ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Toxicological Study of Gbberellic Acid on Liver , Kidney And Brain And Its Apostasy In Adult Abino Rats

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ABSTRACT

The aim of this study investigate the toxicity effect of (GA3) on liver & kideny function, activity of acetylcholine and antioxidant at different doses as well as the possibility of recovery after apostasy GA3 Twenty five adult male albino Rats were .equally divided into five groups. Group 1 served as control. Groups 2&3 received GA3 daily in drinking water in two gradually increasing doses of 100 and 200 ppm, respectively for 8 weeks. Groups 4 &5 received the same treatment of GA3 as the second and third groups then were left without any treatment for another 8 weeks Groups (2& 3) were receivedGA3 in both doses ,showed ahighly significant increase in Alanine aminotransferase (ALT),Aspatate aminotransferase (AST), Alkaline phosphatase (ALP) and Gamma-Glutamyl transferase(GGT) Also, elevation of serum in urea ,creatinine ,uric acid and α fetoprotein increase in MDA but decrease in antioxidant enzyme(glutathione GSH & Superoxide dismutase SOD) and decrease in activity of acetylcholine but the result showed improvement after recovery for another eight weeks specific the dose of 100ppm. the previously mentioned results we can conclude that GA3 inhas adose depedent toxic effect on liver kidney and Acetylcholine activity also, oxidative stress following of another eight weeks without drinking GA3in water the result showed Ameliorative in resuts.

Keywords: Gibberellic acid , Rats, liver , acetylcholine, Kidney.

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ISSN: 0975-8585

INTRODUCTION

Plant growth regulators (PGRs) were commonly used to increase seeds production[1,2] People may be exposed to residues of GA3 in diet derived from consumption of different types in fruits and vegetables treated with GA3 exposure to residues may also be through drinking water [3,4]

Recent reports indicate that this PGR may induce oxidative stress, leading to the generation of free radicals and causing cells damage in many organs, including the heart, kidney, stomach and spleen of adult rats [5] and the liver of GA3 treated suckling rats[6,7]

stated that Abcisic acid (ABA) and GA3 caused[7] a significant decrease in serum LDH and CPK activity with both periods (subacute & subchronic) Also, GA3 significantly decreased serum AST activity with subacute and decreased serum ALT and GGT treated with subchronic periods. The lipid peroxidation end product MDA signifcantly increased in the erythrocyte, liver, brain, and muscle of rats treated with both the period of GA3

After GA3 stoppage, eight weeks period of follow up showed incomplete recovery of these toxic effects. So, gibberellic acid should be used cautionary[8]

Reported[9] that GA3 induced increases in the lipid peroxidation and antioxidant defense- systems in various tissues in rats following 25 days oral administration of GA3.

Gibberellic acid (GA3) is highly persistent and bioactive in soil for months. The Environmental Protection Agency has determined its use to be only allowed in low doses[10]

Gibberellic, acid(GA3) aplant growth regulator, was largely used in agriculture of many countries including Tunisia[6] The study showed that GA3 affected the structure and function of the rat liver[11].Their work was conducted to study the histopathological and histochemical effects of gibberellic acid (GA3) on the liver of albino rats.

The effects of Indole acetic acid(IAA) and kinetin (Kn) were also investigated on human serum enzymes in vitro. IAA was found to inhibit aspartate aminotransferase (AST) and activate . Kn inhibited muscle creatine kinase (CK-MB), while it activated AST and alanin aminotransferase [12](ALT)

That[13] GA3 caused a significant increase in total lipids. A significant increase in serum AST, ALT, urea and creatinine, while, a significant decrease in total protein content in serum was observed in rats given GA3

The extensive use of plant growth hormones as (GA3) in agriculture, make it as an interesting subject to detect its possible harmful effects on the kidney as one of the main target organs for many of different toxins [14]

If gibberellic acid or one of its metabolites is applied to dwarf varieties of peas, broad beans or maize, growth is greatly accelerated[15]

The aim of this study was to exposure on neuro, kidney and liver toxicity from effect of GA3 and also, to determine the effects of GA3 apostasy on the affected biochemical Parameters following eight weeks follow up.

MATERIALS AND METHODS

Chemical

Gibaifar (5% Gibberellic acid) supplied by AIFAR AGROCHIMICA SRL Via Bazzano, 12 6019 Ronco Scrivia (Genoa) Italy. www.aifar.it

Preparation of GA3:

2ml and 4ml of 5 % GA3 (equivalent to 100 mg and 200 mg of GA3, respectively) each other were diluted with tap water until 1000 ml to obtain 100 and 200 ppm of GA3, respectively according to[16,17]

Experimental animals

Adult male albino rats weighing 170-200 g were obtained from animal house in Medical Research center (MRC), Faculty of Medicine, Ain Shams University.

The animals were housed in cages and fed ad libitum with a standard diet and provided with free access to water, being kept under suitable laboratory conditions during the whole period for experimentation. Twenty five Rats were divided into five groups (5 rats each) and treated as follows:

Goup1(control) : Animals of the first group served as control and were received tap water and basic diet
Group2(100ppm) :(GA3 group) the animal received 100ppm of GA3 that 2ml of GA3 completed to 1000 ml of tap water)[16] to be added to drinking water of these animals for eight weeks
Group3(200ppm) : (GA3 group) the animal received 200ppm of GA3 , 4ml of GA3 Completed to 1000 ml of tap water were drinking water of rats for eight weeks [17]
Group4(recovery 100ppm) : (Recovery low dose group) Animals of this group received the same treatment as group2 for eight weeks then, they were left without any treatment for another eight weeks.
Group5(recovery 100ppm) : (Recovery high dose group) Animals of this group received the same treatment as group3 for 8weeks then, they were left without any treatment for another eight weeks.

At the end of experiment the animals rats, were sacrified and blood was collected by carotid bleeding in centrifuge tubes and then centrifuged at 3000 rpm for 15 minutes to separated serum and stored at -20C for biochemical analysis and medulla oblongata were quickly harvested ,then hemogenate

Determination of GA3 Concentration in brain (medulla oblongata) : A residue of GA3 was estimated in brain tissues by thin layer chromatography according to method described by Official Methods of Analysis [18]

Biochemical analysis:

Serum AST and ALT were measured colorimetrically according to [19] **and** Determination of ALP and GGT by method of [20] , [21] respectively. Serum urea, creatinine , and uric acid , α fetoprotein were measured according to [22] , [23 24,25] respectively ,

Brain tissue analysis:

Brain homogenates to analysis ,glutathione(GSH) was assayed spectrophotomertricall by the method of[26] .The activity of brain SOD was determined by assessing the inhibition of pyrogallol auto oxidation[27] Malondialdhyde (MDA) was determined in brain according to method of[28] **.** Brain Acetylcholine activity was measured according to[29]

Statistical analysis:

All data were analyzed using the SPSS for windows version12.0 [30] Analysis of variance (one- way ANOVA) was peformed to test for any significant differences among groups and independent sample t-test was used to calculate statistical significant between the control group and each treated group .The level of significance was set as $P< 0.05$ for all statistical tests[31]

ETHICAL CONSIDERATIONS

This study has approved protocol from the ethical point of view and according to Animal welfare Act Ain Shams University.

ISSN: 0975-8585

RESULTS

Biochemical results:

The our results in rats were showed The higher concentration of GA3 in brain of rats treated with the high dose of GA3(200 ppm) than those in rats treated with the low dose of GA3(100 ppm) . In the recovery groups (G4 & G5) following eight weeks of GA3 withdrawal and with were reduced in GA3 content in brain of both groups mainly G4 in (Table I)

Table (I)): Concentration of of GA3 in Brain

Groups	Concentration of GA3 (Ug /g)
G1 Control	Nile
G2(100 ppm)	17.24 ± 1.80
G3 (200ppm)	54.24 ± 2.55
G4 (recovery) 100 ppm	7.64 ±o.64
G5 (recovery) 200 ppm	16.00 ± 1.10

Mean ± S.E (n= 5 in each group)

The present data in (**TableII)** showed a highly significant increase ($p < o.oo1$) in the G2&G3 that treatment with GA3 in AST ,ALT , ALP and GGT but by the end of the follow up the period (eight weeks) the results in recovery groups were showed little improvement of the mean values of AST,A LT,ALP and GGT compared to control group

Table (II) The Effect of Gibberellic, acid on Serum Liver Function of Male Albino Rats

Groups	AST U/ml	ALT U/ml	Alp U/ml	GGT U/ml
G1 Control	16 .3± 0.64	11.54± 0.7o	88.6 ±1.63	9.200±0.390
G2(100 ppm)	25.34 ± 1.74^{a**}	15.04± 0.34^{a*}	106.48±2.63^{a**}	27.84±2.30^{b***}
G3 (200ppm)	33.18± 1.73^{a***}	19.76 ±0.74^{b**}	123.7 ± 3.86^{a***}	42.40±1.27^{ac***}
G4 (recovery) 100 ppm	23.020 ±1.1^{b**}	22.6 ±1.10^{b***}	101.74±1.014^{a**}	28.80 ±2.23^{ac**}
G5 (recovery) 200 ppm	12.94±0.83^{c***}	16.06±0.47^{ab**}	103.12±1.99^{ab**}	11.82± 0.56^{ab**}

Mean ± S.E (n= 5 in each group) * Significant change at p< 0.05. ** A highly significant change at p < 0.01
*** Very highly significant change at p< 0.001

The data in **(TableIII**) concerned with changes of serum urea ,creatinine, uric acid and α feto-protein since showed, a highly significant increases (p<o.oo1) in the mean value of rats treated with GA3 (G2) in drinking water . After, eight weeks period of recovery the results showed a significant improvement in kidney function and α feto-protein in (G5)Recovery Compared with control and (G4)Recovery

ISSN: 0975-8585

Table (III):The Effect of Gibberellic, acid on Serum kidney Function and α feto-Protein of Male Albino Rats.

Groups	Urea mg/dl	Creatininm g/dl	Uric acid mg/ml	α feto-protein U/ml
G1 Control	22.34±1.67	0.84±0.02	5.24±0.30	7.38± 0.35
G2(100 ppm)	42.18±1.02^{a***}	1.50±0.054^{a***}	7.54± 0.20^{a**}	11.02±0.54^{a**}
G3 (200ppm)	89.78 ±3.17^{a***}	2.20±0.11^{c***}	9.10± 0.45^{b***}	20.94±0.35^{b***}
G4 (recovery) 100 ppm	39.98 ±3.17^{ab**}	1.38±0.04^{b**}	5.54±0.15^{c*}	9.82± 0.22^{ab**}
G5 (recovery) 200 ppm	28.14±1.57^{ab*}	1.27±0.22^{a**}	5.74±0.17^{ab*}	8.32 ±0.26^{c*}

Mean ± S.E (n= 5 in each group)

The data in (**Table IV**) showed a significantly decreased. In brain tissue **GSH** and **SOD** in group 2 and group 3 were treated with GA3 in drinking water while , the result showed a significantly increased in and (**MDA**) Also,a highly significant increased (p<o.oo1) were showed in **Acetylcholine Activity. On the other hand** the recovery groups (G4 & G5) were showed partial amelioration but these values still higher ((p<o.oo1) than those in control group.

Table (IV): The Effect of Gibberellic, acid ON Oxidative Stress(GSH, SOD ,MDA) and Acetylcholine Activity (tissue brain) of Male Albino rats

Groups	GSH U/g wet tissue	SOD U/g wet tissue	MDA tissue U/g wet	Acetylcholine μ M/min /mg pro.
G1 Control	3.88±0.45	10.22±0.26	0.28± 0.03	33.00±1.98
G2(100 ppm)	1.74±0.21^{a**}	17.56±0.66^{a**}	5.86± 0.19^{a***}	11.90±0.73^{ab***}
G3 (200ppm)	0.86± 0.02^{a***}	30.1±1.26^{b***}	10.58±0.25^{ab***}	4.00±0.20^{c***}
G4 (recovery) 100 ppm	1.36±0.05^{ab**}	12.54±0.29^{c**}	4.08±0.07^{c***}	23.38±0.96^{c**}
G5 (recovery) 200 ppm	2.42±0.18^{ac*}	11.24±0.34^{ab*}	2.02±0.11^{b**}	30.72±0.6^{b*}

Mean ± S.E (n= 5 in each group) **Acetylcholine activity**(micro mol/ min /mg protein) **brain**

DISCUSSION

The organ dysfunction have been recently ascribed as one of the causes contributing to various physiological changes induced by plant growth regulators (PGRs) as Gibberellic acid (GA3).

In the present study ,the concentration of GA3 in brain was higher in content (G2 &G3) in (**Table I**) The extraction of GA3 from brain(medulla oblongata) contents was carried out according to[32] .Although GA3 is extensively used in Egypt and other countries, litter is know about its toxic effects on human health [11 ,33]

The present study revealed that change in groups were treated with Low dose induced ahighly significant in ,AST ,ALT , ALP and GGT in (**Table II)** also,a highly significant increase in Urea ,Creatinine , uric acid and , α-fetoprotein (**Table III**) . the results were showed a highly significant decrease in (**Table IV**) (Antioxidant enzyme)SOD, GSH but significant increase in , MDA and Acetylcholine activity in brain,

The animal were taken a high dose G2 (200ppm) of GA3 were induced a very highly significant increase in liver enzyme compared with recovery and control group these result agreement to [11] which were considered hepatocelluar damage measure in evaluating.Also the group was treated with 100ppm of GA3 for eight week ,showed significant increase in urea and creatinine , uric acid and , α- Fetoprotein

Countries little is know about its toxic effect on human heath [11,33] .The present study revealed liver function biomarkers AST ,ALT ,GGT ,and ATP and ,kidney function as urea, creatinine, also, acetylcholine activity ,antioxidant enzyme(SOD ,GSH and MDA level in brain , changes in brain specimens of the adult male albino rats. GA3 treatment with low dose 100 ppm for 8weeks induced ,increase in liver function as ALT,AST ,GGT&ALP but the high dose (200ppm) induced a highly significant increase liver function compared with control group and recovery group these results agreement with to [11] which these were considered to be sensitive measure in evaluating hepatocellular damage .Low dose(100ppm) of GA3 treatment for eight weeks induced a significant increase in urea ,creatinine but take ahigh dose ofGA3 for eight weeks show a highly significant increase in urea and creatinine.In the present study the disturbance
in LFTs

GA3 could exert toxic effect on many soft organs including the liver(Tuluc&Celik,2006) .It is well known that the liver is the first organ in toxicological prospected regarding its role in detoxification ,biotransformation and excretion of xenobiotics[11]

The present data were in line with [6] stated that , liver was damaged by GA3 as demonstration by an increase of AST and ALT level in plasma these biomarkers indicated cellular leakage and loss function integrity of cell membrane in live[34] . Also, they stated that other plant hormones in ***vitro*** using kinetin.Other biomarkers of liver toxicity were also studied ALP & GGT synthesized by liver was increased in serum this agreement with the present work that may be due to explaining inflammatory reaction particularly in dams[35]
.
Other factors include kidney dysfunction which an increased protein catabolism in the mammalian body or more efficient conversion of ammonia to urea as a result of increased synthesis of enzyme involved in urea production) Urea is the end –product of the protein catabolism and this is confirmed by decrease in plasma proteins and referred to kidney dysfunction as proven by enlargement of the relative weight of kidney as suggested by[36]

Stated that[37,38] the brain of the mammalian is very sensitive to oxidative damage due, in part, to its high oxygen requirement and abundance of oxidizable substrates such as polyunsaturated fatty acids and catecholamines were accompanied with disruption of the brain,hepatic ,antioxidant enzymes activities , also accumulation of MDA these indicating that GA3 induced oxidative stress as, lipid peroxidation in treated animal brain.The results were showed significant decrease in SOD and GSH activities but significant increase in MDA level in brain tissue of the rats were taken GA3in drinking water comparison to the control group. These results agreement with the current study[39] reported that GA3 can accelerate lipid peroxidation.

The result of recovery groups were showed statistically significant increase in GSH and SOD activity comparison to low dose of GA3 treated group but the result show decrease in MDA level and increase in acetyl choline activity comparison to the control group plausible to speculate from their results that GA3 received might cause the peroxidation of polyunsaturated fatty acids, leading to the degeneration of phospholipids and cellular deterioration [17]The extracellular concentration of acetylcholine (ACh), in the mammalian brain is typically very low due to its rapid hydrolysis by acetylcholinesterase (AChE)[39]

Moreover, several studies have reported that the central nervous system is the principal target organ for PGRs ,It is especially susceptible to oxidative damage due to its high oxygen consumption rate, high levels of polyunsaturated fatty acids and to its relatively low levels of defense mechanisms against oxidant toxicity[40,41]

Also, GA3 were showed a significantly decreased serum AST, CPK, and LDH activity with subacute periods and also, decreased serum ALT, CPK, LDH, and GGT treated with subchronic periods[42]

They[43,39] found ,this compound as (GA3)can accelerate lipid peroxidation up to 65-fold, and this is attributed to the formation of peroxyl radicals that may react with the lipids

CONCLUSION

From the previously mentioned results we can conclude gibberellic acid (GA3) has potent pro-oxidant and a dose-dependent toxic effect on the kidney, liver function and acetylcholine of adult male albino rats. On the other hand, eight weeks period of follow up and its apostasy was insufficient for complete recovery of these toxic effects special (high dose).

ACKNOWLEDGEMENT

The author is thankful to staff of Medical Research center (MRC), Faculty of Medicine, Ain Shams University for their support and kind help

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