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Antioxidant Effect of Artichoke and Selenium on Potassium Bromate Induced Oxidative Stress in Experimental Rats

[1]Hala E.M. El-Kewawy and [2]Fatma L. Ahmed

[1]Department of Home Economics,
Faculty of Specific Education, Mansoura University, Egypt
[2]Department of Nutrition and Food Science,
Faculty of Home Economic, Helwan University, Egypt

Abstract: Forty-nine adult albino male rats Sprague -Dawley strain were randomly classified into seven groups (7 rats each). The control (-ve) fed on standard diet all over the period of the experiment. The other rats subjected to potassium bromate to induce oxidative stress and classified into control (+ve) and five treated rat groups with artichoke extract, artichoke powder, selenium, artichoke extract with selenium and artichoke powder with selenium. The results revealed that, the control (+ve) rat group showed a significant decrease in final body weight, body weight gain , food efficiency ratio, hemoglobin ,packed cell volume, total protein, albumin and globulin, serum superoxide dismutase (SOD), glutathione peroxidase (GPX) levels, serum catalase ,liver superoxide dismutase (SOD) and liver glutathione peroxidase(GPX) levels but a significant increase in blood free radical , serum ALT and AST, serum lipid peroxidase (LPX), serum nitric oxide (No) and liver monaldialdehyde (MDA) levels in comparing with control (-ve) group. Artichoke extract, artichoke powder, selenium, artichoke extract with selenium and artichoke powder with selenium rat groups showed a significant increase in final body weight, weight gain, food efficiency ratio, hemoglobin , packed cell volume ,serum total protein, serum albumin, serum globulin, serum SOD, serum GPX, liver SOD and liver GPX levels but a significant decrease in serum ALT and AST ,blood free radical, serum LPX, serum No and liver MDA levels in comparing with control (+ve) rat group.

Key wards: Oxidative stress · Artichoke · Selenium · Antioxidant-rats

INTRODUCTION

Oxidative stress is defined as the imbalance between biochemical processes leading to the production of reactive oxygen species (ROS) and those responsible for the removal of ROS called antioxidant cascade. ROS are known to damage all cellular biomacromolecules and this damage can lead to secondary products that can be just as damaging as the initial ROS [1]. Potassium bromate (KBrO3) is a by-product from ozonation of high bromide surface water for production of drinking water and the toxicological effects may be mediated via the induction of oxidative stress. Oxidative stress denotes a shift in the cellular prooxidant /antioxidant balance in favor of the prooxidants and has been implicated in mechanisms of carcinogenesis, aging and neurodegenerative disease [2, 3]. The potential consequence of oxidative damage could be attenuated by the administration of antioxidant compounds in food plants.

Globe artichoke (*Cynara scolymus*) still plays an important role in human nutrition, especially in the Mediterranean region. Nutritional and pharmaceutical properties of both artichoke heads and leaves are linked to their special chemical composition, which includes high levels of polyphenolic compounds and inulin [4]. The antioxidant properties of caffeoylquinic acids present in artichoke leaf extracts are considered to be mainly responsible for the hepatoprotective action [5, 6]. Selenium is an essential dietary trace element that is essential to good health but required only in small amounts. Selenium was incorporated into proteins to make selenoproteins, which were important as antioxidant

Corresponding Author: H.E.M. El-Kewawy, Department of Home Economics,
Faculty of Specific Education, Mansoura University, Egypt.

enzymes. Selenium is an integral part of many proteins with catalytic and structural functions. Its nutritional deficiency leads to muscular dystrophy, endemic fatal cardio myopathy and chronic degenerative diseases in humans that could be prevented by selenium supplementation when used alone or in combination [7].

This study aimed to investigate the antioxidant effect of artichoke and selenium on potassium bromate induced oxidative stress in experimental rats.

MATERIALS AND METHODS

Materials:

Potassium Bromate ($Kbro_3$) and Selenium: Potassium bromate is a white powder odorless, purchased from El-Gomhoria Co. Cairo, Egypt. Potassium bromate was dissolved in deionized water 20 ppm and given to rats by stomach tube all over the period of treatment [3]. Sodium selenite (Na_2SeO_3) was purchased from EL-Nasser Company for Chemical Industry. The rat dose of selenium was 3mg/kgb.w./day by stomach tube whereas Na_2SeO_3 dose was selected based on the clinical application and on results from previous studies on human and experimental animals [8].

Experimental Plants: Artichoke (*Cynara scolymus*) was obtained from local market then dried in dry freezer and crushed into powder. Artichoke powder added to standards diet as 10 % in substitution of fiber.

Experimental Animals: Forty nine rats were purchased from Helwan Farm of Laboratory Animals. The average weight was 121±8 g. The animals were kept under observation for five days before experiment and fed on standard diet and water *ad libitum*. The standard diet comprised of casein (200g/kg), corn starch (497g/kg), sucrose (100g/kg) , cellulose (30 g/kg) ,corn oil (50g/kg), mineral mixture (100g/kg) , vitamin mixture (20g/kg) and DL-methionine (3g/kg).The standard diet was performed according to NRC [9].

Biochemical Kits: BioMeriuex Kits were purchased from Alkan Co. for Chemicals and Biodignostics, Dokki, Egypt.

Methods:

Preparation of the Artichoke Powder and Ethanol Extract: The artichoke was dried in dry freezer and crushed into powder then added to standards diet as 10 % in substitution of fiber.270 g of fresh artichoke were chopped with both 300 ml ethanol by using a blender for 1 min at average speed. The mixture was macerated during 24h at the 4°C. After that, resulting extract was filtered using a 0.45 ìm pore size cellulose acetate membrane filter. The extract was used directly at dose 400 mg/kg of animal [10].

Experimental Design: The rats were randomly classified into seven groups (7rats each). The control (-ve) fed on standard diet all over the period of the experiment. The other groups subjected to potassium bromate to induce oxidative stress and classified into control (+ve) and five treated rat groups with artichoke extract, artichoke powder, selenium, artichoke extract with selenium and artichoke powder with selenium. The food intake was calculated daily and the body weight gain was recorded weekly. Food efficiency ratio (FER) was determined by Chapman *et al.* [11] as following: FER = weight gain (g)/ food intake (g). At the end of the duration period of the experiment (eight weeks), the rats were sacrificed for collection of blood and liver samples for laboratory analysis by BioMeriuex Kits.

Part of blood was heparenized for estimation of hemoglobin (HB) and packed cell volume (PCV) according to Drabkin [12] and Mc Inory [13]. Blood free radical, superoxide dismutase (SOD) and glutathione peroxidase (GPX) levels were estimated according to Borg [14], Beuchamp and Fridovich [15] and Beuther *et al.* [16], respectively. The rest part of blood was left to coagulate then centrifuged at 3000 rpm for 15 minutes to obtain serum. Serum aminotransferase (ALT&AST), total protein and albumin were estimated according to Reitman and Frankel [17], Weichselbaum [18] and Bartholomev and Delany [19], respectively. Serum globulin (G) value was determined by subtracting the albumin from the total proteins according to Coles [20]. Serum catalase, lipid peroxidase (LPX) and nitric oxide (NO) levels were estimated according to Claiborne [21], Botsoglou *et al.* [22] and Green *et al.* [23]. Livers of rats were rapidly removed and parts of them perfuse with 50 to 100 of ice cold 0.9%NaCL solution for estimation of liver superoxide dismutase (SOD), glutathione peroxidase (GPX) and malondialdehyde (MDA) levels which determined according to Beuchamp and Fridovich [15], Weiss *et al.* [24] and Uchiyama and Mihara [25], respectively.

Statistical Analysis: Collected data were subjected to analysis according to SPSS program according to Armitage and Berry [26].

RESULTS AND DISCUSSION

Nutritional Results: The statistical data in Table 1 represented that, control (+ve) rat group showed a significant decrease in final body weight, body weight gain and food efficiency ratio ($p<0.001$) while artichoke extract and artichoke powder with selenium rat groups showed significant decrease in final weight and weight gain ($P<0.05$) but selenium rat group showed only a significant decrease in weight gain compared with control (-ve) rat group. Artichoke extract, artichoke powder, selenium, artichoke extract with selenium and artichoke powder with selenium rat groups showed a significant increase in final body weight, weight gain and food efficiency ratio compared with control (+ve) rat group. Reduction in body weight is used as an indicator for the deterioration of rat general health status resulting from potassium bromate. Globe artichoke has important nutritional values due to its particularly high proportion of dietary fiber, protein, mineral, low percentage of lipids, polyphenolic substances and fructan-oligosaccharides in addition to important source of natural phenolic antioxidants such as hydroxycinnamic acids and flavones [27]. Organic or aqueous extracts of the raw plant, a rich source of polyphenols, demonstrate a strong antimicrobial and antioxidant activity in cell systems, inhibit cholesterol biosynthesis in hepatocytes and modulate vasomotor function [4]. Selenium is a constituent of selenoproteins and nutritionally acts through its enzyme, cytosolic glutathione or membrane bound phospholipids hydroperoxide glutathione peroxidase and thioredoxin reductase to control levels of cellular hydroperoxides and redox tone of cells [28].

Biochemical Results: In Table 2 the control (+ve) rat group showed a significant decrease in hemoglobin and packed cell volume ($P<0.01$&0.001) in compared with control (-ve) group. Artichoke extract, artichoke powder, selenium, artichoke extract with selenium and artichoke powder with selenium rat groups showed a significant increase in hemoglobin and packed cell volume compared with control (+ve) rat group. Artichoke extracts exhibit antioxidant properties in cultured endothelial cells and monocytes, mainly antagonizing lipid peroxidation [29]. Selenium plays an important role in the synthesis of glutathione peroxidase enzymes that catalyzes the reaction of reduced glutathione with hydrogen peroxide and organic peroxide. Selenium increase the concentration of vitamin E which is important antioxidant which acts as a scavenger of free radicals and reduces peroxides [30].

Table 3 showed that the control (+ve) ,artichoke extract and selenium rat groups showed a significant increase in blood free radical and a significant decrease in superoxide dismutase (SOD) and glutathione peroxidase (GPX) levels in compared with control (-ve) group. Artichoke powder showed a significant increase in blood free radical and a significant decrease in SOD while artichoke extract with selenium and artichoke powder with selenium rat groups showed a significant increase in blood free radical in compared with control (-ve) group. Artichoke extract, artichoke powder, selenium, artichoke extract with selenium and artichoke powder with selenium rat groups showed a significant decrease in blood free radical and a significant increase in SOD and GPX levels compared with control (+ve) group.

Table 1: Mean values ± SD of body weight gain, food intake and food efficiency ratio (FER) of the experimental rat groups

Groups Variables	Control (-ve)	Control (+ve)	Artichoke extract	Artichoke powder	Selenium	Artichoke extract+ Selenium	Artichoke powder + Selenium
Initial Weight(g)	121.71±3.11^{a}	123.24±3.61^{a}	122.71±3.27^{a}	124.35±4.01^{a}	125.11±3.69^{a}	124.36±3.67^{a}	121.81±3.89^{a}
Final weight (g)	197.04±5.76^{a}	162.23±6.01^{c**}	189.08±6.71^{b*}	192.46±5.28^{ab}	190.55±6.14^{ab}	196.55±7.37^{a}	182.16±6.81^{b*}
Weight Gain (g)	75.33±3.22^{a}	38.99±3.47^{c**}	66.37±4.11^{b*}	68.11±2.99^{ab}	65.44±2.89^{b*}	72.19±3.91^{a}	60.33±2.71^{b*}
Food intake (g/w)	15.90±1.57^{a}	14.27±1.35^{a}	15.41±1.24^{a}	15.05±1.41^{a}	15.57±1.59^{a}	16.41±1.41^{a}	14.99±1.45^{a}
FER	0.078±0.003^{a}	0.045±0.004^{b**}	0.071±0.002^{a}	0.075±0.001^{a}	0.070±0.003^{a}	0.073±0.001^{a}	0.067±0.005^{a}

Significant with control group * $P<0.05$ ** $P<0.01$ *** $P<0.001$

Mean values in each column having different superscript (a, b, c, d) are significant.

Table 2: The Mean values ± SD of hemoglobin (HB) and packed cell volume (PCV) levels of the experimental rat groups

Groups Variables	Control (-ve)	Control (+ve)	Artichoke extract	Artichoke powder	Selenium	Artichoke extract+ Selenium	Artichoke powder + Selenium
HB(g/dl)	14.75±1.29^{c}	8.21±1.49^{b**}	12.34±2.01^{a}	12.91±1.22^{a}	12.34±1.33^{a}	13.61±1.41^{a}	13.24±1.12^{a}
PCV%	35.41±5.46^{a}	23.24±4.66^{b**}	32.11±6.22^{a}	33.61±5.14^{a}	31.29±5.26^{a}	34.32±5.11^{a}	35.91±6.19^{a}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant.

Table 3: The Mean values ± SD of blood free radical, superoxide dismutase (SOD) and glutathione peroxidase (GPX) levels of the experimental rat groups

Groups Variables	Control (-ve)	Control (+ve)	Artichoke extract	Artichoke powder	Selenium	Artichoke extract+ Selenium	Artichoke powder + Selenium
Free radical	13245.13±4216.35^{c}	43256.91±7851.87^{a***}	20321.83± 2420.45^{b*}	20048.19±2111.33^{b*}	22340.77±3201.36^{b*}	19341.33±2131.14^{b*}	19982.34±1233.88^{b*}
SOD	16.22±2.03^{a}	7.87±1.11^{c**}	10.45±1.54^{b*}	9.78±1.30^{b*}	9.87±1.21^{b*}	14.51±1.17^{a}	14.71±1.81^{b*}
GPX(µg /mg)	6.14±1.11^{a}	2.91±0.56^{c***}	4.98±0.55^{b*}	5.01±0.44^{ab}	4.90±0.33^{b*}	5.77±0.34^{ab}	5.35±0.60^{ab}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant.

Table 4: The Mean values ± SD of serum amino transferase (ALT & AST), enzymes, total protein, albumin and globulin levels of experimental rat groups

Groups Variables	Control (-ve)	Control (+ve)	Artichoke extract	Artichoke powder	Selenium	Artichoke extract+ Selenium	Artichoke powder + Selenium
ALT(µ /ml)	28.77±4.44^{c}	55.16±5.88^{a***}	38.10±4.30^{b*}	40.21±6.17^{b*}	37.87±6.70^{b*}	32.16±3.40^{bc}	53.61±4.66^{bc}
AST(µ /ml)	32.14±5.31^{c}	68.32±9.96^{a**}	51.35±8.31^{b*}	49.37±7.41^{b*}	52.22±9.31^{b*}	47.13±4.66^{b*}	49.17±6.81^{b*}
T. protein ((g/dl))	7.51±1.76^{a}	4.29±0.31^{c**}	5.82±1.66^{ab}	6.35±1.77^{ab}	6.31±1.41^{ab}	7.11±1.81^{ab}	7.21±1.66^{ab}
Albumin (g/dl)	3.69±0.22^{a}	1.99±0.11^{c***}	2.81±0.31^{ab}	3.01±0.55^{a}	3.11±0.65^{a}	3.22±0.68^{a}	3.41±0.55^{a}
Globulin (g/dl)	3.82±0.42^{a}	2.30±0.33^{b*}	3.07±0.27^{a}	3.34±0.23^{a}	3.20±0.22^{a}	3.89±0.17^{a}	3.80±0.15^{a}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant.

Humans have evolved a wide range of defenses against free radical induced damage. They include the enzymes: superoxide dismutase (SOD), catalase and glutathione peroxidase and several low molecular weight compounds that act as antioxidants. Glutathione - peroxidase is a selenium-dependent enzyme that exists as a homo tetramer with each 22-kDa subunit containing a selenium atom incorporated within a catalytically active selenocysteine residure [31]. Artichoke extract is very effective as an antioxidant and its health-protective potential has been attributed to its antioxidant power. It has been found to decrease the production of reactive oxygen species as lipid and protein oxidation and increase the activity of glutathione peroxidase [27, 29].

Table 4 showed that, control (+ve) rat group showed a significant increase in serum ALT and AST and a significant decrease in serum total protein, albumin and globulin (P<0.001&0.05) compared with control (+ve) rat group. Artichoke extract, artichoke powder and selenium rat groups showed a significant increase in serum ALT and AST enzymes activity at P<0.05 while artichoke extract with selenium and artichoke powder with selenium rat groups showed a significant increase in AST at P<0.05 compared with control (-ve) group. Artichoke extract, artichoke powder, selenium, artichoke extract with selenium and artichoke powder with selenium rat groups showed a significant decrease in serum ALT and AST and a significant increase in serum total protein, albumin and globulin compared with control (+ve) group.

In fact, the leakage of hepatic enzymes such as ALT and AST are commonly used as an indirect biochemical index of hepatocellular damage A significant increase in the activities of ALT and AST probably may resulting from hepatocyte membrane damage. If the liver is injured, its cells spill out the enzymes into blood. The decrease in the protein concentration might be due to changes in protein synthesis and/or metabolism [32, 33].Artichoke may have protective effects against liver injury. The major components responsible for the essential effects of the artichoke extract have been identified. Flavonoid luteolin, its glycoside luteolin-7-O-glucoside and the caffeoylquinic acids (particularly cynarine) have been shown to possess hepatoprotective activity. Polyphenol constituents of the extract may be responsible of the higher secretion of cholephilic compounds into the bile canaliculi [34, 35]. Artichoke extract with higher caffeoyl derivatives proved to be more effective in decreasing plasma AST and ALT levels and liver MDA content in carbon tetrachloride-induced liver injury and oxidative stress [36].

Table 5: The Mean values ± SD of serum catalase, lipid peroxidase (LPX) and nitric oxide (No) levels of the experimental rat groups

Groups Variables	Control (-ve)	Control (+ve)	Artichoke extract	Artichoke powder	Selenium	Artichoke extract+ Selenium	Artichoke powder + Selenium
Catalase(μ /ml)	311.35±43.68^{a}	173.22±30.81^{b***}	264.33±45.43^{ab}	240.11±41.17^{b*}	220.11±22.21^{b*}	255.14±33.41^{ab}	261.31±39.24^{ab}
LPX	1.63±0.78^{c}	6.45±1.17^{a**}	1.96±0.11^{c}	2.64±0.14^{bc}	2.71±0.23^{bc}	2.11±0.13^{bc}	2.34±0.28^{bc}
No (μmol/l)	1.87±o.65^{d}	9.73±1.48^{a***}	3.14±0.31^{b*}	3.27±0.55^{b*}	3.45±0.24^{b*}	2.78±0.18^{cd}	2.55±0.31^{cd}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant.

Table 6: The Mean values ± SD of liver superoxide dismutase (SOD), glutathione peroxidase (GPX) and malondialdehyde (MDA) levels of experimental rat groups

Groups Variables	Control (-ve)	Control (+ve)	Artichoke extract	Artichoke powder	Selenium	Artichoke extract+ Selenium	Artichoke powder + Selenium
SOD (μg /mg)	99.88±10.20^{a}	42.36±8.51^{d***}	87.14±9.65^{ab}	77.35±7.64^{c*}	85.24±9.11^{ab}	91.89±10.14^{a}	89.91±11.33^{a}
GPX (μg /mg)	91.14±14.24^{a}	40.17±6.32^{d***}	78.18±10.31^{c*}	96.14±6.71^{c*}	88.34±9.27^{ab}	95.32±14.35^{a}	92.41±12.60^{a}
MDA (nmol/g)	9.91±1.69^{c}	22.66±4.14^{a***}	15.32±2.96^{b*}	16.29±2.49^{b*}	14.13±2.36^{b*}	8.49±1.14^{c}	9.11±1.32^{c}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each column having different superscript (a, b, c, d) are significant.

Table 5 showed that, control (+ve) rat group showed a significant decrease in serum catalase and a significant increase in lipid peroxidase (LPX) and nitric oxide (No) levels (P<0.001) while the artichoke extract showed a significant increase in nitric oxide (No) levels (P<0.05) compared with control (-ve) group. The artichoke powder and selenium showed a significant decrease in serum catalase and a significant increase in nitric oxide (No) levels (P<0.05) compared with control (-ve) group. Artichoke extract, artichoke powder, selenium, artichoke extract with selenium and artichoke powder with selenium rat groups showed a significant decrease in serum lipid peroxidase (LPX) and nitric oxide (No) levels compared with control (+ve) group. It is well known that the key mechanism of endothelial damage is mediated by oxidative stress. The oxidative charge of low density lipoproteins promotes the breakdown of endothelial nitric oxide. Moreover the reduced bioavailability of nitric oxide may also be the consequence of a reduced transcription of nitric oxide synthase, the production of a less bioactive nitric oxide and the increase in asymmetric dimethylarginine levels [37]. The beneficial role of Se in reducing oxidative stress parameters might be related to its mild antioxidant. Potential lipid peroxidation is a basic cellular deteriorating process induced by oxidative stress and occurs readily in the tissues rich in highly oxidizable polyunsaturated fatty acids .Se plays an important role in preventing hydroxyl radicals' formation and in protecting the integrity and the functions of tissues [38] .

Data in Table 6 demonstrated that, control (+ve) and artichoke powder rat groups showed a significant decrease in liver superoxide dismutase (SOD) and glutathione peroxidase (GPX) levels and significant increase in liver monaldialdehyde (MDA) at P<0.001&0.05 compared with control (-ve) group. Artichoke extract showed a significant decrease in liver GPX levels and a significant increase in liver MDA at P<0.05while selenium rat group showed a significant increase in liver (MDA) at P<0.05compared with control (-ve) group. Artichoke extract, artichoke powder, selenium, artichoke extract with selenium and artichoke powder with selenium rat groups showed a significant increase in liver SOD and GPX levels and significant decrease in liver MDA compared with control (+ve) group. Artichoke has antioxidant properties. The leaves of this plant are rich in phenolic compounds, such as mono- and dicaffeoylquinic acids and flavonoids, which have been extracted, isolated and identified as major chemical components [6]. Artichoke extracts reduce oxidative stress in endothelial cells stimulated by tumor necrosis factor alpha and oxidized LDL [29]. On the other hand, the addition of artichoke extract to primary rat hepatocyte cultures, which were exposed to various hydroperoxides induced MDA levels was dose-dependently. The extract was not found to affect cellular GSH but decreased the GSH loss [6]. Artichoke extract alone was found to increase glutathione and glutathione peroxidase and decrease hepatic MDA levels without any change in other antioxidant system parameters [27].

The most important metabolic roles of selenium in mammalian cell are due to its function in the active site of many antioxidant enzymes as thioredoxin reductase and glutathione peroxidase. Moreover, glutathione peroxidase is selenoenzyme does not only protect cells against damages by free radicals, but also protects membrane

lipids against such oxidation generated by peroxides and permits regeneration of membrane lipid molecules [7,38]. Thus, glutathione peroxidase may prevent the harmful effects of free radicals and may also reduce the formation of the reactive metabolites induced by potassium bromate.

It is recommended to consume artichoke and selenium to improve healthy status to overcome the free radical resulting from routine life.

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