The Antioxidant Effects of Flavonoids and non Flavonoid Part Extracted from Ginger  
(Zingiber Officinale) Roots.

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(Received 22 / 10 / 2008 ; Accepted 15 / 6 / 2009)

ABSTRACT

The extraction of Flavonoids from dried ginger (Zingiber officinale Ros.) roots in addition of non-flavonoids parts (oils and defatted) were studied against H2O2 induced oxidative stress in the serum of male rats for 15 day.

Fifty male rats with age of 3-4 months and weight of 225-300 g were divided into 5 groups : group (1): control group received drinking tap water and ideal diet, group (2): received 0.5% H2O2 in drinking tap water, group (3): received 0.5% H2O2 and oral dose of 15 mg/ kg of BW. flavonoids once daily, group (4): received 0.5% H2O2 and oral dose of 30 mg/ kg BW flavonoids once daily. Group (5): received 0.5% H2O2 and the experimental diet (50 g non- flavonoids part / kg diet).

H2O2 treated group showed elevation in serum cholesterol, malondialdehyde (MDA), peroxynitrite radical levels and reduction in glutathione (GSH), vit C, albumin, calcium, and phosphorus levels compared with the control group (p ≤ 0.05). Treatment with ginger extracts counteracted the oxidative stress induced by H2O2 by reducing the levels of cholesterol, MDA and peroxynitrite and enhancing the levels of GSH, vit C, albumin and calcium compared with the H2O2 treated group (p ≤ 0.05). Treatment with non flavonoids part, by means of experimental diet, showed decreasing in the MDA, peroxynitrite, phosphate levels and increasing in vit C level compared with the H2O2 treated group (p ≤ 0.05). The results of the present study provided the protective effects of the ginger extract by increasing antioxidant defense and suppression free radicals production in the serum.

Keywords: ginger, flavonoids, oxidative stress, antioxidants.
INTRODUCTION

The ginger (Zingiber officinale Rosce, Zingiberaceae) approximately 2-3 cm. in length, is one of the more commonly used herbal supplements (Langer et al., 1998) it is taken by many patients to treat a variety of conditions (Ghayur et al., 2005) its root has been used for perhaps thousands of years in the Far East to treat inflammatory diseases (Shen et al., 2003) and also shown to be effective for pregnancy-induced and postoperative nausea and vomiting (White, 2007). Ginger is rich in volatile oils including zingiberene, zingerol, shogoal, phellandrene, Borneol, Cineole, as well as citral, starch,
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mucilage and resin. Different varieties of ginger contain different essential oils (Langner et al., 1998).

Ginger showed an antioxidant activity (Cao et al., 1993). 6- Gingerol is one of the major antioxidant of fresh ginger and has antioxidant, antiapoptotic, anti- inflammatory and pharmacologic activities in vitro and in vivo (Kim et al., 2007). The structure of more than 50 antioxidants isolated from the rhizomes of ginger were determined. The isolated antioxidants divided into two groups: gingerol related compounds with an alkyl group bearing 10- 14 carbon chain length, and diaryl heptanoids (Masuda et al., 2004). The antioxidant activities of these compounds were evaluated by their free radical scavenging and inhibitory of autoxidation of lipids (Ahmed et al., 2000 a).

Ginger extract tested positive for saponins, flavonoids, amines, alkaloids and terpenoids. The hypotensive, vasodilation and cardiosuppressant effects of its aqueous extract has been reported. Stimulation of muscarinic receptors and blockade of Ca$^{+2}$ channels were also been reported (Ghayur et al., 2005).

Few studies have demonstrated the flavonoids activities of ginger. The present study was designed to evaluate the effect of alcohol extracted flavonoids administrated orally on the oxidative stress in H$_2$O$_2$ - induced rats. The effect of nonflavons part in the diet supplement was also investigated.

**MATERIAL AND METHODS**

**Flavonoids extraction:** The dried powdered roots of the ginger was obtained from local markets. Sixty grams were extracted with diethyl ether using soxhlet apparatus for 3 hours to remove fatty contents (Tyler, 1993) . The defatted plant material was dried at 35 C° in an air oven, and then extracted at room temperature with two aliquots of 500 ml of methanol. The extract containing the flavonoids was concentrated to a final volume of approximately 50 ml using rotary evaporator. This extract (Flavonoids part) was dried completely in an air oven at a temperature of 35 C°(Wagner, 1986).

**Experimental diet:** The nonflavons plant materials (oil and defatted plant material ) were mixed with the standard animal diet in concentration of 5% w/w.

**Animals:** Fifty albino male rats (weight of 225- 300 g, age of 3- 4 months) were divided randomly into five groups, each group was housed in communal cage, water in polyethylene bottles was available as drinking water, room temperature was maintained between 22-25 C°. Animals in each group received once daily for 15 day (experimental period ) the following :

Group (1): drinking water and standard diet (control ).
Group (2): 0.5% H$_2$O$_2$ in drinking water.
Group (3): 0.5% H$_2$O$_2$ and 15 mg/ kg BW. oral dose of flavonoids.
Group (4): 0.5% H$_2$O$_2$ and 30 mg/ kg BW. oral dose of flavonoids.
Group (5): 0.5% H$_2$O$_2$ and the experimental diets contain 5% non flavonoid part.

At the end of the experimental period, animals were sacrificed, (5 ml) of blood were collected from each animal and the serum was separated.

**Chemical tests:** Serum cholesterol was estimated enzymatically using Kit manufactured by Syrbio company, the quinineimine complex formed was measured at 500 nm. Malodialdehyde (MDA) was determined in the serum by the method of (Beuge and Aust, 1978) using Thiobarbituric acid (TBA), the color formed was read at 532 nm.
The glutathione content (GSH) was measured in serum by the reaction with [5,5- dithiobis
(2- Nitrobenzoic acid)] (DTNB) using the method of (Burtis and Ashwood, 1999). The
reduction reaction was read at 412 nm.
Peroxynitrite radical was assayed in serum by the nitration of phenol using the method of
(Vanuffelen et al., 1998). the nitrophenol content formed was read at 412 nm.
Albumin concentration was determined in serum by the Bromocresol green method (Kit
manufactured by Sybio Company).
The concentration of ascorbic acid (vit C) was determined in serum by the method of
(Colowick- Kaplan, 1979) using hydrazine, thiourea, copper reagent. The Bis- 2,4-
dinitrophenyl hydrazone complex was read at 520 nm.
Serum uric acid was enzymatically assayed (Kit manufactured by Biolabo SA). The red
quinoneimine complex formed by uricase was measured at 520 nm.

Serum total calcium was determined using commercial Kit (manufactured by BioMerieux
company). Calcium in the sample reacts with O- cresolphthalain complexone (CPC) at
alkaline pH, the colored complex formed was measured at 570 nm.
Serum inorganic phosphorus was determined using commercial Kit (manufactured by
BioMerieux company) The phosphomolybdate complex formed in the presence of a
reducing agent (ferrous sulfate) was measured at 690 nm.

Statistical analysis: standard statistical methods were used to determine the mean and
standard deviation (S.D). Unpaired t- test was used to compare the means of flavonoid-
treated groups with control on one aspect and with H2O2- treated group on the other aspect
for various parameters (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Cholesterol concentration: The results in figure (1) showed a significant reduction in
cholesterol level in groups treated with 15 and 30 mg/ kg of flavonoids compared with the
H2O2 treatment group (p ≤ 0.05) Fig (1). This result was similar to that obtained by (Masuda
et al., 2004) who mentioned that the gingerol related compounds substituted with alkyl
group, might contribute to both radical scavenging effect and inhibitory effect of lipid
autooxidation. (Fuhrman et al., 2000) explained the role of ginger extract in reducing the
development of aortic atherosclerotic lesions, along with an impressive reduction in the
levels of plasma LDL- cholesterol in atherosclerotic mice. Hypolipidemic and
antiatherosclerotic effects of ginger extract were also demonstrated in cholesterol- fed
rabbits (Bhandari et al., 1998, Sharma et al., 1996).
The hypocholesterolemic effect of ginger could have possibly resulted, at least in part, from the inhibition of cellular cholesterol biosynthesis. Reduced cellular cholesterol biosynthesis is associated with increased activity of the LDL-receptors which in turn leads to enhanced removal of LDL from plasma, resulting in reduced plasma cholesterol concentration (Ness et al., 1996). These results are in agreement with data showing that plant foods possess cholesterol-suppressive capacity (O’Brien and Reiser, 1979), also act as hypocholesterolemic agent due to their inhibitory effect of cellular cholesterol biosynthesis (Fuhrman et al., 1997b). Reduction of atherosclerotic lesion development along with reduced LDL oxidation was demonstrated in apolipoprotein E-deficient mice supplemented with the two red wine flavonoids, catechin and quercetin (Hayek et al., 1997) suggested that LDL oxidation can lead to modification of lipoproteins and LDL aggregation, aggregated LDL are taken up by macrophages at enhanced rate, leading to cellular cholesterol accumulation and foam cell formation (Aviram 1993b, Heinecke et al., 1991, Suits et al., 1989).

**MDA concentration:** The MDA level increased significantly following H₂O₂ treatment (p≤0.05) compared with the control group (Fig 2). This result was explain the important role of H₂O₂ as a potent oxidizing agent which cause the oxidative stress by increasing the reactive oxygen species (ROS) or decreasing the antioxidant defense systems. Halliwell explained the oxidative stress statement and the role of free radical in the participation of many diseases (Halliwell, 1993). H₂O₂ was used to induce many diseases including oxidative stress, such as atherosclerosis and diabetes melitus (Aziz, 2001, Wohaieb et al., 1994).
Flavonoids- treated groups reversed the increase of MDA level to a considerable extent, (fig 2). It is now well established that Zingiber officinale rose significantly attenuated lipid peroxidation (Ahmed et al., 2000b) and has antioxidant properties by maintaining the activities of Superoxide dismutase (SOD), Catalase and glutathione Peroxidase (Ahmed et al., 2000a). Also, 200 mg/ kg of ethanolic Zingiber officinale extract administered for 20 day, showed to enhance the antioxidant defense SOD, catalase and tissue glutathione against isoproternol- induced oxidative myocardial necrosis in rats (Ansari et al., 2006).

(Shanmuga- sundaram et al., 1994) explained the effect of Amrita Bindu, the ginger naturally occurring salt, in the protection against free radical and ROS induced tissue lipid peroxidation in blood and liver when exposed to a carcinogenic nitrosamine and N- methyl-N- nitro- N- nitrosoguanidine.

**GSH concentration:** Fig (3) showed a significant decrease in GSH level in H2O2- treated group (p\(\leq\) 0.05) compared with the control group. This result is in agreement with that obtained by (Ahmed et al., 2000b) who explained that dietary feeding of ginger (1% w/w) increased GSH level in whole blood of rats treated with 20 ppm of malathion, a chemical pesticide, for 4 weeks. Also, (Ahmed et al., 2000a) mentioned that dietary ginger (1% w/w) is comparatively as effective as ascorbic acid in increasing blood GSH in rats. The results is also in agreement with previous study showing that the pretreatment of ethanolic zingiber extract (200 mg/ kg) for 20 day, enhance endogenous myocardial antioxidants, GSH, Catalase and Superoxide dismutase against isopropanol- treated rats (Ansari et al., 2006).
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Fig. 3: Effect of ginger extract on GSH level.

A study of (Shanmugasundaram et al., 1994) showed that Amrita- Bindu, increased blood and liver GSH and provide protection against free radical and ROS induced by nitrosamine.

**ONOO⁻ concentration:** The results of Peroxynitrite concentration showed a significant decrease in all Ginger- treated groups compared with the control and H₂O₂- treated groups (p ≤ 0.05). This result was similar to that obtained by (Lippoushi et al., 2003) who explained that the 6- gingerol, a flavonoid present in ginger, is a potent inhibitor of nitric oxide synthesis and an effective protector against peroxinitrite- mediated oxidation and nitration reaction, in activated mouse macrophages (Lishen et al., 2005) explained the inhibitory effects of ginger root extract on the production of inflammatory mediators including nitric oxide (NO) and prostaglandin E2 (PGE2) in chondrocytes isolated from sow cartilage explants.

*significant with control, * significant with H₂O₂ group. (p ≤ 0.05)

Fig. 4: Effect of ginger extract on Peroxynitrite level.
**Albumin concentration:** Group treated with 15 mg/kg flavonoid showed a significant increase in serum albumin, Fig (5) compared to the control and H₂O₂ treated groups (p ≤ 0.05) (Patterson et al., 2007) explained the interaction between the albumin and flavonoids. The quercetin which is a major flavonoid found in most natural food, is bound to human serum albumin at physiologically correlated concentration.

![Graph showing albumin concentration](image)

*significant with control, * significant with H₂O₂ group. (p ≤ 0.05)

Fig. 5: Effect of ginger extract on albumin level.

Spectroscopic studies show that 80% of alpha tocopherol radical (α- Toc o) are repaired over several seconds by quercetin bound to human serum albumin. αTOC O generated by the oxidation of Tryptophan and Tyrosine residues of apolipoproteins AI and AII, in HDL and apolipoprotein E in LDL produces the corresponding species Tyro’ and Try’. This species are repair by endogenous alpha Toc-OH (Patterson et al., 2007). In addition, (Banerjee et al., 2008) in recent investigations report a study on the interactions of the flavone, 7-hydroxyflavone (7HF) with the plasma human albumin and acting against the tryptophan oxidation. On the other hand, (Murota et al., 2007) showed that the quercetin is metabolized in the intestinal mucosa and liver, and is present as glucuronide sulfate conjugates. This quercetin is known to possess strong antioxidant activity when its metabolized, more than 80% of quercetin metabolites were localized in the human plasma albumin and lipoprotein generated antioxidant activity against LDL oxidation.

**Vit C concentration:** H₂O₂ treated group showed a significant reduction in vit C level compared with the control group (p ≤ 0.05). The ginger extracts counteracted the H₂O₂ induced oxidative stress, Fig (6), 30 mg/kg of flavonoid treated group showed a significant increase in vit C level (p ≤ 0.05) this is the same result with that obtained by (Shanmugasundaram, 1994) who explained that ginger supplementation prevented depletion of the antioxidant enzymes and vitamins A, C and E.

The non flavonoids part of the ginger roots showed a significant decrease in MDA and peroxynitrite level, and a significant increase in vit C level compared with the H₂O₂-treated group (p ≤ 0.05). Many studies showed the bioactivity of ginger constituents and is commonly useful for treating an upset stomach, preventing symptoms of nausea and motion sickness (Chrubasik et al., 2005). It's activity to reduce the risk of blood clott formation...
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makes it a useful herb for lowering the risk of cardiovascular diseases (Guh et al., 1995). (Ghayur et al., 2005) showed that ginger is rich in essential fatty acids which can react with the toxins stored in the body. From chemicals found in food. In addition, (Ghayur and Gllani, 2006) showed that is probably to use the whole herb rather than other fractional supplements since there may be other compounds found in the whole herb such as terpenoids, alkaloids, gingerols and diarylheptanoids that possess anti-mutagenic, anti-inflammatory activity and exhibit a strong antioxidant activity similar to that of vit E. Ginger also inhibit various cancers due to the presence of antioxidant curcumin, a substance that reported to stimulate the activity of glutathione-s-transferase, an enzyme which assists in the elimination of cancer-causing substances from the body (Hertog et al., 1995).

Uric acid concentration: H₂O₂-treated group showed a significant increase in uric acid (Fig 7) compared with the control group (p ≤ 0.05). 30 mg/kg ginger flavonoids reduced the level of uric acid compared with H₂O₂-treatment group (p ≤ 0.05), this result is in agreement with the result obtained by (Bliddal et al., 2000) showing that the ginger helps the body to get rid of the excess uric acid and prevents gout attacks. (Chrubasik et al., 2005) mentioned that alcohol inhibits kidney function which results in more uric acid in blood, and quercetin, the major flavonoid in many plants, inhibits uric acid production in a similar fashion to commonly drugs for gout.
Fig. 7: Effect of ginger extract on uric acid level.

The elimination of uric acid from the tissues by ginger was demonstrated by (Ozaki et al., 1991) showing that ginger is high in flavonoids which helpful in overcoming uric acid.

Ca and P concentration: The result in Fig (8 and 9) showed a significant reduction in Ca and P levels in H2O2 – treated group compared with the control group (p ≤ 0.05) Treatment with flavonoids (30 mg/kg) counteracted the effect of H2O2 on the Ca concentration (p ≤ 0.05) Although ginger root has been used for perhaps thousands of years to treat inflammatory diseases including osteoarthritis, however, the effect of ginger root has never been evaluated on the Ca and P levels.

Fig. 8: Effect of ginger extract on calcium level.

Many previous study demonstrated the anti-arthritic effect of ginger root. (Chen et al., 2003) explained its anti-arthritic effect in sow cartilage explants. (Lishen et al., 2005) explained the important role of ginger root extract as an anti-arthritic agent by reducing the
production of inflammatory mediators including nitric oxide (NO) and prostaglandin E2 (PGE2) in normal chondrocytes and osteoarthritic chondrocytes. (Altman and Marcussen 2001) studied its effect in 261 people with osteoarthritis knee pain and suggested that ginger root extract may be considered as a potentially useful in combination with ibuprofen, a medication frequently used in reducing symptoms of osteoarthritis. However, our study is in agreement with (Yamaguchi et al., 2007) who explained the action of various flavonoids (quercetin, myricetin, kaempferol, isorhamnetin and curcumin) on bone calcium content and osteoclastogenesis in rats. This study demonstrated that various flavonoids have a potent inhibitory effect on osteoclastogenesis and bone resorption, and had a stimulatory effect on bone formation when cortical bone and trabecular bone tissues were cultured for 7 days in the presence of parathyroid hormone PTH, a bone-resorbing factor. The decreasing in phosphate level after flavonoids treatment may be explained as a result of parathyroid hormone (PTH) secretion that influence the both calcium and phosphate homeostasis in tissues and extracellular fluids (Bringhurst, 1995). PTH in serum, increase total and free Ca and reduce the concentration of phosphate (Potts et al., 1995).

Fig. 9: Effect of ginger extract on phosphorus level.

Recently the antioxidant properties of natural flavonoids and related phenolic compounds extracted from dietary or herb plants have aroused much attention due to its structure that included aromatic rings and electrons transport. Plants rich in these compounds are ideal sources of natural antioxidants (Van Acker et al., 1996). It has been shown in this study that flavonoid components isolated from the root extract of ginger have the free radical scavenging and protective effects on oxidative responses, recent reports have implied that oxygen free radical is important mediator of tissue injuries and lipid peroxidation reaction. Lam et al., 2007) We suggest that the protective effect of the alcoholic extract of Zingiber officinalis may be due to increasing antioxidant defense and suppression of free radical production in the blood.
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