The Antioxidant effects of Wheat Germ Oil Against Potassium bromate Induced Hepatorenal toxicity in Male Rats.

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Abstract: The present study was carried out to investigate the effect of oral administration of wheat germ oil at three dosage levels (150, 300 and 450 mg/kg.b.wt.) to male rats intoxicated with potassium bromate (KBrO$_3$) after 4 weeks of treatment on blood urea nitrogen (BUN), uric acid (UA), creatinine (Cr), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and oxidative stress markers such as glutathione (GSH), malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) were determined. Histopathological examination of liver was also performed. Forty adult male Wistar rats were divided into five equal groups as follows: group1: negative control group, group 2: positive control group, groups 3, 4 and 5 intoxicated with KBrO3 and orally given wheat germ oil at dosage levels 150, 300 and 450 mg/kg.b.wt., respectively. The results showed that oral administration of wheat germ oil at three dosage levels (150, 300 and 450 mg/kg.b.wt.) to intoxicated rats with potassium bromate for 4 weeks significantly decreased in serum levels of blood urea nitrogen (BUN), creatinine (Cr), and liver enzymes (AST, ALT and ALP) in a dose depended manner when compared to the control positive group. Oxidative stress markers (MDA, GSH, GPx, SOD and CAT) were significantly improved as compared to the control positive group. Histopathological examination of liver sections of rats orally given wheat germ oil showed alleviation of histological degeneration changes caused by KBrO3. The results of this study indicated that oral administration of wheat germ oil induce potent hepatoprotective, nephroprotective and antioxidant effects in rats intoxicated by KBrO3. This study recommends that, intake of wheat germ oil may be beneficial for patients with oxidative stress which is linked as a main cause of many diseases.

Key words: Wheat germ oil, Antioxidant enzymes, Oxidative stress, Potassium bromate.

1. Introduction

Oxidative stress is a phenomenon associated with pathogenetic mechanisms of several diseases including atherosclerosis, neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, cancer, diabetes mellitus, inflammatory diseases, as well as psychological diseases or aging processes **(Durackova, 2010).**

Potassium bromate (KBrO$_3$) is a nephrotoxic and carcinogenic substance used in food and found in drinking water as a by-product of disinfection by ozonaization **(Alli et al., 2013).** In the early **1990's** the World Health Organization **(WHO)** discovered that potassium bromate if added to dough which subsequently is produced as bread has the capacity to cause such diseases as cancer, kidney failure and several other related diseases. Potassium bromate causes renal cell cancer and act as a tumor promoter in carcinogen-initiated animals. Renal cell tumors have been observed in rats after exposure to KBrO$_3$ due to oxidative stress **(Dodd et al., 2013).**

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. When added to foods, antioxidants minimize rancidity, delay the formation of toxic oxidation products, maintain nutritional quality and increase shelf life **(Erukainure et al., 2012).**

Varieties of medicinal plants are recognized as a source of natural antioxidants that can protect from oxidative stress, thus playing an important role in chemoprevention of diseases **(Luqman et al., 2009).**

Wheat germ is an important nutritional natural resource, which contains important fatty acids and minerals **(Kan, 2012).** Wheat germ oil was claimed anti-inflammatory and described as a suitable natural antioxidant due to its high content of vitamin E **(Paranich et al., 2000; Leenhardt et al., 2008).** Wheat germ oil is an excellent source of polyunsaturated fatty acids. It is one of the richest natural sources of α- tocopherol, the type of tocopherol with the greatest vitamin E activity. Wheat germ oil has been attributed to reduced plasma and liver cholesterol in animals and improved physical endurance **(Wang and Johnson, 2001).** Also, wheat germ oil can reduce oxidative stress, improve lipid metabolism **(Singh et al., 2006; Megahed, 2011).**

2. Material and Methods
Material

Wheat germ oil

Wheat germ oil, used in this study was purchased from a local market, Jeddah, Kingdom of Saudi Arabia.

Potassium bromate

Potassium bromate ($KBrO_3$), is potassium salt of bromic acid. It was purchased from Cayman Chemicals and BioVision Incorporated, USA, as a white powder.

Kits for biochemical analysis

Diagnostic commercial kits for biochemical analyses were purchased from Cayman Chemicals and BioVision Incorporated, USA.

Experimental animals

Forty adult male albino rats of Wister strain weighing 200-250 grams body weight and 8-10 weeks old were obtained from the experimental animal unit of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia.

Methods

Feeding of rats

Standard nutritionally balanced diet AIN-93 according to **(Reeves, 1997)** was obtained from King Fahd Center for Medical Research, the diet consists of the following ingredients; crude protein 20.0%, crude fat 4.0 %, crude fiber 5.0 %, vitamin mix 1.0%, mineral mix 3.50%, choline chloride 0.25%, the remained formula up to 100% cornstarch, and its energy equals 2850 kcal/kg. The diet manufactured by Grain Silos and Flour Mills Organization, KSA.

Induction of oxidative stress

Rats were injected intraperitoneally (i.p.) with a single dose ofpotassium bromate 125 mg/kg.b.wt., to induce oxidative stressaccording to the method described by **Khan and Sultana (2004).**

Experimental design and grouping of rats

The experiment was performed on forty male adult Wistar rats distributed randomly into five equal groups. Rats were housed in plastic cages at a room temperature maintained at 24 ± 2 °C, with fixed 12 hour lighting system.

All rats were allowed to free access to basal diet and water for one week before starting the experiment for acclimatization. After acclimatization period all rats were allocated in to the following groups:

Group (1): Rats fed on rat pellets only; Control negative (Con–ve).

While the other four groups were injected by a single intraperitoneal dose of potassium bromate at a dose of 125 mg/kg.b.wt.,to induce oxidative stress. Before starting the experiment, blood samples were taken for measuring the activity of antioxidant enzymes to make sure that all rats were intoxicated with potassium bromate. After that, rats were distributed equally into the following groups:

Group (2): Rats fed on rat pellets only; Control positive (Con +ve).

Group (3): Rats fed on rat pellets and orally givenwheat germ oil in a dose of 150 mg/kg.b.wt./day for 4 weeks.

Group (4): Rats fed on rat pellets and orally given wheat germ oil in a dose of 300 mg/kg.b.wt./day for 4 weeks.

Group (5): Rats fed on rat pellets and orally given wheat germ oil in a dose of 450 mg/kg.b.wt./day for 4 weeks.

Anesthesia and collection of blood samples

At the end of the experimental period, the rats were fasted over night. On the morning of the next day the rats were anesthetized by general volatile anesthesia using ether. Anesthesia was done by placing the rat in a large glass jar with a piece of cotton soaked with ether. After induction of mild anesthesia, the rat was rapidly pulled out and blood was collected. Blood samples were withdrawn by capillary microtubes (Micro Hematocrite Capillaries, Mucaps) from the retro-orbital plexuses of veins in inner canthus of the eye into plain tube with gel. The blood samples were centrifuged at 3000 rpm for 15 minutes. Serum samples were separated and frozen at -80°C untilused for the biochemical analyses **(Margoni et al., 2011).**

After decapitation of the rats, liver was removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. Liver was washed with cold 0.9 % sodium chloride saline solution and dried between two filter papers. Liver was kept in 10% neutral buffered formalin pending for the histopathological examination.

Serum biochemical analysis

Serum samples were used for determination of creatinine according to **Henry (1974)**, urea nitrogen according to **Patton and Grouch (1977),** uric acid according to **Fosssati et al. (1980).** Activities of serum liver enzymes Aspartate aminotransaminase (AST), alanine aminotransferases and alkaline phosphatase (AST, ALT, and ALP) were chemically estimated according to**Tietz (2006)**, The liver homogenate was used for determination of tissue lipid peroxide (MDA), enzymatic (GPx, SOD, and CAT) and non-enzymatic (GSH) antioxidants. Malondialdehyde was determined according to **Yoshioka et al., 1979).** The reduced glutathione (GSH) content in liver homogenate was determined colorimetrically by the method modified by **Foyer et al., 1995).** Activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) antioxidant enzymes were determined chemically according to **Paglia and Valentaine, (1979); Kakkar et al., (1984) and Sinha, (1972)** respectively.

Histopathological examination

The fixed liver specimens were washed and dehydrated in ascending grades of alcohol. Specimens were then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Haematoxylin and Eosin stain for histopathological examination as described by **Bancroft and Cook, 1998),** and examined microscopically.

Statistical analysis

All data obtained results were analyzed using statistical package for the social sciences (SPSS) for windows, version 20 (SPSS Inc., Chicago, IL, USA). Collected data were presented as mean± standard deviation (SD). Analysis of variance (ANOVA) test and Post hoc test were used for determining the significances among different groups according to **Armitage *et al*. (2002).** All differences were considered significant at P-values were < 0.05.

3. Results

Results in Table (1) showed the effect of different doses of wheat germ oil on kidneys function (blood urea nitrogen (BUN), uric acid (UA) and creatinine (Cr) in hepatorenaltoxicity of male rats.

Results of blood urea nitrogen (BUN) and creatinine (Cr) showed that there was a significant (P< 0.05) increase in potassium bromate–intoxicated rats (positive control group) as compared to normal rats (negative control group), as depicted in Table 1. Oral administration of wheat germ oil at three dosage levels 150, 300 and 450 mg/kg.b.wt., significantly (P< 0.05) decreased the serum levels of blood urea nitrogen (BUN) and creatinine (Cr) as compared to the potassium bromate–intoxicated rats (positive control group).

Table (1) Effect of oral administration of wheat germ oil on blood urea nitrogen (BUN), uric acid (UA) and creatinine (Cr) levels in hepatorenaltoxic rats.

Parameters Groups	BUN (mg/dl)	UA (mg/dl)	Cr (mg/dl)
Con (-)	38.02 ± 2.76^c	$1.55 \pm .06^a$	$.78 \pm .03^d$
Con (+)	57.02 ± 4.23^a	$1.50 \pm .02^a$	$1.74 \pm .09^a$
WGO 150 gm/kg	49.68 ± 3.24^b	$1.52 \pm .04^a$	$1.48 \pm .08^b$
WGO 300 gm/kg	41.71 ± 3.78^c	$1.53 \pm .03^a$	$1.09 \pm .06^c$
WGO 450 gm/kg	39.00 ± 2.65^c	$1.53 \pm .02^a$	$.83 \pm .05^d$

Data are presented as means ± standard deviation, (n = 8 for each group).
Values with different superscripts within the column are significantly different at P< 0.05.

Effect of oral administration of wheat germ oil on serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in potassium bromate–intoxicated rats are presented in Table (2).

It is clear from Table (2) that potassium bromate–intoxicated rats (positive control group) had significant (P< 0.05) increases in the level of AST, ALT and ALP enzymes compared to normal rats (negative control group) by 120.69, 73.49 and 15.93 % respectively.

Potassium bromate–intoxicated rats orally given wheat germ oil in doses of 150, 300 and 450 mg/kg.b.wt., had significant (P< 0.05) reduction in the serum level of AST enzyme level by 12.86, 41.73 and

52.15 % respectively when compared to potassium bromate–intoxicated rats (positive control group), as shown in Table (2).

Oral administration of wheat germ oil at three dosage levels 150, 300 and 450 mg/kg.b.wt., when given to potassium bromate–intoxicated rats significantly (P <0.01) lowered the high serum level of ALT enzyme by 16.93, 34.57 and 42.93 % respectively as compared to potassium bromate–intoxicated rats (positive control group) as shown in Table (2). While the percentages of decreased in ALP enzyme were 4.95, 10.77 and 14 % respectively as compared to the potassium bromate–intoxicated rats (positive control group) as shown in Table (2).

Table (2) Effect of oral administration of wheat germ oil on serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in hepatorenaltoxic rats..

Parameters Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Con (-)	41.08 ± 3.24^d	34.36 ± 2.56^d	99.20 ± 7.56^c
Con (+)	90.66 ± 6.23^a	59.61 ± 4.33^a	115.00 ± 9.33^a
WGO 150 gm/kg	79.00 ± 5.24^b	49.52 ± 3.61^b	109.31 ± 8.61^b
WGO 300 gm/kg	52.83 ± 6.53^c	39.00 ± 1.99^c	102.61 ± 9.99^c
WGO 450 gm/kg	43.38 ± 2.42^d	34.02 ± 2.61^d	98.90 ± 8.61^c

Data are presented as means ± standard deviation, (n = 8 for each group).
Values with different superscripts within the column are significantly different at P< 0.05.

Results in Table (3) showed the effect of wheat germ oil at three dosage levels 150, 300 and 450 mg/kg.b.wt., on malondialdehyde (MDA) and reduced glutathione (GSH) in hepatorenaltoxic rats..

Data recorded in Table (3) showed that potassium bromate–intoxicated rats (positive control group) had a significant (P< 0.05) increase in the serum level of malondialdehyde (MDA) as compared to normal rats (negative control group) by 121.05 %. The tested doses 150, 300 and 450 mg/kg.b.wt., caused significant (P< 0.05) decrease in the serum levels of malondialdehyde (MDA) when compared with the potassium bromate–intoxicated rats (positive control group) by 29.76, 51.19 and 53.57 % respectively.

Table (3) Effect of oral administration of wheat germ oil on serum levels of malondialdehyde (MDA) and reduced glutathione (GSH) contents in hepatorenaltoxic rats.

Parameters Groups	MDA (nmol/ml)	GSH (U/ML)
Con (-)	$.38 \pm .02^d$	28.62 ± 1.76^a
Con (+)	$.84 \pm .01^a$	15.22 ± 1.03^c
WGO 150 gm/kg	$.59 \pm .03^b$	20.52 ± 1.54^b
WGO 300 gm/kg	$.41 \pm .02^c$	26.53 ± 2.15^a
WGO 450 gm/kg	$.39 \pm .03^d$	27.53 ± 2.02^a

Data are presented as means ± standard deviation, (n = 8 for each group).
Values with different superscripts within the column are significantly different at P< 0.05.

It is clear from Table (3) that potassium bromate–intoxicated rats (positive control group) had a significant (P< 0.05) decreases in the serum level of reduced glutathione (GSH) when compared to the normal rats (negative control group) by 46.82 %. Oral administration of wheat germ oil in the doses of 150, 300 and 450 mg/kg.b.wt., significantly (P< 0.05) increased serum level of reduced glutathione (GSH) when compared to potassium bromate–intoxicated rats (positive control group) by 34.82, 74.31 and 80.88 %respectively.

From data recorded in Table (4) it could be noticed that potassium bromate–intoxicated rats (positive control group) had significant (P< 0.05) decreases in the serum level of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes when compared to normal rats (negative control group) by 27.96, 44.71 and 27.72 % respectively.

Oral administration of wheat germ oil at doses of 150, 300 and 450 mg/kg.b.wt., showed significant (P< 0.05) increase in SOD enzyme serum level by 19.54, 35.67 and 38.62 % respectively, when compared to potassium bromate–intoxicated rats (positive control group). The corresponding percentages of increased of GPx were 34.04, 61.70 and 78.72 % respectively, when compared to potassium bromate–intoxicated rats (positive control group). Regarding CAT enzyme level, a significant (P< 0.05) increase was recorded in rats orally given wheat germ oil in doses of 150, 300 and 450 mg/kg.b.wt., by 17.29, 34.59 and 37.59 % respectively, as compared to potassium bromate–intoxicated rats (positive control group).

Table (4) Effect of oral administration of wheat germ oil on the serum levels of antioxidant enzymes (superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT)) in hepatorenaltoxic rats..

Parameters Groups	SOD (U/ML)	GPx (U/ML)	CAT (U/ML)
Con (-)	64.28 ± 5.83^a	$.85 \pm .06^a$	$.184 \pm .07^a$
Con (+)	46.31 ± 3.98^c	$.47 \pm .02^d$	$.133 \pm .08^c$
WGO 150 gm/kg	55.36 ± 4.34^b	$.63 \pm 0.04^c$	$.156 \pm 0.04^b$
WGO 300 gm/kg	62.83 ± 5.23^a	$.76 \pm .05^b$	$.179 \pm .06^a$
WGO 450 gm/kg	64.19 ± 4.99^a	$.84 \pm .07^a$	$.183 \pm .05^a$

Data are presented as means ± standard deviation, (n = 8 for each group).
Values with different superscripts within the column are significantly different at P< 0.05.

Histopathological Examination

The histopathological examination of liver sections of rats in the negative (normal) control group showed normal histological structure of hepatic lobule as illustrated in Figure (1). Examination of liver sections of rats injected by a single intraperitoneal dose of potassium bromate at dose of 125 mg/kg body weight (positive control group) revealed bile ductuoles, fibroplasia in portal triad and inflammatory cells in the lumen of bile duct Figure (2) and focal hepatic hemorrhage Figure (3). The examination of liver sections of potassium bromate–intoxicated rats orally given WGO in a dose of 150 mg/kg.b.wt., showed oval cells proliferation Figure (4). Oral

administration of WGO in a dose of 300 mg/kg.b.wt., to potassium bromate–intoxicated rats showed slight congestion of hepatic sinusoids was seen in Figure (5).

The liver section of potassium bromate–intoxicated rats orally given WGO in a dose of 450 mg/kg.b.wt., showed no histopathological changes Figure (6).
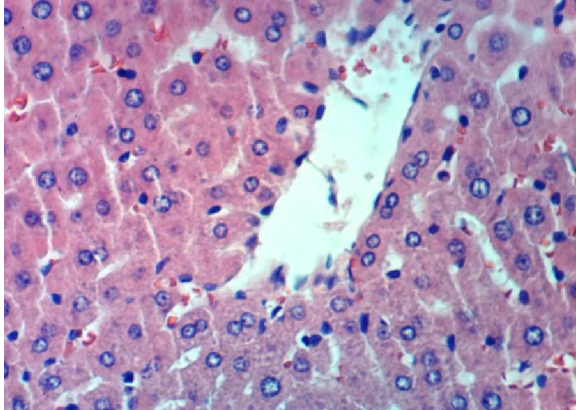

Figure (1) Liver of rat from group G1 showing the normal histological structure of hepatic lobule (H & E X 400).
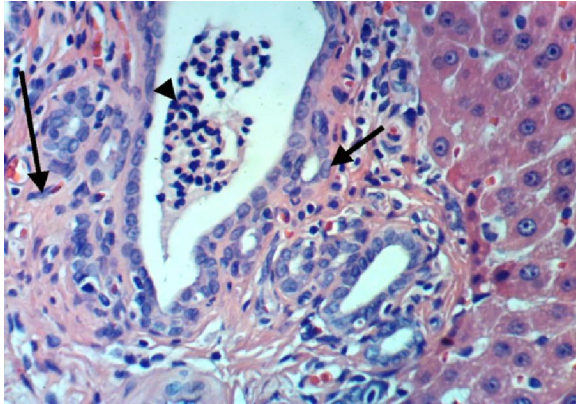

Figure (2) Liver of rat from group G2 showing newly formed bile ductuoles, fibroplasia in portal triad and inflammatory cells in the lumen of bile duct (H & E X 400).
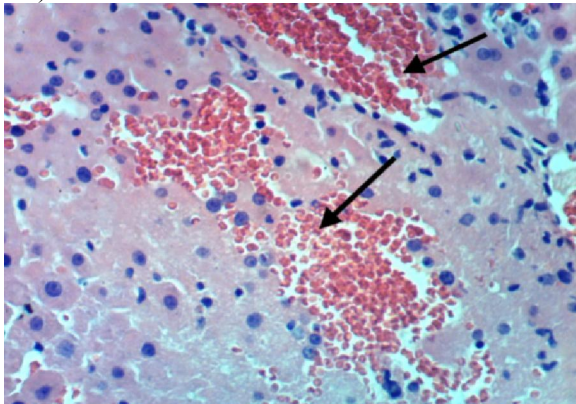

Figure (3) Liver of rat from group G2 showing focal hepatic hemorrhage (H & E X 400).
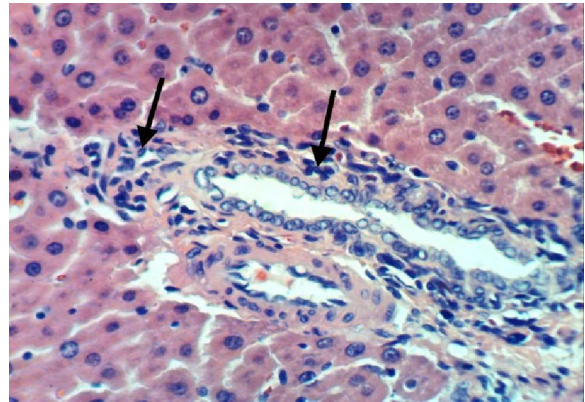

Figure (4) Liver of rat from group G3 showing oval cells proliferation (H & E X 400).
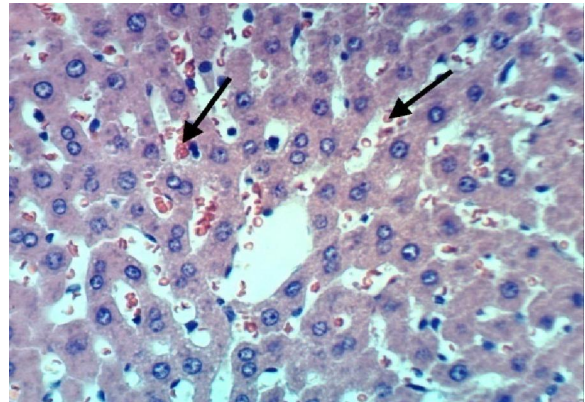

Figure (5) Liver of rat from group G4 showing slight congestion of hepatic sinusoids (H & E X 400).
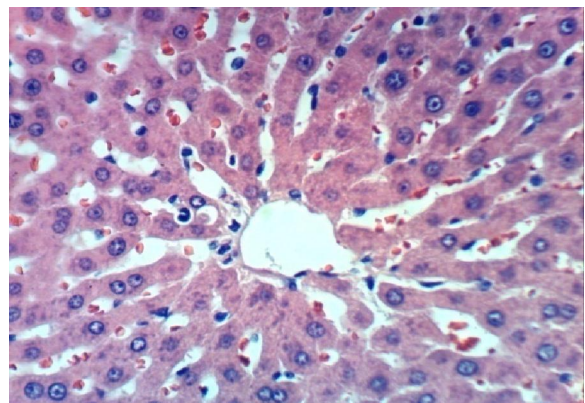

Figure (6) Liver of rat from group G5 showing no histopathological changes (H & E X 400).

4. Discussion

Potassium bromate induces oxidative stress in human erythrocytes through the generation of reactive oxygen species and alters the cellular antioxidant defense system **(Ahmad *et al*., 2014; Waffa and Farida, 2012).** Potassium bromate ($KBrO_3$) has many dangerous effects. It exerts nephrotoxic and ototoxic

effects in experimental animals as well as in man. Potassium bromate is a carcinogen inducing renal cell tumors, mesotheliomas and thyroid follicular cell tumors in rats. It is highly probable that active oxygen radicals are involved in these effects leading to DNA damage. We have also noted its possible toxic effects on platelets **(Rashwan, 2012).**

Wheat germ oil is an excellent source of polyunsaturated fatty acids and vitamin E. It is one of the richest natural sources of α- tocopherol, the type of tocopherol with the greatest vitamin E activity. Wheat germ oil reduced plasma and liver cholesterol in animals, improved physical endurance, and delayed aging **(Vaher *et al*., 2010).** Also, wheat germ oil can reduce oxidative stress, improve lipid metabolism **(Singh *et al*., 2006).** Moreover, it had significantly higher protective levels of vitamin E in the blood and liver, conferring greater antioxidant protection **(Leenhardt *et al*., 2008).**

Regarding biochemical analysis results of the present study revealed that rats intoxicated with potassium bromate resulted in significant increases in serum level of blood urea nitrogen (BUN) and creatinine (Cr) compared to negative control group. Consistent with our results, **Adewole *et al*. (2007)** reported that the significant increases in serum level of blood urea nitrogen (BUN) and creatinine (Cr) might result from considerable leakage due to injuries to glomeruli and tubules induced by KBr O_3. The increases in serum creatinine and urea levels are indication of renal toxicity. This is illustrated by the pathological changes seen in the kidney as reported previously by **Watanabe *et al*. (2004). Abd El-Ghany *et al*. (2011)** who reported that the decline in renal mitochondrial function following sub-chronic and chronic exposure to potassium bromate is based on the oxidative stress mode of action of potassium bromate. Potassium bromate toxicity in male rat kidney included change in energy consumption and utilization in renal cells that involve up-regulation of glycolytic processes, possibly resulting from altered mitochondrial function.

The current results revealed that oral administration of wheat germ oil in doses of 150, 300 and 450 mg/kg.b.wt., to potassium bromate–intoxicated rats during experimental period (4 weeks) significantly ($p<0.05$) decreased serum level of blood urea nitrogen (BUN) and creatinine (Cr) compared to positive control group. The results of the present study are in agreement with previously published authors **Anwar (2010).**

The present result asserted the renoprotective action of wheat germ oil.These findings are in agreement with **Khalifa *et al*. (2011)** who concluded that the decreases in the serum level of blood urea nitrogen (BUN) and creatinine (Cr) could be related to

its ability to scavenge reactive oxygen species, thus preventing further damage to lipids membrane. Our results are also in the same line with previous studies conducted by **Kalender *et al*. (2005)** who have shown that vitamin E (a major costituents of wheat germ oil) exhibits excellent antioxidant property. The improvement of kidney integrity and function may be mediated via the antioxidant activity of wheat germ oil. In many studies, vitamin E neutralizes lipid peroxidation and unsaturated membrane lipids because of its oxygen scavenging effect **(Quiles *et al*., 2002).**

The measurement of the activities of liver 'marker' enzymes in tissues and body fluids can be used in assessing the degree of assault and the toxicity of a chemical compound on organ/tissues **(Yakubu *et al*., 2003).** Measurement of serum enzyme activities can also be used to indicate tissue cellular damage caused by a chemical compound long before histological changes **(Akanji *et al*., 2008).**

The administration of potassium bromate caused intense damage to liver tissue architecture **(Oyewo *et al*., 2013).** High levels of AST indicates liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury, AST catalysis the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver **(Ismet *et al*., 2013).** Serum alkaline phosphatase (ALP) level on other hand is related to the function of hepatic cell. Increase in serum level of ALP is due to increase synthesis, in the presence of increasing biliary pressure **(Ismet *et al*.,2013).** In the present study rats intoxicated with potassium bromate (control positive group) had a significant increase in the serum level of AST, ALT and ALP compared to control negative group. These results were in agreement with **Dimkpa *et al*. (2013)** who reported that there were increases in AST, ALT and ALP enzymes in rats administered with 100 and 200 mg/kg of $KBrO_3$. Remarkable increases in AST and ALT suggests high permeability of hepatocytes due to liver damage which was supported histo-pathologically. Aspartate aminotransferase, ALT and ALP enzymes are consequent with hepatic cell damage and injured cell membrane permeability **(Omer *et al*., 2008).**

Histological examination of tissues could serve as complementary evidence to enzyme studies towards revealing any distortion/damage to the normal structure of the tissue cells. Our results indicated that there was an incidence of congestion of cells displayed by the liver following administration of $KBrO_3$, this may be due to damage to the hepatocyte by the chemical compound. This is used as an indication of

cirrhosis which usually disrupts the normal flow of blood through the liver **(Soliman *et al*., 2014),** congestion of the central vein and sinusoidal dilatation as well as cell necrosis was recorded by **Omer *et al*. (2008).** Our results demonstrated that potassium bromate might lead to labialization of the cell plasma membrane due to the presence of high oxygen content per molecule of potassium bromate. Such disruption of the ordered lipid bilayer of the plasma membrane has resulting in leakage of the enzymes to the extracellular fluid, the serum has corroborated by the histological studies. These alterations may account for the various adverse effects associated with potassium bromate administration.

Oral co-administration of wheat germ oil in doses of 150, 300 and 450 mg/kg.b.wt.,to potassium bromate–intoxicated rats during experimental period (4 weeks) showed significant decreases of AST, ALT, ALP enzymes compared to control positive group this result could be attributed to the presence of phenolic compounds in WGO that may have free radical scavenging activity **(Soliman *et al*., 2015),** an alleviation of liver damage could result from reducing hepatic lipid peroxidation **(Lin and Yin, 2008).** Additionally, in vitro studies results showed that plant sterols are effective in preventing hyper-proliferation. In addition wheat germ oil contains the highest tocopherol content of all vegetable oil, and also the highest content of α-tocopherol, which represents around 60% of the total content **(Piras *et al*., 2009).** Vitamins E is the most important among many low molecular weight compounds which can act as biological antioxidants **(Abdel-Rahim, 2007).** Our data agreement with the finding of **Abd El- Hameed *et al*. (2013)** who stated that wheat germ oil (WGO) significantly reduce the oxidative stress by altered the hepatic enzyme activities and thus can be considered a potential hepatoprotective agent.

Abdel Fattah *et al*. (2011) found that, rats that received combined treatment with wheat germ oil and panax ginseng supplement showed significantly less severe damage and remarkable improvement in all of the liver measured parameters when compared to irradiated rats and also demonstrated that, wheat germ oil and panax ginseng supplement might be a useful candidate against radiation-induced oxidative stress and metabolic disorders without any toxicity.

The biochemical results of our study were confirmed by histopathological findings, which seen in liver sections. The histological findings of liver of treated rats with wheat germ oil in doses of 300 and 450 mg/kg.b.wt., showed almost completely normal structure with mild fibroblastic proliferation and sporadic cell necrosis. Karyomegaly and binucleated hepatocytes were also seen, thus may be explained by antioxidant activity of wheat germ oil that may be attributed to its constituents of Vitamin E. These histological findings agreed with the study of **Piras *et al*. (2009)** who demonstrated that pretreatment with wheat germ oil significantly improved the structure of hepatic cells.

The major components of the antioxidant system in mammalian cells consists of three enzymes, namely, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). These enzymes constitute a mutually supportive team for defense against ROS **(Venukumar and Latha, 2002).** The decreased activities of the antioxidative enzymes probably occurred as a result of free radical production and the excessive use of these enzymes. These results were in accordance with the result previously reported by **Baravalia *et al*. (2011).** Evidence of lipid peroxidation by increased MDA level is one of the primary means of associated oxidative processes with an overall decrease in cellular function **(Rosenblat *et al*., 2006).** The increase in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals **(Thanga Krishna kumari *et al*., 2012).** In the present study, a significant elevation of malondialdehyde (MDA) (lipid peroxidation product) accompanied by lower activities of the antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) had been observed in potassium bromate–intoxicated rats (control positive group) compared to control negative group. The increased of lipid peroxidation due to oxidative stress induced by potassium bromate was previously reported by **Khan and Sultana (2004), Abd El-Ghany *et al*. (2011) and Ahmed and Mahmood (2012)** who stated that peroxidation of lipids membrane initiate the loss of membrane integrity, membrane bound enzyme activity and consequently causes cell lyses.

On the other hand, **Hafez *et al*. (2012)** reported that oxidative damage in tissues can be limited by the body defense system. These defenses appear to be inducible by nutrients/non-nutrients in the diet. Low levels of tissue antioxidant enzymes are likely to result in high levels of tissue damage that are reflected as lipid peroxides. Conversely, elevated levels of antioxidant enzymes would reduce this oxidative damage to tissues. Several studies suggested that disorders of lipid metabolism, hyperlipidemia and obesity are associated with overproduction of oxygen free radicals **(Rehman *et al*., 2003).** The enhanced accumulationof these free radicals and dysfunction of antioxidant defence system resulted in oxidative stress **(Giao *et al*., 2008).** These radicals can bind covalently to the macromolecules and induce peroxidative degradation of the membrane lipids rich in

polyunsaturated fatty acids, leads to the formation of lipid peroxides followed by multiple pathological changes **(Shyamala *et al*., 2003).**

Results of the current study revealed that oral administration of wheat germ oil showed significant increase in CAT, SOD, GPx and GSH enzyme activites and decreased in MDA. These findings might be due to antioxidant agents such as vitamins (tocopherol - Vit. E), which have been shown to scavenge free radicals **(Karaoz *et al*., 2002).** Wheat germ oil is unique among dietary supplements; it is highly rich in the most biologically active forms of naturally occurring vitamin E and mixed tocopherols **(Khalifa *et al*., 2011).** Vitamin E acts as an inhibitor of oxidative processes in body tissues, it protects the unsaturated fat in the body from oxidation. It has been reported that oral administration of wheat germ oil efficiently saturates the body of rats with vitamin E and inhibits oxidation **(Yousef *et al*., 2006). Singh *et al*. (2006) and Katiyar *et al*. (2011)** reported that wheat germ oil can reduce oxidative stress, improve lipid metabolism. Moreover, it had significantly higher protective levels of vitamin E in the blood and liver, conferring greater anti-oxidant protection **(Leenhardt *et al*., 2008).**

Navarro *et al*. (1998), Liu *et al*. (2008), Abd El-Hameed *et al*. (2013) and Soliman *et al*. (2015) demonestrated that wheat germ oil decrease MDA level and improve antioxidant enzymes activity. Tocopherol is one of the structural components of WGO that considered a strong antioxidant due to its radical scavenging activity preventing lipid peroxidation of the cell membranes. Phenolic compounds found in WGO may also have free radical scavenging activity **(Zhu *et al*., 2011).** Wheat germ oil contribute to the antioxidant effect as a result of their incorporation into the structure of cell membrane as well as by inhibiting certain enzymes, which mediate the generation of free radicals, thus reducing the amount of free radicals generated **(Alessandri *et al*., 2003).**

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