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Effect Indol acetic acid On liver of Albino Rats

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Abstract.

The study aims to clarify the toxic effect of plant hormones, which are widely used in agriculture. One of these is the plant hormones (indole acetic acid); has been giving the hormone to rats at 100 ppm salt solution of 0.2 per day after day for a period of forty days before conception until the fourteenth day or sixteenth or childbirth. Treatment brought about a marked shortage in the rate of increase in the weight of mice., And a percentage of the weight of the liver there was a distinct increase in the relative weight of the liver. As well as the increase in pathological changes and increase the size of the nuclei and Kupffer cell, as noted widespread and dense clusters of inflammatory cells accompanied by about the erosion of liver tissue and blood infiltration. Biochemical analyzes showed a marked decrease of the liver in antioxidant enzymes and an increase in the rate of free radicals. It was also noted an increase in cases of abortion. The owner of so many birth defects. It was also noted the lack of body weight in fetuses and increase the absorption rate of embryos in fetuses of mothers treatment compared to the control group. Showed microscopic examinations of the liver of mice born in the transaction and the decay in the presence of hepatic cells and edema, blood vessels and increase the rate of cell death.

Keywords: Indol acetic acid, liver, pathological changes.

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1. INTRODUCTION AND AIM OF THE WORK

Are chemicals applied by a horticulturist to regulate plant growth. In plant propagation, cuttings are dipped in a rooting hormone to stimulate root development. In greenhouse production, many potted flowering plants may be treated with plant growth regulators to keep them short. Seedless grapes are treated with plant growth regulators to increase the size of the fruit. Because plant growth regulators are effective in parts per million or parts per billion, they have little application in home gardening (1). They are classified into six classes; Plant growth regulators, gibberellins, cytokinins, ethylene generators, growth inhibitors and growth retardants .

Indole acetic acid is a plant hormone detected in human urine (2), blood plasma (as a metabolite of tryptophan) (3) and central nervous system (4). It is also, found in cerebrospinal fluid and in several organs such as liver, kidney, lungs and brain (5). IAA is predominantly formed as a result of the mono amin oxidase - mediated oxidative deamination of tryptamine, a putative neurotransmitter or neuromodulator in CNS (6). The plasma levels of IAA and its metabolites were elevated in some human diseases such as insulin dependent diabetes mellitus (7), renal dysfunction and phenylketonuria (2). The combination of IAA and horseradish peroxidase (HRP) was found to cause cytotoxic toward mammalian cells (8) and increase lipid peroxidation (9;8).

The effects of IAA were also investigated on human serum enzymes in vitro. IAA was found to inhibit aspartate aminotransferase (AST) and activate amylase, creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). KN inhibited muscle creatine kinase (CK-MB), while it activated AST and alanine aminotransferase (ALT) (10;11). suggested that kinetin has effective free radical-scavenging activity in vitro and antithrombotic activity in vivo. Also, (12) determined that incubation for 24 h in the presence of IAA (1 mM) showed increase in the activities of superoxide dismutase (SOD), CAT, and glutathione peroxidase. The addition of exogenous antioxidant enzymes (SOD and CAT) prevented the loss of cell membrane integrity induced by IAA. (13) found that the PGRs treatments caused different effects on the content of MDA and antioxidant defense system in comparison to those of control rats. The sub chronic treatments of IAA caused significant decrease in the GSH concentration and CAT activity in erythrocyte. MDA concentration in brain was increased significantly by IAA. IAA decreased the liver GST activity and increased CAT activity .

2. AIM OF THE WORK

From the previous points of views the present study was aimed to investigate the toxicities and teratogenicities of IAA on albino rats of the Wistar strain through investigating the following parameters :

- Body weight gain of pregnant.
- Histopathological effects on maternal liver .
- Body of weight, size, and crown-rump length of delivered new born.
- Histological investigation of liver, as well as histogenesis of fore-& hind limb of 14 & 16 days prenatal .
- Biochemical assessments of liver functions, antioxidant defenses and lipid peroxidation in mothers and their newborn.

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3. MATERIALS AND METHODS

All experiments were conducted in accordance with the national laws for the use of animals in research and approved by the local ethical committee.

Applied plant growth hormones : one applied plant hormones were used in the present work ; belongs to auxin (indole acetic acid). they purchased from Sigma Chemical Company (St. Louis, MO 6, USA) and of highest purity. Both compounds were dissolved in tap water at doses of 100 mg/kg B.wt for indole acetic acid. Each individual was received indole acetic acid of the mentioned corresponding dose.

Experimental work: thirty fertile male and virgin female rats weighing approximately 125g body weight were obtained. Free access of standard diet composed of 50 % grinding barley, 10% grinding yellow Maize, 20% milk and 10% vegetables was supplied. Barley is a very useful grain source for growing, gestating, and lactating dairy cattle, providing more protein than the most other grains as well as showed a highly digestible starch (energy) and useful fiber. Barley is an economical nutrient source that should be strongly considered in formulating rations for dairy animals (14). Free excess of water was allowed ad-libitum. They were kept under good ventilation with 12 hour light and dark cycle. Females weighing approximately 123-125 gm body weight were made pregnant by keeping them with healthy fertile male rat overnight (at a ratio of 1male-3 females). On the next morning, vaginal plugs were examined. Vaginal smears were carried out to give a precise determination of the onset of gestation.

The rats were arranged in to Two groups, each was composed of 10 members in the first group and 20 in the placebo group:

- Control pregnant females: the animals received basal diet and tab water.
- Indole acetic acid (IAA)-treated pregnant: Each individual received oral doses of 100 mg/Kg B.wt for 40 days every other day before pregnancy and from the 1st to 14th day of gestation according to (15).

Investigated parameters: At parturition, fasted pregnant rats were sacrificed by cervical dislocation and blood samples were collected into non-heparinized tubes. Blood was centrifuged at 3000 RPM for 20 min at 4°C. The separated sera were frozen at – 20°C for biochemical analysis. Maternal liver of both control and experimental groups were separated into two parts ; the first was kept frozen at – 20°C for biochemical analysis and the second preserved immediately in 10 % phosphate buffered formalin for histological investigation.

Body weight gain of pregnant: The percentages of increased body weight gains were determined at intervals of gestation of 6, 14, 16 & 20 days for both control and experimental groups.

- **Biochemical investigations:** In case of blood analysis, at the end of the treatment five rats of both control and experimental groups were sacrificed and blood was collected in nonheparinized tube and centrifuged at 3000 RPM and serum was collected. For liver, of both mothers and their pups one-day old of control and experimental groups were weighed and homogenized a 10 fold volume of ice-cold distilled water. After the homogenates had been rapidly frozen and thawed at room temperature (25°C) to ensure a thorough release of all soluble components from particulate matter they were centrifuged at 10000 rpm for 60 min. the clear

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supernatants were decanted and either analyzed at once or stored at 4°C. All procedures except thawing of extracts were carried out at 0-4°C, according to (16).

- **Determination of Total bilirubin concentration:** The concentration of total bilirubin in serum was estimated by colorimetric method of (17), using total bilirubin kit purchased from Blood Diagnostic Comp., Taher St., Dokki, and Giza, Egypt. The reaction between bilirubin and the diazonium salt of sulphanilic acid produced azobilirubin which shows a maximum absorption at 535 nm in an acid medium. In the presence of dimethylsulfoxide (DMSO) the total bilirubin participate in the reaction and in the absence of (DMSO) only conjugated bilirubin react.
- **Light microscopic investigations:** The liver of pregnant as well as their prenatal embryos (14&16 days old) of both control and experimental groups were incised immediately and fixed in 10 % formal saline and processed for histological investigation. Serial 5µm histological sections were cut and stained with hematoxylin and eosin and examined under bright field light microscopy.
- **Determination of serum calcium content:** Serum calcium content was evaluated using the atomic absorption apparatus. Investigated samples of both control and experimental groups was digested with nitric acid then diluted to a constant volume. Mention parameters were calculated as mg/ml.
- **Biostatistics:** Statistical methods are used to make inferences about populations, based on samples from those populations. In most toxicity and bioaccumulation tests, samples of exposed organisms. The response from the samples is usually compared with the response to a reference, or with control. In any toxicity or bioaccumulation test, summary statistics such as means and standard errors for response variables (e.g., survival, contaminant levels in tissue) are provided for each treatment (e.g., elutriate concentration, sediment). In the tests described here in, samples or observations refer to replicates of treatments. Sample size n is the number of replicates (i.e., experimental units, test containers) in an individual treatment, not the number of organisms in a test container. Overall sample size N is the total number of replicates in all treatments combined, i.e., $N = n_1 + n_2 + n_3 + \dots + n_k$. Where k is the total number of treatments in the experiment. Mean, standard deviation and analysis of variance were used (18).

4. RESULTS

Effects on maternal tissues:

Absolute & relative liver weight: indole acetic acid (IAA) treatment lead to a decrease absolute liver weight at parturition; however relative liver weights were non-significantly (Table 1).

Table 1: Absolute (g.) and relative liver weight (g. /100g.) of pregnant female albino rats treated with 100 mg /Kg B. wt from either indole acetic acids (IAA).

group	Control	Indole Acetic acid (IAA)
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	Absolute liver weight	Relative liver weight	Absolute liver weight	Relative liver weight
1	5.70	3.70	5.30	4.37
2	6.70	4.70	4.71	3.69
3	4.70	2.90	5.68	4.76
4	5.90	3.70	4.26	3.38
Mean ± SD	5.750± 0.82	3.750±0 .74	4.99±0.6 3*	4.05±0.63 *

* Significant at $p < 0.05$, # Non significant at $p > 0.05$

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Histological observations of maternal tissues:

Liver: In control, the hepatic tissue is composed of polygonal hepatocytes joined to one another in anastomosing plates, with borders that face either the sinusoids or adjacent hepatocytes.

The hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids containing Kupffer cells. They are regular and contain a large spheroidal nucleus with a distinctly marked nucleolus and peripheral chromatin distribution. Some cells have two nuclei each. Few spaced hepatic sinusoids were arranged in between the hepatic cords and contained fine arrangement of Kupffer cells (Fig.2 A&B).

In experimental indole acetic acid –treatment (100 mg/kg B.wt) exhibited similar histopathological alterations characterized by perivascular leukocytic infiltration associated with erosion of endothelial cells lining the blood vessels. There was a marked increase of dissolution of hepatic cords with prominent dilated blood sinusoids and ill defined cell boundaries of hepatocytes. Numerous hypertrophied Kupffer cells were detected in the sinusoidal wall. Many of hepatocytes exhibited either karyolysis or pyknosis of their nuclei as well as possessed ill defined cell boundaries. The blood vessels appeared either congested or edematous (Fig.3 A-D).

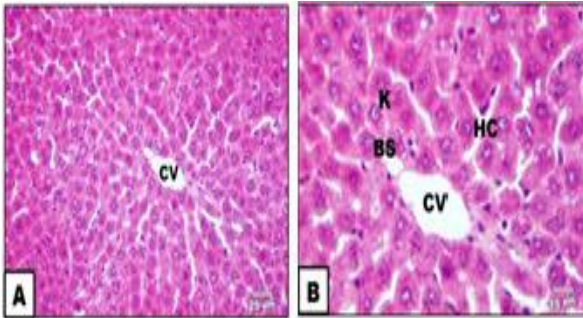


Fig. 2(A&B): Photomicrographs of histological sections of control maternal liver showing radially arranged cords of hepatocytes around the central vein. (Abbreviation: CV: Central vein, Bs: blood sinusoid, Hc: Hepatic cell and K: Kupffer cell.) HX.-E.

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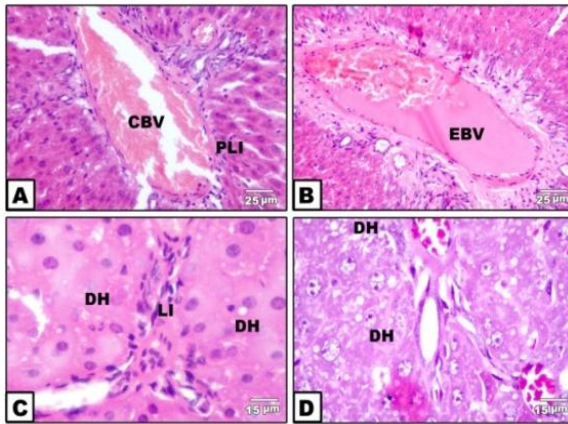


Fig. 3(A-D): Photomicrographs of histological sections of maternal liver treated with 100 mg/kg B.wt of IAA.

Fig.A: Showing congested blood vessel and Perivascular leukocytic infiltration.

Fig. B: Showing edematous blood vessel.

Fig. C: Showing leucocytic infiltration and degenerated hepatocytes.

Fig. D: Showing fatty degeneration and round cell infiltration.

(Abbreviation: DH: degenerated hepatocytes, PLI: Perivascular leukocytic infiltration, CBV: congested blood vessel, EBV: edematous blood vessel.) HX.-E.

Biochemical observations: Table (2) illustrates the serum biochemical analysis of liver function tests, antioxidant enzymes and lipid peroxidation and free radicals of mothers intoxicated indole acetic acid. plant growth hormones significantly decreased alanine transaminase and increased asparate transaminase. The serum albumen and billirubin contents as well as the activities of arginase and D-L- Fucosidae were markedly increased in both treatments. Highest susceptibility and intensified effects were detected in indole acetic acid-treatment. On the other hand, the assayed antioxidant enzymes; super oxide dismutase, catalase, glutathione S-transferase, peroxidase and reductase showed a considerable reduction post-applied plant growth hormone treatment especially after indole acetic acid-treatment. The observed drastic effects of the used plant hormones was reflected by marked increase of lipid peroxidation malonadialdehydes and free radicals especially of those subjected to indole acetic acid treatment .

Table (2): Biochemical Analysis of Serum of Mother treated with 100 mg /Kg B. wt from either gibberellic (GA_3) or indole acetic acids (IAA).

	C	IAA
ALT(U / ml)	3.2 ± 0.01	$1.3 \pm 0.01^*$

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AST (U / ml)	29.79 ± 1.62	65.64 ± 2.3*
Albumin (mg / dl)	12.43 ± 1.31	23.56 ± 16.73*
Bilirubin (mg / dl)	1.02 ± 0.01	2.4 ± 0.09*
SOD (U /ml)	17.23 ± 1.60	1440. ± 1.26 *
CAT (U/ L)	29.79 ± 0.72	65.64 ± 1.03 *
GST (U /ml)	1.02 ± 0.0	2.1 ± 0.09*
GSPase (U/L)	17.23 ± 3.59	1540. ± 2.81
GSH (mg / dl)	12.43 ± 0.58	17.12 ± 7.48 *
MDA (n mol / m)	1.02 ± 0.01	1.4 ± 0.04 *
H2O2 (m mol / L)	25 ± 0.01	40 ± 0.01 *
Arginase (U/L)	4.08 ± 1.22	111.55 ± 27.31*
α-L-Fucosidase (U/L)	21.81 ± 10.39	75.48 ± 5.15*

Each result represents the mean ± SD of 10 replicates.

* Significant at $p < 0.05$, # Non significant at $p > 0.05$.

MDA, Malondialdehyde concentration, SOD, SuperOxide Dismutase activity, H₂O₂, Hydrogen Peroxide. CAT, Catalase activity. Glutathione -S-Transferase activity, GSPase , Glutathione Peroxidase activity, GSH Glutathione Reduced concentration, Albumin, Bilirubin, GOT, Glutamic - Oxaloacetic Transaminase. GPT, Glutamic – Pyruvic Transaminase. Arginase, and α-L- Fucosidase.

Table (3) illustrated the biochemical analysis of antioxidant enzymes and lipid peroxidation and free radicals of liver of mothers intoxicated with indole acetic acid. Except glutathione s-transferase the assayed antioxidant enzymes; super oxide dismutase, catalase, glutathione peroxidase and reductase showed a considerable reduction post-applied plant growth hormone treatment. In liver, meanwhile the other assayed antioxidants were markedly affected by indole acetic acid treatment (Figs.6-7).

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The observed drastic effects of the used plant hormones was reflected by marked increase of lipid peroxidation malonadialdehydes and free radicals in both liver regions especially of those subjected to indole acetic acid treatment .

Table (3): Biochemical analysis of liver of Mother treated with 100 mg /Kg B. wt from indole acetic acids (IAA).

	Liver	
	C	IAA
MDA (n mol / g tissue)	10.47 ±0.97	19.24 ±0.12*
H2O2(m mol / g tissue)	166.9 ±17.77	382.03 ±15.8*
SOD (U / g tissue)	19.25 ±0.13	8.48 ±0.98*
CAT (U/ g tissue)	362.08 ±6.56	353.20 ±25.5*
GSH (mg / g tissue)	1.85 ±0.07	0.58 ±0.20*
GST (U / g tissue)	87.51 ±8.35	144.8 ±12.8*
GSPase (U / g tissue)	41.05 ±7.17	25.29 ±0.96*

Each result represents the mean ± SD of 10 replicates. * Significant at $p < 0.05$ after studying paired sample t- test statically analysis. Malondialdehyde concentration, SOD, SuperOxide Dismutase activity, H2O2, Hydrogen Peroxide. CAT, Catalase activity. Glutathione -S- Transferase activity, GSPase , Glutathione Peroxidase activity, GSH Glutathione Reduced concentration.

Effects on pregnancy & prenatal embryos and newly delivered pups:

From table (4), treating pregnant with indole acetic acid on pregnant mothers revealed 5/25 of pregnant mothers failed to complete pregnancy especially of those subjected to indole acetic acid treatment. There was a marked reduction of both body weights and crown-rump size of prenatal embryos at 14 & 16 days old.

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Examining the gross morphology of pups of experimental treated groups revealed the presence of different pattern of congenital malformations. Superficial haematomas and fore limb deformation were detected in delivered newborn of studied experimental groups. Highest incidence was recorded in experimental group receiving indole acetic acid-treatment (Table (5-7)).

Table (4): Effect of experimental applied of Indole Acetic acid (IAA) treated female albino rats during pregnancy on pregnant mothers and their fetuses and pups.

	Control	(IAA)
Total number of mothers	20 (100%)	25 (100%)
Total number and percentage of aborted mothers	0 (0%)	5 (20%)
Total number and percentage of pregnant	20 (1000%)	20 (80%)
Total number of fetuses and newborn	120 (1000%)	75(100 %)
% of numerical reduction of fetuses & newly born from control	0 (0%)	(62.5%)
Total number (% of fetal mortality)	0 (0%)	21 (28%)
Total number & % of alive fetuses & newly born	120 (1000%)	54 (72%)

Control (C) Indole Acetic acid (IAA)

Table (5): Body weight (gm) and size (Kg B. wt.) of fetuses (14&16 -days) and pups maternally treated with 100 mg /Kg B. wt from indole acetic acids (IAA).

Pregnancy Day	14 day prenatal		16 day prenatal		pups	
	C	IAA	C	IAA	C	IAA
weight	4.01±0.041	3.10±0.018*	5.2±0.066	4.1±0.068*	8.1±0.016	6.7±0.041*
size	3.9±0.07	2.10±0.056*	5.6±0.051	4.5±0.052*	15.8±0.094	9.2±0.121*

Each result represents the mean ± SD of 10 replicates.

* Significant at $p < 0.05$. Control (C) Indole Acetic acid (IAA)

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Table (6):crown-rump length (cm.) of fetuses (14&16) days and pups maternally treated with 100 mg /Kg B. wt from indole acetic acids (IAA).

Pregnancy Day	14 day prenatal		16 day prenatal		21 day	
	C	IAA	C	IAA	C	IAA
Mean+SD	1.98±0.08	1.6±0.053*	2.60±0.223	2.0±0.108*	4.1±0.047	3.8±0.107*

Each result represents the mean ± SD of 10 replicates.

* Significant at $p < 0.05$. Control (C) Indole Acetic acid (IAA)

Table (7): Incidence of gross morphological and skeletal abnormalities of 14&16 days fetuses and pups maternally treated with 100 mg /Kg B. wt from indole acetic acids (IAA).

	Control	IAA
Total number & % of alive fetuses & newly born	120 (100%)	54 (72%)
Superficial haematomas	0 (0%)	10 (18.51%)
Abnormal fore limb		
-Unilateral	0 (0%)	15 (27.8%)
-Bilateral	0 (0%)	25 (46.29%)
Abnormal hind limb		
-Unilateral	0 (0%)	7 (12.96%)
-Bilateral	0 (0%)	30(55.55%)
Incomplete ossification of sternum	0 (0%)	6(11.11%)
Kyphotic body	0 (0%)	5(9.25%)
Kinky tail	0 (0%)	14(29.92%)

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Missing of caudal vertebrae	0 (0%)	13(24.07%)
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Each result represents the mean \pm SD of 10 replicates.

* Significant at $p < 0.05$. Control (C) Indole Acetic acid (IAA)

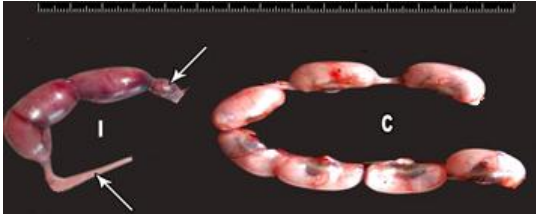


Fig. 12 (C-I): photomicrographs of uterus of 16 days of gestation

C. control uterus showing symmetric distribution of implantation

I. Mother's uterus treated with 100 mg/kg B.wt of indole acetic acid (IAA) showing asymmetric



Fig. 13 (C-I2): Photomicrographs of lateral view gross morphology of 14-days old fetuses.

C. Control 14-days old fetuses.

I1-I2. 14 days old fetuses Maternal treated with 100 mg/kg B.wt of indole acetic acid (IAA) showing

deformation of limbs.

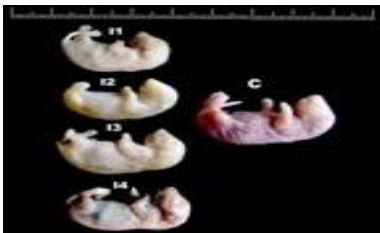


Fig. 14 (C-I4): Photomicrographs of lateral view gross morphology of 16-days old fetuses.

C. Control 16-days old fetuses.

I1-I4. 16 days old fetuses Maternal treated with 100 mg/kg B.wt of indole acetic acid (IAA) showing

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marked reduction in size, deformation of fore and hind limb and non demarcation of trunk region.



Fig. 15 (C-I3): Photomacrographs of lateral view gross morphology of pups.

C. Control pups.

I1-I3. Pups Maternal treated with 100 mg/kg B.wt of indole acetic acid (IAA) showing marked deformation in size, deformation of fore and hind limb, exencephaly and superficial haematomes.

distribution of implantation and resorption of embryo.4. Histological observations on pups:

Liver: The control liver consisted of irregular cords of hepatocytes separated by islets of haemopoietic cells and primitive vascular sinusoids containing erythrocytes. The hepatocytes were not arranged in lobules. Fibroblasts were present between them. The thickness of hepatic strands one or two cell layers thick (Fig.16 A-B).

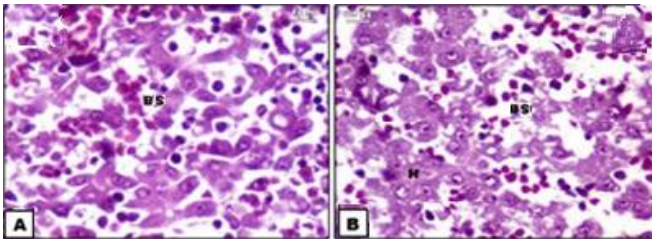


Fig.16 (A-B): Photomicrograph of histological sections of liver of control pups showing normal hepatocytes. (Abbreviations: BS: blood sinusoid and H: hepatocytes.) HX-E

On the other hand, the liver of pups maternally treated with indole acetic acid exhibited apparent clumping of nuclear chromatin of hepatocytes. Many of the hepatocytes appeared eosinophilic manifesting cell death. Megakaryocytes were abundant in necrotic tissues. The vascular bed of the hepatic tissue was distorted. Indole acetic acid showed the highest drastic effect and most of the blood became congested and edematous with apparent degeneration of their endothelial lining cells (Fig.17 A-C).

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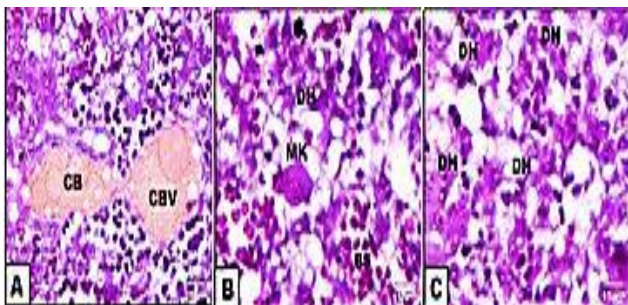


Fig.17 (A-C): Photomicrograph of histological sections of liver of Pups maternally treated with 100 mg/kg B.wt of indole acetic acid (IAA).

Fig. A: Showing edematous and congestion of blood vessels.

Fig. B: Showing breakdown of hepatocytes and presence of megakaryocyte.

Fig. C: Showing degeneration of hepatocytes.

(Abbreviations: CBV: congested blood vessels DH: degenerated hepatocytes, and MK: megakaryocyte.) HX-E

5. DISCUSSION

Recently plant growth hormones are among those widely used chemicals in agriculture accelerated the growth of fruits and vegetables (19;20). The amounts of its placed into the environment exceed those of the insecticides (21). Although, the consumption of contaminated food products led these growth hormones to find their ways in our body, however, the toxicological aspects are very limited (22; 23; 24; 12; 15; 11).

From the present findings, oral administration of either indole acetic acid at doses of 100 mg/Kg for 40 days every other day and from the 1st to 14th day of gestation led to alterations in maternal hepatic tissues. Liver hepatitis was characterized by either cytoplasmic vacuolization of the hepatocytes with increased incidence of pyknotic nuclei. There was a marked increase of dissolution of hepatic cords with prominent dilated blood sinusoids and ill-defined cell boundaries of hepatocytes. Numerous hypertrophied kupffer cells were detected in the sinusoidal wall. The blood vessels become either congested or hyalinized with apparent degeneration of their lining endothelium as well as had perivascular leukocytic infiltration. Drastic effects of maternal livers were reflected on the liver of their pups of almost identical histopathological lesions in the form of massive cell death characterized by massive necrosis of hepatocytes, distortion of blood sinusoids and abnormal congestion of blood vessels with apparent degeneration of their endothelial lining cells.

Although there is no further explanation of the mammalian toxicities of indole acetic acid, it might be interacting primarily with the liver, resulting in structural damage. The applied PGRs may lead to breakdown of hepatocyte enzymes and leakage their enzymes into the plasma. These enzymes are mainly localized in the cytoplasm and any damage in

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hepatic cells may result in alteration in the serum levels. The decrease in the activities of these enzymes in the serum may result only consequent to impairment of the synthesis in cell of tissues with subsequent liberation of the enzymes into the circulation from the damaged tissue. Transaminases are intracellular enzymes which exist in only a small amount of the serum. Therefore, damage to liver cell may result in leakage of the enzymes into the plasma due to a large concentration gradient (25).

In addition the observed decrease of hepatic serum marker enzymes were coincides with that of the hepatic tissues of mothers and their pups as well as lead to drastic effects on the antioxidant defense systems as a result of the applied plant growth hormones. This was evidenced from our observation that, upon PGRs treatment in vivo, the concentration of MDA and the antioxidant defense markers in liver differ from that of control rats. The present study showed that the lipid peroxidation end product MDA significantly increased in the liver of mothers treated with either GA3 or IAA. The reasons for such affect of applied PGRs are not understood at the present. But, the increased content of MDA may result from an increase of hydroxyl radicals (+OH).

MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation (26). It is known that +OH can initiate lipid peroxidation in tissues (27) and may intern illustrate both liver .

The observed oxidative stress in hepatic tissues lead to the depletion of the assayed antioxidant enzyme activities catalase, superoxide dismutase, glutathione -, peroxidase , reductase and s-transferase and consequently lead to the production of oxygen radicals which exceeds their antioxidant capacity. Excess of free radicals damage essential macromolecules of the cell, leading to abnormal gene expression, disturbance in receptor activity, proliferation or cell death, immunity perturbation, mutagenesis, protein or lipofuscin deposition (28). Antioxidant enzymes catalyze decomposition of reactive oxygen species (ROS). The three major antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) differ from each other in structure, tissue distribution and cofactor requirement (29).

Disturbances in the antioxidant system could play a role in pathogenesis of chronic liver disease (30; 31). The etiology of liver disease in pups may be associated with increased free radical generation as assessed by indirect depletion of our assayed antioxidant enzymes especially superoxide dismutase which have certain respect in fetal liver (32).

Similar reduction of antioxidant enzymes as well as concomitant increase of lipid peroxidation were reported in rats intoxicated with indole acetic acid (33), indole butyric acid (34) and fenthion (35).

The authors finally concluded that to advise farmers to reduce application of plant growth hormones in their green house to reduce their impacts on health along run of life.



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