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Research Article
Antioxidant Activities of Parsley (*Petroselinum crispum*) on the Induced Biochemical and Histopathological Changes of Potassium Bromate-Fed Rats

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Abstract
Background and Objective: Parsley including flavonoids, tocopherol, coumarins carotenoids and ascorbic acid offers healthy amounts of potassium and iron. These phytochemicals improve total antioxidants capacity and these suppress destructive oxygen free radicals and prevent oxidative stress which is due to potassium bromate. The present study aimed to clarify the effect of adding parsley as an antioxidant on Potassium Bromate-fed Wister albino rat. **Materials and Methods:** A total of twenty four rats 60 day sold were randomly assigned to four treatment groups. Group 1 was fed untreated diet and served as negative control group. Potassium bromate was thoroughly mixed with the normal diet and fed to rats at 600 mg kg^{-1} (Group 2) and served as positive control group. Parsley was thoroughly mixed with the normal diet and fed to rats at 20 mg kg^{-1} (Group3); while Group 4 was fed a diet containing 600 mg kg^{-1} potassium bromate and 20 mg kg^{-1} parsley. All the four groups were regularly observed for 60 days. **Results:** The results revealed that there was no death throughout the experimental period (60 days). There was no significant change in the body and organs weights of all groups during the first 30 days but there was a significant decrease in body weight and a significant increase in kidney, liver and brain weights for the group treated with 600 mg kg^{-1} of potassium bromate only. There was no significant lesion seen in the growth of liver, kidney, spleen and brain in rats fed diet containing 20 mg kg^{-1} of parsley (Group 3) and in rats fed a diet containing 20 mg kg^{-1} of parsley mixed with 600 mg kg^{-1} potassium bromate (Group 4) compared with the negative control Group (Group 1). Significant increases of urea, creatinine and albumins levels besides significant decreases in Packed Cell Volume (PCV), Hemoglobin (HB) and Red Blood Cell (RBC) were observed in group treated with 600 mg kg^{-1} body weight of potassium bromate diet. Besides, there were significant increases ($p<0.05$) in Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in the group treated with 600 mg kg^{-1} body weight of potassium bromate diet. On the other hand, PCV, RBC and Hb were significantly increased ($p<0.05$) by adding 20 mg kg^{-1} of parsley (Group 3) and 20 mg kg^{-1} parsley with 600 mg kg^{-1} potassium bromate (Group 4) compared with the control (Group 1). However, cholesterol, creatine and urea were significantly decreased ($p<0.05$) for Group 3 and Group4 as compared to Group 1. The level of total protein and albumin as well as AST did not change within 60 days. Histopathological changes showed that there was a congestion of liver beside a marked demyelination changes in the brain (brain oedema) of rats fed with 600 mg kg^{-1} of potassium bromate but there was no congestion in liver of rats fed with a diet containing 600 mg potassium bromate plus 20 mg kg^{-1} parsley. **Conclusion:** Parsley is a good source of phenolic compound and vitamin C; hence, the antioxidant activity of parsley played an important role in the protection against disorders caused by oxidative damage through delaying or inhibiting the initiation or propagation of oxidative chain reactions.

Key words: Parsley, potassium bromate, body weight, organs weight, hematological changes, biochemical changes, histopathological changes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antioxidants are now known to play an important role in protection against oxygen driven disorders. They can delay or inhibit the initiation or propagation of oxidative chain reactions and thus prevent or repair body's cells which are damaged by oxygen[1]. Typical phytochemical compounds that possess antioxidant activity include phenols, phenolic acids and their derivatives, ascorbic acid, flavonoids and many sterols. As antioxidants, these species are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals and inhibit oxidases[2].

Parsley (*Petroselinum crispum*) is a member of Apiaceous family that has been employed in the food, pharmaceuticals, perfumes and cosmetic industries[3]. Parsley has several classes of flavonoids[4]. Flavones and flavones derivatives which occur as glycosidic form in nature, are major flavonoids found in parsley and other apiaceous vegetables[5]. Active antioxidants within parsley including flavonoids, tocopherol and coumarines[4] carotenoids[6] and ascorbic acid[7]. These phytochemicals improve total antioxidants capacity, suppress destructive oxygen free radicals and prevent oxidative stress damage[8].

Potassium bromate ($KBrO_3$) is an oxidizing agent that has been used as a food additive. It has long been used to increase the volume of bread with a fine crumb structure[9-11]. The maximum amount of potassium bromate allowed in bread is 0.02 mg g^{-1} or 20 mg kg^{-1} by the Food and Drug Administration[12]. All of these aspects explain the increasing interest in plant phenolics that has been manifested in the past few years. In this context, Parsley (*Petroselinum crispum*) has been explored for its antioxidant potential. Therefore, the main objective of this study was to clarify the antioxidant activity of Parsley (*Petroselinum crispum*).

MATERIALS AND METHODS

Experimental animals: Twenty four 60-day-old, clinically healthy male Wister rats were allocated at random to four groups each of 6 rats. Group 1 was fed untreated diet and served as a negative control. Potassium bromate (BDH, England) was thoroughly mixed with the normal diet and fed to rats at 600 mg kg^{-1} (Group 2) as a positive control. Parsley well was thoroughly mixed with the normal diet and fed to rats at 20 mg kg^{-1} (Group 3) and 20 mg kg^{-1} with 600 mg kg^{-1} potassium bromate (Group 4) for 60 days.

Three rats from each group were slaughtered after30 days and another three rats from each group were slaughtered at the end of experiment. Specimens of the liver, intestines, kidneys, spleen, stomach, bone marrow and heart were immediately fixed in 10% neutral buffered formalin for histopathology examination. Blood samples were collected at slaughter for hematology and serum analysis.

Hematological parameters: The parameters measured were Hemoglobin (Hb), Package Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC), differential WBC counts, erythrocyte series, Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH) and Mean Cell Hemoglobin Concentration (MCHC). These measurements were performed using an automatic Hematology Analyzer (Sysmex kx-21, Japan).

Biochemical analysis: The parameters measured were aspartate amino-trantransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, cholesterol and urea. These parameters were measured by a Hitachi 902 analyzer using commercial kits (Bio-system Chemicals, Barcelona , Spain)(Germany,1996). Globulin concentration was obtained by subtracting albumin concentration from that of the total protein and T-bilirubin and D-bilirubin.

Histopathological findings: Necropsy was conducted to identify gross lesions on specimens of the liver, kidneys, heart, spleen, intestines and stomach which were immediately fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 μm and stained routinely with haematoxylin and eosin (H and E)[13].

Statistical analysis: All obtained data were statistically analyzed by using SPSS program, 2002. Descriptive values of data were represented as means$\pm$standard deviations and with $p\leq 0.05$ being considered statistically significant.

RESULTS AND DISCUSSION

Hematological changes: The effects of various treatments on hematological constituents are presented in Table 1 and 2. Results indicated that WBC values were significantly lower in Groups 2 in 60 days of the experimental period. However, PCV values were higher in Group 2 through the 60 days. The RBC, Hb and PCV were significantly lower in Group 2 after 60 days. Potassium bromate treatment was found to have significant effects on hemopoiesis at the dose of 600 mg kg^{-1} potassium bromate in all periods of feeding resulting in anaemia.

Table 1: Haematological changes in rats fed on Potassium bromate, Parsley or their mixture for 30 days

	Treatment groups			
Parameters	1 (Control)	2 ($KBrO_3$) 600 mg kg^{-1}	3 (Parsley) 20 mg kg^{-1}%	4 ($KBrO_3$) 600 mg kg^{-1} +20 mg kg^{-1} (Parsley)
Hemoglobin (Hb) g dL^{-1}	13.76±0.54	12.33±0.84	14.90±0.47*	14.23±0.33*
Red blood cell (RBC) (×10^6 mm)	9.30±0.84	7.05±0.43	10.00±0.12*	9.88±0.33*
Package cell volume PCV (%)	62.70±0.59	48.23±3.53	76.02±1.25*	75.50±2.02*
Mean cell volume (MCV) (m^3)	73.67±2.90	68.40±1.30	72.00±1.73	71.50±0.77
Mean cell hemoglobin concentration (MCHC) (pg)	14.90±0.90	16.67±0.12	16.00±0.37	15.97±0.26
Mean cell hemoglobin concentration (MCHC) (%)	18.90±0.58	21.27±1.08	18.17±0.38	18.00±0.05
White blood cell (WBC) (×10^3 mm)	7.76±0.82	2.10±0.12*	6.98±0.30	6.25±0.11
Lymphocytes (LY)	56.73±1.99	35.30±1.12*	36.83±1.65	35.73±1.22
Monocytes (MO)	5.63±1.88	6.33±1.34	7.01±0.21	6.73±2.51
Granulocytes (GR)	37.63±3.02	58.37±2.37*	35.73±1.40	34.43±10.04

Data are the Means±SD, *Mean values in the same column indicates significance at $p\leq 0.05$

Table 2: Hematological changes in rats fed Potassium bromate, Parsley or their mixture for 60 days

	Treatment groups			
Parameters	1 (Control)	2 ($KBrO_3$) 600 mg kg^{-1}	3 (Parsley) 20 mg kg^{-1}%	4 ($KBrO_3$) 600 mg kg^{-1} +20 mg kg^{-1} (Parsley)
Hemoglobin (Hb) g dL^{-1}	14.80±0.57	12.63±0.23*	15.00±0.37*	14.31±0.60*
Red blood cell (RBC) (×10^6 mm)	10.53±0.67	8.98±0.55*	11.01±0.11*	10.90±0.50*
Package cell volume (PCV) (%)	60.47±3.79	34.90±0.95*	63.90±1.45*	62.80±1.13*
Mean cell volume (MCV) (m^3)	57.33±0.33	54.67±1.45	58.67±1.33	55.67±1.44
Mean cell hemoglobin concentration (MCHC) (pg)	14.10±0.38	15.47±0.32	14.30±0.13	14.11±0.10
Mean cell hemoglobin concentration (MCHC) (%)	24.57±0.61	27.400±0.06	25.53±0.60	24.57±0.00
White blood cell (WBC) (×10^3 mm)	11.77±0.32	7.17±0.15*	11.60±0.52	10.97±0.11
Lymphocytes (LY)	39.17±11.17	69.80±6.62	37.17±5.20	35.23±5.00
Monocytes (MO)	24.10±3.71	13.50±4.54*	21.37±1.61	20.37±5.22
Granulocytes (GR)	36.73±13.31	16.70±10.32*	42.47±6.03	40.40±0.04

Data are the Means±SD, *Mean values with (*) in the same column indicates significance at $p\leq 0.05$

Chipman *et al.*[14] reported that an induction of methaemoglobinaemia in rats was evident when potassium bromate was fed. They also claimed that oxidation of ferrous ion to ferric by reactive species was generated from potassium bromate ingestion. The study also revealed that there was no statistically significant difference in the test and control samples for MCHC and haematocrit values.

In a report of a two and half years, a boy who swallowed a neutralizing solution containing potassium bromate, the only haematological finding was a change in hemoglobin concentration from 11.4-10.7 g dL^{-1} in a period of two months[15].

Table 1 and 2 shows that, in Group 3 and 4, values of PCV, RBC and Hb were significantly higher than the control during the whole experimental period. These results indicated that rats fed with diet containing 20 mg kg^{-1} parsley and 20 mg kg^{-1} parsley+600 mg kg^{-1} potassium bromate had no anemia because parsley is a good source of iron, beta-carotene and vitamin C[16]. Parsley is an excellent digestion restorative remedy. It improves the digestion of protein and fats, therefore, promoting intestinal absorption, liver assimilation and storage[17].

Biochemical changes: Table 3 and 4 shows the effects of various doses of Potassium bromate, Parsley and Parsley with potassium bromate on biochemical changes in rats. The activities of serum AST and ALP were significantly higher in Group 2. ALT was higher with no significant difference in Group 2 within 60 days. The urea, creatinine and albumin levels significantly increased in Group 2 after 30 days compared with the control Group 1. On the other hand, cholesterol levels increased in Groups 2 after 30 days. Measurement of enzymatic activities for AST and ALT is of clinical and toxicological importance, as changes in their activities are indicative of liver damage by toxicants or in diseased conditions[18].

The observed decrease in the activities of liver followed by concomitant increase in serum AST and ALT activities

Table 3: Biochemical changes in rats fed on Potassium bromate and Parsley or their mixture for 30 days

Parameters	Treatment groups 1 Control	2 ($KBrO_3$) 600 mg kg^{-1}	3 (Parsley) 20 mg kg^{-1}%	4 ($KBrO_3$) 600 mg kg^{-1} +20 mg kg^{-1} (Parsley)
Aspartate aminotransferase (AST) (IU)	140.33±0.89	167.50±3.75*	142.50±0.87	161.00±0 .02*
Alanine aminotrantransferas (ALT) (IU)	30.00±3.46	39.33±1.45	32.67±0.88	37.67±4.98
Alkaline Phosphatase (ALP) (IU)	264.00±2.37	304.50±7.79*	140.33±0.09*	180.00±2.1.3
Total protein (g dL^{-1})	6.13±0.22	6.33±0.29	6.37±0.23	6.37±0.32
Albumin (g dl^{-1})	3.70±0 .58	3.90±0.06	3.90±0.32	3.87±0.17
Globulin (g dl^{-1})	2.43±0.22	2.53±0.23	2.47±0.12	2.50±0.15
Cholesterol (mg dL^{-1})	71.00±2 .89	65.33±2.19	62.67±4.67*	60.33±1.86*
D-bilirubin	0.02±0.01	0.02±0.01	0.01±0.00	0.03±0.00
T-bilirubin	0.04±0. 01	0.03±0.00	0.03±0.00	0.03±0.01
Urea (mg dL^{-1})	42.33±2.19	43.00±4.51	37.33±1.45*	38.33±2.96*
Creatin (mg dL^{-1})	0.80±0 .06	0.83±0.03	0.73±0.03*	0.77±0.03

Data are the Means±SD, *Mean values with (*) in the same column indicates significance at $p \leq 0.05$

Table 4: Biochemical changes in rats on Potassium bromate and Parsley or their mixture for 60 days

Parameters	Treatment groups 1 Control	2 ($KBrO_3$) 600 mg kg^{-1}	3 (Parsley) 20 mg kg^{-1}%	4 ($KBrO_3$) 600 mg kg^{-1} +20 mg kg^{-1} (Parsley)
Aspartate aminotrantransferas (AST) (IU)	140.00±0.05	159.50±0.05.48*	135.50±0.00*	136.00±0.05*
alanine aminotrantransferas (ALT) (IU)	14.00±1.15	17.00±2.31	12.63±0.00*	13.00±1.00*
alkaline phosphatase (ALP) (IU)	65.00 2.89	80.00±5.77*	55.00±5.88*	56.00±5.77*
Total protein (g dL^{-1})	7.67±0.22	7.20±0.30	7.77±0.15	8.17±0.46
Albumin (g dL^{-1})	3.47±0.13	4.17±0.09*	3.67±0.07	3.33±0.03
Globulin (g dL^{-1})	4.20±0.35	4.50±0.46	4.69±0.15	4.50±0.12
Cholesterol (mg dL^{-1})	59.67±2.03	63.33±1.76	52.67±3.76*	53.00±2.89*
D-bilirubin	0.02±0.00	0.01±0.00	0.02±0.00	0.02±0.000
T-bilirubin	0.03±0.01	0.02±0.00	0.03±0.01	0.03±0.000
Urea (mg dl^{-1})	50.00±0.58	63.50±4.33*	43.33±3.06*	46.67±0.20*
Creatin (mg dL^{-1})	0.87±0.03	1.01±0.03*	0.63±0.03*	65.00±0.00*

Data are the Means±SD, *Mean values with (*) in the same column indicates significance at $p \leq 0.05$

suggested that there may be a leakage of these enzymes from the liver to the serum[19]. Abdel-Tawab *et al*.[20] described the reduction in transaminases activity to liver necrosis caused by the toxicants and possible damage to the hepatocytes. The reduction in the activities of ALT and AST in the liver may be due to the interference with protein metabolism in the cells or inhibition of the enzyme[21]. The significant increase in ALP activity observed in the serum of rats fed on bromate-containing diet compared with the control may be attributable to the loss of membrane components due to a possible reaction between potassium bromate and the membranes of liver and kidney cells causing leakage of the enzyme into the serum. This observation was supported by Fleischer and Schwartz[22] who reported that any damage to the cell membrane may lead to leakage of ALP, which is a marker enzyme in the plasma membrane into extracellular fluid. Increased activities of serum enzymes have been reported in case of tissue damage[19]. The increase in serum levels of urea and creatinine in this study are indication of renal toxicity. This is in agreement with previous study conducted by Khan *et al*.[23] who stated that 125 mg kg^{-1} potassium bromate given intra-peritoneally to rats resulted in marked elevation of BUN and creatinine. Similar findings were also reported by Watanabe *et al*.[24]. El-Sokkary[25] reported that potassium bromate caused degeneration and necrotic change.

Table 3 and 4 shows that the rats treated in Groups 3 and 4 showed lower values of AST, ALP, Cholesterol, Creatin and urea than control Group 1. The level of globulin and albumin did not change within the 60 days. The protective role of parsley may be attributed to its higher content of the flavonoids which scavenge free radicals[4]. Parsley has a high enzyme content that is good for liver assimilation and storage[17].

Histopathological changes: Histopathological findings revealed congestion in the liver (Fig. 1) beside marked demyelination changes in brain (brain edema) of rats treated with 600 mg kg^{-1} for 30 days (Fig. 2). In this study, however, no congestion was observed in the liver of rats treated with a

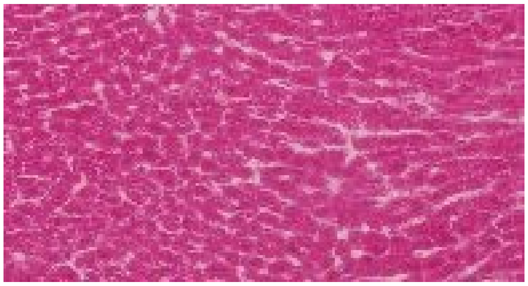

Fig. 1: liver of rats treated with 600 mg kg^{-1} Potassium Bromate +20 mg kg^{-1} Parsley
Note normal p40 power

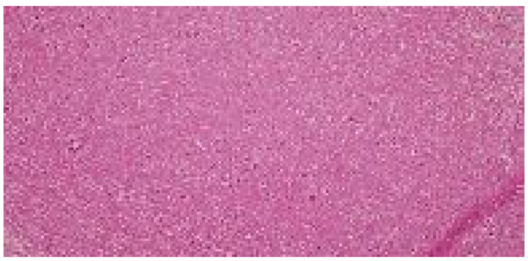

Fig. 2: Brain of rats treated with 600 mg kg^{-1} Potassium Bromate +20 mg kg^{-1} Parsley
Note normal p40 power

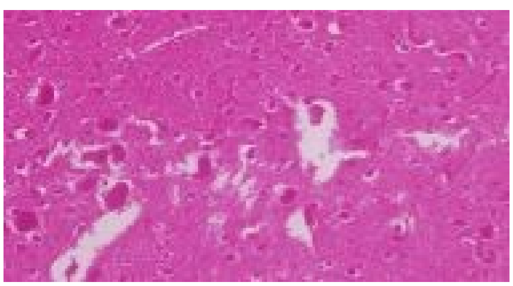

Fig. 3: Brain of rats treated with 600 mg kg^{-1} Potassium Bromate
Note mild demyelination p40 power

diet containing 600 mg potassium bromated plus 20 mg parsley during 60 days (Fig. 1) and no changes in brain tissues (Fig. 3).

It has been shown that $KBro_3$ induced methemoglobinemia in mice due to the reduction of glutathione peroxide activity in the blood with an increase in superoxide and nitrous oxide[24]. Methemoglobinemia was not observed in $KBro_3$ fed rats. There was an evidence that potassium bromate had an effect on the brain. The lesion in the brain indicates that potassium bromate may cross the brain barrier and exerts its effects on the endothelium permeability as well as brain tissue. This may indicate that it has a neurotoxic effect. Zheng *et al.*[26] reported that parsley is rich in myristicin which showed high activities as inducer of the detoxifying enzyme glutathione S-transferase in the liver and small intestinal mucosa of mice. As Parsley leaf is also used for the treatment of edema and diseases of liver[27], supplementation of the diets with fresh parsley leaf can significantly increase antioxidant capacity[28].

CONCLUSION

It was clear that potassium bromate is a health risk. Efforts to stop the use of potassium bromate in preparation of food should be intensified. National Agency for Food and Drug Administration and Control (NAFDAC) should also focus its attention not only on bread but on other bakery products in which bromated flour is used as a raw material. The physical properties of $KBrO_3$ make it easy to be taken or administered as a poison to human, thus its use and handling should be highly regulated by the relevant authorities. The WHO encouraged using medicinal herbs and plants to substitute or minimize the use of chemicals through the global trend to go back to nature. Antioxidants are now known to play an important role in protection against oxygen driven disorders.

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