Cytogenetic, histological and histochemical studies on the effect of gibberllin A_3 in albino rats

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Abstract: Gibberllic acid (GA_3) is an endogenous plant growth regulator used worldwide (particularly in Egypt) in agriculture. The goal of this work was to screen the possible genotoxic and cytotoxic effects of GA_3 in adult male albino rats. The frequency of chromosome aberrations (CA), micronuclei (MN) and sperm abnormalities were used as endpoints for genotoxicity. In addition, bone marrow activity has been investigated. Kidney histological and histochemical studies were performed to detect the cytotoxic effect of GA_3. Image analysis was used to quantify the histochemical detection of protein as a bioindicator for GA_3 toxicity in the renal tissue. A single daily close of 500 mg GA_3 (⅓LD_{50})/kg body weight was orally administrated in male albino rats for 6 continuous days. An increase in the frequency of chromosomal aberrations (structural & numerical), micronuclei production and sperm abnormalities was observed in most treatments with GA_3 with a comparable increase in bone marrow activity. Also, administration of GA_3 induced many histopathological alterations in the kidney such as glomerular vacuolization, shrinkage and degeneration, necrosis and atrophy in the epithelia of the renal tubules leading to increased intertubular spaces. Congested and damaged blood vessels leading to concomitant hemorrhage were also observed. Histochemical observations supported by image analysis revealed a significant decrease in the total protein content of the renal epithelia as compared to controls. Therefore, kidney histological and histochemical studies confirmed the cytogenetical parameters to conclude that the exposure of rats to GA_3 has a genotoxic and cytotoxic effects. The increase in the genotoxic effect corresponds to a decrease in the mitotic activity of bone marrow cells.

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Key words: Gibberllin A_3, Cytotoxic, Genotoxic, Albino rats.

1. Introduction

Many chemicals are currently used in agriculture and plant growth regulators (PGRs) were among those widely employed and the most of these substances placed into the environment **(Mickel, 1978).** Gibberellic acid is a type of plant hormones which regulate growth. 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). Weaver (1961)** reported that the gibberellic acid (GA_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 cauliflowers).

Gibberellic acid was found to induce chromosomal aberrations in human lymphocytes **(Zalinian *et al.*, 1990)** and mice (**Bakr *et al.*, 1999).** Also**, Sakr *et al.* (2009)** showed that gibberellic acid induced cytogenetic changes in human lymphocytes culture in which treating cultures with GA_3 induced chromosomal aberrations, sister chromatid exchanges and DNA damage. The increase of GA_3 dosage increased the chromosomal aberration. They also reported that when green tea and GA_3 were simultaneously applied in the culture media, the mutagenic changes induced by GA_3 were significantly reduced.

The micronuclei are fragments of chromosomes or whole chromosomes that are not incorporated in the main nuclei during the mitosis and, therefore, appear in those cells which have gone through a division process**. Johnson and Sharma (2001)** cleared that the micronucleus test is a valid indicator of chromosomic breakdown and dysfunction of the spindle. Also **Ortize *et al.* (2000)** observed the presence of micronuclei in mouse bone marrow cells and peripheral lymphocytes treated with high dose of paraquat.

A significant increase in sperm motility was recorded by **El komy (2003)** adult cocks treated with GA_3 conducted to a significant increase in ejaculate volume and sperm concentration and this increase was correlated with increase in serum testosterone level. Also, **Kamel *et al.* (2009)** recorded that the male rabbits treated with gibberellic acid caused a significant increase in semen ejaculate volume, sperm concentration, total sperm out-put, seminal plasma

proteins and sperm motility percentage and has direct androgenic-like action on testes compared to control group. GA_3 also increase percentage of live sperm and decrease percentage of dead and abnormal sperm. Feeding toads (*Bufo regularis*) with GA_3 induced neoplasms in 16% of the experimental animals (**El-Mofty and Sakr, 1988)** These results showed that GA_3 has a carcinogenic effect in the liver, kidneys and ovaries of the Egyptian toads. Moreover, **Hanan *et al.* (2010)** revealed that kidney sections of gibberellic acid-treated rats suffered from areas of interstitial fibrosis which appeared as segmental and global glomerular sclerosis and tubule-interstitial injury.

Gibberellin A3 was also found to be involved in the synthesis of RNA and proteins (**Williams and Weisburger, 1991).** These authors showed that chemical carcinogens, by themselves or after activation, interact with cellular macro-molecules such as DNA, RNA, and proteins, and these interactions result in the development of neoplasia. **Hanan *et al.* (2010)** reported that, rats which received 75 ppm of GA_3 in drinking water for 50 days. GA_3 produced non-significant alterations in plasma total protein, albumin, globulin, total lipids, total cholesterol, calcium and glucose.

Aim: The present work was conducted to study the cytogenetic, histological and histochemical effects of gibberllin A_3 in albino rats.

2. Material and methods:

Animals and treatment:

Thirty five adult male albino rats were obtained from the animal house of the Faculty of Veterinary Medicine, Zagazig University, Egypt having an average age of 2.5 - 3 months and their weight average was 150 - 200 gm. The animals were housed in plastic cages and supplied with enough food (standard pellets) and water and observed daily for a period of two weeks before any experimental action for acclimatization. Animals were classified into 2 groups. The first group: five rats given 0.5ml of distilled water orally by oro-gastric tube for 6 successive days and served as control. Second group: thirty rats subdivided into six equal subgroups, 5 rats in each one. Each rat of these subgroups received a daily dose of 500mg (1/3 LD_{50} according to **Dresbach, 1987**) of gibberellic acid /Kg b.w. (a product of VALENT BioSciences corporation, USA, imported by SOFEZCo and registered at Egyptian Ministry of Agriculture N 4127) dissolved in 0.5 ml distilled water and administrated by orogastric tube for 6 continuous days. The treated animals and their controls were sacrificed after the 1st, 2nd and 3rd weeks post GA_3 administration.

Chromosomal aberration assay:

Cytogenetic analysis of chromosomal aberration (CA) of bone marrow cells was carried out according to the CA technique modified by **Carbonell *et al.*** (1996).

Micronucleus test:

The frequency of micronucleated polychromatic erythrocytes (MNPCEs) in femoral bone marrow preparations was scored and evaluated according to the method of **Schmid (1975)** modified by **Hayashi *et al*. (1994).**

Estimation of the frequencies of abnormally shaped sperm cells:

The sperm smears were obtained from the cauda epididymes of the testes of adult control and treated male rats. The cauda epididymes were cut into small pieces in 1 ml of 0.9% saline solution. Sperm smears were obtained from the resulting suspension. They were stained by Feulgen nuclear stain and counter-stained in 1% light green (1 gm dissolved in 100 ml methyl alcohol. Approximately 1000 sperm cells were microscopically examined for each animal. A binocular microscope with X 10 eyepieces and X 100 oil immersion objective lenses were used for this study. Abnormally shaped sperms were recorded randomly and microphotographs were taken whenever necessary. Statistical analysis was evaluated by a dispersion test according to **Snedecor and Cochran (1976).**

Histological studies:

Tissue specimens of kidney from control and treated animals were removed and fixed in 10% neutral formalin, dehydrated, cleared and sectioned at 3 µ to be stained with H&E for light microscopic examination **(Bancroft and Gamble, 2002).**

Histochemical studies:

The total protein content of the renal tissue in control and treated animals was visualized qualitatively by the application of "Mercury Bromophenol Blue" (MBB) method of **Mazia *et al.* (1953)**.

Protein quantification:

All the histochemical sections of kidney for protein in control and treated animals were subjected to the image analysis. The method was used for the quantitative measurements of the optical density of protein using IP win image proplus version 4.5. Data were statistically analyzed using Microsoft Excel 2007.

3. Results:

Chromosomal pattern in bone marrow cells of the control and GA_3-treated animals:

Examination of control animals showed that metaphases with aneuploidy cells could be demonstrated and could be divided into more & less than 42 chromosomes. Less ones could be spotted on

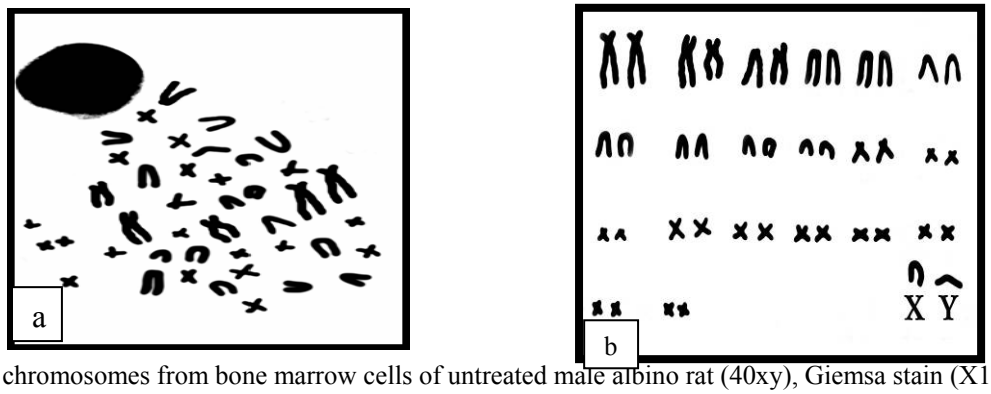
15 metaphases, while the more ones could be spotted on 7 out of 250 metaphases. No polyploid cells were seen in control metaphases. As regards the structural aberrations in examined 10500 chromosomes belonging to 250 metaphases only 2 ring chromosomes and 2 acentric fragments were seen (Table 1). Totally the chromosomal abnormalities were 26 metaphases giving a percentage of 10.4%.

Various chromosomal aberrations were observed in spreads of bone marrow cells of GA_3-treated rats. Chromosomal aberrations induced by GA_3-treatment included numerical and structural aberrations. The numerical ones were represented by metaphases with more or less number of 42 chromosome (aneuploidy) and metaphases with more than 2 haploid sets of chromosomes (polyploidy). However, the structural chromosomal aberrations were in the form of dicentric chromosomes, deletions, ring chromosomes, exchange figures, centromeric fusions and acentric fragments (Fig. 2).

The total chromosomal aberrations in bone marrow cells after GA_3 treatment exhibited significant values; 104, 95 and 84 metaphases after the 1st, 2nd and 3rd weeks post-treatment respectively. While, total aberration in the control group was 26 among 250 metaphases. Therefore, the average percentages of these values of total chromosomal aberrations were 41.6%, 38% and 33.6% respectively, compared to 10.4% in the control group. The higher percentage of chromosomal aberrations after GA_3 administration was noted after the first week post-treatment while this percentage was decreased gradually by the time factor after GA_3 administration (Table 1).

In the current investigation the mitotic index of bone marrow cells which reflect their growth rate and measured by the proportion of cells undergoing mitosis to those of the non- dividing cells at a given time was also calculated. The cytogenetic toxicity of GA_3 in bone marrow cells affected directly their growth rate i.e. GA_3 reduced the mitotic index which recorded 4.8% in control group and 4.0%, 3.6% and 3.2% after 1st, 2nd and 3rd weeks post-treatment respectively (Table 1).

In comparison between the frequencies of chromosomal aberrations during all time intervals after treatment of rats with GA_3 showed that the numerical aberrations were more frequent than the structural aberrations. Also, referring to the time of treatment, the total aberration was more affected after the first week post treatment and then decreased gradually towards the end of the experimental time (Fig. 3).



a- Metaphase chromosomes from bone marrow cells of untreated male albino rat (40xy), Giemsa stain (X1000).
b- A karyotype of the previous spread of the control animal.

Table (1): Comparison between the frequencies of chromosomal aberrations from bone marrow cells of control and GA_3-treated rats:

Time of treatment	No. of rats	No. of scored cells 50/Rat	Chromosomal aberrations										
			Numerical aberrations			Structural aberrations			Total aberrations			Mitotic index	
			No.	%	Mean ± S.E.	No.	%	Mean ± S.E.	No.	%	Mean ± S.E.	No. of metaphases /1000 cells	%
Control	5	250	22	8.8	4.4 ± 0.5	4	1.6	0.8±0.37	26	10.4	5.2±0.8	48	4.8
1 st week	5	250	82	32.8	16.4±0.6**	22	8.8	4.4±0.67**	104	41.6	20.8±0.73**	40	4.0
2 nd week	5	250	73	29.2	14.6±0.87**	22	8.8	4.4±0.5**	95	38.0	19.0±0.7**	36	3.6
3 rd week	5	250	68	27.2	13.6±0.6**	16	6.4	3.2±0.37*	84	33.6	16.8±0.37**	32	3.2

*Significant at $P < 0.05$ ** Significant at $P < 0.01$

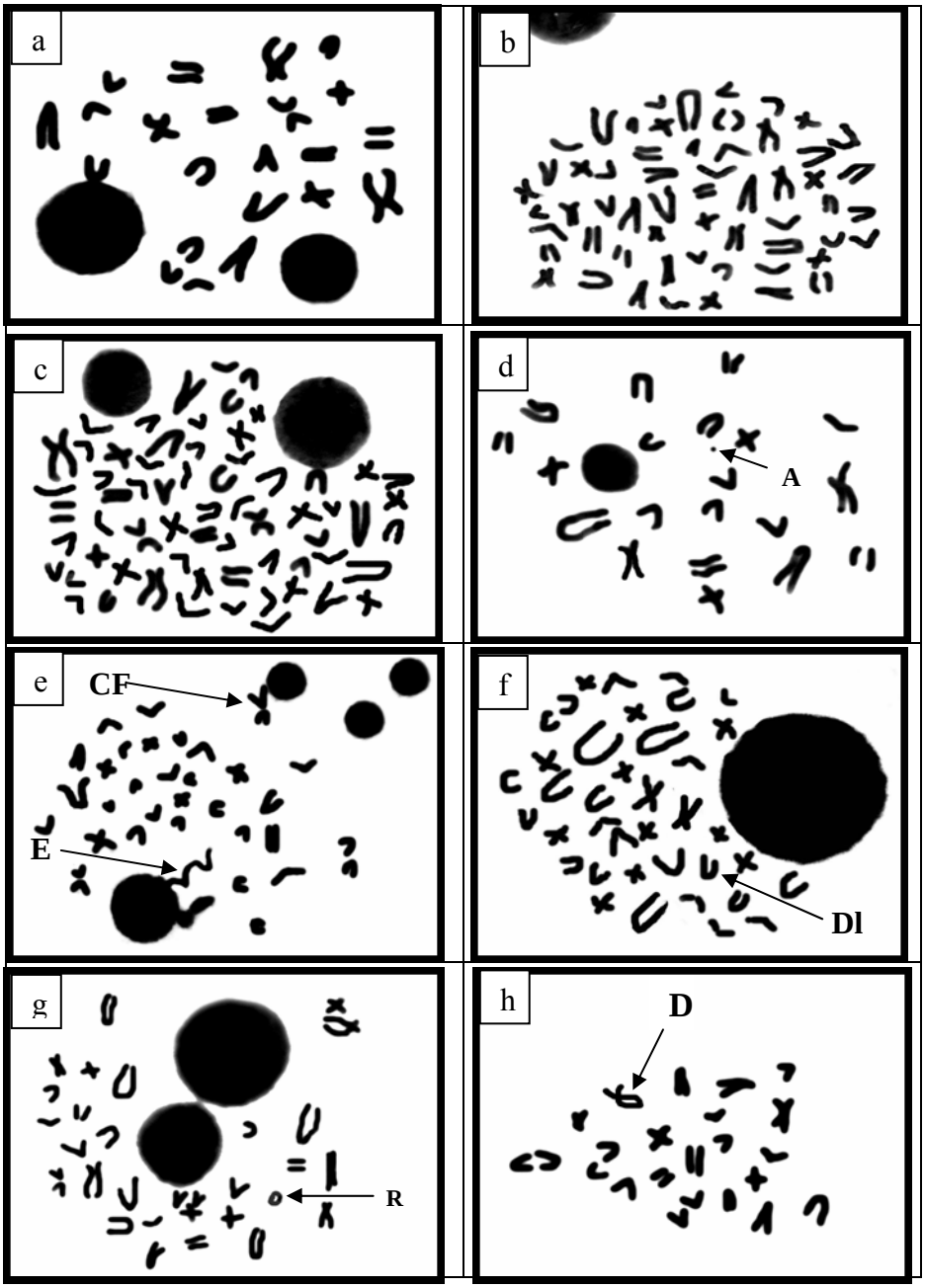


Fig. (2): A micrograph of metaphase chromosomes from bone marrow cells of control and GA_3-treated animals (Giemsa stain, X1000) illustrating numerical and structural aberrations as follows:

a) A decreased number of chromosomes (< 42).
b) An increased number of chromosomes (> 42).
c) Polyploid chromosomes (> 60 chromosomes).
d) d) Acentric fragments (A).
e) Exchange figure (E) and centromeric fusion (CF).
f) Deletion chromosome (Dl).
g) g) Ring chromosome (R).
h) Dicentric chromosome (D).

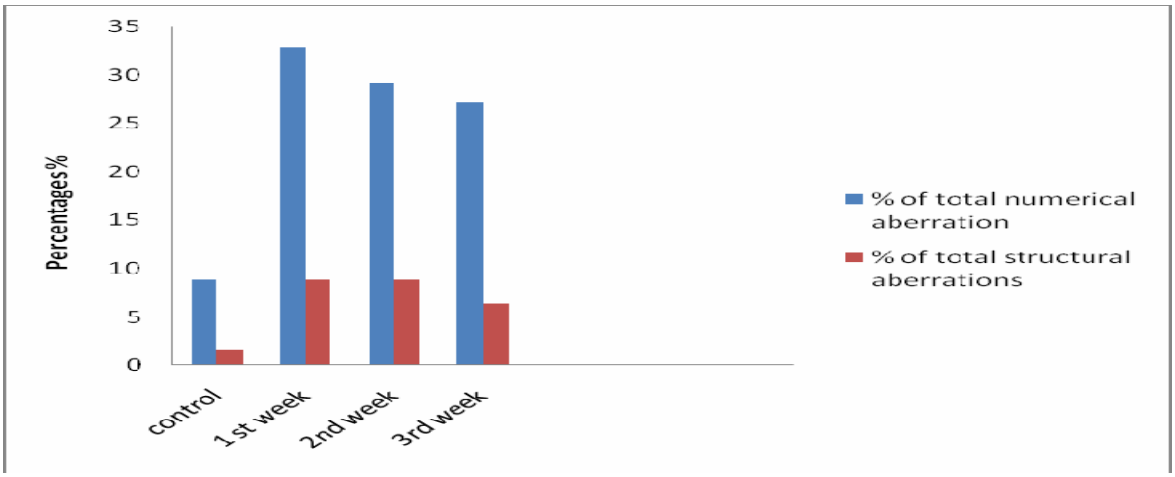


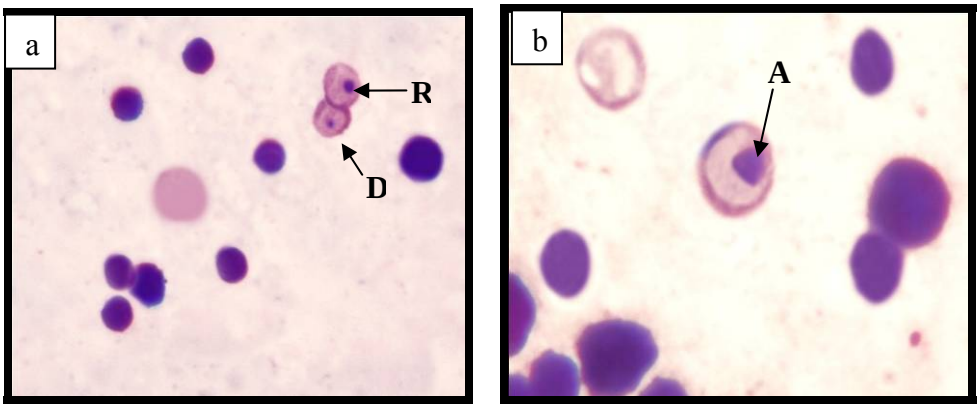
Fig. (3) Comparison between the percentage of frequency of numerical and structural aberrations from bone marrow cells of control and GA_3-treated rats.

Micronucleated polychromatic erythrocyte (MNPCEs) production in bone marrow cells of control and GA_3-treated animals.

The light microscopic examination of the bone marrow cells of control as well as GA_3-treated animals revealed the production of micronuclei which appeared in different shapes and variable numbers. Concerning the shape, the micronuclei appeared in the form of accumulated rounded masses or as dot-like structures or almond shape or they may be of rod-like (Fig. 4: a,b,c). As regards the number of these micronuclei produced in the bone marrow cells of the treated animals they appeared as single-micronucleated cells or bi-micronucleated cells or in a multi-micronucleated cells as shown in (Fig. 4: d,e,f).

Statistically, in the control group, the total micronucleated polychromatic erythrocytes (MNPCEs) was 60 among 5000 examined PCEs including only mono-nucleated cells with an average mean of 12 and the percentage was 1.2%. In addition, the bone marrow activity which equals to the mean of MNPCEs per 1000 examined cells and reflects the ability of bone marrow cells to form micronuclei reached 0.012 in control group while it was 0.072, 0.0564 and 0.038 after the 1st, 2nd and 3rd weeks post treatment with GA_3 respectively. Therefore, the bone marrow activity was increased at all time intervals as compared with that of the control animals. The total number of micronuclei produced in bone marrow cells of the GA_3-treated animals recorded a highly significant value ($P< 0.01$) at all studied intervals as compared to that of the control (Table 2).

Therefore, the data obtained after the oral administration of rats with 1/3 LD_{50} of gibberellic acid illustrated that gibberellic acid (GA_3) increased the number of MNPCEs and bone marrow activity to produce these nuclei as compared with that of the control animals (Fig. 5).



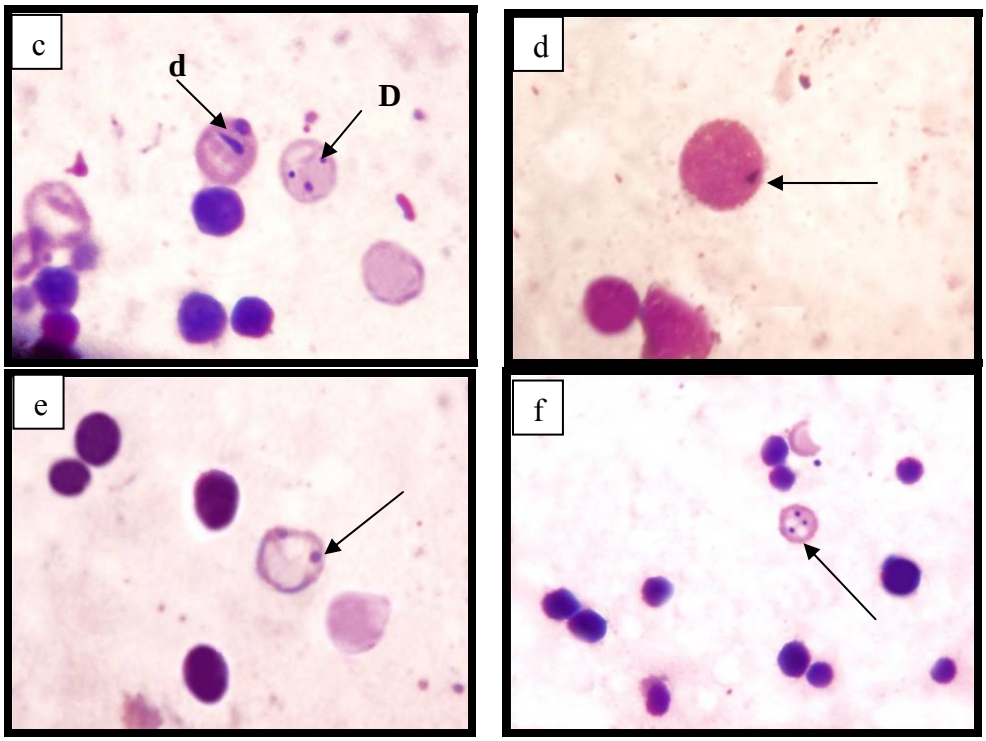


Fig. (4): Photomicrographs of bone marrow cells of control and GA_3-treated animals illustrating micronuclei with various shapes and different numbers (Giemsa stain, X1000):

a) An accumulated single-rounded mass (R) and a single-dot like structure (D).
b) Almond shape (A).
c) Rod-like structure (d) and Dot-like structure (D).
d) Single micronucleus ( ).
e) Bi-micronuclei ( ).
f) Multi-micronuclei ( ).

Table (2): Comparison between the frequencies of Micronuclei polychromatic Erythrocytes (MNPCEs) from bone marrow cells of control and GA_3-treated rats.

Time of treatment	No. of rats	No. of scored cells 1000/Rat	Micronuclei aberrations			Bone marrow activity(Mean / 1000)
			Total aberrations			
			No.	%	Mean ± S.E.	
Control	5	5000	60	1.2	12.0 ± 1.3	0.012
1 st week	5	5000	360	7.2	72.0±1.51**	0.072
2 nd week	5	5000	273	5.5	54.6±1.66**	0.0564
3 rd week	5	5000	190	3.8	38.0±1.14**	0.038

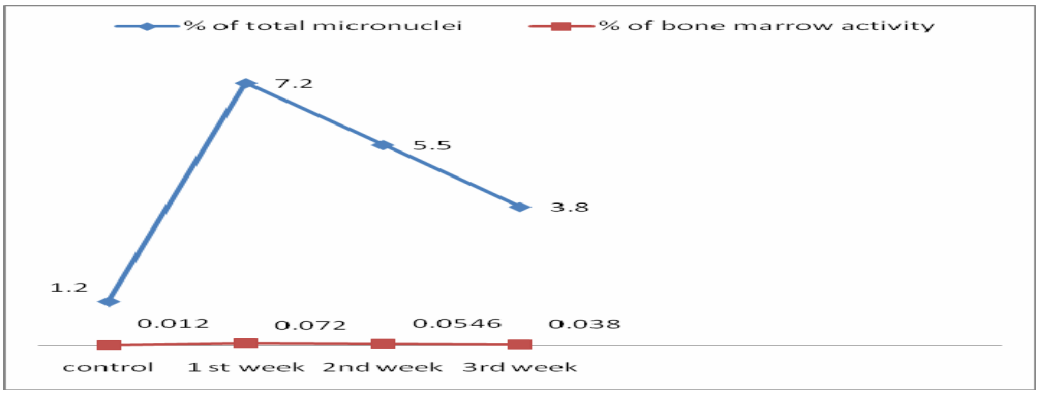


Fig. (5): Comparison between the frequencies of micronucleated polychromatic erythrocytes (MNPCEs) and the percentage of bone marrow activity from the bone marrow cells of control and GA_3-treated animals.

Sperm abnormalities in control and GA_3-treated animals:

The morphology of the sperm cell was examined in the present study microscopically and statistically in the testicular tissue of the control and GA_3-treated animals. The sperm abnormalities, as affected by GA_3-treatment, involved the head or the tail region and/or head and tail together. Head abnormalities presented in various forms. Sometimes the apical part is seen with an unusual curvature, it might be abnormally acute or abnormally straight. In some cases, the size of the head was within the normal limits, while in some others the size was abnormal or it may have an irregular outline which was rather difficult to describe. The sperm abnormalities in the tail region were more frequent in most of the studied slides of the treated animals which include the form of head without tail and tail with abnormal length. Also the abnormalities may be occurring in both head and tail together (Fig. 6).

Statistically, in the control group the total count of the deformed sperms was 144 among 5000 examined sperm cells including 82 with deformed head and 62 with deformed tail, but no sperms with deformed head and tail together were demonstrated. The total mean of the deformed sperms was 28.8 with a total percentage of 2.88%. The total number of sperms with deformed head was found to be highly significant after the first day and second weeks post-treatment. However, it was non-significant in the first week post-treatment. Also, it was found that the frequency of sperms with deformed tail was increased with highly significant value as compared to that of the control group at all studied intervals. However, the total number of sperms with deformed head and tail was found to be highly significant after the first day, first week and second week post-Treatment. Also, it was found that the frequency of the total deformed sperms in $GA_3$was increased with highly significant value as compared to that of the control in all studied intervals (Table 3).

Table (3): Comparison between the frequencies of sperm cell abnormalities of control and GA_3-treated rats.

Time of treatment	No. of rats	No. of scored cells 1000/Rat	Deformed sperm cells morphology											
			Deformed head			Deformed tail			Deformed head & tail			Total deformed sperms		
			No.	%	Mean ± S.E.	No.	%	Mean ± S.E.	No.	%	Mean ± S.E.	No.	%	Mean ± S.E.
Control	5	5000	82	1.64	16.4±0.68	62	1.24	12.4±0.93	0	0	0.0±0.0	144	2.88	28.8±1.36
1st week	5	5000	155	3.1	31.0±3.03**	419	8.38	83.8±2.39**	29	0.58	5.8±0.58**	603	12.06	120.6±2.01**
2nd week	5	5000	129	2.58	25.8±4.32	423	8.46	84.6±5.33**	17	0.34	3.4±0.51**	569	11.38	113.8±1.36**
3rd week	5	5000	261	5.22	52.2±3.2**	256	5.12	51.2±3.1**	23	0.46	4.6±0.68**	540	10.8	108.0±4.06**

*** Significant at P< 0.05** **** Significant at P< 0.01**

Histopathological findings:

The light microscopy evaluation of kidney in the control group showed normal renal parenchyma with will-defined glomeruli and tubules (Figs. 7 & 8).

Inspection of kidney of rats after one week of GA_3-treatment had resulted in different histopathological lesions both in the renal corpuscles and the tubular elements. The renal epithelia of the proximal and distal convoluted tubules were ill defined and their nuclei appeared overlapping each other. They also appeared hydrobic undergoing cloudy swelling on a large scale of the uriniferous tubules. The renal blood vessels appeared congested and dilated. Also, the glomerular tuft inside the renal corpuscle exhibited intensive vacuolization. Pyknotic dense nuclei belonging to necrotic desquamated epithelial cells were abundant particularly inside the glomerular tuft. Some renal corpuscles appeared degenerated and shrunken with pyknotic nuclei. The interstitial tissue suffered from severe haemorrhage especially around the damaged blood vessels at the vascular Poole of the renal corpuscle (Figs.9&10). After two week of GA_3 treatment of rats, the histopathological alterations were persisted and increased to include ruptured Bowman's capsules around the renal corpuscles. The renal corpuscles appeared shrunken, atrophied and the endothelia of their capillary tufts became hyperplastic with concomitant haemorrhage. Also, most of the cell nuclei in the tubular elements appeared swollen and faintly stained. Necrosis of the renal tubules was developed on a large scale. Increased intertubular spaces became evident due to renal tubular atrophy (Fig. 11). On the third week post treatment of rats, the histological picture of kidney revealed more adverse effects of this PGR on the renal tissue. The glomeruli appeared highly degenerated and invaded by many pyknotic and damaged nuclei. The renal blood vessels were markedly affected where the renal vein appeared congested and dilated while the renal

artery appeared with thickened wall and invested with damaged nuclei. In addition, leucocytic infiltration of plasma and lymphocytic cells could be demonstrated in the renal parenchyma (Fig.12). These histopathological changes denoted a cytotoxic and destructive effect of GA_3 on the histological structure of the kidney.

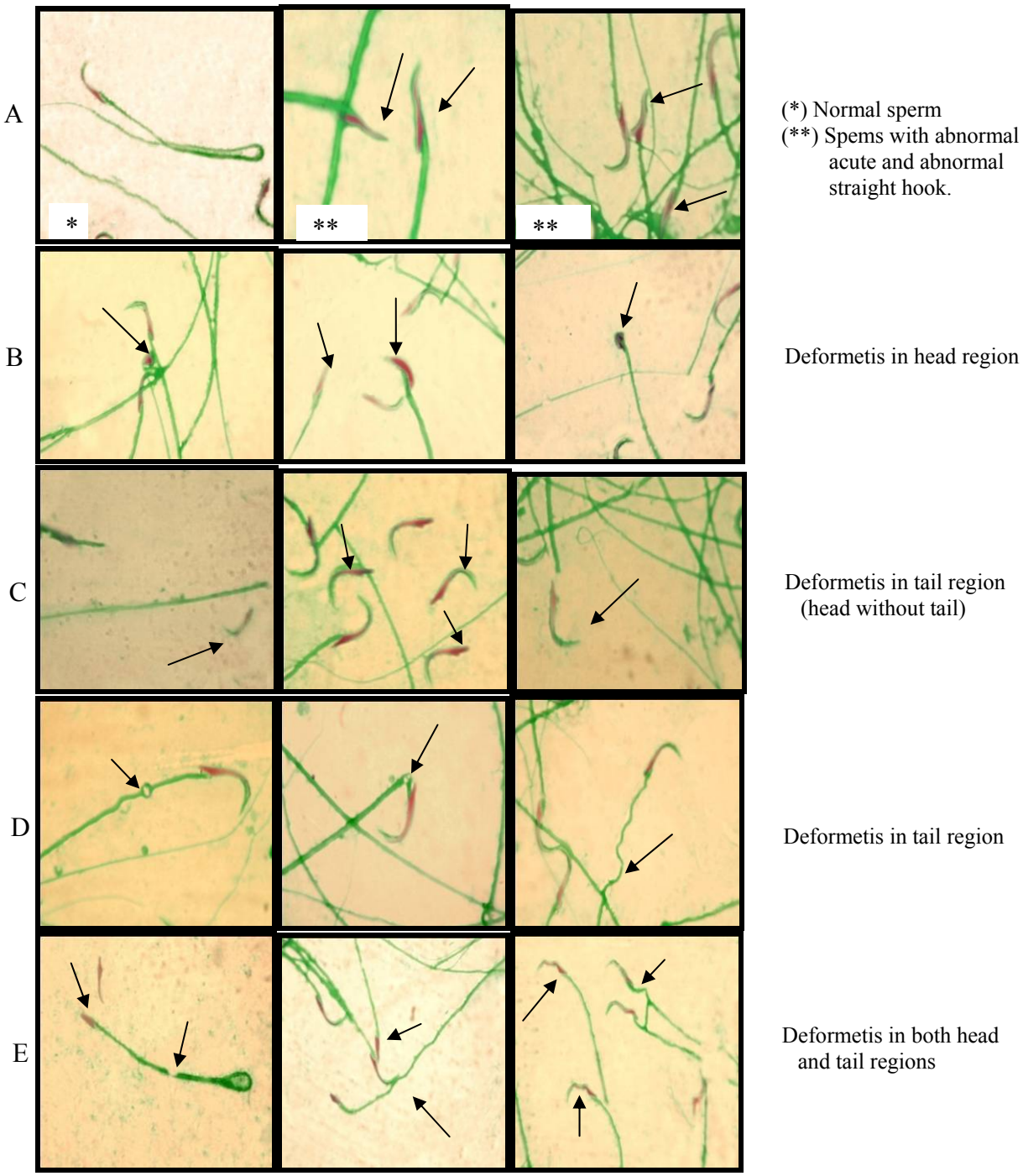
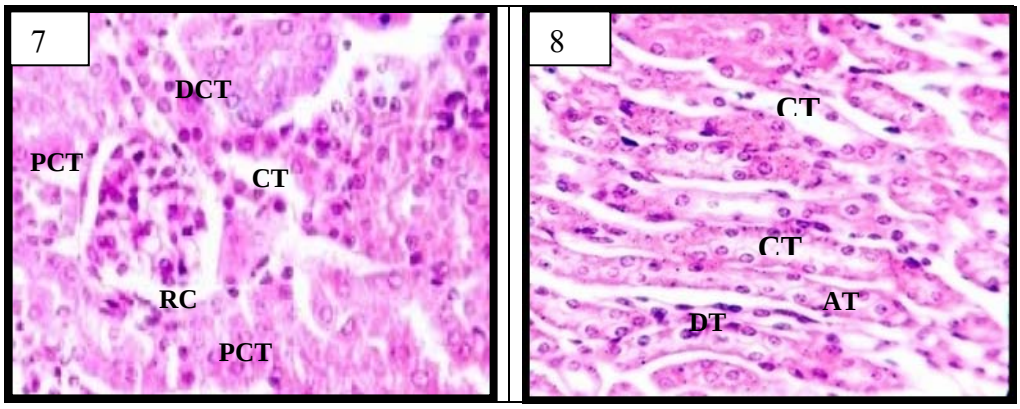
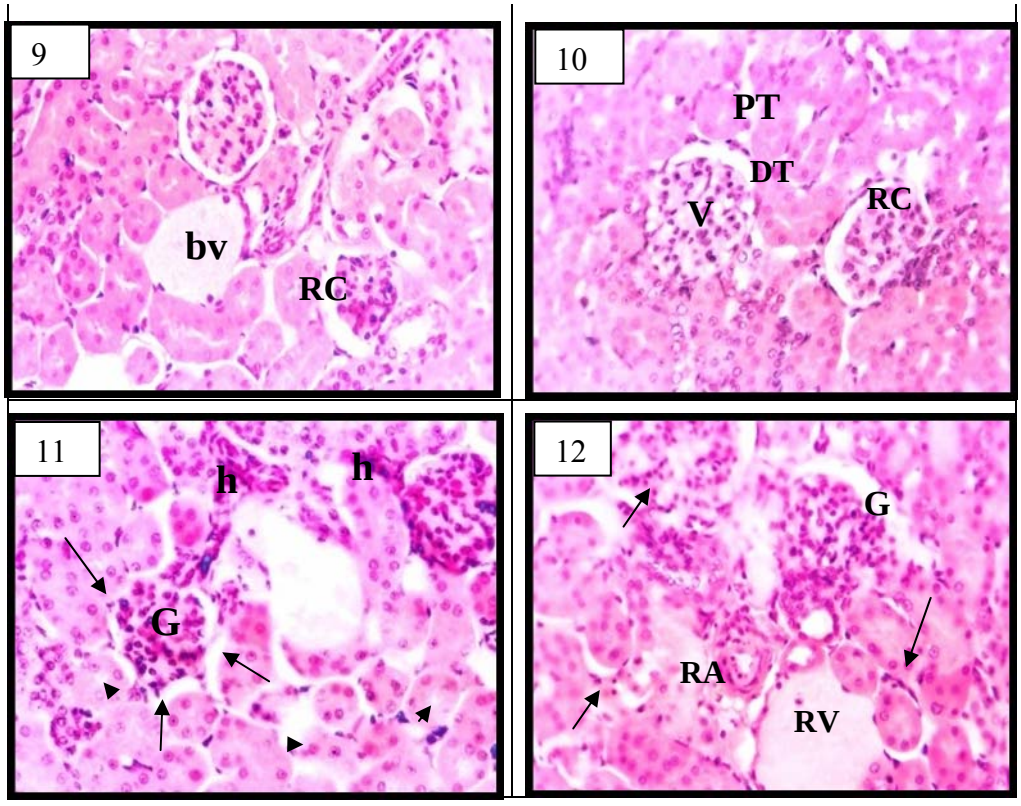


Fig. (6: A, B, C, D & E): Micrographs of sperm cells from the testis of control and GA_3-treated rats illustrating various abnormalities (Feulgen satin & light green, X1000).



(Figs. 7, 8) T.S. in kidney of normal male albino rat illustrating the elements of the renal cortex and renal meduella respectively showing; renal corpuscle (RC), proximal convoluted tubule (PCT), distal convoluted tubule (DCT), collecting tubule (CT), descending tubules (DT), ascending tubules (AT) and collecting tubules (CT)(H&E, x400) .



(Figs. 9, 10) T.S. in the kidney of GA_3-treated rat after the first week post-treatment showing ill defined proximal and distal (PT, DT) convoluted tubules with overlapping nuclei , congested and dilated blood vessel (bv), cloudy swelling in the cells of proximal (PT) and distal tubules (DT) and vacuolation (V) in glomeruli with pyknotic nuclei, degenerated and shrunken renal corpuscles (RC) (H&E, x400) .
(Fig. 11) T.S. in the kidney of GA_3-treated rat after the second week post-treatment showing ruptured Bowman's capsule (arrows), glomeruli (G) with hyperplasia, haemorrhage (h) and tubular necrosis with faintly stained nuclei (arrow heads) (H&E, x400).
(Fig. 12) T.S. in the kidney of GA_3-treated rat after the third week post-treatment showing highly degenerated glomeruli (G), leucocytic infiltration (arrows), dilated and congested renal vein (RV) and renal artery (RA) with thickened wall (H&E, x400).

Histochemical observations:

In normal animals, the total protein content of the renal tissue was determined histochemically using the mercury bromophenol blue (MBB) method. The glomeruli of the renal corpuscles exhibited a strong MBB reaction while the epithelia of the tubular elements disclosed their proteinic content in the form of deeply-stained blue granules homogenously distributed throughout their nuclei and cytoplasm (fig. 13).

In GA_3-treated rats (one week post-treatment), the histochemical observation resulted in a marked decrease in the positivity of the protein material inside the damaged renal elements (Fig.14). This decrease was statistically proved to be a significant one ($P \leq 0.032$) and the percentage of change was 5.2%. On the second week post GA_3-treatment the renal tissue revealed an obvious and sharp reduction in the MBB positive material inside the renal elements (Fig. 15) concomitant to the deteriorated phase of degeneration due to GA_3-toxicity. This sharp reduction in the proteinic content was proved to be a highly significant one where the P value was ≤ o.ooo86 and the percentage of change in this histochemical component was 8.62% (Table 4 & Fig. 17). On the third week post GA_3-treatment the histochemical localization of proteins inside the elements of the renal tissue revealed a persisted decrease in the MBB positive material (Fig. 16). This was also supported by the quantitative measurements (image analysis) which recorded this decrease in a value less than that of the control one but slightly more than that of the preceeding treatment (Table 4 & Fig. 17). It was statistically a significant decrease where its P value equals ≤ 0.0065 and the percentage of change was 6.10 %. These results give us an additional confirmation for the hazardous effect of GA_3 –administration from the histochemical point of view.

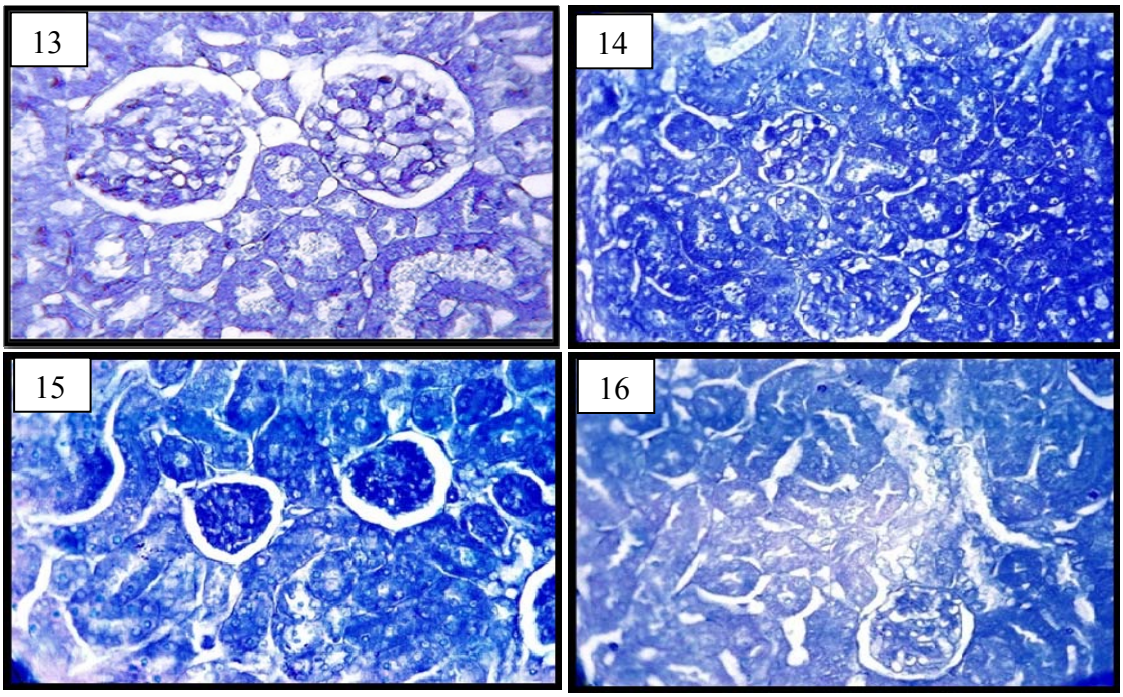


Fig (13): Section in kidney from control rat showing the normal distribution of total protein as a strong (+++) mercury bromophenol blue reaction (MBB, x400).

Fig. (14): Section in kidney of GA_3-treated rat after one week post-treatment showing a marked decrease (++) in the protein content of the renal elements (MBB, x400).

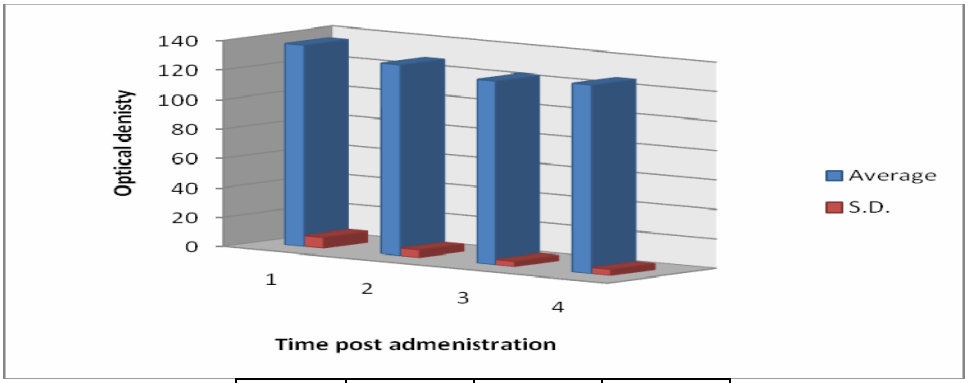
Fig. (15): T.S. in kidney of GA_3-treated rat 2 weeks post-treatment revealing an obvious and sharp reduction (+) in protein content (MBB, x400).

Fig. (16): T.S. in kidney of GA_3-treated rat 3 weeks post-treatment showing persistent decrease (++) in protein content of the renal elements (MBB, x400).

Table (4): The quantitative measurements (image analysis) of the optical density (OD) of total protein content in the renal tissue in control and GA_3 treated groups.

	C	T1	T2	T3
	134.1	121.1	127.1	130.6
	128.9	131.1	124	131.1
	138.6	133.8	126.2	128.7
	143.3	132.3	129.9	125.5
	128.4	135.6	129.1	133.1
	130.4	136.4	121	125.5
	144.9	129.3	119.4	126.3
	145.4	125.7	121.2	136.2
	145.3	123.1	128.5	125
	127.9	127.8	122.9	121.7
Average	136.72	129.62	124.93	128.37
S.D.	7.584165	5.190333	3.74286	4.382047
t test		0.032405	0.000869	0.006582
Probalbility (P)	Sign.	h-Sign.	Sign.	Sign.
% of change		-5.1931	-8.62346	-6.10737

- **C: represents the values of the OD of the protein content in control animals**
- **T_1 –T_3 represents the Values of the OD of the protein content in the 1st, 2nd and 3rd weeks post-treatment, respectively.**



C	T1	T2	T3
136.72	**129.62**	**124.93**	**128.37**

Fig.(17): A histogram illustrating the values of the quantitative measurements of the optical density of total protein content together with its standard deviation in control and GA_3-treated rats.

4. Discussion

Gibberellic acid GA_3 was the stress factor in this investigation which is one of the plant growth regulators used in Egypt on a vast range of vegetables and fruits. On the basis of the present experiments, the genotoxic effect of GA_3 was manifested by the induction of chromosomal aberration, the occurrence of micronuclei in bone marrow cells and the sperm abnormalities in testicular tissue. Histopathological and histochemical alterations in the renal tissue were also observed due to GA_3 toxicity.

The chromosomal aberration observed in this study included numerical and structural aberrations, the numerical aberrations (aneuploidy) were more affected after GA_3 treatment of animals and the obtained data are highly significant at the statistical level after 1st, 2nd and 3rd weeks of treatment, at the same time the polyploid metaphase was not affected by treatment. These results are in accordance with those of **Sakr *et al.* (2009)** who reported that gibberellic acid induced chromosomal aberrations in human lymphocyte cultures. Gibberellic acid in this demonstration induced a significant damage of DNA. Also, data recorded by **Abou-Eisha (2001)** showed that gibberellic acid induced a significant increase in the level of DNA breakage in human blood cells. 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, or may be due to accumulation of nucleases as reported by **Fath *et al.* (1999).**

Concerning the structural aberration, the most structural aberrations were dicentric, deletion, ring chromosomes which more affected by GA_3 treatment than other aberrations, these data refer to the GA_3

interaction with DNA leading to deletion in the terminal end of chromosomes or chromatids, this deletion causes instability of chromosomes or chromatids, then lead to dicentric and ring chromosomes, these results are concurrent with previous data **(Abou-Eisha 2001)** who reported that the mechanism of gibberellic acid to induce DNA damage may be attributed to elevation of oxidative stress markers such as (ROS) and glutathione (GSH), Bcl-2 protein expression, mitochondrial membrane potential and caspase-3 activity. The sequels of these events lead to mitochondrial membrane depolarization and caspase-3activation followed by apoptosis. Also **Hassab-Elnabi and Sallam (2002)** reported that gibberellic acid induced total genomic damage of DNA especially at higher doses. **Jacqmard (1968)** has proposed that one of the effects of GA_3 is to promote the onset of DNA synthesis in cells which are arrested in the G1 phase of the cell cycle. It has also been reported that replication of DNA with carcinogen-induced lesions is an essential step in the initiation of carcinogenesis. Gibberellin A_3 was found to have a carcinogenic effect in amphibians **(El-Mofty and Sakr, 1988)** and mammals **(El-Mofty *et al*., 1994)**.

The total chromosomal aberrations (numerical and structural) recorded in the current investigation were significant after treatment with GA_3 at all time intervals post-treatment. These data are in agreement with previous data obtained by **Bakr et al. (1999)** who reported a significant increase in the incidence of total chromosomal aberrations induced by gibberellic acid in bone marrow cells of albino mice and these chromosomal aberrations can be explained on the basis that the ability of the animal enzyme system to break down the hormone is higher at all studied periods. This makes the effect of the gibberellic acid on the DNA synthetic system during the S-phase of the earlier cell cycles more drastic than the later ones. It seems likely to conclude and confirm the genotoxicity of GA_3 and its inductive effect in the increase of the number of total chromosomal aberrations in bone marrow cells of albino rats. Also, the illustrated results are fully agreed with that recorded by **Zalinian *et al.* (1990)**.

In addition the mitotic index, which gives information about the frequency of mitotic cells (mitotic activity) had decreased gradually in the present investigation from the 1st week towards the 3rd week post administration of GA_3. Similar data were recorded by **Jovtchev *et al.* (2010)** who attributed the increase in the frequency of chromosomal aberrations and micronuclei in bone marrow cells of rats to the decrease in the mitotic activity of these cells.

In the present work, the value of the total number of micronuclei produced in bone marrow cells of the GA_3-treated animals recorded a highly significant value at all studied intervals as compared to that of the control animals. Also the size of micronuclei varied and some cells had 2 or more micronuclei, these results indicate that the GA_3 administration has an adverse effect on the chromatin migration at anaphase stage which may cause breaks at different sides of chromatin and/or on the protein of the spindle fibers leading to different sizes of micronuclei. These data are complementary to types of damage in chromosomes. The large and almond micronuclei represent whole chromosome lost during anaphase stage or during degeneration of erythrocyte nucleus. It seems likely to report that the formation of micronuclei in bone marrow cells of GA_3-treated rats in the current investigation is either due to chromosomal breakage or spindle disruption, or it may be due to toxic effects excreted by the used chemical. This comes in agreement with the results of **Lerda *et al.* (2005)**. In contrast the **EPA (1995)** reported that mouse-micronucleus assay on Gibberellins (GA_4 + GA_7) indicated that no increased incidence of micronucleated-polychromatic erythrocytes (MNPCE) were found at levels up to 1200 mg/kg. Also **Lerda *et al.* (2005)** noticed that maleic hydrazide (a plant growth regulator) failed to influence the incidence of micronuclei or the ratio of poly to normo-chromatic erythrocytes.

Another principal focus of the present study was the deformities in the sperm morphology where the frequency of deformed sperms of GA_3-treated rats was increased. Furthermore, the abnormalities of sperms included abnormal head or tail and/ or head and tail together. The head abnormality presented in various forms. The abnormality of head referred to the sensitivity of the component of head region (DNA) to GA_3 and these data indicated the genotoxicity of GA_3 on germ cell and caused some sort of risk on future generation. On the other hand, the tail regions of sperms were more affected by GA_3 administration than head region. Mostly, the cases of the head without tail and those of the tail with abnormal length were more frequent. These alterations may affect the motility of sperm and consequently affect the fertilization process. Therefore, it was found conclusively that the frequency of total deformed sperms of GA_3-treatment rats was increased and highly significant at statistical level as compared to that of the control in all studied intervals. Nearly similar data were noticed by **Rupa *et al* (1991)** who illustrated that using ethephone as a plant growth regulator induced a significant increase in the number of the abnormal sperms in mice and they were recorded that these results were due to the reduction in several biochemical parameters after the treatment with this phsphorylating agent. **Nada and Saleha (2008)** were in accordance with the present results where they recorded an increase in abnormal sperm morphology after treatment with various doses of ethephon. Also,

Sovkovic *et al*. (1983) recorded a harmful effect of one of these plant growth regulators where they recorded that the effect of ethephon on fertility is a dominant lethal mutations, because of its effect on chromosomal translocations at the first meiotic metaphase in male mice. Moreover, the results of the current investigation come in accordance with those of **Yousef (1995)** who reported that a recent study in rabbits showed a significant adverse effect on libido, ejaculate volume and sperm concentration, with increased abnormal or dead sperms by using glyphosate which is herbicidal and used as plant growth regulator.

The results obtained from this investigation revealed an obvious nephrotoxic effect of GA_3. Nephrotoxicity was confirmed and objectified by many histopathological lesions which could be recorded in the renal tissue of the treated animals throughout the period of experimentation. These histopathological changes appeared in the first week after GA_3 administration and persisted and increased progressively towards the third week post administration. These findings are in agreement with those of **Troudi *et al.* (2011)** who concluded that, the exposure of rats to GA_3 induced oxidative stress and histopathological changes in kidneys of suckling rats and their mother's during late pregnancy and early postnatal periods. The authors recorded an inflammatory cell infiltration, a widened tubular lumen, a degeneration of tubular epithelial cells and vascular congestion. Among the histopathological criteria observed in the present work was the remarkable damage occurred in the renal corpuscles. These renal corpuscles appeared, in the first week after GA_3-treatment, shrunken, degenerated with vacuolated glomeruli having necrotic nuclei and eroded or ruptured Bowman's capsules. These renal corpuscles underwent a progressive phase of degeneration towards the third week post exposure to exhibit remarkable signs of glomerular atrophy. **Katzung (1990)** attributed the sensitivity of the glomeruli to the large surface area of the glomerular capillaries which renders them susceptible to damage from circulating toxins and immune complexes. Glomerular atrophy in the treated animals which was recorded in this work may be regarded as a participation of the kidney in the elimination of GA_3 from the body. **Saly (1998)** reported that GA_3 was highly injurious to the kidney of albino rats. The treated animals revealed destruction in the renal tubules, loss of their architecture, epithelial cells with vacuolated cytoplasm and dense nuclei and leucocytic infiltration in the interstitium. **Sakr *et al.* (2002)** recorded many histopathological changes in the kidney after GA_3 administration such as glomerular degeneration, congested blood vessels, cytoplasmic vacuolation in the epithelia of the renal tubules and renal tissue impairment.

Lesions in renal sections of GA_3-treated animals in the present study also revealed proximal and distal convoluted tubules with hydrobic degeneration leading to cloudy swelling and ill-defined cell boundaries. These pathological criteria in the renal epithelia appeared in the earlier days (first week) of experimentation and persisted till the end of the experimental time where they went to necrosis leading to tubular atrophy. These findings and hydrobic degenerations may offer a support to the view speculated by **Elwi (1967)** who demonstrated an impairment of the oxidative phosphorylation processes and consequently reduced release of energy necessary for the regulation of the concentration of ions in the cells. A loss of intracellular potassium ions is followed by the entry of more sodium ions into the cells. The tendency of the cell to become hypertonic is balanced by the entry of water and the injured mitochondria become swollen and vacuolated and presumably the site of water accumulation. The concurrent previous findings recorded in the renal tissue due to GA_3 poisoning are in agreement with those of several investigators **(El-Hady, 1994; Abdeen *et al.* 1994; Abdel Mageid, 1994 and Saly, 1998)**. **El-Hady (1994)** recorded swelling in the cells of the renal tubules and disappearance of their brush borders due to aldicarb (pesticide) toxicity of kidney of *Arvicanthis niloticus*. **Abdeen *et al.* (1994)** reported that fenvalerate (insecticide) induced renal damage of the epithelial lining of the renal tubules, ruptured the distal tubules and enlarged the glomeruli with hydrobic degeneration. The karyolysed nuclei of the renal epithelia and the widened intertubular spaces which observed in the renal tissue due to GA_3 toxicity are considered as earlier and concomitant events prior to tubular necrosis and tubular atrophy.

The diffused masses of inflammatory leucocytic infiltration together with the haemorrhagic foci recorded inbetween the tubular elements in the current study are considered as signs of toxicity and consequent activation of the defensive mechanism of the treated animals. **Abdel Mageid (1994)** was in accordance with this explanation. **Abdel-Rahman and Zaki (1992)** considered this response as a prominent reaction of the body tissue facing any injurious impact and **Sakr *et al.* (2002)** accepted also this explanation. Blood system of the renal organs appeared to be very sensitive to GA_3 toxicity in the current investigation where congested, dilated and damaged blood vessels were noticed throughout the experimental time. In addition thickness of the arterial walls and obviously eroded walls of the renal veins could be also demonstrated. This may be due to the direct toxic effects of GA_3 on the blood vessels and accumulation of its metabolites in blood. This explanation is in agreement with **Abdel Mageid (1994)** and **Kohen** and

Chevin (1985) who suggested that the toxicity of paraquat (herbicide) is due to oxygen-derived free radicals such as superoxide anion or related to complex pharmacokinetics of paraquat **(Damian *et al.*, 1991).**

In the present investigation the fluctuations of the protein material inside the cells of the renal tissue of control and GA_3 -treated rats was taken as a bioindicator to reflect the GA_3 toxicity in this target organ. The choice is based upon the fact that proteins are considered as the core of the histochemical components and being included in all vital activities of the cell, so they reflect the metabolic status of the cell. The present histochemical observations of the total protein content of the renal tissue supported the previously mentioned histopathological alterations both qualitatively and quantitatively. The current data of the image analysis confirmed the gradual and significant decrease in the protein content from the first week to the third week after GA_3- treatment. This is occurred to match the cytotoxic and destructive effect of GA_3 at these intervals post administration. The presently recorded results of proteins are in consistence and agreement with those of several investigators **(Jacobsen, 1977, Bewley and Black, 1978, Abdelhamid *et al*., 1994; Moussa *et al*., 1987, Abdel-Rahman and Zaki, 1992; De Marco *et al*., 1992; Williams and Weisburger, 1991; Haux *et al*., 2000 & 2002** and **Sakr *et al*., 2002). Jacobsen (1977)** and **Bewley and Black, (1978)** reported that GA_3 reduces the duration of the cell cycle by nearly 30% and it does so primarily by reducing the length of G1 phase by 30% and that of S by 36%. It was well established that plant hormones and growth regulators affect the synthesis of RNA and protein. **Moussa *et al.* (1987)** and **Abdel-Rahman and Zaki, (1992)** attributed the reduction in protein content partially to the decreased level of protein synthesis in the renal cells suffering from pathological changes due to the hyperactivity of hydrolytic enzymes. Chemical carcinogens – by themselves or after activation – interact with cellular macromolecules such as DNA, RNA, and proteins and these interactions result in the development of neoplasia **(Williams and Weisburger, 1991). Abdel-Hamid *et al.* (1994)** indicated that GA_3 induced a reduction of muscular proteins in chickens. A reduction in total proteins was also observed in kidney of some animals exposed to insecticides. Maleic hydrazide is a pyridazine which inhibits the synthesis of nucleic acids and proteins **(De Marco *et al*., 1992** and **Haux *et al*., 2000 & 2002)** observed a decrease in nucleic acid, protein and cholinesterase contents after treatment with different doses of ethephon which may be attributed to its pesticidal and phosphorylating activities. **Sakr *et al.* (2002)** after 2 weeks of treatment with GA_3 recorded a noticeable decrease in the total protein content of both tubular and glomerular elements of rat kidney. After 3 weeks treatment with GA_3, many tubules showed signs of degeneration and their linging cells appeared with cytoplasmic vacuolation. These cells showed a marked decreased in total proteins. Also, **Nada (2006)** recorded a reduction in plasma protein levels in ethephon-treated mice correlated with dose increase. This is may be due to changes in protein content of blood plasma. It seems reasonable to agree with those authors in their explanations.

In conclusion, the oral administration of albino rats to GA_3 significantly increased the frequency of chromosomal aberration, micronuclei production and sperm cell abnormalities particularly on the first week post-administration. At the same time, the exposure of rats to GA_3 manifested several histopathological and histochemical alterations to confirm and support the genotoxic and cytotoxic effects of GA_3 toxicity.

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