinine (mg/dl)	0.346 ± 0.16	0.636±0.09*

Total protein (gm/dl)	8.82 ± 1.61	8.91±1.55
Albumin (gm/dl)	4.42 ± 0.54	4.35±0.52
Globulin (gm/dl)	4.37 ± 0.33	3.61±0.21
Total lipids (gm/l)	4.208 ± 0.59	4.34± 0.18
Total cholesterol (mg/dl)	72.58 ± 5.78	75.89±5.48
Glucose (mg/dl)	138.24 ± 10.95	145.35±7.51
calcium (mg/dl)	1.66 ± 0.33	2.15±0.05
Catalase (nmol/min/ml)	18.77 ± 1.91	15.04±0.33*
Superoxide dismutase (u/ml)	105.28 ± 10.58	85.28±5.71*
Glutathione peroxidase (nmol/min/ml)	90.48 ± 10.84	74.33±5.49*
Malondialdehyde (nmol/dl)	4.33 ± 1.63	6.85±0.35*

*significant at $p < 0.05$

Total genomic damage of DNA :

Figure (1) shows the total genomic DNA electrophoretic pattern of lymphocytes deprived from control and Gibberellic acid treated groups. Gibberellic acid revealed strong and obvious DNA damage at the tested concentrations as represented by a lot of fragments migrated from the wells. On the other hand, control group did not reveal any damage of DNA .



Fig(1): Effect of Gibberellic acid-A3 treatment on DNA damage of DNA of rats, **arrows** indicate that fragmented DNA bands in Gibberellic acid treated group.

Histological Examination

Kidney sections of Gibberellic acid treated rats which stained with periodic acid-Schiff (PAS) stain and Masson trichrome stain to reveal histological changes and areas of interstitial fibrosis appear as segmental and global glomerular sclerosis tubulointerstitial injury (tubular dilatation, atrophy of tubular epithelial cells) (Photo , 1) .

Histopathological examination of Hematoxylin and eosin-stained liver section of normal and Gibberellic acid treated rats with magnification -400 X , revealed that Gibberellic acid induced liver fibrosis; fatty metamorphosis and necrosis (Photo, 2).

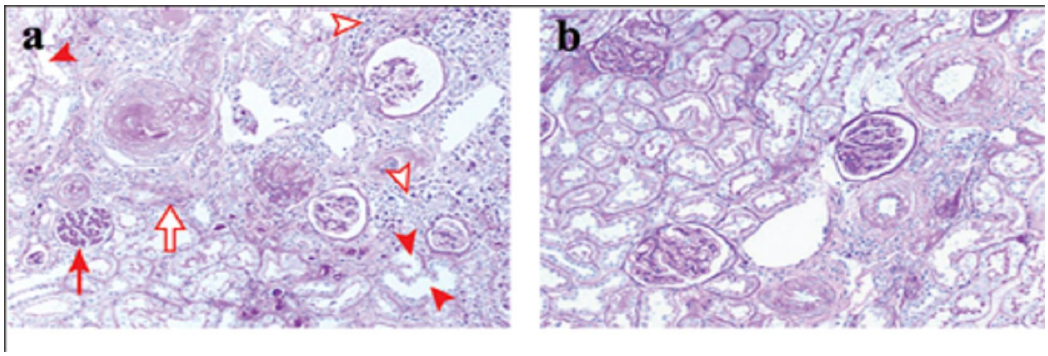


Photo (1): Rat renal tissue sections with PAS staining arrows indicating global glomerular sclerosis and tubulointerstitial injury: arrow heads; fibrosis: open arrow; and infiltration of inflammatory cells: open arrow heads) in Gibberellic acid treated (a) and control (b).

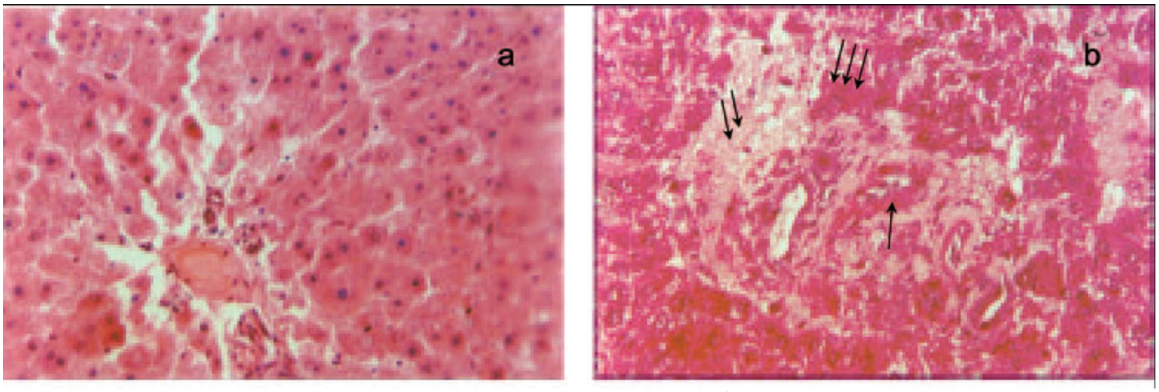


Photo (2):Liver tissue sections were stained with haematoxylin and eosine for light microscope observation . **(a**) control . (**b**) Gibberellic acid treated. ↓↓, fibrosis; ↓↓↓, fatty metamorphosis; ↑, necrosis

DISCUSSION

Gibberellic acid is a type of plant hormone which regulates growth. There are 126 known Gibberellic acids, divided into two classes, and many more may be discovered in the future. Plants produce these hormones naturally through biosynthesis as they grow, ensuring that they have the hormones they need to develop normally, and these hormones can also be applied to plants by gardeners and farmers to achieve specific desired outcomes (**Fernandez** and **Rodriguez**,1979).

In the present study, Gibberellic acid induced significant elevations of plasma AST, ALT , alkaline phosphatase, creatinine and malondialdehyde. However, Gibberellic acid produced non significant alterations in plasma total protein, albumin globulin, total lipids, total cholesterol, calcium and glucose .on the other hand, Gibberellic acid exposure elucidate significant reductions of catalase, superoxide dismutase and glutathione peroxidase in comparison with control group.

The histopathological findings revealed that Kidney sections of Gibberellic acid treated rats suffered from areas of interstitial fibrosis appear as segmental and global glomerular sclerosis tubulointerstitial injury .

On the similar ground , liver section of Gibberellic acid treated rats with magnification -400 X , revealed that Gibberellic acid induced liver fibrosis; fatty metamorphosis and necrosis.

The previous findings were in accordance with the recorded findings of Sakr et al.(2003), who recorded that Gibberellic acid induced 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.

On the same hand, Celik et al. (2007) recorded that gibberellic acid (GA3) has deleterious effect on the antioxidant defense systems [reduced glutathione (GSH), glutathione reductase (GR), superoxide dismutase (SOD), glutathione-S-transferase (GST) and catalase (CAT)] and lipid peroxidation level (malondialdehyde = MDA) in various tissues of the rat were investigated during treatment as a drinking water 75 ppm of ABA and GA3 in drinking water were continuously administered orally to rats for 50 days. The

lipid peroxidation end product MDA significantly increased in the lungs, heart and kidney of rats treated with GA3. The GSH levels were significantly depleted in the spleen, lungs and stomach of rats treated with GA3. SOD significantly decreased in the spleen, heart and kidney. While CAT activity significantly decreased in the lungs of rats treated with GA3. The drug metabolizing enzyme GST activity significantly decreased in the lungs of rats treated with ABA but increased in the stomach of rats treated with GA3. the authors concluded that GA3 produced substantial systemic organ toxicity in the spleen, lungs, stomach, heart and kidney during a 50-day period of subchronic exposure.

The present study disclose that the total genomic DNA electrophoretic pattern of lymphocytes deprived from control and Gibberellic acid treated groups. Gibberellic acid revealed strong and obvious DNA damage at the tested concentrations as represented by a lot of fragments migrated from the wells. On the other hand, control group did not reveal any damage of DNA .

These data on the same way of the findings of Hassab-Elnabi and Sallam (2002).
They recorded that higher concentration of Gibberellic acid induced total genomic damage of DNA. By the application of modified comet assay (single cell gel electrophoresis) technique, DNA damage was found at all applied doses. Also, Bakr et al. (1999) reported a significant increase in the incidence of total chromosomal aberrations induced by gibberellic acid in bone marrow cells of albino mice.

The illustrated results is fully agreed with that recorded by Zalinian *et al.* (1990). They reported that gibberellic acid induced chromosomal aberrations in human lymphocyte cultures. Gibberellic acid in this demonstration induced a significant damage of DNA. This result agreed with the data that declared by Abou-Eisha (2001). He showed that Gibberlic acid induces a dose-dependent increase in the level of DNA breakage in human blood cells , this increase attaining statistical significance at the highest concentrations tested (25, 100, 150µg/ml), which would confirm its genotoxicity.

So, the DNA damage may attributed to the direct attack of DNA by gibberellic acid causing alkali labile and single strand breaks and total genomic damage as revealed by our demonstration, or may be due to accumulation of nucleases as reported by Fath *et al.* (1999). The mechanism of gebrilic acid to induce DNA damage may be attribute to elevation of oxidative stress markers such as (ROS and GSH), Bcl-2 protein expression, mitochondrial membrane potential and caspase-3 activity. The sequel of these events lead to mitochondrial membrane depolarization and caspase-3 activation followed by apoptosis(Abou-Eisha , 2001).

From the recoded data , it could be concluded that Gibberellic acid(75 ppm) produce hepatonephrotoxicity , subsequently has oxidative stress role and DNA damage in albino rats 50 days post treatment.

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