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Short term effect of 28-homobrassinolide on serum, liver and kidney marker enzymes and other biochemical parameters of male albino rats

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Abstract

Brassinosterols (BS) are ubiquitous pluripotent growth regulator present in plants. They exist in isoforms of epi and homobrassinolides (HB). BS act as potent stimulators of root and shoot elongation, cell division, DNA and RNA polymerase activity, ethylene production and of stress tolerance to temperature, water scarcity and salinity in plants. It is also used to increase the yield of crop and to protect the plants against pesticides. Consumption of plant material as diet and used as growth regulator in animals, and application of BS in agriculture would increases its availability to the host tissues. In the present study, the effect of 28-HB, an isomer of brassinosterol on serum, liver and kidney marker enzymes, lipid peroxidation, tissue histology and the blood parameters of rat were investigated. The rats were given the compound by intradermal mode at the concentration of 75µg, 150µg and 300µgas single dose and the effects were observed after 4 hr to study the immediate response of the animal.

The treatment of rats with 28-HB, caused different effects on the serum, liver and kidney parameters of this study.

In conclusion, the present study showed that 28-HB affects the structure and function of rat tissues in a dose dependent manner.

Keywords: Brassinosterols; pluripotent; plant growth regulators

Introduction

The use of plant growth regulators (PGRs) in agriculture for raising plant food production employing a wide variety of crops is increasing at a steady rate. The amount of these substances deposited into the environment may soon exceed those of insecticides (1). Hence, the probability of assimilating PGRs from environment, plant derived foods and traditional medicine into animal tissues including human is significant. Although many studies have been conducted on the effect of PGRs on animal cell metabolism, there is no study on the effect of 28-HB on animal cell metabolism. The present study therefore, aims to investigate the immediate effects of 28-HB at different doses on male albino rats by estimating the activities of selected metabolic marker enzymes like Aspartate transaminase(AST), Alanine transaminase(ALT), Acid phosphatase (ACP), Alkaline phosphatase(ALP), Gamma glutamyl transpeptidase(GGT) amylase(AMY), Hexokinase (HK) and certain biochemical parameters and histological changes in liver, kidney and serum of male rats.

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28-Homobrassinolide (28-HB) is a naturally occurring polyhydroxy steroid lactone growth regulator with pleiotropic effects in plants (2). It is an ubiquitous hormone in the plant kingdom and plays an important role in cellular processes such as anti stress activity, seed germination, flowering, senescence, abscission, maturation in plants and for protection of plants against environmental stress (3). The studies of Guarra (4), Visscher (5), Visscher (6), De man et al. (7) and Alonso (8) reported that the fecundity, longevity and egg viability of insects were changed by PGR treatment. Olson et al. (9) reported that the PGRs caused an increase in the number of splenic plaque forming cells, circulating WBCs, hematocrit values, and thymus weight in young deer mice.

Celik.I et al. (10) reported that some PGR affects the oxidative defense system and MDA content in rats. Lipid peroxidation is one of the molecular mechanisms of PGRs induced toxicity (11). Oxidative stress produces DNA damage, enzyme inactivation and lipid peroxidation of cell constituents, especially when antioxidant defenses were impaired or overcome (12). John et al (13) reported that the gibberellic acid (GA_3) induce liver neoplasm in Egyptian toads. Sexual differentiation and physiological parameters of mice was affected by GA_3 and Abscissic acid (14). Alteration of hematological and biochemical parameters of rats treated with indole acetic acid and kinetin was also reported by Celik et al (15). Indole acetic acid (IAA) was found to inhibit AST and activate amylase in rats, whereas it activated AST and ALT in human blood serum (16). The levels of marker enzymes in tissues and biological fluids may get altered following administration of foreign agents and that such alterations could be used to assess the metabolic assault induced in the tissue of rat. It has been shown**,** that hexokinase gene expression is a realistic possibility employing 28-HB as the effector molecule during in vivo studies in rats (17).

Though there are several research reports available on the effect of PGR on animal cell metabolism, the study on the immediate effects of 28-HB on animal cell function is absent. Therefore, this study is of important and unique in view of mode of entry, duration and organs structure and toxicity relationships on selected parameters. Acute and short term toxicity study and toxicological evaluation using experimental animals should be done in all preparations marketed for medicinal use (18).

Materials And Methods

Materials

28-Homobrassinolide was a gift from Dr.Vyas Godrej Agrovet Chemicals Ltd., Mumbai, India. All chemicals used for this study were of analytical grade purchased from manufacturers in India. Glass distilled water was used for the preparations of all reagents.

Animals

Male albino wistar strain rat in the weight range 160-180g (10-12 weeks old) were obtained from JIPMER, Puducherry, India. The rats were housed at 20±2°C in a daily light/dark cycle. All rats were fed Kamadhenu (India) rat feed pellet (composition analysed and established) and were given water ad libitum. The care of rats was as per the `Guide for the care and use of Laboratory Animals' during experiments.

Treatment of rats

The rats were housed in 4 groups of 6 each. A control group was given 50µl of 50% absolute alcohol subcutaneously to serve as control. The experimental group of rats was given 28-HB in 50µl of alcohol subcutaneously in a single dose at the dose levels of 75µg, 150µg and 300µg/kg body weight. 4 hours following the treatment, the rats were anaesthetized with anesthetics ether. Blood samples were collected by cardiac puncture. Blood samples were allowed to clot

and were centrifuged for the preparation of serum used for biochemical analysis. Selected tissues of the rat were surgically removed and immediately rinsed in ice cold physiological saline. The tissues were initially cut into small pieces and taken for homogenization employing several stroke in a potter-Elvehjem homogenizer using a Teflon pestle to obtain 10% W/V tissue homogenate in 1.15% KCl solution. Throughout the homogenization process, the tissue homogenates were maintained on crushed ice and then centrifuged at 10,000xg for 10 minutes in a refrigerated Hitachi high speed centrifuge at 4°C. Supernatant and serum obtained were used for biochemical analysis.

Measurement of enzyme activities

AST and ALT was assayed by the method of Reitman and Frankel (19). ACP was assayed by the method of Tennis wood et al (20). ALP was assayed by the method of Bessey et al (21). GGT activity was measured by Orlowski and Meister (22). Amylase activity was assayed by the method of Bernfeld (23) and the Hexokinase activity was measured by the standard method (24).

Biochemical analysis

Glucose content was measured by the modified procedure of Asatoor and King method (25). Cholesterol content was measured by the modified method of Zak et al (26). Triglyceride content was measured by the method of Gruddy and Benjamin (27). The serum quantitative colorimetric determination of LDL, HDL and VLDL level was done using EnzyChromTM HDL and LDL/VLDL Assay Kit (EHDL-100). Protein content was measured by the method of Lowry et al. (28). The quantity of albumin was measured by the method of Reinhold (29). Urea content was measured by the method of Netelson et al. (30). The content of uric acid was measured by the method of Caraway (31). The end product of lipid peroxidation, thiobarbutric acid reactive substance (TBARS) as MDA, was measured by the method of Nichens and Samuelson (32).

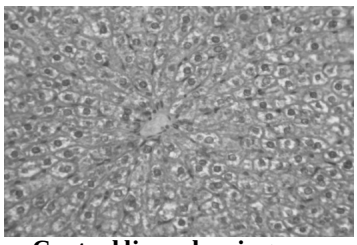
Analysis of data

The data was expressed as mean ± Standard Error Mean (SEM) for six rats. SPSS program was used for statistical analysis. The difference between the control and treated values was found using one way ANOVA and the significance was noted at $p<0.05$* and $p<0.01$** levels for all the tests.

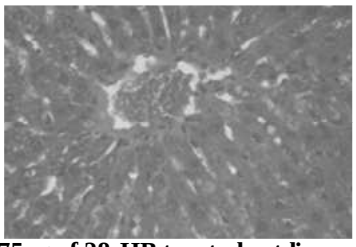
Results

The results of the present study showed that the treatment of rat with 28-HB caused changes in the specific activities of metabolic marker enzymes (Table-1), concentration of biomarkers (Table-2) in serum, liver and kidney and histological architecture (Figure1-8) in dose dependent manner. A 19% decrease and 12% increase in serum AST activity was observed, respectively at 150 and 300µg of treatment without any significant change in liver and kidney. An increment in the activity of ALT was noted in serum at 75µg 28-HB treatment alone. On the other hand, the activities of ACP, ALP, GGT, amylase and hexokinase were significantly increased in liver at 150 and 300µg doses after 4 hrs of treatment. There was a significant decrease in ALP activity was noted in serum for all the doses of 28-HB treatment against the corresponding control rats for the same period. In contrast, the activities of amylase and hexokinase were significantly increased in serum, liver and kidney irrespective of the dose.

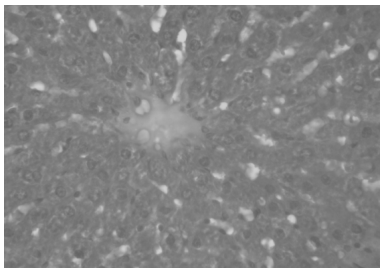
Analysis of serum parameters showed a significant reduction (37-41%) in serum glucose level for all the doses of 28-HB. The rat liver tissue cholesterol content was reduced quantitatively by 28-HB (20-27%). In contrast, the serum and kidney cholesterol content was increased (14%) for all the dose of 28-HB.



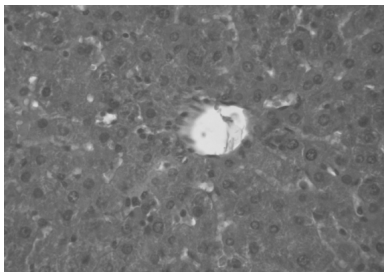
Control liver showing normal architecture



75µg of 28-HB treated rat liver showing congestion of central vein

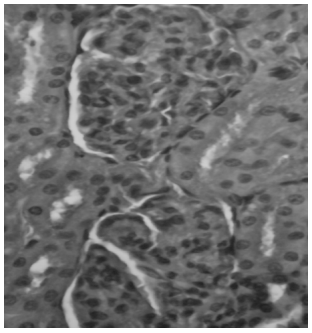


150µg of 28-HB treated rat liver showing congestion of central vein loss of blood cells

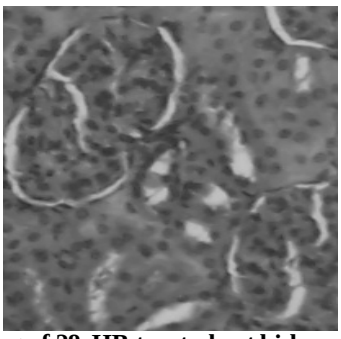


300µg of 28-HB treated rat liver showing congestion of central vein and and loss of blood cells

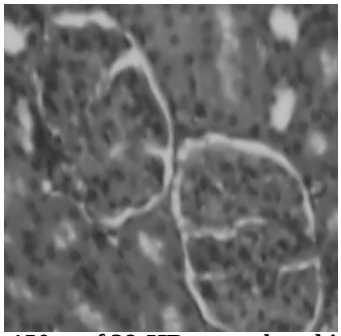
Fig. 1: Section through liver of rat 4 hr following 28-HB administration (H & E x 40).



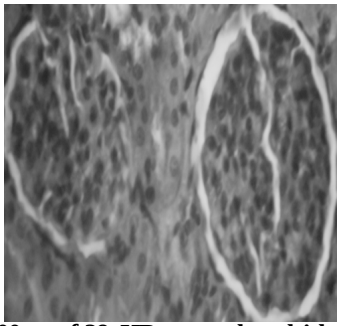
Control kidney showing normal architecture



75µg of 28-HB treated rat kidney showing moderate tubular dilation



150µg of 28-HB treated rat kidney showing moderate tubular dilation



300µg of 28-HB treated rat kidney showing high tubular dilation

Fig 2: Section through kidney of rat 4 hr following 28-HB administration (H & E x 40).

Table 1: Effect of 28-Homobrassinolide on the metabolic marker enzymes of serum, liver and kidney in male rat.

Tissues	Enzymes	Control	28-homobrassinolide (µg/kg body wt.)		
			75µg	150µg	300µg
Serum	**AST**	3.39 ± 0.159	2.75 ± 0.160	3.41 ± 0.121	3.78 ± 0.182*
	ALT	0.94 ± 0.063	0.73 ± 0.022**	0.90 ± 0.043	0.94 ± 0.035
	ALP	6.48 ± 0.380	3.82 ± 0.133**	4.15 ± 0.238**	4.90 ± 0.205**
	GGT	0.101 ± 0.007	0.063 ± 0.004**	0.062 ± 0.003**	0.086 ± 0.003*
	AMY	10.42 ± 0.233	10.48 ± 0.220	11.82 ± 0.409*	12.47 ± 0.515*
Liver	**AST**	4.88 ± 0.217	4.77 ± 0.247	4.97 ± 0.280	4.95 ± 0.270
	ALT	8.16 ± 0.580	8.07 ± 0.680	8.08 ± 0.613	8.10 ± 0.370
	ACP	8.32 ± 0.615	8.28 ± 0.537	9.45 ± 0.399*	10.20 ± 0.453**
	ALP	4.04 ± 0.376	4.42 ± 0.171	4.50 ± 0.254	4.68 ± 0.121*
	GGT	7.44 ± 0.480	7.94 ± 0.550	10.37 ± 0.401**	11.93 ± 0.520**
	AMY	4.29 ± 0.168	4.34 ± 0.177	5.15 ± 0.160*	5.50 ± 0.110*
	HK	1.42 ± 0.082	1.98 ± 0.106**	2.16 ± 0.128**	1.76 ± 0.063**
Kidney	**AST**	2.63 ± 0.092	2.23 ± 0.102*	2.24 ± 0.102*	2.57 ± 0.201
	ALT	3.26 ± 0.220	3.12 ± 0.145	3.00 ± 0.100	3.27 ± 0.108
	ACP	5.61 ± 0.307	4.51 ± 0.204*	4.99 ± 0.200	5.18 ± 0.311
	ALP	33.8 ± 1.450	41.4 ± 2.17**	42.5 ± 1.90**	46.6 ± 2.07**
	GGT	257.0 ± 11.30	228.0 ± 11.4	231.0 ± 7.10	242.0 ± 12.4
	AMY	2.40 ± 0.135	3.81 ± 0.169**	4.09 ± 0.180**	4.19 ± 0.207**
	HK	1.22 ± 0.072	1.62 ± 0.099**	1.92 ± 0.121**	1.67 ± 0.092**

p<0.05*, p<0.01**

Serum enzyme activities were expressed as IU/mg protein x 10^{-3}

Tissue enzyme activities were expressed as IU/mg protein/gm tissue x 10^{-2}

Table 2: Effect of 28-homobrassinolide on serum, liver and kidney biochemical parameters of male rats.

Tissues	Parameters	Control	28-Homobrassinolide (µg/kg body wt)		
			75µg	150µg	300µg
Serum	**Glucose(mg/dl)**	110.0 ± 6.73	65.0 ± 2.11**	69.0 ± 3.18**	70.0 ± 3.61**
	TC(mg/dl)	78.0 ± 4.44	84.0 ± 4.92	86.0 ± 3.46	89.0 ± 4.49*
	TG(mg/dl)	56.0 ± 3.92	58.0 ± 3.64	60.0 ± 5.04	62.0 ± 3.02
	HDL(mg/dl)	17.1 ± 0.69	22.1 ± 1.03**	22.3 ± 1.06**	22.1 ± 1.11**
	LDL(mg/dl)	39.0 ± 1.12	46.0 ± 2.04*	46.0 ± 2.69*	48.0 ± 2.04**
	VLDL(mg/dl)	22.1 ± 1.21	16.2 ± 0.92**	16.3 ± 0.74**	21.2 ± 1.02
	Protein(g/dl)	6.00 ± 0.444	6.20 ± 0.411	6.10 ± 0.553	6.00 ± 0.567
	Albumin(g/dl)	3.60 ± 0.21	4.00 ± 0.139*	3.90 ± 0.29	3.90 ± 0.20
	Urea (mg/dl)	33.0 ± 1.38	30.0 ± 2.83	29.0 ± 1.06*	23.0 ± 1.09**
	Uric acid(mg/dl)	9.70 ± 0.687	9.50 ± 0.516	10.60 ± 0.475	11.20 ± 0.404*
Liver	**TC(mg/gm)**	4.50 ± 0.381	3.30 ± 0.165**	3.40 ± 0.111**	3.60 ± 0.168**
	TG(mg/gm)	1.30 ± 0.173	0.80 ± 0.045**	0.80 ± 0.032**	0.80 ± 0.033**
	Protein(mg/gm)	150.0 ± 9.97	150.0 ± 9.09	150.0 ± 11.1	150.0 ± 7.41
	Albumin(g/gm)	30.0 ± 2.70	40.0 ± 2.27**	30.0 ± 1.29	30.0 ± 2.78
	Urea (mg/gm)	1.95 ± 0.214	1.89 ± 0.074	1.99 ± 0.138	2.60 ± 0.133**
	Uric acid(mg/gm)	1.21 ± 0.057	1.25 ± 0.061	1.25 ± 0.051	1.47 ± 0.058*
	MDA (nmol/mg of protein)	1.45 ± 0.060	1.74 ± 0.071**	1.82 ± 0.070**	1.91 ± 0.040**

Kidney	**TC(mg/gm)**	5.22 ± 0.317	4.50 ± 0.169	5.47 ± 0.365	5.47 ± 0.415
	TG(mg/gm)	1.90 ± 0.145	2.50 ± 0.112**	2.70 ± 0.129**	2.80 ± 0.100**
	Protein(mg/gm)	100.0 ± 7.73	112.0 ± 7.28	110.0 ± 6.62	100.0 ± 8.32
	Albumin(g/gm)	31.0 ± 2.27	52.0 ± 1.90**	51.0 ± 1.86**	36.0 ± 1.84*
	Urea (mg/gm)	5.30 ± 0.441	6.50 ± 0.203**	6.60 ± 0.309**	7.00 ± 0.263**
	Uric acid(mg/gm)	2.29 ± 0.139	2.34 ± 0.099	2.35 ± 0.161	2.50 ± 0.110
	MDA (nmol/mg of protein)	1.52 ± 0.105	2.06 ± 0.086**	2.08 ± 0.073**	2.10 ± 0.076**

$p<0.05$*, $p<0.01$**

The content of triglyceride reduced in liver for all dose (20-27%), but showed elevation in kidney for all dose and in serum for 300µg 28-HB alone. A significant increase in HDL (29%) and LDL (18-23%), and decrease in VLDL (4-27%) was observed in serum in a dose dependent manner. 28-HB does not produced any remarkable changes in protein and albumin content of serum and liver, with significant depletion (65-68%) in kidney albumin content only.

The quantity of urea was significantly decreased (12-30%) in serum and increased (23-32%) in kidney at all dose treatment. While, the uric acid content was significantly improved in serum, liver and kidney at 300µg dose. MDA, the end product of lipid peroxidation was significantly elevated in liver (20-31%) and the kidney (36-38%) in a dose dependent manner. The figure 1-8 shows the histological structure of the liver and kidney of a control and 28-HB treated rat tissue. Examination of liver of rat after 4 hr of treatment with 28-HB, showed a degenerative changes in hepatocytes such as cytoplasmic vacuolation and mild congestion of central and portal veins. The examination of the kidney of rats after the same period of treatment showed moderate tubular dilation and other alterations.

Discussion

The effect of human health of chemicals that are modified through the endocrine system has generated huge interest and investment. It has also been recognized that endocrine active substances are potentially present in food as natural compounds (33). The present study is therefore, directed to understand the short-term effect of a brassinosteroid isoform, 28-homobrassinolide, whose positive/negative effects on higher animals, remains unknown, when it was exposed in higher doses. The data collected in this study relates to experiments carried out for a single time point, but using different doses. Till date no study had been conducted to investigate the short term influence of 28-HB in vivo on rat serum, tissue marker enzyme activities, biomarkers and on their MDA content and their histological changes. Therefore, the present result could not be compared with the previous results. The measurement of the activities of the various enzymes in the animal tissues and body fluids plays a critical role in disease investigation and diagnosis and also for the identification of drug toxicity including that of natural compounds (34).

Treatment of rat with 28-HB induced an up and down regulation in the activities of AST and ALT in serum, liver and kidney in a dose dependent manner. These changes may possibly due to the imbalance in an amino acid metabolism (transamination) by this compound in an animal system. ALP activity also decreased remarkably in serum and increased in liver and kidney. Such an increase in liver and kidney ALP activity may due to the induction of synthesis of this enzyme in liver and kidney by

28-HB. It may also be due to a reduction in concentration or total absence of specific phospholipids required by this membrane bound enzyme to express its full activity in liver (34). A mixed response was noted for the GGT activity showing reduction trend in serum and kidney in a dose dependent manner. It indicates that 28-HB affected the kidney alone by altering its glutamate metabolism and amino acid absorption. Taken together with the changes in the AST and ALT specific activity levels observed in the rat serum and liver and the changes in GGT activity pointed to a possible hepato-biliary disturbance (35) and myocardial infraction like symptoms in the experimental rats. In contrast, the amylase activity was elevated in all tissues for all doses of 28-HB. Although no specific reasons for such changes are clearly understood, it possible that 28-HB exerted effect similar to other pesticides/xenobiotics causing structural damage to membranes and protein leakage in cells. The effect of 28-HB on rat on the other hand is similar to of the effect of analgesics on the sphincter of Oddi, causing spasmodic contraction of this muscular wall resulting in impedance of bile and other juice. Hexokinase activity was also found to increase 28-HB at all dose in liver and kidney in a dose dependent pattern. This may be to increase the rate of glucose metabolism (glycolysis). Serum enzymes are generally derived from tissues through extrusion and damage to cell membranes (36). Increase in the hexokinase enzyme specific activity encountered during the study was also statistically significant, and had an important bearing on the physiology of the animal. Since, hexokinase is primarily an enzyme that phosphorylated circulating free glucose that entered the cells, increased activity of this enzyme induced by 28-HB points to the greater phosphorylation potential that developed in an animal cell causing it to utilize the cellular glucose at greater rates, thereby regulating glucose homeostasis in normal rats. It has been reported that 28-HB was capable of inducing hexokinase mRNA expression in rat tissues when administered orally.

28-HB treatment of rat resulted in a significant reduction in serum glucose. The exact mechanism for such a decrease of glucose is not known clearly. But it may be possibly due to the enhanced glucose metabolism as a result of the 28-HB effect on animal cells. Plant products may act on blood glucose through different mechanisms. Some of them may have insulin-like substances. Decreased blood glucose has been reported following consumption of some plant extracts (37).

Decrease in liver cholesterol content was observed following 28-HB treatment without any significant change in serum and kidney tissues. Cholesterol is seen generally increased in liver diseases and contributed to the disease of arthrosclerosis, when deposited within the arterial endothelium. An increase in cholesterol level is a sign of liver damage. The level of triglycerides in serum and kidney was increased. Serum triglyceride level is a biomarker to asses the risk of cardiovascular diseases in man. Elevated triglycerides levels were indicative of high risk and can be due to obesity as well as drugs such as β-blockers, retinoids, estrogens and cholesteramine. A significant increase in HDL (29%) and LDL (18-23%), and decrease in VLDL (4-27%) was observed in a dose dependent manner without any increment in the HDL/LDL ratio. Cholesterol and LDL are important lipids associated with cardiovascular diseases. Plasma VLDL + LDL- cholesterol represent mobilization of fats from the liver to adipose tissue. LDL carries 60%-70% of the total cholesterol in the serum and also VLDL is the main carrier of triglycerides. High HDL/LDL ratios are associated with a decreased risk of vascular diseases (38).

Treatment of rat with 28-HB increased the content of albumin in kidney. It is therefore probable that the kidney albumin fractions

increased as a result of the plant hormone impact. The content of urea was increased in serum, liver and kidney for 75µg 28-HB. Elevation of urea content in serum, liver and kidney of 28-HB treated rats is considered to be an indicator of haemo, hepato and nephrotoxicity. Altered serum urea level noted in the experimental rat in the present study possibly indicated influence of 28-HB on the tissue urea cycle. Higher blood urea or NH_3 concentration was considered as an indication of urinary obstruction or hepatic dysfunction (39).

Our results also indicate that 28-HB affected MDA content in the liver and kidney tissues of rats. MDA is a major oxidation product of peroxidised fatty acids as an indicator of lipid peroxidation. MDA content was significantly increased in liver and kidney at all of 28-HB. These results were in agreement with the earlier reports from this laboratory. The observed histological changes in the liver and kidney of 28-HB treated rats' shows that this compound is capable of altering the histology of rats in a dose dependent manner. These changes were similar to the earlier reports though the compounds are different. GA_3 has been reported to induce micro abscesses and hydropic degeneration in the liver, while causing nuclear inflammatory infiltration in the kidney of mice and in the liver of rat. Moreover, gibberellin A_3 also has been reported to induce breast and lung adenoma in mice. Feeding of chicken with GA_3 led to numerous histological lesions in different organs including liver, but returned to normal following withdrawal (40). In conclusion, these results suggest that the 28-HB having the potential to modulate the metabolism of rats like other PGRs, pesticides and xenobiotics by altering different enzymes, MDA, and biomarkers and histology which is being evidenced by this study. Judicial use of the natural products is necessary though it is a component of natural origin.

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