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

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ABSTRACT

The extraction of Flavonoids from dried ginger (*Zingiber officinale Ros.)* roots in addition of non-flavonoids parts (oils and defatted) were studied against H_2O_2 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 tab water and ideal diet, group (2): received 0.5% H_2O_2 in drinking tab water , group (3): received 0.5% H_2O_2 and oral dose of 15 mg/ kg of BW. flavonoids once daily, group (4): received 0.5% H_2O_2 and oral dose of 30 mg/ kg BW flavonoids once daily. Group (5): received 0.5% H_2O_2 and the experimental diet (50 g non- flavonoids part / kg diet).

H_2O_2 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 ($p \leq 0.05$). Treatment with ginger extracts counteracted the oxidative stress induced by H_2O_2 by reducing the levels of cholesterol, MDA and peroxynitrite and enhancing the levels of GSH, vit C, albumin and calcium compared with the H_2O_2 treated group ($p \leq 0.05$). 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 H_2O_2 treated group ($p \leq 0.05$). 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.

التأثيرات المضادة للأكسدة للفلافونويدات والجزء غير الفلافونويدي المستخلصة من جذور الزنجبيل

الملخص

تضمن البحث استخلاص الفلافونويدات من جذور نبات الزنجبيل الجافة ودراسة تأثير هذا الجزء من النبات فضلا عن الجزء غير الفلافونويدي (الزيتي والمنزوع الدهن) على الإجهاد التأكسدي المحدث بواسطة بيروكسيد الهيدروجين في مصل ذكور الجرذان البالغة لمدة 15 يوما.

تم استخدام 50 ذكرا من ذكور الجرذان البالغة بعمر 3-4 أشهر وبأوزان تراوحت بين 225- 300 غم. قسمت عشوائيا إلى 5 مجاميع متساوية: (1) مجموعة سيطرة استهلكت ماء الشرب الاعتيادي والغذاء القياسي، (2) مجموعة عوملت ببيروكسيد الهيدروجين (H_2O_2) 0.5% في ماء الشرب، (3) مجموعة عوملت ببيروكسيد الهيدروجين 0.5% و15 ملغم/ كغم من وزن الجسم من الفلافونويدات جرعة يومية عن طريق الفم، (4) مجموعة عوملت ببيروكسيد الهيدروجين 0.5% و 30 ملغم/ كغم من الفلافونويدات جرعة يومية عن طريق الفم، (5) مجموعة عوملت ببيروكسيد الهيدروجين 0.5% والغذاء المضاف إليه 5% من الجزء غير الفلافونويدي من النبات.

أظهرت المجموعة المعاملة بـ H_2O_2 ارتفاعا في مستويات الكولستيرول، المالونداياألديهايد وجذر بيروكسي نتريت وانخفاضا في مستويات الكلوتاثيون، فيتامين C ، الألبومين، الكالسيوم والفوسفور في مصل الدم مقارنة مع مجموعة السيطرة ($p\leq 0.05$) . كان تأثير المعاملة بالمستخلصات معاكسا لتأثير الإجهاد التأكسدي المحدث ببيروكسيد الهيدروجين من خلال خفض مستويات الكولستيرول، المالونداياألديهايد وجذر بيروكسي نتريت وزيادة مستويات الكلوتاثيون، فيتامين C ، الألبومين والكالسيوم مقارنة مع المجموعة المعاملة ببيروكسيد الهيدروجين ($p\leq 0.05$) . أدى إعطاء الجزء غير الفلافونويدي عن طريق الغذاء إلى انخفاض تراكيز المالنداياألديهايد و جذر بيروكسي نتريت والفسفور وارتفاع في تركيز فيتامين C مقارنة مع المجموعة المعاملة ببيروكسيد الهيدروجين. إن هذه النتائج توضح دور الزنجبيل في الحماية من أصناف الأوكسجين الفعالة عن طريق زيادة إنتاج مضادات الأكسدة وكبح الجذور الحرة في الدم.

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, zingiberole, gingerol, shogoal, phellandrene, Borneol, Cineole, as well as citral, starch,

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, antiapoptitic, 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 autooxidation 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_2O_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_2O_2 in drinking water.

Group (3): 0.5% H_2O_2 and 15 mg/ kg BW. oral dose of flavonoids.

Group (4): 0.5% H_2O_2 and 30 mg/ kg BW. oral dose of flavonoids.

Group (5): 0.5% H_2O_2 and the experimental diets contain 5% non flavonoid part.

At the end of the experimental period, animals were sacrified, (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 Syrbio 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.

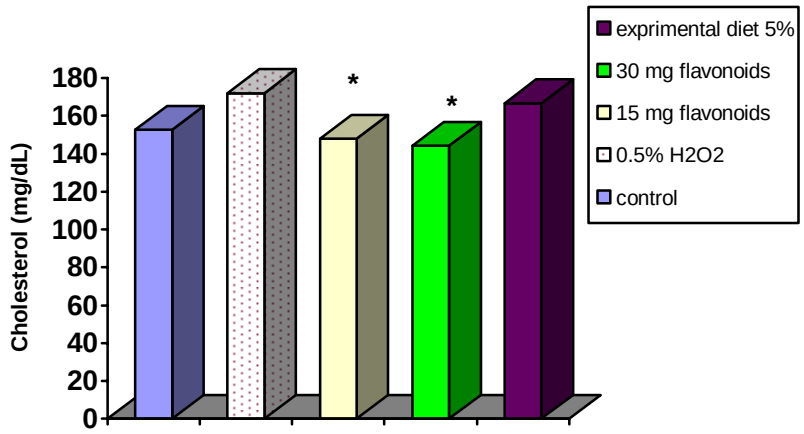
Serum total calcium was determined using commercial Kit (manufactured by BioMerieux company). Calcium in the sample reacts with O- cresolphthalin complexone (CPC) at alkaline pH, the colored complex formed was measured at 570 nm.

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 H_2O_2 treatment group ($p \leq 0.05$) 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).

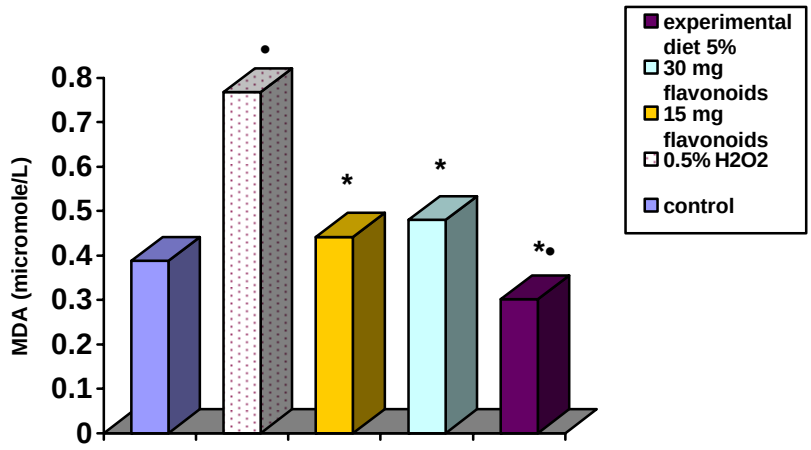


• significant with control, * significant with H_2O_2 group. ($p \leq 0.05$)

Fig.1: Effect of ginger extracts on cholesterol level.

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*., 1997**b**). 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 H_2O_2 treatment ($p \leq 0.05$) compared with the control group (Fig 2). This result was explaine the important role of H_2O_2 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 participitation of many diseases (Halliwell, 1993). H_2O_2 was used to induce many diseases including oxidative stress, such as atherosclerosis and diabetes melutus (Aziz, 2001, Wohaieb *et al*., 1994).

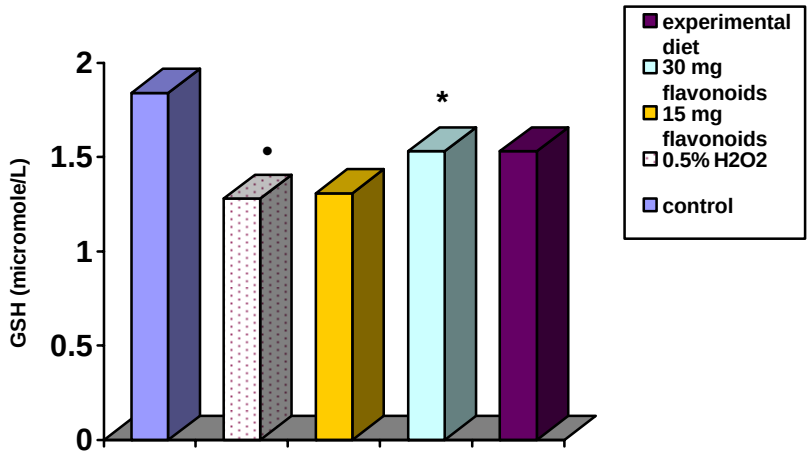


• significant with control, * significant with H_2O_2 group. ($p \leq 0.05$)

Fig. 2: Effect of ginger extract on MDA level.

Flavonoids- treated groups reversed the increase of MDA level to a considerable extent, (fig 2). It is now well established that *Zingiber officineles* rose significantly attenuated lipid peroxidation (Ahmed *et al*., 2000**b**)and has antioxidant properties by maintaining the activities of Superoxide dismutase (SOD), Catalase and glutathione Peroxidase (Ahmed *et al*., 2000**a**). 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 H_2O_2- treated group ($p \leq 0.05$) compared with the control group.. This result is in agreement with that obtained by (Ahmed *et al*., 2000**b**) 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*., 2000**a**) 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).

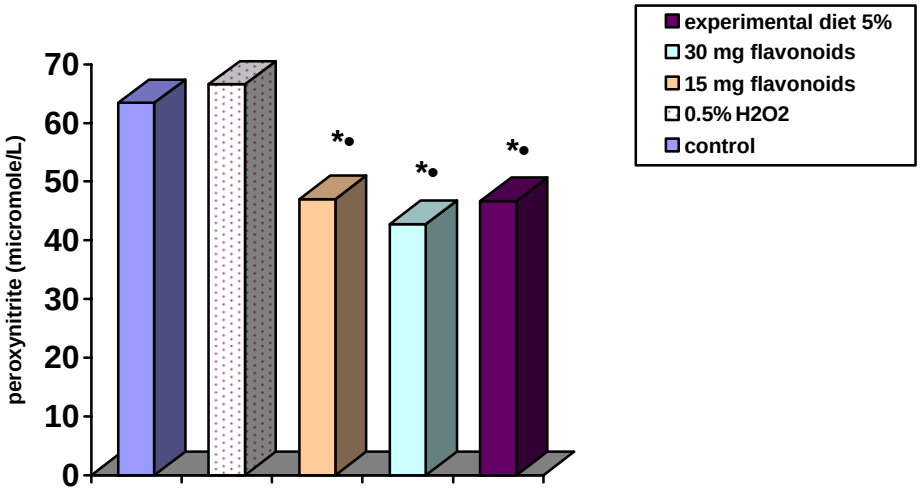


• significant with control, * significant with H_2O_2 group. ($p \leq 0.05$)

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.

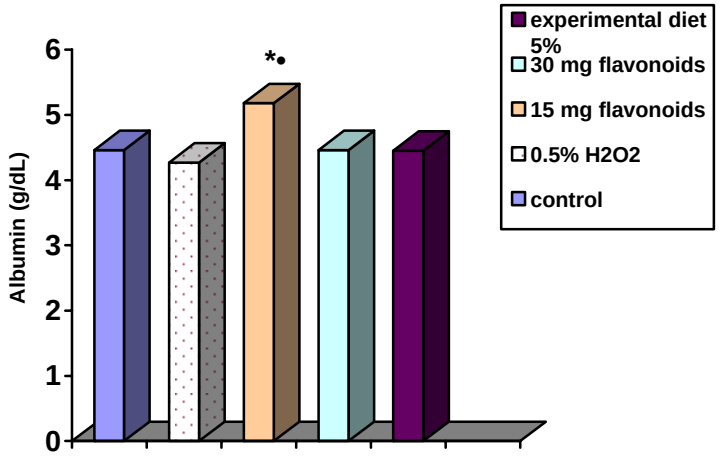
$ONOO^-$ concentration: The results of Peroxynitrite concentration showed a significant decrease in all Ginger- treated groups compared with the control and H_2O_2- treated groups ($p \leq 0.05$). This result was similar to that obtained by (Lippoushi *et al*., 2003) who explained that the 6- gingerol, 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 H_2O_2 group. ($p \leq 0.05$)

Fig. 4: Effect of ginger extracts 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 H_2O_2 treated groups ($p \leq 0.05$) (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.



• significant with control, * significant with H_2O_2 group. ($p \leq 0.05$)

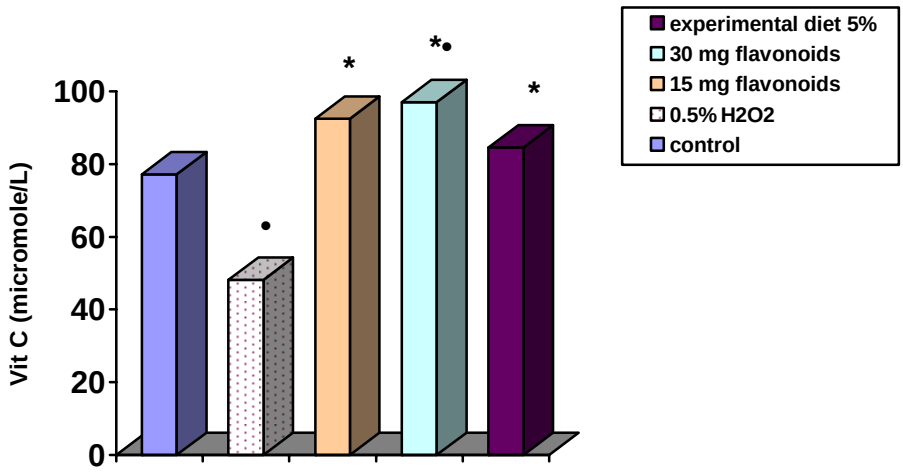
Fig. 5: Effect of ginger extract on albumin level.

Spectroscopic studies show that 80% of alpha tocopherol radical (α- Toc o$^{\cdot}$) are repaired over several seconds by quercetin bound to human serum albumin. αTOC O$^{\cdot}$ 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$^{\cdot}$ and Try$^{\cdot}$**,** 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: H_2O_2 treated group showed a significant reduction in vit C level compared with the control group ($p \leq 0.05$). The ginger extracts counteracted the H_2O_2 induced oxidative stress, Fig (6), 30 mg/ kg of flavonoid treated group showed a significant increase in vit C level ($p \leq 0.05$) this is the same result with that obtained by (Shanmuga sundaram, 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 H_2O_2- treated group ($p \leq 0.05$). Many studies showed the bioactivity of ginger consitituents 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

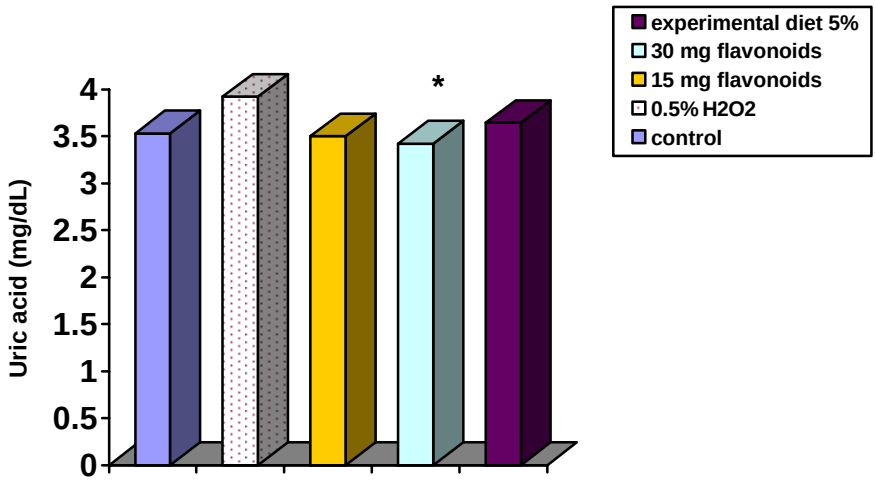
makes it a useful herb for lowering the risk of cardiovascular diseases (Guh *et al.,* 1995). (Ghayur *et al.,* 2005) showed that ginger is rich in essential fatty acids which can react with the toxins stored in the body From chemicals found in food. In addition, (Ghayur and Gllani, 2006) showed that 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-inflammatoryactivity 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).



• significant with control, * significant with H_2O_2 group. ($p \leq 0.05$)

Fig. 6: Effect of ginger extract on vitamin C level.

Uric acid concentration: H_2O_2- treated group showed a significant increase in uric acid (Fig 7) compared with the control group ($p \leq 0.050$. 30 mg/ kg ginger flavonoids reduced the level of uric acid compared with H_2O_2- treatment group ($p \leq 0.05$), this result is in agreement with the result obtained by (Bliddal *et al*., 2000) showing that the ginger helps the body to get ride 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.

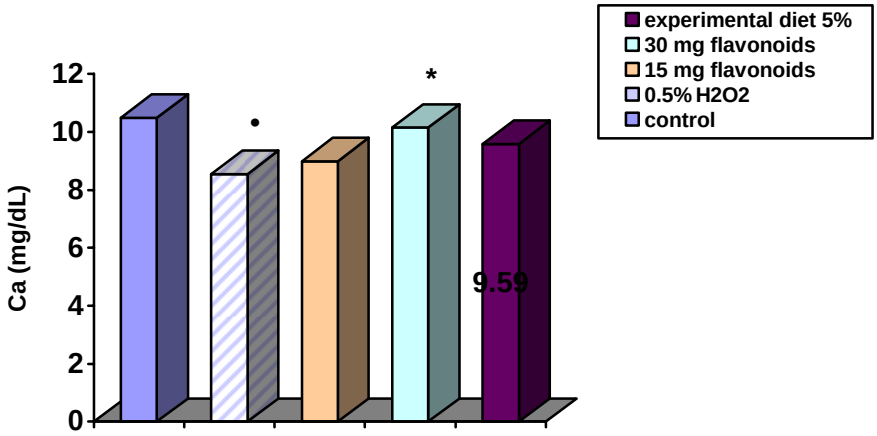


• significant with control, * significant with H_2O_2 group. ($p \leq 0.05$)

Fig. 7: Effect of ginger extract on uric acid level.

The eleimination 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 H_2O_2 – treated group compared with the control group ($p \leq 0.05$) Treatment with flavonoids (30 mg/ kg) counteracted the effect of H_2O_2 on the Ca concentration ($p \leq 0.05$) 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.

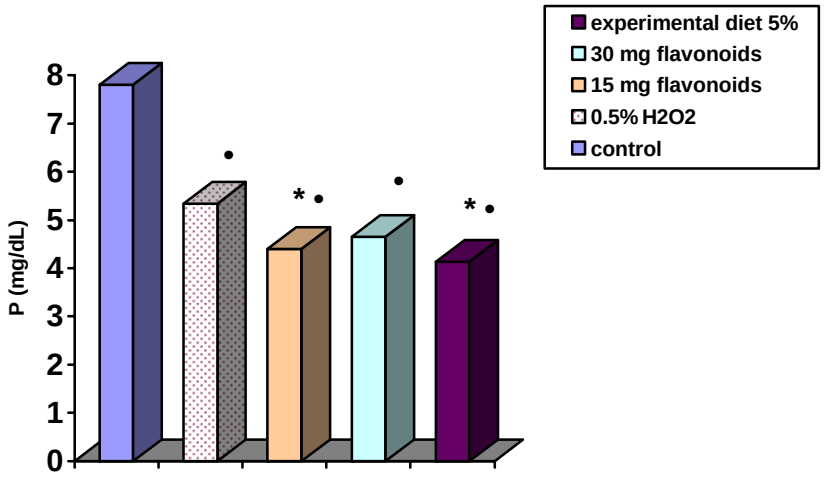


• significant with control, * significant with H_2O_2 group. ($p \leq 0.05$)

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 osteoarthrotic 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 extracelular fluids (Bringhurst, 1995). PTH in serum, increase total and free Ca and reduce the concentration of phosphate (Potts *et al.,* 1995).



• significant with control, * significant with H_2O_2 group. ($p \leq 0.05$)

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 scavengering 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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