The Ability of Blackberry Juice Compared to Anthocyanin and Gallic Acid to Reduce the Harmful Effects of Acrylamide in Rats' Kidneys

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Abstract

Background & Objective(s): Blackberry (BB) ranks highly among fruits with strong antioxidant activities. It contains appreciable levels of phenolic compounds, mainly gallic acid and anthocyanin that have been related to antioxidant activity in these fruits. Acrylamide (ACR) is a chemical with a very wide range of uses in industry. It has been detected in a widely consumed food item and accounts as one of the major health concern. The aim of this study was to compare the effect of BB juice as a natural source of antioxidant with either anthocyanin or gallic acid on the acrylamide harmful effect on kidneys in rats.

Methods: A total of 50 adult male Albino rats equally allocated in five groups were used in this study. Rats in group 1 served as untreated control, group 2 rats were given ACR at dose of 50mg/kg BW, group 3 rats were given anthocyanin at a dose of 5 mg/kg BW + 50 mg/kg BW ACR, group 4 rats were given 3µg/kg gallic acid + 50 mg/kg ACR and group 5 rats were treated with 50 mg/kg BW ACR + 1.6 g/kg BW of BB juice. Rats were administered their respective doses on daily basis for eight weeks.

Results: The results showed that there was a significant difference in body weight gain between experimental groups. It was noticed that ACR caused a significant decrease in some hematological parameters [red blood cell (RBC) count (2.2×10^6/L), hemoglobin level (9.0 g/dl) and platelet count (206×10^6/L)] and some antioxidants mea levels [glutathione (GSH) (6.1 μmol/ml), superoxide dismutase (SOD) (302.4 μmol/ml) and total antioxidant capacity (TAC) (1.3 μmol/ml)] compared to the control group. On the contrary, ACR caused a significant increase in white blood cell (WBC) count, kidney functions [uric acid (5.1 mg/dl), creatinine (1.5 mg/dl) and urea (76.4 mg/dl)], thiobarbituric acid reactive substances (TBARS), nitric oxide (NO) and tumor markers [Interleukin-6 (IL-6) (209.3 pg/ml), tumor necrosis factor- alpha (TNF-α) (120.5 pg/ml), and tumor suppressor gene (p53) (25.5 pg/ml)] than in the control group. On the other hand, we found that polyphenols caused a significant increase in white blood cell (WBC) count, kidney functions [uric acid (5.1 mg/dl), creatinine (1.5 mg/dl) and urea (76.4 mg/dl)], thiobarbituric acid reactive substances (TBARS), nitric oxide (NO) and tumor markers [Interleukin-6 (IL-6) (209.3 pg/ml), tumor necrosis factor- alpha (TNF-α) (120.5 pg/ml), and tumor suppressor gene (p53) (25.5 pg/ml)] than in the control group. On the other hand, we found that polyphenols caused a significant increase in RBC, hemoglobin, platelets and the best effect was observed with BB juice. The latter significantly increased the levels of GSH, SOD, and TAC, while polyphenols decrease WBC, uric acid, creatinine, urea, TBARS, NO, p53, TNF and IL6 levels compared to the ACR group. Histological examination for kidney tissue showed that ACR can damage the kidney structure but presence of polyphenols especially BB Juice may save kidney from damage.

Conclusion: the result of this study suggested that BB Juice as a natural source of antioxidants is more protective than either anthocyanin or gallic acid alone against ACR toxicity on rat’s kidneys.

Keywords: Blackberry, Anthocyanin, Gallic acid, Acrylamide, Kidney, tumor markers

INTRODUCTION

Acrylamide (ACR) is a great public health concern due to its proved carcinogenicity in animals as was declared by the International Agency for Research on Cancer (IARC) (1). It is used in the industry, especially in the form of polyacrylamides utilized as flocculants for wastewater treatment, in adhesives and grouts, soil stabilizers and in laboratory gels (2). ACR is formed in any carbohydrate rich food items cooked at high temperatures.
ACR is a reactive, small organic molecule with very high water solubility. These properties facilitate its rapid absorption and distribution through the body (4). Once absorbed, ACR may be conjugated by glutathione-S-transferase (GST) to N-acetyl-S-(3-amino-3-oxopropyl) cysteine or it reacts with cytochrome P450 (CYP450) to produce glycxlidamidate. Several metabolic studies have been conducted that focused on the interaction of ACR with CYP450 and GST in rats and mice. The results of these studies indicated that ACR may inhibit GST, resulting in increased metabolism of glycxlidamide by the CYP450 pathway (5, 6). The resulting metabolite is an epoxide derivative, glycxlidamate, which is more reactive towards DNA and proteins than the parent compound, ACR (7).

A growing body of evidence pointed to the significant binding capacity of liver, kidney, brain and erythrocyte GST with ACR (8). Increasing attention has been therefore paid to naturally occurring antioxidants derived from vegetables and fruits for protection against ACR induced toxicity (8, 9). Dietary polyphenols predominate among the most common phytochemical agents for which anticarcinogenic, including photochemopreventive properties, have been described (10,11,12). These botanical agents exhibit antioxidant, anti-inflammatory, anti-proliferative and DNA repair properties with potential photoprotective and/or photochemopreventive applications (13).

Blackberry (BB) Morus nigra, is well known to contain abundant polyphenols that contribute to its high antioxidant capacity (14,15). BBs are rich sources of anthocyanin and other phenolic compounds such as gallic acid. They possess great health-promoting functions including anti-hyperglycemic, anti-obesity, anti-inflammatory, anticancer, and neuroprotective effects as well as, blood pressure lowering and antioxidant properties (14-18). Anthocyanin is the most important natural active ingredients in berries and has been reported to have anticanter, antioxidant and other physiological activities (19-23).

The aim of this study was to compare the effect of BB juice as a natural source of antioxidant with either anthocyanin or gallic acid on the acrylamide harmful effect on kidneys in rats

METHODS

Study setting and reagents: All reagents used in this study were of analytical grade. ACR (99.9%), anthocyanin and gallic acid were purchased from Sigma Company. Fresh BB was obtained from local market in Alexandria, Egypt. BB was washed, homogenized and its juice (hereafter BBJ) was freshly prepared on daily basis. BBJ was analyzed to estimate its content of Anthocyanin according to Prieto et al., (1999)(24) and gallic acid according to Singleton et al., (1999)(25) As 1 kg of BBJ had approximately 3170 mg anthocyanin, so 1.6 g of BBJ contain 5 mg anthocyanin. Also, 1 kg of BBJ had approximately 2 mg gallic acid, so 1.6 g BBJ contains 3 µg gallic acid. The recommended doses of BBJ, anthocyanin and gallic acid were 1.6 g/kg BW, 5 mg/kg BW and 3 µg/kg BW respectively. For ACR, 1/3 LD50 (50mg/kg BW) was used (26).

Experimental animals: Male albino rats (n= 50) averaging 250±5g were obtained from the animal house of the Institute of Graduated Studies and Researches, Alexandria University, Egypt. Animals received optimum care, and had adequate stable diet and water ad libitum. Animals were acclimatized to the laboratory conditions for two weeks before being experimented.

Experimental design: After two weeks of acclimation, animals were allocated into five equal groups (n=10). Rats in group 1 served as untreated control (negative control group), group 2 rats were given ACR dissolved in distilled water at a dose of 50mg/kg BW intraperitoneally (positive control group), group 3 rats were given anthocyanin at a dose of 5 mg/kg BW orally throw oral gavages + 50 mg/kg BW ACR, group 4 rats were given 3 µg/kg gallic acid + 50 mg/kg ACR and group 5 rats were treated with 50 mg/kg BW ACR + 1.6 g/kg BW of BBJ. Rats were administered their respective doses on daily basis for eight weeks.

Body weight and kidney weight: Body weight of rats was recorded at the beginning, weekly and at the end of the experimental period. Animals were sacrificed by decapitation, and their kidneys were immediately removed and weighed. Relative kidney weight was calculated as g/100g BW.

Blood sampling: Blood samples were collected from the sacrificed animals in heparinized tube. Plasma samples were obtained by centrifugation at 3000 xg for 20 minutes, and then samples were stored at -20ºC until used for further analyses.

- Antioxidant enzymes activity and free radicals: The activity of thiobarbituric acid reactive substances (TBARS), nitric oxide (NO) glutathione (GSH), and total antioxidant capacity (TAC) were assayed in kidney homogenates using commercial kits of Biodiagnostic Company (27-31).

- Kidney functions: Plasma samples were analyzed for the determination of uric acid, creatinine and urea concentrations according to the method described by Lamb et al., (2006) (31).

Tumor Markers: Commercially available Enzyme-Linked Immuno Sorbent Assay (ELISA) kits were used for the in vitro quantitative measurement of Interleukin-6 (IL-6) (Kamiya Biomedical C., 12779 Gateway Drive, Seattle, WA 98168), tumor necrosis factor-alpha (TNF-α) (Abcam co., UK), and the tumor suppressor gene p53 (Active Motif co. 1914, Palomar Oaks Way, Suite 150, Carlsbad, CA 92008 USA).

Histological examination: Kidney specimen used for histological study was fixed in neutral formalin for a week at room temperature, dehydrated then cleared in xylene and embedded in paraffin wax. The paraffin sections were
cut at 20 microns thickness and stained with hematoxylin and eosin for histological examination using the light microscope (32).

**Statistical analysis**
All data were expressed as mean ± SD. Statistical Analyses System (SAS) software program version 9.1 (SAS, 2003)(33) was used for one way analysis of variance (ANOVA), at ≤ 0.05 to compare the statistically significant difference between groups.

**Ethical considerations**
The study protocol was approved by the institutional review board and the ethics committee of the Institute of Graduated Studies and Researches, Alexandria University, Egypt. The research conformed to the international guidelines on research ethics of animal experimentation. All laboratory biological specimens and hazardous waste were disposed of safely.

**RESULTS**
Table (1) showed that there was a significant difference in body weight gain between experimental groups, although the change in relative kidney weights was negligible. Hematological parameters for ACR and experimental groups are detailed in table (2). It was noticed that ACR caused a significant decrease in RBC count (2.2×10⁶ vs 5.1×10⁶/l), hemoglobin concentration (9.0 vs 14.8 g/dl) and platelets count (208×10³ vs 328×10³/l) but significantly increased the WBC count (17250×10³ vs 6350×10³/l) as compared to the control group. On the other hand, it was found that polyphenols increased total RBC count, hemoglobin concentration and platelets counts than in ACR group; and the best effect was achieved by BBJ (3.8×10⁶/l, 11.4 g/dl and 250×10³/l) respectively. BBJ also significantly decreases the level of WBC (9750×10³/l) than in ACR group.

**Table 1: Body weight gain and relative kidney weight in acrylamide treated rats compared to polyphenols treated groups**

| Parameters                  | Control | ACR         | ACR + Anthocyanin | ACR + Gallic Acid | ACR + BBJ |
|-----------------------------|---------|-------------|-------------------|-------------------|-----------|
| Initial weight (g)          | 255.0 ± 7.0⁰ | 248.3 ± 3.6⁰ | 252.5 ± 9.2⁰     | 248.3 ± 10.1⁰   | 245.6 ± 13.3⁰ |
| Final weight (g)            | 275.5 ± 10.4⁰ | 246.1 ± 4.7⁰ | 277.8 ± 4.2⁰     | 264.4 ± 8.5⁰   | 266.9 ± 7.9⁰ |
| Body weight gain (g)        | 20.5 ± 11.5⁰ | -2.2 ± 2.3⁰ | 25.3 ± 9.5⁰      | 16.1 ± 8.8⁰    | 21.3 ± 9.8⁰ |
| Kidney g/100g BW            | 0.70 ± 0.02⁰  | 0.76 ± 0.02⁰ | 0.69 ± 0.04⁰     | 0.76 ± 0.04⁰  | 0.77 ± 0.03⁰ |

*Data are expressed as mean ± SE (n= 10). Means which share the same superscript are not significantly different; significance level at p ≥ 0.05.*

**Figure 1:** (A-E): light photomicrographs of rat renal cortex sections showing: (A) Control group, (B) ACR group and (C) Anthocyanin group, (D) Gallic acid group and (E) Blackberry juice group (H&E X 100, 200 & 400).
Table 2: Hematological parameters in acrylamide treated rats compared to polyphenols treated groups

| Parameters | Treated groups | Control | ACR | ACR + Anthocyanin | ACR + Gallic acid | ACR + BBJ |
|------------|----------------|---------|-----|-------------------|-------------------|-----------|
| RBC (×10^6/L) |                | 5.1 ± 0.12a | 2.2 ± 0.06a | 2.7 ± 0.1b | 3.2 ± 0.2c | 3.8 ± 0.21b |
| WBC (×10^3/L) |                | 6350 ± 260d | 17250 ± 837d | 14950 ± 375b | 8800 ± 231c | 9750 ± 375c |
| Hemoglobin g/dl |                | 14.8 ± 0.34a | 9.0 ± 0.3c | 9.5 ± 0.4b | 11.4 ± 0.36b | 11.4 ± 0.6b |
| Platelets (×10^3/L) |          | 328 ± 19.1a | 208 ± 7.1c | 225 ± 13.6bc | 231 ± 7.3bc | 250 ± 10.7b |
| PCV % |               | 48.7 ± 1.2a | 29.3 ± 1.2c | 30.3 ± 2.2c | 38.0 ± 1.5b | 39.0 ± 1.5b |

Data are expressed as mean ± SE (n= 10), Means which share the same superscript are not significantly different; significance level at \( p \geq 0.05 \).

Table (3) describes kidney functions in the experimental groups. It was obvious that ACR increased the levels of uric acid, creatinine and urea (5.1, 1.5, and 76.4 mg/dl respectively) than in control group (3.7, 1.0, and 43 mg/dl respectively). Treatment of rats with polyphenols along with ACR decreased the levels of uric acid, creatinine and urea and the best effect was noticed with BBJ (3.9, 1.1, and 49.2 mg/dl respectively) compared to the ACR group.

Regarding the effect on antioxidant levels, ACR increased the levels of TBARS (74.0 μmol/ml) and NO (70.3 μmol/ml) than in control group (39.5 and 25.4 μmol/ml respectively). Likewise, ACR decreased the level of GSH (6.1 μmol/ml), SOD (302.4 μmol/ml), TAC (1.3 μmol/ml) compared to the control group (6.4, 355.2 and 1.5 μmol/ml respectively).

On the other hand, it was found that the presence of polyphenols along with ACR decreased the levels of TBARS than in ACR group and the best effect was noticed with gallic acid (50.3 μmol/ml). Polyphenols also decreased the level of NO compared to the ACR group, which was more evident with anthocyanin (37.3 μmol/ml). Moreover, polyphenols increased the levels of GSH, SOD and TAC compared to the ACR group (Table 4). Remarkably, ACR increased the levels of tumor markers [p53 (25.5 pg/ml), TFN-α (120.5 pg/ml) and IL-6 (209.3 pg/ml) respectively] than in the control group (6.2, 41.5 and 100.5 pg/ml respectively), whereas polyphenols decreased the mean tumor markers levels compared to the ACR group, with the best effect being achieved by BBJ (9.4, 50.0 and 91.0 pg/ml respectively) (Table 5).

Table 3: Kidney functions in acrylamide treated rats compared to polyphenols treated groups

| Parameters | Treated groups | Control | ACR | ACR + Anthocyanin | ACR + Gallic acid | ACR+BBJ |
|------------|----------------|---------|-----|-------------------|-------------------|---------|
| Uric acid (mg/dl) |                | 3.7 ± 0.06b | 5.1 ± 0.1a | 3.9 ± 0.06b | 3.9 ± 0.04b | 3.9 ± 0.05b |
| Creatinine (mg/dl) |                | 1.0 ± 0.02d | 1.5 ± 0.02b | 1.3 ± 0.02b | 1.2 ± 0.05c | 1.1 ± 0.04cd |
| Urea (mg/dl) |               | 43.0 ± 0.8a | 76.4 ± 0.9b | 59.1 ± 0.6b | 57.1 ± 1.1b | 49.2 ± 0.9b |

Data are expressed as mean ± SE (n= 10), Means which share the same superscript are not significantly different; significance level at \( p \geq 0.05 \).

Table 4: Antioxidant enzymes activities and free radicals in acrylamide treated rats compared to polyphenols treated groups

| Parameters | Treated groups | Control | ACR | ACR + Anthocyanin | ACR + Gallic acid | ACR + BBJ |
|------------|----------------|---------|-----|-------------------|-------------------|-----------|
| TBARS (μmol/ml) |                | 39.5 ± 0.6b | 74.0 ± 0.5b | 57.3 ± 0.6b | 50.3 ± 0.9d | 53.3 ± 0.8b |
| NO (μmol/ml) |                | 25.4 ± 0.3d | 70.3 ± 1.4b | 37.3 ± 0.44c | 42.9 ± 0.45c | 39.0 ± 0.03b |
| GSH (μmol/ml) |               | 6.4 ± 0.06b | 6.1 ± 0.09h | 7.0 ± 0.03h | 6.5 ± 0.06b | 6.6 ± 0.1b |
| SOD (μmol/ml) |               | 355.2 ± 2.1d | 302.4 ± 2.6e | 400.9 ± 3.6h | 430.9 ± 2.1e | 391.7 ± 1.5b |
| TAC (μmol/ml) |               | 1.5 ± 0.03b | 1.3 ± 0.03b | 1.3 ± 0.00f | 1.3 ± 0.00f | 1.4 ± 0.00f |

Data are expressed as mean ± SE (n= 10), Means which share the same superscript are not significantly different; significance level at \( p \geq 0.05 \).
Histologic changes in kidney tissue are depicted in Figure 1 (A-E). Light photomicrographs of rat renal cortex sections showed that controls had normal renal corpuscle surrounded by sections of proximal and distal tubules with its classical brush borders (A). The tissues in ACR treated group revealed massive destruction of normal cortex architecture as matted renal corpuscles dilated vacuolated tubules and loss of prominent brush border. Extravasation of blood and cellular infiltration was also noticed (B). Polyphenols treated groups showed marked preservation of normal looking renal corpuscle while mild vacuolation were illustrated in some renal tubules with restoration of its brush border (C, D, and E).

Table 5: Interleukin-6, tumor necrosis factor- alpha and tumor suppressor gene p53 in acrylamide treated rats compared to polyphenols treated groups

| Parameters | Control | ACR | ACR + Anthocyanin | ACR + Gallic acid | ACR + BBJ |
|------------|---------|-----|-------------------|-------------------|-----------|
| IL6 (pg/ml)| 100.5 ± 1.6^a | 209.3 ± 2.7^a | 124.5 ± 4.9^b     | 133.5 ± 3.8^c    | 91.0 ± 5.2^c |
| TFN (pg/ml)| 41.5 ± 1.4^d | 120.5 ± 4.9^a | 71.0 ± 1.2^b      | 70.0 ± 2.3^b     | 50.0 ± 0.6^c |
| p53 (pg/ml)| 6.2 ± 0.2^d | 25.5 ± 0.7^a   | 10.7 ± 0.3^c      | 14.0 ± 0.4^b     | 9.4 ± 0.6^c |

Data are expressed as mean ± SE (n= 10), Means which share the same superscript are not significantly different; significance level at p< 0.05.

**DISCUSSION**

BBs is considered as one of the important fruits with strong antioxidant activities (34,35). It contains appreciable levels of phenolic compounds, mainly gallic acid and anthocyanin (36,37), two of the most common phenolic compounds in berries with potent antioxidant activity (38).

In the current work, it was found that presence of polyphenols along with ACR can enhance the toxic effect of ACR on RBC and hemoglobin while BBJ restored this activity. Mansour et al., (2008) also found that ACR produced a significant decrease in RBC counts, hemoglobin concentration, and hematocrit values in rats that received ACR (39). Likewise, the data of Benziane's study revealed changes in several hematological parameters (40).

We found that ACR affected kidney function as it caused significant increase in the level of creatinine, urea and uric acid. Benziane et al., (2018) found that ACR can affect various biochemical parameters particularly creatinine and urea, and concluded that oral exposure to ACR induces kidney damage (40). ACR causes oxidative stress by inducing the generation of reactive oxygen species (ROS), reducing the antioxidant defense systems of cells via depleting non enzymatic antioxidant system (vitamins and glutathione) and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition (41,42).

The results of the present study showed that ACR increased the level of TBARS and the best effect found with gallic acid. Polyphenols also reduced the level of NO and the best effect was achieved by with anthocyanin. This could be attributed to the increased levels of GSH, SOD and TAC by polyphenols. Li et al., (2018) concluded that anthocyanin can inhibit the decrease in SOD activity as induced by ACR (44). He suggested that anthocyanin can restore the antioxidant enzyme activity and possibly reduce the generation of free radicals.

In the present study, ACR increased the levels of tumor markers mainly p53, TNF-α and IL-6. Histological evidence in ACR treated group showed a massive destruction of normal cortex architecture as matted renal corpuscles dilated vacuolated tubules and loss of prominent brush border. There was also extravasation of blood and cellular infiltration. These observations are in agreement with a study conducted by Mahmood et al., (2015) who found that the kidney of the groups treated with ACR showed degeneration of the glomerular tuft with infiltration of lymphocytes. The renal tubules became vacuolated and lost their brush borders and degenerative changes were observed in their epithelial lining followed by rupture of the cells, necrosis, and finally congestion of the interstitial blood vessels (45). Özer et al., (2014) also found that ultra-structural alterations were detected in epithelial cells of proximal tubules in kidney sections of the rats treated with 50 mg/kg ACR. Enhanced vacuolization and widely distributed peroxisomes were detected in the cytoplasm of epithelial cells (43). Similarly, Mansour et al., (2008) found that the kidney revealed an interstitial hemorrhage and edema (39). Vascular degeneration of the convoluted tubular epithelium, glomerular degeneration with albuminous material in the capsular space and hyaline cast within tubular lumen was also seen. In the present study, we noticed that polyphenols can decrease the levels of tumor markers, and the best effect was found with BBJ.
Histological study also showed marked preservation of normal looking renal corpuscle while mild vacuolation were illustrated in some renal tubules with restoration of its brush border.

CONCLUSION AND RECOMMENDATIONS

the result of this study suggested that BBJ as a natural source of antioxidants had more protective effect than either anthocyanin or gallic acid alone in reducing the toxic effects of ACR on kidney in experiment rats.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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