Testicular disorders induced by plant growth regulators: cellular protection with proanthocyanidins grape seeds extract

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Abstract The present study aims to investigate the adverse effects of plant growth regulators : gibberellic acid (GA₃) and indoleacetic acid (IAA) on testicular functions in rats, and extends to investigate the possible protective role of grape seed extract, proanthocyanidin (PAC). Male rats were divided into six groups; control group, PAC, GA₃, IAA, GA₃ + PAC and IAA + PAC groups. The data showed that GA₃ and IAA caused significant increase in total lipids, total cholesterol, triglycerides, phospholipids and low-density-lipoprotein cholesterol in the serum, concomitant with a significant decrease in high-density-lipoprotein cholesterol, total protein, and testosterone levels. In addition, there was significant decrease in the activity of alkaline phosphatase, acid phosphatase, and gamma-glutamyl transferase. A significant decrease was detected also in epididymal fructose along with a significant reduction in sperm count. Testicular lipid peroxidation product and hydrogen peroxide (H₂O₂) levels were significantly increased. Meanwhile, the total antioxidant

capacity, glutathione, sulphahydryl group content, as well as superoxide dismutase, catalase, and glucose-6-phosphate dehydrogenase activity were significantly decreased. Moreover, there were a number of histopathological testicular changes including Leydig's cell degeneration, reduction in seminiferous tubule and necrotic symptoms and sperm degeneration in both GA₃- and IAA-treated rats. However, an obvious recovery of all the above biochemical and histological testicular disorders was detected when PAC seed extract was supplemented to rats administered with GA₃ or IAA indicating its protective effect. Therefore it was concluded that supplementation with PAC had ameliorative effects on those adverse effects of the mentioned plant growth regulators through its natural antioxidant properties.

Keywords Plant growth regulators · Gibberellic acid · Indoleacetic acid · Proanthocyanidin · Grape seed extract

Abbreviations

PGRs	Plant growth regulators
GA ₃	Gibberellic acid
IAA	Indoleacetic acid
PAC	Proanthocyanidin
TL	Total lipids
TC	Total cholesterol
TG	Triglycerides
PL	Phospholipids
LDL-C	Low-density-lipoprotein cholesterol

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HDL-C	High-density-lipoprotein cholesterol
TP	Total protein
ALP	Alkaline phosphatase
ACP	Acid phosphatase
γ -GT	Gamma-glutamyl transferase
MDA	Malondialdehyde
H ₂ O ₂	Hydrogen peroxide
TAC	Total antioxidant capacity
GSH	Glutathione
SH	Sulphahydryl
SOD	Superoxide dismutase
G6PD	Glucose-6-phosphate dehydrogenase

Introduction

PGRs are chemicals used to alter the growth of plants. They are commonly used to increase seed production (Ashikari et al. 2005). Some of them are endogenous plant hormones, which are included in the diet of all herbivorous and omnivorous animals (Celik et al. 2002). PGRs fall into six major classes: auxins, gibberellins, cytokinins, ethylene generators, growth inhibitors, and growth retardants. One of the gibberellins, gibberellic acid-3 (GA₃) accelerates and improves the yield of a wide variety of plants by increasing cell division (Silverstone and Sun 2000), promoting stem elongation, overcoming dormancy in seeds and buds, involved in parthenocarpic fruit development, flowering and mobilization of food reserves in grass seed germination. In addition, it is used against harmful agriculture pests by accelerating the growth of fruits and vegetables (Arous et al. 2001).

Additionally, indoleacetic acid (IAA), the principal natural auxin, affects numerous plant processes, e.g., cell division and elongation, autumnal loss of leaves and the formation of buds, roots, flowers and fruits. It is widely used commercially to cause more vigorous plant growth, and promotes root formation in plants not easily propagated by stem cuttings, to retard fruit drop and to produce seedless varieties by parthenogenetic fruiting (Audesirk and Audesirk 1986). Since animals, and humans consume GA₃ and IAA in their diet, it was hypothesized that metabolic events in their cells could influence such dietary ingredients (Srikumar et al. 2009). Therefore, GA₃ and IAA could pose a risk to those exposed to them because of their profession, as well as the general population, by the excessive consumption of such food products.

Recent trends in controlling and treating diseases tend to prefer natural compounds. Medicinal plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years, and have served humans well as their valuable components (Monobe et al. 2012). Proanthocyanidin (PAC) is considered one of the natural and safe compounds that have beneficial effects on health (Serrano et al. 2009). It is extracted from grape seeds and their basic structure units are the catechins. PAC contains catechin, monomers, dimers, and trimers, all of which are water-soluble molecules and contain a number of phenolic hydroxyls (Bagchi et al. 2002). Oligomeric PAC compounds are naturally occurring antioxidants which have been reported to possess a broad spectrum of biological, pharmacological and therapeutic activity against free radicals and oxidative stresses (Bagchi et al. 2000). Beside the antioxidant activity, PAC compounds have been described as anti-microbial, anti-cancer, anti-inflammatory and anti-fatigue agents (Shan et al. 2010) with cardio-protective properties (Serrano et al. 2009). Therefore, the present work aims at investigating the possible protective role of PAC against the testicular adverse effects induced by two widely used plant growth regulators, GA₃ and IAA.

Materials and methods

Chemical used

GA₃ and IAA were purchased from Sigma Chemical Company (St. Louis, MO, USA). PAC was purchased from the Arab Co. for Gelatin and Pharmaceutical Products (Alexandria, Egypt), under license of Nulife International USA

Animal grouping and experimental design

Thirty-six adult male albino rats (*Rattus rattus*) weighing 130–140 g were used in this study. The animals were housed in ventilated stainless-steel cages in an artificially illuminated and thermally controlled room (22–25 °C and 12-h light dark cycle) at the Animal House Lab., Faculty of Science, Mansoura University (Mansoura, Egypt). They were fed on basal diet, and offered tap water for drinking, for 1 week for adaptation purposes. All animals received humane

care in compliance with the guidelines of the Mansoura University, and the protocol conformed to the guidelines of the National Institutes of Health. They were divided into six groups, six rats each; negative control group (no treatment at all), proanthocyanidin (PAC) positive control group, gibberellic acid (GA₃) group, indoleacetic acid (IAA) group, gibberellic acid and proanthocyanidins (GA₃ + PAC) group and indoleacetic acid and proanthocyanidins (IAA + PAC) group. PGR-only treated animals daily received oral treatment of GA₃, 3.85 mg/kg body weight (Celik et al. 2007), and IAA 5 mg/kg body weight (Celik et al. 2006). While, PAC was freshly prepared, and daily given to rats at a dose of 100 mg/kg body weight (Karthikeyan et al. 2007). PGR-PAC treated animals received the same doses of each compound combined together. All treatments were given using gastric intubation, and continued for 60 days.

Sampling

At the end of the experimental period, overnight-fasting rats were sacrificed, and blood samples were collected into chilled heparin-free tubes, and centrifuged at 860 G for 20 min. at 4 °C. The separated sera were frozen at –20 °C for biochemical analysis. In addition, tissue samples from a known portion of the right testis were accurately weighed and homogenized in a ten-fold volume of ice-cold distilled water for later biochemical analysis. Other samples of the left testes were fixed in neutral formalin solution for histo-pathological studies. Cauda epididymis and specimens of the testis were accurately weighed and homogenized manually in 0.5 mL of 0.9 % NaCl solution. The homogenates were diluted with 1.5 mL of the same solution and spermatozoa were counted at a 400× magnification in a Neubauer hemocytometer. Three counts per sample were averaged (Majumder and Biswas 1979; Liobet et al. 1995).

Biochemical analysis

Total lipid (TL) level was measured according to the method of Frings et al. (1972). Total cholesterol (TC), triglycerides (TG), and high-density-lipoprotein cholesterol (HDL-C) levels were determined by kits from Spinreact Co. S.A. Ctra (Santa Coloma, Spain) using the method of Young (1995). Low-density-lipoprotein cholesterol (LDL-C) level was calculated according to the method of Friedewald et al. (1972). The level of

Phospholipids (PL) was determined using kits from Bio-diagnostic Co. Dokki (Giza, Egypt) (Connerty et al. 1961). Alkaline phosphatase (ALP), acid phosphatase (ACP), gamma glutamyl transferase (GGT), hydrogen peroxide (H₂O₂), total antioxidant capacity (TAC), glucose-6-phosphate dehydrogenase (G6PDH) and fructose were determined according to the methods of Belfield and Goldberg (1971), Kind and King (1954), Szasz (1969), Aebi (1984), Koracevic et al. (2001), Kornberg (1955) and Foreman et al. (1973), respectively. Total protein (TP) was determined according the method of Henry (1964). Testis lipid peroxidation product, malondialdehyde (MDA), glutathione (GSH), sulphahydryl (SH) catalase (CAT) and superoxide dismutase (SOD) were estimated according to the method of Ohkawa et al. (1982), Prins and Loose (1969), Ellman (1959), Bock et al. (1980), Nishikimi et al. (1972), respectively. Serum testosterone level was determined using in vitro diagnostic radioimmunoassay with the Immulite 2000 analyzer for quantitative measurement (Wilson and Foster 1992).

Histopathological studies

Testis samples were carefully fixed in 10 % formalin solution, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in a paraffin wax, sectioned at 5–7 µm and stained with hematoxylin and eosin. The stained sections were examined and photographed under a research light microscope to detect the histopathological changes (Drury and Wallington 1980).

Statistical analysis

The obtained results in the present work were evaluated by One Way ANOVA (analysis of variance) test and post comparison was carried out with Duncan test. The results were expressed as mean ± standard error (SE). The values of $P \leq 0.05$ were considered statistically significant (Snedecor and Cochran 1982).

Results

Biochemical studies

As shown in Tables 1, 2 and 3; the present data recorded that the oral administration of PAC for

Table 1 Serum and testis lipid profile in different rat groups

	Animal groups						ANOVA
	Control	PAC	GA ₃	IAA	GA ₃ + PAC	IAA + PAC	<i>P</i>
<i>Serum</i>							
TL (mg/dL)							
Mean ± SE	331.5 ± 3.85 ^a	328.3 ± 2.11 ^a	483.2 ± 3.70 ^b	485.8 ± 3.14 ^b	383.7 ± 1.83 ^c	383.1 ± 3.02 ^c	<i>P</i> < 0.05 S
TC (mg/dL)							
Mean ± SE	77.1 ± 0.94 ^a	76.9 ± 0.61 ^a	109.6 ± 0.93 ^b	110.6 ± 1.16 ^b	86.1 ± 1.45 ^c	88.4 ± 0.85 ^c	<i>P</i> < 0.05 S
TG (mg/dL)							
Mean ± SE	61.6 ± 1.39 ^a	59.4 ± 0.58 ^a	101.9 ± 1.43 ^b	102.3 ± 1.59 ^b	82.7 ± 0.91 ^c	83.3 ± 0.69 ^c	<i>P</i> < 0.05 S
PL (mg/dL)							
Mean ± SE	108.9 ± 1.19 ^a	108.4 ± 1.64 ^a	160.3 ± 0.96 ^b	160.2 ± 0.78 ^b	116.2 ± 1.55 ^c	112.1 ± 1.55 ^{a & d}	<i>P</i> < 0.05 S
HDL-C (mg/dL)							
Mean ± SE	43.2 ± 0.98 ^a	44.1 ± 0.94 ^a	30.6 ± 0.60 ^b	30.5 ± 0.50 ^b	38.2 ± 0.89 ^c	42.2 ± 0.59 ^{a & d}	<i>P</i> < 0.05 S
LDL-C (mg/dL)							
Mean ± SE	21.50 ± 0.51 ^a	20.80 ± 0.56 ^a	58.50 ± 0.78 ^b	59.60 ± 0.75 ^b	31.30 ± 0.90 ^c	29.50 ± 0.97 ^c	<i>P</i> < 0.05 S
<i>Testis</i>							
TL (mg/g)							
Mean ± SE	57.3 ± 1.42 ^a	56.7 ± 1.54 ^a	99.5 ± 1.22 ^b	102.9 ± 1.53 ^b	71.7 ± 1.48 ^c	74.3 ± 1.47 ^c	<i>P</i> < 0.05 S
TC (mg/g)							
Mean ± SE	13.5 ± 0.76 ^a	13.4 ± 0.81 ^a	22.7 ± 1.05 ^b	24.2 ± 1.01 ^b	16.1 ± 0.63 ^c	16.4 ± 0.44 ^c	<i>P</i> < 0.05 S
TG (mg/g)							
Mean ± SE	15.5 ± 0.82 ^a	15.4 ± 0.63 ^a	29.4 ± 0.38 ^b	30.1 ± 0.59 ^b	20.8 ± 0.67 ^c	21.1 ± 0.70 ^c	<i>P</i> < 0.05 S
PL (mg/g)							
Mean ± SE	23.2 ± 0.75 ^a	23 ± 0.69 ^a	43.9 ± 0.78 ^b	43.7 ± 0.96 ^b	29.8 ± 0.68 ^c	29.8 ± 0.70 ^c	<i>P</i> < 0.05 S

Data are expressed as mean ± SE of 6 rats in each group. Within each row, means with different superscript (a, b, c or d) are significantly different at *P* < 0.05. Whereas means superscripts with the same letters mean that there is no significant difference (*P* > 0.05)

GA₃ gibberellic acid, IAA indoleacetic acid, PAC proanthocyanidins, S significant

60 days alone did not induce pronounced changes in most of the analyzed parameters, and their values were found near normal values. Also, the data indicated significantly increased levels of TL, TC, TG, PL and LDL-C, and a significant decrease in HDL-C concentration in rats having received GA₃ or IAA when

compared to the control groups. While, in rats having received PAC besides GA₃ or IAA, the data showed an improvement in the lipids profile compared to the control groups (Table 1). The results revealed also significant decrements in ALP, ACP and GGT activity, in rats having received GA₃ or IAA compared to

Table 2 Serum and testis enzyme activities in different rat groups

	Animal groups						ANOVA
	Control	PAC	GA ₃	IAA	GA ₃ + PAC	IAA + PAC	P
<i>Serum</i>							
ALK (U/L)							
Mean ± SE	127.4 ± 1.39 ^a	130.3 ± 1.28 ^a	75.6 ± 1.58 ^b	70.8 ± 1.52 ^c	119.4 ± 1.12 ^d	113.1 ± 1.01 ^c	P < 0.05 S
ACP (U/L)							
Mean ± SE	40.1 ± 0.65 ^a	41.1 ± 0.65 ^a	26.6 ± 0.49 ^b	24.6 ± 0.68 ^c	38.3 ± 0.53 ^d	37.5 ± 0.58 ^d	P < 0.05 S
<i>Testis</i>							
ALP (IU/g)							
Mean ± SE	29.8 ± 0.63 ^a	30.9 ± 0.71 ^a	13.7 ± 0.56 ^b	12.1 ± 0.57 ^b	27.8 ± 0.63 ^c	26.7 ± 0.51 ^c	P < 0.05 S
ACP (U/g)							
Mean ± SE	12 ± 0.62 ^a	12.5 ± 0.61 ^a	5.7 ± 0.29 ^b	5.5 ± 0.33 ^b	12.5 ± 0.59 ^{a & c}	12.3 ± 0.59 ^{a & c}	P < 0.05 S
GGT (U/g)							
Mean ± SE	11.3 ± 0.52 ^a	11.1 ± 0.65 ^a	4.5 ± 0.35 ^b	4.9 ± 0.35 ^b	10.1 ± 0.63 ^{a & c}	10.4 ± 0.59 ^{a & c}	P < 0.05 S

Data are expressed as mean ± SE of 6 rats in each group. Within each row, means with different superscript (a, b, c or d) are significantly different at $P < 0.05$. Whereas means superscripts with the same letters mean that there is no significant difference ($P > 0.05$)

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the control groups. However, the activity of serum enzymes was ameliorated significantly with the addition of PAC to the PGR treated groups (Table 2). Additionally, the data in Table 3 showed significant decline in serum TP content, sperm count, serum testosterone, and epididymal fructose levels in rat treated with GA₃ or IAA compared to control groups. However, in the PGR-PAC groups, serum TP, sperm count, serum testosterone and epididymal fructose levels were all improved. Moreover, the results in Table 4 indicated significant increase in testes MDA, and H₂O₂ levels and significant decrease in testes TAC, GSH and total SH content as well as CAT, SOD and G6PDH activity in rat treated with GA₃ or IAA compared to the control groups. However, the administration of PAC to the PGR-treated animals succeeded to induce a significant improvement in these parameters through overcoming the oxidative stress and up-regulate the endogenous antioxidant defence system. As shown in Tables 1, 2, and 3, the present data recorded that, the oral administration of PAC alone did not induce pronounced changes in most of the analyzed parameters, and their values were found near normal values.

Histo-pathological examination of the testicular tissues

Histo-pathological observation of the testes in both control and proanthocyanidin (PAC) groups showed normal testicular structure, where each seminiferous tubule (ST) was lined with a germinal epithelium and contained several rows of successive stages of spermatogenesis supported with scattered Sertoli cells. Numerous rounded or hemispherical spermatogonia were lying adjacent to the basal lamina. The primary spermatocytes were the largest among different cell stages, whereas the spermatids were the smallest. Mature spermatozoa heads appeared directed towards the elongated Sertoli cells (Fig. 1a, b). On the other hand, histopathological investigation of sections of the testes in GA₃- or IAA-treated rats showed disturbances in spermatogenesis process including degenerative changes in the testes with sever disintegration of spermatocytes, spermatogenic arrest with moderate tubular necrosis, and Leydig's cell degeneration. In addition, there was reduction in the size of the seminiferous tubules with some spermatogonia detached from the basement membrane, tunica

Table 3 Serum total protein (TP) and testosterone, epididymal fructose and sperm count (SC) in different rat groups

	Animal groups						ANOVA
	Control	PAC	GA ₃	IAA	GA ₃ + PAC	IAA + PAC	<i>P</i>
<i>Serum</i>							
TP (g/dL)							
Mean ± SE	7.60 ± 0.17 ^a	7.70 ± 0.15 ^a	5.70 ± 0.08 ^b	5.70 ± 0.08 ^b	6.90 ± 0.18 ^c	7.10 ± 0.17 ^c	<i>P</i> < 0.05 S
<i>Serum</i>							
Testosterone (ng/mL)							
Mean ± SE	2.4 ± 0.07 ^a	3.1 ± 0.41 ^b	0.7 ± 0.03 ^c	0.7 ± 0.03 ^c	1.7 ± 0.04 ^d	1.8 ± 0.08 ^d	<i>P</i> < 0.05 S
<i>Epididymys</i>							
Fructose (mg/dL)							
Mean ± SE	370.3 ± 1.32 ^a	382 ± 1.66 ^b	180.6 ± 1.68 ^c	191.5 ± 1.66 ^d	355.8 ± 1.68 ^e	365.6 ± 1.26 ^f	<i>P</i> < 0.05 S
SC (10 ⁴ /g)							
Mean ± SE	5.1 ± 0.35 ^a	5.3 ± 0.39 ^a	2 ± 0.12 ^b	2.1 ± 0.15 ^b	4.2 ± 0.21 ^c	4.4 ± 0.22 ^{a & c}	<i>P</i> < 0.05 S

Data are expressed as mean ± SE of 6 rats in each group. Within each row, means with different superscript (a, b, c or d) are significantly different at *P* < 0.05. Whereas means superscripts with the same letters mean that there is no significant difference (*P* > 0.05)

GA₃ gibberellic acid, IAA indoleacetic acid, PAC proanthocyanidins, S significant

albuginea (TA) and some were degenerated (Fig. 1c–f). Furthermore, in some cases, the treatment with IAA showed complete absence of all spermatogenesis stages. Combined treatment of GA₃ + PAC or IAA + PAC revealed some improvements in spermatogenesis process as judged by the presence of all stages of sperm formation, quite normal testicular histo-architecture and seminiferous tubules tightly connected together with inter-tubular connective tissue (IT) (Fig. 1g, h).

Discussion

In the present study, the obtained lipids profile alteration in rats having received PGRs demonstrates the adverse effect of PGRs on lipid metabolism. Increased total lipids and TC level may be due to that GA₃ activates fat metabolism and mobilization of this compound from membrane stores due to increased lipid peroxidation. In addition, the activation of 3-hydroxy-3-methyl-glutaryl-Coenzyme A reductase may be responsible for increased synthesis of cholesterol (Muthuraman et al. 2010). Human male infertility

might be associated with altered lipid metabolism in seminal plasma (Khalili et al. 2009). It was found earlier that high levels of lipids were shown to be common in azoospermic males, as high lipid levels exert direct adverse effects on the testicular levels. This situation may alter the sperm maturation process in male reproductive tract (Padron et al. 1989). Moreover, epididymal dysfunction in hypercholesterolemic animals may have detrimental effects on the cytostructural modifications and biochemical changes that occur during sperm epididymal maturation, and may result in both decreased sperm motility and sperm morphometric abnormality (Glander et al. 2002).

The administration of PAC caused marked improvements in lipid profiles, as evidenced by decreased levels of serum TL, TC, TG, PL, and LDL-C, in contrast to increased HDL-C. The decreased TG by PAC may be due to that the endogenous synthesis of TG was reduced and the activity of lipid protein lipase increased promoting the clearance of TG (Clausen 2003). Indeed, PAC has a strong ability of fat removal, and can increase the numbers of mitochondria in muscle and improve its

Table 4 Testis oxidative stress and antioxidants markers in different rat groups

	Animal groups						ANOVA
	Control	PAC	GA ₃	IAA	GA ₃ + PAC	IAA + PAC	<i>P</i>
<i>MDA (n mol/g)</i>							
Mean ± SE	45.7 ± 0.78 ^a	43.4 ± 0.50 ^b	77.3 ± 0.94 ^c	72.7 ± 0.63 ^d	56.7 ± 0.48 ^c	53.9 ± 0.62 ^f	<i>P</i> < 0.05 S
<i>H₂O₂ (m mol/g)</i>							
Mean ± SE	0.55 ± 0.04 ^a	0.40 ± 0.05 ^a	3.3 ± 0.15 ^b	3.1 ± 0.15 ^b	1.2 ± 0.12 ^c	1.2 ± 0.13 ^c	<i>P</i> < 0.05 S
<i>TAC (m mol/g)</i>							
Mean ± SE	0.72 ± 0.007 ^a	0.75 ± 0.010 ^a	0.41 ± 0.016 ^b	0.35 ± 0.015 ^c	0.68 ± 0.012 ^d	0.65 ± 0.011 ^d	<i>P</i> < 0.05 S
<i>GSH (mg/g)</i>							
Mean ± SE	0.31 ± 0.006 ^a	0.33 ± 0.008 ^a	0.19 ± 0.007 ^b	0.20 ± 0.006 ^b	0.29 ± 0.007 ^{a & c}	0.29 ± 0.009 ^{a & c}	<i>P</i> < 0.05 S
<i>SH (m mol/g)</i>							
Mean ± SE	4.2 ± 0.06 ^a	5 ± 0.09 ^b	2.4 ± 0.12 ^c	2.3 ± 0.10 ^c	3.2 ± 0.05 ^d	3.2 ± 0.05 ^d	<i>P</i> < 0.05 S
<i>CAT (μ mol/s/g)</i>							
Mean ± SE	7.6 ± 0.28 ^a	8 ± 0.31 ^a	3.2 ± 0.27 ^b	3.4 ± 0.25 ^b	7.1 ± 0.26 ^{a & c}	7.4 ± 0.28 ^{a & c}	<i>P</i> < 0.05 S
<i>SOD (U/g)</i>							
Mean ± SE	149.6 ± 1.62 ^a	151.5 ± 1.47 ^a	102.1 ± 1.55 ^b	103.1 ± 1.32 ^b	138.1 ± 1.42 ^c	140 ± 1.15 ^c	<i>P</i> < 0.05 S
<i>G6P DH (n mol/min)</i>							
Mean ± SE	63.2 ± 0.49 ^a	64.4 ± 0.65 ^a	36.2 ± 0.63 ^b	36 ± 0.59 ^b	53.1 ± 0.72 ^c	52.7 ± 0.66 ^c	<i>P</i> < 0.05 S

Data are expressed as mean ± SE of 6 rats in each group. Within each row, means with different superscript (a, b, c or d) are significantly different at *P* < 0.05. Whereas means superscripts with the same letters mean that there is no significant difference (*P* > 0.05)

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ability of oxidizing and utilizing fatty acids (FA) then it can speed up the clearance of plasma FA (Shan et al. 2010). PAC also inhibits the absorption of cholesterol and bile acids by decreasing micellar cholesterol solubility, and can diminish chylomicron secretion by enterocytes (Blade et al. 2010). As far as cholesterol absorption is concerned, elevated excretion of neutral steroids and bile acids have been described in rats supplemented with PAC (Tebib et al. 1994).

The observed disturbances in testicular enzymes due to GA₃ or IAA administration may result also as a consequence to impairments of their synthesis in cells of tissues with subsequent liberation into the circulation from the damaged tissues and their breakdown (Tuluze and Celik 2006). Significant correlations have been found between semen volume and sperm concentration and γ-GT, ALP and ACP activities, which proposes a primary testicular and

epididymal origin of these enzymes. Significant correlation between γ-GT and sperm motility may be a sign of its function for cell protection against free radicals and spermatozoa from oxidative stress. ALP is essential for metabolic processes, which provide energy for survival, motility and fertility of spermatozoa (Ciereszko et al. 1992). While the obtained enzymatic improvement by administration of PAC may be due to protecting the cell membranes by scavenging the free radicals by the anti-oxidative phyto-chemicals present in PAC, such as flavonoids (Yousef et al. 2009).

The recorded decline in total proteins may be associated with dietary factors influenced by the metabolic events in animals and humans consuming GA₃ in their diet (Srikumar et al. 2009). Alternatively, it may be due to impaired enzymatic antioxidants due to increased oxidative stress induced by

Fig. 1 Histopathological comparison of testicular tissues of untreated control and different treatment groups. Detailed description of groups and explanation of abbreviations are below. Photomicrograph of a 5 μm testis section stained with H&E representative of **a** control group, **b** proanthocyanidin only group, **c**, **d** gibberellic acid group, **e**, **f** indoleacetic acid group,

g gibberellic acid and proanthocyanidin group, and **h** indoleacetic acid and proanthocyanidin group (Scale bar represents 250 μm). *C Sp* cluster of sperms, *IT* inter-tubular connective tissue, *S* Sertoli cell, *SpG* spermatogonia, *ST* seminiferous tubule, *TA* tunica albuginea

the used plant growth regulators (Herken et al. 2001). The adverse effect of the PGRs, GA_3 or IAA, may also be due to the inhibition of protein and enzymes synthesis in the tissues, because of mRNA

breakdown or cellular transcription disruption mechanisms (Celik et al. 2007).

On the other hand, the administration of PAC with GA_3 or IAA enhanced total serum proteins

concentration. This improvement may be due to the antioxidant effects of PAC, which improved the liver cells function and consequently increased protein synthesis by the liver. In addition, PAC directly interferes with free radicals and restores the balance of oxidants/antioxidants leading to improvement of liver functions (Shin et al. 2010).

The current reductions in serum testosterone, epididymal fructose, and sperm count in rats treated with GA_3 and IAA were attributed to the direct testosterone-like action of GA_3 , which inhibits testosterone secretion. This effect of GA_3 and IAA is supported by the observed decrease in ALP activity, which is proposed to have a role in the synthesis of testosterone, and is necessary for the early stages in spermatogenesis (Moussa and Abdel-Hafez 1983). Alternatively, the decreased sperm count may be due to decreased testosterone secretion as spermatogenesis process depends on the action of testosterone (Sharpe et al. 1988). The initiation of spermatogenesis at puberty and the maintenance of this process in adults are also depended on testosterone. It is required for the completion of meiosis and differentiation of the spermatids and maturation and conversion of round to elongated spermatids by promoting the stages VII and VIII of the spermatogenic cycle (O'Donnell et al. 1994). It is confirmed that the conversion of round spermatids to their elongated forms was suppressed when testosterone in the interstitial fluid was only 5 % of the control group concentration (Sun et al. 1990). Additionally, the decreased sperm count may be a result of decreased ACP and ALP enzymes activity. The above enzymes play a pivotal role in the energy generating cycles in sperm metabolism, fertilization process, maintenance of constant osmotic pressure during preservation (Dhami and Kodagali Dhami and Kodagali 1978), and in transamination and phosphorylation process in sperm metabolism, thus explaining the decreased sperm count (Dhami et al. 1994).

Decreased fructose content may be due to decreased testosterone concentration. As, fructose synthesized by seminal vesicles and accessory sex organs under the influence of testosterone, low levels of testosterone may alter the secretion activity of the seminal vesicles affecting the synthesis of fructose (Rocha 1999). Additionally, most of the energy needed by spermatozoa for motility comes from fructose oxidation in the process of anaerobic glycolysis, which produces lactic acid. So, decreased

fructose content may be related to decreased sperm count and quality (Dogan et al. 2009). Moreover, H_2O_2 even at physiological concentrations affects Leydig's cell function and induces oxidative stress and apoptosis (Gautam et al. 2006) and this is proposed by increased H_2O_2 in this study. On the other hand, the administration of PAC increased serum testosterone and epididymal fructose and sperm count. This beneficial effect was attributed to the reduction in lipid peroxidation potential and subsequently improved the testicular function (Hassan 2005). Additionally, the positive effects of PAC on sperm count could be linked to its antioxidative properties (Eid 2008). The higher seminal quality index of the PAC-treated groups is linked also to higher ACP activity in their seminal plasma (Kamel 2005). The mechanisms by which PAC exerts its protective action could be by modulating the estrogen response systems which is involved in male reproductive biology, enhancing sperm production by stimulating the hypothalamic-pituitary-gonadal axis and increasing the secretion of gonadotrophins, leading in turn to an increment in testosterone and sperm output (Juan et al. 2005). Also enhanced sperm motility and quality with PAC administration could, in part, be attributed to the concomitant induction in semen fructose, as high quality semen should contain a high percentage of vigorous and active sperms as well as having higher glycolytic or fructolytic rates than weak immobile sperms (Yousef et al. 2003).

In this context, the obtained elevation in testis MDA and H_2O_2 levels due to GA_3 or IAA may be due to that PGRs are associated with the generation of reactive oxygen species (ROS) which interacts with tissues leading to numerous pathophysiological alterations (Soliman et al. 2010). ROS could damage every major cellular component, including membranes, lipids, carbohydrates and DNA (Bhalla and Dhawan 2009; Chen et al. 2012). Therefore, GA_3 treatment might cause peroxidation of polyunsaturated fatty acids leading to the degeneration of PL, and cellular deterioration (Troudi et al. 2011). In addition, the increased content of MDA may result from an increase of hydroxyl radicals that can accelerate lipid peroxidation and this is attributed to the formation of peroxy radicals that may react with the lipids possibly by hydrogen abstraction leading to male infertility (Herken et al. 2001). Furthermore, ROS can modify the spermatozoon cytoskeleton and axoneme resulting

in a reduction of sperm motility and the inhibition of sperm oocyte fusion, which in turn leads to reduced fertility (Eid 2008). The decline in the antioxidants effect may also be due to the antioxidants utilization in challenging the prevailing oxidative stress, under the excessive influence of ROS generated by the PGRs. The PGRs, IAA and GA₃, also might have interacted with the tissues, resulting in fluctuated enzymes activities by the way of increased free radicals as a result of stress condition in rats (Celik et al. 2007).

However, the diminished MDA and H₂O₂ concentrations by administration of PAC may be related to the capacity of PAC to trap reactive oxygen species in aqueous series such as plasma and interstitial fluid of the arterial wall (Yamakoshi et al. 1999). Moreover, PAC has demonstrated inhibition of oxidative stress through modulation of metabolic functions, enhancement of detoxification pathways, and/or prevention of the interaction of xenobiotics with biological molecules (Bagchi et al. 2000). However, PAC contains mainly flavonoids, all involved in ameliorating the oxidative stress in vitro and in vivo by increasing the endogenous antioxidant status (Hassan 2005; Hassan and Abdel-Aziz 2010). PAC ingestion increased GSH level may ultimately increase G6PDH level to near normal values and this may be due to the protection of SH groups in GSH from oxidative damage via free radical quenching action of di-HO (catechol) structure in the B ring of PAC (Ishige et al. 2001). The improvement in SOD activity may be due to the potential quenching of free radicals by PAC through the formation of resonance stabilized phenoxyl radical, which significantly decrease the levels of superoxide radicals (Rice-Evans et al. 1996). This may be attributed to the antioxidant activity of phenolic PAC that act as hydrogen or electron donor and stabilize radical intermediates, thus preventing oxidation of various food ingredients (Cetin et al. 2008).

Furthermore, the obtained adverse effects of GA₃ and IAA on the histological structure of the testis may be attributed to the current oxidative stress evidenced by increased lipid peroxidation product, MDA. The obtained results including disturbances in spermatogenesis including spermatogenic arrest, degenerative changes in the testis, moderate tubular necrosis, Leydig's cell degeneration and reduction in the size of the seminiferous tubules, with some spermatogonia detached from the basement membrane may be all related to free radicals production as well. It was

evidenced that the reactive oxygen resulting from oxidative stress in the tissue cause a significant alteration in the cellular structure resulting in tissue injury and cell damage. This is in accordance with Ravikumar and Srikumar (2005). On the other hand, the administration of PAC caused significant histopathological improvement in testicular tissues. This result may be related to the reduction of lipid peroxidation through scavenging ROS and its antioxidative activity (Jiao et al. 2005).

Conclusion

The obtained results revealed the adverse effects of the plant growth regulators, gibberellic acid or indoleacetic acid, on the testicular functions through increasing the oxidative stress and inhibiting the endogenous antioxidant system. However, supplementation with proanthocyanidins (grape seed extract) had ameliorative effects on those negative effects of the mentioned plant growth regulators through its natural antioxidant properties.

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