Nephroprotective effect of Kale (*Brassica oleracea*) against potassium bromate induced renal injury in rats

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Abstract: The present study was performed to evaluate the nephroprotective effects of Kale (***Brassica oleracea***) leaves, Juice and seeds on potassium bromate, KBrO3 (200 mg/kg BW gavaged once) induced renal injury in rats. Forty adult male rats were assigned to five groups (n=8) for a four-weeks experimental period; group (1) normal control, group (2) $KBrO_3$-induced control, groups (3) administrated 150 mg/kg BW kale Juice (KJ) by gastric tube, group (4) treated with 15 % kale leaves powder (KLP) in diet and group (5) treated with 15% kale seed powder (KSP) in diet. Total phenolic content and total antioxidant activity from the extract were identified. The serum lipid profiles, serum kidneys biomarkers and lipid peroxidation marker MDA, non enzymatic antioxidant reduced glutathione (GSH), enzymatic antioxidant superoxide dismutase (SOD), Catalase (CAT) in kidneys were estimated. Total phenolic content was high 64.3 mg in methanolic extract of kale seeds (MEKS) followed by methanolic extract of KL then aqueous extract of KJ with 53.4 mg and 35.1 mg respectively. Moreover, total antioxidant capacity was high in KJ then KL and KS at levels 0.83, 0.22 and 0.13 (mmol/g) respectively. Results of KBrO3-induced renal injury rats showed significant ($p<0.05$) elevation levels of serum cholesterol, triglycerides, low density lipoprotein (LDL-C) and very low density lipoprotein (VLDL-C), kidney function markers uric acid, urea nitrogen, creatinine and total protein in serum and (MDA) levels in kidneys tissue, whereas they showed significantly decreased level of HDL-C and all kidneys tissue enzymatic and non enzymatic antioxidants (SOD, CAT and GSH). Oral administration of KJ with 150 mg/kg BW to nephrotoxicity rats were showed brought back in serum lipid profiles and hepatic biomarkers, tissue lipid peroxidation product (MDA), enzymatic, and non-enzymatic antioxidants to near normal followed by 15 % seed powder (KSP) group compared to 15% KLP group. Thus results showed that the most effective results revealed from 150mg KJ dose and 15 % KLP and 15 % KSP. Moreover, the histological evaluation of kidney approved the amelioration of the previous parameters and confirms the effective treatments were dried leaves, juice and seeds consequently. In conclusion, the present study discloses the ameliorative and protective effects of ***Brassica oleracea*** against renal injury that is at least, partly mediated by its antioxidant and phenolic properties as indicated by increase of antioxidant status and decrease of lipid peroxidation markers.
Keywords: *Brassica oleracea*, $KBrO_3$, Renal injury, Kidney functions, Antioxidants, Rats.

Introduction

Potassium bromate ($KBrO_3$) has been used widely for water disinfection, hair-coloring solutions, cosmetics, and in food as a food additive in the bread-making process. Toxicological studies have suggested that $KBrO_3$ is: an oxidizing agent; causes hepatotoxicity, neurotoxicity, and induces the development of mesothelioma tumors in experimental animals as well as renal carcinomas in animals and humans. (Kurokawa *et al.*, 1990; Deangelo *et al.*, 1998). Antioxidant enzymes as well as nonenzymatic

compounds such as reduced glutathione (GSH), ascorbic acid, and α-tocopherol all help to cope with the potential damage caused by oxidative stress. (Farombi *et al.*, 2003). ROS are largely generated from mitochondrial energy metabolism through oxidative phosphorylation and mostly removed by endogenous antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) (Sang-Hyun *et al.*, 2013). The increase in oxygen free radicals could be due to their overproduction and/or decreased antioxidant reserve and antioxidant enzyme activity (Jeon *et al.*, 2002).

Dietary intake of antioxidants and phenolic compounds (flavonoids) can inhibit or delay the oxidation of susceptible cellular substrate to prevent oxidative stress. (Rice-Evans et al., 1996).

Kale is a green leafy vegetable that belongs to the brassica family, a group of vegetables including cabbage, collards and brussels sprouts that have widespread attention due to their health promoting, containing phytonutrients. (Emebu and Anyika, 2011)

The most widespread and diverse group of polyphenols in Brassica species are flavonoids (mainly flavonols), the major polyphenolic constituents of Brassica foods, flavonols such as quercetin and kaempferol. (Rice-Evans et al., 1996) This health promoting activity seems to be related to the antioxidant (free-radical scavenging) activity of these compounds (Rice-Evans and Miller, 1996).

No detailed investigation has been addressed the efficacy of Kale leaves in treating KBrO3-induced nephrotoxicity in rats. Thus, the present study was planned to addresses the ameliorative and protective activities of Kale (*Brassica oleracea*) against potassium bromate induced renal injury in rats.

Material and Methods

Materials

Fresh ***Brassica oleracea*** leaves were obtained from local markets in KSA and seeds from traditional herbal store. The leaves were washed with tap water and grounded in a blender and filtering by using funnel and filter paper to obtain juice while other dried with hot air oven (50–60^{o}C) and grinded to powder.(AOAC, 1995)

$KBrO_3$ and biochemical kits were pursued of analytical grade from Sigma Aldrich Company for chemicals and Biodiagnostics, USA. The basal diet was prepared using AIN-93 according to Reeves *et al.*, (1993).

Samples extraction

A fresh, dried leaves and dried seeds were chopped, mixed, powered by blender and extracted by soxhlet apparatus with the aqeuous and methanolic extracts, The extracts were then filtered and evaporated under vacuum by using Rotary Vacuum Evaporator. Dry weight of these materials was determined and stored at -20^{o}C until use in experimental protocols. (Birgül *et al.*, 2011)

Estimation of total phenolics

Total phenolic content of the extracts was determined using the Folin–Ciocalteu micro-method (Slinkard and Singleton, 1977). Briefly, 20 µl of ethanol extract was mixed with 1.16 ml distilled water and 100 µl of Folin–Ciocalteu reagent, followed by 300 µl of Na2CO3 solution (20%) after 1 min and before 8 min. Subsequently, the mixture was incubated in a shaking incubator at 40 C^{o} for 30 min and its absorbance was measured at 760 nm in a Cintra 20 (GMBH, Germany) double beam spectrophotometer. The phenolic

content was expressed as gallic acid equivalents using the following linear equation based on the calibration curve: A = 0.98C + 9.925 x 10-3; R^2 = 0.9996, where A is the absorbance and C is concentration as gallic acid equivalents (µg/g).

Determination of total antioxidant activity

The total antioxidant activity of the Kale extract was evaluated using the phosphomolybdenum complex method (Prieto et al., 1999); 0.4 mL of sample solution (KLP extract) (100 µL/mL methanol) was combined with 4 mL of phosphomolybdenum complex containing 0.6 M sulphuric acid, 2 mM sodium phosphate, and 4 mM ammonium molybdate. Test tubes were capped and placed in hot water for 90 min at 95°C. Samples were cooled to room temperature and the absorbance was measured at 695 nm on a spectrophotometer (TU-1800; Human Corporation). Antioxidant activity was expressed as the mg ascorbic acid equivalent per mL (mg AE/mL).

Experimental animals

Forty male Wistar albino rats weighted 180 ± 20 gm were procured from college of Pharmacy, King Saud University, KSA, and they were maintained in an air-conditioned room (26 ± 1°C) with a 12-hour light/12-hour dark cycle. Feed and water were provided *ad libitum* for one week for adaptation before the start of experiment (4 weeks).

$KBrO_3$ induced renal injury

Solution of KBrO3 was prepared in drinking water and given orally (200 mg/kg bwt gavaged once) to the rats. After adaptation, rats were randomly divided into four groups of eight animals each. Group (1) normal control, group (2) KBrO3-induced control, groups (3) treated with 15 % kale leaves powder (KLP) in diet, groups (4) administrated 150 mg/kg BW kale Juice (KJ) by gastric tube and group (5) treated with 15 % kale seed powder (KSP) in diet. At the end of the experimental period, the animals were anesthetized by anesthetic ether. Blood was collected and the kidney dissected out, and washed in ice-cold saline for removal of blood. Tissues were sliced into pieces and homogenized in an appropriate buffer (pH 7.0) in cold condition to give 20% homogenate (w/v). The homogenates were centrifuged at 1000 rpm for 10 min at 0°C in a cold centrifuge. The supernatants were separated and used for various biochemical estimations.

Assessment of nephroprotective activity

The blood was allowed to clot and serum was separated at 2500 rpm for 15 min. Serum urea nitrogen, uric acid and creatinine were determined according to the methods described (Patton and Grouch, 1977; Fossati et al., 1980; Husdan and Rapoport, 1968). Serum albumin was estimated by Biuret method (Reinholdm, 1953). Serum cholesterol was determined according to the enzymatic method described by Allain et al. (1974), serum triglycerides were colorimetrically determined according to the method described by Wahlefeld (1974), HDL-C was determined according to the method described by Albers et al. (1983), while concentration of VLDL-C was estimated according to the method described by Friedewald's equation (1972). According to the method described by Friedewald et al. (1972), Low density lipoprotein cholesterol can be calculated as follows: LDL-C = Total cholesterol – (HDL-C) – (VLDL-C).

Lipid peroxidation and antioxidant biomarkers determinations in kidneys tissue

Lipid peroxidation (LPO) was determined by quantifying malondialdehyde (MDA) that formed in terms of thiobarbituric acid reactive substances (TBARS) by the method of Niehaus and Samuelson (1968). Determination of antioxidant biomarkers' levels as reduced glutathione (GSH) was estimated by Ellman, 1959. From other hand, the activities of tissues superoxide dismutase (SOD), catalase (CAT) were determined calorimetrically according to Spitz and Oberley (1989) and Sinha (1972); respectively.

Histopathological studies

For histological studies, the kidney tissues were fixed with 10% phosphate buffered neutral formalin, dehydrated in graded (50-100%) alcohol and embedded in paraffin. Thin sections (5M) were cut and stained with routine hematoxylin and eosin stain for photo microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in kidney tissue.

Statistical analysis

Data were analyzed by one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) using SPSS version 11 (SPSS, Chicago, IL). The limit of statistical significance was set at $P<0.05$.

Results

Data from determination of total phenolic contents and total antioxidants of Kale aqueous and methanolic extracts of Kj, KL and KS are shown in Table (1). The mean value of total phenols expressed as gallic acid equivalent per 100 ml kale seeds methanolic extract (KSME) was the highest compared to others with 64.3 mg/g dw, 53.4 mg of kale leaves methanolic extract (KLME) and the lowest was the aqueous extract of kale juice (AEKJ) with 35.1 mg. On other hand, the total antioxidant activity in kale aqueous and methanolic extracts was 0.83, 0.22 and 0.13 mg/mL of KJ, KL and KS respectively, expressed as ascorbic acid equivalent.

Table (1). Total phenolic compounds and antioxidant capacity of aqueous and methanolic extracts of Kale.

Extracts	Total phenolic content (mg/g dw)*	Total antioxidant capacity (m mol/g dw)**
Aqueous extract (KJ)	35.1 ± 1.2	0.83 ± 0.02
Methanolic extract (KL)	53.4 ± 3.5	0.22 ± 0.02
Methanolic extract (KS)	64.3 ± 2.7	0.13 ± 0.03

* Expressed as gallic acid equivalent.
** Expressed as Ascorbic acid equivalent.

Data in Table (2) depicts the effects of kale juice, dried leaves and dried seeds levels on serum lipid profiles of KBrO3 induced renal injury in rat. Administrating KBrO3 (200 mg/kg/ bwt) resulted in a significant ($p<0.05$) increase in total cholesterol, triglycerides, low density lipoprotein (LDL-c), and very low density lipoprotein (VLDL-c) while the level of high density lipoprotein (HDL-c) showed a significant ($p<0.05$) decrease compared with normal control. Treatment with 150 mg KJ, 15% KL and 15% KS to animals prior to KBrO3 treatment resulted in significant ($p<0.05$) attenuation in the KBrO3- induced alterations in all lipid parameters compared to normal control.

Significant alterations in uric acid (1.5-fold), UN (2.1- fold), creatinine (1.5-fold), and total protein (3.5-fold) levels were seen after treatment with KBrO3 alone compared to the control group showing the induction of nephrotoxicity (Table 3). Administration of kale juice; leaves; and seeds to animals prior to KBrO3 treatment resulted in significant ($p<0.05$) attenuation in the KBrO3- induced alterations in kidney parameters' levels near normal.

Table (4) showed the effect of kale parts on kidney tissues lipid peroxidation MDA, GSH, SOD and CAT activities of KBrO3- induced renal injury rats. In the current study there was a significant decreased in the mean value of kidney SOD, GSH and CAT in KBrO3- induced control ($p<0.05$) and significant increased in MDA ($p<0.05$) compared to normal control group. KJ treated group showed significant ($p<0.05$) increase in the mean value of kidney enzymes, and significant ($p<0.05$) difference in MDA compared to induced control, while KL group showed significant increased in the mean value of kidney MDA ($p<0.05$) and significant decreased in SOD, GSH and CAT ($p>0.05$) compared to normal control group. KS treated group showed lower values of kidney antioxidant enzymes and MDA compared with other treatments. All the treatment groups showed increase in kidney antioxidant enzymes and decrease MDA levels compared to $KBrO_3$-induced group.

Groups	Total cholesterol (mg /dl)	Triglycerides (mg/dl)	HDL-c (gm /dl)	LDL-c (mg /dl)	VLDL-c (mg/dl)
Normal control	80.31 ± 2.14^{a}	62.34 ± 1.88^{a}	49.26 ± 2.98^{a}	18.59 ± 1.85^{a}	12.46 ± 0.376^{a}
Induced control	144.42 ± 1.98^{d}	118.25 ± 2.11^{d}	29.95 ± 1.58^{d}	90.82 ± 1.79^{e}	23.65 ± 0.422^{c}
KBr03 + KJ (150ml/kg bwt)	86.11 ± 1.96^{ab}	68.61 ± 1.99^{a}	42.77 ± 0.97^{b}	29.62 ± 1.62^{b}	13.72 ± 0.398^{a}
KBr03 + KL (15% diet)	92.46 ± 1.25^{b}	76.27 ± 1.93^{bc}	38.53 ± 1.10^{c}	38.68 ± 1.97^{c}	15.25 ± 0.386^{ab}
KBr03 + KS (15% diet)	101.12 ± 1.52^{c}	91.03 ± 2.17^{c}	32.51 ± 1.53^{cd}	50.41 ± 2.13^{d}	18.20 ± 0.434^{b}

Table 2. Effects of kale parts on serum lipid profiles of KBr03-induced renal injury in rats.
Values are expressed as mean ± S.D. n= 8 rats/group.
Values not sharing a common superscript differ significantly at $p< 0.05$ (DMRT).

Groups	Uric acid (mg/ dl)	Urea nitrogen (mg/dl)	Creatinine (mg/dl)	Total protein (mg/dl)
Normal control	1.73 ± 0.13^{a}	24.45 ± 2.34^{a}	0.77 ± 0.03^{a}	0.63 ± 0.07^{a}
Induced control	2.56 ± 0.10^{d}	51.56 ± 2.46^{d}	1.12 ± 0.02^{d}	2.23 ± 0.10^{d}
KBr03 + KJ (150ml/kg bwt)	1.76 ± 0.15^{a}	29.35 ± 0.67^{b}	0.81 ± 0.03^{ab}	0.68 ± 0.02^{b}
KBr03 + KL (15% diet)	1.96 ± 0.12^{bc}	36.63 ± 0.75^{c}	0.85 ± 0.06^{b}	0.72 ± 0.04^{bc}
KBr03 + KS (15% diet)	1.84 ± 0.16^{b}	34.66 ± 0.88^{bc}	0.90 ± 0.02^{c}	0.85 ± 0.01^{c}

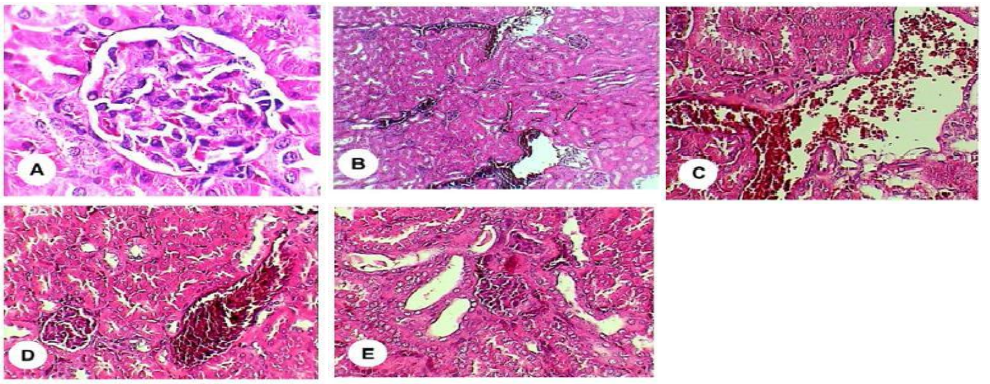
Table 3. Effect of kale parts on serum kidneys functions of KBr03-induced renal injury in rats.
Values are expressed as mean ± S.D. n=8 rats/group.
Values not sharing a common superscript differ significantly at $p< 0.05$ (DMRT).

Table 4. Effect of kale parts on kidneys tissues Lipid peroxide MDA, GSH, SOD, and CAT of KBr03-induced renal injury in rats.

Groups	Lipid Peroxidation (µmol / MDA/mg protein)	GSH (mmol /min/mg protein)	SOD (U /mg protein)	CAT (U /mg protein)
Normal control	3.01 ± 0.04^{a}	15.61 ± 2.12^{c}	28.37 ± 1.26^{d}	42.12 ± 2.42^{d}
Induced control	6.12 ± 0.02^{c}	8.55 ± 2.71^{a}	11.64 ± 1.65^{a}	21.13 ± 1.98^{a}
KBr03 + KJ (150ml/kg bwt)	3.42 ± 0.02^{b}	9.43 ± 2.23^{ab}	22.36 ± 1.37^{c}	27.52 ± 2.30^{b}
KBr03 + KL (15% diet)	3.13 ± 0.03^{a}	11.74 ± 2.67^{b}	15.28 ± 1.41^{b}	36.21 ± 1.99^{cd}
KBr03 + KS (15% diet)	3.96 ± 0.03^{b}	12.81 ± 2.01^{b}	18.17 ± 1.32^{bc}	31.11 ± 2.27^{c}

Values are expressed as mean ± S.D. n= 8 rats/group.
Values not sharing a common superscript differ significantly at p< 0.05 (DMRT).

Microscopic examination of normal control group, showed the normal histological structure or renal parenchyma (Fig. A). The kidney of $KBrO_3$-induced rats showed more severe degeneration alteration, vacuolar degeneration of endothelial lining glomerular tufts and epithelial lining renal tubules (Fig. B). In addition, microscopic examination of kidneys for KL (15% diet) group showed slight hypertrophy of glomerular tuft as well as mild presence of eosinophilic protein cast in the lumen of some renal tubules (Fig. C). In same context, histological structure of kidney tissues of KJ (150 mg/kg bwt) treated rats showed apparent normal histological structure (Fig. D), while, microscopic examination of kidney tissues of rats administrated KS (15% diet) showed congestion of glomerular tufts and granularity of epithelial lining renal tubules (Fig. E).



Figs 1: 1. Kidney microscopic examination of normal control rat group, showing the normal histological structure; 2. Kidney's tissue microscopic examination of KBr03 treated control rats showing gross necrosis of nephrocytes with nuclear pyknosis, marked vascular degeneration and congestion; 3. Kidney microscopic examination of KBr03+KJ (150ml/kg bwt) treated rats showing marked improvement in vacuolar degeneration of focal nephrocytes over induced control group; 4. Kidney's cells microscopic examination of KBr03+KL (15% diet) treated rat, showing good histological structure near to normal architecture of kidney cells. 5. Kidney's cells microscopic examination of KBr03+KS (15% diet) treated rat, showing mild structure for kidney's tissue.

Discussion

In the current study, ameliorative and protective activities of *Brassica oleracea* juice, dried leaves and dried seeds on $KBrO_3$-induced renal injury in rats were investigated. *Brassica oleracea* is having several biological functions and exhibits anti-bacterial and anti-inflammatory activities due to its anti-oxidant activities (Ferreres *et al.*, 2007; Kusznierewicz *et al.*, 2008; Soengas *et al.*, 2012]. Total phenolic content was high 64.3 mg in methanolic extract of kale seeds (MEKS) followed by methanolic extract of KL then aqueous extract of KJ with 53.4 mg and 35.1 mg respectively. Moreover, total antioxidant capacity was high in KJ then KL and KS at levels 0.83, 0.22 and 0.13 (m mol/g) respectively.

Elevated plasma total cholesterol and triglyceride concentration is seen in KBrO3-induced toxicity due to triglyceride over-production and /or underutilization and liver tissue injury. Lipoprotein lipase activity is markedly impaired, besides, a significant improvement in LDL internalization and degradation suggesting that chemical modification of LDL particle like nonenzymatic glycation of LDL itself might result in its increased incorporation in the arterial wall via a receptor independent pathway. Studies have strongly suggested an inverse relationship of HDL cholesterol with atherosclerosis to be independent of other lipid abnormalities (Taylor and Agius, 1988).

HDL cholesterol, the smallest of the lipoprotein species containing approximately 20% cholesterol ester and very little triglyceride is strongly and independently related to Coronary heart disease (CHD). But, unlike LDL, the relationship is inverse, a low HDL level being an important predictor of CHD and high HDL level protecting against CHD (Gordon *et al.*, 1997). A decrease in HDL turnover has been shown in KBrO3-induced toxicity rats.

In the present study, total cholesterol, triglycerides, LDL and VLDL were brought down significantly by *Brassica oleracea* treatment in $KBrO_3$-induced toxicity rats. This effect could be partly due to the control of liver and kidney toxicity. Decreased HDL cholesterol concentrations in KBrO3-induced toxicity rats appear to be markedly altered favorably by *Brassica oleracea* supplementation. All the lipid abnormalities developed in KBrO3-induced toxicity rats were effectively countered by feeding *Brassica oleracea*.

Urinalysis provides important clues about acid–base balance and kidney function [Free and Free, 2002]. Urobilinogen is a conjugated product of bilirubin, which passes through the bile duct and is metabolized in the intestine (Pels *et al.*, 1989; Simerville *et al.*, 2005). High levels of urobilinogen, urea, creatinine, protein and albumin in urine reflect the kidney dysfunction and renal injuries induced by $KBrO_3$ treatment (Ogeturk *et al.*, 2005 and Ozturk *et al.*, 2003). The oxidative stress induced by $KBrO_3$ might promote the formation of various vasoactive mediators that can affect renal function directly by initiating renal vasoconstriction or decreasing the glomerular capillary ultrafiltration coefficient. This action will reduce the glomerular filtration rate, leading to proteinuria. In our study the level of urea nitrogen, uric acid and creatinine increased in KBrO3 administered rats as reported previously (Ozturk *et al.*, 2003). Treatment of *Brassica oleracea* prevented $KBrO_3$-induced toxicity, and that the levels of urea nitrogen, uric acid and creatinine could be decreased to near control group.

Studies have shown that the plant extracts has been reduced the renal injuries against BrO3 intoxication (Simerville *et al.*, 2005 and Khan *et al.*, 2003). In our study, the activity of the antioxidant enzymes CAT, SOD, GSH-Px and GST were lowered in the

$KBrO_3$-induced rats when compared with that of the control group. Lowered activities of these antioxidant enzymes with $KBrO_3$ in in-vivo experimental models have been reported [Khan *et al.*, 2003]. However, the treatment of *Brassica oleracea* with $KbrO_3$ modified the biochemical changes caused by $KBrO_3$ in rat. In the present study, concluded that the *Brassica oleracea* treated group had a potential protective effect against KbrO3 induced rats.

GSH is a vital extracellular and intracellular protective antioxidant against oxidative stress. It reduces hydrogen peroxides and hydroperoxides by its redox and detoxification reactions, and protects protein thiol groups from oxidation. This tripeptide is present in high concentrations in kidney cells. In our study, the level of GSH was depleted on KBrO3 treatment rats when compared with that of control group. Treatment with *Brassica oleracea the level* of GSH were found to be increased with an accompanying increase in the mean activities of GSH-Px, GST and GSR with rutin to that of the KBrO3-treated group.

Increased TBARS concentration of renal tissues in $KBrO_3$ treated rat may be result of increased oxidative stress. TBARS, the final metabolite of peroxidized polyunsatured fatty acids (Dotan *et al.*, 2004), considered as a late biomarker of oxidative stress (Kim *et al.*, 2000), not only translate reactive oxygen species into active chemicals but also magnifies the function of reactive oxygen species through the chain reaction, inducing alterations in cellular and functional impairment (Cheeseman, 1993), and serves to indicate the presence of free radicals, lipid peroxide formation (Banerjee *et al.*, 2003). In our study was found to be increased concentration of TBARS in KBrO3 induced rats. This may be the consequence of an increment in the formation of oxygen free radicals (generated by $KBrO_3$) since antioxidant defense systems are compromised (Simerville *et al.*, 2005). Treatment with *Brassica oleracea* to $KBrO_3$ induced rats, the lower concentration of TBARS was observed which indicates the ameliorating effects of this extract against the oxidative stress induced with $KBrO_3$ in rats. *Brassica oleracea* significantly improved the alteration of antioxidant status caused by $KBrO_3$ in male rat which might be associated due the presence of flavonoids.

Histological examinations of rats' kidney for all groups also revealed the improvement in damaged tissues with the type of *Brassica oleracea* administration (KL 15% diet then KJ (150 mg/kg BW)) due to elevating the level of its antioxidants and phenolic contents (Wu *et al.*, 2006 and Yu Wang *et al.*, 2015).

In conclusion, all previous results suggests that supplementation of *Brassica oleracea* even with dried leaves, juice or seeds significantly improved the antioxidants status and reduce the risk of oxidative stress and dyslipidemia in $KBrO_3$-induced toxicity in rats.

References

A .O. A. C. (1995): Official Methods of Analysis, Association of Official Analytical Chemists, 18th Ed., Virginia, U.S.A.

Albers N, Benderson V, Warnick G. Enzymatic determination of high density lipoprotein cholesterol: Selected methods. Clin Chem 1983;10:91-99.

Allain CC, Poon LS, Chan CS. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470-475.

Banerjee SK, Sood S, Dinda AK, Das TK, Maulik SK. Chronic oral administration of raw garlic protects against isoproterenol-induced myocardial necrosis in rat. Comp Biochem Physiol Part C. 2003;136:377–86.

Birgül Vanizor Kural, Nurçin Küçük, Fulya Balaban Yücesan and Asim Örem. (2011). Effects of kale (Brassica oleracea L. var. acephala DC) leaves extracts on the susceptibility of very low and low density lipoproteins to oxidation.Indian Journal of Biochemistry & Biophysics; 48: pp 361-364.

Birgül Vanizor Kural, Nurçin Küçük, Fulya Balaban Yücesan and Asim Örem. (2011). Effects of kale (Brassica oleracea L. var. acephala DC) leaves extracts on the susceptibility of very low and low density lipoproteins to oxidation.Indian Journal of Biochemistry & Biophysics; 48: pp 361-364.

Cheeseman KH. Mechanisms and effects of lipid peroxidation. Mol Aspects Med. 1993;14:191–197.

Deangelo A, George M, Kilburn S, Moore T, Wolf D: Carcinogenicity of potassium bromate administered in the drinking water to male B6C3F1 mice and F344/N rats. Toxicol Pathol 1998, 26:724–729.

Dotan Y, Lichtenberg D, Pinchuk I. Lipid peroxidation cannot be used as a universal criterion of oxidative stress. Prog Lipid Res. 2004;43:200–227.

Ellman GL. Tissue sulfhydrl groups. Arch Biochem Biophys 1959;82:70-77.

Emebu, P.K. and Anyika, J.U. (2011). Vitamin and antinutrient composition of kale (Brassica oleracea) grown in Delta State, Nigeria. Pakistan Journal of Nutrition 10 (1): 76-79

Farombi EO, Alabi MC, Akuru TO: Kolaviron modulates cellular redox status and impairment of membrane protein activities induced by potassium bromate (KBrO3) in rats. Pharmacol Res 2003, 45:63–68.

Ferreres F, Souza C, Valentão P, Seabra RM, Pereira JA, Andrade PB. Tronchuda cabbage (Brassica oleracea L. var. costata DC) seeds: Phytochemical characterization and antioxidant potential. Food Chem. 2007;101:549–58.

Fossati P, Prencipe L, Berti G. Uric acid measurements with enzymatic colorimetric method. Medicinal Clin Chem 1980;26:227-273.

Free AH, Free HM. Urine analysis, critical discipline of clinical science. Critical Review Clinic Lab Sci. 2002;3:481–531.

Friedewald WT, Leve RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein. Clin Chem 1972;18:499-502.

Gordon T, Castelli WP, Hjortland MC. High density lipoprotein as a protective factor against coronary heart disease. Am J Med. 1997;62:707–14.

Husdan H, Rapoport A. Estimation of creatinine by Jaffe reaction. Clin Chem 1968;14:222-228.

Jeon SM, Bok SH, Jang MK, Kim YH, Nam KT, Jeong TS, Park YB, Choi MS. Comparison of antioxidant effects of naringin and probucol in cholesterol-fed rabbits. Clin Chim Acta 2002;317:181–190.

Khan N, Sharma S, Sultana S. Nigella sativa (black cumin) ameliorates potassium bromate-induced early events of carcinogenesis: diminution of oxidative stress. Human Exp Toxicol. 2003;23:193–203.

Kim YH, Mun KC, Lee SS, Seo SH, Kwak CS, Park SB, Kim HC. Oxidative damage in renal transplant patients. Transplant Proc. 2000;32:1777–1778.

Kurokawa, Y., A. Maekawa, M. Takahashi and Hayashi, Y. (1990): Toxicity and carcinogenicity of potassium bromate, a new renal carcinogen. Environ. Health Perspect. 87:309-335.

Kusznierewicz B, Bartoszek A, Wolska L, Drzewiecki J, Gorinstein S, Namieśnik J. Partial characterization of white cabbages (Brassica oleracea var. capitata f. alba) from different regions by glucosinolates bioactive compounds total antioxidant activities and proteins. LWT-Food Sci Technol. 2008;41: 1–9.

Niehaus WG, Samuelson B. Formation of MDA from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem 1968; 6: 126– 130.

Ogeturk M, Kus I, Colakoglu N, Zararsiz I, Ilhan N, Sarsilmaz M. Caffeic acid phenyl ester protects kidney against carbon tetrachloride toxicity in rats. J Ethnopharmcol. 2005;97:273–280.

Ozturk F, Ucar M, Ozturk IC, Vardi N, Batcioglu K. Carbon tetrachloride induced nephrotoxicity and protective effect of betaine in Sprague dawley rats. Urology. 2003;62:353–356.

Patton C, Grouch SR. Enzymatic determination of urea. Anal Chem 1977;49:464-468.

Pels RJ, Bor DH, Woolhandler S, Himmelstein DU, Lawrence RS. Dip sticks urinalysis screening of asymptomatic adults for urinary tract disorders. J Am Med Assoc. 1989;262:1221–1224. doi: 10.1001/jama.262.9.1221.

Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999;269:337-341.

Reeves PG, Nielson FH, Fahey GC. Reports of the American Institute of Nutrition, adhoc Willing Committee on Reformulation of the AIN 93 Rodent diets. J Nutr 1993; 123: 1939-1951.

Reinholdm J. G. Manual determination of serum total protein, albumin and globulin fractions by Biuret method. In: Reiner M (Ed.), Standard Methods in Clinical Chemistry, Academic Press, New York. 1953; p. 88.

Reitman S, Frankel S. A colorimetric method for the determination of serum glutamate oxaloacetate transaminase. Am J Clin Pathol 1957;28:53-56.

Rice-Evans CA, Miller NJ (1996). Antioxidant activities of flavonoids as bioactive components of food. Biochem Soc Trans 24:790–795

Rice-Evans, C.A., N.J. Miller and Paganga, G. (1996): Structure- antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. (20): 933- 956.

Sang-Hyun S, Si-Kwan K, Young-Ock K, Hyung-Don K, Yu-Su S, Seung-Ok Y, Seung-Yu K, Sang-Won L. A comparison of antioxidant activity of Korean White and Red Ginsengs on H2O2-induced oxidative stress in HepG2 hepatoma cells. J Ginseng Res 2013;37:442–450.

Simerville JA, Maxted WC, Pahira JJ. Urinalysis: A Comprehensive Review. Am. Family Physician. 2005;71:1153–1162.

Sinha KA. Colorimetric assay of catalase enzyme. Anal Biochem 1972; 47:328-330.

Slinkard K, Singleton VL. Total phenol analysis; automation and comparison with manual methods. Am J Enology & Viticulture 1977;28:49–55.

Soengas P, Cartea ME, Francisco M, Sotelo T, Velasco P. New insights into antioxidant activity of Brassica crops. Food Chem. 2012;134:725–33.

Spitz DR, Oberley LW. An assay for superoxide dismutase activity in mammalian tissue homogenates. Anal Biochem 1989;179:8-18.

Taylor R, Agius L. The biochemistry of diabetes. Biochem J. 1988;250:625–40.

Wahlefeld AW. Enzymatic Determination of triglycerides. methods of enzymatic analysis. HU. Bergmeyer, Ed. Academic Press, New York, 1974;5:1831-1835.

Wu, Y., Wang, F., Zheng, Q., Longxi, L., Hongtian, Y., Changxin, Z., Xiumei, Wu., and Yu, Z. (2006). Hepatoprotective effect of total flavonoids from Laggera alata against carbon tetrachloride-induced injury in primary cultured neonatal rat hepatocytes and in rats with hepatic damage. Journal of Biomedical Science, vol. 13, no. 4, pp. 569–578.

Yu Wang, Changyun Tang, Hao Zhang. (2015). Hepatoprotective effects of kaempferol 3-O-rutinoside and kaempferol 3-O-glucoside from Carthamus tinctorius L. on CCl4-induced oxidative liver injury in mice. journal of food and drug analysis, 23: 310-317.

التأثير الوقائي على الكلى لنبات الكرنب تجاه بروميد البوتاسيوم المسبب للإصابة الكلوية لفئران التجارب

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الملخص

أجريت هذه الدراسة لتقييم التأثير الوقائي على الكلى لمسحوق أوراق أو عصير أو بذور نبات الكرنب على بروميد البوتاسيوم (200 ملج/كجم وزن الجسم/ جرعة واحدة) المسبب لإصابة كبد الفئران. استخدمت الدراسة 40 فأر من الذكور قسمت إلى 5 مجموعات /8 فئران لمدة 4 أسابيع، المجموعة الأولى هي الطبيعية تغذت على غذاء قياسي، الثانية هي المريضة (الغير معالجة)، المجموعة الثالثة والرابعة والخامسة هم المريضة المعالجة ب 150 ملج/كجم وزن من عصير الكرنب ثم 15% مسحوق الأوراق ثم 15% مسحوق البذور والكل مع الغذاء القياسي. المحتوى الكلى للمركبات الفينولية كان الأكثر ارتفاعا 64.3 ملج في المستخلص الكحولى للبذور (MEKS) تلاه مستخلص الأوراق ثم العصير بنسب 53.4 ملج و 35.1ملج على الترتيب، القدرة الكلية لمضادات الأكسدة تم تقديرها وكانت الأعلى في العصير تلاه الأوراق ثم البذور بنسب 0.83، .22، 0.13 ملل مول/جرام. أظهرت نتائج المجموعة الضابطة المريضة إرتفاعا معنويا في مؤشرات وظائف الكلى ومشتقات الدهون الثلاثية والكولسترول والدهون المنخفضة الكثافة ومؤشر البيروكسيد الدهنى MDA في أنسجة الكلى مع إنخفاض معنوي في الدهون مرتفعة الكثافة ومضادات الأكسدة الإنزيمية والغير إنزيمية (SOD, CAT, GSH) في أنسجة الكلى. جرعات العصير بالفم بنسبة 150 ملج/كجم وزن الجسم كانت الأكثر فعالية في إعادة مؤشرات الدهون وقياسات الكبد لمعدلات تقترب من الطبيعية، وكانت نسبة 15% من مسحوق الأوراق الأكثر فعالية لمؤشرات البيروكسيد الدهني (MDA) ومضادات الأكسدة الإنزيمية والغير إنزيمية في أنسجة الكلى مع نسبة 15% من مسحوق البذور، كما توافقت نتائج فحص أنسجة عينات الكلى مع النتائج السابقة حيث دلت على تحسين القياسات الكيميائية الحيوية ومضادات الأكسدة وإظهار الدور الوقائي لعصير ومسحوق الأوراق والبذور للكرنب. توصي الدراسة باستخدام عصير الكرنب ومسحوق الأوراق والبذور إما بتناولها مباشرة أو بتدعيم المخبوزات والعجائن بها لما لها من تأثيرات حيوية وصحية جيدة في الوقاية من الإصابة الكلوية الناتجة عن التعرض لبروميد البوتاسيوم.

الكلمات المفتاحية: الكرنب، بروميد البوتاسيوم، إصابة الكلى، وظائف الكلى، مضادات الأكسدة، فئران.