ISSN 1996-3351

Asian Journal of Biological Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

OPEN ACCESS
Asian Journal of Biological Sciences
ISSN 1996-3351
DOI: 10.3923/ajbs.2019.9.16

Research Article
Utilization of Black Berry Juice to Reduce the Oxidative Stress in Rats Treated with Acrylamide

[1,3]Rehab M. Ibrahim, [2]Mokhtar Yousef, [3]Isis Nawar, [1]Amal Hassanein and [4]Dalia Hikal

[1]Department of Specialist Foods and Nutrition, Food Technology Research Institute, ARC, Giza, Egypt
[2]Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt
[3]Department of Home Economics, Faculty of Agriculture, Alexandria University, Alexandria, Egypt
[4]Department of Home Economics, Specific Education Faculty, Mansoura University, Mansoura Egypt

Abstract
Background and Objective: Acrylamide is carcinogenic and mutagenic to experimental animals. Also, it has neurotoxic effect of animals and humans. The aim of study was to investigate the effects of different doses of acrylamide and black berry on free radicals and antioxidant enzyme activities in male rats. **Materials and Methods:** Animals were assigned at random to one of the following treatments: group 1 served as control, group 2 was treated with black berry juice 9 mL kg^{-1} b.wt., while groups 3, 4 and 5 were treated with 120, 600 and 1200 µg kg^{-1} b.wt., of acrylamide, respectively in drinking water for 10 weeks. Groups 6, 7 and 8 were treated with those doses of acrylamide and black berry juice. **Results:** The results showed that acrylamide increase thiobarbituric acid reactive substances (TBARS), while decrease the activities of GSH and antioxidant enzyme (Catalase, SOD, GST and GPX) in liver, kidney, brain, lung and testes compared to control group. Black berry decrease of acrylamide risk. **Conclusion:** The present results showed that different doses of acrylamide exerted deterioration effects on enzyme activities and free radicals in a dose-dependent manner. Black berry protection in all organs effects of acrylamide.

Key words: Acrylamide, black berry, lipid oxidation, antioxidant enzymes

Citation: Rehab M. Ibrahim, Mokhtar Yousef, Isis Nawar, Amal Hassanein and Dalia Hikal, 2019. Utilization of black berry juice to reduce the oxidative stress in rats treated with acrylamide. Asian J. Biol. Sci., 12: 9-16.

Corresponding Author: Rehab Mohamed Khalaf Allah, Department of Specialist Foods and Nutrition, Food Technology Research Institute, Alexandria, Egypt Tel: (020) 1225707447

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In April, 2002, the Swedish National Food Administration (SNFA) and the University of Stockholm together reported that processed carbohydrate-rich foods that are fried or baked at relatively high temperature may contain considerable levels of acrylamide[1,2]. Potato products (such as potato chips, french fries and hash browns), bread, biscuits and cereal were among the food items containing the highest amounts of acrylamide[1]. Acrylamide contains an α, β-unsaturated system that reacts with nucleophilic compounds via a Michael addition. The major site of reaction is in reduced glutathione and in proteins, cysteine with sulfhydryl groups, although acrylamide may also react with other amino groups to a lesser extent such as those at the N-terminal position of the protein and amino acids[3,4]. After ingestion, acrylamide is rapidly distributed through the whole body via the bloodstream. It can be found in the thymus, liver, heart, brain, kidneys[5] and even in human breast milk[6]. Acrylamide has been classified as a group 2A carcinogen by the International Agency for Research on Cancer[7] and a Category 2 carcinogen and Category 2 mutagen by the European Union. This finding has caused worldwide concern[8,9]. Several studies in rodents support the evidence that acrylamide is a multi-organ carcinogen, being able to cause tumors to many organs such as lung, uterus, skin, mammalian gland, brain etc[10]. Black berry (Morus nigra) contain large amounts of flavonoid pigments (anthocyanins) that give blackberries their characteristic red to blue color[11]. Black berry is rich in antioxidants: (vitamin C, vitamin E, anthocyanins, ellagitannins and some minor proanthocyanidins). However, anthocyanins have the biggest contributions to the antioxidant capacity[12-15]. They have anti-oxidant and anti-inflammatory properties and, therefore, may be potentially used to combat oxidative stress[16,11]. On the other hand, tropical red-black berries contain cyanidin-3-glucoside[17,18], which has one of the highest Oxygen Radical Absorbance Capacity (ORAC)[19]. In addition, seed extract enhances the levels of reduced glutathione and the activity of natural antioxidant enzymes, such as glutathione S-transferase, superoxide dismutase and catalase[20]. Fresh fruit of blackberry has a good effect on human body through protection the integrity of cells and the internal structure of cells, avoiding destruction of some enzymes and internal components of cells. These contents have antioxidants and it can improve immunity, play an antagonistic role of protective agent from toxic substances[21]. Furthermore, previous studies examined the anti-oxidative activity of phenolic extracts from edible and non-edible plant materials (berries, fruits, vegetables, herbs, cereals, tree materials, plant sprouts and seeds) and reported remarkable high anti-oxidant activity and high total phenolic contents especially berries[22,23]. Due to the harmful effects of acrylamide, which have been studied in many previous studies, this study find that the available data on the effect of acrylamide on the body's internal organs are very few, as well as the role of antioxidants in reducing the negative effects of acrylamide on the internal organs of the body. Therefore, the present study was carried out to investigate the antioxidant effect of blackberry juice on experimental animals treated with different doses of acrylamide.

MATERIALS AND METHODS

Chemicals: Acrylamide (C_3H_5NO, >99% purity) was used in the present study. Acrylamide was purchased from Sigma Chemical Company. Fresh black berry fruits were obtained from local market (Alexandria, Egypt), washed, homogenized and its juice was daily freshly prepared. The dose of black berry juice (BBJ) (1.6 g kg^{-1} b.wt., equal to 9 mL kg^{-1} b.wt., which containing 5 mg active constituent; anthocyanin) was selected according to the previous studies of Hassan and Yousef[24].

Animals: Sixty-four male albino rats, ten weeks old and weighing 127$\pm$3.87 g were used. Animals were obtained from the animal house of the Institute of Graduate Studies and Research (IGSR), Alexandria University, Alexandria, Egypt. The local committee in Alexandria University, Egypt, approved the design of the experiments and the protocol conforms to the guidelines of the National Institutes of Health (NIH).

Experimental design: Animals were housed 4 per cage and kept on basal diet (consisted of 37.5% dried skim milk, 9% corn oil, 30% corn starch, 13.5% sucrose, 5% cellulose, 1% vitamin mixture and 4% mineral mixture[25]) and tap water was provided *ad libitum*. The animals were housed in plastic cages with sawdust bedding and maintained in an air-conditioned animal house at a controlled temperature (22$\pm$2°C) and relative humidity (60$\pm$10%) with a photoperiod of 12 h light/12 h dark[26]. After 2 weeks of acclimatization, the animals were divided into 8 equal groups, 8 animals in each group (Fig. 1).

- Group 1 was used as a control
- Group 2 was daily orally treated with blackberry juice by (9 mL kg^{-1} b.wt.)
- Group 3, 4 and 5 were treated with acrylamide (120, 600 and 1200 µg kg^{-1} b.wt., respectively) dissolved in tap water

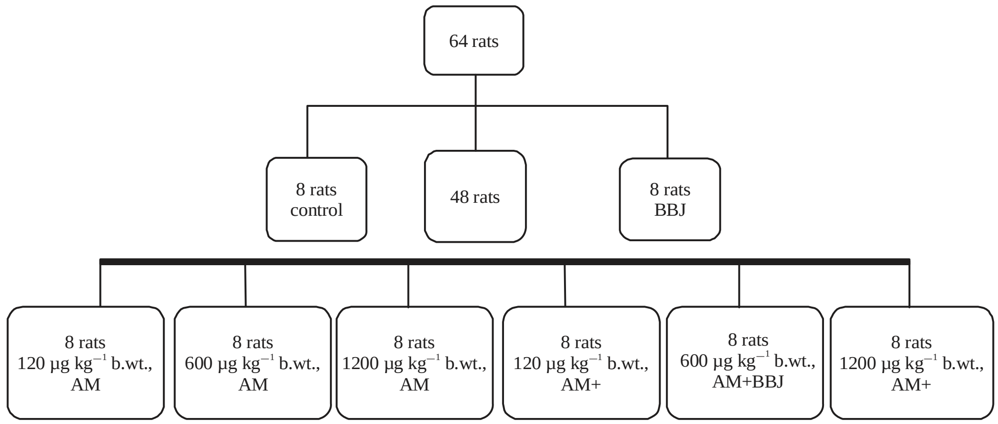

Fig. 1: Experiment design

- Group 6, 7 and 8 were treated with acrylamide solution (120, 600 and 1200 µg kg^{-1} b.wt.) plus orally given black berry juice (9 mL kg^{-1} b.wt.). Animals were daily treated with the tested doses of acrylamide and black berry juice for 10 weeks

Measured parameters: At the end of the experimental period (after 10 weeks) rats were anesthetized with ether and sacrificed. Brain, kidney, liver, lung and testes were removed and washed with saline solution and homogenized (10%, w/v) in ice-cold sodium, potassium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl in a Potter-Elvehjem type homogenizer. The homogenate was centrifuged at 10,000×g for 20 min at 4°C and the resultant supernatant was kept for further analysis. Brain, kidney, liver, lung and testes kidney thiobarbituric acid-reactive substances (TBARS) were measured at 532 nm by using 2-thiobarbituric acid (2, 6-dihydroxypyrimidine-2-thiol; TBA). An extinction coefficient of 156,000 M^{-1} cm^{-1} was used for calculation[27]. Brain, kidney, liver, lung and testes glutathione reduced (GSH) was determined according to the method of Jollow *et al.*[28]. Catalase (CAT; EC 1.11.1.6) activity was determined using the Luck method involving the decomposition of hydrogen peroxide[29]. Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured in different tissue organs according to Mishra and Fridovich[30]. Glutathione S-transferase (GST; EC 2.5.1.18) activity was determined according to Habig *et al.*[31], using para-nitrobenzyl chloride as a substrate. The activity of Glutathione peroxidase (GPx, EC. 1.1.1.9) was assayed by the method of Chiu *et al.*[32].

Statistical analysis: Data were analyzed as a completely randomized design[33] using the General Linear Model procedure of SAS[34]. Dunnett *post hoc* analysis was used to compare means of treatment groups against the control. The p<0.05 were accepted as significant.

RESULTS

General toxicity of acrylamide exposure: The present results showed that no mortality was observed on rats after exposure to all tested doses of acrylamide (120, 600 and 1200 µg kg^{-1} b.wt.). Clinical signs of general toxicity were observed at the 8th week of exposure to 1200 µg acrylamide kg^{-1} b.wt. and at the 10th week of exposure to both 1200 µg acrylamide kg^{-1} b.wt., combined with black berry juice together. Also, the rats became weak and dragged their hind legs of both groups of 1200 µg acrylamide kg^{-1} b.wt., alone and the combination group. While, no clinical signs were observed after exposure to 120 and 600 µg acrylamide kg^{-1} b.wt. and 120 and 600 µg AM kg^{-1} b.wt., combined black berry juice during the entire period of treatment.

Effect of acrylamide and black berry on the levels of free radicals in tissues of rats: Table 1 presented the results of thiobarbituric acid reacting substance (TBARS) in all organs of rats treated with acrylamide, blackberry and their combination. Treatment with acrylamide alone caused significant (p<0.05) increase in the level of TBARS in brain, liver, lung and testes with all doses (120, 600 and 1200 µg kg^{-1} b.wt.) and caused significant (p<0.05) increase in kidney with the doses of 600 and 1200 µg kg^{-1} b.wt., acrylamide. On the other hand, the results showed that the TBARS levels were significant (p<0.05) decrease in rats received blackberry juice alone compared to control. While, the treatment with black berry and acrylamide together in the

Table 1: Effect of different doses of acrylamide (AM) and black berry (BBj) on TBARS (nmol g^{-1} tissue) in tissues of male rats

	Experimental groups							
Parameters	Control	Black berry juice (BBj)	120 (µg AM/kg BW)	600 (µg AM/kg BW)	1200 (µg AM/kg BW)	120 (µg AM/kg BW+BBj)	600 (µg AM/kg BW+BBj)	1200 (µg AM/kg BW+BBj)
Brain	52.1±3.19^{d}	41.0±3.09^{e}(-21)*	59.9±3.92^{bc}(15)*	65.3±3.46^{b}(25)*	74.2±3.45^{a}(42)*	55.3±2.63^{cd}(6)*	58.3±2.46^{bcd}(12)*	64.7±2.95^{b}(24)*
Kidney	55.3±3.17^{c}	43.7±2.98^{d}(-21)*	60.0±2.52^{bc}(9)*	65.9±2.22^{b}(19)*	75.7±3.31^{a}(37)*	57.1±3.15^{c}(3)*	60.7±2.27^{bc}(10)*	66.4±3.45^{b}(20)*
Liver	54.7±2.15^{e}	43.7±3.58^{f}(-20)*	62.9±3.14^{cd}(15)*	71.5±2.86^{b}(31)*	79.3±3.30^{a}(45)*	56.7±2.90^{de}(4)*	63.7±2.34^{cd}(16)*	68.6±4.29^{bc}(25)*
Lung	63.2±4.27^{d}	51.2±2.65^{e}(-19)*	71.2±2.56^{bc}(13)*	76.9±2.63^{b}(22)*	86.1±3.27^{a}(36)*	65.1±3.27^{cd}(3)*	70.3±2.60^{bcd}(11)*	72.7±3.91^{b}(15)*
Testes	56.5±2.35^{c}	46.0±2.69^{d}(-19)*	64.6±2.81^{b}(14)*	73.5±4.98^{ab}(30)*	79.3±2.53^{a}(40)*	58.6±2.26^{c}(4)*	66.7±4.06^{b}(18)*	70.5±3.57^{b}(25)*

Values are expressed as Means±SE, n = 8 for each treatment group, Mean values within a row not sharing a common superscript letters (a, b, c, d, e) were significantly different, p<0.05, *Percentage of control group, TBARS: Thiobarbituric acid reacting substance

Table 2: Effect of different doses of acrylamide (AM) and black berry (BBj) on GSH in tissues of male rats

	Experimental groups							
Parameters	Control	Black berry juice (BBj)	120 (µg AM/kg BW)	600 (µg AM/kg BW)	1200 (µg AM/kg BW)	120 (µg AM/kg BW+BBj)	600 (µg AM/kg BW+BBj)	1200 (µg AM/kg BW+BBj)
Brain	28.3±1.08^{bc}	34.1±2.23^{a}(20)*	25.0±1.60^{cd}(-12)*	22.6±1.40^{ed}(-20)*	19.4±1.30^{e}(-31)*	28.8±1.30^{b}(2)*	25.8±1.48^{bcd}(-9)*	25.0±1.32^{cd}(-12)*
Kidney	27.4±1.34^{b}	33.7±2.50^{a}(23)*	22.7±1.21^{c}(-17)*	21.6±1.67^{cd}(-21)*	18.8±1.26^{d}(-31)*	25.2±1.53^{bc}(-8)*	24.3±1.56^{bc}(-11)*	22.7±1.60^{c}(-17)*
Liver	27.0±1.20^{b}	33.4±2.81^{a}(24)*	23.7±1.24^{bc}(-12)*	21.0±1.95^{c}(-22)*	16.3±1.31^{d}(-40)*	25.1±1.34^{b}(-7)*	23.3±1.87^{bc}(-14)*	20.0±1.56^{cd}(-26)*
Lung	28.4±1.68 ^{b}	36.7±2.45^{a}(29)*	26.4±1.28^{bc}(-7)*	23.9±1.36^{cd}(-16)*	20.3±1.06^{d}(-29)*	28.2±1.64^{b}(-1)*	25.8±1.59^{bc}(-9)*	24.4±1.20^{c}(-14)*
Testes	30.4±1.26^{b}	38.1±2.19^{a}(25)*	27.4±1.10^{bc}(-10)*	24.3±1.40^{cd}(-20)*	18.5±1.08^{e}(-39)*	30.0±1.89^{b}(-1)*	27.0±1.55^{bc}(-11)*	22.1±1.12^{d}(-27)*

Values are expressed as Means±SE, n = 8 for each treatment group, Mean values within a row not sharing a common superscript letters (a, b, c, d, e) were significantly different, p<0.05, *Percentage of control group, GSH: Reduced glutathione

Table 3: Effect of different doses of acrylamide (AM) and black berry (BBj) on catalase in tissues of male rats

	Experimental groups							
Parameters	Control	Black berry juice (BBj)	120 (µg AM/kg BW)	600 (µg AM/kg BW)	1200 (µg AM/kg BW)	120 (µg AM/kg BW+BBj)	600 (µg AM/kg BW+BBj)	1200 (µg AM/kg BW+BBj)
Brain	54.7±2.85^{b}	67.1±3.52^{a}(23)*	51.8±3.65^{b}(-5)*	44.5±2.51^{c}(-19)*	36.9±2.08^{d}(-33)*	54.8±1.70^{b}(0)*	49.6±2.69^{bc}(-9)*	43.5±2.90^{c}(-21)*
Kidney	66.4±2.04^{b}	77.8±2.25^{a}(17)*	60.7±3.09^{bc}(-9)*	52.3±3.70^{de}(-21)*	44.8±2.96^{e}(-33)*	65.6±3.76^{b}(-1)*	60.5±3.80^{bc}(-9)*	54.8±4.75^{cd}(-18)*
Liver	45.2±2.94^{b}	57.6±4.04^{a}(27)*	43.0±2.42^{bc}(-5)*	36.6±2.60^{cd}(-19)*	29.7±2.76^{e}(-34)*	45.1±3.26^{b}(0)*	40.9±2.51^{bcd}(-9)*	36.1±2.09^{de}(-20)*
Lung	56.0±3.92^{b}	72.4±3.72^{a}(29)*	52.6±2.94^{b}(-6)*	43.4±2.40^{cd}(-22)*	37.6±2.34^{d}(-33)*	55.7±2.09^{b}(0)*	50.5±3.87^{bc}(-10)*	43.1±2.84^{d}(-23)*
Testes	60.0±3.79^{b}	75.0±3.95^{a}(25)*	57.2±2.30^{b}(-5)*	49.7±2.47^{c}(-17)*	39.4±2.32^{d}(-34)*	59.5±2.15^{b}(-1)*	55.0±3.50^{bc}(-8)*	48.6±2.61^{c}(-19)*

Values are expressed as Means±SE, n = 8 for each treatment group, Mean values within a row not sharing a common superscript letters (a, b, c, d, e) were significantly different, p<0.05, *Percentage of control group

combination group showed reduced the increase in TBARS levels compared to control. The protective effect of black berry was pronounced as follows: Low>medium>high doses of acrylamide.

Effect of acrylamide and black berry on the levels of glutathione reduced in tissues of rats: Data presented in Table 2 showed the changes in glutathione reduced (GSH) levels after treatment with acrylamide, blackberry juice and their combination. Animals treated with acrylamide alone exhibited a significant (p<0.05) decrease in GSH with all doses of acrylamide (120, 600 and 1200 µg kg^{-1}) in brain and kidney and caused significant (p<0.05) decrease in GSH with the doses of 600 and 1200 µg kg^{-1} b.wt., acrylamide in liver, lung and testes. Meanwhile, the results showed that there was a significant (p>0.05) increase in rats received blackberry juice alone compared to control. While, the presence of black berry with acrylamide in the combination group showed that blackberry reduced the decrease in GSH compared with control except the high dose (1200 µg kg^{-1} b.wt.). Black berry showed capability to alleviate the toxic effect of acrylamide with the low dose more than the medium and high dose.

Effect of acrylamide and black berry on the levels of the activities of antioxidant enzymes in tissues of rats: Table 3 showed that catalase activity was significantly (p<0.05) decreased in brain, kidney, liver, lung and testes in rats treated with 600 and 1200 µg acrylamide kg^{-1} b.wt. On the other side, no significant (p>0.05) differences in rats received blackberry juice alone compared to control. While, the presence of black berry with acrylamide in the

Table 4: Effect of different doses of acrylamide (AM) and black berry (BBj) on SOD (Units/mg protein) in tissues of male rats

	Experimental groups							
Parameters	Control	Black berry juice (BBj)	120 (µg AM/kg BW)	600 (µg AM/kg BW)	1200 (µg AM/kg BW)	120 (µg AM/kg BW+BBj)	600 (µg AM/kg BW+BBj)	1200 (µg AM/kg BW+BBj)
Brain	19.5 ± 1.54^{b}	24.2 ± 1.20^{a}(24)*	18.3 ± 1.12^{bc}(-7)*	15.3 ± 1.22^{de}(-21)*	13.1 ± 1.03^{e}(-33)*	19.3 ± 1.30^{b}(-1)*	17.4 ± 1.05^{bcd}(-11)*	15.4 ± 1.23^{cde}(-21)*
Kidney	15.8 ± 1.07^{b}	19.5 ± 1.38^{a}(24)*	14.5 ± 1.08^{bc}(-8)*	12.1 ± 0.90^{cd}(-23)*	10.3 ± 0.71^{d}(-35)*	15.4 ± 1.00^{b}(-2)*	14.0 ± 1.10^{bc}(-11)*	13.4 ± 1.10^{bc}(-15)*
Liver	16.1 ± 1.35^{b}	21.4 ± 1.75^{a}(33)*	15.2 ± 1.42^{b}(-6)*	14.0 ± 1.01^{bc}(-13)*	11.4 ± 1.08^{c}(-29)*	16.0 ± 1.33^{b}(-1)*	15.1 ± 1.25^{b}(-6)*	13.4 ± 1.18^{bc}(-17)*
Lung	27.0 ± 1.43^{b}	35.3 ± 1.50^{a}(30)*	25.5 ± 1.85^{bc}(-6)*	23.5 ± 1.08^{cd}(-13)*	18.5 ± 1.08^{e}(-32)*	26.9 ± 1.64^{bc}(-1)*	25.4 ± 1.47^{bc}(-6)*	21.2 ± 1.39^{de}(-21)*
Testes	27.6 ± 1.16^{b}	35.7 ± 2.23^{a}(29)*	25.6 ± 1.24^{bc}(-7)*	22.7 ± 1.23^{c}(-18)*	18.6 ± 1.08^{d}(-33)*	27.0 ± 1.21^{b}(-2)*	25.3 ± 1.34^{bc}(-8)*	23.1 ± 1.16^{c}(-16)*

Values are expressed as Means±SE, n = 8 for each treatment group, Mean values within a row not sharing a common superscript letters (a, b, c, d, e) were significantly different, p<0.05, *Percentage of control group, SOD: Superoxide dismutase

Table 5: Effect of different doses of acrylamide (AM) and black berry (BBj) on GST (µmol/h/mg protein) in tissues of male rats

	Experimental groups							
Parameters	Control	Black berry juice (BBj)	120 (µg AM/kg BW)	600 (µg AM/kg BW)	1200 (µg AM/kg BW)	120 (µg AM/kg BW+BBj)	600 (µg AM/kg BW+BBj)	1200 (µg AM/kg BW+BBj)
Brain	1.02 ± 0.048^{b}	1.37 ± 0.044^{a}(33)*	0.94 ± 0.043^{b}(-9)*	0.80 ± 0.070^{c}(-22)*	0.66 ± 0.034^{d}(-36)*	1.01 ± 0.057^{b}(-1)*	0.90 ± 0.075^{bc}(-12)*	0.78 ± 0.053^{cd}(-24)*
Kidney	1.29 ± 0.050^{b}	1.71 ± 0.085^{a}(33)*	1.16 ± 0.047^{bc}(-10)*	1.02 ± 0.080^{cd}(-21)*	0.90 ± 0.045^{d}(-31)*	1.25 ± 0.075^{b}(-3)*	1.15 ± 0.042^{bc}(-11)*	1.08 ± 0.052^{c}(-17)*
Liver	1.59 ± 0.051^{b}	2.12 ± 0.190^{a}(33)*	1.38 ± 0.059^{bc}(-13)*	1.22 ± 0.050^{de}(-24)*	1.06 ± 0.069^{e}(-34)*	1.50 ± 0.076^{bc}(-6)*	1.38 ± 0.075^{cd}(-14)*	1.36 ± 0.073^{cd}(-15)*
Lung	1.24 ± 0.077^{b}	1.56 ± 0.070^{a}(26)*	1.09 ± 0.079^{bc}(-12)*	0.960 ± 0.0678^{cd}(-22)*	0.843 ± 0.0573^{d}(-32)*	1.23 ± 0.062^{b}(-1)*	1.10 ± 0.059^{bc}(-11)*	0.984 ± 0.0580^{cd}(-21)*
Testes	1.48 ± 0.063^{b}	1.81 ± 0.050^{a}(22)*	$1.36\pm0.^{057bc}$(-9)*	1.18 ± 0.053^{d}(-20)*	0.945 ± 0.0557^{e}(-36)*	1.46 ± 0.046^{b}(-1)*	1.36 ± 0.080^{bc}(-8)*	1.27 ± 0.050^{cd}(-15)*

Values are expressed as Means±SE, n = 8 for each treatment group, Mean values within a row not sharing a common superscript letters (a, b, c, d, e) were significantly different, p<0.05, *Percentage of control group, GST: Glutathione–S transferases

Table 6: Effect of different doses of acrylamide (AM) and black berry (BBj) on GPX in tissues of male rats

	Experimental groups							
Parameters	Control	Black berry juice (BBj)	120 (µg AM/kg BW)	600 (µg AM/kg BW)	1200 (µg AM/kg BW)	120 (µg AM/kg BW+BBj)	600 (µg AM/kg BW+BBj)	1200 (µg AM/kg BW+BBj)
Brain	232.0 ± 13.7^{b}	304.0 ± 20.3^{a}(31)*	209.0 ± 11.0^{bcd}(-10)*	184.0 ± 10.9^{de}(-21)*	160.0 ± 10.1^{e}(-31)*	225.0 ± 11.0^{bc}(-3)*	209.0 ± 11.3^{bcd}(-10)*	200.0 ± 12.1^{cd}(-14)*
Kidney	237.0 ± 12.5^{b}	287.0 ± 17.5^{a}(21)*	220.0 ± 13.7^{bc}(-7)*	193.0 ± 12.2^{cd}(-18)*	168.0 ± 11.7^{d}(-29)*	233.0 ± 14.1^{b}(-1)*	216.0 ± 13.1^{bc}(-9)*	199.0 ± 12.5^{c}(-16)*
Liver	341.0 ± 12.4^{b}	455.0 ± 23.9^{a}(33)*	300.0 ± 25.0^{bcd}(-12)*	256.0 ± 20.8^{d}(-25)*	207.0 ± 10.2^{e}(-39)*	321.0 ± 20.2^{bc}(-6)*	288.0 ± 20.0^{cd}(-16)*	273.0 ± 24.9^{d}(-20)*
Lung	292.0 ± 18.3^{b}	377.0 ± 13.9^{a}(29)*	262.0 ± 15.2^{bc}(-10)*	222.0 ± 13.7^{d}(-24)*	175.0 ± 12.1^{e}(-40)*	285.0 ± 15.3^{bc}(-2)*	252.0 ± 12.1^{cd}(-14)*	223.0 ± 11.8^{d}(-23)*
Testes	263.0 ± 13.0^{b}	338.0 ± 18.9^{a}(28)*	233.0 ± 13.5^{cd}(-11)*	206.0 ± 9.5^{d}(-22)*	171.0 ± 8.6^{e}(-35)*	257.0 ± 11.9^{bc}(-2)*	235.0 ± 14.0^{bcd}(-11)*	214.0 ± 10.9^{d}(-19)*

Values are expressed as Means±SE, n = 8 for each treatment group, Mean values within a row not sharing a common superscript letters (a, b, c, d, e) were significantly different, p<0.05, *Percentage of control group, GPX: Glutathione peroxidase

combination group showed reduced the decrease in the catalase activity compared with control except the high dose (1200 µg kg^{-1} b.wt.). Black berry showed capability to alleviate the toxic effect of acrylamide with the low dose more than the medium and high dose. Table 4 presented the effect of treatment with acrylamide, blackberry and their combination on superoxide dismutase (SOD) activity in rats organs tissues. Animals treated with acrylamide alone caused significant (p<0.05) decrease in SOD with the doses of 600 and 1200 µg kg^{-1} b.wt., acrylamide in brain, kidney, lung and testes and caused significant (p<0.05) decrease in liver with high doses of acrylamide (1200 µg kg^{-1}). On the other hand, SOD activity was significant (p>0.05) increase in rats received blackberry juice alone compared to control. While, when the black berry combined with acrylamide reduced the decrease in SOD compared with control except at high dose (1200 µg kg^{-1} b.wt., in brain, lung and testes). The protective effect of blackberry was pronounced with the low dose of acrylamide then with the medium dose and then with the high dose.

Table 5 presented the results of glutathione-S transferases (GST) in all organs tissues of rats treated with acrylamide, blackberry and their combination. Animals treated with acrylamide alone caused significant (p<0.05) decrease in GST with the doses of 600 and 1200 µg kg^{-1} b.wt., acrylamide. On the other hand, the treatment with blackberry juice alone significantly (p>0.05) increased GST activity compared to control. The presence of blackberry in the combination group improved GST activity compared with control at low acrylamide dose (120 µg kg^{-1} b.wt.). From this result it can be concluded that the black berry showed capability to alleviate the toxic effect of acrylamide with the low dose more than the medium and high dose. The effect of treated with acrylamide black berry on glutathione peroxidase (GPX) of rats organs were showed in Table 6. Animals treated with acrylamide alone caused significant (p<0.05) decrease in GPX with all doses of acrylamide. There were a significant (p<0.05) decrease in GSH values in rats organs after treatment with all doses of acrylamide. On the other hand, the results showed that there is a significant ($p\leq0.05$) increase in rats received blackberry juice alone compared to control. While, the oral intake of black berry in combination group reduced the decrease in GPX activity in rats organs at all doses of acrylamide.

DISCUSSION

Acrylamide contents are high in fried and starch based foods. Such foods constitute the bulk of dietary global intake. Consequently, concern for acrylamide has become priority issue for most countries[35]. In this study we investigate the effect of giving blackberry juice on experimental animals treated with different doses of acrylamide. The obtained results are in agree with Anonymous[36], who reported that occupational or experimental exposure to acrylamide produces neurotoxicity characterized by ataxia, weight loss and nerve damage. Also, Yousef and El-Demerdash[37] found that clinical signs of general toxicity were observed at the 7th week of exposure to 250 μg acrylamide kg^{-1} b.wt. and at the 8th week of exposure to 500 μg kg^{-1} b.wt. The rats became weak and dragged their hind legs. While, no clinical signs were observed after exposure to 0.5, 5, 25 or 50 μg acrylamide kg b.wt., during the entire period of treatment. Barber and LoPachin[38] reported that the neurological defects associated with acrylamide intoxication are mediated by impaired neurotransmission at central and peripheral synapses. Free radicals are continuously produced *in vivo* and there are number of protective antioxidant enzymes (SOD, catalase, GST, GPX and antioxidant glutathione) for dealing with these toxic substances. Acrylamide is oxidized to glycidamide, a reactive epoxide and undergoes conjugation with glutathione. DNA adducts from glycidamide have been reported following administration of acrylamide[39]. Also, Lamy *et al.*[40] suggested that conjugation to glutathione would lead to a depletion of GSH store, increasing cell oxidative stress and thereby affecting gene expression. The GSH concentration in the human liver is high, ranging from 3 to 5 μmol g^{-1} of liver. Conditions that can decrease GSH levels and hence increase the toxicity of acrylamide at much lower exposure include oxidative stress, which may result in oxidation of GSH to oxidized glutathione (GSSG) and liver damage associated with alcoholic hepatitis and other malignant disorders. According to Odland *et al.*[41], the rate of protein synthesis as well as GSH levels of neuroblastoma cells decreased on exposure to acrylamide. The resulting depletion GSH could lead to less protection of cell membranes against oxidative stress. Superoxide dismutase, a metallic antioxidant enzyme play an active role during appearance of stress as a result of free radical generation was found increased in tissues. The increase in SOD activity could be to combat free radical generation during acrylamide toxicity[42]. Yousef and El-Demerdash[37] found significant decrease in the antioxidant enzymes and increase in the level of TBARS of rats treated with acrylamide in different tissue homogenates and this effect was dose-dependent. In that study, the authors detected a reaction between acrylamide and serum albumin or GSH, two of the most abundant thiols in the human body. They demonstrated that reactions with these thiols appear to account for most of acrylamide's elimination from the body. Halliwell[43] had demonstrated that the total pigment extract from black berries (TPEB), which is commonly used as a natural food colorant, is also an excellent natural antioxidant and free radical scavenger. Also, Jiao *et al.*[23] reported that the TPEB may have some antioxidant activities and health benefits related to its high anthocyanin and phenolic content. Also, the present study (Table 1-6) showed that blackberry juice reduced the levels of TBARS and increased the activities of antioxidant enzymes and GSH. Each polyphenol, such as anthocyanin, has different free radical-scavenging activities depending on their chemical structure (position, number and types of substitutions)[44]. The chemical activities of anthocyanins in terms of their reducing properties occur through the donation of electrons or hydrogen to free radicals. They also can act as metal chelators, inhibiting the formation of free radicals catalyzed by transition metals[45]. Tropical red–black berries had one of the highest oxygen radical absorbance capacity (ORAC)[19]. Antioxidants-rich black berry (vitamin C, vitamin E, anthocyanins, ellagitannins and some minor proanthocyanidins). However, anthocyanins have the biggest contributions to the anti-oxidant capacity[12,13]. They have anti-oxidant and anti-inflammatory properties and, therefore, may be potentially used to combat oxidative stress[11]. The anthocyanidins in the blackberry have shown a higher antioxidant activity than vitamins C and E. These compounds are able to capture free radicals by donation of phenolic hydrogen atoms; this is the reason for its anticarcinogenic activity[46]. It has also been reported, a linear correlation between the values of the antioxidant capacity and the anthocyanins content in blackberries it has been described that the berry extracts possess a high scavenging activity towards reactive oxygen species chemically generated[47]. The antioxidant activity of berries is directly proportional to the anthocyanins content[48]. The observed protective effect of blackberry juice in the present study (Table 1-6) may be attributed to synergistic effect of both vitamins C and E for preventing oxidative stress and cell destruction Hassan and Yousef[24]. Vitamin C and vitamin E are key synergistic anti-oxidants; when vitamin E quenches free radicals, it becomes a vitamin E radical, which then uses vitamin C to return it to its anti-oxidant state and acts as chelating agent[49]. Hassan and Yousef[24] found that the treatment with black berries juice alone significantly decreased the levels of TBARS and nitric oxide (NO) as well as increased total antioxidant capacity (TAC), GSH, SOD and catalase. Also, the presence of black berry juice with sodium fluoride (NaF) alleviated the harmful effects of NaF on most of the measured parameters. This effect may be related to the antioxidant properties of black berry juice. Administration of black berry juice alone caused significant ($p<0.05$) increase in the activities of SOD and catalase and the levels of GSH of TAC compared to control rats. In addition, a significant recovery relating to SOD, CAT, GSH and TAC was observed in response to the presence of black berry juice with NaF. Oxidative damage to cellular components such as lipids and cell membranes by free radicals and other reactive oxygen species is believed to be associated with the development of a range of degenerative diseases including heart disease, cancer, inflammation, arthritis, immune system decline, brain dysfunction and cataracts[43].

CONCLUSION

Animals treated with acrylamide alone caused significant decrease in catalase, superoxide dismutase (SOD), glutathione-S transferases (GST) and glutathione peroxidase (GPX) activities and reduced glutathione (GSH). On the other hand, the results showed that there was significant increase in rats received blackberry juice alone compared to control. While, the presence of black berry with acrylamide in the combination group showed that blackberry reduced the decrease in the antioxidant enzymes activities and GSH compared with control except the high dose of acrylamide.

REFERENCES

1. SNFA., 2002. Analysis of acrylamide in food. Swedish National Food Administration. http://www.slv.se/acrylamide.
2. Tareke, E., P. Rydberg, P. Karlsson, S. Eriksson and M. Tornqvist, 2002. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J. Agric. Food Chem., 50: 4998-5006.
3. Barber, D.S., J.R. Hunt, M.F. Ehrich, E.J. Lehning and R.M. LoPachin, 2001. Metabolism, toxicokinetics and hemoglobin adduct formation in rats following subacute and subchronic acrylamide dosing. Neurotoxicology, 22: 341-353.
4. Dybing, E., P.B. Farmer, M. Andersen, T.R. Fennell and S.P.D. Lalljie *et al*., 2005. Human exposure and internal dose assessments of acrylamide in food. Food Chem. Toxicol., 43: 365-410.
5. Abramsson-Zetterberg, L., J. Wong and N.G. Ilback, 2005. Acrylamide tissue distribution and genotoxic effects in a common viral infection in mice. Toxicology, 211: 70-76.
6. Sorgel, F., R. Wiessenbacher, M. Knizig-Schippers, A. Hofmann, M. Illauer, A. Skott and C. Landersdorfer, 2002. Acrylamide: Increased concentrations in homemade food and first evidence of its variable absorption from food, variable metabolism and placental and breast milk transfer in humans. Chemoteraphy, 48: 267-274.
7. IARC., 1994. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 60, International Agency for Research on Cancer, Lyon, France, pp: 389-433.
8. WHO., 2002. FAO/WHO consultation on the health implications of acrylamide in food. Summary Report, Geneva, June 25-27, 2002. World Health Organization, Geneva, Switzerland.
9. EC., 2002. Opinion of the scientific committee on food on new findings regarding the presence of acrylamide in food. July 3, 2002. European Commission, Belgium.
10. Rice, J.M., 2005. The carcinogenicity of acrylamide. Mutat. Res./Genet. Toxicol. Environ. Mutagen., 580: 3-20.
11. Felgines, C., O. Texier, C. Besson, D. Fraisse, J.L. Lamaison and C. Remesy, 2002. Blackberry anthocyanins are slightly bioavailable in rats. J. Nutr., 132: 1249-1253.
12. Stintzing, F.C., A.S. Stintzing, R. Carle, B. Frei and R.E. Wrolstad, 2002. Color and Antioxidant properties of cyanidin-based anthocyanin pigments. J. Agric. Food Chem., 50: 6172-6181.
13. Pantelidis, G.E., M. Vasilakakis, G.A. Manganaris and G. Diamantidis, 2007. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. Food Chem., 102: 777-783.
14. Stoner, G.D., 2009. Foodstuffs for preventing cancer: The preclinical and clinical development of berries. Cancer Prev. Res., 2: 187-194.
15. Seeram, N.P., 2010. Recent trends and advances in berry health benefits research. J. Agric. Food Chem., 58: 3869-3870.
16. Rice-Evans, C.A., N.J. Miller, P.G. Bolwell, P.M. Bramley and J.B. Pridham, 1995. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic. Res., 22: 375-383.
17. Akter, M.S., S. Oh, J.B. Eun and M. Ahmed, 2011. Nutritional compositions and health promoting phytochemicals of camu-camu (*Myrciaria dubia*) fruit: A review. Food Res. Int., 44: 1728-1732.
18. Celli, G.B., A.B. Pereira-Netto and T. Beta, 2011. Comparative analysis of total phenolic content, antioxidant activity and flavonoids profile of fruits from two varieties of Brazilian cherry (*Eugenia uniflora* L.) throughout the fruit developmental stages. Food Res. Int., 44: 2442-2451.

19. Wang, H., G. Cao and R.L. Prior, 1997. Oxygen radical absorbing capacity of anthocyanins. J. Agric. Food Chem., 45: 304-309.
20. Arun, R., M.V.D. Prakash, S.K. Abraham and K. Premkumar, 2011. Role of *Syzygium cumini* seed extract in the chemoprevention of *in vivo* genomic damage and oxidative stress. J. Ethnopharmacol., 134: 329-333.
21. Dunn, W.B. and D.I. Ellis, 2005. Metabolomics: Current analytical platforms and methodologies. TrAC Trends Anal. Chem., 24: 285-294.
22. Kahkonen, M.P., A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala and M. Heinonen, 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem., 47: 3954-3962.
23. Jiao, Z., J. Liu and S. Wang, 2005. Antioxidant activities of total pigment extract from blackberries. Food Technol. Biotechnol., 43: 97-102.
24. Hassan, H.A. and M.I. Yousef, 2009. Mitigating effects of antioxidant properties of black berry juice on sodium fluoride induced hepatotoxicity and oxidative stress in rats. Food Chem. Toxicol., 47: 2332-2337.
25. Walters, C.L., 1980. The exposure of humans to nitrite. Oncology, 37: 289-296.
26. Childs, A.C., S.L. Phaneuf, A.J. Dirks, T. Phillips and C. Leeuwenburgh, 2002. Doxorubicin treatment *in vivo* causes cytochrome C release and cardiomyocyte apoptosis, as well as increased mitochondrial efficiency, superoxide dismutase activity and Bcl-2:Bax ratio. Cancer Res., 62: 4592-4598.
27. Tappel, A.L. and H. Zalkin, 1959. Inhibition of lipide peroxidation in mitochondria by vitamin E. Arch. Biochem. Biophys., 80: 333-336.
28. Jollow, D.J., J.R. Mitchell, N. Zampaglione and J.R. Gillette, 1974. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology, 11: 151-169.
29. Luck, H., 1974. Catalase. In: Method of Enzymatic Analysis. Bergmayer, M.V. (Ed.)., Verlag Chemic. Academic Press, New York, pp: 885.
30. Misra, H.P. and I. Fridovich, 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247: 3170-3175.
31. Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.
32. Chiu, D.T.Y., F.H. Stults and A.L. Tappel, 1976. Purification and properties of rat lung soluble glutathione peroxidase. Biochem. Biophys. Acta Enzymol., 445: 558-566.
33. Steel, R.G.D. and J.H. Torrie, 1981. Principle and Procedure of Statistics. A Biometrical Approach. 2nd Edn., McGvaus-Hill Book Company, New York, US.
34. SAS., 1986. SAS User's Guide: Statistics. Version 5. Statistical Analysis System Institue Inc., Cary, NC., USA.
35. Naewbanij, J.O., 2002. Acrylamide in food: A serious but not a public health emergency issue. A Report of the Swedish National Food Institute (NFA). Department of Information Services.
36. Anonymous, 2002. Overview of acrylamide toxicity and metabolism. Proceedings of the JIFSAN/NCFST Workshop on Acrylamide in Food, October 28-30, 2002, Toxicology and Metabolic Consequences Working Group, pp: 1-35.
37. Yousef, M.I. and F.M. El-Demerdash, 2006. Acrylamide-induced oxidative stress and biochemical perturbations in rats. Toxicology, 219: 133-141.
38. Barber, D.S. and R.M. LoPachin, 2004. Proteomic analysis of acrylamide-protein adduct formation in rat brain synaptosomes. Toxicol. Applied Pharmacol., 201: 120-136.
39. Dybing, E. and T. Sanner, 2003. Risk assessment of acrylamide in foods. Toxicol. Sci. 75: 7-15.
40. Lamy, E., Y. Volkel, P.H. Roos, F. Kassie and V. Mersch-Sundermann, 2008. Ethanol enhanced the genotoxicity of acrylamide in human, metabolically competent HepG2 cells by CYP2E1 induction and glutathione depletion. Int. J. Hyg. Environ. Health, 211: 74-81.
41. Odland, L., L. Romert, C. Clemedson and E. Walum, 1994. Glutathione content, glutathione transferase activity and lipid peroxidation in acrylamide-treated neuroblastoma N1E 115 cells. Toxicol. *In Vitro*, 8: 263-267.
42. Sridevi, B., K.V. Reddy and S.L.N. Reddy, 1998. Effect of trivalent and hexavalent chromium on antioxidant enzyme activities and lipid peroxidation in a freshwater field crab, *Barytelphusa guerini*. Bull. Environ. Contam. Toxicol., 61: 384-390.
43. Halliwell, B., 1997. Antioxidants and human disease: A general introduction. Nutr. Rev., 55: S44-S49.
44. Wallace, T.C., 2011. Anthocyanins in cardiovascular disease. Adv. Nutr., 2: 1-7.
45. Rice-Evans, C.A., N. Miller and G. Paganga, 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci., 2: 152-159.
46. Kamei, H., T. Kojima, M. Hasewaga, T. Koide, T. Umeda, T. Yukawa and K. Terabe, 1995. Suppression of tumor cell growth by anthocyanins *in vitro*. Cancer Invest., 13: 590-594.
47. Wang, S.Y. and H.S. Lin, 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry and strawberry varies with cultivar and developmental stage. J. Agric. Food Chem., 48: 140-146.
48. Heinonen, I.M., A.S. Meyer and E.N. Frankel, 1998. Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. J. Agric. Food Chem., 46: 4107-4112.
49. Chinoy, N.J. and M.R. Memon, 2001. Beneficial effects of some vitamins and calcium on fluoride and aluminium toxicity on gastrocnemius muscle and liver of rat. Fluoride, 34: 21-33.