



## BIOCHEMICAL EFFECT OF PLANT GROWTH PROMOTERS ON MINERAL, IMMUNITY AND FERTILITY

Ali, H.A., El-Far, A.H., Hassan, A.E.

Department of Biochemistry, Fac. Of Vet. Med., Benha University

### ABSTRACT

The aim of the present study is to find a relationship between plant promoters and serum trace element level, antioxidant activity, immunoglobulins, allergic factors, kidney, liver function and level of sexual hormones. In order to achieve this aim 50- Rats (25 male-25 female) 4 - 6 weeks old weighting 120-190 grams used in the experiment. They classified to 5 groups one control group and 4 injected groups .Each group consisted of 10 rats (5 male -5 female). Five groups were abdominal injected twice a week with 100mg of either gibberellic acid GA<sub>3</sub>, Auxins or ethephone (2 -chloroethyle phophonic acid) alone or in combination /kg body weight for 6 weeks. The results of the present study showed significance association between control and injected rats . In addition, the following were found: Low Immunity (IgG, IgM , IgA,) level, low antioxidant (SOD, G6PD , GSH ) activity, low total cholesterol level , high allergic factors (cortisol, Histamine , total IgE) level, increase in NO level, increase in Blood urea and creatinine level followed by alteration in Na, K, total protein, and albumin; and disturbance in sexual hormones (testosterone, progesterone follicle stimulating hormone FSH and lutinizing hormone LH) levels between male and female rats. These parameters may all be regarded as predictors or risk factors for injected rats. The findings of the present study suggest that mineral imbalance, immunity dysfunction, oxidative stress, ,and low fertility are primary interacting mediators in the pathogenesis of injected rats.

**Key words:** Gibberellic acid, Auxins, Ethephone, Blood, Reproduction.

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### 1. INTRODUCTION

Plant growth regulators have been widely employed in recent decades to improve crops quality and yield. Gibberellic acid-3 (GA<sub>3</sub>) is a naturally occurring plant growth regulator which may cause a variety of effects. GA<sub>3</sub> is produced commercially by growing *Gibberella fujikuroi* fungus cultures. Gibberellic acid-3 is heat resistant ,not losing its activity after 4 hours at 100°C. GA<sub>3</sub> induced sub acute and sub chronic adverse effects on serum Markers enzymes and erythrocytes in rats according to Tuluze and Celik [30]. Auxins are plant growth hormones that are involved plant-cell

elongation. A well-known natural auxin extracted from plants is Indole Acetic Acid (IAA). Research shows that auxins have fetal hormonal action. They also stimulate cellular growth and provide better resistance to disease. The IAA in auxins acts as an antiinflammatory and helps tissue to regenerate. Also, when used in combination with the plant enzyme peroxidase, Indole Acetic Acid can produce byproducts that are toxic to cancer cells, yet harmless to healthy cells. Residues of many plant growth regulators such as cytokinines ,auxines , gibberelline and ethylene in or in all food commodities due to their low toxicities on mammals (toxicity category III or IV). The

agency added the phrase (when used as plant regulators) to the guidelines of usage which means should be used in suitable times and control quantities according to EPA [31]. Ethephone (2-chloroethylphosphonic acid) (CAS Number: 16672-87-0, molecular weight: 144.5) acts via liberation of ethylene, which is absorbed by the plant and interferes in the growth process. It is also used in the acceleration of ripening of fruits and vegetables. Products containing ethephon carry the Signal Words "Danger", "Warning", or "Caution" depending on the product according to El-Okazy [8].

## 2. MATERIAL AND METHODS

### 2.1. Animals

This study conducted on 50 rats (25 male-25 female) Sprague-Dowley rats, 4-6 weeks old weighting 120-190 grams were used in the experimental investigation of this study. Rats were obtained from El-Nasr Pharmaceutical Chemical Company, Abou Zaabal, Qalubia, Egypt. Rats were housed in Special Laboratory Animals Rome in Biochemistry Department; Faculty of Veterinary Medicine Moshtohor in stainless-steel cages limited their movements.

They classified to (5) groups each group contain (10) rats (5) male-(5) female. Group I : control healthy rats ( injected with distilled H<sub>2</sub>O)

Injected groups:

- a) Group II: - Consisted from 10 rats ((5) male- (5) female) injected with gibberelline.
- b) Group III: - Consisted from 10 rats ((5) male - (5) female) Injected with Auxines.
- c) Group IV: - Consisted from 10 rats ((5) male - (5) female) Injected with Ethephone.
- d) Group V: - Consisted from 10 rats (5) male - (5) female) Injected with Mixture (gibberelline, Auxines and Ethephone).

### 2.2. Sampling

Blood is collected every two weeks for 6 weeks from median canthus by capillary tube. Blood samples divided in to two portions the first one poured in tubes contain 20 IU Heparin as anti-coagulant/1 ml blood used for preparation of haemolysed by digitonine according to Kornburg [16]. After washing erythrocytes by physiological saline, this haemolysed used for estimation of Erythrocytes total super oxide Dismutase (t. SOD) according to Misra [19] · reduced glutathione (GSH) according to Bergmayer [3], glucose 6 phosphate dehydrogenase (G6PD) according to Nishikimi *et al* [22]. The second portion poured in tubes with out anticoagulants for isolation of serum for nitric oxide (NO) according to Bories [5] , cortisone according to Mullner *et al* [21] , histamine according to Hermann *et al* [12] , T. IgE according to Plebani *et al* [24], total protein according to Henry [11] , immunoglobulin Igs according to Whicher *et al.*, [33] , Albumin according to Young *et al.*, [35], sodium according to Schwechheimer [29], potassium according to Hillmann [13], B.urea according to Kaplan [14], creatinine according to Bonsens [4] , testosterone according to Tateiki [28], progesterone according to Radwanska [25], LH according to Knobil [15] , FSH according to Marshall [18], cholesterol according to Ellefson [7] .

### 2.3. Statistical analysis:

All values were expressed as mean  $\pm$  standard error (SE). All statistical analyses were performed using SPSS (version 19). Statistical differences among the experimental groups were assessed by ANOVA. Duncan's test was used as a follow-up test and significance was defined at  $p < 0.05$ .

## 3. RESULTS

The recorded data in table (1, 2, 3, 4, 5, 6, 7 and 8) revealed that injected rats are accompanied by decrease in the mean values of

serum immunoglobulins (IgG, IgM, IgA) in table 1. Decrease in serum protein in males but increase in females, alternation in serum albumin and serum cholesterol between males and females in table 2. Decrease in serum testosterone level in males but increase in females and decrease in serum LH (Lutinizing Hormone) level in both sex in table 3. Decrease in serum FSH hormone but increase in Serum Histamine and Serum cortisone level in both male and female in all groups in table 4. Increase in Total IgE level but decrease in Erythrocyte SOD, G6PD in table 5. Decrease in erythrocyte reduced GSH but it found increase in serum Nitric oxide level (NO) in male and female in table 6. Increase in serum Blood urea and serum creatinine levels in both sex along the experimental period in table 7. Alternation in serum sodium and potassium levels between male and female in table 8. These results in comparison with the mean values recorded in the control healthy individual group.

#### 4. DISCUSSION

Gibberellic acid (GA3) is produced by a naturally occurring fungus in large vats [26]. GA3 is extensively used in Egypt and other countries, little is known about its toxic effects in mammals as well as its potential hazardous effects on human health [10]. In agreement with Wafaa *et al.* [32] disruption of the hepatic antioxidant enzymes activities with accumulation of MDA indicating GA3 induced oxidative stress and lipid peroxidation in the treated animal livers. As there were statistically significant decreases in the mean values of SOD, CAT, GSHPx enzymes activities and significant increase in the mean values of MDA level in the hepatic tissues of the treated rats in comparison to that of the control group. Said that GA3 causes oxidative Stress in erythrocytes of lactating rats and their suckling pups. Total SOD, CAT, and GPx activities significantly decreased in suckling rats as well as in their

mothers treated with GA3 [1]. In this study showed a decrease in hepatic enzymatic and non enzymatic antioxidant inhibitors (SOD, GSH, G6PD, ) and increase in NO level in both male and female parts in all injected groups for 6 weeks of injection. In agreement with El-Okazy [8]. Animals received oral sub-acute doses of gibberellic acid and ethephon together showed mild toxicity. These growth regulators should be strictly used as prescribed regarding the quantity and the time of administration. Higher blood urea were recorded in groups treated with the combination. These undesirable effects increased in an additive manner when gibberellic acid accompanied ethephon. This might be due to the acidic effect of gibberellic acid which might together with stomach acidity keep ethephon from rapid degradation because ethephon is stable at pH less than or equal to 3 and decomposing to liberate ethylene and phosphonic acid at higher pH values. In this study we showed that increase in B.urea and Creatinine in groups injected with GA3 alone or combined with Ethephon and Auxines. Gibberellic acid caused histological, immunohistochemical and biochemical changes [20]. In present study found that significant decrease in immunoglobulin Igs (IgG, IgM, IgA) in injected groups with all plant promoters when compared to control group which demonstrated that these promoters interact with immune cell and cause disturbance in immune system. In agreement with S. Muthu 1 *et al.* [27]. GA3 treatment also were reduced the content of serum minerals. The reason for the reduction in the quantity of sodium, potassium, calcium and phosphorus noted was not clearly understood. It may be due to alterations in the permeability of electrolytes across cell membranes/ or due to aberrations in the membrane ATPase function. A decrease in these ions may lead to severe complications in body functions. In present study we agree with this result we

Table 1. Serum immoglobulin (IgG), (IgM) and (IgA) (mg/dl) in control and injected groups of male and female rats.

	Sex	Duration periods (weeks)	Control	Gibberellic acid	Groups Auxins	Ethephon	Mixture	Average data of sex
Immoglobulin (IgG) (mg/dl)	Male	Two	493.4±4.31	475.8±1.02	407.2±3.31	373.8±3.84	382.2±3.25	374.35±10.02 <sup>a</sup>
		Four	517.8±3.34	316.6±1.40	372.2±2.48	362.6±4.14	307.2±3.53	
		Six	503.4±3.93	272.2±1.24	301.4±2.20	274.4±2.23	255.0±2.55	
	Female	Two	471.4±4.23	462.0±1.52	432.4±2.87	340.8±2.20	295.8±12.69	
		Four	474.0±3.89	433.4±1.36	329.0±2.92	330.4±2.42	278.0±2.86	
		Six	518.4±4.19	380.8±1.88	307.8±3.34	262.8±3.34	259.2±3.37	
	Average data of groups		496.40±3.80 <sup>E</sup>	390.13±13.94 <sup>D</sup>	358.33±9.26 <sup>C</sup>	324.13±7.85 <sup>B</sup>	296.23±8.22 <sup>A</sup>	
Immoglobulin (IgM) (mg/dl)	Male	Two	56.77±0.22	41.45±0.29	38.95±0.36	45.96±0.58	29.91±0.39	36.02±1.77 <sup>b</sup>
		Four	58.96±0.04	30.32±0.33	20.98±0.02	40.86±0.74	21.59±0.39	
		Six	65.99±0.08	21.30±0.39	17.69±0.37	32.68±0.68	16.91±0.42	
	Female	Two	50.84±0.15	37.38±0.24	33.04±0.30	41.47±0.28	31.60±0.61	
		Four	61.94±0.07	37.61±0.25	30.94±0.37	39.96±0.51	21.21±0.50	
		Six	50.17±0.05	23.28±0.36	17.98±0.49	38.66±0.56	18.57±0.39	
	Average data of groups		57.45±1.05 <sup>E</sup>	31.89±1.41 <sup>D</sup>	26.60±1.52 <sup>C</sup>	39.93±0.76 <sup>B</sup>	23.30±1.04 <sup>A</sup>	
Immoglobulin (IgA) (mg/dl)	Male	Two	39.05±0.04	24.23±0.56	30.13±0.31	21.43±0.33	19.81±0.36	21.79±0.85 <sup>a</sup>
		Four	30.94±0.36	20.93±0.51	19.99±0.34	17.26±0.20	18.31±0.50	
		Six	27.24±0.18	16.77±0.41	18.63±0.46	11.15±0.23	11.04±0.32	
	Female	Two	30.13±0.03	25.68±0.24	30.96±0.47	20.65±0.51	22.70±0.56	
		Four	33.04±0.03	22.23±0.20	19.64±0.36	18.21±0.23	17.23±0.51	
		Six	31.07±0.05	19.21±0.23	17.64±0.52	11.57±0.31	9.65±0.36	
	Average data of groups		31.91±0.68 <sup>D</sup>	21.51±0.57 <sup>C</sup>	22.83±1.04 <sup>B</sup>	16.71±0.76 <sup>A</sup>	16.46±0.88 <sup>A</sup>	

Data are presented as (Mean ± S.E). S.E = Standard error. a, b & c: There is no significant difference ( $P>0.05$ ) between any two means, within the same column. A, B & C: There is no significant difference ( $P>0.05$ ) between any two means, within the same raw.

Table 2. Serum total protein, albumin and lipid biomarkers serum cholesterol in control and injected groups of male and female rats.

	Sex	Duration periods (weeks)	Control	Gibberellic acid	Groups Auxins	Ethephon	Mixture	Average data of sex	
Total protein (mg/dl)	Male	Two	5.31±0.12	6.10±0.05	6.30±0.10	6.55±0.11	6.39±0.07	6.35±0.06 <sup>a</sup>	
		Four	5.98±0.05	6.18±0.05	6.15±0.06	6.46±0.09	6.83±0.05		
		Six	7.09±0.08	6.08±0.06	6.03±0.06	6.46±0.04	7.31±0.04		
	Female	Two	5.74±0.09	7.09±0.06	7.10±0.27	7.32±0.06	7.69±0.02		7.01±0.06 <sup>b</sup>
		Four	6.65±0.11	7.23±0.07	6.58±0.21	6.98±0.04	7.48±0.13		
		Six	7.23±0.21	7.20±0.11	6.38±0.04	7.46±0.04	7.02±0.05		
	Average data of groups			6.33±0.14 <sup>A</sup>	6.65±0.10 <sup>B</sup>	6.42±0.09 <sup>A</sup>	6.87±0.08 <sup>C</sup>		7.12±0.08 <sup>D</sup>
Albumin (mg/dl)	Male	Two	3.27±0.08	3.28±0.06	3.34±0.04	2.82±0.03	3.09±0.04	3.54±0.06 <sup>a</sup>	
		Four	3.22±0.02	3.27±0.07	3.82±0.04	3.56±0.03	3.57±0.04		
		Six	3.16±0.07	3.46±0.09	4.25±0.08	4.82±0.07	4.16±0.03		
	Female	Two	3.87±0.05	3.41±0.10	3.20±0.07	3.11±0.04	3.33±0.05		3.81±0.06 <sup>b</sup>
		Four	3.61±0.05	3.50±0.13	3.69±0.06	4.08±0.05	4.01±0.04		
		Six	3.49±0.04	3.70±0.14	4.50±0.09	5.10±0.06	4.54±0.05		
	Average data of groups			3.44±0.05 <sup>A</sup>	3.44±0.05 <sup>A</sup>	3.8±0.09 <sup>B</sup>	3.91±0.16 <sup>C</sup>		3.78±0.09 <sup>B</sup>
Cholesterol (mg/dl)	Male	Two	121.4±1.17	150.0±0.55	146.4±0.68	154.6±0.68	174.6±0.60	97.24±4.78 <sup>a</sup>	
		Four	65.2±0.92	76.6±0.60	53.0±0.63	45.0±1.38	58.6±0.51		
		Six	79.0±3.78	81.2±0.37	72.8±0.97	64.6±0.93	115.6±1.47		
	Female	Two	118.4±0.75	155.8±0.97	151.0±0.45	162.2±0.66	183.8±0.73		98.80±5.16 <sup>b</sup>
		Four	76.0±0.55	56.4±0.68	63.0±0.95	40.8±0.73	51.8±0.66		
		Six	108.2±0.49	72.6±0.51	83.8±0.37	57.0±0.84	101.2±1.24		
	Average data of groups			94.70±4.15 <sup>B</sup>	98.77±7.26 <sup>C</sup>	95.00±7.27 <sup>B</sup>	87.37±9.45 <sup>A</sup>		114.27±9.49 <sup>D</sup>

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Table 3. Serum testosterone progesterone and LH hormones concentration in control and injected groups of male and female rats.

	Sex	Duration periods (weeks)	Groups				Average data of sex		
			Control	Gibberellic acid	Auxins	Ethephon		Mixture	
Testosterone	Male	Two	8.48±0.16	7.69±0.16	7.83±0.11	2.49±0.09	2.08±0.07	3.99±0.37 <sup>b</sup>	
		Four	8.64±0.13	4.05±0.06	4.13±0.10	1.02±0.02	1.21±0.02		
		Six	8.52±0.14	1.88±0.10	1.19±0.01	0.20±0.01	0.38±0.01		
	Female	Two	0.16±0.01	0.19±0.02	0.29±0.01	0.20±0.00	0.72±0.02		0.80±0.09 <sup>a</sup>
		Four	0.17±0.01	0.33±0.02	0.99±0.04	0.5±0.020	1.36±0.02		
		Six	0.18±0.01	0.55±0.02	1.68±0.06	2.62±0.01	2.08±0.09		
Average data of groups			4.36±0.78 <sup>E</sup>	2.45±0.50 <sup>C</sup>	2.68±0.48 <sup>D</sup>	1.17±0.19 <sup>A</sup>	1.30±0.12 <sup>B</sup>		
Progesterone	Male	Two	1.94±0.05	1.18±0.07	1.56±0.03	3.89±0.09	3.49±0.24	2.86±0.18 <sup>a</sup>	
		Four	2.04±0.07	4.77±0.15	1.86±0.04	2.84±0.10	2.66±0.17		
		Six	1.98±0.07	7.11±0.11	2.09±0.04	3.86±0.04	1.71±0.16		
	Female	Two	2.23±0.09	2.74±0.15	8.14±0.10	4.39±0.02	2.86±0.07		3.04±0.20 <sup>b</sup>
		Four	2.30±0.11	1.96±0.03	4.88±0.12	3.78±0.04	2.36±0.10		
		Six	2.32±0.12	1.02±0.02	1.39±0.02	3.15±0.04	2.04±0.04		
Average data of groups			2.13±0.04 <sup>A</sup>	3.13±0.40 <sup>C</sup>	3.32±0.46 <sup>D</sup>	3.65±0.10 <sup>E</sup>	2.52±0.12 <sup>B</sup>		
LH hormones	Male	Two	0.48±0.08	0.13±0.00	0.11±0.00	0.32±0.01	0.36±0.02	0.23±0.02 <sup>b</sup>	
		Four	0.46±0.08	0.13±0.01	0.10±0.00	0.19±0.02	0.25±0.02		
		Six	0.46±0.08	0.14±0.01	0.10±0.00	0.12±0.01	0.17±0.01		
	Female	Two	0.16±0.01	0.11±0.01	0.09±0.01	0.27±0.01	0.29±0.01		0.15±0.01 <sup>a</sup>
		Four	0.15±0.01	0.11±0.00	0.07±0.01	0.19±0.01	0.21±0.01		
		Six	0.16±0.02	0.10±0.01	0.10±0.00	0.09±0.00	0.11±0.00		
Average data of groups			0.31±0.04 <sup>D</sup>	0.12±0.00 <sup>A</sup>	0.10±0.00 <sup>A</sup>	0.20±0.02 <sup>B</sup>	0.23±0.02 <sup>C</sup>		

Table 4. Serum FSH hormone concentration, histamine level and cortisone level in control and injected groups of male and female rats.

	Sex	Duration periods (weeks)	Control	Gibberellic acid	Groups Auxins	Ethephon	Mixture	Average data of sex	
FSH hormone (mIU/ml)	Male	Two	0.26±0.02	0.13±0.01	0.14±0.01	0.19±0.01	0.49±0.03	0.22±0.02 <sup>b</sup>	
		Four	0.26±0.02	0.12±0.01	0.12±0.01	0.15±0.01	0.30±0.01		
		Six	0.25±0.02	0.45±0.21	0.14±0.01	0.14±0.00	0.18±0.01		
	Female	Two	0.25±0.07	0.11±0.00	0.12±0.00	0.15±0.01	0.43±0.02		0.18±0.01 <sup>a</sup>
		Four	0.25±0.09	0.09±0.00	0.10±0.00	0.14±0.01	0.28±0.02		
		Six	0.26±0.07	0.08±0.01	0.11±0.00	0.12±0.00	0.14±0.01		
	Average data of groups		0.26±0.02 <sup>B</sup>	0.16±0.04 <sup>A</sup>	0.12±0.00 <sup>A</sup>	0.15±0.00 <sup>A</sup>	0.30±0.02 <sup>B</sup>		
Histamine level (ng/ml)	Male	Two	0.97±0.04	3.02±0.05	3.20±0.22	2.11±0.13	3.5±0.26	3.45±0.21 <sup>a</sup>	
		Four	0.97±0.05	4.00±0.23	3.51±0.28	3.10±0.29	4.54±0.24		
		Six	1.20±0.17	4.02±0.09	4.71±0.37	4.91±0.35	7.95±0.36		
	Female	Two	0.80±0.02	2.80±0.26	4.07±0.25	3.35±0.27	3.12±0.12		3.51±0.19 <sup>a</sup>
		Four	0.75±0.02	3.78±0.16	5.48±0.28	5.56±0.23	3.60±0.34		
		Six	0.87±0.13	3.55±0.23	5.13±0.27	5.62±0.21	4.17±0.25		
	Average data of groups		0.93±0.04 <sup>A</sup>	3.53±0.11 <sup>B</sup>	4.35±0.19 <sup>CD</sup>	4.11±0.26 <sup>C</sup>	4.48±0.32 <sup>D</sup>		
Cortisol level (Ug/dl)	Male	Two	2.49±0.25	7.46±0.25	5.73±0.37	7.97±0.25	9.17±0.24	14.09±1.33 <sup>b</sup>	
		Four	1.99±0.07	10.49±0.26	8.47±0.29	15.51±0.36	19.5±0.38		
		Six	2.71±0.28	21.8±0.32	24.47±0.37	30.21±0.37	43.42±0.39		
	Female	Two	2.54±0.26	6.23±0.38	5.01±0.37	6.91±0.36	6.92±0.24		12.56±1.23 <sup>a</sup>
		Four	1.99±0.04	8.43±0.28	10.55±0.38	11.87±0.34	17.32±0.45		
		Six	1.97±0.05	22.8±0.57	16.73±0.38	39.15±0.23	30.00±0.36		
	Average data of groups		2.28±0.09 <sup>A</sup>	12.87±1.27 <sup>C</sup>	11.83±1.28 <sup>B</sup>	18.60±2.23 <sup>D</sup>	21.06±2.32 <sup>E</sup>		

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Table 5. Mean values  $\pm$  S.E of serum total IgE level, antioxidant SOD activity and antioxidant G6pD activity in control and injected groups of male and female rats.

	Sex	Duration periods (weeks)	Control	Gibberellic acid	Groups Auxins	Ethephon	Mixture	Average data of sex	
Total IgE level (IU/ml)	Male	Two	2.81 $\pm$ 0.14	4.00 $\pm$ 0.09	3.90 $\pm$ 0.25	3.85 $\pm$ 0.27	4.82 $\pm$ 0.26	6.61 $\pm$ 0.43 <sup>a</sup>	
		Four	2.92 $\pm$ 0.12	8.01 $\pm$ 0.39	5.52 $\pm$ 0.27	4.51 $\pm$ 0.28	9.95 $\pm$ 0.32		
		Six	2.99 $\pm$ 0.25	12.58 $\pm$ 0.43	10.62 $\pm$ 0.36	8.53 $\pm$ 0.37	14.16 $\pm$ 0.76		
	Female	Two	2.87 $\pm$ 0.16	4.54 $\pm$ 0.26	3.61 $\pm$ 0.25	4.59 $\pm$ 0.35	4.51 $\pm$ 0.29		7.53 $\pm$ 0.50 <sup>b</sup>
		Four	2.91 $\pm$ 0.07	11.41 $\pm$ 0.37	4.88 $\pm$ 0.23	10.6 $\pm$ 0.38	10.58 $\pm$ 0.33		
		Six	3.10 $\pm$ 0.07	15.75 $\pm$ 0.37	8.71 $\pm$ 0.23	11.22 $\pm$ 0.44	13.71 $\pm$ 0.54		
	Average data of groups		2.93 $\pm$ 0.06 <sup>A</sup>	9.38 $\pm$ 0.80 <sup>D</sup>	6.21 $\pm$ 0.49 <sup>B</sup>	7.22 $\pm$ 0.58 <sup>C</sup>	9.62 $\pm$ 0.73 <sup>D</sup>		
Antioxidant SOD (U/mg protein) activity	Male	Two	23.30 $\pm$ 0.36	12.07 $\pm$ 0.56	12.90 $\pm$ 0.56	10.05 $\pm$ 0.33	10.53 $\pm$ 0.37	11.68 $\pm$ 0.85 <sup>b</sup>	
		Four	24.11 $\pm$ 0.66	8.35 $\pm$ 0.24	8.31 $\pm$ 0.23	7.28 $\pm$ 0.20	7.09 $\pm$ 0.04		
		Six	28.55 $\pm$ 0.51	6.41 $\pm$ 0.40	6.29 $\pm$ 0.25	5.22 $\pm$ 0.11	4.73 $\pm$ 0.23		
	Female	Two	22.15 $\pm$ 0.61	14.48 $\pm$ 0.52	10.91 $\pm$ 0.38	8.78 $\pm$ 0.24	10.65 $\pm$ 0.39		10.66 $\pm$ 0.67 <sup>a</sup>
		Four	19.95 $\pm$ 0.33	10.38 $\pm$ 0.27	6.82 $\pm$ 0.23	5.83 $\pm$ 0.30	6.41 $\pm$ 0.27		
		Six	20.87 $\pm$ 0.39	6.33 $\pm$ 0.35	5.72 $\pm$ 0.29	5.38 $\pm$ 0.27	5.23 $\pm$ 0.21		
	Average data of groups		23.15 $\pm$ 0.55 <sup>D</sup>	9.67 $\pm$ 0.57 <sup>C</sup>	8.49 $\pm$ 0.50 <sup>B</sup>	7.09 $\pm$ 0.35 <sup>A</sup>	7.44 $\pm$ 0.45 <sup>A</sup>		
Antioxidant G6pD (U/gm Hb) activity	Male	Two	3.95 $\pm$ 0.05	1.90 $\pm$ 0.10	2.46 $\pm$ 0.23	3.22 $\pm$ 0.26	1.78 $\pm$ 0.05	2.13 $\pm$ 0.15 <sup>a</sup>	
		Four	3.89 $\pm$ 0.03	1.10 $\pm$ 0.07	1.12 $\pm$ 0.05	2.90 $\pm$ 0.07	0.94 $\pm$ 0.05		
		Six	4.42 $\pm$ 0.24	0.81 $\pm$ 0.06	0.77 $\pm$ 0.06	2.00 $\pm$ 0.02	0.67 $\pm$ 0.03		
	Female	Two	5.09 $\pm$ 0.44	2.25 $\pm$ 0.22	2.71 $\pm$ 0.23	3.32 $\pm$ 0.21	1.97 $\pm$ 0.02		2.37 $\pm$ 0.17 <sup>b</sup>
		Four	3.94 $\pm$ 0.36	1.89 $\pm$ 0.10	1.03 $\pm$ 0.05	2.54 $\pm$ 0.27	0.86 $\pm$ 0.02		
		Six	5.03 $\pm$ 0.36	1.01 $\pm$ 0.02	0.85 $\pm$ 0.04	2.49 $\pm$ 0.24	0.62 $\pm$ 0.02		
	Average data of groups		4.39 $\pm$ 0.14 <sup>D</sup>	1.49 $\pm$ 0.11 <sup>B</sup>	1.49 $\pm$ 0.15 <sup>B</sup>	2.74 $\pm$ 0.11 <sup>C</sup>	1.14 $\pm$ 0.1 <sup>A</sup>		



Table 6. Antioxidant reduced glutathione (GSH) activity and antioxidant serum nitric oxide (NO) in control and injected groups of male and female rats.

	Sex	Duration periods (weeks)	Control	Gibberellic acid	Groups Auxins	Ethephon	Mixture	Average data of sex
GSH (Umol/gm protein) activity	Male	Two	40.31±0.75	30.47±0.37	27.40±0.37	25.26±0.24	18.55±0.35	21.40±1.32 <sup>b</sup>
		Four	40.00±0.03	20.03±0.36	17.61±0.56	18.70±0.31	9.37±0.19	
		Six	38.18±0.76	8.80±0.23	9.77±0.22	9.15±0.22	7.38±0.23	
	Female	Two	33.71±0.57	22.57±0.61	19.99±0.03	19.50±0.40	19.54±0.35	17.66±1.06 <sup>a</sup>
		Four	30.30±0.52	13.83±0.52	15.64±0.29	10.19±0.21	8.98±0.04	
		Six	35.31±0.61	9.47±0.25	10.83±0.37	8.49±0.28	6.48±0.24	
	Average data of groups			36.30±0.70 <sup>E</sup>	17.53±1.43 <sup>D</sup>	16.88±1.11 <sup>C</sup>	15.22±1.18 <sup>B</sup>	11.72±0.98 <sup>A</sup>
NO (Umol/ml)	Male	Two	13.70±0.54	17.88±0.49	20.77±0.2	14.94±0.39	21.55±0.37	26.83±1.35 <sup>b</sup>
		Four	16.16±0.02	24.75±0.39	28.01±0.32	29.53±0.36	28.18±0.21	
		Six	14.70±0.54	31.40±0.68	45.70±0.46	43.68±0.76	51.49±0.35	
	Female	Two	11.53±0.38	19.88±0.37	19.65±0.38	14.87±0.39	19.73±0.32	25.17±1.25 <sup>a</sup>
		Four	12.76±0.47	18.51±0.38	31.72±0.26	30.35±0.21	30.69±0.68	
		Six	12.39±0.36	29.85±0.34	40.51±0.27	45.13±0.55	39.91±0.31	
	Average data of groups			13.54±0.33 <sup>A</sup>	23.71±1.01 <sup>B</sup>	31.06±1.78 <sup>D</sup>	29.75±2.25 <sup>C</sup>	31.93±2.04 <sup>E</sup>

Biochemical effect of plant growth promoters on mineral, immunity and fertility

Table 7. Serum B. urea and creatinine in control and injected groups of male and female rats.

	Sex	Duration periods (weeks)	Control	Gibberellic acid	Groups Auxins	Ethephon	Mixture	Average data of sex	
B. urea (mg/dl)	Male	Two	15.22±0.18	29.72±0.24	29.60±0.27	28.43±0.12	29.57±0.20	31.64±0.92 <sup>b</sup>	
		Four	21.63±0.22	31.91±0.46	33.28±0.54	35.39±0.25	33.21±0.21		
		Six	23.28±0.13	34.13±0.24	37.65±0.59	44.77±0.38	46.81±0.42		
	Female	Two	14.98±0.36	29.08±0.10	28.66±0.16	29.95±0.20	29.06±0.43		30.50±0.80 <sup>a</sup>
		Four	22.62±0.48	27.98±0.28	31.50±0.32	36.24±0.16	32.72±0.28		
		Six	24.30±0.16	31.41±0.27	34.33±0.32	39.51±0.48	45.19±0.38		
	Average data of groups		20.34±0.71 <sup>A</sup>	30.71±0.39 <sup>B</sup>	32.50±0.58 <sup>C</sup>	35.72±1.03 <sup>D</sup>	36.09±1.34 <sup>E</sup>		
Creatinine (mg/dl)	Male	Two	0.53±0.02	1.02±0.03	0.97±0.00	0.95±0.00	1.27±0.02	1.13±0.03 <sup>a</sup>	
		Four	0.74±0.01	1.08±0.01	1.19±0.02	1.24±0.02	1.32±0.02		
		Six	0.78±0.00	1.22±0.02	1.55±0.04	1.48±0.04	1.53±0.03		
	Female	Two	0.50±0.01	1.03±0.03	0.95±0.00	1.00±0.01	1.19±0.01		1.13±0.04 <sup>a</sup>
		Four	0.75±0.02	0.95±0.01	1.31±0.02	1.17±0.03	1.41±0.01		
		Six	0.81±0.00	1.14±0.02	1.66±0.04	1.43±0.03	1.65±0.03		
	Average data of groups		0.69±0.02 <sup>A</sup>	1.07±0.02 <sup>B</sup>	1.27±0.05 <sup>D</sup>	1.21±0.04 <sup>C</sup>	1.40±0.03 <sup>E</sup>		

Table 8. Serum sodium (Na) level and serum potassium (K) level in control and injected groups of male and female rats.

	Sex	Duration periods (weeks)	Control	Gibberellic acid	Groups Auxins	Ethephon	Mixture	Average data of sex	
Na level (mEq/L)	Male	Two	147.0±0.71	137.8±0.86	145.6±0.51	139.4±0.51	145.6±0.51	145.93±0.56 <sup>b</sup>	
		Four	148.2±0.97	148.0±0.71	142.2±0.37	143.4±0.51	147.6±0.51		
		Six	147.6±0.51	155.6±0.87	139.4±0.40	150.4±0.51	151.2±0.37		
	Female	Two	146.4±0.51	148.0±0.71	148.0±0.45	144.2±0.37	146.6±0.51		
		Four	147.2±0.58	141.6±0.68	150.4±0.51	137.0±0.55	151.6±0.51		145.17±0.77 <sup>a</sup>
		Six	146.8±0.8	134.6±0.51	152.2±0.58	130.0±0.45	153.0±2.02		
	Average data of groups			147.20±0.28 <sup>D</sup>	144.27±1.34 <sup>B</sup>	146.30±0.85 <sup>C</sup>	140.73±1.20 <sup>A</sup>	149.27±0.62 <sup>E</sup>	
K level (mEq/L)	Male	Two	10.22±0.1	9.35±0.12	10.37±0.18	7.02±0.02	9.08±0.06	9.18±0.15 <sup>b</sup>	
		Four	9.96±0.09	8.14±0.08	10.22±0.10	7.81±0.10	9.72±0.16		
		Six	10.16±0.09	6.36±0.22	10.54±0.22	8.26±0.19	10.42±0.27		
	Female	Two	8.07±0.04	9.60±0.13	10.71±0.13	5.40±0.12	6.18±0.11		
		Four	8.09±0.05	8.62±0.17	11.69±0.16	5.99±0.04	7.87±0.10		8.45±0.24 <sup>a</sup>
		Six	8.13±0.04	7.73±0.13	12.48±0.17	6.46±0.15	9.76±0.17		
	Average data of groups			9.11±0.19 <sup>D</sup>	8.30±0.21 <sup>B</sup>	11.00±0.16 <sup>E</sup>	6.83±0.19 <sup>A</sup>	8.84±0.27 <sup>C</sup>	

found alternation in sodium and potassium level in male and female rats in injected groups with 3 types of plant promoters not only gibberelline. Gibberellic acid can have testosterone biological effects on male chicks, whereas, gibberellic acid treatments induced effects on chick comb and testes' weights that was similar to testosterone effects, as both reduced testes weight significantly. Also, GA was capable of inducing testosterone secretion in male chicks [6]. In agreement with Ozok and Celik [23]. Synthetic Auxines like  $\beta$ -NOA and 4-CPA caused a significant alteration in the activities and levels of serum parameters selected as TPRO, TALB, TCHOL, TBIL, AST, ALT and LDH biomarkers for liver damage. Namely,  $\beta$ -NOA and 4-CPA caused a significant fluctuate the level serum biomarker for hepatotoxicity with both periods and dosages. Also demonstrated that the rats treated with both doses of  $\beta$ -NOA and 4-CPA could have affected the antioxidant markers in erythrocyte, liver, brain, kidney and heart tissues differ from that of control rats. PGRs and 4-CPA may also have detrimental effects on reproductive organs and endocrine system. It has been reported that prepubertal exposure to 4-CPA may have a dose dependent apoptosis in reproductive organs for both male and female rats. They determined change in the biochemical hormone levels of female rats, but dose dependent cell death in the ovarian tissue, epithelium and stroma of the uterus was observed that suggests the cause of apoptosis is oxidative stress [34]. In agreement with Anant and Avinash [2]. The acute toxicity of ethephon ( $C_2H_6ClO_3P$ ) was evaluated in blood serum biochemical in European rabbit. The data obtained showed that the depletion in protein, cholesterol, creatinine. Many agricultural pesticides – Including some previously untested and commonly found in food – disrupt male hormones, according to new tests conducted by British scientists. to check if they block

testosterone and other androgens, the hormones critical to a healthy reproductive system for men and boys [17]. In agreement with Main. et al [9]. investigate whether occupational pesticide exposure during pregnancy causes adverse effects on the reproductive development in the male infants Boys of pesticide - exposed mothers showed decreased penile length, testicular volume, serum concentrations of testosterone, and inhibin B. Serum concentrations of sex hormone-binding globulin, follicle-stimulating hormone, and the luteinizing hormone: testosterone ratio were increased compared with boys of non-exposed mothers. In present study according to data results we found that decrease in immunoglobulin Igs , erythrocyte antioxidant SOD, GSH, G6PD, and increase allergic factors such as serum total IgE, Histamine and Cortisone also increase B.urea and creatinine with variables values of minerals sodium , potassium , total protein , albumin and cholesterol level. We also study biochemical effect of these promoters on fertility and we can noted that through data results decrease in testosterone level in male rats but increase in female rats in all injected groups with decrease in LH and FSH hormones in both sex with variable in progesterone level in both male and female rrats in injected groups with 3 types of plant promoters compaired to control group.

### *Conclusion*

These results suggest that plant growth promoters such as in gibberellic acid, Auxins and Ethephone having the potential to modulate the metabolism of rats like other PGRs, pesticides and xenobiotics by altering different enzymes, and biomarkers, which is being evidenced by this study.

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## التأثير الكيميائي الحيوي لمنشطات نمو النباتات على الاملاح المعدنية، المناعة والخصوبة

حسين عبد المقصود على، علي حافظ الفار، أسماء السيد حسن السيد

قسم الكيمياء الحيوية - كلية الطب البيطري - جامعة بنها

### الملخص العربي

تم اجراء هذا البحث لدراسة التأثير الكيميائي الحيوي للمنشطات النباتية وعلاقتها بنسبة الصوديوم، البوتاسيوم، البروتينات المناعية، الهرمونات الجنسية بالإضافة الي بعض وظائف الكلي والكبد، مضادات الأكسدة وبعض عوامل الحساسية. وقد استخدم لهذا الغرض 50 فأراً "معمليا" 25 ذكور -25 إناث تتراوح اعمارهم من 5-6 اسابيع وأوزانهم بين 120-190 جرام تم تقسيمهم الي 5 مجموعات كل مجموعة تحتوي علي 10 فئران (5 من الذكور - 5 من الإناث) المجموعة الاولي المجموعة الضابطة تم حقنها بالماء المقطر وال4 مجموعات الباقية تم حقنهم بـ 100مجم /كجم من وزن الجسم بالـ (الجبريلين أو الاوكسين أو الاثيون أو خليط منهم) تم جمع عينات الدم بعد 2-4-6 اسابيع من الحقن وتم قياس نسبة الصوديوم والبوتاسيوم و IgA, IgG, IgM. البروتين والألبومين والبولينا والكرياتين والكوليسترول والهستامين والكورتيزون ، T. IgE، والتستوستيرون والبروجستيرون FSH, LH, أكسيد النيتريك جميعهم تم تعينهم في السيرم. بالإضافة الي ذلك تم قياس بعض مضادات الاكسدة كأنزيم سوبر أكسيد ديسميوتيز، جلوكوز 6 فوسفات ديهيدروجيناز، جلوتاثيون في كرات الدم الحمراء. وقد أظهرت النتائج نقصاً "معنوياً" في نشاط مستوي البروتينات المناعية، السوبر أكسيد ديسميوتيز، الجلوتاثيون، جلوكوز 6 فوسفات ديهيدروجيناز، الكوليسترول بينما ازداد نشاط كل من الكورتيزول، الهستامين، T. IgE أكسيد النيتريك، مع ارتفاع طفيف في البولينا والكرياتين مصاحباً له تغير في نسبة الصوديوم والبوتاسيوم بالإضافة الي البروتين والألبومين وقد أظهرت النتائج أيضاً "اختلال في نسبة الهرمونات بين الذكور والإناث بالمقارنة بالمجموعة الضابطة ومن هذه النتائج يمكن استخلاص أن استخدام المنشطات النباتية بدون رقابة قد يؤدي الي اختلال الجهاز المناعي وعدم التوازن في نسبة الاملاح المعدنية مما قد يؤثر علي وظائف الكلي والكبد وبدوره قد يكون له تأثير علي الخصوبة والإنجاب.

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