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Effect of Leek and Zinc Consumption on Renal Oxidative Stress in Rats

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Abstract: Forty-nine adult albino male rats, Sprague Dawley strain were randomly classified into seven groups (7 rats each). The first group kept as normal control fed standard diet only. The other six groups injected with potassium bromate and reclassified into positive control, leek, leek extract, leek with zinc, leek extract with zinc and zinc groups. The results revealed that, the positive control group showed significant decrease in final weight, weight gain, food efficiency ratio (FER), protein efficiency ratio(PER), hemoglobin (HB), packed cell volume(PCV), plasma glutathione transferase(GST), plasma catalase, plasma superoxide dismutase(SOD), kidney SOD, kidney glutathione peroxidase (GPX) and kidney GST but significant increase in serum aspartate and alanine amino transferase(AST&ALT)enzymes, alkaline phoshatase(AP),creatinine, uric acid, nitric oxide(NO) and kidney malondialdehyde (MDA) compared to normal control group. The leek group showed significant decrease in PCV, plasma GST, kidney GPX and GST while leek extract showed significant decrease in plasma GST but zinc group showed significant decrease in PCV compared to normal control group. The leek, leek extract, leek with zinc, leek extract with zinc and zinc groups showed significant increase in serum uric acid compared to normal control group. On the other side, they showed significant increase in final weight, weight gain, FER, HB, plasma (GST, SOD &catalase) and kidney (SOD, GPX and GST) but showed significant decrease in serum AST, ALT &AP enzymes, creatinine, uric acid, NO and kidney MDA compared to positive control group.

Key wards: Renal oxidative stress · Potassium bromate · Leek · Zinc · Rats

INTRODUCTION

Potassium bromate ($KBrO_3$) is widely used as a food additive in food products, fish paste, fermented beverages, bread-making and as a neutralizer in cold-wave hair lotions. It is also found in drinking-water samples as a by-product of ozone disinfection [1].Pharmacokinetic studies on $KBrO_3$ have shown that this compound is degraded in vivo and in vitro to BrO_3- and contact with renal tubular epithelium $KBrO_3$ is oxidative stress which enhances kidney cellular proliferation and induces renal cell tumors [2]. Nowadays, there is a growing interest in plant foods to promote health and wellness. The health-promoting affects of plant foods to their comprehensive array of vitamins, minerals and fiber. Phytonutrients are active compounds in plants that have been shown to prevent cancer and lowering the risk of health problems [3, 4]. *Allium* genus has over 500 members, each differing in maturing, color and taste, but with similar biochemical, phytochemical and neutraceutical content. *Alliums* were revered to possess anti-bacterial and anti-fungal activities because of the powerful antioxidants, sulfur and other numerous phenolic compounds [5].

Leeks (*Allium ampeloprasum var. porrum*) belongs to the Alliaceae family, look a bit like a huge spring onion and have a mild onion flavor but have flat leaves instead of tubular and relatively little bulb development. The thick leaf bases and slightly developed bulb look like a giant green onion and are eaten as a cooked vegetable. Today leeks are grown as an excellent substitute for onions and for its own unique mild onion flavor in soups and other dishes. Since leeks are related to garlic and onions, they contain many of the same beneficial compounds. Leeks are a good source of allyl sulfides which have been shown to modify certain pathways associated with the growth of malignant tumors. Leeks are also rich in the saponins, kaempferol, carotenoids and chlorophyll mainly in the green tops [6]. Zinc is essential for all life forms and has structural and regulatory roles in many enzymes and gene transcription systems that are essential for human metabolism, growth and production. Zinc is present in every part of the body and has a wide range of functions.

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It helps with the healing of wounds and is a vital component of many enzyme reactions. It is particularly important for healthy skin and is essential for a healthy immune system and resistance to infection. Zinc is present in a wide variety of foods, particularly in association with protein foods. A vegetarian diet often contains less zinc than a meat based diet and so it is important for vegetarians to eat plenty of foods that are rich in this vital mineral [7, 8].

This study aimed to investigate the effect of leek and zinc consumption on renal oxidative stress induced by potassium bromate in experimental rats.

MATERIALS AND METHODS

Materials

Potassium Bromate ($KBrO_3$) and Zinc: Potassium bromate is a white powder odorless, purchased from El-Gomhoria Co., Cairo, Egypt. The rats received a single intra-peritoneal injection of potassium bromate ($KBrO_3$) at a dose level 125 mg /kg body weight [9]. Octozinic capsules produced by October Pharma S.A.E and contain 110 zinc sulphate heptahydrate. The human therapeutic dose of zinc sulphate heptahydrate was converted to rat dose according to Paget and Barnes [10] that was 20 mg/Kg body weight, dissolved in distilled water and given to rats by oral intubations. Biochemical kits were purchased from Alkan Co. for Chemicals and Biodignostics, Dokki, Egypt.

Leek Leaves: Leek leaves were obtained from the local market in Cairo City, Egypt.

Experimental Animals: Forty-nine male rats were purchased from Helwan Farm of Laboratory Animals. The average weight was 110±5 g. The animals were kept under observation for five days before experiment and fed on standard diet according to NRC [11] 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), vitamins mixture (20g/kg) and DL-methionine (3g/kg).

Methods: The leek leaves were dried at 60°C and then crushed to powder. The leek powder was added to the diet as 10% of the diet. The other part was used for preparation of methanol extract. 100 g of leek leaves powdered were soaked in 500 ml of 80% ethanol with frequent agitation. Clarification was then carried out using vacuum filtration through filter paper watman number 2. The resultant extract was concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 40°C. The rat dose of leek leaves extract was 40 mg/kg b.wt according to Irkin and Korukluoglu [12].

The rats were randomly classified into seven groups (7 rats each). The first group kept as normal control fed standard diet only. The other six groups injected with potassium bromate to induce renal stress. One group served as non treated positive control while others groups treated with leek powder, leek extract, leek powder with zinc, leek extract with zinc and zinc. The study was assigned for eight weeks. The food intake was calculated daily and the body weight gain was recorded weekly. Food and protein efficiency ratio (FER&PER) were calculated according to Chapman *et al.* [13].

At the end of the experiment, the rats were sacrificed to obtain blood samples. Heparenized blood was analyzed for estimation of hemoglobin (HB) and packed cell volume (PCV) according to Drabkin [14] and Mc Inory [15], respectively. Serum alanine and aspartae aminotransferase (ALT, AST), alkaline phosphates (AP) enzymes, creatinine and uric acid were estimated according to Reitman and Frankel [16], Kind and King [17], Hare [18] and Fossati *et al.* [19], respectively. Plasma glutathione transferase (GST), catalase and superoxide dismutase enzymes (SOD) and nitric oxide (NO) were estimated according to Habig [20], Claiborne [21], Beuchamp and Fridovich [22] and Green *et al.* [23], respectively.

Two kidneys of each rat were rapidly removed and perfuse with 50 to 100 of ice cold 0.9%NaCL solution for estimation of superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione S-transferase (GST) and malondialdehyde (MDA) according to Beuchamp and Fridovich [22], Weiss *et al.* [24], Ellman [25] and Uchiyama and Mihara [26], respectively.

Statistical Analysis: Differences between groups were analyzed using Dunnet's *t*-test followed by analysis of variance (ANOVA).

RESULTS

Data recorded in Table 1 illustrated that the positive control group showed a significant decrease in final weight, weight gain, food protein efficiency ratio (FER) and protein efficiency ratio (PER) at $p< 0.01$ compared to normal control group. The leek powder, leek extract, leek powder with zinc, leek extract with zinc and zinc groups showed non significant decrease in final weight, weight gain, food intake, FER and PER at $p>0.05$ compared to normal control group. The leek powder, leek extract, leek powder with zinc, leek extract with zinc and zinc groups

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

	Groups						
Variables	Normal control	Positive control	Leek powder	Leek extract	Leek powder +zinc	Leek extract + zinc	Zinc
Initial weight	110.55± 3.67^{a}	110.41± 3.50^{a}	109.14±2.45^{a}	108.33± 2.99^{a}	110.22±3.11^{a}	110.34±3.14^{a}	109.14±3.41^{a}
Final Weight (g)	203.47±13.01^{a}	154.71±12.13^{b**}	189.71± 11.22^{a}	199.41±13.78^{a}	201.14±12.35^{a}	205.11±14.41^{a}	190.11±12.38^{a}
Weight Gain (g)	92.92±11.33^{a}	44.30±7.71^{b**}	80.57±8.17^{a}	91.08±10.22^{a}	90.92±11.11^{a}	94.77±11.21^{a}	80.97±10.14^{a}
Food Intake(g/d))	16.65±2.11^{a}	14.20±2.17^{a}	15.90±2.11^{a}	16.35±2.91^{a}	16.55±2.18^{a}	16.75±2.81^{a}	15.95±1.99^{a}
FER	0.093±0.001^{a}	0.051±0.002^{b**}	0.084±0.003^{a}	0.092±0.001^{a}	0.091±0.001^{a}	0.094±0.003^{a}	0.084±1.002^{a}
PER	0.46±0.03^{a}	0.25±0.01^{b**}	0.42±0.03^{a}	0.46±0.02^{a}	0.45±0.04^{a}	0.47±0.03^{a}	0.42±0.02^{a}

Significant with control (-ve) group * P<0.05 **P<0.01***P<0.001

Mean values in each raw having different superscript (a,b,c) denote significant difference

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

	Groups						
Variables	Normal control	Positive control	Leek powder	Leek extract	Leek powder +zinc	Leek extract +zinc	Zinc
HB(gm/dl)	12.08±2.18^{a}	7.99±1.39^{b**}	10.14±1.4^{a}	10.55±1.98^{a}	11.11±2.01^{a}	11.51±1.82^{a}	10.35±1.03^{a}
PCV%	38.61±3.82^{a}	29.81±3.55^{b*}	33.79±3.47^{b*}	34.14±4.01^{ab}	36.71±4.11^{a}	36.81±3.17^{a}	35.32±3.16^{b*}

Significant with control (-ve) group *P<0.05 **P<0.01 ***P<0.001

Mean values in each raw having different superscript (a,b,c) denote significant difference

Table 3: Mean values ± SD of serum amino transferase (AST&ALT), alkaline phosphotase enzymes (Alk-Pho), creatinine and uric acid of the experimental rats groups

	Groups						
Variables	Normal control	Positive control	Leek powder	Leek extract	Leek powder +zinc	Leek extract +zinc	Zinc
AST(µ/ml)	41.17±5.81^{b}	72.39±9.61^{a**}	49.37±6.01^{b}	51.14±8.10^{b}	48.21±6.15^{b}	40.21±4.13^{b}	43.19±4.61^{b}
ALT(µ/ml)	13.35±1.12^{b}	28.55±3.35^{a**}	15.71±1.81^{b}	16.28±2.01^{b}	18.13±3.51^{b}	14.11±3.65^{b}	15.31±3.66^{b}
Alk-Pho(µ/ml)	30.17±5.66^{b}	50.38±5.81^{a**}	37.80±4.11^{b}	38.73±4.37^{b}	38.34±5.01^{b}	32.11±3.11^{b}	35.30±2.99^{b}
Creatinine(mg/dl)	0.77±0.01^{b}	1.95±0.11^{a**}	0.99±0.02^{b}	0.88±0.12^{b}	0.75±0.13^{b}	0.70±0.15^{b}	0.98±0.18^{b}
Uricacid(mg/dl)	1.83±0.26^{c}	4.41±1.01^{a***}	2.11±0.81^{b*}	2.41±0.77^{b*}	2.17±0.67^{b*}	1.74±0.74^{c}	2.25±0.16^{b*}

Significant with control (-ve) group *P<0.05 **P<0.01 ***P<0.001

Mean values in each column having different superscript (a,b,c) denote significant difference

Table 4: Mean values ± SD of plasma glutathione transferase (GST), catalase, superoxide dismutase (SOD) enzymes and nitricoxide (NO) of the experimental rats groups

	Groups						
Variables	Normal control	Positive control	Leek powder	Leek extract	Leek powder +zinc	Leek extract +zinc	Zinc
GST(µ/l)	271.31±33.27^{a}	77.85±8.40^{c***}	188.35±22.17^{b*}	211.31±23.81^{b*}	240.21±23.71^{a}	278.15±31.71^{a}	231.25±34.25^{a}
Catalase(µ/l)	385.21±55.14^{a}	115.55±10.14^{c***}	230.77±32.11^{ab}	291.61±31.61^{a}	277.11±30.91^{a}	384.11±39.11^{a}	245.18±41.11^{ab}
SOD(µ/l)	70.13±5.22^{a}	21.25±3.47^{b***}	63.14±7.16^{a}	68.33±6.35^{a}	71.31±9.23^{a}	73.14±7.81^{a}	62.45±7.11^{a}
NO(µmol/l)	2.17±0.33^{b}	13.99±1.44^{a***}	4.33±1.11^{b}	3.22±1.03^{b}	3.11±1.05^{b}	2.01±1.21^{b}	3.21±1.22^{b}

Significant with control (-ve) group *P<0.05 **P<0.01 ***P<0.001

Mean values in each column having different superscript (a,b,c) denote significant difference

Table 5: Mean values ± SD of kidney superoxid dismutase (SOD), glutathione peroxidase (GPX), glutathione transferase (GST) and malondialdehyde (MDA) of the experimental rat groups

	Groups						
Variables	Normal control	Positive control	Leek powder	Leek extract	Leek powder +zinc	Leek extract +zinc	Zinc
SOD(µ/mg)	140.81±21.17^{a}	35.81±3.81^{b***}	110.15±11.15^{a}	131.25±22.61^{a}	118.82±17.34^{a}	143.32±25.16^{a}	115.94±17.48^{a}
GPX(µ/mg)	121.33±17.13^{a}	29.14±4.19^{c***}	89.59±7.95^{b*}	118.41±11.18^{a}	114.38±13.21^{a}	120.33±21.35^{a}	109.25±13.31^{a}
GST(µ/mg)	4.14±0.66^{a}	1.51±0.19^{c***}	2.99±0.88^{b*}	3.45±0.97^{a}	3.29±0.77^{a}	4.11±0.98^{a}	3.41±0.55^{a}
MDA(nmol/g)	9.45±1.98^{b}	19.34±3.14^{a***}	10.14±2.61^{b}	9.11±2.16^{b}	10.33±1.69^{b}	8.22±1.91^{b}	11.12±1.69^{b}

Significant with control (-ve) group *P<0.05 **P<0.01 ***P<0.001

Mean values in each column having different superscript (a,b,c) denote significant difference

showed a significant increase in final weight, weight gain, FER and PER compared to positive control group. As shown in Table 2, the positive control group showed a significant decrease in hemoglobin and packed cell volume at $p<0.01$&0.05 compared to normal control group. The leek powder and zinc groups showed significant decrease in packed cell volume at $p<0.05$ compared to normal control group. The leek powder, leek extract, leek powder with zinc, leek extract with zinc and zinc groups showed a significant increase in hemoglobin while the leek powder with zinc and leek extract with zinc groups showed significant increase in packed cell volume compared to positive control group.

As shown in Table 3, the positive control group showed a significant increase in serum aspartate and alanine amino transferase (AST&ALT) enzymes, alkaline phoshatase(AP), creatinine and uric acid at $p<0.01$&0.001 compared to normal control group. The leek powder, leek extract, leek powder with zinc and zinc groups showed a significant increase in serum uric acid at $p<0.05$ compared to normal control group. The leek powder, leek extract, leek powder with zinc, leek extract with zinc and zinc groups showed a significant decrease in serum AST, ALT&AP enzymes, creatinine and uric acid compared to positive control group.

As shown in Table 4, the positive control group showed a significant decrease in plasma glutathione transferase (GST), catalase and superoxide dismutase (SOD) and a significant increase in nitric oxide (NO) at $p<0.001$ compared to normal control group. The leek powder and leek extract groups showed a significant decrease in plasma GST at $p<0.05$ compared to normal control group. The leek powder, leek extract, leek powder with zinc, leek extract with zinc and zinc groups showed a significant increase in GST, catalase, SOD and significant decrease NO compared to positive control group.

As shown in Table 5, the positive control group showed a significant decrease in kidney superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione transferase (GST) at $p< 0.001$ and a significant increase in kidney malondialdehyde (MDA) at $p< 0.001$ while the leek powder group showed a significant decrease in GPX and GST at $p<0.05$ compared to normal control group. The leek powder, leek extract, leek powder with zinc, leek extract with zinc and zinc groups showed a significant increase in kidney SOD, GPX and GST and significant decrease in MDA compared to positive control group.

DISCUSSION

There is considerable evidence that chronic exposure to potassium bromate acts as oxidative stress causing toxicity in male rat kidney includes changes in energy consumption and utilization in renal cells that involve up-regulation of glycolytic processes, possibly resulting from altered mitochondrial function [27]. There are early alterations in protein and gene expression in rat kidney following bromate exposure. Renal cell injury in response to oxidant chemical exposure have demonstrated a wide range of cytotoxicities, However, these toxicities are reversible with full renal cell function being restored several days after withdrawal of the toxicant [28, 29]. $KBrO_3$ is reduced by glutathione and results in the generation of short-lived intermediates that can react with DNA and cause single and double strand breaks. Bromine radicals (Br•) or oxides (BrO•, BrO_2•) are the species directly responsible for the observed cellular and cell free DNA damage in the kidney that is responsible for the proliferative response [30, 31].

Intraperitoneal injection of $KBrO_3$ to rats caused reduction in renal glutathione content and activities of renal antioxidant enzymes that are glutathione peroxidase, glutathione reductase, catalase, glucose-6-phosphate dehydrogenase and phase-II metabolizing enzymes such as glutathione-*S*-transferase and with enhancement in lipid peroxidation [9]. Modification of cellular detoxification enzymes could be a major mechanism for protecting against the toxic and neoplastic effects. Glutathione-*S*-transferase detoxifies a number of carcinogenic electrophiles by catalysis of the conjugation with reduced glutathione and ameliorated malondialdehyde levels [32]. It is interesting to note that leeks contain excellent amounts of vitamin C, as well as folate and some useful amounts of vitamins B, vitamin E, copper, potassium and iron. Leeks are easier to digest than standard onions. Leeks have laxative, antiseptic, diuretic and anti-arthritic properties. Leeks energize the human body to perform many types of biological functions like digestion and metabolism. Leeks contain indigestible carbohydrates which serve as fuel for probiotic bacteria in the digestive tract. These probiotic bacteria keep invading pathogens at bay, fortifying immune systems and keeping digestive processes running smoothly [6]. Onion, one of the richest and most common antioxidant quercetin sources that present in onion mainly as glycosides the consumption of a flavonoids rich diet might decrease the risk of degenerative changes and

certain diseases [33]. The antioxidant properties of *Allium* vegetables might therefore result from the contributions of various sulfur components at different steps of the process [34, 35].

The ALT and AST are enzymes involved in amino acid metabolism and used as a marker in liver diseases. Antioxidant enzymes are involved in the defense system against free radical-mediated tissue or cellular damage. They metabolize either free radicals or reactive oxygen intermediates to nonradical products. These enzymes include a family of glutathione-dependent enzymes, superoxide dismutase and catalase. The increase of GST activity possibly is an indication of a cellular failure in compensating the induced oxidative stress [36]. GST is a family of proteins that catalyzes the conjugation of glutathione with various electrotrophils, many of which are toxic. Also, GST plays a key role in cellular detoxification by catalyzing the reaction of glutathione with toxicants to form an S-substituted glutathione. Creatinine is a nitrogenous waste product formed from the metabolism of creatine in skeletal muscle. It is filtered from the extracellular fluid by the kidney. The level of creatinine is used as a test of renal function [37]. It was reported that the mechanism by which zinc functions as an antioxidant is through the prevention of free radical formation by other metals, such as iron and copper and protects of proteins and enzymes against free radical attack or oxidation. Zn is involved in the maintenance of gut structure and function and helps to maintain the integrity of intestinal mucosa to reduce or prevent fluid loss and also is importantly related to gut immune function [38]. Zn supplementation leads to accelerating regeneration of the mucosa and increased levels of brush border enzymes. Zinc significantly attenuated indomethacin induced oxidative stress, mitochondrial dysfunction and changes in the lipids in mitochondrial membranes in the kidney. Zinc seemed to hasten functional recovery of the kidney [8, 39]. Zn is a cofactor for proper functioning as carbonic anhydrase, alkaline phosphatase, superoxide dismutase and alcohol dehydrogenase and carboxy peptidase. Zinc plays an important role in the structure of proteins and cell membranes. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their function. Zinc confers protection against hepatotoxicity induced by various drugs and other xenobiotics [40, 41]. A marked reduction in the levels of serum creatinine and uric acid, marker parameters of kidney damage, in leek and zinc treatment groups shows that prophylaxis of the plant is effective in improving kidney function in $KBrO_3$-treated group.

From the present study, it is concluded that leek, zinc and a combination of them in diet have an antioxidant effect on potassium bromate induced renal oxidative stress in experimental rats.

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