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Biological Study on the Effect of Arginine and Parsley on Renal Toxicity in Rats

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Abstract: Forty eight adult male of white albino rats (Sprague Dawley Strain) were injected by a single dose of potassium bromate at dose 130 mg/kg body weight intraperitoneal to induce renal toxicity. Rats were classified into six groups that were control (+ ve), arginine (100 mg/kg), parsley powder (10% parsley powder), parsley extract (25 mg /kg), parsley powder + arginine (10% parsley powder and 5 g/kg of arginine) and parsley extract with arginine group (25 mg /kg parsley extract with 5 g/kg of arginine) rat groups. The study period was 60 days. Results showed that consumption of arginine and parsley group showed improvement of nutritional values and had significant decrease in serum creatinine, urea, uric acid and malondialdehyde. Moreover, they showed significant increase in serum total protein, globulin, hemoglobin, packed cell volume and serum and kidney superoxide dismutase, glutathione-peroxidase and glutathione S-transferase compared with control (+ve) group. The best treatment effect of kidney injured was appeared in parsley powder or extract with arginine groups. These results may be attributed to nutritional component of parsley and antioxidant activity.

Key words: Parsley · Arginine · Potassium bromate · Renal toxicity · Rats

INTRODUCTION

Kidneys are responsible for removing waste products and extra water from the body in the form of urine. Structural and functional damage to the kidneys can lead to a variety of conditions, including kidney failure and kidney stones. Factors such as diabetes, high blood pressure and family history increase the risk of kidney diseases [1]. Potassium bromate (KBrO3) is an oxidizing agent that has been used as a food additive, mainly in the bread-making process. Although adverse effects are not evident in animals fed bread-based diets made from flour treated with $KBrO_3$, the agent is carcinogenic in rats and nephrotoxic in both man and experimental animals when given orally. $KBrO_3$ has inhibitory effects on inducing lipid peroxidation in the rat kidney. Active oxygen radicals generated from $KBrO_3$ were implicated in its toxic and carcinogenic effects, especially because $KBrO_3$ produced 8-hydroxy deoxyguanosine in the rat kidney [2-4]. Treatment of injured kidney depends on their condition and might include medications and surgery. Certain herbs such as parsley might help maintain kidney health. Parsley (*Petroselinum crispum*) is a plant that reaches 30cm in height, with composed leaves of bright green color, they can be either flat or curly, with white small flowers in bunches with small seeds. Parsley tea is essentially made with the green leaves and is one of the medicinal herbs. Parsley is rich with an antioxidant arsenal that includes luteolin, flavonoid that searches out and eradicates free radicals in the body that cause oxidative stress in cells [5-7]. Arginine is a conditionally non essential amino acid, meaning most of the time it can be manufactured by the human body and does not need to be obtained directly through the diet. The biosynthetic pathway however does not produce sufficient arginine and some must still be consumed through diet. Individuals who have poor nutrition or certain physical conditions may be advised to increase their intake of foods containing arginine. Arginine is found in a wide variety of foods. Arginine plays an important role in cell division, the healing of wounds, removing ammonia from the body, immune function and the release of hormones [8].

So, this study was carried out to investigate the effect of arginine and parsley on kidney stress induced by potassium bromate in experimental rats.

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MATERIALS AND METHODS

Materials: The fresh parsley was obtained from local market in Cairo. Forty eight adult male of white albino rats (Sprague Dawley Strain) weighing 115±6 g, were obtained from the Laboratory Animal Colony, Helwan and Cairo, Egypt. Potassium bromate ($KBrO_3$) and arginine were purchased from El-Gomhorya Company, Cairo, Egypt. Kits used for biochemical analysis were obtained from Gama Tread Company, Cairo, Egypt.

Methods: The basal diet was prepared according to NRC [9]. The fresh parsley was washed with tap water, chopped into small pieces, dried with hot air oven (40–60°C) and grinded to powder [10]. The aqueous extract was prepared by boiling 5 g of parsley powder in 100 ml of distilled water for 10 min and left for 15 min to infuse then cooled and filtered before use to remove particular matter. After adaptation period (one week), rats were injected by a single dose of potassium bromate at dose 130 mg/kg body weight intraperitoneal to induce renal toxicity [11]. Rats were classified into control (+ve) and five treatment groups as following:

Control (+ ve): Administered basal diet and water *ad libitium*.

Arginine Group: Administered basal diet containing 5% L-arginine.

Parsley Powder Group: Administered basal diet containing 10% parsley powder.

Parsley Extract Group: Administered basal diet and 5 mg /kg body weight daily parsley extract by stomach tube.

Parsley Powder + Arginine Group: Administered basal diet containing 10% parsley powder and 5% L-arginine.

Parsley Extract with Arginine Group: Administered basal diet containing 5% L-arginine and 5 mg/kg body weight daily parsley extract by stomach tube. The food intake (F I) was calculated daily and the body weight gain was recorded weekly. Food efficiency ratio (FER) was calculated according to Chapman *et al*. [12]. After 60 days, the rats were anesthetized, blood sample were collected in clean centrifuge tubes to obtain serum. Kidneys were immediately removed, rinsed with saline, blotted on filter paper and stored at -70°C for biochemical analyses. Serum creatinine, urea and uric acid were estimated according to Bonsens -and Taussky [13], Patton and Crouch [14] and Fossati *et al*. [15], respectively. Serum total protein, albumin and globulin were determined as described by the method of Weichselbaum [16], Bartholomev and Delany [17] and Coles [18], respectively. Hemoglobin (HG) and packed cell volume (PCV) were estimated in heparinized blood according to Drabkin [19] and Mc Inory [20], respectively. In addition, serum superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione transferase (GST) and malondialdehyde (MDA) were determined as described by the method of Dechatelet *et al*. [21], Beutler *et al*. [22], Habig *et al*. [23] and Placer *et al*. [24], respectively. Kidney superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase, glutathione transferase (GST) and malondialdehyde (MDA) were determined according to Beuchamp and Fridovich [25], Tapple [26], Cohen *et al*. [27], Moran *et al*. [28] and Uchiyama and Mihara [29], respectively.

Statistical Analysis: Collected data are expressed as mean ±SE. Statistical analysis was done by using analysis of variance (ANOVA) followed by student's t-test and P values of 5% and less were considered to be significant [30].

RESULTS

The effect of parsley either powder or extract and also arginine on the nutritional results was tested in Table 1. The treated groups showed significant increase in body weight gain ($P<0.001$), food intake ($P<0.05\&0.001$), FER ($P<0.01\&0.001$) and PER ($P<0.01\&0.001$) compared with control (+ve) group. However, the rat groups which consumed parsley powder or extract with arginine showed the best nutritional results compared with other treated groups.

Consumption of parsley either powder or extract only or with arginine showed a significant decrease the value of creatinine ($P<0.001$), urea ($P<0.01\&0.001$) and uric acid ($P<0.01\&0.001$) in all treated groups compared with control (+ve) group. There was non- significant difference in creatinine value among treated experimental groups while parsley extract with arginine rat group showed the lowest value in urea and creatinine among treated groups as shown in Table 2.

Arginine group showed a significant increase in total protein and globulin ($P<0.05\&0.001$) while parsley powder group showed a significant increase in globulin ($P<0.001$) compared with control (+ve) group. Parsley extract,

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

Groups variables	Control (+ve)	Arginine	Parsley powder	Parsley extract	Parsley powder + Arginine	Parsley extract + Arginine
Body weight(g)	23.61±2.61^{d}	40.21±5.31^{c***}	44.21±4.11^{c***}	55.71±6.14^{b*}**	61.31±7.21^{a***}	71.36±8.29^{a***}
F I (g/w)	11.31±1.36^{c}	13.99±1.35^{b*}	14.70±1.22b*	14.96±1.36^{b*}	15.01±1.11^{a**}	16.20±1.21^{a**}
FER	0.034±0.001^{e}	0.047±0.001^{d**}	0.050±0.002^{d**}	0.062±0.002^{c***}	0.068±0.03^{b***}	0.073±0.001^{a***}
PER	0.174±0.020^{c}	0.240±0.04^{b**}	0.250±0.03^{b**}	0.310±0.01^{a***}	0.340±0.05^{a***}	0.367±0.01^{a***}

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

Values with the same letters indicate non- significant difference (P<0.05) and vice versa.

Table 2: Mean values ± SD of creatinine, urea and uric acid of the experimental rat groups

Groups variables	Control (+ve)	Arginine	Parsley powder	Parsley extract	Parsley powder + Arginine	Parsley extract + Arginine
Creatinine (mg/dl)	3.41±0.33^{a}	1.57±0.12^{b***}	1.95±027^{b***}	1.81±0.16^{b***}	1.66±0.24^{b**}	1.76±0.35^{b***}
Urea (µ/mg)	78.81±8.14^{a}	49.71±5.01^{b**}	50.19±6.11^{b**}	48.27±4.20^{b**}	41.17±4.05^{bc***}	39.37±4.61^{c***}
Uric acid (mg/dl)	6.80±0.77^{a}	4.11±0.42^{b**}	4.01±0.44^{b**}	4.51±0.43^{b**}	3.96±0.55^{bc***}	3.41±0.55^{c***}

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

Values with the same letters indicate non- significant difference (P<0.05) and vice versa.

Table 3: Mean values ± SD of total protein, albumin and globulin of the experimental rat groups

Groups variables	Control (+ve)	Arginine	Parsley powder	Parsley extract	Parsley powder + Arginine	Parsley extract + Arginine
Total protein(g/dl)	5.13±0.88^{b}	7.41±1.30^{a*}	6.89±1.21^{ab}	6.99±1.19^{a*}	7.57±1.21^{a*}	7.36±1.14^{a*}
Albumin (g/dl)	3.71±0.74^{a}	3.68±0.54^{a}	3.51±0.66^{a}	3.54±0.77^{a}	3.70±0.55^{a}	3.71±0.63^{a}
Globulin (g/dl)	1.42±0.42^{b}	3.73±0.71^{a***}	3.38±0.75^{a***}	3.45±0.83^{a***}	3.87±0.35^{a***}	3.65±0.55^{a***}

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

Values with the same letters indicate non- significant difference (P<0.05) and vice versa.

Table 4: Mean values ± SD of HG and PCV of the experimental rat groups

Groups variables	Control (+ve)	Arginine	Parsley powder	Parsley extract	Parsley powder + Arginine	Parsley extract + Arginine
HG	9.07±0.88^{b}	12.71±1.24^{a**}	12.33±1.14^{a**}	12.11±1.36^{a**}	13.55±1.50^{a***}	13.19±1.49^{a***}
PCV	21.17±2.60^{c}	31.71±3.75^{b**}	33.30±3.11^{ab**}	34.21±4.10^{ab**}	37.22±3.24^{a***}	38.14±3.71^{a***}

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

Values with the same letters indicate non- significant difference (P<0.05) and vice versa.

parsley powder with arginine and parsley extract with arginine rat groups showed a significant increase in total protein (P<0.05) and globulin (P<0.001) compared with control (+ve) group. There were non significant differences in the previous parameters among all treated groups as shown in Table 3.

Arginine group showed a significant increase in hemoglobin (P<0.01) while other treated groups which consumed parsley either powder or extract only or with arginine showed a significant increase in HG and PCV (P<0.01&0.001) compared with control (+ve) group. There was non-significant difference in HG and PCV among treated groups as shown in Table 4.

Arginine group showed a significant increase in serum SOD, GPX, catalase and GST (P<0.001&0.01) and a significant decrease in serum MDA (P<0.01) while other treated groups which consumed parsley either powder or extract only or with arginine showed a significant increase in serum SOD, GPX, catalase and GST (P<0.001&0.01) and a significant decrease in serum MDA (P<0.001) compared with control (+ve) group. There was non significant difference in SOD, GPX and catalase among arginine; parsley powder and parsley extract rat groups. The highest values of serum SOD, catalase and GST were recorded in parsley powder with arginine and parsley extract with arginine compared with other treated groups. There was non significant difference among treated groups as shown in Table 5.

Arginine group showed a significant increase in kidney SOD,GPX and GST (P<0.01&0.001) and a significant decrease in kidney MDA(P<0.001) while other treated groups which consumed parsley either powder or extract only or with arginine showed a significant increase in kidney SOD and GPX (P<0.001) and a significant decrease in kidney MDA(P<0.001) compared with control (+ve) group. Parsley extract, parsley powder with arginine and parsley extract with arginine rat groups showed a significant increase in kidney GST (P<0.01&0.001) compared with control (+ve) group. The highest values of kidney SOD, GPX and GST were recorded in parsley powder with arginine and parsley extract with arginine compared with other treated groups as shown in Table 6.

Table 5: The Mean values ± SD of serum SOD, GPX, catalase, GST and MDA of the experimental groups

Groups variables	Control (+ve)	Arginine	Parsley powder	Parsley extract	Parsley powder + Arginine	Parsley extract + Arginine
SOD (mmol/l)	0.29 ± 0.10^{c}	$0.61\pm0.16^{b***}$	$0.79\pm0.20^{ab***}$	$0.71\pm0.13^{ab***}$	$0.81\pm0.12^{a***}$	$0.88\pm0.11^{a***}$
GPX (mmol/l)	0.20 ± 0.01^{d}	$0.87\pm0.42^{bc***}$	$0.92\pm0.23^{b***}$	$0.91\pm0.24^{b***}$	$1.01\pm0.54^{a***}$	$0.99\pm0.14^{b***}$
Catalase (µ/l)	0.54 ± 0.24^{d}	$0.88\pm0.22^{c**}$	$0.85\pm0.30^{c**}$	$0.99\pm0.33^{bc**}$	$1.30\pm0..53^{a***}$	$1.17\pm0.44^{a***}$
GST (mmol/l)	0.87 ± 0.08^{c}	$0.99\pm0.22^{b**}$	$1.11\pm0.12^{a***}$	$1.20\pm0.25^{a***}$	$1.65\pm0.34^{a***}$	$1.54\pm0.44^{a***}$
MDA (mmol/l)	9.67 ± 1.12^{a}	$7.14\pm1.11^{b**}$	$6.71\pm1.20^{b***}$	$6.21\pm1.11^{b***}$	$5.61\pm1.03^{bc***}$	$5.33\pm1.01^{bc***}$

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

Values with the same letters indicate non- significant difference (P<0.05) and vice versa.

Table 6: The Mean values ± SD of kidney SOD, GPX, GST and MDA of the experimental groups

Groups variables	Control (+ve)	Arginine	Parsley powder	Parsley extract	Parsley powder + Arginine	Parsley extract + Arginine
SOD (µ/mg)	29.61 ± 3.41^{d}	$55.11\pm5.17^{c***}$	$59.64\pm6.60^{bc**}$	$63.18\pm5.91^{b***}$	$75.60\pm7.11^{a***}$	$80.41\pm8.40^{a***}$
GPX (µ/mg)	20.01 ± 3.11^{d}	$45.11\pm5.36^{c***}$	$51.14\pm5.30^{b***}$	$54.39\pm6.17^{b***}$	$65.36\pm6.80^{a***}$	$69.45\pm7.11^{a***}$
GST (µ/mg)	1.56 ± 0.24^{c}	$2.01\pm0.23^{b**}$	1.96 ± 0.20^{c}	$2.66\pm0.32^{b**}$	$3.04\pm0.45^{a***}$	$3.29\pm0.44^{a***}$
MDA (mmol/g)	17.33 ± 1.71^{a}	$10.11\pm1.17^{b***}$	$9.41\pm1.03^{b***}$	$9.41\pm1.21^{b***}$	$9.76\pm1.05^{b***}$	$8.87\pm1.21^{bc***}$

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

Values with the same letters indicate non- significant difference (P<0.05) and vice versa.

DISCUSSION

It is known that $KBrO_3$ has been reported to be a potent nephrotoxic agent that can mediate renal oxidative stress. It also enhances renal lipid peroxidation and hydrogen peroxide formation with reduction in renal antioxidant enzymes. Also, potassium bromate contributes to the cellular redox status and impairment of membrane protein activities in rats [31]. Arginine and parsley groups showed better nutritional results that were agreed with previous study. L-Arginine is a precursor for the synthesis of biologically important molecules, including nitric oxide, polyamines, creatine, agmatine, proline and glutamate. Available evidence shows that physiological levels of arginine and NO promote fat oxidation and decrease fat synthesis in a tissue-specific manner [7, 32]. Arginine infusion induced a 2.3-fold increase in growth hormone mRNA expression, which could result from the Arginine -mediated inhibition of somatostatin release in parallel to its recognized growth hormone releasing activity [33]. Parsley contains calcium, B-complex vitamins and iron, which nourish the glands that help regulate the uptake of calcium. It is a source of magnesium, calcium, potassium, vitamin A, beta-carotene and vitamin K. Furthermore, two tablespoons of parsley contain 16% of the recommended daily allowance (RDA) of vitamin C and over 12% of the RDA of vitamin A - two powerful antioxidants. Parsley and parsley juice are a great source of natural beta-carotene, chlorophyll, essential fatty acids, folic acid and iron [34]. $KBrO_3$ treatment also induced blood urea nitrogen, serum creatinine, uric acid levels and superoxide anion. The reduction of creatinine, urea and uric acid in the obtained results was attributed to the effect of consumption of arginine and parsley. The bong-term administmation of L-arginine in the drinking water ameliorates the progression of renal disease in rats with subtotal nephrectomy by significant decrease of the glomerular capillary hydraulic pressure gradient level of systemic hypertension and decrease the post glomerular resistance and increase the K, which presumably reflect the preservation of glomerular wall structural integrity [35]. Parsley serves as a diuretic and is beneficial to the kidneys. Its additional efficacy as a laxative doubly assists the body's elimination of toxins. The mechanism of action of parsley extract seems to be mediated through an inhibition of the Na+K+ pump that would lead to a reduction in Na+ and K+ reabsorption leading thus to an osmotic water flow into the lumen and dieresis. Parsley owes its diuretic effect to the presence of two ingredients, apiol and myristicin, which are found in parsley oil [36]. $KBrO_3$ can reduce renal glutathione content, activities of renal anti-oxidant enzymes, viz., glutathione peroxidase, glutathione reductase, catalase, glucose-6-phosphate dehydrogenase and glutathione S-transferase [37]. Parsley is rich with an antioxidant arsenal that includes luteolin, a flavonoid that searches out and eradicates free radicals in the body that cause oxidative stress in cells. Luteolin also promotes carbohydrate metabolism and serves the body as an anti-inflammatory agent [38]. $KBrO_3$ induces oxidative stress in human erythrocytes through the generation of reactive oxygen species and alters the cellular antioxidant defense system [39, 40]. Parsley is also used a treatment for anemia due to its high concentration of iron. Myristicin, an organic compound found in the essential oil of parsley

activates the enzyme glutathione-S-transferase, which helps the molecule glutathione attach to and fight against, oxidized molecules. Apigenin, one of the main flavonoids in parsley showed strong antioxidant effects, increasing the activities of antioxidant enzymes and, in turn, decreasing the oxidative damage to tissues [41, 42].

In summary, this study confirmed that potassium bromated has renal toxicity in rats and the consumption of arginine and parsley can improve efficiency of kidney function because of their antioxidant effect.

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