Original Article

Effect of Betaine on Blood Parameters Related to the Iron Status in Acrylamide-Treated Rats

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Abstract

It has been well documented that acrylamide (ACR) is a human carcinogen. One of the consequences of ACR exposure is central and peripheral nervous system damage, which may lead to hallucinations, drowsiness, and numbness in the hands, as well as legs. Betaine (BET) plays an active role in methylation reactions, including DNA methylation, the integrity of cell membranes, and memory development. It has been approved that BET protects the liver from oxidative stress-inducing substances, such as ethanol, and nonalcoholic fatty liver, brain, kidney, stomach, as well as ovaries, in rats. Therefore, this study was designed to investigate how BET influences iron-related blood parameters and biomarkers in acrylamide-treated rats. Twenty adult rats, weighing 180-200 g and aging 6-7 weeks, were randomly divided into four equal groups and given the following treatments every day for 30 days. The control group was identified as G1. Animals in the G2 group were intubated with BET [250 mg/kg body weight (B.W.)]. Animals in the G3 group were intubated with ACR (1 mg/kg B.W.), while animals in the G4 group were intubated with both BET and ACR at the same doses as animals in G2 and G3 groups. At the end of the experiment, blood samples were collected from anesthetized rats using the cardiac puncture technique for measuring white blood cell count, as well as their differential count, red blood cells count, hemoglobin, and their related parameters, including mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, hematocrit, as well as red cell distribution width. Additionally, serum was taken to measure serum iron concentration, ferritin, total iron-binding capacity, and transferrin saturation percentage. The results showed significant changes in some hematological parameters and the iron status correlated with anemia in the G3 group, treated with ACR. Such changes, accompanied by histopathological changes in the spleen oral intubation of BET alone, as well as its combination with ACR, caused the alleviation of anemia through correcting previous parameters. In conclusion, the findings revealed that BET positively influences anemia in rats treated with ACR.

Keywords: Acrylamide, Betaine, Hemoglobin, Iron, White blood cells

1. Introduction

Betaine (BET) or trimethylglycine is an amino acid-like substance, which is widely distributed in plants, animals, and microorganisms. It was firstly discovered in sugar beet (_Beta Vulgaris_ subspecies) and was later called glycine betaine. Chemically, its small N-trimethyl amino acid is found in commonly consumed meals, such as cereals, spinach, and beets. In human nutrition, wheat is the major source of BET (1). The BET plays an active role in methylation reactions, including DNA methylation (2), the integrity of cell membranes, and memory development (3). It has been approved that BET protects the liver from oxidative stress-inducing substances, such as ethanol (4), as well as nonalcoholic fatty liver, brain, kidney, stomach, and ovaries in rats (5).

It has been well documented that acrylamide (ACR) is a human carcinogen. One of the main consequences of ACR exposure is central and peripheral nervous system damage, which may lead to hallucinations, drowsiness, and numbness in the hands and legs. The ACR is created in high-temperature baking, roasting, or frying starchy meals (6). Foods that are highly affected by ACR include bread, crisps, coffee, and fried potatoes (7). Biscuits also include a significant amount of ACR. Humans are exposed to ACR mostly at the workplace; however, it can also come from food, water supply, and smoke (8, 9). The ACR is neurotoxic in humans, as well as laboratory animals (10), and it has mutagenesis and carcinogenic properties (11). Moreover, ACR was reported to induce hepatotoxicity (12), DNA damage (13), and reproductive toxicity (14, 15). The BET and ACR have influenced blood lipid profile and the antioxidant status (16). Considering different applications and usages of the ACR in human and animal fields, as well as the great chance of exposure to ACR toxicity, this study aimed to alleviate the deleterious effects of ACR on some blood parameters and the iron status using BET.

2. Materials and Methods

2.1. Animals and Housing

This experiment was carried out on 20 female adult Wistar albino rats (weighing 180-200 g and aging 7-8 weeks). Animals were gathered for the experiment after two weeks of acclimation in the animal house of the College of Veterinary Medicine at Baghdad University (Baghdad, Iraq) during February and March 2020. Throughout the experiment, they were kept in individual cages (five rats per container) in a well-ventilated room and were supplied a regular meal feeding, as well as water. The heat in the room was maintained at 23.2°C for 12 h. During the study, there was a light/dark cycle with the lighting on from 6:00 a.m. to 6:00 p.m.

2.2. Acrylamide and Betaine Preparation and Force Feeding

The ACR (Shandon Southern Products, England)

was dissolved in distilled water and orally administered to female rats at a dose of 1 mg/kg/0.5 ml a day using gastric intubation (17). The BET (Sigma Aldrich, USA) was dissolved in distilled water and orally administered to female rats at a dose of 250 mg/kg/0.5 ml a day using oral gavage (OG) (18).

2.3. Design of the Experiment

Twenty female adult rats were divided into four groups and intubated orally at random for 30 days as follows: 1) Animals in the control group (G1) received physiological saline solution (0.5 mL) by OG; 2) Animals in the BET group (G2) received BET at a dose of 250 mg/kg B.W. by OG; Animals in the ACR group (G3) received ACR at a dose of 1 mg/kg B.W. by OG; and 4) Animals in the ACR+BET group (G4) received ACR at a dose of 1 mg/kg B.W. and BET at a dose of 250 mg/kg B.W. by OG.

2.4. Blood Sampling and Biochemical Evaluation

At the end of the experiment, animals were sedated with intramuscular injections of xylazine (40 mg/kg B.W.) and ketamine (90 mg/kg B.W.) using sterile syringes while blood samples were obtained by the cardiac punctured procedure. Blood samples were placed in non-heparinized containers and allowed to stand for 30 min before being centrifuged for 15 min at 3,000 rpm to get the serum, which was then locked in securely sealed vials for subsequent use at -20°C. An electronic automated blood analyzer was used to evaluate the counts of white blood cells (WBCs), WBCs differential count, red blood cells (RBCs), hemoglobin (Hb), platelet count (PCT), red cell distribution width-coefficient of variation (RDW-CV) percentage, packed cell volume (PCV) percentage, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) (Tables 1 and 2). Serum levels of the following measures were then evaluated: ferritin percentage, iron density, transferrin saturation percentage (TS%), and total Iron-binding capacity (TIBC) (Table 3).

Table 1. Influence of betaine, acrylamide, and their combination on total leukocyte counts, differential leukocyte counts, and platelets counts in adult male rats

Parameters	Groups				
	G1	G2	G3	G4	LSD value
White blood cells (10³/ul)	12.00±0.82ᵇ	16.07±0.49ᵃ	7.90±0.41ᶜ	12.56±0.81ᵇ	1.971**
Neutrophil (%)	7.05±0.35ᵇᶜ	9.10±1.02ᵃᵇ	4.90±0.55ᵇ	10.14±0.88ᵃ	2.245**
Lymphocyte (%)	3.86±0.25ᵇᶜ	6.54±0.65ᵃ	2.54±0.29ᶜ	4.64±0.51ᵇ	1.373**
Monocyte (%)	0.70±0.28ᵃ	0.75±0.12ᵃ	0.20±0.05ᵇ	0.175±0.04ᵇ	0.473*
Basophil (%)	0.174±0.04ᵇ	1.15±0.21ᵃ	0.774±0.24ᵃ	0.824±0.10ᵃ	0.505**
Eosinophil (%)	0.170±0.04ᵃᵇ	0.150±0.02ᵇ	0.274±0.05ᵃ	0.224±0.04ᵃᵇ	0.009*

Means with different letters in the same row differed significantly at *= (P<0.05) and **= (P<0.01) levels. G1 is the control group, and G2 is the group of rats treated with betaine at a dose of (250 mg/kg B.W.) for 30 days. G3 is the group of rats treated with acrylamide at a dose of (1 mg/kg B.W.) for 30 days. G4 is the group of rats treated with acrylamide at a dose of (1 mg/kg B.W.) and betaine (250 mg/kg B.W.) for 65 days. LSD stands for lysergic acid diethylamide.

Table 2. Effect of betaine, acrylamide, and their combination on complete blood counts in adult male rats

Parameters	Groups				
	G1	G2	G3	G4	LSD value
RBCs (10⁶/ul)	5.58±0.09ᵃ	6.06±0.21ᵃ	3.48±0.17ᵇ	6.06±0.43ᵃ	0.780**
Hb (g/dl)	12.98±0.56ᵇ	14.56±0.57ᵃ	7.35±0.31ᶜ	14.00±0.23ᵃᵇ	1.334**
PCV (%)	36.76±0.79ᵇ	59.35±4.66ᵃ	19.00±0.55ᶜ	40.92±2.35ᵇ	7.969**
MCV (fL)	66.50±1.06ᵃᵇ	85.63±11.62ᵃ	65.19±6.72ᵇ	67.87±1.95ᵃᵇ	20.397*
MCH (pg)	23.26±1.11ᵃ	24.09±1.21ᵃ	21.17±1.94ᵃ	23.38±1.41ᵃ	4.352 NS
MCHC (g/dl)	35.34±1.54ᵃ	27.22±1.88ᵇ	38.76±2.32ᵃ	34.56±1.64ᵃ	5.612**
PCT (10³/ul)	636.00±48.85ᵇ	807.00±58.37ᵃ	571.20±22.90ᵇ	678.00±43.09ᵃᵇ	135.55**
RDW-CV (%)	12.88±0.20ᶜ	13.60±0.27ᶜ	17.70±0.35ᵃ	16.52±0.25ᵇ	0.826**

Means with different letters in the same row differed significantly at *= (P<0.05) and **= (P<0.01) levels. NS means non-significant. G1 is the Control group, and G2 is the group of rats treated with betaine at a dose of (250 mg/kg B.W.) for 30 days. G3 is the group of rats treated with acrylamide at a dose of (1 mg/kg B.W.) for 30 days. G4 is the group of rats treated with acrylamide at a dose of (1 mg/kg B.W.) and betaine (250 mg/kg B.W.) for 30 days. LSD stands for lysergic acid diethylamide. RBC: red blood cell; Hb: hemoglobin; PCV: packed cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean cell hemoglobin concentration; PCT: platelet count; and RDW-CV: red cell distribution width-coefficient of variation.

Table 3. Effect of betaine, acrylamide, and their combination on Ferritin, Iron, TIBC, and Transferrin Saturation in adult male rats

Parameters	Groups				
	G1	G2	G3	G4	LSD value
Ferritin (ng/ml)	2.47±0.34ᵇ	3.70±0.25ᵃ	1.92±0.24ᵇ	2.63±0.32ᵇ	0.869**
Iron (µg/dl)	200.20±6.60ᶜ	294.80±16.57ᵃ	162.20±7.07ᵈ	238.60±10.48ᵇ	32.786**
TIBC (µg/dl)	435.00±14.74ᵃᵇ	424.40±27.08ᵇ	486.20±19.77ᵃ	401.80±16.20ᵇ	60.036**
Transferrin saturation (%)	45.99±1.52ᵇ	65.84±5.12ᵃ	33.42±1.21ᶜ	60.30±4.17ᵃ	10.322**

Means with different letters in the same row differed significantly at *=(P<0.05) and **=(P<0.01). G1 is the Control group, and G2 is the group of rats treated with betaine at a dose of (250 mg/kg B.W.) for 30 days. G3 is the group of rats treated with acrylamide at a dose of (1 mg/kg B.W.) for 30 days. G4 is the group of rats treated with acrylamide at a dose of (1 mg/kg B.W) and betaine at a dose of (250 mg/kg B.W.) for 30 days. TIBC: Total iron-binding capacity. LSD stands for lysergic acid diethylamide.

2.5. Histopathology

The histopathological analysis was performed following the dissection of animals. In this phase, the spleen samples with appropriately sized portions were obtained and fixed in 10% neutral buffered formalin for histopathological evaluations. The fixed specimens of the spleen were subjected to an overnight process for dehydration, clearing, and impregnation using an automatic tissue processor (Sakura, Japan). By using an embedding station (Sakura, Japan), the spleen samples were embedded in paraffin blocks, and the serial sections of 4 µm thickness were cut using a microtome (ModelRM2245, Leica Biosystems, Wetzlar, Germany). In this study, an Autostainer (Model 5020, Leica Biosystems, Wetzlar, Germany) was used for the Hematoxylin and Eosin staining of the spleen sections. The mounted specimens were then observed and scored under light microscopy.

2.6. Statistical Analysis

To investigate the impact of different treatments on different physiological parameters, the Statistical Analysis System (version 9.1, 2012) was utilized. In this experiment, the least significant difference test (ANOVA) was run to analyze the means.

3. Results

At the end of the experiment (on day 30), a significant increase was observed in the total WBC count (neutrophil, lymphocyte, and monocyte) in the G2 group, compared to the G3 and G4 (except for neutrophil percentage) groups. Furthermore, a significant decrease was recorded in the number of eosinophils in the G2 group, in comparison with the G3 group. The results of the evaluation in the G4 group showed that blood parameters (WBC count, lymphocyte, and eosinophil percentage) tended toward normalization, compared to the G3 group (Table 1).

After the BET administration, there was a significant increase in the RBC count, Hb, PCV percentage, MCV, and PTC in G1, G2, and G4 compared to the G3 group. On the other hand, the results showed a significant decrease in MCHC and RDW-CV percentage in the G4 group, which caused the normalization of most of the blood parameters similar to that of the G1 (except for RDW-CV percentage) and G2 groups, except for PCV percentage, MCHC, and RDW-CV percentage.

The results of the current study revealed a significant increase in serum ferritin, iron concentration, and TS%, accompanied by a significant decrease in TIBC in the G2 group, compared to the G3 and G1 groups. The recorded data in the G4 group showed that BET treatment after ACR administration caused normalization in the value of ferritin and TIBC similar to that of the G1 group.

The results of the histopathological analysis of the animals' spleen in the G1 (Figure 1) and G2 (Figure 2) groups showed a normal histological structure and no clear lesions, respectively, compared to the section obtained from the spleen of the G3 group, which showed congested blood vessels with hemorrhage in red pulps (Figure 3). On the other hand, in the G4 group, which was treated with ACR and BET, the histopathological evaluations indicated a moderate proliferation in white pulps (Figure 4).
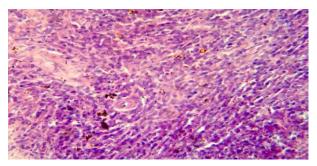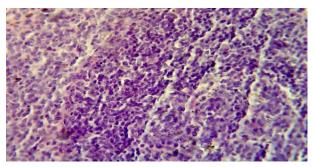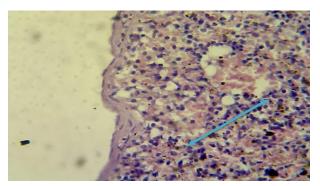

Figure 1. Histopathological segment of the animals' spleen (G1 group) showed no clear lesions (hematoxylin and eosin stain 400×)
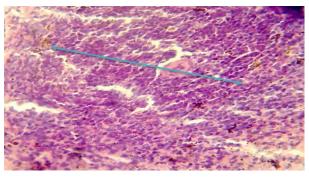

Figure 2. Histopathological segment of the animals' spleen treated with betaine (250 mg/kg body weight) for 30 days showed no clear lesions (hematoxylin and eosin stain 400×)

Figure 3. Histopathological segment of the animals' spleen treated with acrylamide (1 mg/kg body weight) for 30 days showed congested blood vessels and hemorrhage in the red pulp (hematoxylin and eosin stain 400×)

Figure 4. Histopathological segment of the animals' spleen treated with acrylamide (1 mg/kg body weight) and betaine (250 mg/kg B.W.) for 30 days showed a moderate proliferation of white pulp (hematoxylin and eosin stain 400×)

4. Discussion

The findings of the present study demonstrated a substantial rise in the overall WBC count and the differential leukocyte percentage in the G2 groups, compared to the G3 sections. The BET has an antioxidant property, as evidenced by an increase in the RBC count, Hb, and PCV percentage. The BET controls cell volume by regulating erythrocyte (red blood cell) membranes ATPase through conformational changes (19). Furthermore, the anti-hemolytic activity of the BET might be linked to the hydrophobicity of the three methyl groups (20).

The increase in MCHC after the BET administration may be attributed to the defense reaction against ACR, which occurred due to the stimulation of erythropoiesis (21). Barber, Hunt (22) identified a declining trend in hematological values, including Hb content,

erythrocyte count, and hematocrit level following the ACR treatment, showing the onset of microcytic anemia. (23) revealed considerable declines in Hb, RBCs, and hematocrit in the ACR-treated animals, which is compatible with the recent findings. The ACR is extremely reactive and may bind permanently with cysteine residues on the erythrocyte, producing a duct with sulfhydryl units. Therefore, heme is lost due to a decrease in the quantity of Hb in the plasma, resulting in anemia (24).

After the ACR treatment, WBC count decreased, which could be due to the decreased generation and transfer from peripheral blood into tissues, or the fast destruction of WBCs. Oxidative stress could be the mechanism of hematotoxicity induced by ACR (25). The observed changes in the iron concentration following the BET admiration could be attributed to the antioxidant effect of BET, which caused a reduction in the gene expression of reactive oxygen species leading to the reduction in homocysteine levels and the correction of iron homeostasis (26). It has been documented that a higher intake of BET correlated with a lowered plasma Homocysteine level by denoting the Methyl group and converting Homocysteine to methionine (27). To the best of the present researchers' knowledge, after reviewing previously published scientific studies, the present study is the first that directly investigates the association between iron homeostasis and ACR treatment. Oxidative stress could be the major contributor to iron deficiency anemia. Additionally, the shortened erythrocyte survival due to hemolytic caused depression in the iron level, as well as hyperhomocysteinemia, which is regarded as the biomarker of the early stages of iron deficiency (28). An inverse correlation was found between hyperhomocysteinemia and serum ferritin, Hb, and iron level (29, 30).

Accordingly, it can be concluded that hyperhomocysteinemia and oxidative stress accompanied by ACR could distribute iron homeostasis. Furthermore, ACR forms an adduct with

Hb; therefore, it leads to a disturbance in serum iron concentrations.

Authors' Contribution

Study concept and design: R. A. A.

Acquisition of data: M. H. Y.

Analysis and interpretation of data: M. H. Y.

Drafting of the manuscript: A. T. V. A.

Critical revision of the manuscript for important intellectual content: R. A. A.

Statistical analysis: A. T. V. A.

Administrative, technical, and material support: M. H. Y.

Ethics

All the procedures were approved by the ethics committee of the University of Baghdad, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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