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TOXIC,PATHOLOGICAL AND REPRODUCTIVE EFFECT OF INDOLE-3- BUTYRIC ACID (PLANT GROWTH HORMONE) ON MALE ALBINO RATS

By

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SUMMARY

Indole-3-butyric acid (IBA) is a plant growth regulator .It used on fruit and vegetable crops to increase crop yields. Such substance induces hazard for human and animal health, through consuming forage-containing residue of these substances. IBA residue was detected in Apple, Peach and Plum fruit samples taken either from farms or market. To study such hazard effect twenty-four male Albino rats divided into three groups each of eight rats. First and second groups administered 2.5and 1.9 PPM indole-3-butyric acid orally by stomach tube three time /week for 65 consecutive days. Third group kept as control. Half animals in each group were slaughtered after last dose .The other half was slaughtered after 2weeks from last dose. When compared with control group Indole-3-Butyric acid (IBA) increased serum transaminases even at 2weeks from last dose in first and second group. IBA caused increase serum urea and creatinine of the first group in comparison to control group after 2weeks from last dose. Pronounced decrease in serum testosterone in first and second group was detected at last dose and 2 weeks from last dose compared to control group. High significant decrease in percentage of normal spermatozoa in IBA received groups were recorded compared to control group. IBA residues were detected in the liver, kidney and testis even after 2 weeks from last dose. Concerning to pathological finding indole -3- butyric acid induced degenerative changes in liver and kidney. The mostly affected organ was the testes especially in rats received indole -3-butyric acid by a large dose.

NTRODUCTION

Growth regulating substances are widely used in agricultural field crops. They classified nto growth promoters as Auxins (natural as indole acetic acid and synthetic as indole - 3- butyric acid) and growth inhibitors **(Mehia and Mathai, 1975).** The plant growth hytohormones has been studied intensively for many years by plant biologists **Engvild, 1989).**

ndole -3-butyric acid (IBA) is similar in structure and function to the naturally occuring plant hormone (indole-3- acetic acid). The difference being that the aliphatic side

chain contains two additional carbon atoms. Indole -3- butyric acid classified as biochemical pesticide **(EPA1992 and EPA 2001)**. Products containing IBA were first registered in October 1960 for use on ornamental plant cutting and transplant to promote root growth and to reduce transplanting shock. In1990, new IBA products were registered for use on fruit and vegetable crops, field crops and ornamental turf to promote growth and development of flowers and fruit to increase crop yields. Thirty-one pesticide products containing IBA with or without other active ingredients currently are registered **(EPA, 1992)** where **EPA (2001)** reported more than 40 product containing IBA as active ingredient. IBA applied to soil or plant as spray or as dip for cutting. IAA (Indole acetic acid) induced cell elongation in wheat and tylosis formation in xylem of tomato cutting **(Lane, et al.1978 and Bollalico and Graniti, 1974).**

Common name of indole-3 butyric acid is IBA; while chemical name is indole-3-butyric acid and the empirical formula is $C_{12}H_{13}NO_2$ **(EPA, 1992)**. Indole injected into soil at 1-9 gm/kg was decomposed within 19-135 days **(Medveder, et al.1981)**. Indole and its derivative form a class of toxic environmental pollutant **(Kamath and Vaidyanathan, 1991)**. People may be exposed to IBA during mixing loading and application activities **(EPA, 1992).**

Sawada, et al (1999) introduce compounds that have stronger inhibitory activity against human prostatic 5 alpha - reductase enzymes such compounds contain indole-3-butyric acid in their structures (Fig.1a&b). The same author added that several derivatives were prepared from IBA that has to be useful for treatment of benign prostatic hyperplasia. Spermatogenesis were significantly suppressed to 20% of control by co-administration of 5alpha - reductase inhibitor because of a reduction in the number of round spermatid progressing **(O'Donnell, et al 1999).Inami, et al (1997)** reported that one of these compound (FK143) reduced prostatic volume of normal male dog to about 60% of the initial value and caused atrophy of glandular epithelial.

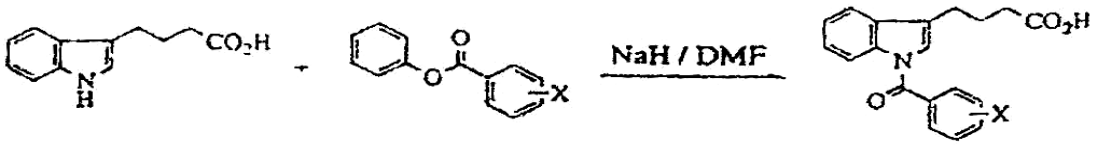

Fig. (1a): Synthesis of compound used for treatment of benign prostatic hyperplasia.

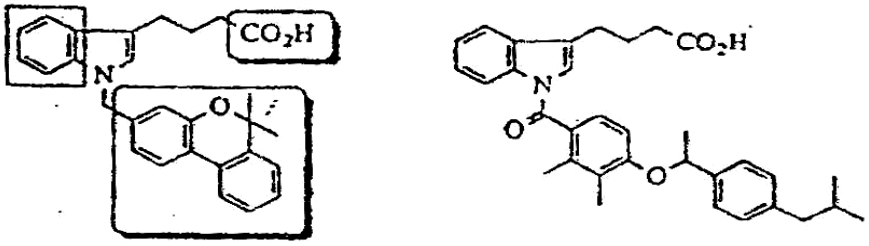

Fig. (1b): Inhibition of prostatic 5 alpha - reductase enzymes.

Indole acetic acid and some derivatives are toxic after oxidative decarboxylation. Toxicity is thought to be due to formation of 3 methylene-2 -oxindole, which may conjugate with DNA bases and protein thiols **(Folkes and Wardman 2001).** Elimination of compounds that contains IBA from liver, kidney, lung, epididymis, seminal vesicle and prostate were slower than from blood **(Katashima, et al, 1997).** Indole acetic acid is moderately toxic to cultured rat neutrophils **(Pires deMelo et al. 1997and 1998).** Plant growth hormones caused coagulative necrosis, chronic inflammatory reaction and leucocytic infiltration in liver of rat and chicken **(Abd EL Hamid et al, 1994 and Tawfik,1997).**Kidney showed acute focal interstitial nephritis ,coagulative necrosis ,and depletion in lymphocytes of white pulb of spleen. Testes showed congestion, degeneration of spermatogonial cell, degenerated spermatid and interstitial cells **(Tawfik, 1997).**

AIM OF THE WORK: plant growth hormone was widely used during last years in plant crops as mentioned by Environmental Pollution Agency .In addition IBA used in

a novel series of compounds used for treatment of benign prostatic hyperplasia. The aim of this investigation was to study the chronic toxic and reproductive effect of indole-3- butyric acid on male rat.

MATERIAL AND METHODS

Test material

A) Fruit samples: Apple, Peach and Plum fruit samples were obtained from Kafer Mansoer farms Kaliobia, EL-Nobaria and from the market respectively.

B) Indole -3-butyric acid (IBA): Fine Powder 99% burity obtained from WINLAB. Chemical Formula is $C_{12}H_{13}NO_2$ and structure formula in fig. (2).

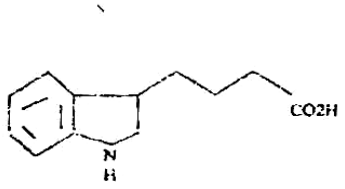

Fig.(2) Structure formula of Indole-3-butyric acid.

C): Animals: Twenty four male Albino rats with average weight100-120 gm were obtained from laboratory animal house, faculty of veterinary Medicine zagazig university, Benha branch. Rats were kept under hygienic conditions and fed on balanced ration. Rats were divided into three groups each of eight rats.

Determination of indole-3 butyric acid in fruit: 5gm from each sample were freshly macerated and extracted with methanol for 72 h. The compound extract was used for determination of IBA at 530 mu using spectrophotometer according to **(Larsen et al., 1962).**

Experimental design:

First group: Indole-3- butyric acid was dissolved in methanol and corn oil and given orally by stomach tube at 2.5 PPM 3 time a week (mean level of IBA in marketed Plum fruit) for 65 consecutive days.

Second group: This group was given 1.9 PPM IBA dissolved in methanol &corn oil 3 time a week (Mean level of IBA of Peach fruit) for 65 consecutive days.

Third group: This group was given an equivalent volume of methanol and corn oil that was given to first and second group 3 time /week for 65 consecutive days and kept control group.

Half number of rats from each group were slaughtered after last dose. These 65 days cover, the period of spermatogenic cycle, which range between from 56 and 60 days in rat (**Hershberger et al 1969**). Other rats slaughtered after 2weeks from last dose.

Samples:

Blood samples were obtained and allowed to clot then serum were separated. Serum value of aspartate amino transferase, alanine amino transferases were determined cloro-metrically according to **Reitman and Frankel (1957).** Serum urea &creatinine levels were determined according to **Pattonn and Crouch(1977)** and **Henry(1974)** respectively. Serum testosterone were determined according to **Wilson and Foster(1992).**

Drop from the epididymal fluid was mixed with two drops of Eosin (5%) and Nigrosin 10% stains. A slid smear was made and air-dried. A total number of 100 normal and abnormal spermatozoa were counted and percentage of normal spermatozoa was calculated **(EL-Azab, 1977).**

Liver, kidney and testes were used for determination of IBA residue according to **(A.O.A.C .1975).** Same specimen were taken and fixed in 10% buffered neutral formalin solution for histopathological examination according to **(Dury & Wallington ,1984)**

Statistical analysis: The data obtained were statistically calculated by the student's (t) test according to **Scan (1986).**

RESULTS

Table (1) illustrate the IBA levels in apple, peach and plum fruit from Kafer Mansor Kaliobia, EL-Nobaria and Kalioba markets respectively. The highest mean level of the plant growth hormone (Indole-3- Butyric acid) was detected in peach and plum fruit .The mean IBA levels were 1.9+ 0.62 and 2.5+ 0.55ppm respectively.

Table (1): Concentration of Indole butyric acid (ppm fresh weight) in Apple, peach & plum fruit.

Samples	Locations	No.	Min.	Max.	Median	Mean
Egyptian apple	Farms in Kafr mansour Kaliobia	50	0.2	3.3	0.4	1.1± 0.25
Peach	El-Nobaria Behera	25	0.1	4.3	0.7	1.9± 0.62
Plum	Kaliobia markets	50	0.9	4.5	2.3	2.5± 0.55

Table (2) shows the effect of oral administration of indole-3- butyric acid on serum ALT, AST, urea and creatinine in comparison to control group. First group (taken 2.5 PPM IBA) showed increase in ALT and AST in comparison to control group. Second group (taken1.9 PPM. IBA) showed increase in level of AST as compared to control group. Serum ALT were 63.07± 1.02, 56.63 ± 0.30 and 54.6± 2.29 u/l for first, second and control groups respectively. Serum level of AST for first and second group were 162.56± 4.66, 158.14± 2.36 and 136.76± 4.99 U/l respectively. Urea and creatinine levels were not affected in first and second groups just after last dose as compared to control one.

Serum levels of ALT at 2 weeks after last dose showed high significant increase in first and second group as compared to control group. Serum ALT level were 55.59± 0.915,55.57± 1.29 and 49.82 ± 3.33 U/l. Urea and creatinine were increased in serum of first group at 2 weeks from last dose in comparison to control group. Second group showed non-significant effect on serum urea and creatinine.

Table (2): Serum ALT. AST (U/l), urea and creatinine mg/dl levels of rats given 2.5and 1.9ppm Indole butyric acid orally (3 time/ week for 65 consecutive days) after last dose & 2 weeks after last dose (Mean + S.E).

Groups	Doses	After last dose				2 weeks after last dose			
		ALT (U/l)	AST (U/l)	Urea (mg/dl)	Creatinine (mg/dl)	ALT (U/l)	AST (U/l)	Urea (mg/dl)	Creatinine (mg/dl)
Group 1	2.5ppm IBA	63.07* ±1.02	162.56** ±4.66	28.9± 0.54	0.65± 0.26	54.59** ±0.915	146.1± 2.27	173.4** ±5.16	3.97**± 0.25
Group 2	1.9ppm IBA	56.63 ±0.3	158.14** ±2.36	29.4 ± 1.51	0.72± 0.03	55.57** ±1.29	149.5± 3.3	37.325± 2.55	0.925± 0.036
Group3	Control group	54.6 ±2.29	136.76 ±4.99	29.7± 3.3	0.8± 0.14	49.82± 3.33	138.63 ±3.67	34.4± 4.45	1 ±0.09

*Significant at p≤ 0.05
**High significant at p≤ 0.01

Table (3) shows the effect of indole -3- butyric acid in percentage of normal spermatozoa. Within the different treated groups there was highly significant decrease in percentage of normal spermatoza from 85.2± 2.15 %to 78±1.75% in first group after last dose & two weeks from last dose respectively. A decrease from90.7+1.64% to 81± 0.99%were detected in second group at last dose and 2 weeks from last dose. In an overall mean, the present results showed highly significant decrease in percentage of normal spermatoza of rats in first and second group when compared to control group (Fig.3, 4, 5& 6).

Table (3):Percentage(%) of normal spermatozoa of rats received 2.5 &1.9 ppm indole butyric acid orally (3 time/ week for 65 consecutive days) after last dose & 2weeks after last dose (Mean + S.E).

Groups	Doses	After last dose	2 weeks after last dose	2 weeks after last dose
Group 1	2.5ppm IBA	85.2± 2.15	78.00± 1.75	82± 3.8**
Group 2	1.9ppm IBA	90.7± 1.64	81.0± 0.99	85.9± 5.1**
Group3	Control	96.6± 1.51	97± 1.10	96.75± 1.29

*Significant at p≤ 0.05
**High significant at p≤ 0.01

Fig. (3): Rat sperm stained by Eosin. Nigrosin. The sperm has an abnormal head and thin middle piece X 1000.

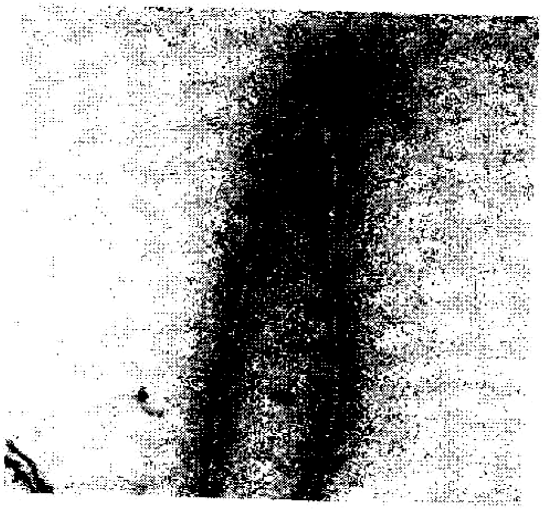

Fig. (4): Rat sperm stained by Eosin Nigrosin.The sperm is normal as control X1000

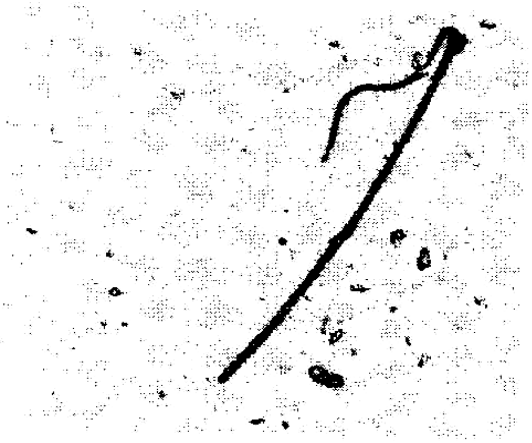

Fig.(5): Rat sperm stained by spermac stain.The sperm has an abnormal head & retro-axillor tail displacement X1000.

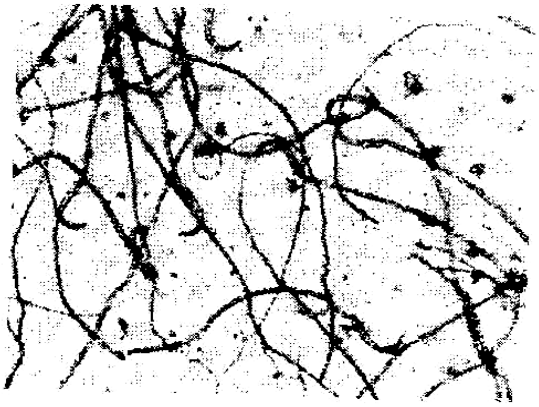

Fig (6): Rat spermatozoa stained by spermac stain. Arrow shows an abnormal sperm with reversibly directed head X1000

Table (4) and fig.(7) show the effect of indole-3-butyric acid on serum testosterone level. First and second group given (2.5 and 1.9ppm respectively) showed high significant decrease in serum testosterone level at last or 2 weeks after last dose. Serum testosterone was 0.23±0.019, 0.34±0.47 and 3.28±0.27ng/ml. For first , seconds and control group respectively just after last dose. Serum testosterone at 2 weeks from last dose were 0.037 ± 0.01, 0.135±0.02ng/ml in comparison to 2.97±0.26ng/ml respectively for first second and control group respectively.

Table (4): Serum testosterone(Orally 3 time/ week for 65 consecutive days) after last dose & 2weeks after last dose (Mean ± S.E)

Groups	Doses	Testosterone level (ng/ml)	
		After last dose	2 weeks after last dose
Group 1	2.5ppm IBA	0.23** ±0.019	0.037** ±0.01
Group 2	1.9ppm IBA	0.34** ±0.47	0.135** ±0.02
Group3	Control group	3.28 ±0.27	2.9 ±0.26

*Significant at p≤ 0.05
**High significant at p≤ 0.01

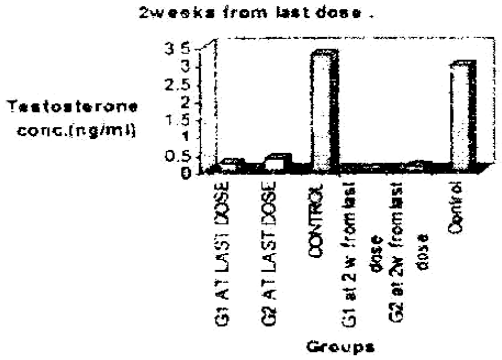

Fig.(7): Effect of oral adminestration of 2.5 ; 1.9 PPM IBA on serum testosterone (ng/ml) of male Albino rats after last dose & 2 weeks from last dose.

Table (5) &fig. (8) showed residue of indole-3- butyric acid in liver, kidney and testis of male Albino rats. Non significant variation was detected between liver, kidney and testis of rats after last dose and that after 2weeks from last dose. There is only significant decrease in residue of IBA in testes of second and first group at 2 weeks from last dose.

Table (5): Indole-3- butyric acid concentration (ug/1000gm) in liver, kidney and testis of male rats given 2.5and 1.9ppm Indole butyric acid orally (3 time/ week for 65 consecutive days) after last dose & 2weeks after last dose (Mean ± S.E).

Groups	Doses	After last dose			2 weeks after last dose		
		Liver	Kidney	Testis	Liver	Kidney	Testis
Group 1	2.5ppm IBA	0.480± 0.19	0.691± 0.29	0.751± 0.13	0.277± 0.38	0.609± 0.16	0.368± 0.33
Group 2	1.9ppm IBA	0.436± 0.039	0.262± 0.071	0.567± 0.11	0.270± 0.057	0.308± 0.043	0.167** ±0.17

*Significant at p≤ 0.05
**High significant at p≤ 0.01

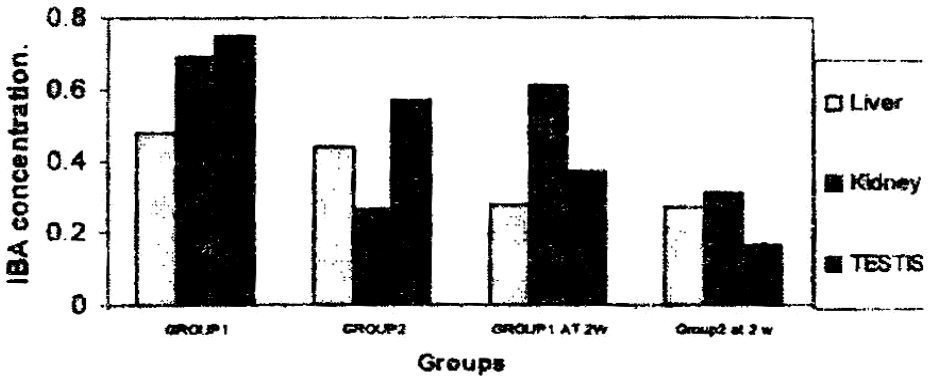

Fig (8): Concentration of IBA (µg/1000gm) in liver, kidne y & testes in firs t & second group at last & and 2 weeks post last dose

Histopathological examination: Liver of rats received 2.5 PPM indole-3- butyric acid showing severe congestion of hepatic blood vessels and sinusoids. The hepatocytes were suffered from degenerative changes in the form of vacuolar and hydropic degeneration (Fig.9). Meanwhile, after 2 weeks liver showing perivascular mononuclear infiltration. Hepatocytes were suffered from degenerative changes or even necrosis. Focal areas of coagulative necrosis represented by structurless homogeneous eosinophilic

substance infiltrated with leukocytes mainly mononuclear and eosinophilic type (Fig. 10&11). Liver of all examined cases dosing 1.9ppm IBA showing mild degenerative changes in the form of vacuolar and hydropic degeneration (Fig.12) Severe congestion of hepatic blood vessels and central blood vessel. After 2 weeks from last dosing the liver showing congestion of hepatic blood vessels.

Kidney of rats received 2.5ppm IBA showing focal areas of coagulative necrosis infiltrated with mononuclear cells (Fig.13). Kidneys showing severe congestion of the intertubular blood capillaries and renal blood vessels. The glomeruli showing either shrinkish of their glomerular tuft, morcover complete degeneration with presence of eosinophilic debris in the glomerular space (Fig.14). Meanwhile, after 2 weeks from last dose kidney showing in addition to previous findings, showing presence of hyaline casts in the lumina of the renal tubules kidney of rat received dose of 1.9ppm IBA showing severe congestion of intertubular blood capillaries and renal blood vessels. Moreover, the glomeruli are showing shrinkish of glomerular tuft with presence of eosinophilic debris in their lumen. Meanwhile, after 2 weeks from last dosing kidneys showing chronic inflammation in the form of severe dilatation of renal tubules. Peritubular fibrosis was also seen (Fig.15&16).

Testes of rat dosing of 2.5ppm IBA showing congestion of testicular blood vessels .The semniferous tubules showing degenerative changes in primary and secondary spermatocytes. Moreover tubular edema was also detected (Fig.17). Meanwhile, after 2weeks from last dosing semniferous tubules showing hyalinization with mononuclear infiltration (Fig.18). Testes of rat received dose of 1.9 PPM IBA showing intertubular edema and degenerative changes of semniferous tubules with few spermatozoa (Fig.19). After 2 weeks from last dosing testes showing degenerative changes together with hyalinization of semniferous tubules (Fig. 20).

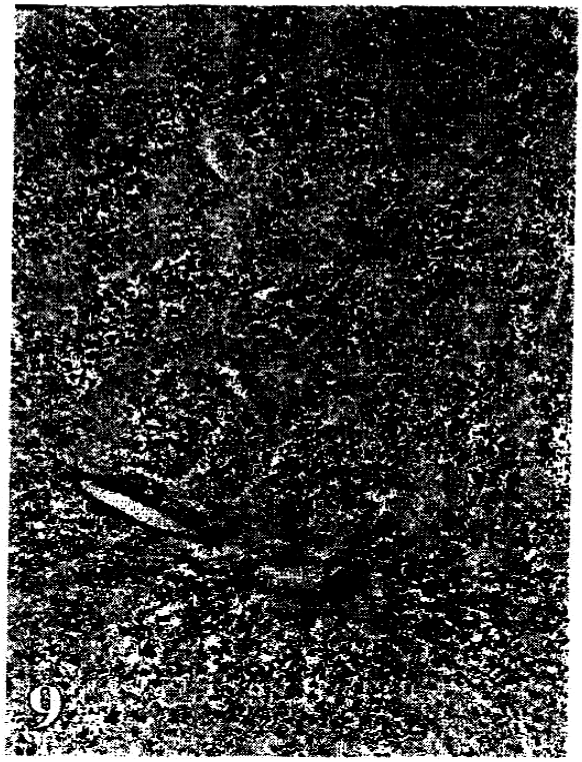

Fig.(9): Liver of rat received (2.5ppm Indole - 3-butyric acid) showing degenerative changes in the form of vacuolar and hydropic H&E Stain X300

Fig. (10): liver of rat after 2 weeks from last dose (2.5ppm indole-3-butyric acid) showing necrotic areas represented by structurless homogeneous eosinophilic substance infiltrated with leucocytes. H&E Stain X 300

Fig. (11): High power of previous figure.H&E stain X800

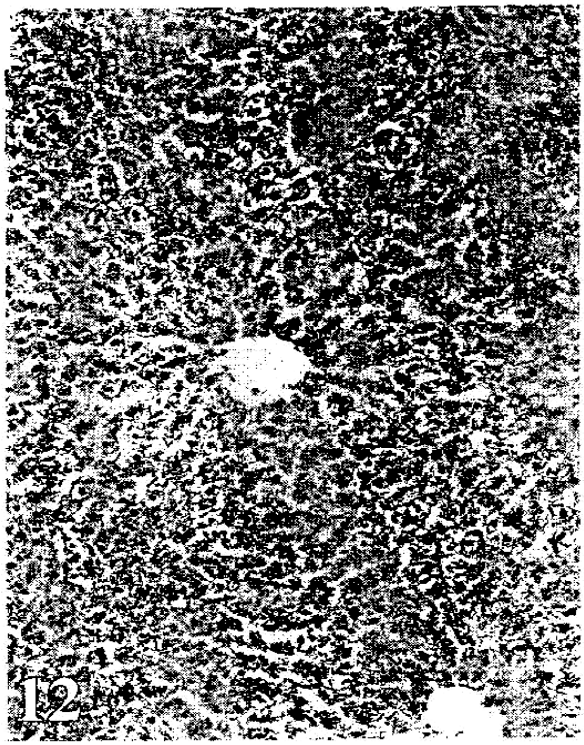

Fig. (12): Liver of rat received 1.9ppm Indole-3-butyric acid, showing mild degenerative changes in the form of vacuolar degeneration. H&E X300

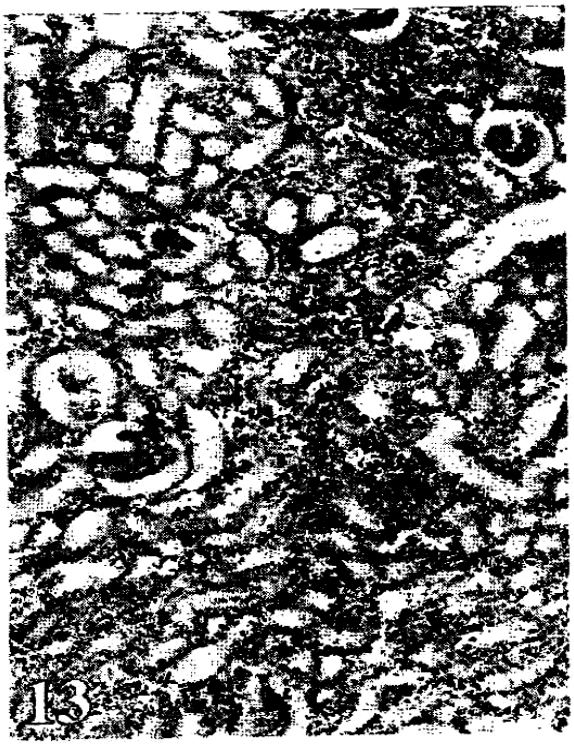

Fig.(13): Kidneys of rat received the dose of 2.5ppm Indole -3-butyric acid, showing focal areas of coagulative necrosis with leucocytic infiltration especially mononuclear type. H&E stain X 300

Fig. (14): Kidneys of rat received (2.5ppm Indole - 3-butyric acid) showing complete degeneration of the glomerular tuft. H&E stain

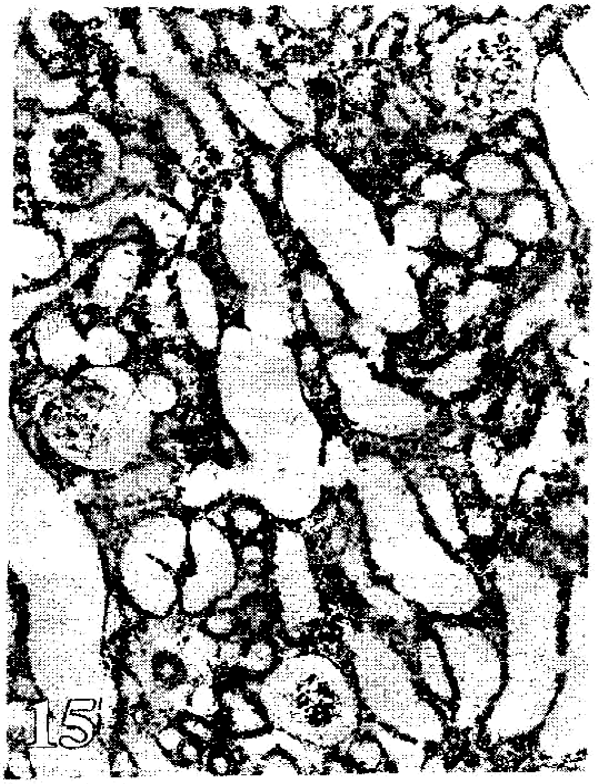

Fig. (15): Kidneys of rat scarified after 2 weeks dose (1.9ppm Indole -3-butyric acid) showing shrinkish of glomerular tuft with severe dilatation of renal tubules. H&E stain X 250

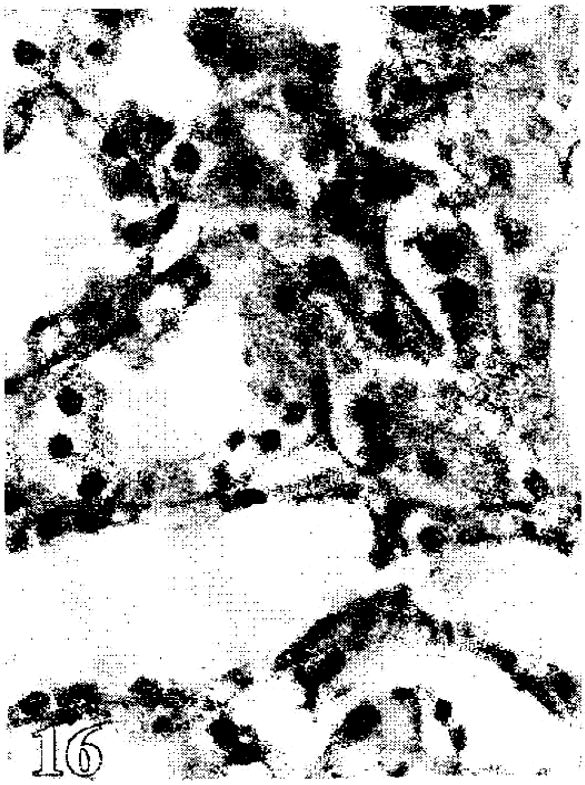

Fig. (16): High power of previous figure. H&E from last stain X800.

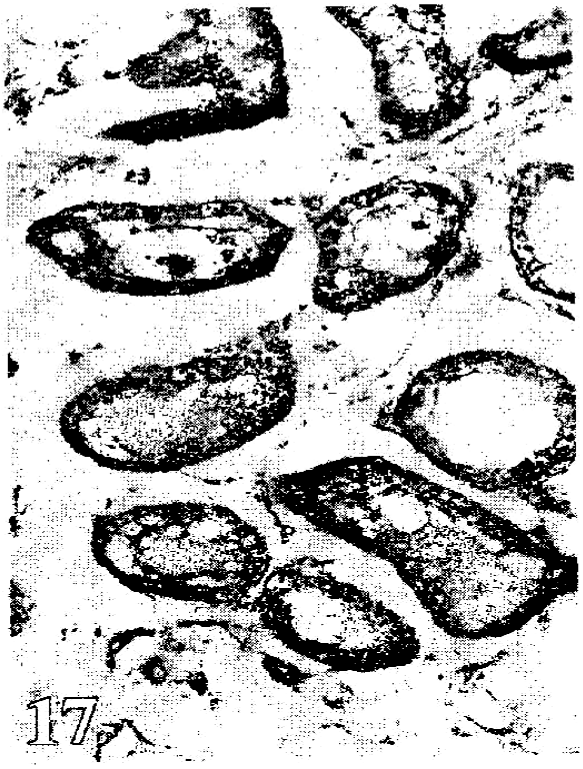

Fig. (17): Testes of rat received (2.5ppm Indole -3-butyric acid) showing cytoplasm destruction of spermatozoa.H&E stain X 250

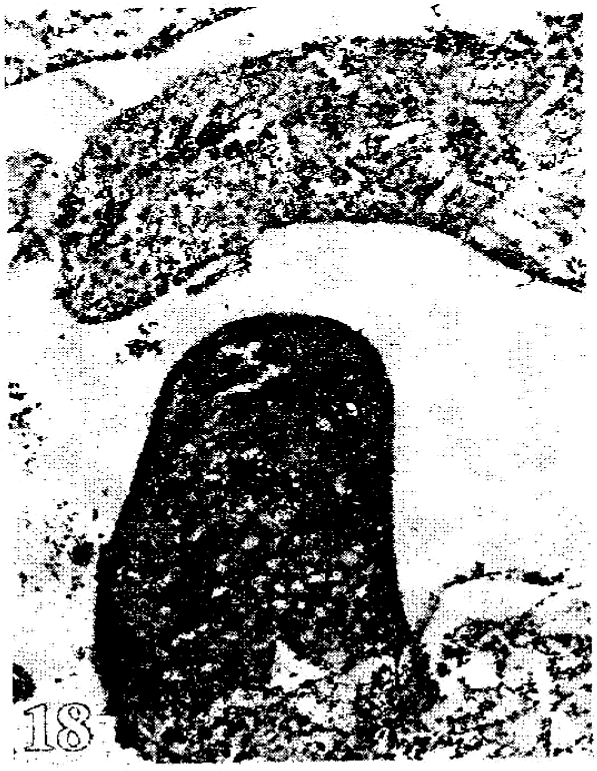

Fig. (18): Testes of rat scarified after 2 weeks from last dose (of 2.5ppm) Indole -3-butyric acid showing complete hyalinization of seminfrous tubules with mononuclear infiltration. H&E stain X 400.

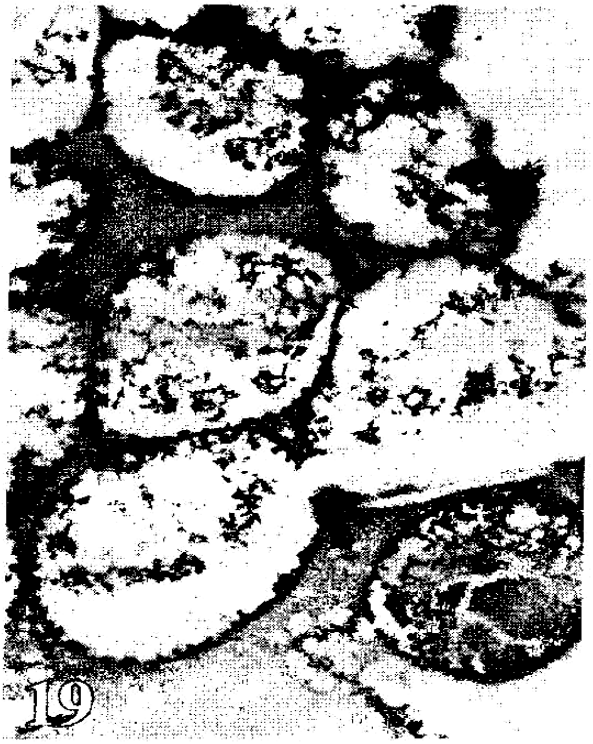

Fig. (19): Testes of rat received (1.9 PPM of Indole -3-butyric acid) showing intertubular edema and degenerative changes of seminferous tubules. H&E stain X 300.

Fig. (20): The testes of rat scarified after 2 weeks from last dose (1.9ppm Indole -3-butyric acid) showing degenerative changes of seminferous tubules with hyalinization .H&E stain X 300.

DISCUSSION

Growth regulating hormones are widely used in agricultural and field crops to increase plant growth. Products containing Indole-3-Butyric acid were first registered in October 1960. It is originally registered for use on a variety of non food ornamental plants shrubs and shade trees to promote and accelerate root formation of plant clipping and to reduce transplant shock .On 1990 additional uses were registered for IBA which include fruit and vegetable crops, field crops and ornamental turf **(EPA, 1992)**. Different levels of IBA residue were detected in the apple, peach and plum fruits. This indicates uses of IBA (synthetic plant growth hormone) in large scale in last years, where **EPA (2001)** reported that more than 40 product used in plant field containing IBA as an active ingredient.

Table (2) shows the effect of indole-3-butyric acid on some serum parameters of intoxicated rats. A dose related increase in serum AST and ALT was detected due to indole -3-butyric acid toxicity even at two weeks after last dose .The increased values of AST &ALT could be related to oxidative decarboxylation of indole-3- butyric acid and formation of 3-methylene -2-oxindole which conjugate with DNA bases and protein thiol **(Folkes and Wardman, 2001)**. Toxicant enter the body via gastrointestinal tract are absorbed and carried to liver that contain a high concentration of xenobiotic metabolizing enzymes **(Bridges, et al 1983).** Serum AST and ALT have been used as indicators of hepatic injuries **(Frank, 1987)** .Our results concerning the pathology were confirmed this damage in liver (Fig.9, 10,11&12). Liver of rats received indole 3- butyric acid showed severe congestion of blood vessels, degenerative changes of hepatocytes even after 2 weeks from last dose. This results are in agreement with **Abd EL Hamid et al (1994) and Tawfik, (1997).** They reported hepatocelluler changes in the liver of chicken & rats received plant growth hormone. These findings may be attributed to chronic toxic effect of indole -3 butyric acid on hepatocyte.

Regarding to the effect of indol-3- butyric acid on serum urea and creatinine values, high significant increase in serum urea indicates glomerular damage and hepatotoxicity. However elevation of creatinine level indicates impaired kidney function **(Frank, 1987)** .The increased levels at two weeks from last dose in the first group could be augmented by residue of indole-3-butyric acid in kidney tissue. That came in accordance

with the results of **Katashima, et al (1997)** who mentioned that elimination rate in the kidney is slow. Also severity of kidney tissue damage was seen in fig. (13&14) could augment this results. Kidney lesions reported due to indole 3- butyric acid toxicity are agreed with **Tawfik, (1997).**

The effect of indole butyric acid in male fertility was pronounced and manifested by reduction in percentage of normal spermatozoa. Such reduction is a dose dependent. Toxicant can interfere to the reproductive system either directly or via certain endocrine organs **(Frank, 1987).** Decrease in percentage of normal spermatozoa in first and second group might be due to inhibition of prostatic 5 alpha - reductase and reduction of round spermatid progressing due to IBA administration. These results are agreed with **O'Donnell, et al (1999)** who found that spermatogenesis were suppressed to 20%. These results were augmented by decreasing the level of testosterone might explain the reduction of normal spermatozoa, where testosterone is responsible for the maintenance of gametogenesis. Also histpathological lesions in testes as clear in fig. (17,18,19&20). Testicular degeneration and degenerative changes of spermatocyte recorded in this study are in agreement with result of **Tawfik, (1997)** and partially with **Inami, et al (1997).** This may be attributed to inhibition of 5 alpha reductase enzymes by indole 3-butyric acid **(O'Donnell, et al 1999).**

The noticed decrease in serum testosterone might be due to the strong inhibitory activity of IBA to the prostatic enzyme (5 & reductase) that responsible for formation of dihydrotestosterone (the active form testosterone) **(Sawada, et al, 1999).** Partially agree with **Inami, et al (1997)** who reported atrophy in prostate volume. These results augmented by residual of IBA in testes table (4)&fig. (4). Congestion of testicular blood vessels, degenerative change of primary and secondary spermatocyte as in fig. (17,18,19& 20).

Data of table (5)&fig. (8) revealed IBA residue in liver, kidney and testes. No variations on liver or kidney IBA residue values after last dose or at 2 weeks from last dose. Only decrease in IBA level in testes of second group was detected at 2 weeks in comparison to that just after last dose. These results might possible due to slow elimination rate of indole-3-butyric acid. That came in accordance with the reported results of **Katashima, et al, (1997)** who reported that elimination rate of compounds contains In-

dole-3-butyric acid from liver, kidney, seminal vesicle and prostate were slower than from the blood.

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