Phytic Acid Ameliorates Acrylamide-Induced Genotoxicity and Biochemical Disturbance in Mice

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Abstract: Acrylamide (AA) has sparked renewed interest in assessing human health hazards in addition to the long-term risk associated with exposure to vinyl compounds. For this reason, this study aims to evaluate the chemo-protective efficacy of Phytic Acid (PA) against AA- induced genotoxicity and biochemical disturbance in mice *in vivo*. PA is found in wheat and rice bran, legumes and virtually every kind of mammalian cell. It plays an important role in regulating vital cellular functions, including cell proliferation and differentiation. For genotoxicity, using of different cytogenetic endpoints such as Micronucleus test (MN) and chromosome aberrations in somatic and germ cells as well as sperm abnormalities were evaluated in the tested mice. The biochemical assays included ALT, AST, AlP, cholesterol, triglycerides, TBARS, GSH and testosterone. Male Swiss mice were treated orally with AA and/or PA at doses 10 and/or 30mg/ kg b. wt/ day for 1, 7 and 14 days respectively. The percentages of MNPCE and chromosome aberrations in somatic and germ cells were increased significantly with the duration of time after treatment with AA. Consecutive administration of PA with AA for 1, 7 and 14 days reduced these percentages statistically in high significance. AA also induced a significant increase in the percentage of sperm shape abnormalities. This percentage was reduced with animals administered PA. For the biochemical analysis, AA induced elevation in ALT, AST, ALP, total cholesterol, triglycerides and TBARS. Also, significant decrease in GSH content and testosterone were recorded after 14 days from treatments with AA. Administration of PA with AA regulated the biochemical disturbance induced by AA. In conclusion, the results demonstrate the protective role of PA against the genotoxicity and biochemical disturbance of AA. [Nature and Science 2010;8(9):168-177]. (ISSN: 1545-0740).

Key words: Acrylamide, Phytic acid, Micronuclei, Chromosome aberrations, Sperm abnormalities, Oxidative stress, Liver function.

1. Introduction

Acrylamide (AA) is a high-production vinyl compound whose polymeric form is used in the construction and oil industry, in the manufacture of paper, plastics and textiles, as a flocculant in the treatment of waste water, and in cosmetics (Friedman, 2003). Worldwide public concern has been caused by the finding that the same compound is generated in many common foods during cooking at high temperatures (Svensson et al., 2003). In addition, AA detected in arrange of fried and over cooked carbohydrate rich food items (Tareke et al, 2002) is formed during the Maillard browning reaction from a heat-induced reaction between the amino acid asparagine and the carbonyl group of glucose (Stadler et al., 2002). AA caused world wide concern as it has neurotoxic, carcinogenic, genotoxic and other toxic properties in laboratory animals (Shipp et al, 2006). For genotoxic studies, AA has been shown to be clastogenic and mutagenic in rodent *in vivo* (Dearfield et al., 1995) and induced DNA damage in the PCCl3 and FRTL5 rat thyroid cell lines, as well as in human lymphoblastoid TK6 cells in the Comet assay (Koyama et al., 2006). AA produces genotoxic effect through its reactive metabolite, glycidamide, generated by catalysis with cytochrome p450 (Sumner et al, 1999). The resulting metabolite is an epoxide derivate i.e glycidamide which is more reactive toward DNA and proteins than the parent compound AA (Besartinia and Pfeifer,2007).Glycidamide is regarded as a cancer initiating species reacting with DNA to cause mutagenicity and clastogenic (Adler et al, 2000). The clastogenic potential is responsible for dominant lethality targeting male gonadal spermatocytes to result in reproductive toxicity in rodent (Xiao and Tates, 1994; Adler et al, 2000). Several authors reported that AA has the ability to induce biochemical changes and hormone disturbances (Park et al., 2002; Yousef and El-Demerdash, 2006; Parzefall, 2008).

Phytic acid (PA) or Inositol hexaphosphate is a sugar molecule attached to six phosphate molecules. It is found in wheat and rice bran, legumes and virtually every kind of mammalian cell (Vucenik and

Shamsuddin, 2003). PA plays an important role in regulating vital cellular functions, including cell proliferation and differentiation, thus it controls the number and growth of cells. It helps in keeping cells multiplying out of control and overwhelming the immune system (Saied and Shamsuddin, 1998; Vucenik et al., 1998a and b). Same authors added that PA might be responsible for the anti-oxidant and anti-cancer properties of green tea and grains. In laboratory and animal experiments, inositol has proved to be beneficial in preventing and slowing the spread of cancer (Shamsuddin et al., 1996 and Lee et al., 2005). PA also appears to be a natural anti-oxidant that can reverse the effects of damaging free radicals fight tumor formation and enhance the body's natural disease resistance (Huang, 1997; Saied and Shamsuddin, 1998 and Lee et al., 2005). PA prevents pathological calcification and kidney stone formation and reduces pathological platelet activity (Vucenik and Shamsuddin, 2006).

Thus, the present study is focusing on the possible ameliorative effect of PA to enhance the genotoxic effects and biochemical disturbances induced in mice by AA.

2. Materials and Methods

2.1.1. Animals

Laboratory-bred strain Swiss albino male mice of 8-10 weeks old with an average weight of 27.5±2.5 g obtained from the National Research Center, Cairo, Egypt, were used. Animals were housed in groups and maintained under standard conditions of temperature, humidity and light. The animals were given standard food and water *ad libitum*.

2.1.2. Chemicals

Acrylamide was purchased from SISCO Research Laboratories, PVT LTD, Mumbai, India. Phytic Acid was purchased from Agriculture Research Institute, Egypt. All other chemicals used were of analytical grade.

2.1.3. Doses and experimental design

The experimental design was carried out in three groups. The first groups were treated with 10mg AA/ kg b. wt, the second groups were treated with 30mg PA/ kg b. wt and the third groups were treated with consecutive administration of PA and AA. Control groups were treated with vehicle. The tested materials were administered AA and/or PA daily orally by gavage for 1, 7 and 14 days. Samples were collected 24h from the last treatment.

2.2.1. Micronucleus test

The epiphyses were cut and the bone marrow was flushed out by gentle flushing and aspiration with fetal calf serum (Valette et al., 2002). The cell suspension was centrifuged at 1000 rpm for 10 min and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on to clean glass slides and air-dried. The bone marrow smears were made in five replicates and fixed in absolute methanol for 10 minutes and stained with May-Grünwald/ Giemsa at pH 6.8 (D'Souza et al., 2002). Scoring the polychromatic erythrocytes and the percentage of micronucleated polychromatic erythrocytes (MNPCEs) was determined by analyzing the number of MN cells from 1000 PCEs per animal.

2.2.2. Chromosome aberrations

The groups of animals which are used in chromosome aberrations in somatic and germ cells were injected with colchicine (0.6 mg/kg b. wt) for 3h prior to collecting samples. Bone-marrow metaphases were prepared according to Yosida and Amano (1965). The diakinase-metaphase I cells collected from the spermatocytes were made following the air-drying technique of Evans et al. (1964). Slides were stained with 7% Giemsa stain in phosphate buffer (pH6.8). 500 well spread metaphases (5 animals / group) were scored for chromosome aberrations in both somatic and germ cells. The types of aberrations in bone-marrow cells including gaps, breaks, deletions, fragments, centric fusions and tetraploidy were scored. The types of aberrations in spermatocytes including XY univalents, autosomal univalents and fragment were scored.

2.2.3-Sperm-shape abnormalities

The epididymides were excised and, minced in isotonic sodium citrate solution (2.2%). Smears were prepared and sperms were stained with Eosin Y (Wyrobek and Bruce, 1978). At least 1000 sperm per animal (5 animals/ group) were assessed for morphological abnormalities of the sperm shape.

2.2.4. Biochemical analysis

Liver from six animals per group was dissected out, washed, washed with saline solution, weighed and homogenized in phosphate buffer pH 7.4 Alanine amino transferase (ALT) and aspartate amino transferase (AST) content were determined after Reitman and Frankel (1957). Alkaline phosphatase (ALP) was measured according to Belfield and Goldberg, (1971). Total cholesterol content in liver homogenates was determined according to Allain et al., (1974). Triglycerides were measured according to Fossati and Principe (1982). Lipid peroxide content was determined by quantifying the thiobarbituric acid reactive substance (TBARS) in liver homogenate after the method described by Yoshioka et al. (1979). Reduced glutathione (GSH) content was determined according to

Table 1: Number and mean percentage of MNPCE and chromosome aberrations induced after treatment for 1, 7 and 14 days with Acrylamide and/or Phytic Acid simultaneously in mice bone-marrow cells.

Dose	Treatment/Day	MNPCE [a]		Total Abnormal Metaphases Excluding Gaps [b]		No. of different types of aberrations					
		No.	Mean (%)±SE	No.	Mean (%)±SE	Gaps	F and/or Br	Del	C F	M A	Tetrap
I. Control		27	0.54±0.52	14	2.8±0.39	7	9	5	0	0	0
II.PA (30mg/kg)											
	1	29	0.58±0.51	13	2.6±0.45	10	12	1	0	0	0
	7	26	0.52±0.48	22	4.4±0.37	8	19	2	0	1	0
	14	31	0.62±0.31	19	3.8±0.50	6	16	2	0	0	1
III. AA (10mg/kg)											
	1	41	0.82±0.55	41	8.2±0.45***	16	32	5	1	2	1
	7	152	3.04±0.40***	48	9.6±0.57***	12	37	4	2	2	3
	14	201	4.02±0.51***	61	12.2±0.52***	13	38	12	3	5	3
IV. PA+AA											
	1	30	0.60±0.40	34	6.8±0.44	11	30	3	1	0	0
	7	81	1.62±0.38•	25	5.0±0.54•••	10	20	1	0	2	2
	14	112	2.24±0.42•	27	5.4±0.41•••	9	23	3	0	0	1

a. The total number of scored cells is 5000 (5 animals/ group) b. The total number of scored metaphases is 500 (5 animals/ group); *** p<0.001: Significance compared to Control. •p<0.05, •••p<0.001: Significance compared to treatment with AA (t-test). F: Fragments, Br: Breaks, Del: Deletions, C F: Centric Fusions, M A: Multiple Aberrations, Tetrap: Tetraploidy.

Beutler et al. (1963). Serum testosterone was determined using commercial radioimmunoassay kit purchased from Diagnostic Systems Laboratories (DSL), INC, USA.

2.3. Statistical analysis

The significance of the results from the control data was calculated using (t- test) for micronucleus polychromatic erythrocytes, chromomose aberrations in bone marrow cells and spermatocytes, and sperm- shape abnormalities. Data for biochemical parameters were examined using one way analysis of variance (ANOVA) followed by Duncan multiple range test (Duncan, 1955).

3. Results

3.1. Micronucleus test

From the results presented in table (1) we found that the percentage of MNPCE increased significantly by increasing time of treatment with AA (Fig. 1A,B). The occurrence of MNPCE was statistically significant after all treatments compared to control (except with treatment for 1 day). The protective effect of PA at 30 mg/kg b. wt consecutively with AA reduced MNPCE compared with AA alone with all the durations of times (Table 1).

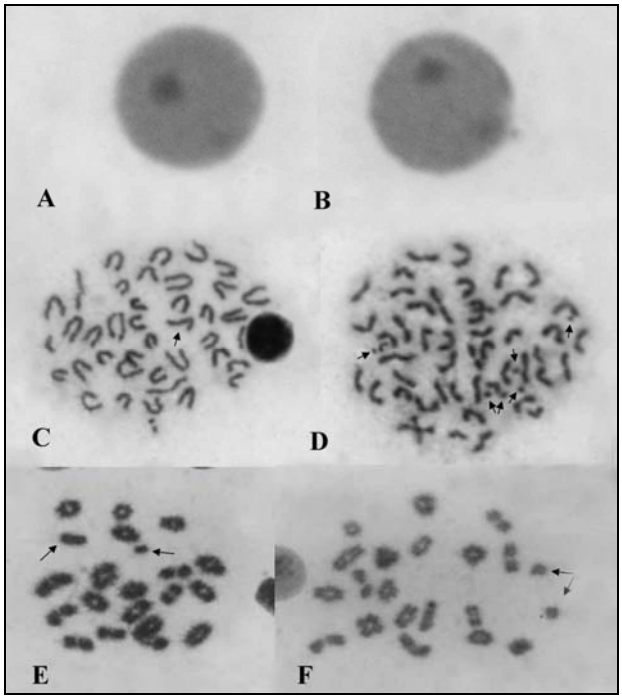

Fig. 1: The cytogenetic changes induced with AA administration in male mice bone-marrow cells; (A,B) micronuclei in PCEs, (C) deletion, (D) fragments, and in spermatocyte; (E) XY univalent, (F) autosomal univalent.

Table 2: Number and mean percentage of chromosome aberrations induced after treatment for 1, 7 and 14 days with Acrylamide and/or Phytic Acid simultaneously in mice spermatocytes.

Dose	Treatment/Day	Total Abnormal Metaphases		No. of different types of aberrations			
		No.	Mean (%)±SE	XY Uni	Auto. Uni	XY+Auto Uni	Fragment
I. Control		16	3.2±0.24	12	4	0	0
II.PA (30mg/kg)							
	1	17	3.4±0.30	13	4	0	0
	7	15	3.0±0.20	10	5	0	0
	14	18	3.8±0.28	13	5	0	0
III. AA (10mg/kg)							
	1	26	5.20±0.40	17	6	3	0
	7	48	9.60±0.54***	27	17	4	0
	14	58	11.6±0.58***	35	20	2	1
IV. PA+AA							
	1	20	4.0±0.32	15	5	0	0
	7	31	6.2±0.32•••	18	10	3	0
	14	28	5.6±0.54•••	20	8	0	0

The total number of scored metaphases is 500 (5 animals/ group); ***p<0.001: Significance compared to Control. •••p<0.001: Significance compared to treatment with AA (t-test). XY Uni: XY Univalents, Auto. Uni: Autosomal Univalents, XY+ Auto. Uni: XY Univalents plus Autosomal Univalents.

3.2. Chromosome aberrations

Table (1) represents chromosome aberrations induced in bone marrow cells after repeated oral treatments, with AA up to 14 days. The results show that the tested dose of AA induced a statistically significant increase in the percentage of chromosome aberrations even after excluding gaps. Such percentage was found to be time-dependent. The results, also, demonstrate that the percentage of chromosome aberrations in bone marrow cells was significantly reduced when animals treated with PA at 30 mg/kg b. wt with AA at the tested dose level. The main aberrations observed were breaks, fragments and deletion (Fig. 1C,D).

The percentage of chromosome aberrations in spermatocytes was time dependant after treatment with AA. PA ameliorated these genotoxicity and reduced the aberrations statistically in high significant ($p<0.001$) with increasing the time of treatment (Table 2). XY-univelents and autosomal univelents were the main aberrations observed in diakinase metaphase I cells (Fig. 1E,F).

3.3. Sperm-shape abnormalities

The mean percentage of sperm shape abnormalities for animals treated with AA alone was increased with time response. This significance increased from $p<0.05$ to $p<0.001$ after extending the time of treatments from 1 to 14 days respectively. PA reduced the response of AA with no significance after 1 day, but its effect was observed to be highly significant ($p<0.001$) after 7 and 14 days. Table (3) represents the number and mean percentage of sperm shape abnormalities and the main types demonstrated with head abnormalities (Fig. 2).

3.4. Biochemical analysis

The effects of AA on liver enzymes are shown in Table (4). The results illustrated that AST ,ALT and ALP were significantly increased in AA treated group after 14 days of treatment as compared with control group, while ALP showed significant increase after 7days when compared to PA group. Also, significant increase in the liver homogenate content of TBARS and triglycerides were recorded after 7 and 14 days of treatment with AA, while total cholesterol increased numerically not significantly after 7 days of treatment. GSH content and serum testosterone level decreased significantly after 14 days of treatment with AA. At all the experimental period the treatment with PA revealed significant improvement in all tested parameters.

4. Discussion

The present study illustrates the genotoxic effects and biochemical disturbances induced by AA in mice after different periods of time. Because AA molecule is small and hydrophilic, it passively diffuses throughout the body (Friedman, 2003). For this reason, all tissues are theoretically targets for AA carcinogenesis. Hogervorst et al. (2008) investigated the relation between dietary AA and risk of cancer of the kidney, bladder, and

Table 3: Number and mean percentage of sperm shape abnormalities induced after treatment for 1, 7 and 14 days with Acrylamide and/or Phytic Acid simultaneously in mice.

Dose	Treatment/Day	No. Of Scored sperms	Abnormal sperms		No. of different types of abnormal sperms					
			No.	Mean (%) ± SE	Amor	Triang	W Hook	Big Head	Small Head	Coiled Tail
I. Control		5029	97	1.92±0.10	23	47	17	1	-	9
II.PA (30mg/kg)										
	1	5013	101	2.01±0.10	25	49	17	-	-	10
	7	5062	102	2.02±0.08	29	53	9	1	-	10
	14	5015	83	1.65±0.10	15	52	9	1	-	6
III. AA (10mg/kg)										
	1	5025	141	2.80±0.20	39	62	20	1	-	19
	7	5025	294	5.85±0.21***	78	149	25	4	2	36
	14	5011	422	8.42±0.50***	114	222	26	4	3	53
IV. PA+AA										
	1	5012	116	2.31±0.23	26	57	19	-	-	14
	7	5029	226	4.49±0.14•	51	114	32	1	-	28
	14	5024	286	5.69±0.27•••	47	203	19	3	2	12

***p<0.001: Significance compared to Control. •p<0.05, •••p<0.001: Significance compared to treatment with AA (t-test). Amor: Amorphous, Triang: Triangular, W Hook: Without Hook.

prostate. However, the mechanism by which acrylamide may cause cancer in laboratory animals and humans was unclear. Besaratinia and Pfeifer (2007) demonstrated that AA and its metabolite glycidamide were clastogenic and glycidamide formed DNA adducts. Therefore, we sought to use an edible rich food has the efficacy to reduce the genotoxic and biochemical disturbance such as PA. This ameliorative effect is back to the fact that PA plays an important role in regulating vital cellular functions, including cell proliferation and differentiation (Shamsuddin et al., 1996; Vucenik et al., 1998a and b). Also, they found that PA might be responsible for the anti-oxidant and anti-cancer properties of green tea and grains, in addition to its vital role in enhancing the functional capabilities of nervous system and reproductive functions.

The present study clarifies that AA has the ability to induce MNPCE in bone marrow cells and chromosome aberrations in somatic and germ cells in time response. These observations were confirmed by other previous studies (Husøy et al., 2005; Witt et al., 2008; Yener et al., 2009). Consumption of fried carbohydrate-rich food contains AA caused an increased level of micronucleated young erythrocytes in humans (Abramsson-Zetterberg *et al*., 2008). Also, AA and its metabolite induced elevation in the percentage of chromosome aberrations in somatic and germ cells (Gassner, 1996; Rice, 2005). Assessment of sperm shape abnormalities by any agent is of great significance, because it has the potential of allowing identification of chemicals that induce spermatogenic dysfunction and perhaps heritable mutation (Wyrobek *et al*., 1983). In relation to mutagenicity, sperm shape is reported to be genetically controlled by numerous genes (Ito *et al*., 2004). The results showed that AA induced significant sperm shape abnormalities after 7 and 14 days of treatments. Both head and tail abnormalities were recorded in this study. These morphological abnormalities due to AA affected negatively the development and movement of sperm causing testicular atrophia (Burek et al., 1980). Wang et al. (2007) demonstrated that AA had toxic effects on seminiferous tubules and decreased the production of sperm in male rats. Besides, it decreased the weights of the testis and epididymis and the sperm concentration in the cauda of the epididymis after administration and the histopathological lesions were presented in the testes of the treated rats, and the number of Leydig cells around the apoptosis seminiferous tubules increased significantly. Furthermore, subchronic exposure to AA affected the normal development of sperm, caused changes of the activity of some enzymes in the testis and significantly influenced hindlimb motor coordination and it directly damaged Leydig cells and affected the endocrine function of the testis (Song et al., 2008). Wang et al. (2010) demonstrated that AA

decreased epididymal sperm reserves suggesting partial depletion of germ cells. Furthermore, they observed histopathologic lesions and distinct expression patterns of sGC heterodimers suggesting different physiologic roles for sGC subunits in spermiogenesis and steroidogenesis.

Administration of PA with AA all over the duration time of treatment effectively reduced the DNA damage, chromosome aberrations in somatic and germ cells as well as sperm shape abnormalities induced by AA alone. The ameliorative effect of PA was clear after 14 days from consecutive treatments with AA. The efficacy of PA to ameliorate the genotoxicity of different carcinogens was illustrated in many studies. PA had a potential effect in regulating vital cellular functions, including cell proliferation and differentiation (Shamsuddin et al., 1996; Vucenik et al., 1998a and b). PA controls the number and growth of human mammary cancer cell lines *in vitro*. It helps in keeping cells multiplying out of control and overwhelming the immune system. In laboratory and animal experiments, inositol has proved to be beneficial in preventing and slowing the spread of cancer (Shamsuddin et al., 1996; Vucenik et al., 1997). Also, the efficacy of PA to improve the histopathological features of carcinogens was clarified in Abu El-Saad and Mahmoud, (2007) studies. They demonstrated that rats post-treated with PA After aflatoxinB1 (AFB1) for 1 week showed a marked regenerative effect upon the histopathologic features of the seminiferous tubules. Also, testis sections of rats treated with AFB1–PA mixture showed regenerative features after 2 weeks of treatments, in spite of the absence of spermatids.

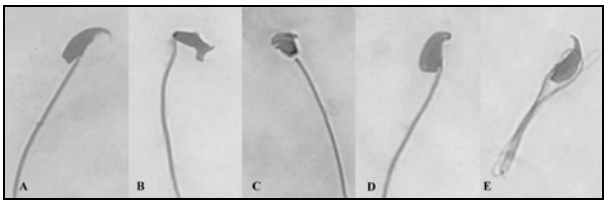

Fig. 2: Sperm shape abnormalities induced in male mice treated with AA showing (A) normal, (B) amorphous, (C) triangular, (D) without hook and (E) coiled tail.

The genotoxic effects induced by AA may be resulted from decreasing of oxidative defense system in the cells (Zamorano-Ponce et al., 2006), as well as, releasing the reactive oxygen species (ROS) (Liping et al., 2007). Thus, it was important to measure some of the biochemical parameters which are sensitive index to changes due to xenobiotics and can constitute important diagnostic tool in toxicological studies. The present study demonstrates that AA exerts deterioration effects on enzyme activities and lipid peroxidation in a time response. These results are in agreement with Yousef and El- Demerdash (2006), who demonstrated that different doses of AA perturbations effects on enzyme activities and lipid peroxidation in a dose-dependent manner. In our study, significant increase in ALT and AST content was recorded in AA treated group. However, the bioactivity of PA is represented with improvement the biochemical disturbances induced with AA at different time intervals. The decrease in liver homogenate activities of AST, ALT and ALP in the mice indicates the improvement of liver function and protection from the toxicity of AA. These results are in agreement with Abu El-Saad and Mahmoud (2007). They found that PA ameliorated the activity of enzymatic and non enzymatic antioxidants in the testis' homogenate of aflatoxin B1 (AFB1) -treated rats.

Free radicals impair liver functions and can be a major reason of hormone imbalance. This imbalance induces hyperlipidemia through its multiple effects on lipid metabolism including increased synthesis of cholesterol and triglycerides (Bowden et al., 1989). In the present study, a marked significant increase in total cholesterol content was recorded after 14 days of AA treatment while significant increase of triglycerides was obtained after 7 and 14 days. This increase of triglycerides may be related to the stimulation of liver enzymes responsible for the biosynthesis of fatty acids.

The observed changes in cholesterol and triglycerides stake place in the liver due to the imbalance between the normal rates of lipid synthesis, utilization and secretion (Glaser and Mager, 1972). PA effectively reduced this hyperlipidemic effect by reducing the total cholesterol content and triglycerides after concurrent treatment with AA for 14 days (Vucenik and Shamsuddin, 2006). The ameliorative effect of PA was obviously clear when Midorikawa et al. (2001) demonstrated that PA inhibited oxidative DNA damage by chelating Cu(II) as well as Fe(II) and Fe(III). Also, PA inhibited the generation of highly reactive species from H_2O_2 by chelating transition metal ions, resulting in chemoprevention of cancer (Midorikawa et al., 2001).

The present studies show also that AA increased the level of TBARS (the marker of extent of lipid peroxidation) after one and two weeks of treatments, which accompanied with decreased the reduced glutathione (GSH) content in liver homogenate. This indicates that the primary effects of AA on the liver are mediated via damage cell membrane. The increased lipid peroxidation is due to an inhibition or changing the activity of non enzymatic and enzymatic components of the antioxidant system (reduced glutathione) GSH (Wu and Cederbaum, 2003). While, the decrease in glutathione content in the liver of animals treated with

Table 4: Changes in different biochemical parameters and testosterone hormone after treatment for 1, 7 and 14 days with Acrylamide and/or Phytic Acid simultaneously in mice.

Dose	Treatment /Day	AST U/g tissue	ALT U/g tissue	ALP IU/g tissue	Cholesterol mg/g tissue	Triglycerides mg/g tissue	TBARS µmol/ g tissue	GSH µg/ g tissue	Testosterone ng/ml serum
I. Control									
	1	**29.50±0.92^{a}**	**25.0±0.82^{a}**	**9.16±1.07^{a}**	**14.34±0.94^{a}**	**37.27±1.68^{a}**	**7.23±0.74^{a}**	**10.71±0.92^{a}**	**0.93±0.29^{a}**
	7	**32.83±1.0^{a}**	**33.5±1.06^{a}**	**13.29±1.40^{a}**	**21.85±0.41^{a}**	**36.34±1.01^{a}**	**9.04±1.08^{a}**	**14.77±0.52^{a}**	**0.98±0.01^{a}**
	14	**24.0±1.65^{a}**	**36.08±0.67^{a}**	**11.64±1.09^{a}**	**22.09±0.65^{a}**	**32.23±0.63^{a}**	**9.63±1.36^{a}**	**13.84±0.82^{a}**	**0.81±0.05^{a}**
II.PA (30mg/kg)									
	1	**30.0±0.36^{a}**	**23.20±0.73^{a}**	**7.96±0.92^{a}**	**13.04±1.76^{ab}**	**34.82±1.05^{a}**	**7.25±0.31^{ab}**	**10.50±0.66^{a}**	**1.09±0.19^{a}**
	7	**31.0±1.07^{ab}**	**31.66±1.05^{ab}**	**11.86±0.99^{ab}**	**19.83±0.81^{b}**	**37.08±0.86^{a}**	**9.50±1.08^{a}**	**14.17±0.75^{a}**	**1.18±0.34^{a}**
	14	**24.99±0.79^{ac}**	**36.25±0.92^{ac}**	**13.97±0.76^{a}**	**20.11±1.41^{a}**	**34.51±1.56^{a}**	**8.65±0.61^{a}**	**14.99±0.42^{a}**	**0.79±0.05^{a}**
III. AA (10mg/kg)									
	1	**30.83±1.41^{a}**	**22.17±0.54^{a}**	**9.88±0.69^{a}**	**10.61±0.43^{bc}**	**38.51±1.52^{a}**	**8.66±0.34^{a}**	**9.04±0.32^{a}**	**0.91±0.06^{a}**
	7	**35.75±1.36^{a}**	**37.71±2.29^{a}**	**15.32±1.02^{ac}**	**23.98±1.48^{a}**	**67.85±1.80^{b}**	**13.06±0.56^{b}**	**10.56±0.93^{b}**	**0.83±0.11^{a}**
	14	**32.75±1.22^{b}**	**48.58±1.61^{b}**	**18.39±1.54^{b}**	**26.81±0.98^{b}**	**71.54±0.77^{b}**	**17.17±1.15^{b}**	**11.53±0.56^{b}**	**0.35±0.07^{b}**
IV. PA+AA									
	1	**30.33±0.96^{a}**	**22.83±0.65^{a}**	**9.09±1.35^{a}**	**16.32±0.99^{ab}**	**37.46±0.87^{a}**	**8.41±0.34^{a}**	**9.76±0.48^{a}**	**0.95±0.19^{a}**
	7	**32.90±1.85^{a}**	**34.66±1.96^{ab}**	**11.86±1.32^{abc}**	**15.65±1.54^{c}**	**58.21±1.51^{c}**	**7.59±1.26^{a}**	**13.96±0.72^{a}**	**1.13±0.38^{a}**
	14	**27.33±0.65^{a}**	**38.81±0.78^{c}**	**12.41±1.37^{a}**	**21.91±1.47^{a}**	**68.65±1.38^{cb}**	**7.13±1.09^{a}**	**14.14±0.39^{a}**	**0.73±0.09^{a}**

The data are represented as mean ±SE (ANOVA test)

The mean values bearing different small letters in the same column at the same time are significantly different at ($P<0.05$)

AA may be releated to the absorption and metabolization of AA by pathways: inactivation by glutathione conjugation or bioactivation through cytochrome P450 (cyp2E1) dependent epoxidation to glycidamide. The majority of AA is conjugated with glutathione and lesser is activated via glycidamide (Parzefall, 2008). Glycidamide is detoxified by glutathione or hydrolyzed to glyceramide (Fennell et al., 2006). In the present study, PA reduced the TBARS content significantly and increased GSH content. Many studies illustrated the efficacy of PA in preventing free radical damage to DNA. Unbound iron (ferritin); it can be the catalyst for the deadly Fenton reaction in which iron reacts with the relatively harmless hydrogen peroxide (H_2O_2) and forms the highly reactive hydroxyl radical (Porres et al., 1999). Besides, it increase GSH, an essential component of antioxidant system serves as a cofactor for glutathione transferase, which helps to remove certain drugs and chemicals as well as other reactive molecules from the cells (Wu and Cederbaum, 2003).

Serum testosterone level was decreased significantly after 14 days of treatment with AA. Ali et al., (1983) reported that AA decreased the testosterone and prolactin levels in blood serum. Subchronic exposure to AA affected in activity of some enzymes and the endocrine function of the testis. The level of testosterone was markedly reduced in the serum and testis homogenate (Song et al., 2008). PA administration regulated the testosterone level with all time intervals. Abu El-Saad and Mahmoud (2007) observed that PA improved the sex hormone levels in AFB1-treated rats, and decreased testicular lipid peroxidation product levels and significantly increased testicular glutathione content, catalase and total peroxidase and superoxide dismutase activities.

In conclusion, the present study from the first studies illustrates the protective efficacy of PA in reducing the genotoxic and biochemical disturbances induced by AA in mice. Besides, it demonstrates the importance of edible riches with PA to merit the potential for a beneficial application in the chemoprevention of AA genotoxicity and, possibly, human carcinogenesis. Thus it is necessary to increase PA in foods in order to minimize the side effects of AA. This can obviously achieved by increasing PA intake in food as AA is formed during cooking of carbohydrate-rich foods at higher temperature.

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