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# Amelioration of liver and kidney functions disorders induced by sodium nitrate in rats using wheat germ oil

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ABSTRACT

The purpose of this study was to evaluate the effect of sodium nitrate administration on some biochemical parameters and to explore the ability of Wheat germ oil (WGO) as a natural source of antioxidants to minimize the deleterious effects of sodium nitrate.

The results showed significant increase in alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and significant decrease in butyryl cholinestersea (BChE) content in hepatic tissue in nitrate group as compared to control and wheat germ oil groups through the experimental period. Furthermore, there was a significant increase in thiobarbituric reactive substances (TBARS) accompanied by significant decrease in reduced glutathione (GSH) content in rat renal tissue after 28 and 42 days of treatment with drinking water containing sodium nitrate. Significant decrease was also observed in serum estradiol (E2) in group treated with nitrate through the experimental period. In addition, microscopically examination of renal tissue showed atrophy of glomerular tuft and congestion of renal blood vessels in nitrate treated group. Administration of WGO to rats with sodium nitrate suggesting role of WGO as a natural protective antioxidant agent in hepatic and renal tissues. WGO also stimulates estrogen secretion and inhibits oxidative damage that may be attributed to the presence of biologically active components (unsaturated fatty acids, unsaponifible matters and sterols matters) as antioxidant and cyto-protective activities.

It can be concluded that WGO offers a great advantage for therapeutic purpose to minimized sodium nitrate free radical induced cell damage.

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## 1. Introduction

In recent years, considerable attention has been paid to the problem of nitrate due to the intensive use of nitrates as agricultural fertilizers which reach to humans and animals by different routs (Manassaram, Backer, & Moll, 2006; Mande et al., 2012). Nitrate is a naturally occurring form of nitrogen and is an integral part of the nitrogen cycle in the environment. It is formed from fertilizers, decaying plants, manure and other organic residues. Nitrate is found in air, soil, water, and vegetables food and is produced naturally within the human body (Ogur et al., 2005). In fact certain foodstuffs such as maize, guinea corn, carrots, potatoes, sunflower, pumpkins and cabbage are known to accumulate large quantity of nitrates even at the normal fertilizer application rate of 150 kg/ha (Awodi, Ayo, Nwude, & Dzenda, 2005; Manassaram et al., 2006). It is also used as a food additive, mainly as a preservative and antimicrobial agent (Speijers & van den Brandt, 2003). Due to the increased use of synthetic nitrogen fertilizers and livestock manure in intensive agriculture, vegetables and drinking water may contain higher concentrations of nitrate than in the past (Manassaram et al.,2006).

The major source of nitrate in the human body is through intake of food and water (IPCS, 1999). Vegetables may account for more than 70% of the nitrates in a typical human diet (ATSDR, 2001). Drinking water may contain variable amounts of nitrates which accounts for up to 21% of total nitrates intake in a typical human diet (Manassaram et al., 2006). The presence of nitrate in vegetable as in water and generally in other foods is a serious threat to man's health. Nitrate per se is relatively non toxic (Mensinga, Speijers, & Meulenbelt, 2003), but approximately 5% of all ingested nitrate is converted by microflora in the gastrointestinal tract to the more toxic nitrite (Pannala et al., 2003). Nitrite and N- nitroso compounds which form when nitrite binds to other substances before or after ingestion are toxic and can lead to severe pathologies in humans (Speijers & van den Brandt, 2003). The ability of animals to resist the toxic effects of environmental agents is dependent on the detoxication and antioxidant systems. Recently, several nutrients and other chemicals are effective antioxidants, such as vitamins, trace elements, amino acids and their derivatives, fatty acids and plant phenolics (Ayo, Minka, & Mamman, 2006; Son, Mo, Rhee, & Pyo, 2004; Suteu et al., 2007).

WGO is extracted from the germ of the wheat kernel and is particularly high in policosanol contents specially octacosanol (Irmak & Dunford, 2005) which has been shown to increase physical performance (Kim, Park, Han, & Park, 2003) to be helpful in cholesterol management, chronic inflammatory reactions and neurological disorders (Reddy et al., 2000). Therefore, in recent decades WGO has received much attention in treatment of diseases involving oxidative damage (Blommers, Elisabeth, Deklerk, Piter, & Maiyer, 2002). WGO is a valuable source of essential fatty acids including linoleic acid and alpha lionlenic acid which may be beneficial by increasing endurance, lowering cholesterol levels, and assisting muscular dystrophies and other neuromuscular disorders. WGO may be also changed the intensity of lipid peroxidation processes by stimulating the tocopherol redox-system (Leenhardt, Fardet, Lyan, Gueux, & Remesy, 2008).

WGO is a rich source of natural antioxidant toco-pherols and sterols and also is a rich source of B complex vitamins which may have significant implications in chemoprevention (Jensen, Koh-Banerjee, Hu, Franz, & Sampson, 2004; Lui, 2007). In addition, WGO is a source of easily assailable vitamin E which acts as an inhibitor of oxidation processes in body tissues and protects cells against the effects of free radicals which are potentially damaging by products of the body's metabolism. It is well know that free radicals can cause cell damage that may contribute to the development of cancer (Traber, Vitamin, Shil, Olson, & Shike, 1999). Moreover, WGO not only prevents autoxidation of unsaturated fatty acids but also, generates DNA protective properties (Gelmeza, Kineal, & Yener, 2009).

In view of this consideration, the current study has been designed to investigate the effect of nitrate in a short term experiment (for 42 days i.e 6 weeks) on albino rats and the role of WGO as an antioxidant to counteract the toxic effect of nitrates was taken into consideration.

## 2. Material and methods

### 2.1. *Experimental animal*

Forty eight female albino rats weighing 130 ± 10 g obtained from the animal house of Biological Applications Department, Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt were used in experiment. Animals were maintained under normal requirement ventilation, illumination conditions and adequate stable balanced diet and water.

### 2.2. *Wheat germ oil preparation*

Wheat germ oil (WGO) was packaged as a liquid and was purchased from Arab company for Pharm-Medicinial plants, (MEPACO) Egypt. WGO was given to animals by gavages using stomach tube and was prepared as water emulsion (1.0 ml emulsion 900 mg wheat germ oil/Kg body weight/rat).

### 2.3. *Experimental design*

Animals were randomly divided into 4 equal groups ($n = 12$)

1 Group 1: Control group: rats received 1.0 ml tap water through stomach tube (placebo treatment) through the experimental period.
2 Group 2: Wheat germ oil group: rats received WGO orally (900 mg WGO/Kg body weight/rat) for five consequence days per week through the experimental periods (28 and 42 days) (Mohamed & Anwar, 2010).
3 Group 3: Sodium nitrate group: rats were supplied with 500 mg sodium nitrate/L in drinking tap water every day through the experimental period (Mohamed & Anwar, 2010; Zaki et al., 2004). Sodium nitrate (NaNo3) was produced from Sigma Chemicals Company, Egypt.
4 Group 4: Wheat germ oil plus sodium nitrate group: rats were supplied with 500 mg sodium nitrate/L in drinking tap water and treated with 900 mg WGO/Kg body weight/rat for five consequence days per week through the experimental periods.

Six animals from each group were randomly sacrificed 24 h after termination of treatment processing. Blood samples were withdrawn by cardiac puncture after light anesthesia. Sera were then separated and kept frozen for subsequent biochemical analysis. The liver and one kidney were taken, washed with saline solution, weighed then homogenized in phosphate buffer (pH 7.4) to yield 10% homogenate. The other kidney was dissected out and imbedded immediately in 10% formalin for histo pathological examination.

### 2.4. Biochemical analysis

Determination of alanine transaminase (ALT) and aspartate transaminase (AST) activities, alkaline phosphatase (ALP) and butyrylcholinesterase (BChE) were determined in hepatic tissue by the method of Reitman and Frankel (1957), Kind and King (1954) and Knedel and Bottger (1967) respectively. Estimation of thiobarbituric acid reactive substance (TBARS) and reduced glutathione (GSH) content in the kidney homogenates were determined according to the methods of (Beutler, Duran, & Kelly, 1963; Yoshioka, Kawada, Shimada, & Mori, 1979) respectively. Serum estradiol (E2) was estimated using commercial radioimmunoassay (RIA) kits, purchased from Immunotech A Beckman Coulter Company. The level of estradiol represents the mean levels of eostrous cycle.

### 2.5. Histological preparation

For the histological preparation, animals were dissected to remove kidney through the experimental period. Kidney tissues were cut into small pieces and then fixed in 10% neutral buffered formalin. The tissue was routinely processed and sectioned at 5–6 μm thickness with a microtome and stained with haematoxylin and eosin for histo-pathological studies.

### 2.6. Statistical analysis

The data were expressed as mean ± standard error for 6 animals. Results of control and the experimental groups were statistically analyzed using one way analysis of variance (ANOVA) according to Snedecor and Cochran (1982).

Differences between means of treatments were tested according to Duncan (1955).

## 3. Results

### 3.1. Effect of administration of WGO with sodium nitrate on liver function

The data in Table 1 showed that daily administration of WGO with sodium nitrate significantly decreased the elevation of ALT, AST and ALP and increased BChE as compared to the corresponding values of sodium nitrate treatment.

### 3.2. Effect of administration of WGO with sodium nitrate on TBARS, GSH and serum estradiol

It was noticed from Table 2 that administration of sodium nitrate in drinking water resulted in significant increase in TBARS and significant decrease in GSH content in renal tissue as compared to the control and wheat germ oil groups. The data showed also that the daily administration of wheat germ oil with sodium nitrate significantly decrease the elevation of renal TBARS and increased GSH content. Table 2 revealed also that nitrate treatment in drinking water caused a significant decrease in serum estradiol (E2) level as compared to the control group at both time intervals.

### 3.3. Effect of administration of WGO with sodium nitrate on kidney histo-pathological examination

Microscopically, kidney of rat from control group revealed normal histological structure (Plate 1: Fig. 1). Similarly, kidney of rat from WGO group for 28 and 42 days of administration revealed no histo-pathological changes (Plate 1: Fig. 2 and 5).

On the other hand, kidney of rat from group administrated sodium nitrate for 28 days showed congestion of renal blood vessels and some sections showed congestion of inter-tubular renal blood vessels (Plate 1: Fig. 3). After 42 days of treatment, renal tissue of rat showed focal renal hemorrhage and some sections showed vacillations of renal tubular epithelium and atrophy of some glomerular tuft (Plate 1:Fig. 6). Some sections from group 4 of rats administrated with WGO with sodium nitrate for 28 days showed no histo-pathological changes (Plate 1: Fig. 4). After 42 days of treatment congestion of renal blood vessel was the only histo-pathological finding observed

**Table 1 – Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and butyryl cholinestersea (BChE) levels in hepatic homogenate rats of four experimental groups.**

| Parameters | Experimental periods (days) | Control | WGO | Nitrate | Nitrate + WGO |
|---|---|---|---|---|---|
| ALT U/g wet tissue | 28 | $10.27 \pm 1.51^{c}$ | $11.40 \pm 1.06^{c}$ | $19.90 \pm 0.72^{a}$ | $15.65 \pm 1.0^{b}$ |
| | 42 | $12.00 \pm 0.99^{c}$ | $12.73 \pm 0.99^{bc}$ | $18.80 \pm 1.14^{a}$ | $15.13 \pm 0.58^{b}$ |
| AST U/g wet tissue | 28 | $9.16 \pm 1.18^{b}$ | $10.26 \pm 0.92^{b}$ | $16.92 \pm 1.13^{a}$ | $11.13 \pm 0.64^{b}$ |
| | 42 | $12.46 \pm 0.66^{b}$ | $11.82 \pm 0.86^{b}$ | $21.33 \pm 1.78^{a}$ | $12.27 \pm 0.58^{b}$ |
| ALP IU/g wet tissue | 28 | $8.89 \pm 3.13^{b}$ | $10.55 \pm 2.88^{b}$ | $29.61 \pm 2.81^{a}$ | $14.44 \pm 1.33^{b}$ |
| | 42 | $7.82 \pm 1.48^{b}$ | $7.97 \pm 1.61^{b}$ | $24.86 \pm 5.52^{a}$ | $10.20 \pm 2.22^{b}$ |
| Butyryl cholinesterase | 28 | $36.06 \pm 1.73^{a}$ | $37.55 \pm 0.87^{a}$ | $23.77 \pm 1.14^{c}$ | $30.12 \pm 0.64^{b}$ |
| U/g wet tissue | 42 | $39.18 \pm 0.99^{a}$ | $40.27 \pm 1.32^{a}$ | $22.89 \pm 1.62^{c}$ | $35.19 \pm 1.11^{b}$ |

Data presented mean values ± S.E. in particular groups of 6 rats.
Values in the same row with different superscripts differ significantly ($P < 0.05$).

**Table 2 – Thiobarbituric acid reactive substance (TBARS), reduced glutathione (GSH) contents in kidney homogenate and serum estradiol in adult female rats of four experimental groups.**

| Parameters | Experimental periods (days) | Control | WGO | Nitrate | Nitrate + WGO |
|---|---|---|---|---|---|
| TBARS nmol/g wet tissue | 28 | $13.08 \pm 1.05^{c}$ | $13.97 \pm 1.24^{c}$ | $24.21 \pm 1.04^{a}$ | $17.26 \pm 0.59^{b}$ |
| | 42 | $13.97 \pm 1.08^{c}$ | $10.68 \pm 0.75^{d}$ | $26.99 \pm 0.76^{a}$ | $15.84 \pm 0.88^{b}$ |
| GSH mg/g wet tissue | 28 | $20.37 \pm 1.46^{b}$ | $24.11 \pm 0.85^{a}$ | $11.80 \pm 0.80^{d}$ | $18.01 \pm 0.86^{b}$ |
| | 42 | $22.26 \pm 0.90^{b}$ | $28.86 \pm 1.97^{a}$ | $10.68 \pm 0.74^{d}$ | $15.70 \pm 0.91^{c}$ |
| Serum E2 (ng/ml) | 28 | $19.20 \pm 0.37^{a}$ | $18.02 \pm 0.66^{ab}$ | $17.56 \pm 0.62^{b}$ | $16.81 \pm 0.23^{cb}$ |
| | 42 | $16.89 \pm 0.79^{a}$ | $16.93 \pm 0.36^{a}$ | $13.69 \pm 0.39^{b}$ | $17.91 \pm 0.75^{a}$ |

Data presented mean values ± S.E. in particular groups of 6 rats.
Values in the same row with different superscripts differ significantly ($P < 0.05$).

in renal tissue of rat treated with WGO and sodium nitrate (Plate 1: Fig. 7).

## 4. Discussion

### 4.1. *Effect of administration of WGO with sodium nitrate on liver function*

Nitrate salt is partially converted to nitrite by oral bacteria and by stomach acids, helping to reduce gastrointestinal tract infection (Combs, 2000). Nitrate is in high concentration in drinking water and meat products which induced methemoglobinemia in infinite. It has been implicated in the formation of methemoglobin and carcinogenic nitrosamine in human (Chan, 2011; Kapor, 2004). Information available shows that nitrates and nitrites are both oxidation products and ready sources of NO, that NO reacts rapidly with superoxide to form highly reactive peroxynitrite ($ONOO^{-}$) and such products may increase lipid peroxidation (LPO) which can be harmful to different organs including kidney (Hassan et al., 2009; Rocha et al., 2012).

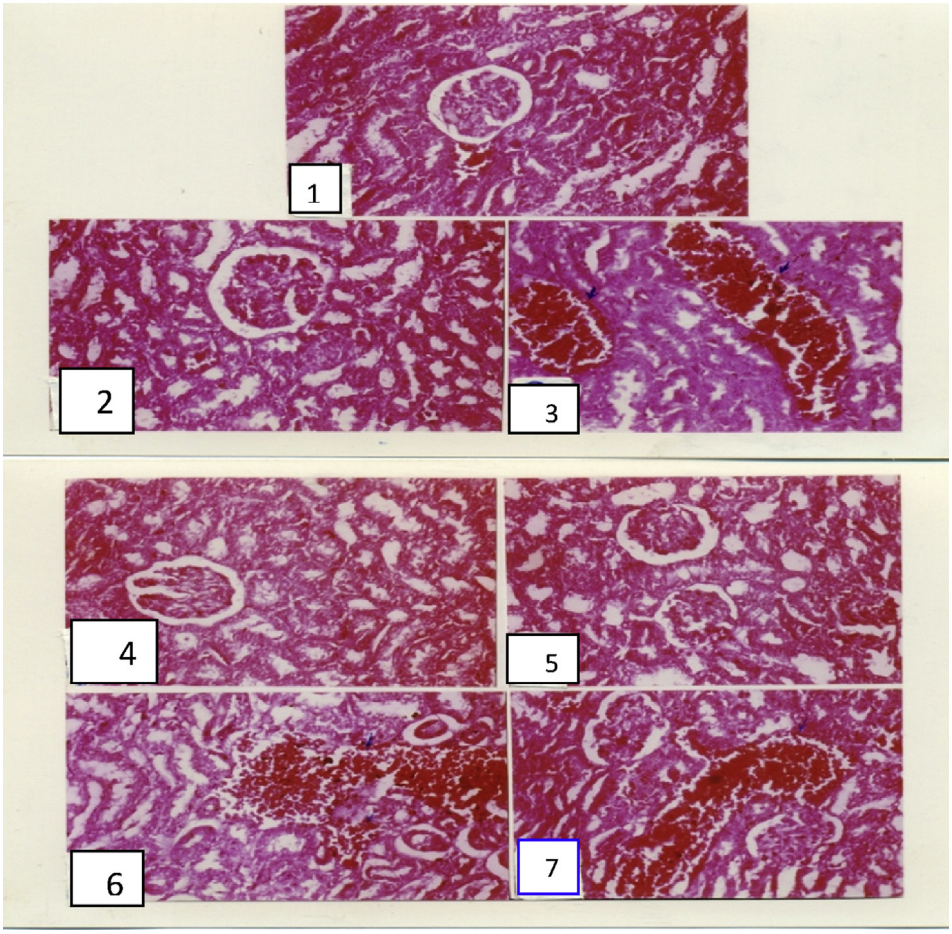


**Plate 1 – Light photomicrographs of kidney sections of rats (H and E X 400). Fig. (1): Control. Fig. (2): WGO group (28 days). Fig. (3): Sodium nitrate group (28 days). Fig. (4): WGO + sodium nitrate group (28 days). Fig. (5): WGO group (42 days). Fig. (6): sodium nitrate group (42 days). Fig. (7): WGO + sodium nitrate group (42 days).**

Antioxidant supplementation may become more important when intake from the diet is insufficient or where the production of oxidative free radicals is greater. Butyryl cholinesterase (BChE) is one class of cholinesterase enzyme which is synthesized by the liver and is abundant in the serum (Massoulié, Pezzementi, Bon, Krejci, & Vallette, 1993). The decrease in BChE is used as an indicator of liver disorders (Ogunkeye & Roluga, 2006). This hypothesis is supported by the increase in AST, ALT and ALP activities in hepatic tissue in treated group with nitrate.

According to the result of this study, WGO has protective properties that can prevent undesired consequences of repeated nitrate administration on ALT, AST, ALP and BChE in hepatic tissue. This finding is in agreement with to the finding of (Mohamed & Anwar, 2010) who recorded that the intake of WGO ameliorating the effect of nitrate. Co-administration of WGO with nitrate compensated the imbalance of the AST, ALT, ALP and BChE in hepatic tissue caused by sodium nitrate to the control level. This may be attributed to that WGO is a valuable source high amount of unsaturated fatty acid and high amount of unsapounfible matter special sterols and essential fatty acids including lenoleic acid and alpha linolic acid which stimulate fatty acid oxidation (Vecera et al., 2003) as well as octacosanol and natural vitamin E (Gu et al., 2002).

### 4.2. *Effect of administration of WGO with sodium nitrate on TBARS, GSH and serum estradiol*

The present results revealed significant increase in TBARS and significant decrease in GSH in renal tissue in treated group with nitrate. This results became in accordance with Farombi and Onyema (2006) and Mohamed and Anwar (2010). One of the establish mechanisms of toxicity of nitrate is their ability to induce oxidative stress through the generation of free radicals (Manassaram, Backer, & Moll, 2007), therefore, the significant increase in TBARS may be attributed to the damaging effects of free radicals which were initiated by the peroxidation of poly unsaturated fatty acids of blood lipoprotein, phospholipids and cholesterol ester moiety of cell membrane. This oxidation damage of different organs was reflected by the accumulation of abnormal products (Microalwski, 1994). Nitric oxide (NO) an unstable molecule is synthesized from L-arginine by the enzyme nitric oxide synthase and reacts with oxygen species and biological molecules to form a varity of end products including nitrite, nitrate radicals and S-nitrosothiols (Mehta et al., 2009).

In the present study treatment with WGO alone revealed non significant changes in the investigated parameters indicating its safe in use. WGO is a source of easily assimilable vitamin E which represented by 89.3% and acts as inhibitor of oxidation processes in body tissues (Abd El-Aziem, Abdou, & Nasr, 2005). Vitamin E has antioxidant and free radical scavenging activities, which suggests that this vitamin may modulate oxidative DNA damage in mammalian cells (Jacobs, Connell, Rodriguez, & Seymour, 2001). Vitamin E neutralizes the increased ROS production induced by toxic substances (Nielsen, Skionsberg, & Lyberg, 2008). Vitamin E is the primary liposoluble antioxidant which may have an important role in scavenging free oxygen radicals and in stabilizing the cell membranes thus maintaining its permeability (Navarro, Arroyo, Martin, Bello, & Villalba, 1999).

Co-administration with WGO cause significant decrease in TBARS and significant increase in GSH content in renal tissue. The obtained results became in accordance with Alessandri et al. (2006), Hargrove, Greenspan, and Hartle (2004) and Singh, Li, and Porter (2006) who reported that WGO decreases oxidative stress probably due to vitamin E in WGO which is a potent peroxyl radical scavenger that prevents the propagation of free radical damage in biological cell membranes (Leppala et al., 2000). The intake of WGO resulted in a rapid increase in vitamin E content in the brain, liver, heart, lungs, kidneys and spleen (Paranich, Cherevko, Frolova, & Paranich, 2000). The obtained results are in agreement with that of Alessandri et al. (2006) who reported that WGO decrease the oxidative stress, so it protects the renal cellular structure.

Nitrate treatment in drinking water caused a significant decrease in serum estradiol (E2) level as compared to control. However, the estradiol level presented herein recorded the means which is the average of estradiol concentration at the different stages of oestrous cycles. The administration of WGO in combination with nitrate exhibited a significant increase in the level of E2 at both two time intervals. This improvement attributed to the content of WGO of vitamin E (Mohamed & Anwar, 2010; Paranich et al., 2000) reported that WGO consists of refractive index 1.4780, acid value 2.13 mg KOH/g oil, peroxide value 8.0 meq/oil, iodine number 133.5 and saponification number 184, fatty acids composition of WGO consisted of unsaturated fatty acid (80.0%) (palmitic oil, stearic, oleic, linoleic, linolenic) and total saturated fatty acid (19.72%), unsaponifiable matter components of WGO composed of 77.0% hydrocarbons and 23.0% total sterols. It contains the other plant materials such as phytosterols (Lewis et al., 2003; Nakari, 2005) which have estrogenic activity and can alter cholesterol metabolism (Ostlund, Racette, Okeke, & Stenson, 2002). However, cholesterol is a major precursor of the ovarian hormones synthesis. Co-administration of WGO with nitrate eliminated the effect of nitrate on estradiol hormone as well as enzymes and this results are in accordance with Mehranjani, Abnosi, Naderi, and Mahmodi (2007).

### 4.3. *Effect of administration of WGO with sodium nitrate on kidney histo-pathological examination*

In the present study, it was found that sodium nitrate causes atrophy of glomerular tuft and congestion of renal blood vessels which may be due to nitric oxide formations, which causes vascular smooth muscle relaxation. This leads to dilatations of their lumens and increases their blood flow.

Finally, the present study reveals toxic effects of sodium nitrate on the liver and renal functions. Therefore, more researches must be done on other organs of the body to highlight its effects on these organs.

## 5. Conclusion

It could be concluded that supplementation of WGO can protect against free radical damage induced by sodium nitrate and improve liver and kidney functions.

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## REFERENCES

Abd El-Aziem, S. H., Abdou, H., & Nasr, E. S. (2005). The protective effect of wheat germ oil against genotoxicity and pathological changes induced by mutagenic drug in mice. *Journal of Genetic Engineering and Biotechnology, 1*(2), 409–422.

Alessandri, C., Pignatelli, P., Loffredo, L., Lenti, L., Del Ben, M., Carnevale, R., et al. (2006). Alpha-linolenic acid rich wheat germ oil decreases oxidative stress and CD40 ligand in patients with mild hypercholesterolemia. *Arteriosclerosis Thrombosis and Vascular Biology, 26*(11), 2577.

ATSDR(Agency for Toxic Substances and Disease Registry). (2001). Nitrate/nitrite toxicity. In P. S. Wigington (Ed.), *Case studies in environmental medicine*. USA: Department of Health and Human Services, DTEM, 22.

Awodi, S., Ayo, J. O., Nwude, C. I., & Dzenda, T. (2005). Effects of sodium nitrite and ascorbic acid on the erythrocyte osmotic fragility in red Sokoto goats. In *Proceedings of 10th Annual Conference of the Animal Science Association of Nigeria (ASAN), University of Ado-Ekiti, Nigeria, 65–68*.

Ayo, J. O., Minka, N. S., & Mamman, M. (2006). Excitability scores of goats administered ascorbic acid and transported during hot- dry conditions. *Journal of Veterinary Science, 7*(2), 127–131.

Beutler, E., Duran, O., & Kelly, B. (1963). Improved method of blood glutathione. *Journal of Laboratory and Clinical Medicine, 61*(51), 852.

Blommers, M. D., Elisabeth, S. M., Deklerk, M. D., Piter, D., & Maiyer, M. D. (2002). Evening primrose oil and fish oil for severe chronic mastalgia: a randomized, double blind controlled trial. *American Journal of Obstetrics and Gynecology, 187*, 1389.

Chan, T. Y. K. (2011). Vgetable-borne nitrate and nitrite and the risk of methaemoglobinaemia. *Toxicology Letters, 200*(1–2), 107–108.

Combs, G. F. (2000). Food system based approaches to improving micronutrient nutrition, the case for selenium. *Biofactors, 12*, 39–43.

Duncan, D. B. (1955). Multiple range and multiple F-test. *Biometrics, 11*(1).

Farombi, E. O., & Onyema, O. O. (2006). Monosodium glutamate induced oxidative damage and genotoxicity in the rar, modulatory role of vitamin C, vitamin E and quercetin. *Human and Experimental Toxicology, 25*(5), 251.

Gelmeza, N., Kineal, N. S., & Yener, M. E. (2009). Optimization of supercritical carbon dioxide extraction of antioxidants from roasted wheat germ based on yield, total phenolic and tocopherol contents and antioxidant activities of the extracts. *The Journal of Supercritical Fluids, 48*, 217–224.

Gu, Y., Hasegawa, T., & Oshima, M. (2002). Radioprotection effects of TMG to radical scavenger effect of the mice in radiation. In , *Proce. 1stAsian and Oceanic Cong.Rad. Protec. (AOCRP-) Seoul, Korea, 20–24 Oct, P.13*.

Hargrove, J. L., Greenspan, P., & Hartle, D. K. (2004). Nutritional significance and metabolism of very long chain fatty alcohols and acids from dietary waxes. *Experimental Biology and Medicine, 229*(3), 215.

Hassan, H. A., El-Agm, S. M., Gaur, R. L., Fernando, A., Raj, M. H. G., & Ouhtit, A. (2009). In vivo evidence of hepato- and reno- protective effect of garlic oil against sodium nitrite-induced oxidative stress. *International Journal of Biological Sciences, 5*(3), 249–255.

IPCS (International Programme on Chemical Safety). (1999). Nitrates and nitrites. In M. Ruse (Ed.), *Poisons, information Monograph (Group Monograph) Go 16, chemical* (Vol. 35).

Irmak, S., & Dunford, N. T. (2005). Policosanol contents and compositions of wheat varities. *Journal of Agricultural and Food Chemistry, 53*(14), 5583.

Jacobs, E., Connell, C., Rodriguez, C., & Seymour, J. (2001). Vitamin C and E supplementation and colon cancer mortality in a large American society cohort. *Cancer Epidemiology, 10*, 17–23.

Jensen, M. K., Koh-Banerjee, P., Hu, F. B., Franz, M., & Sampson, L. (2004). Intakes of whole grains, bran and germ and the risk of coronary heart disease in men. *The American Journal of Clinical Nutrition, 80*, 1492–1499.

Kapor, A. (2004). Nitrite removal from drinking water,review. *Journal of Environmental Engineering Assoiation, 124*, 378–384.

Kim, H., Park, S., Han, D. S., & Park, T. (2003). Octaosanol supplementation increases running endurance time and improves biochemical parameters after exhaustion in trained rats. *Journal of Medicinal Food, 6*(4), 345.

Kind, D. P., & King, E. G. (1954). In practical clinical biochemistry. In H. A. Varly, H. Gownlock, & M. Bell (Eds.), *William Heinmann medical books* (5th ed.). (p. 892). London: LTD.

Knedel, M., & Bottger, R. (1967). A kinetic method for determination of the activity of pseudocholinesterase. *Wiener Klinische Wochenschrift, 45*, 325–327.

Leenhardt, F., Fardet, A., Lyan, B., Gueux, E., & Remesy, C. (2008). Wheat germ supplementation of low vitamin E diet in rats affords effective antioxidant protection in tissues. *Journal of the American College of Nutrition, 27*(2), 228.

Leppala, J. M., Virtamo, J., Vogelholm, R., Huttunen, J. K., Albanes, D., Taylor, P. R., et al. (2000). Controlled trail of alpha-tocopherol and beta- carotene supplements on stroke incidence and mortality in male smokers. *Arteriosclerosis, Thrombosis, and Vascular Biology, 20*, 230.

Lewis, R. W., Brooks, N., Milburn, G. M., Soames, A., Stone, S., Hall, M., et al. (2003). The effects of the phytoestrogen genistein on the postnatal development of the rat. *The Journal of Toxicological Sciences, 71*, 74–83.

Lui, R. H. (2007). Whole grain phytochemicals and health. *Journal of Cereal Science, 46*, 207–219.

Manassaram, D. M., Backer, L. C., & Moll, D. M. (2006). A review of nitrates in drinking water :Maternal exposure and adverse reproductive and developmental outcomes. *Environmental Health Perspectives, 114*, 320.

Manassaram, D. M., Backer, L. C., & Moll, D. M. (2007). A review of nitrates in drinking water: maternal exposure and adverse reproductive and developmental outcomes. *Cien Saude Colet, 12*, 153–163.

Mande, S. A., Liu, M., Djaneye-Boundjou, G., Liu, F., Moctar Limam Bawa, M. L., & Chen, H. (2012). Nitrate in drinking water: a major polluting component of groundwater in gulf region aquifers, south of Togo. *International Journal of the Physical Sciences, 7*(1), 144–152.

Massoulié, J., Pezzementi, L., Bon, S., Krejci, E., & Vallette, F. M. (1993). Molecular and cellular biology of cholinesterases. *Progress in Neurobiology, 41*, 31–91.

Mehranjani, M. S., Abnosi, M. H., Naderi, A., & Mahmodi, M. (2007). Preventing effects of wheat germ oil on sex hormones, liver enzymes, lipids and proteins in rat serum following treatment with p-nonylphenol. *Journal of Biological Sciences, 7*(8), 1406–1411.

Mehta, A., Verma, R. S., & Srivastava, N. (2009). Chloryrifos induced alterations in the levels of hydrogen peroxide, nitrate

and nitrite in rat brain and liver. *Pesticide Biochemistry and Physiology, 94*, 55–59.
Mensinga, T. T., Speijers, G. J. A., & Meulenbelt, J. (2003). Health implications of exposure to environmental nitrogenous compounds. *Toxicological Reviews, 22*, 41.
Microalwski, A. S. (1994). Radiation damage to normal tissues and its treatment. *Acta Oncologia, Stockholm, 33*(2), 139.
Mohamed, N. E., & Anwar, M. M. (2010). Efficacy of wheat germ oil in counteracting of some biochemical hazards induced by sodium nitrate in rats. *Isotope and Radiation Research, 42*(1), 211–227.
Nakari, T. (2005). Estrogenicity of phytosterols evaluated in vitro and in vivo. *Journal of Environmental Sciences, 12*, 87–97.
Navarro, F., Arroyo, A., Martin, S., Bello, R., & Villalba, J. (1999). Protective role of uniquinone in vitamin E and selenium deficient plasma membranes. *Biofactors, 9*, 163.
Nielsen, H. G., Skionsberg, O. H., & Lyberg, T. (2008). Effect of antioxidant supplementation on leucocyte expression of reactive oxygen in species athletes. *Scandinavian Journal of Clinical & Laboratory Investigation, 18*(1).
Ogunkeye, O. O., & Roluga, A. I. (2006). Serum cholinesterase activity helps to distinguish between liver disease and non-liver disease aberration in liver function tests. *Pathophysiology, 13*, 91–93.
Ogur, R., Coskun, O., Korkmaz, A., Oter, S., Yaren, H., & Hasde, M. (2005). High nitrate intake impairs liver functions and morphology in rats; protective effects of a- toicopoherol. *Environmental Toxicology and Pharmacology, 20*(1), 161.
Ostlund, R. E., Racette, S. B., Okeke, A., & Stenson, W. F. (2002). Phytosterols that are naturally present in commercial corn oil significantly reduce cholesterol absorption in humans. *The American Journal of Clinical Nutrition, 75*, 1000–1004.
Pannala, A. S., Mani, A. R., Spencer, J. P. E., Skinner, V., Bruckdorfer, K. R., Moore, K. P., et al. (2003). The effect of dietary nitrate on salivary, plasma and urinary nitrate metabolism in humans. *Free Radical Biology & Medicine, 34*, 576.
Paranich, V. A., Cherevko, O. L., Frolova, N. A., & Paranich, A. V. (2000). The effect of wheat germ oil on the antioxidant system of animals. *Lik Sprava., 2*, 40.
Reddy, B., Hirose, Y., Cohen, L., Simi, B., Cooma, I., & Rao, C. (2000). Preventive potential of wheat bran fractions against experimental colon carcinogenesis: implications for human colon cancer prevention. *Cancer Research, 60*, 4792.
Reitman, S., & Frankel, S. A. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology, 28*, 56–63.
Rocha, B. S., Gago, B., Barbosa, R. M., Lundberg, J. O., Radi, R., & Laranjinha, J. (2012). Intragastric nitration by dietary nitrite: implications for modulation of protein and lipid signaling. *Free Radical Biology & Medicine, 52*(3), 693–698.
Singh, D. K., Li, L., & Porter, T. D. (2006). Policosanol inhibits cholesterol synthesis in hepatoma cells by activation of AMP-Kinase. *Journal of Pharmacology and Experimental Therapeutics, 318*(3), 1020.
Snedecor, G. W., & Cochran, W. G. (1982). *Statistical methods* (8th ed.). USA: Louis State, University Press.
Son, E. W., Mo, S. J., Rhee, D. K., & Pyo, S. (2004). Vitamin C blocks TNF- alpha- induced NF kappa B activation and ICAM-1Expression in human neuroblastoma cells. *Archives of Pharmacology Research, 27*(10), 1073–1079.
Speijers, G. J. A., & van den Brandt, P. A. (2003). Nitrate (and potential endogenous formation of N- nitroso compounds). Online. In *WHO food additives series 50*. Available http://www.inchem.Org.
Suteu, R., Altuntas, L., Buyukvanli, B., Aktrurk, O., Koylu, H., & Delibas, N. (2007). The effects of diazozia on lipid peroxidation and antioxidant enzymes in rats erythrocytes: role of vitamins E. and C. *Toxicology and Industrial Health, 23*(1), 13–17.
Traber, M. G., Vitamin, E., Shil, S. M. E., Olson, J. A., & Shike, M. (1999). *Modern nutrition in health and disease* (10th ed.). (pp. 347–362). Baltimore: Williams & Wilkins.
Vecera, R., Skottova, N., Vana, P., Kazdova, L., Chmela, Z., Svagera, Z., et al. (2003). Antioxidant status, lipoprotein profile and liver lipids in rats fed on high cholesterol diet containing currant oil rich in n-3 and n-6 polyunsaturated fatty acids. *Physiological Research, 52*(2), 177–187.
Yoshioka, T., Kawada, K., Shimada, T., & Mori, M. (1979). Lipid peroxidation in maternal and cord blood and protective mechanism against activated oxygen toxicity in the blood. *American Journal of Obstetrics & Gynecology, 135*(3), 372–376.
Zaki, A., Aitchaoul, A., Talibi, A., Derouiche, A. F., Aboussaouira, T., Zarrouck, K., et al. (2004). Impact of nitrate intake in drinking water on the thyroid gland activity in male rat. *Toxicology Letters, 147*(1), 27–33.