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Black berry juice attenuates neurological disorders and oxidative stress associated with concurrent exposure of aluminum and fluoride in male rats

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ABSTRACT

The objective of this study was to assess the protective effect of black berry juice (BBJ) on the neurological disorders and oxidative stress induced by co-exposure to $ALCL_3$ and NaF in male albino rats. Administration of either AlCl3 (200 mg/kg bw) or NaF (10 mg/kg bw) or both of them caused a significant increase in serum and brain TL, TC, TG as well as serum LDLC and VLDLC levels while serum HDLC level was decreased significantly. Additionally, brain neurotransmitter (DA and 5-HT) levels, AChE, Na-K ATPase activity and ATP values were decreased significantly but NE level was increased in rats administered Al or F alone or in combination. Moreover, a significant increase in brain MDA, NO, H2O2 and free radical enzyme (xanthine oxidase (XO)) and a significant decrease in the level of TAC, SOD and GSH were recorded in $AlCl_3$ or NaF intoxicated rats. In addition, the levels of serum Na, Ca, Cu and zinc (Zn) were significantly diminished, while the level of K was significantly increased. However $ALCL_3$ appears to enhance the neurotoxic hazards caused by NaF. On the other hand, the administration of BBJ (1.6 g/kg bw) showed a marked neuroprotective effect against the biochemical abnormalities that occurred and oxidative stress of the brain induced by co-exposure to $AlCl_3$ and NaF. So, it can be concluded that the consumption of BBJ might be useful for alleviating the neurological disorders and oxidative stress associated with concurrent exposure of $ALCL_3$ and NaF indicating its free radical scavenging and potent antioxidant activity.

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Abbreviations: $AlCl_3$, Aluminum chloride; NaF, sodium fluoride; BBJ, black berry; TL, total lipid; TC, total cholesterol; TG, triglycerides; LDLC, low density lipoprotein; VLDLC, very low density lipoprotein; HDLC, high density lipoprotein cholesterol; DA, dopamine; 5-HT, serotonin; AChE, acetyl choline esterase; Na-K ATPase, sodium potassium adenosine tri phosphatase; NE, nor-epinephrine; MDA, malondialdehyde; NO, nitric oxide; H_2O_2, hydrogen peroxide; XO, xanthine oxidase; TAC, total antioxidant capacity; SOD, super oxide dismutase; GSH, reduced glutathione

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1. Introduction

Aluminum and fluoride are the most widely distributed toxic metals in the environment. The exposure of the human to these metals causes various adverse physiological effects particularly neuropathological changes. Aluminum occurs naturally and makes up around 8% of the surface of the earth. It constantly exists in combination with other elements like fluorine, oxygen and silicon. Everybody is exposed to aluminum from the air, water and food at low levels. It is frequently utilized as a part of building materials, cooking utensils, appliances and containers. It is additionally utilized as a component in fireworks and paints, to produce glass, rubber, and ceramics, and in consumer products such as buffered aspirin, astringents, antacids, food additives, and antiperspirants [1]. Production of free radicals seems to be the catalytic activity of aluminum. Additionally, aggregation of beta-amyloid protein in the brain of Alzheimer's patients induces more free radicals production [2]. Aluminum (Al) can induce oxidative damage through its ability to attach to negatively charged phospholipids of the brain, which include polyunsaturated fatty acids. So the reactive oxygen species (ROS) including H_2O_2, $O^{2\bullet-}$, $OH^{\bullet}$, and OH^- can easily attack these fatty acids [3]. Furthermore, the most electronegative element in nature is fluoride anion, its accumulation leads to fluorosis, a common disorder in developing countries, where human's main source of drinking water is usually polluted with fluoride [4]. Fluoride easily distributes throughout the body through the blood stream, penetrates the cellular membranes and its subsequent intoxication leads to cellular injury [5]. Also, production of free radical is considered to be one of the most crucial mechanisms of fluoride toxicity [4]. Brain tissues are highly susceptible to oxidative damage, probably because of high oxygen consumption rate (20%), the presence of abundant polyunsaturated fatty acids in cell membranes, high iron (Fe) content, and low antioxidative enzyme activities [6]. Actually, ingestion of diets containing large amounts of natural antioxidants including vegetables and fruits were considered to diminish specific age related neurological disorders such as dementia and macular degeneration [7].

Black berry (*Rubus spp.*) is one of the most important natural diets with anti-oxidant properties, contains large amounts of anthocyanins, and these flavonoid pigments give black berries their special red to blue color. Numerous studies have demonstrated the health benefits and antioxidant activities of the anthocyanins which naturally occur in various vegetables and fruits [8,9]. Anthocyanins, water soluble pigments found in plants, are polyphenols that have health promoting benefits including antioxidant and anti-inflammatory effects [10]. The antioxidative properties of anthocyanins arise from their high reactivity and ability to scavenge free radicals. Some reports have confirmed that anthocyanins are good antioxidants and can effectively eliminate free radicals [10]. So the present study was designed to elucidate the neurological disorders and oxidative stress related to exposure to fluoride alone or in conjugation with aluminum and the possible protective effect of black berry as a natural antioxidant against their adverse effects in male albino rats.

2. Materials and methods

2.1. Chemicals

Aluminum chloride (98%; anhydrous) and sodium fluoride (99%) were obtained from Sigma Chemical Company (St. Louis, USA). Fresh black berry fruits (Rubus spp.) were purchased from the local market (Mansoura, Egypt). The fruits were washed, homogenized and their juice was freshly prepared daily. The doses administered were prepared from the LD_{50} values of each compound; LD_{50} for NaF is 52 mg/kg for rat, while LD_{50} for $AlCl_3$ is 200–1000 mg/kg in rats. The tested doses of aluminum chloride ($AlCl_3$; 200 mg/kg bw) and sodium fluoride (NaF; 10 mg/kg bw) was chosen according to previous studies conducted by our group [11] also, black berry dose (1.6 g/kg bw equal to 9 ml/kg bw) was selected based on earlier studies [8,12]. All kits used through the experiment were obtained from Biodiagnostic Company, Egypt. All used reagents and chemicals were of analytical grade.

2.2. Experimental animals

Forty-eight adult male Wistar albino rats (*Rattus rattus*) with weight of 120–140 g, were obtained from the holding company for biological product and vaccines (VACSERA), Cairo, Egypt. The local committee approved the design of the experiments and the protocol follows the guidelines of the National Institutes of Health (NIH). Animals received human care, were kept under good ventilation, had adequate stable diet and water were allowed *ad libitum*. They were maintained on normal light/dark cycle throughout the experimental period. The animals were acclimatized to the laboratory conditions for two weeks before being experimented.

2.3. Experimental design

After 2 weeks of acclimation, rats were classified into eight groups comprising six rats in each. Group 1: served as untreated control (C). Group 2: rats were given orally black berry juice (BBJ) at dose of 1.6 g/kg bw. Group 3 ($AlCl_3$): rats were given orally $AlCl_3$ dissolved in distilled water at dose of 200 mg/kg bw. Group 4 (NaF): rats were given orally NaF dissolved in distilled water at dose of 10 mg/kg bw. Group 5 ($AlCl_3$+NaF): rats were given orally $AlCl_3$ followed by NaF at the same mentioned doses. Group 6 (BBJ+$AlCl_3$): rats were given orally BBJ followed by $AlCl_3$ at the same mentioned dose. Group 7 (BBJ+NaF): rats were given orally BBJ followed by NaF at the same mentioned dose. Group 8 (BBJ+$AlCl_3$+NaF): rats were given orally BBJ followed by $AlCl_3$ then NaF at the same mentioned doses. Rats were given their respective doses daily for 5 weeks.

2.4. Blood collection and tissue homogenate

At the end of the experimental period (5 weeks), blood samples were collected from the retro-orbital venous plexus of overnight fasted rats [13] in clean tubes and then centrifuged at 860 × *g* for 20 minutes. The separated sera were stored at –20 °C for subsequent analysis. Then rats of each group were sacrificed by decapitation and brain specimens were carefully