



Ameliorative effects of phycocyanin against gibberellic acid induced hepatotoxicity



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ABSTRACT

Gibberellic acid (GA3) was used extensively unaware in agriculture in spite of its dangerous effects on human health. The current study was designed to investigate the ameliorative effects of the co-administration of phycocyanin with GA3 induced oxidative stress and histopathological changes in the liver. Forty male albino rats were randomly divided into four groups. Group I (control group) received normal saline for 6 weeks, Group II (GA3 treated group) received 3.85 mg/kg body weight GA3 once daily for 6 weeks, Group III (phycocyanin treated group) received Phycocyanin 200 mg/kg body weight/day for 6 weeks orally dissolved in distilled water and Group IV was treated with GA3 and phycocyanin at the same doses as groups 2 and 3. All treatments were given daily using intra-gastric intubation and continued for 6 weeks. Our results revealed significant downregulation of antioxidant enzyme activities and their mRNA levels (CAT, GPx and Cu-Zn, SOD) with marked elevation of liver enzymes and extensive fibrous connective tissue deposition with large biliary cells in hepatic tissue of GA3 treated rats, while treatment with phycocyanin improved the antioxidant defense system, liver enzymes and structural hepatocytes recovery in phycocyanin treated group with GA3. These data confirm the antioxidant potential of Phycocyanin and provide strong evidence to support the co-administration of Phycocyanin during using GA3.

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1. Introduction

Plant growth regulators (PGRs) are widely used in Egypt to increase plant size, production and to increase plant availability all year round [1]. Gibberellic acid (GA3) is one of the most important growth stimulating plant hormones that belongs to gibberellins and is used for promoting cell elongation, cell division and growth of many plant species [2].

A growing amount of evidence indicates that GA3 alters the antioxidative systems in the rat's tissues as it induces oxidative stress leading to generation of free radicals and causes lipid peroxidation. The most important enzymes, superoxide dismutase (SOD), glutathione peroxidases (GPx) and catalase (CAT), are also affected [3]. GA3 exerts toxic effects on many soft organs including the liver [4]. GA3 treated cells lose their ability to scavenge reactive oxygen species

(ROS) and this loss ultimately results in oxidative damage and cell death [5]. GA3 also induces microabscesses and hydropic degeneration in the liver and mononuclear inflammatory infiltration in the kidneys of laboratory mice [6].

Phycocyanin is a biliprotein pigment found in blue-green algae *Spirulina platensis*, which have attracted attention because of their nutritional value and medicinal properties. This pigment has antioxidant, anti-inflammatory and hepatoprotective activities in different experimental models [7]. The antioxidant properties of Phycocyanin come from its ability to scavenge free radicals and also react with other oxidants of pathological relevance [8]. It is well known that ROS are the main cause of many important pathological processes including inflammatory, neurodegenerative diseases, atherosclerosis, cancer and reperfusion injury [9].

Antioxidant, free radical scavenging and other beneficial effects are reported for phycocyanin, which could justify testing phycocyanin as a potential approach to alleviating the biochemical, molecular and pathological effects of GA3 on liver tissue.

2. Materials and methods

2.1. Chemicals

Gibberellic Acid, phycocyanin, epinephrine, DTNB (5,5 Dithiobis 2-nitrobenzoic acid) and NADPH were purchased from Sigma Aldrich Chemical Co., USA. Potassium dichromate, hydrogen peroxide, glacial

Abbreviations: ALP, alkaline phosphatase; ALT, alanine amino transferase; AST, aspartate amino transferase; CAT, catalase; cDNA, complementary deoxy ribonucleic acid; CU-ZN SOD, superoxide dismutase; GA3, gibberellic acid; γ GT, gamma glutamyl transferase; GPx, glutathione peroxidase; GSH, reduced glutathione; MDA, malondialdehyde; PGRs, plant growth regulators; RNA, ribonucleic acid; ROS, reactive oxygen species; RT-PCR, reverse transcriptase polymerase chain reaction.

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acetic acid and EDTA were purchased from El-Nasr Company, Cairo, Egypt. Kits of alanine amino transferase (ALT), Aspartate amino-transferase (AST), Alkaline phosphatase (ALP) and gamma glutamyl transferase (γ GT) were obtained from spectrum kits, Egyptian Company for Biotechnology, Cairo, Egypt.

2.2. Animal management

Healthy adult forty male albino rats, weighing 120–150 g were obtained from the Animal House in Faculty of Veterinary Medicine, Zagazig University, Sharkia, Egypt. Rats were given free access to food and water with 12 h/12 h dark light cycle. All animals were left 2 weeks for adaptation in standard cages under controlled conditions with free access to food and water. The European Community Directive (86/609/EEC) and national rules on animal care have been followed.

2.3. Experimental design

After the period of acclimation, rats were randomly segregated into four groups (10 rats per group). Group I (control group) received normal saline for 6 weeks. Group II (GA3 treated group) received 3.85 mg/kg body weight GA3 once daily for 6 weeks [10]. Group III (phycocyanin treated group) received Phycocyanin 200 mg/kg body weight/day for 6 weeks orally dissolved in distilled water [7]. Group IV was treated with GA3 and phycocyanin at the same doses as groups 2 and 3. All treatments were given daily using intra-gastric intubation and continued for 6 weeks.

2.4. Sampling

At the end of experimental period, rats from all groups were fasted overnight and blood samples were collected into chilled non-heparinized tubes and centrifuged at $860 \times g$ for 20 min at 4°C . The separated sera were frozen at -20°C for biochemical analysis. After the collection of blood samples, animals were sacrificed and samples from the liver tissue were accurately weighed and homogenized using tissue homogenizer (Potter–Elvehjem) using chilled potassium chloride (1.17%) for measuring antioxidant status. To follow up changes in antioxidant enzymes gene expression, liver samples were collected in liquid nitrogen container until the time of RNA extraction. Meanwhile, small parts of the liver were fixed in neutral formalin solution for histopathological examination.

2.5. Biochemical analysis

The sera were used for the determination of some liver enzymes such as ALT (EC 2.6.1.2) and AST (EC 2.6.1.1) activities using commercial assay kits according to the methods described by Breuer [11]. ALP (EC 3.1.3.1) and γ -GT (EC 2.3.2.2) were measured using commercial kits according to Moss et al. [12]. The post mitochondrial fraction of liver was prepared according to Kim et al. [13]. In brief, liver samples were homogenized by a tissue homogenizer using chilled potassium chloride (1.17%). The nuclear debris were separated by centrifugation at $8000 \times g$, 4°C for 5 min. The supernatant was again centrifuged for 20 min at $10\,500 \times g$, 4°C to get the post-mitochondrial supernatant which was used to assay malondialdehyde (MDA), Lipid peroxidation marker, CAT (EC 1.11.1.6), SOD (EC 1.15.1.1), GPx (EC 1.11.1.9) and reduced glutathione (GSH). The level of MDA in liver homogenate was determined spectrophotometrically according to Nair and Turner [14]. The activity of SOD in the liver tissues was determined spectrophotometrically at a wavelength of 480 nm by epinephrine method and expressed in units of enzyme activities per gram of tissues wet wt [15]. CAT activity in the liver was determined spectrophotometrically at a

wavelength of 570 nm according to the method of Sinha [16]. GPx activity was determined by the method of Paglia and Valentine [17]. GSH levels were determined according to the method of Beutler [18]. The optical densities of the given parameters were measured by Shimadzu type spectrophotometer (UV 120-02).

2.6. Semi-quantitative RT-PCR

Total RNA was extracted from the liver with RNeasy Mini kit (Qiagen) following the manufacturer's instructions. The amount of extracted RNA was quantified and qualified using NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA). The purity of RNA was checked and ranged between 1.8 and 2.1, demonstrating the high quality of the RNA. The cDNAs were synthesized using Revert-Aid™ First Strand cDNA Synthesis Kit (Qiagen, Hilden, Germany) [19]. Equal amounts of the reverse transcriptional products (1 μg cDNA) were subjected to PCR amplification in a volume of 50 μL using gene specific primers [20]. (Table 1), 2X PCR Master Mix from (Fermentas, Cairo/Egypt) following the manufacturer's instructions. β -actin was used as an internal control for PCR. Amplification of cDNA was started with 5 min of denaturation at 94°C followed by 28 cycles consisted of 60 sec at 95°C , 55°C , 1 min; 72°C , 1 min for all genes were placed in 2720 thermocycler (Applied Biosystems, USA). The final extension was for 7 min at 72°C . A single major band for each gene was detected by electrophoresis on a 1.5% agarose/ethidium bromide (0.25 $\mu\text{g}/\text{mL}$) gel. The intensity of the bands was compared to those collected from the control group using the public domain NIH Image program (National Institutes of Health, Bethesda, Maryland). mRNA expression levels of antioxidant enzymes were expressed as mean \pm standard error (SE).

2.7. Histopathological examination

The liver of male albino rats was collected from the different groups after 6 weeks. The samples were fixed in Bouin's solution, then dehydrated in ascending grades of alcohols, cleared in xylene and embedded in paraffin. The samples were casted, then sliced into 5 μm thickness and placed onto glass slides. The slides were stained by general and specific stains [21].

2.8. Statistical analysis

Data are expressed as mean values \pm SE of 10 rats. Statistical analysis was performed using one way analysis of variance (ANOVA) using SPSS statistical version 18 software package (SPSS, Inc, USA). Duncan's test was used for making multiple comparisons among the groups to test the inter-grouping homogeneity.

Table 1
Primer sequences of rat's GPx, CAT, Cu Zn SOD and β -actin with its product size.

Gene		Oligonucleotide sequences	Product length (bp)	Gene ID
CAT	F	5'-GTCCGATTCTCCACAGTCGC-3'	272	S50336.1
	R	5'-CGCTGAACAAGAAAGTAACCTG-3'		
Cu-Zn SOD	F	5'-ATGGGGACAATACACAAGGC-3'	225	AH004967.1
	R	5'-ATGGGGACAATACACAAGGC-3'		
GPx	F	5'-CACAGTCCACCGTGTATGCC-3'	292	Z21917.1
	R	5'-AAGTTGGGCTCGAACCCACC-3'		
β -actin	F	5'-CATAGCTCTTCTCCAGGGAG-3'	600	NM_007393
	R	5'-AGGGTGTGATGGTGGGTATG-3'		

F indicates forward primer; R indicates reverse primer.

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