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RESEARCH ARTICLE

A comparative study on the effect of homobrassinolide and gibberellic acid on lipid peroxidation and antioxidant status in normal and diabetic rats

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Abstract

Dietary content of phytohormones may potentially influence metabolic processes in animal cells. This study therefore aimed to investigate the effect of two plant growth regulators homobrassinolide (HB) and gibberellic acid (GBA) on the antioxidant defense status and lipid peroxidation level in the tissues of normal and streptozotocin- induced diabetic rats. Normal and diabetic rats (Albino –wistar strain) were administered 50µg HB and GBA intradermally each day for seven days and their tissue and blood levels of malondialdehyde (MDA), 4-hydroxy-2-nonenol (4-HNE), reduced glutathione (GSH) content and catalase (CAT) activity were determined. Subchronic treatment of rats with HB reduced lipid perioxidation and elevated antioxidant defense whereas GBA caused enhancement of lipid peroxidation and reduction of antioxidant defense in treated animals compared to the control rats.

Keywords: *Diabetes mellitus; lipid peroxidation; antioxidant defense status; plant growth regulators; male rats; gibberellic acid; homobrassinolide*

Introduction

Diabetes mellitus is a clinical disorder that exhibits multiple symptoms and causes several complications in humans. Cellular lipids and proteins are subjected to various structural modifications though a variety of disease conditions. Peroxidative changes in lipids are a consequence of structural modifications brought about by oxygen free radicals generated during the course of disease progression. The lipid peroxidation/antioxidant status of animal tissues that are normal and those that are under duress is generally determined by monitoring the endogenous content of malondialdehyde (MDA), 4-hydroxy -2- nonenol (4-HNE), reduced glutathione (GSH) and the activity of catalase enzyme present in a tissue. Oxidative stress induced by hyperglycemia or hyperlipidemia or by the generation of advanced glycated end (AGE) products is considered as an important indicator of diabetes related complications. Generation of oxygen free radicals and reduction in antioxidative defense mechanisms observed in the diabetics is believed to cause late diabetic complications [1]. Diabetic levels of glucose content in the plasma and tissues reportedly correlate with increased production of reactive oxygen metabolites in a subject [2]. Consequently, the reactive oxygen metabolites cause damage to cellular proteins, lipids, and DNA. A state of increased oxidative stress is thus indicated in diabetes based on the observed increase in lipid peroxidation and on the reduced antioxidant reserves found in animal and human models [3]. Furthermore, activation of stress-sensitive signaling pathways that regulated specific gene expression also caused cellular damage [4]. Therefore it becomes necessary to identify novel and potent antidiabetic agents.

Knowledge on the effects of plant growth regulators (PGRs) on animal cell function is limited. Though effects of different PGRs on insects had been investigated earlier [5, 6, 7] reports concerning vertebrates are very limited [8, 9, 10]. Ozmen et al [11] observed that absicisic acid and gibberellic

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acid affected sexual differentiation and certain other physiological parameters in laboratory mice. PGRs reportedly [12] caused increase in the number of splenic plague forming cells, circulating white blood cells, hemotocrit values, and thymus weight in young deer mice. El-Mofty and Sakr [13] found that gibberellin induced liver neoplasm in Egyptian toads and suggested that tumors could be diagnosed as hepatocellular carcinomas. Gibberellins induced microabscesses and hydrophic degeneration in the liver and caused mononuclear inflammatory infiltration in the kidneys of laboratory mice [14]. A rational understanding of PGRs effect is therefore required to be achieved for which the mechanism of toxicity needs to be clarified from a structure-function point of view.

In our previous work we had shown that the PGR, brassinosteroid isoform homobrassinolide (HB) possessed an antidiabetic effect on rats [15]. However the **antidiabetic** potency of this PGR compared to other PGRs is not already known. Hence, in the present study we aimed at comparing the antidiabetic efficiency of HB with Gibberellic acid (GBA) in influencing lipid peroxidation on normal and diabetes-induced male rats, employing streptozotocin as a diabetogenic agent due to its ability to reduce the synthesis of insulin. Treatment of animals with these phytochemicals was done orally to establish the direct effect of HB and GBA for a well characterizable in vivo toxicity model system, choosing specific tissues due to their important individual role in animal metabolism.

Materials

Male Wistar strain-albino rats weighing 150–200g were used for the investigation. The animals were housed under controlled temperature and hygiene conditions in propylene cages. Commercial rat chow with free access to drinking water ad libitum was provided for the animals. Rats were fasted for 16 h [16]. Animals were then injected streptozotocin at a dose of 60mg/kg of body weight intravenously. Streptozotocin induced diabetes within 3 days by destroying the beta cells [17]. Animals with blood glucose concentration > 250 mg/dl was considered diabetic, and were used for the experiment. Hypoglycemia occurring within 24 h following streptozotocin administration was prevented by feeding 5% glucose solution orally to the diabetic rats. Experiments were carried out in accordance with internationally accepted ethical guidelines for the care of laboratory animals. Six groups of six animals each were designated as, Group I: Normal control rat, Group II: Normal rat treated with HB, Group III: Diabetic control rat, Group IV: Diabetic rat treated with HB, Group V: Normal rat treated with GBA and Group VI: Diabetic rat treated with GBA. The content of MDA was measured as thiobarbituric acid-reactive substance (TBARS), 4-HNE, GSH and erythocyte CAT activity was determined using the washed cells.

The rats were given 50μg of HB and 50 μg GBA orally for seven days. At the end of the treatment, rats were anesthetized with diethyl ether. Blood was collected from heart and transferred immediately into disposable silicon coated tubes with EDTA as anticoagulant. The blood samples were used for the determination of peroxidised lipid content. Packed erythocytes were obtained by centrifuging the blood sample at 4000xg for 15 min at 4°C, followed by washing the cells with thee times physiological saline. Rat tissues were taken in physiological saline, homogenized for 15min in 1.15% ice cold KCl solution, and centrifuged at 7000xg for 15min to obtain the supernatant for use as sample source.

Methods

MDA assay

The erythrocyte and tissue MDA concentration was determined using the method of Jain et al [18] based on thiobarbituric acid reactivity. Briefly, 0.2 mL of packed erythocytes or supernatant obtained from tissues, 0.8 mL of phosphate buffer (pH 7.4), 0.025 mL of butylated hydroxytoluene and 0.5 mL of 30% trichloroacetic acid were added to the reaction tubes, mixed and incubated at –20 °C for 2 h. The mixture was then centrifuged (400*g*) for 15 min. Following this, 1 mL of the supernatant was added to 0.075 mL of 0.1 M EDTA and 0.25 mL of 1% thiobarbituric acid (TBA) contained in a set of tubes, with Teflon-lined screw caps and were incubated at 90 °C in a water bath for 15 min, cooled to room temperature. Erythocyte MDA content was measured at 532 and 600 nm in a spectrophotometer, and tissue MDA content was measured at 532nm.

4-HNE assay

The erythocyte and tissue 4-HNE concentration was determined using the method of Kinter [19]. 2 mL of packed erythocytes or tissue homogenate supernatant was treated with 1.5 mL of 10% TCA, and centrifuged at 3000 rpm for 30 min and filtered. The filtrate (2 mL) was treated with 1 mL of 2, 4 dinitrophenyl hydrazine and allowed to stand for 1 h at room temperature. The samples were then extracted with hexane, and the extract was evaporated at 40°C. After cooling to room temperature, 2 mL of methanol was added to each sample, mixed thoroughly, and the absorbance of the sample was measured at 350 nm against methanol as blank. A series (300, 400, 500 and 600 μl) of primary standards of 4-HNE were diluted with 4.70, 4.60, 4.50 and 4.40 mL of phosphate buffer, and 2 mL of each diluted sample was pipetted out and taken for assay as before. Samples were extracted with 2 mL of hexane thee times. All extracts were collected in stoppered test tubes. The extract was evaporated to dryness under argon at 40°C and the residue was reconstituted in 1mL methanol. The absorbance of the reconstituted residue was measured at 350nm against a blank. The nonenol content of the test sample was estimated employing the relationship 4-HNE= $(A_{350}$ - 0.005603185)/0.003262215 and from a standard plot.

GSH assay

The erythocyte and tissue GSH concentration was measured using the method of Beutler et al [20]. Erythocyte pellet or supernatant (0.2 mL) was initially added to 1.8 mL of distilled water. Three milliliters of a precipitating solution (1.67 g metaphosphoric acid, 0.2 g EDTA and 30 g NaCl in

100 mL distilled water) was then mixed with the haemolysate. The mixture was allowed to stand for 5 min and then filtered. Two milliliters of the filtrate was mixed with 8 mL of the phosphate solution and 1 mL of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). A blank was prepared with 8 mL of phosphate solution, 2 mL diluted (thee parts to two parts distilled water) precipitating solution and 1 mL DTNB reagent. The absorbance of each sample was measured at 412nm in a spectrophotometer. A standard (40 mg/100 mL) solution of the glutathione was prepared and employed as reference.

Catalase assay

Catalase activity was also determined by the method of Beutler [21], 1 M Tris–HCl, 5 mM EDTA (pH 8), 10 mM H_2O_2, and H_2O were mixed and the rate of H_2O_2 consumption at 37°C and at 240nm was used for quantitative determination of catalase activity.

Analysis of data

Values are presented as mean ± S.E.M. Statistical differences between the relevant control and treated samples were evaluated by one way ANOVA and p value *<0.05* was considered statistically significant.

Results

Tables 1 and 2 shows the levels of MDA, 4-HNE, GSH and the activity of catalase in the erythrocyte and tissues such as brain, heart, liver, kidney and testis of control, diabetic and treated rats.

Erythocyte

MDA content was reduced to 33and 42% in erythocyte of normal and diabetic rats treated with HB while, 13 and 14% marginal increment was found in normal and diabetic rats treated with GBA. 4-HNE content of HB treatment declined to 12% in normal and 38% in diabetic rats. 18 and 9% enhancement was noted in GBA treated experiment. Catalase activity was found to be mildly elevated in normal rats, while 43% increase was noted in diabetic. A reverse trend (11 and 15% reduction) in catalase activity was observed in normal and diabetic rats treated with GBA. GSH content of HB treated rats was increased slightly in normal and 38% in diabetic rats, while in GBA-treated normal and diabetic rats showed 20 and 10% reduction respectively.

Brain

Brain MDA content was reduced to 11 and 22% in normal and diabetic rats treated with HB respectively, whereas 38 and 34% enhancement was found when GBA was administered. 4-HNE content was diminished to 33% in the diabetic and moderately in normal rats treated with HB. In GBA rats 26 and 31% increment respectively was noted. Catalase activity was elevated to 17 and 29% in normal and diabetic rats respectively following HB administration, whereas GBA treatment brought about 13 and 40% reduction of catalase activity in these animals. While HB treated normal and diabetic rats

Table 1. Effect of homobrassinolide (50ug) on lipid peroxidation and antioxidant defense in erythocytes and tissues of normal and diabetic rats.

Tissue	Studies	Parameters	Normal rat	HB-normal rat	Diabetic rat	HB-diabetic rat
Erythocyte	LP	MDA	1.5 ± 0.1	1.1± 0.1	2.8 ± 0.2	1.6 ± 0.1**
	AD	4-HNE	1.7± 0.2	1.5± 0.1**	3.4 ± 0.2	2.1± 0.2***
Brain	LP	CAT	190 ± 8	210± 12	107 ± 11	125 ± 10
		GSH	80 ± 9	90± 15	60 ± 4	83 ± 7
		MDA	56 ±7	50±7	95 ± 9	74 ± 11
	AD	4-HNE	42± 4	40±3	63 ± 4	42± 2**
		CAT	30 ± 4	35±2	21 ± 1	27 ± 2
		GSH	80 ± 7	90± 6	60 ± 4	75 ± 4***
Liver	LP	MDA	67± 5	44±4**	90 ± 7	63 ± 4***
		4-HNE	40± 2	35±7	72± 5	42± 3***
	AD	CAT	15 ± 2	19±3	11 ± 2	15 ± 2
		GSH	86 ± 5	95±5	60 ± 4	80± 5**
Kidney	LP	MDA	49 ± 4	33±4*	76 ± 7	51± 4*
		4-HNE	46±3	33±2**	70± 4	52± 4*
	AD	CAT	26 ± 2	28±4	18 ±3	27 ± 3*
		GSH	62 ±2	72±3*	41 ± 3	59± 4*
Heart	LP	MDA	31± 4	27±3	53 ± 4	29 ± 3***
		4-HNE	28± 2	24±2	68 ± 6	49± 4**
	AD	CAT	20 ± 3	27±2	11 ± 2	16 ± 4
		GSH	60 ± 5	68 ±5	46 ± 2	51 ± 4
Testis	LP	MDA	26 ±3	18 ±2*	35 ± 3	29 ± 3**
		4-HNE	32 ± 2	26 ± 2	63 ± 4	48± 2*
	AD	CAT	14 ± 3	16 ± 2	11 ± 1	13 ± 2
		GSH	45 ± 5	49 ± 5	36 ± 1	42 ± 4

MDA and 4-HNE expressed nmol/mL (erythocytes) and nmol/g (tissue). Catalase activity U/mL (erythocytes) and U/g (tissue). GSH content mg/g. p<0.05 *, p<0.01**, p<0.001***. LP: Lipid peroxidation, AD: Antioxidant defense

Table 2. Effect of giibberellic acid (50μg) on lipid peroxidation and antioxidant defense in erythocytes and tissues of normal and diabetic rats.

Tissues	Studies	Parameters	Normal rat	GBA-normal rat	Diabetic rats	GBA-diabetic rat
Erythocyte	LP	MDA	1.5 ± 0.1	1.7± 0.1	2.8 ± 0.1	3.2 ± 0.3**
		4-HNE	1.7± 0.2	2± 0.1	3.4± 0.1	3.7± 0.2
	AD	CAT	190 ± 8	169 ± 7	100± 11	85 ± 9
		GSH	80 ± 9	68± 5*	70± 4	63 ± 7
Brain	LP	MDA	56 ±7	68±6*	90 ± 9	124 ± 5*
		4-HNE	42± 4	53±4**	61± 4	80±4**
	AD	CAT	30 ± 4	26±3*	20 ± 1	12 ± 2**
		GSH	80 ± 7	68± 6	58 ± 4	53 ± 4
Liver	LP	MDA	67± 5	89±3	87 ± 7	99 ± 5
		4-HNE	40± 2	52±5**	72 ± 5	84± 7
	AD	CAT	15 ± 2	13 ±2	11 ± 1	7 ± 1*
		GSH	86 ± 5	65 ± 6*	58 ± 4	43 ± 3**
Kidney	LP	MDA	49 ± 4	55±4	70 ± 7	90 ± 6
		4-HNE	46±3	66 ±3**	71 ± 4	102 ±7**
	AD	CAT	26 ± 2	16± 1	20 ±3	8 ±1*
		GSH	62 ±2	45 ± 3*	41 ± 2	28 ± 4
Heart	LP	MDA	31± 4	48± 2*	53 ± 4	89 ± 8**
		4-HNE	28± 2	35 ± 4	63 ± 6	75± 5
	AD	CAT	20 ± 3	11±2*	12 ± 2	7 ± 1
		GSH	60 ± 5	39 ± 4*	46 ± 1	31 ± 4**
Testis	LP	MDA	26 ±3	34±4	43 ± 4	76 ± 6***
		4-HNE	32 ± 2	54 ± 4**	63± 4	74± 7
	AD	CAT	14 ± 3	10 ± 2	13 ± 1	8 ±1
		GSH	45 ± 5	39 ± 4	36 ± 2	30 ± 4

MDA and 4-HNE expressed nmol/mL (erythocytes) and nmol/g (tissue). Catalase activity U/mL (erythocytes) and U/g (tissue). GSH content mg/g. p<0.05 *, p<0.01**, p<0.001***. LP: Lipid peroxidation, AD: Antioxidant defense

exhibited 13 and 25% increase in GSH content. GBA treated rats showed only 15 and 9% reduction respectively.

Liver

MDA content of liver was reduced 34 and 30% in HB treated normal and diabetic rats, while in GBA treated rats, 32 and 14% enhancement was found respectively. 4-HNE content of HB treated rats was declined to 13 and 42% in normal and diabetic rats respectively, whereas 30 and 17% enhancement was noted with GBA treatment. Catalase activity was found to be elevated to 27 and 36% in HB treatment, whereas with GBA treatment its value was 13 and 42% reduced in normal and diabetic rats respectively. GSH content of HB treated rats was increased to 33% in diabetic and milder in normal, while in GBA treatment showed 24 and 9% reduction.

Kidney

MDA content in kidney was reduced to 33% in HB treated normal and diabetic rats, whereas an increase of 12 and 29% was observed in GBA treated animals respectively. 4-HNE content was reduced to 28 and 26% respectively, whereas 43% enhancement was noted in GBA treated rats. Catalase activity was elevated to 8 and 50% in normal and diabetic HB rats, but was reduced to 38 and 60% in normal and diabetic GBA rats respectively. GSH content in normal and treated HB rats increased to 16 and 44% respectively, while GBA rats showed 27 and 26% reduction.

Heart

Heart MDA concentration was reduced to 13 and 45% in normal and diabetic HB rats, while 55 and 68% increase in content of this compound was found in GBA treated rats. 4-HNE content of HB treated rats was diminished to 14% in normal and 28% percent in diabetic, whereas a corresponding 25 and 19% increase was noted in GBA rats. Catalase activity was elevated 35 and 45% in normal and diabetic HB treated respectively rats, while 45 and 42% reduction in catalase activity was observed in normal and diabetic GBA rats. GSH content of HB treated rats increased moderately, while 35 and 33% reduction in GSH was noted in normal and diabetic GBA treated rats respectively.

Testis

MDA content of testis was reduced to 31 and 17% in normal and diabetic HB treated rats respectively, whereas a corresponding 31 and 71% enhancement was found in GBA treated rats. 4-HNE content was declined to 19 and 24% in normal and diabetic HB rats, whereas 68 and 17% enhancement was noted in GBA rats. Catalase activity was found to be elevated 14 and 18% in normal and diabetic HB rats respectively. GBA treatment resulted in 38 and 28% reduction in catalase activity with normal and diabetic rats. GSH content of diabetic HB rats increased to 17% while 17% reduction of GSH content was noted in GBA diabetic rats. A very moderate increase of GSH was noted in normal HB rats although 13% reduction of GSH content was noted in normal GBA treated rats.

Restorative effect

HB caused significant reduction of MDA and 4-HNE content in erythocyte, brain, liver, heart, kidney and testis. GSH content and activity of catalase were found to be elevated in all tissues including erythocytes of normal and diabetic HB treated rats. The restorative effect of HB on lipid peroxidation and antioxidant status of diabetic rats was found to be statistically significant while in the normal rat, these were subnormal (Table 1).

GBA administration in normal rats caused significant increase in the MDA and 4-HNE content, but a graded increase in all tissues of the diabetic rats. The GSH content and CAT activity was very low in all tissues of normal and diabetic rats treated with GBA. No restorative effect on lipid peroxidation or on antioxidant status of the diabetic rat was observed when GBA was employed. In normal rats, lipid peroxidation and the antioxidant status was abnormal (Table 2).

Discussion

The principal aim of this study was to investigate if homobrassinolide and gibberellic acid could affect lipid peroxidation in normal and diabetic rats. Information on their in vivo influences on cellular metabolism in higher animals is very limited [5]. The present study indicated that gibberellic acid increased the production of lipid peroxides in normal and diabetic rats and homobrassinolide yielded an enhanced antioxidant defense and reduced lipid peroxidation in erythocytes and in the various tissues of normal and diabetic rats.

No study examining their effect in vivo has been made on normal and diabetic rat erythocytes or of rat tissue MDA, 4-HNE content and antioxidant enzyme activities. Hence opportunity does not exist to compare our results with previous studies. Upon gibberellic acid treatment in vivo, the content of MDA and 4-HNE had been increased in erythocytes and in the tissues of normal and diabetic rats. In addition, gibberellic acid exerted a significant decrease in erythocyte GSH and catalase activities. Gibberellic acid also caused significant changes in certain tissue GSH and CAT activities. Therefore, effects of GBA added to the problem of diabetic side effects.

It should be recognized that a decrease in the antioxidant defense may result in an increase in superoxide radical generation. The increased content of MDA and 4-HNE may result from an increase in hydroxyl radicals ($^{\cdot}OH$). However, it is conceivable that gibberellic acid caused erythocyte and tissue lipid peroxidation by way of generating increased superoxide radicals. The reasons for such an effect by gibberellic acid are not understood at present. Since, it is known that $^{\cdot}OH$ can initiate lipid peroxidation in tissues [22] and that MDA and 4-HNE are major oxidation products of polyunsaturated fatty acids, increased MDA and 4-HNE contents are considered as important indices of lipid peroxidation [23]. It is not general rule that increase in a pollutant concentration induced antioxidant activity. Doyotte et al [24] had pointed out that a diminished response may accompany a first exposure to pollutants that can be followed by an induction of the antioxidant systems. Further experiments are needed to be performed to elicit what factor is responsible for the elevation of MDA and 4-HNE content in rat tissues.

There has been considerable debate over the extent to which increased oxidative stress contributed to the development of diabetic complications. Increased membrane rigidity, decreased cellular deformability, reduced erythocyte survival and lipid fluidity caused by peroxidation of membrane lipids have been implicated as consequences in diabetes mellitus [25]. Hyperglycemia resulted in the generation of free radicals that exhausted antioxidant defenses within a cell and contributed to the disruption of cellular function though oxidative damage to cell membranes and susceptibility to lipid peroxidation (26,27). Our observations indicate that the administered dose level of HB reduced lipid peroxidation, whereas GBA augmented peroxidation of lipids and therefore reduced the antioxidant status of a tissue in experimental animals. We therefore suggest that HB served to regulate lipid peroxidation in animal tissues.

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***Declaration of interest*:** The authors report no conflicts of interest.

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