Lowering Effect of Ginger and Selenium on Oxidative Stress in Experimental Rats

Dalia A. Hafez[1*], Abor M. Abd El Rahman[1**] and Safaa M. Faid[1**]

[1]Nutrition and Food Science Department, Faculty of Design and Home Economics, Taif University Kingdom of Saudi Arabia

[*]Home Economics Department, Faculty of Education, Suez Canal University, Ismailia, Egypt [**]
Home Economic Department Faculty of specific Education, Ain Shams University, Cairo, Egypt
daliaij_2000@yahoo.com

Abstract: The effect of combination of ginger and selenium on oxidative stress in rats was studied. Oxidative stress was induced in rats by single intraperiotoneal injection of potassium bromate ($KBrO_3$) at a dose 125 mg/kg b.wt. Seven groups (n=7) of rats were used in this study. One of them was kept negative control and the others were given $KBrO_3$. One was kept positive control and the others were given orally ginger, selenium and their combination for 8 weeks.

Results showed that rats with oxidative stress had significant decreases in weight gain and feed efficiency, blood hemoglobin and packed cell volume; serum GSH, SOD and CAT and liver SOD, GST and GPX. There were also increases in liver AST and ALT, serum uric acid, urea nitrogen, creatinine, MDA and NO concentrations. Administration of ginger powder or extract in combination with selenium increased weight gain, feed efficiency, hemoglobin content and packed cell volume. It reversed the biochemical markers of oxidative stress, lipid peroxidation and liver and kidney functions to normal levels

In conclusion, combination of ginger plant with selenium produces a protective effect in rats with oxidative stress. Therefore, intake of ginger with selenium may be useful for patients who suffer from oxidative stress.

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1. Introduction

Oxidative stress arises from an imbalance between the production of reactive oxygen species and the ability of the system to remove or repair the damage caused and restore the prevailing reducing environment (Halliwell and Gutteridge, 1999). Free radicals such as superoxide and nitric oxide (NO) are also produced as second messengers, particularly by immune cells. Superoxide reacts rapidly with nitric oxide by nitric oxide synthase to produce peroxynitrite, whereas hydrogen peroxide slowly decomposes to the highly reactive hydroxyl radical. Both peroxynitrite and hydroxyl radicals are highly reactive oxidizing agents, capable of damaging proteins, lipids, and DNA. Such damage includes changing protein conformation, altering cellular membrane dynamics by oxidation of unsaturated fatty acids, and alterations in DNA and RNA species (Beckman and Koppenol, 1996). Potassium bromate (KBrO3) is widely used as a food additive in the bread-making process and found in drinking water samples as a by-product of ozone disinfection. KBrO3 induces renal carcinogenesis and act as a tumor promoter in carcinogen-initiated animals. Renal cell tumors have been observed in male and female rats after exposure to this compound due to oxidative stress generated by KBrO3 (Fuji *et al.*,1984 and Kurokawa *et al.*, 1990).

Dietary intake of antioxidants can inhibit or delay the oxidation of susceptible cellular substrate so prevent oxidative stress. Phenolic compounds such as flavonoids, phenolic acids, diterpenes and tannins have received much attention for their high antioxidative activity (Rice-Evans *et al*., 1996).

Ginger (*Zingiber officinale Roscoe*) is one of the most commonly used herbal supplements and its substantial use in folk remedies for different medical conditions has been documented. Traditionally, ginger has been used to treat a wide range of ailments. Phytochemical studies showed that ginger is rich in a large number of biologically active substances, including gingerols and shogaols (White, 2007). Ginger contains antioxidants such as gingerols, shogaols and some related phenolic ketone derivatives. Ginger extract contains monoterpens and sesquiterpene. Ginger extract has antioxidative properties and scavenges superoxide anion and hydroxyl radicals (Cao *et al.*, 1993 and Krishnakanta and Lokesh, 1993).

Selenium is a trace element that that is essential to good health but required only in small amounts. Selenium acts by several mechanisms, including detoxifying liver enzymes, exerting anti-

inflammatory effects, and providing antioxidant defense. Selenium is found in minute amounts in foods, with the richest sources being from meats, fish, whole grains, and dairy products. The selenium content of vegetables is dependent on the soil in which they are grown is a trace mineral (Thomson, 2004 and Yiming *et al.*, 2005).

The objective of the present study was to evaluate the antioxidant activity of ginger and selenium on potassium bromate - induced oxidative stress in rats.

2. Material and Methods

A – Material:

Forty-nine adult male rats of Sprague Dawley Strain weighing 175±10 gm were obtained from Helwan farm, Cairo, Egypt. The basal diet was prepared according to NRC (1995). Fresh ginger plant was obtained from local market in Cairo. Selenium powder and potassium bromate ($KBrO_3$) were purchased from El-Gomhoryia Company, Cairo Egypt.

B- Methods:

1-Preparation of ginger powder and ethanolic extract:

Ginger plant were dried with hot air (40–60^{o} C) and grinded into powder and added to the diet as 5 % of the constituent of fiber. 270 g of fresh ginger were chopped with 300 mL ethanol by using a blender for 15 min. at average speed. The mixture was macerated during 24hrs at 4ºC. The resultant extract was filtered using a 0.45 µm pore size cellulose acetate membrane.

2- Biological design:

After adaptation period (one week), the rats were randomly allocated into seven groups, of seven rats each. The first group was fed on basal diet only and kept as a control (-ve). The other six groups were injected by a single intraperitoneal dose of potassium bromate at dose 125 mg/kg body weight to induce oxidative stress (Khan and Sultana, 2004). One group of rats was left without treatment served as a control (+ve). The other groups were treated by ginger powder, ginger extract, selenium; ginger powder with selenium and ginger extract with selenium.

The food intake was calculated daily and the body weight gain was recorded weekly (Chapman *et al.*, 1950). Feed efficiency ratio (FER): FER = weight gain (g)/ feed intake (g) was then calculated. At the end of experiment (8 weeks), the rats were anesthetized, blood sample were collected into clean centrifuge tubes to obtain serum. Livers were immediately removed and rinsed with saline, blotted on filter paper and stored at -70^{o}C pending for biochemical analyses.

Hemoglobin (Hb) and packed cell volume (PCV) were estimated in heparenized blood according to Drabkin (1949) and Mc Inory (1954) respectively. Activity of serum ALT and AST enzymes and concentrations of creatinine, uric acid and urea were estimated according to Reitman and Frankel (1957), Bonsens and Taussky (1984), Fossati *et al.* (1980) and Kanter (1975.), respectively. Blood glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO) and free radicals were determined according to Afzal *et al.* (2002) , Placer *et al.*(1966), Green *et al.* (1981) and Borg (1976), respectively. Catalase and superoxide dismutase (SOD) were estimated according to Luck (1965) and Winterbourn *et al.* (1975), respectively. Liver superoxide dismutase (SOD), glutathione transferase (GST) and glutathione peroxidase (GPX) were determined according to Beauchamp and Fridovich (1971), Habig *et al.* (1974) and Tapple (1978), respectively.

C-Statistical analysis

The collected data were subjected to statistical analysis using computerized SPSS program (Version 15) according to Godfrey (1986).

3. Results

Table (1) showed that control (+ve) rat group had significant (p< 0.5 and 0.01) decreases in weight gain, food intake and FER when compared to the control (-ve) group. In ginger powder, ginger extract and selenium groups there were significant (p < 0.5 and 0.01) decreases in weight gain and FER, while ginger powder with selenium and ginger extract with selenium groups showed a significant(p< 0.5) decrease in FER when compared to the control (- ve) group. In all treated groups there were significant increase in final weight, weight gain, food intake and FER compared to the control (+ ve) group.

Table (2) revealed that control (+ve) rat group had significant (p < 0.01) decreases in Hb and PCV values compared to control (- ve) group. All treated groups had no significant decreases in Hb and PCV values compared to control (-ve) group, but showed a significant increase in other parameters compared to control (+ ve) group.

Table (3) showed that control (+ve) rat group had significant (p < 0.001) increases in serum AST, ALT, creatinine, uric acid and urea nitrogen values compared to control (- ve) group. Ginger powder, ginger extract and selenium groups showed a significant (p < 0.01) increases in serum ALT, creatinine, uric acid, AST and urea nitrogen (p < 0.05) values compared to control (- ve) group. Ginger powder with selenium group showed a significant (p < 0.05) increases in creatinine and uric acid, while ginger extract with selenium group showed a significant increase in uric acid (p <

0.05)when compared to the control (- ve) group. All treated groups showed significant decreases in AST, uric acid and urea concentrations, but ginger powder or extract with selenium groups had a significant decreases in serum ALT and creatinine compared to control (+ ve) group.

Table (4) showed that control (+ve) rat group had significant increases in blood MDA, NO and free radical ($p < 0.001$) and a significant decrease in GSH ($p < 0.001$). Ginger powder and selenium groups caused a significant ($p < 0.05$) decrease in GSH at compared to control (- ve) group. All treated groups showed a significant ($p < 0.05$) increase in NO level compared to control (- ve) group. There were a significant increase in GSH and significant decreases in MDA, NO and free radicals when compared to the control (+ ve) group.

Table (5) showed that control (+ve) rat group had significant ($p < 0.001$) decreases in activity of serum SOD and catalase, but ginger powder and selenium groups showed a significant ($p < 0.05$) decrease in serum SOD and catalase at compared to control (- ve) group. All treated groups showed significant increases in serum SOD and catalase when compared to the control (+ve) group.

Table (6) showed that control (+ve) rat group had significant ($p < 0.001$) decreases in liver SOD, GST and GPX. Ginger powder and selenium groups had significant ($p < 0.05$) decreases in liver SOD, GST and GPX, while ginger extract group showed a significant ($p < 0.05$) decrease in liver GPX compared to the control (- ve) group. All treated groups showed significant increases in liver SOD, GST and GPX compared to the control (+ve) group.

Table (1): Mean values ± SD of body weight gain, food intake and food efficiency ratio (FER) of the experimental groups

Groups / Variables	Control (-ve)	Control (+ve)	Ginger powder	Ginger extract	Selenium	Ginger powder+ selenium	Ginger extract+ Selenium
Initial weight(g)	175.77± 5.14 ^{a}	179.61± 6.31 ^{a}	181.31± 5.21 ^{a}	183.67± 4.99 ^{a}	182.31± 4.36 ^{a}	180.35± 4.66 ^{a}	183.35± 5.11 ^{a}
Final weight(g)	263.27± 17.76 ^{a}	215.36± 3.71 ^{b*}	242.06± 13.88 ^{a}	247.72± 16.21 ^{a}	244.19± 15.66 ^{a}	252.49± 14.28 ^{a}	263.87± 12.77 ^{a}
Weight gain (g)	87.50± 8.65 ^{a}	35.75± 1.21 ^{c***}	60.75± 6.11 ^{b*}	64.05± 7.18 ^{b*}	61.88± 7.13 ^{b*}	72.14± 8.11	80.52± 12.77
Food intake(g/w)	19.31± 1.36 ^{a}	17.74± 1.21 ^{b*}	19.15± 1.31 ^{a}	19.55± 1.26 ^{a}	18.88± 1.21 ^{a}	18.96± 1.25 ^{a}	19.59± 1.14 ^{a}
FER	0.075± 0.003 ^{a}	0.033± 0.004 ^{d***}	0.052± 0.001 ^{c**}	0.054± 0.002 ^{c**}	0.054± 0.001 ^{c**}	0.063± 0.003 ^{b*}	0.068± 0.004 ^{b*}

Significant with control group * $P<0.05$ ** $P<0.01$ *** $P<0.001$
Mean values in each raw having different superscript (a, b, c, d) are significant

Table (2): The Mean values ± SD of blood Hb and PCV of the experimental rats

Groups / Variables	Control (-ve)	Control (+ve)	Ginger powder	Ginger extract	Selenium	Ginger powder+ selenium	Ginger extract+ Selenium
Hb (g/dl)	13.20± 1.21 ^{a}	8.99± 0.88 ^{c**}	11.14± 1.27 ^{ab}	12.21± 1.11 ^{a}	11.21± 1.30 ^{ab}	12.36± 1.31 ^{a}	12.59± 1.27 ^{a}
PCV %	37.36± 3.14 ^{a}	28.16± 2.96 ^{b**}	33.77± 4.17 ^{a}	35.61± 4.96 ^{a}	34.21± 5.21 ^{a}	35.51± 6.11 ^{a}	35.75± 5.61 ^{a}

Significant with control group * $P<0.05$ ** $P<0.01$ *** $P<0.001$
Mean values in each raw having different superscript (a, b, c, d) are significant

Table (3): The Mean values ± SD of serum ALT, AST, creatinine, uric acid and urea nitrogen of the experimental groups

Groups / Variables	Control (-ve)	Control (+ve)	Ginger powder	Ginger extract	Selenium	Ginger Powder+ Selenium	Ginger extract+ Selenium
AST (µ /ml)	35.36± 3.71 ^{c}	59.11± 5.91 ^{a***}	43.71± 4.98 ^{b*}	45.21± 5.11 ^{b*}	47.31± 3.98 ^{b*}	39.67± 3.61 ^{c}	33.67± 3.51 ^{c}
ALT (µ /ml)	22.16± 3.14 ^{bc}	33.18± 4.11 ^{a**}	31.41± 4.18 ^{a**}	32.36± 3.21 ^{a**}	30.14± 4.26 ^{a**}	25.14± 5.51 ^{b}	26.17± 6.59 ^{b}
Creatinine (Mg/dl)	0.77± 0.02 ^{c}	1.55± 0.33 ^{a**}	0.96± 0.18 ^{ab*}	0.98± 0.17 ^{ab*}	1.21± 0.12 ^{a**}	0.88± 0.06 ^{b*}	0.79± 0.08 ^{c}
Uric acid (Mg/dl)	2.11± 0.54 ^{d}	5.35± 1.21 ^{a***}	3.25± 0.78 ^{bc*}	3.67± 0.77 ^{bc*}	4.78± 0.88 ^{b**}	3.21± 0.89 ^{bc*}	3.17± 1.01 ^{bc*}
Urea nitrogen (Mg/dl)	32.14± 3.61 ^{c}	63.14± 5.69 ^{a***}	45.67± 4.35 ^{b*}	41.33± 5.31 ^{b*}	49.29± 6.11 ^{b*}	35.67± 4.14 ^{c}	33.21± 3.81 ^{c}

Significant with control group * $P<0.05$ ** $P<0.01$ *** $P<0.001$
Mean values in each raw having different superscript (a, b, c, d) are significant

Table (4): The Mean values ± SD of blood GSH, MDA, NO and free radicals of the experimental groups

Groups Variables	Control (-ve)	Control (+ve)	Ginger powder	Ginger extract	Selenium	Ginger Powder+ Selenium	Ginger extract+ Selenium
GSH (mmol/l)	7.58± o.78 ^{a}	3.11± 0.16 ^{c**}	5.99± 0.28 ^{b*}	6.31± 0.43 ^{ab}	5.36± 0.82 ^{b*}	6.37± 0.77 ^{ab}	6.59± 0.76 ^{ab}
MDA (mmol/l)	3.03± o.44 ^{b}	6.11± 0.67 ^{a***}	4.11± 0.55 ^{b}	4.36± 0.65 ^{b}	4.21± 0.54 ^{b}	3.66± 0.38 ^{b}	3.76 ±0.54 ^{b}
NO (mmol/l)	2.67± 0.78 ^{d}	15.65± 2.81 ^{a***}	7.36± 1.36 ^{b*}	5.26± 1.01 ^{bc*}	6.14± 1.21 ^{b*}	5.11± 1.11 ^{bc*}	4.19± 0.55 ^{c*}
Free radicals	25430.11± 336.78^{c}	75365.43± 547.81^{a***}	34361.31± 272.36^{bc}	27353.20± 301.27^{c}	30411.31± 411.14^{bc}	26732.20± 317.21^{c}	24351.71± 273.71^{c}

Significant with control group * P <0.05 ** P <0.01 *** P <0.001

Mean values in each raw having different superscript (a, b, c, d) are significant

Table (5): The Mean values ± SD of serum SOD and catalase (CAT) of the experimental groups

Groups Variables	Control (-ve)	Control (+ve)	Ginger powder	Ginger extract	Selenium	Ginger Powder+ Selenium	Ginger extract+ Selenium
SOD (mmol/l)	214.25± 25.17^{a}	120.71± 12.14^{d***}	178.16± 13.61^{c*}	196.21± 11.17^{ab}	175.31± 12.61^{c*}	199.36± 13.21^{ab}	204.36± 15.55^{a}
CAT (µ/l)	60.36± 6.37^{a}	19.86± 2.16^{c***} *	41.16± 4.71^{b**}	53.21± 6.21^{a}	49.21± 4.59^{b**}	55.61± 6.03^{a}	54.25± 5.21^{a}

Significant with control group * P <0.05 ** P <0.01 *** P <0.001

Mean values in each raw having different superscript (a, b, c, d) are significant

Table (6): The Mean values ± SD of some liver SOD, GST and GPX of the experimental groups

Groups Variables	Control (-ve)	Control (+ve)	Ginger powder	Ginger extract	Selenium	Ginger Powder+ Selenium	Ginger extract+ Selenium
SOD (µ /mg)	105.87± 9.14 ^{a}	41.26± 3.22 ^{c**} *	77.11± 8.08 ^{b*}	91.14± 9.29 ^{a}	76.31± 7.85 ^{b*}	99.88± 9.43 ^{a}	110.14± 10.21 ^{a}
GST (µ /mg)	2.41± 0.24 ^{a}	0.65± 0.08 ^{c**} *	1.36± 0.22 ^{b*}	1.78± 0.33 ^{ab}	1.03± 0.25 ^{b*}	1.96± 0.32 ^{a}	2.49± 0.43 ^{a}
GPX (µ /mg)	99.67± 9.26	35.36± 4.21 ^{c**} *	66.38± 7.28 ^{b*}	70.11± 8.14 ^{b*}	69.36± 7.19 ^{b*}	82.13± 9.17	95.45± 9.18 ^{a}

Significant with control group * P <0.05 ** P <0.01 *** P <0.001

Mean values in each raw having different superscript (a, b, c, d) are significant

4. Discussion

Free radicals are natural by -products of oxygen metabolism that may contribute to the development of chronic diseases such as cancer and heart diseases. Lipid peroxidation is a complex process that damages the cell structure and function. Peroxidation of membrane lipids initiates the loss of membrane integrity; membrane bound enzyme activity and cell lyses (Pryor and Squadrito, 1995). Oxidative damage in tissues can be limited by the defense system of the host. 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, protein carbonyls, etc. Conversely, elevated levels of antioxidant enzymes would reduce this oxidative damage to tissues. The presence of selenium helps induce and maintain the glutathione antioxidant system. Selenium is incorporated into proteins to make selenoproteins, which are important antioxidant enzymes. The antioxidant properties of selenoproteins help prevent cellular damage from free radicals. Other selenoproteins help regulate thyroid function and play a role in the immune system (Combs and Gray, 1998 and McKenzie *et al.*, 1998). Selenium is a component of the enzymes glutathione peroxidase. It is also found in three deiodinase enzymes, which convert one thyroid hormone to another. Selenium is a component of the unusual amino acids selenocysteine and selenomethionine. In humans, selenium is a trace element nutrient that functions as cofactor for reduction of antioxidant enzymes, such as glutathione peroxidase and certain forms of thioredoxin reductase found in animals and some plants (Gladyshev and Hatfield, 1999 and Burk, 2002). Antioxidant effects of selenium can also be accounted for by its role in the selenium- dependent thioredoxin reductases. These enzymes reduce intermolecular disulfide bonds and regenerate ascorbic acid from dehydroascorbic

acid. Selenium also plays a role in the functioning of the thyroid gland and in every cell that uses thyroid hormone, by participating as a cofactor for the three known thyroid hormone deiodinases, which activate and then deactivate various thyroid hormones and their metabolites (Petit *et al*.,2003, Ko¨hrle *et al*.,2005 and Dennert *et al.*,2011).

It is well known that spices possess antioxidant activity and prevent oxidation of lipids in foodstuffs. Kikusaki and Nakatani (1993) reported that chemical constituents like gingerols and shogaols present in ginger exhibited high antioxidative activity. Sekiwa *et al.* (2000) reported that a novel glucoside related to gingerdiol from ginger had antioxidative activity using the linolenic acid model system. Consumption of ginger stimulated liver tissue of rats to increase defenses enzymes such as superoxide dismutase, catalase and glutathione. In addition, these enzymes modulate the oxidative damage within the tissues which can be quantitated in terms of lipid peroxidation and protein oxidation. The significant reduction of MDA in liver and kidney tissues of ginger - fed rats confirmed that ginger enhanced antioxidant system (Nirmala *et al.*, 2008). Ginger-free phenolic and ginger hydrolyzed phenolic fractions exhibited free radical scavenging, inhibition of lipid peroxidation, DNA protection and reducing power abilities indicating strong antioxidant properties (Khanom *et al*., 2003). The pretreatment for 20 days with ethanolic Z. officinale extract enhances the antioxidant defense (catalase, superoxide dismutase, and tissue glutathione) in isoproterenol-induced oxidative myocardial necrosis in rats and exhibited cardio protection property (Ansari *et al.*, 2006). Moreover, Ginger extract has antioxidative properties and scavenges superoxide anion and hydroxyl radicals (Cao *et al.*, 1993). Gingerol derived from ginger, at high concentrations, inhibits ascorbate–ferrous complex that in turn induces lipid peroxidation (Rice-Evans *et al*., 1996). The effects of oral ingestion of bromobenzene-induced hepatotoxicity on the liver function tests of male rats have been demonstrated. El-Sharaky *et al.* (2009) reported that activities of AST and ALT were significantly elevated in bromobenzene- induced hepatotoxicity treated rats compared to the control group. Serum levels of bilirubin and total proteins measure the excretory and synthetic functions of liver. Serum bilirubin was greatly increased while the total proteins were significantly decreased in bromobenzene treated rats compared to untreated control group. This indicates an injury, impaired functions and damage of liver because of oral ingestion of bromobenzene. In addition, the decline in renal mitochondrial function following subchronic and chronic exposure to potassium bromate based on the oxidative stress mode of action of bromate. Bromate toxicity in male rat kidney included changes in energy consumption and utilization in renal cells that involve up-regulation of glycolytic processes, possibly resulting from altered mitochondrial function (Abd El-Ghany *et al.*, 2011).

In conclusion, the present study demonstrates that ginger powder and extract exert significant antioxidant effects and these effects were amplified with coadministration of selenium. Therefore, intake of ginger plant in combination with selenium may be beneficial for amelioration of oxidative stress which is a predisposing risk factor of many diseases.

Corresponding author
Dalia A. Hafez
Home Economics Department, Faculty of Education,
Suez Canal University, Ismailia, Egypt
daliaij_2000@yahoo.com

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