Proanthocyanidin as a cytogenetic protective agent against adverse effects of plant growth regulators supplementation in rats

Hanaa A. Hassan · Wafaa M. El-Kholy ·
Samar E. Nour

Received: 14 January 2013 / Accepted: 12 June 2013 / Published online: 31 July 2013
© Springer Science+Business Media Dordrecht 2013

Abstract The aim of the present study was to investigate the protective role of grape seed extract (containing proanthocyanidin) against the adverse effects of plant growth regulators (GA₃ (gibberellic acid) and IAA (indoleacetic acid)). The present data showed that the administration of either GA₃ and IAA caused undesirable changes in both hepatic and testicular structure. This was evidenced by a disturbed hepatic strands, pyknotic nuclei, central vein with collapsed endothelium, dilatation in bile sinusoids, congested blood vessel, binucleated hepatocytes, lymphocytic infiltration, vacuolation, giant hepatic cells, increased Kupffer cells and karyorrhexis. Additionally, it was shown that degenerative changes in the testis, spermatogenic arrest, moderate tubular necrosis, Leydig cell degeneration and reduction in the number and size of the seminiferous tubules with some spermatogonia detached from the basement membrane. Concerning flow cytometric study of the liver a significant decrease in G0/1 % and a significant increase in S phase %, G2/M %, P⁵³ % and apoptosis % (sub G1) were detected. However, in testis the data recorded a significant decrease in the percentage of mature sperm (percentage of haploid cells) and a significant increase in the percentage of spermatide, diploid cells, P⁵³ and of

apoptotic cells. On the other hand, a distinct recovery of the mentioned hepatic and testicular histopathological and cytogenetic disorders was observed when proanthocyanidin was supplemented to rats administered either of the plant growth hormones (GA₃ and IAA).

Keywords Plant growth regulators · Gibberellic acid · Indoleacetic acid · Proanthocyanidin

Abbreviations

PGRs Plant growth regulators
GA₃ Gibberellic acid
IAA Indoleacetic acid
GSE Grape seed extract
PAC Proanthocyanidin

Introduction

Plant growth regulators (PGRs) or phytohormones are not nutrients, but chemicals that promote and influence the growth, development and differentiation of cells and tissues (Helgi and Stephen 2005). There are six major classes of growth promoting hormones—Abscissic Acid, Auxins, Cytokinins, Ethylene, Gibberellins and Polyamines. However, many chemicals are currently used in agriculture, and plant growth regulators (PGRs) are among those widely employed. The amounts of these substances placed into the environment may soon exceed those of insecticides. Although

H. A. Hassan (✉) · W. M. El-Kholy · S. E. Nour
Physiology Division, Zoology Department, Faculty
of Science, Mansoura University, Mansoura, Egypt
e-mail: drhanaahassan@yahoo.com;
drhanaahassan@mans.edu.eg

PGRs are used for pest control and their use on a wide variety of crops is increasing, there are hazardous physiological effects on different mammalian organs (Celik et al. 2006). Among PGRs, gibberellin A₃ (GA₃), it is widely used as a plant growth and flowering regulator in many countries, however, it has potential hazardous effects on human health (Chaari-Rkhis et al. 2006). It could exert toxic effects on many soft organs including the liver (Tuluze and Celik 2006). Furthermore, several studies demonstrated that, the chronic consumption of GA₃ increased tumor formation and oxidative stress (Erin et al. 2008). Alternatively, indole-3-acetic acid (IAA) is the most abundant naturally occurring auxin, well known for its regulatory function in plant growth (Rapparini et al. 2002). A toxic effect of IAA on animal organisms was showed through generation of reactive oxygen species (ROS) and excited oxygen intermediates resulting in renal dysfunction, hypoglycemia, and myotonia (Yilmaz and Yuksel 2004).

As a prophylactic action, a large number of synthetic and natural antioxidants have been demonstrated to induce beneficial effects on human health and disease prevention. However, the structure–activity relationship, bioavailability and therapeutic efficacy of the antioxidants differ extensively. Grapes (*Vitis vinifera* L.) belong to the world's largest fruit crops with a global production of around 69 million tons in 2006 (Faostat 2007). Proanthocyanidin contain catechin monomer, dimer and trimer, all of which are water-soluble molecules and contain a number of phenolic hydroxyls (Bagchi et al. 2002; Serrano et al. 2009). Oligomeric proanthocyanidin, a naturally occurring antioxidant has been reported to possess a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress (Bagchi et al. 2000; Spranger et al. 2008; Hassan et al. 2013). Besides antioxidant activity, proanthocyanidin has been described as antimicrobial compound, anti-cancer agent, anti-inflammatory agent with cardioprotective properties (Serrano et al. 2009) and as anti-fatigue (Shan et al. 2010) hepato-reno protective agent (Hassan and Al-Rawi 2012).

Therefore, the present study aims to investigate the possible protective role of PAC against the hepatic and testicular adverse effects induced by two widely used plant growth regulators, GA₃ and IAA.

Materials and methods

Chemicals

GA₃, IAA and all chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA). PAC was purchased from the Arab Company for Gelatin and Pharmaceutical Products (Alexandria, Egypt) under licence of Nulife International U.S.A.

Experimental animals

Adult male albino rats (*Rattus rattus*) weighing about 130–140 g were used in this study. Experimental animals were housed in cages with free access to drinking water and diet and were kept at 22–24 °C with 12 h light/dark cycle. All animals received human care and our study complies with the institutional guidelines.

Animal grouping and design

The animals were divided into six groups, 6 rats each; control group, proanthocyanidin (PAC) group, gibberellic acid (GA₃) group, indoleacetic acid (IAA) group, gibberellic acid and proanthocyanidin (GA₃ + PAC) group and indoleacetic acid and proanthocyanidin (IAA + PAC) group. The animals received GA₃ or IAA in drinking water at a dose of 75 ppm/L (Celik et al. 2007) and 100 ppm/L (Celik et al. 2006) respectively. While, PCA was freshly prepared and orally given daily to rats at a dose of 100 mg/kg bw (28 g of PAC was dissolved in 1 L of water) equivalent to the human therapeutic dose (Ghosh 1971) using gastric intubation (Karthikeyan et al. 2007). All treatments were continued for 60 days 6 days a week.

At the end of the experimental period, overnight fasted rats were sacrificed and both liver and testis were quickly cleaned, dissected, rinsed with isotonic saline. Samples were taken for histopathological and flowcytometric studies.

Histopathological studies

Liver and testis samples were carefully fixed in neutral formalin solution 10 %, 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 et al. 1976).

Flow cytometric analysis

Flow cytometric analysis of cell cycle DNA and percentage of apoptotic cells by propidium iodide: Frozen tissues of liver and testis were suspended in 800 μL PBS. Hundred microliters RNase (1 mg/mL PBS) and 100 μL propidium iodide (400 $\mu\text{g/mL}$) were added and incubated at 37 °C for 30 min. The analysis of apoptotic cell death was performed by measuring the DNA contents using the method described by Nicoletti et al. (2001) using a FACS calibur flow cytometer (Becton–Dickinson, Sunnyvale, CA, USA) equipped with a compact air cooled low power 15 m watt argon iron laser beam (488 nm). The average number of the evaluated nuclei per specimen was 20,000 and the number of nuclei scanned was 120 per second. DNA histogram derived from flow cytometry was obtained with a computer program for mathematical analysis (Dean and Jett 1974). Flow cytometry P^{53} protein expression was performed using fluorescence threshold using a FACS Calibur Flow Cytometer (Becton Dickinson, Sunnyvale, CA, USA) as described by Brothrick et al. (1995).

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 the *Duncan test*. The results were expressed as mean $\pm$ standard error (SE). The values of $p \leq 0.05$ were considered statistically significant (Snedecor and Cochran 1982).

Results

Histopathological observation of liver

As shown in Fig. 1a, b, the histopathological observation of livers in control (A) and proanthocyanidin (B) groups showed normal liver cells (hepatocytes)

(HC), hepatic strands (HS), hepatic sinusoids, central vein (CV), endothelium (EN), and Kupffer cells (KC). However, treatment with either GA_3 or IAA exerted histopathological effects on the hepatic tissue illustrated in; disturbed hepatic strands (DHS), pyknotic nuclei (PKN), central vein with collapsed endothelium (CE), dilatation in bile sinusoids (DBS), congested blood vessel (CBV), binucleated hepatocytes (BN), lymphocytic infiltration (LI), vacuolation (V), giant hepatic cells (GH), more Kupffer cells (KC) and karyorrhexis (K) (Fig. 1c–e). On the other hand, combined treatment of GA_3 + PAC or IAA + PAC revealed quitenormal hepatic strands and hepatocytes, normal central vein without congestion, little dilatation of bile sinusoids, few binucleated hepatocytes and few Kupffer cells (Fig. 1f, g).

Histopathological observation of testis

Histopathological observation of testis 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 the successive stages of spermatogenesis with supporting cells of Sertoli scattered in between. Numerous rounded or hemispherical spermatogonia were lying adjacent to the basal lamina. The primary spermatocytes were the largest among different stages whereas the spermatids were the smallest spermatogenic cells and constituted the most superficial layers of the germinal epithelium. Mature spermatozoa heads appeared directed towards the elongated Sertoli cells (Fig. 2a, b). However, histopathological investigation of sections of the testis in GA_3 or IAA treated rats showed disturbances in spermatogenesis process including degenerative changes in the testis with sever disintegration of spermatocytes, spermatogenic arrest with moderate tubular necrosis and Leydig cell degeneration. In addition, there was a reduction in the size of the seminiferous tubule, detached spermatogonia from the basement membrane; tunica albuginea (TA) and sperm degeneration (Fig. 2c–f). Furthermore, the treatment with IAA showed in some cases the complete absence of all spermatogenesis stages. On the other hand, combined treatment of GA_3 + PAC or IAA + PAC revealed some improvement in

◀ **Fig. 1** Photomicrograph of a 5 μ m liver section stained with H&E, representative of (a) control group, b proanthocyanidin group, c, d gibberellic acid group, e indoleacetic acid group, f gibberellic acid and proanthocyanidin group and g indoleacetic acid and proanthocyanidin group (Scale bar represents 400 μ m). BN binucleated hepatocytes, BS bile sinusoids, CBV congested blood vessel, CV central vein, DBS dilatation in bile sinusoids, DHS disturbed hepatic strands, EN normal endothelium, GH giant hepatic cells, HC hepatocytes, HS hepatic strands, K karyorrhexis, KC Kupffer cells, LI lymphocytic infiltration, PKN pyknotic nuclei, V vacuolation

spermatogenesis process as judged by the presence of all stages of sperm formation, quite normal testicular histoarchitecture and seminiferous tubules tightly connected together with intertubular connective tissue (IT) (Fig. 2g, h).

Flow cytometric observation of liver and testis

Regarding flow cytometric observation of the liver (Table 1), the data indicated that there was significant decrease in the percentage of G0/1 and significant increases in the percentage of S phase, the percentage of G2/M, of P^{53} and of apoptosis in rats treated with GA_3 and IAA compared to the control group. Meanwhile, there was significant increase in the percentage of G0/1 and significant decreases in the percentage of S phase, of G2/M, of P^{53} and of apoptosis in rats treated with GA_3 or IAA and PAC compared to the matching control groups. G0/1 % is still lower, while S phase % and G2/M % are still higher. However, in testis (Table 2), the flow cytometric data recorded a significant decrease in the percentage of mature sperm (percentage of haploid) and significant increase in the percentage of spermatide, of diploid, of P^{53} and of apoptosis. On the other hand, a distinct recovery of the mentioned hepatic and testicular cytogenetic disorders was observed when proanthocyanidin was supplemented to rats administered either of the plant growth hormones (Figs. 3, 4).

Discussion

There is growing evidence that the presence of various chemical contaminants in the natural environment and food including plant growth regulators can adversely affect health. Their potential hazardous effects on human health are relatively unexplored (Troudi et al.

2010). In the present study, gibberellic acid (GA_3) and indoleacetic acid (IAA) were chosen because information on their toxicological biological effects in higher animals is very limited. Proanthocyanidin is the most abundant polyphenol in human diets (Blad et al. 2010). Recent studies suggest that in vivo grape seeds proanthocyanidin exposure may protect multiple organs from a variety of toxic materials (Yilmaz and Toledo 2004).

The current results showed that GA_3 or IAA exerted histopathological effects on the liver (disturbed hepatic strands, pyknotic nuclei, central vein with collapsed endothelium, dilatation in bile sinusoids, congested blood vessel, binucleated hepatocytes, lymphocytic infiltration, vacuolation, giant hepatic cells, increased Kupffer cells and karyorrhexis). This is in accordance with Troudi et al. (2009) who observed that intrahepatic blood vessels, central and portal veins are congested and their lining epithelia are eroded. This is besides an inflammatory leucocytic infiltrations, which were more pronounced in the livers of treated mother rats than in their offspring when mother rats were given daily 200 ppm GA_3 in drinking water from the 14th day of pregnancy until day 14 after delivery. Obtained results also revealed that GA_3 or IAA exerted histopathological effects on the testis (disturbances in spermatogenesis process, degenerative changes in the testis, spermatogenic arrest, moderate tubular necrosis, Leydig cell degeneration and reduction in the size of the seminiferous tubule with some spermatogonia detached from the basement membrane). Such results are in accordance with Ravikumar and Sri Kumar (2005).

The leucocytic infiltrations were considered as a prominent response of the body tissue facing any injurious impacts (Abdel-Rahaman and Zaki 1992). The interpretation of vacuolar formation may be due to the increased amount of ROS product which might attack membranes and enhance their permeability leading to vacuolization, the primary response to all forms of cell injury or to the marked disturbances in lipid inclusions as a result of injurious treatments as shown by Sherlock and Doely (1993). According to these authors, the enhanced permeability of cell membranes leads to an increase in intracellular water which sufficiently accumulates within the cell and produces cytoplasmic vacuolization.

Moreover, oxidative stress is presumed to play an important role in hepatic fibrogenesis and several

- ◀ **Fig. 2** Photomicrograph of a 5 μ m testis section stained with H&E, representative of (a) control group, b proanthocyanidin 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 μ m). C.Sp cluster of sperms, IT intertubular connective tissue, S Sertoli cell, Sp.G spermatogonia, ST seminiferous tubule, TA tunica albuginea

reports of liver pathology show a significant alteration of cellular structure (Penteado et al. 2002; Sarandol et al. 2005).

The administration of proanthocyanidin (PAC) caused significant histopathological improvement in hepatic and testicular tissues. This is in agreement with Hassan (2005), Juan et al. (2005), Dulundu et al. (2007), Sehri et al. (2008) and Shin et al. (2010). The last authors found that PAC remarkably reduced extensive hemorrhagic, necrosis and disruption of tissue architecture of hepatocytes due to dimethylnitrosamine administration and may be useful in preventing the development of hepatic fibrosis of rats.

The hepatoprotective effects of PAC may decrease paracrine stimuli which lead to hepatic fibrosis via activated hepatic stellate cells (HSCs), so the antifibrotic effect of PAC may be due to suppressed HSC activation via hepatoprotective effect. Also, the mechanism for the hepatoprotective effects of PAC in the development of liver fibrosis may be related to the reduction of lipid peroxidation by its antioxidative activity (Shin et al. 2010). Moreover, it can exert its effect by scavenging ROS (Hemmati et al. 2008).

Additionally, the protective effect of PAC on testis occurred probably through preventing the damage of testicular tissue induced by free radicals due to its enhanced degradation (Hassan et al. 2013) as well as to enhancement of the antioxidant defense system.

The recorded increment in the percentage of apoptosis and of P^{53} may be due to the fact that IAA produces free radicals including indolyl, skatolyl, peroxy radicals and ROS, such as, O_2^- and H_2O_2 (Kawano et al. 2001). These ROS are well known to cause structural changes in plasma membranes via lipid peroxidation and to initiate cellular damage and

Table 1 Liver DNA cell cycle analysis, P^{53} % and apoptosis % (sub G1) in control and different treated rat groups

	Animal groups						ANOVA
	Control	PAC	GA ₃	IAA	GA ₃ + PAC	IAA + PAC	<i>p</i>
<i>DNA cell cycle</i>							
<i>M1 (G0/1 %)</i>							
Mean ± SE	61.90	63.00	21.50	19.50	47.90	47.40	<0.05
	0.61 ^a	0.66 ^a	0.86 ^b	1.16 ^b	0.57 ^c	0.34 ^c	
<i>S phase %</i>							
Mean ± SE	31.70	31.00	50.00	54.00	37.90	39.20	<0.05
	0.67 ^a	0.54 ^a	0.78 ^b	0.58 ^c	0.67 ^d	0.32 ^d	S
<i>M2 (G2/M %)</i>							
Mean ± SE	6.40	6.00	28.50	26.50	14.20	13.40	<0.05
	0.48 ^a	0.27 ^a	1.06 ^b	0.65 ^c	0.43 ^d	0.58 ^d	S
<i>P⁵³ %</i>							
Mean ± SE	9.00	8.40	19.10	21.10	11.60	11.90	<0.05
	0.23 ^a	0.31 ^a	0.45 ^b	0.66 ^c	0.21 ^d	0.31 ^d	S
<i>Apoptosis %</i>							
Mean ± SE	1.10	0.00	20.90	28.00	4.00	6.80	<0.05
	0.41 ^a	0.00 ^a	0.65 ^b	0.58 ^c	0.23 ^d	0.32 ^e	S

Data are expressed as mean $\pm$ SE of 6 rats in each group. Within each row, means with different superscripts (a, b, c, d, e) were 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

Table 2 Testis DNA cell cycle analysis, P⁵³ % and apoptosis % (sub G1) in control and different treated rat groups

	Animal groups						ANOVA
	C	PAC	GA ₃	IAA	GA ₃ + PAC	IAA + PAC	<i>p</i>
MS %							
Mean ± SE	81.1	91.8	21.7	26.5	69	75	<0.05
	0.68 ^a	0.87 ^b	0.66 ^c	0.47 ^d	0.50 ^e	0.67 ^f	S
Sp %							
Mean ± SE	8.4	5.9	67.6	58.9	19.8	15.4	<0.05
	0.26 ^a	0.23 ^b	0.56 ^c	0.52 ^d	0.56 ^e	0.53 ^f	S
H %							
Mean ± SE	75.1	86.5	27.5	33.1	66.7	71.9	<0.05
	0.62 ^a	0.34 ^b	0.33 ^c	0.67 ^d	0.78 ^e	0.54 ^f	S
M3 (D %)							
Mean ± SE	24.9	13.5	72.5	66.9	33.3	28.1	<0.05
	0.62 ^a	0.34 ^b	0.33 ^c	0.67 ^d	0.78 ^e	0.54 ^f	S
P ⁵³ %							
Mean ± SE	6.4	5.3	18.4	16.4	10.9	9.5	<0.05
	0.33 ^a	0.38 ^b	0.28 ^c	0.31 ^d	0.19 ^e	0.31 ^f	S
Apoptosis %							
Mean ± SE	0.9	0.0	55.3	36.1	13.1	10.4	<0.05
	0.39 ^a	0.00 ^a	0.82 ^b	0.62 ^c	0.56 ^d	0.35 ^e	S

Data are expressed as mean ± SE of 6 rats in each group. Within each row, means with different superscripts (a, b, c, d, f) were 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

MS % Mature sperm %, H % haploid %, Sp % spermatide %, D % diploid %

apoptosis (Bongarzone et al. 1995). However, the cytotoxic effect of IAA has been postulated to be caused by the products of the peroxidation of this acid leading to the formation of ROS (Pires de Mel et al. 1992). Alternatively, reactive oxygen molecules (ROM) can trigger several of the apoptotic mechanisms activated by GA₃ and IAA. For example, ROM can activate P⁵³, alter mitochondria permeability, release cytochrome C into the cytosol and even directly activate the caspases. The caspase pathway plays a key role in apoptotic cell death (Hengartner 2000), and the activation of caspase-3 mediates the cleavage of protein substrates, such as poly-(ADP-ribose) polymerase (PARP) and the nuclear lamins (Nicholson et al. 1995). IAA/HRP induces both caspase-3 activation and PARP cleavage, both of which were blocked by catalase/NADPH treatment. Thus, these results indicated that H₂O₂ is intimately

involved in the IAA/HRP-induced activations of JNK and p38, caspase-3 activation and PARP cleavage (Kim et al. 2006).

In contrast, the present study showed that the percentage of apoptosis (sub G1) and of P⁵³ in liver and testis of rats treated with GA₃ or IAA were significantly decreased by the administration of proanthocyanidin (PAC). This beneficial effect may be due to the fact that PAC has remarkable scavenging activities especially for hydrophilic radicals such as peroxy radicals and also scavenges superoxide radical, quenches singlet oxygen and chelates iron (Ariga 2004; Hassan and Abdel-Aziz 2010). Moreover, grape seed extract (GSE) can block cell death signaling mediated through the proapoptotic transcription factors and genes such as c-jun or in other words by suppressing the proapoptotic protein c-jun (Feng et al. 2007). Above all, proanthocyanidin has a strong

Fig. 3 Liver DNA cell cycle analysis in control and different treated rat groups. *M1* Percent of G0/1, *M2* Percent of G2/M

inhibitory effect towards hydrogen peroxide (Zhu et al. 2002) which is an important cause for apoptosis process, so, decreasing hydrogen peroxide concentration leads to decreased percentage of apoptosis.

Conclusion

The results obtained in the current study revealed a highly adverse action of plant growth regulators (GA₃

Fig. 4 Testis DNA cell cycle analysis in control and different treated rat groups. *M1* Percent of sperm, *M2* Percent of spermatide, *M3* Percent of diploid

and IAA) on the liver and testis functions and structure. However, proanthocyanidin administration has an ameliorative role against these risks by scavenging and reducing the ROS generated by GA₃ and IAA as well as through enhancing the antioxidant

defense mechanism. Therefore, the present study recommends to minimize the use of the plant growth hormones and use proanthocyanidin, as a natural antioxidant component to ameliorate the hazards of such plant growth regulators.

Acknowledgments The authors of this manuscript express their deepest gratitude and appreciation to Professor Dr Shreef Abdeen, Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt for his financial assistance in histopathological examination in the present work.

References

- Abdel-Rahaman M, Zaki TZ (1992) Cytotoxic action of malathion on renal and hepatic tissues of mice. *J Egypt Germ Soc Zool* 8:105–114
- Ariga T (2004) The antioxidant function, preventive action on disease and utilization of proanthocyanidins. *BioFactors* 21:197–201
- Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, Joshi SS, Pruess HG (2000) Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. *Toxicology* 148:187–197
- Bagchi D, Bagchi M, Stohs SJ, Ray SD, Sen CK, Pruess HG (2002) Cellular protection with proanthocyanidins derived from grape seeds. *Ann New York Acad Sci* 957:260–270
- Blad C, Arola L, Salvado MJ (2010) Hypolipidemic effects of proanthocyanidins and their underlying biochemical and molecular mechanisms. *Mol Nutr Food Res* 54:37–59
- Bongarzone ER, Pasquini JM, Soto EF (1995) Oxidative damage to proteins and lipids of CNS myelin produced by in vitro generated reactive oxygen species. *J Neurosci Res* 41:213–221
- Brotherick I, Shenton BK, Cowan WK, Angus B, Horne CHW, Higgs MJ, Lennard TWJ (1995) P53 expression measured by flow cytometry. A comparison of three monoclonal antibodies and the relationship with grade and DNA ploidy in breast cancer. *Cancer Immunol Immunother* 41:146–150
- Celik I, Tuluce Y, Turker M (2006) Antioxidant and immune potential marker enzymes assessment in the various tissues of rats exposed to indoleacetic acid and kinetin: a drinking water study. *Pestic Biochem Physiol* 86:180–185
- Celik I, Tuluce Y, Isik I (2007) Evaluation of toxicity of abscisic acid and gibberellic acid in rats: 50 days drinking water study. *J Enzyme Inhib Med Chem* 22:219–226
- Chaari-Rkhis A, Maalej, OuledMessaoud S, Drira N (2006) In vitro vegetative growth and flowering of olive tree in response to GA3 treatment. *Afr J Biotechnol* 5:2097–2302
- Dean PN, Jett JH (1974) Mathematical analysis of DNA distributions derived from flow microfluorometry. *J Cell Biol* 60:523–527
- Drury RA, Wallington EA, Cancerson R (1976) *Carlton, s histopathological techniques*, 4th edn. Oxford University Press, Oxford
- Dulundu E, Ozel Y, Topaloglu U (2007) Grape seed extract reduces oxidative stress and fibrosis in experimental biliary obstruction. *J Gastroenterol Hepatol* 22:885–892
- Erin N, Afacan B, Ersoy Y, Ercan F, Balci MK (2008) Gibberellic acid, a plant growth regulator, increases mast cell recruitment and alters substance p levels. *Toxicology* 254:75–81
- Faostat (2007) FAO statistical database. <http://www.faostat.fao.org/>
- Feng Y, Liu Y, Leblanc MH, Bhatt AJ, Rhodes PG (2007) Grape seed extract given three hours after injury suppresses lipid peroxidation and reduces hypoxic ischemic brain injury in neonatal rats. *Pediatr Res* 61:295–300
- Ghosh MN (1971) *Fundamentals of experimental pharmacology*. Academic Press, New York, p 85
- Hassan HA (2005) The ameliorating effect of black grape juice on fluoride induced testicular toxicity in adult albino rats. *Egypt J Zool* 44:507–525
- Hassan HA, Abdel-Aziz AF (2010) Evaluation of free radical-scavenging and anti-oxidant properties of black berry against fluoride toxicity in rats. *Food Chem Toxicol* 48:1999–2004
- Hassan HA, Al-Rawi MM (2012) Evaluation Grape seeds proanthocyanidin extract as a hepatic-reno-protective agent against gibberellic acid induced oxidative stress and cellular alterations. *Cytotechnology* (in press)
- Hassan HA, Isa AM, El-Kholy WM, Nour SE (2013) Testicular disorders induced by plant growth regulators: cellular protection with proanthocyanidins grape seeds extract. *Cytotechnology*. doi:10.1007/s10616-012-9525-3
- Helgi Ö, Stephen AR (2005) *The physiology of flowering plants*. Published Cambridge University Press Plant physiology, p 191
- Hemmati AA, Nazari Z, Samei M (2008) A comparative study of grape seed extract and vitamin E effects on silica induced pulmonary fibrosis in rats. *Pulm Pharm Ther* 21:668–674
- Hengartner MO (2000) The biochemistry of apoptosis. *Nature* 407:770–776
- Juan ME, Gonzalez-Pons E, Munuera T, Ballester J, Rodriguez-Gil JE, Planas JM (2005) Trans-resveratrol, a natural antioxidant from grapes, increases sperm output in healthy rats. *J Nutr* 135:757–760
- Karthikeyan K, Sarala Bai BR, Niranjali Devaraj S (2007) Cardioprotective effect of grape seed proanthocyanidins on isoproterenol induced myocardial injury in rats. *Int J Cardiol* 115:326–333
- Kawano T, Kawano N, Hosoya H, Lapeyrie F (2001) Fungal auxin antagonist hypaphorine competitively inhibits indole-3-cetic acid dependent superoxide generation by horseradish peroxidase. *Biochem Biophys Res Commun* 288:546–551
- Kim DS, Jeon SE, Jeong YM, Kim SY, Kwon SB, Park KC (2006) Hydrogen peroxide is a mediator of indol-3-acetic acid/horseradish peroxidase induced apoptosis. *FEBS Lett* 580:1439–1446
- Nicholson DW, Ali A, Thornberry NA, Vaillancourt JP, Ding CK (1995) Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature* 376:37–43
- Nicoletti I, Mannucci R, Miglioni G, Riccardi C, Grignani F (2001) Common method for measuring apoptotic cell death by flow cytometry. *Purdue Cytom CD-ROM Ser* 3:1–9
- Penteado FC, Ferreira HH, Calafati SC, Bernasconi G, Piovesana H, Pedrazzoli J (2002) Neutrophil migration during liver cirrhosis in rabbits. *Clin Exp Pharmacol Physiol* 29:285–290
- Pires de Mel M, Escobar JA, Metodiewa D, Dunford HB, Cilento G (1992) Horseradish peroxidase catalyzed oxidation

- of indole-3-acetic acid II: oxygen uptake and chemiexcitation. *Arch Biochem Biophys* 296:34–39
- Rapparini F, Tam YY, Cohen JD, Slovin JP (2002) Indole-3-acetic acid metabolism in *lemna gibba* undergoes dynamic changes in response to growth temperature. *Plant Physiol* 128:1410–1416
- Ravikumar S, Srikumar K (2005) Metabolic dysregulation and inhibition of spermatogenesis by gibberellic acid in rat testicular cells. *J Environ Biol* 26:567–569
- Sarandol E, Ta S, Dirican M, Serdar Z (2005) Oxidative stress and serum paraoxonase activity in experimental hypothyroidism: effect of vitamin E supplementation. *Cell Biochem Funct* 23:1–8
- Sehirli O, Ozel Y, Dulundu E (2008) Grape seed extract treatment reduces hepatic ischemia reperfusion injury in rats. *Phytother Res* 22:43–48
- Serrano J, Puupponen-Pimia R, Dauer A, Aura AM, Sauracalixto F (2009) Tannins: current knowledge of food sources, intake, bioavailability and biological effects. *Mol Nutr Food Res* 53:310–329
- Shan Y, Ye X, Xin H (2010) Effect of the grape seed proanthocyanidin extract on the free radical and energy metabolism indicators during the movement. *Sci Res* 5:148–153
- Sherlock S, Doely J (1993) Diseases of the liver and biliary system, 9th edn. Blackwell Scientific Publication, Cambridge
- Shin MO, Yoon S, Moon JO (2010) The proanthocyanidins inhibit dimethylnitrosamine induced liver damage in rats. *Arch Pharm Res* 33:167–173
- Snedecor GW, Cochran WG (1982) Statistical methods, 7th edn. The State University Press American, Iowa, p 593
- Spranger I, Sun B, Mateus AM, Freitas V, Ricardo-da-Silva JM (2008) Chemical characterization and antioxidant activities of oligomeric and polymeric procyanidin fractions from grape seeds. *Food Chem* 108:519–532
- Troudi A, Samet AM, Zeghal N (2009) Hepatotoxicity induced by gibberellic acid in adult rats and their progeny. *Exp Toxicol Pathol* 62:637–642
- Troudi A, Amara IB, Soudani N, Bouaziz H, Boudawara T, Zeghal N (2010) Oxidative stress induced by gibberellic acid in bone of suckling rats. *Ecotoxicol Environ Saf* 74:643–649
- Tuluze Y, Celik I (2006) Influence of subacute and subchronic treatment of abscisic acid and gibberellic acid on serum marker enzymes and erythrocyte and tissue antioxidant defense systems and lipid peroxidation in rats. *Pestic Biochem Physiol* 86:85–92
- Yilmaz Y, Toledo RT (2004) Health aspects of functional grape seed constituents. *Trends Food Sci Technol* 15:422–433
- Yilmaz HR, Yuksel E (2004) The effect of Indole-3-acetic acid on some metabolic enzymes in kidney of the second cross maternal mice and their offsprings. *East J Med* 9:1–3
- Zhu QY, Holt RR, Lazarus SA, Orozco TJ, Keen CL (2002) Inhibitory effects of cocoa flavanols and procyanidin oligomers on free radical induced erythrocyte hemolysis. *Exp Biol Med* 227:321–329