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Ginger Attenuates Blood Pressure, Oxidant–antioxidant Status and lipid profile in the Hypertensive Patients

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Ginger had reportedly been used in folk medicine for the management or prevention of hypertension and other cardiovascular diseases. Therefore, this present study sought to investigate the inhibitory effect of aqueous extract of ginger on key enzyme linked to hypertension ,Angiontensin I Converting Enzyme(ACE), and oxidant/antioxidant status and lipid profile in hypertension patients. Oxidative stress parameters malondialdehyde (MDA), antioxidant markers including, superoxide dismutase (SOD), glutathione peroxidase (GP_X**)** and serum lipid status parameters were measured in 75 hypertension patients by standard procedures and the values were compared with 50 age, sex and socioeconomically matched normotensive control subjects. Aqueous extracts of white ginger (Z. officinale Roscoe) was prepared and the ability of the extracts to inhibit ACE was determined by *in vitro* studies. The results revealed that ginger extracts inhibited ACE in a dose dependent manner. Furthermore, these protective properties of the ginger varieties could be attributed to their polyphenol contents. Thus, the possible mechanism through which ginger exert its antihypertensive properties could be through inhibition of ACE activity and prevention of lipid peroxidation .All the lipid fractions TC, TG, LDL-C, VLDL, TC/HDL-C ratio were higher in hypertensive patients than those in the healthy controls.,MDA,ACE levels were significantly raised in hypertensive patients compare to controls. Thus, monitoring lipid level and maintaining oxidative balance in hypertensive patients would be helpful in preventing the diseases associated with hypertension.

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Introduction

Ginger (zingiber officinale) is one of the commonly used flavoring food agents(**1**). In ancient times ginger is used for taste, smell and for its therapeutic value in a wide variety of diseases, mainly gastrointestinal disorders, nausea, vomiting and motion sickness(**1,2**). Ginger is a herb which contain a chemical component named zingiberene. It has anti-inflammatory, analgesic, antipyretic, antimicrobial, hypoglycemic, anti-migraine, antischistosomal, anti motion sickness, anti oxidant, hepatoprotective and antithermic properties(**3**). Ginger chemical component helps to lower overall blood cholesterol components, which can reduce heart diseases. Because of anti thrombic potential of ginger, it may interact with blood thinning drugs such as warfarin. Ginger is a pungent herb has been shown to reduce hypertension or high blood pressure when taken regular in tea form. The crude extract of ginger induced a dose dependent fall in the arterial blood pressuretherfore, ginger has a diuretic and blood pressure lowering effect(**4,5**).

Ginger acts as a hypolipidaemic agent in cholesterol-fed rabbits (**6**). Other researchers reported that ginger treatment significantly decreased both serum cholesterol and triglyceride(**7,8**) In addition, Fuhrman and co-workers

reported that ginger decreased LDL-cholesterol, VLDL-cholesterol and triglycerides levels in apolipoproteins-E deficient mice(**9)**. Furthermore,Bhandari *al*., (2005) found that, the ethanolic extract of ginger significantly reduced serum total cholesterol and triglycerides and increased the HDL-cholesterol levels; also, the extract can protect tissues from lipid peroxidation and exhibit a significant lipid lowering activity in diabetic rats(**10)**.

Interestingly a few studies have been carried out to explore the blood pressure lowering potential of ginger extract and its active constituents (**10,11,12**]. Ginger helps to lowering the blood pressure through blockade of voltage dependent calcium channels**(13**). Ginger blocks a calcium channel which would normally induce the contraction of the smooth muscle tissue found in organs and arterial walls. The reduction of the smooth muscle contraction results in more relaxes arterial walls that allow blood to flow more freely and at a lower pressure.Hydroethanolic extract of the rhizome ginger is known for its strong free-radical reducing efficacy (**14**). It is mediated mostly by its phenolic constituents that may be divided into two groups: gingerol ,gingeron and shogaol related group and diarylheptanoids. A mixture of non-phenolics (sesquiterpene hydrocarbons, carbonyl compounds, monoterpene hydrocarbons and esters) contributes to the antioxidative activity and is responsible for the strong aroma of ginger in food, beverages and dietary supplements (**14**). However, most of the studies are based on exploring the curative effect rather than the preventive effect on risk factors of ischemic heart disease like hypertension and hyperlipidemia which have received very little attention. In view of this deficiency, the present work was undertaken in order to investigate the preventive effects of ginger on blood pressure and serum lipid profile, to know the association of oxidative stress and lipid profile with hypertensive patients and the role of intake ginger extract for prevention hypertension and monitoring lipid level and maintaining oxidative balance in hypertensive patients would be helpful in preventing the diseases associated with hypertension.

Materials and methods :

In this cross sectional study which conducted at Al- sheikh Zaeed hospital teaching hospital in the period from July 2012 to January 2013. Fifty healthy normotensive subjects served as the control group(G1) and 75 hypertensive subjects were recruited. The hypertensive subjects were further subdivided into three sub-groups: The subjects of G2 hypertensive were treated with ACE inhibitor 5 mg Captopril for once time daily for 6 months. The subjects of G3 hypertension were given antihypertensive folk medicine treatment(ginger extract supplied in capsules containing one gm daily) for 6 months ,while group three included 25 hypertension patient who were newly diagnosed in the medicine outpatients department not received any treatment during the period of this study except changed lifestyle habits. The fresh rhizomes of *Zingiber officinale* were obtained from local market and identified by the herbarium staff of the Botany Department, Baghdad University, Iraq .Ginger juice was prepared using the method of Akhani(**15**).

Lipid profile was measured with the help of enzymatic kits. Cholesterol was determined by the enzymatic method as described by Richmond(**16**).Triglycerides were determined by the enzymatic colorimetric method as described by manufacturer. Low-density lipoproteins (LDL) and very low density lipoproteins (VLDL) in sample precipitate with phosphotungestate and magnesium ions(**17**). After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined ,then VLDJ was determined according to Friedwald (**18**).Malondialdehyde (MDA) is determined by a colorimetric reaction with thiobarbituric acid (TBA) according to (**19**).Superoxide dismutase (SOD) activity is measured by method of (**20**).Glutathione peroxidase (GPx) is measured by method of (**21**). All kits are supplied by commercial analytical kits from Sigma (St Louis, MO)..

Body mass index (BMI): Following written informed consent, body weight and height were measured with men in light clothes and without shoes to the nearest 0.5kg of weight and the nearest 0.5cm of height. Exactly the same equipment was used for all studies, and the weighing scales (Seca, Germany) were calibrated regularly. Body mass index was calculated as: BMI = weight (kg) / height $(m)^2$ .Blood pressure was taken by trained personnel using stethoscope with a mercury sphygmomanometer and Measurements were taken from the left upper arm after subjects had been sitting for **> 5** min in accordance with the recommendation of the American Heart Association .

ACE (Human)ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for ACE has been precoated onto 96-well plates. Standards(NSO,L30-L1261) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for ACE is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human ACE

amount of sample captured in plate. For calculation, (the relative O.D.450) = (the O.D.450of each well) –(the O.D.450of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X).The human ACE concentration of the samples can be interpolated from the standard curve. Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Statistical analysis

The statistical analysis is carried out using the SPSS (Statistical Package for Social Sciences) software, version 16.0 for Windows. Results are expressed in mean ± SD. The two tail ANOVAs p values which are<0.001 where considered as highly significant

Results and Discussion:

Blood pressure andBody Mass Index :

The clinical and biochemical characteristics of the study participants were shown in Table 1. The hypertensive subjects and the controls were matched for age and sex. The mean age of the subjects (54±3.9 years) was comparable to that of the controls (55 ± 3.4 years).The mean values of systolic blood pressure (SBP) in severe hypertension group , newly diagnosed without any treatment were 164± 11 mmHg (G4 group).

Table 1: Shows the demographic, clinical, and biochemical profile of the cases under study.

Parameter	Group 1	Group 2	Group 3	Group 4
Age (Years)	55±3.40	58±2.77	53±3.82	57.7 ± 2.94
Height (m)	1.56±0.81	1.57 ± 0.64	1.60±0.43	1.61±0.53
Weight(Kg)	65±1.77	64±1.92	66±1.48	67±1.54
SBP mm Hg	116±10	138±12	145 ± 12	164± 11
DBPmm Hg	75±9	87±10	91± 11	110± 10
BMI	26.6±3.3	25.3 ± 2.2	25.53 ± 3.0	26.83 ± 3.2
MDA(µM)	0.82±0.22	1.7±0.72*	2.3±0.83*	3.5±0.92**
GSH(µM)	15±1.2	13.8±1.3*	11.7±1.7**	8.2±1.9**
SOD(U/ml)	12±1.22	5.7±0.78*	4.5±0.62*	3.1±0.56***
GPx(U/ml)	22±1.9	16±1.7*	14±1.9**	12±1.6***
Trig.(mg/dl)	100±12	156±16*	180±18**	200±22**
Chol.(mg/dl)	166±12	213±16*	223±18*	240±23**
HDL(mg/dl)	45±7.5	42±4.8	43±3.6	40±2.1*
LDL(mg/dl)	101±12	140±10*	142±12	160±13*
VLDL(mg/dl)	20 ± 2.8	31±3.3*	35±4.4	40±5.2*
ACE (ng/ml)	19.6±3.9	21.3 ± 1.9	28.0 ± 5.3*	32.7±8.1**

* G1 =Normotensive group , G2= Hypertension group treated with ACE inhibitor G3= Hypertension group treated with ginger G4= Hypertension group without treatment.

The mean values of SBP in a moderate hypertension group who were intake some traditional plants were 145 ± 12 mmHg(G3 group), while in mild hypertension group who treated with antihypertensive drugs such as ACE inhibitor(Captopril) were 138 ±12 mmHg (G2 group) against 116±10 in normotensive healthy control group (G1 group). There was a significant difference in SBP values ($P<0.01$) between three hypertensive groups compared with healthy normotensive subjects. The Diastolic Blood Pressure (DBP) mean values in severe ,moderate and mild hypertension groups were 110±8, 91± 9 and 87±10 mmHg respectively, there was a significant difference ($P<0.01$) between those three hypertensive groups compared with healthy normotensive subjects. While the mean BMI of all hypertension groups was not significant against healthy normotensive subjects ($P>0.05$). Regardless of the hypertension, none of the patients had any chronic disease or clinical complication

Oxidant and antioxidant status in hypertension

The clinical characteristics and the serum levels of the markers of oxidative stress in the control and the case groups have been shown in table(1).The anti-oxidant enzymes (SOD, and GP_X) were significantly decreased ($p<0.05$) in all hypertension as compared to those in the control group as shown in figure(1).The MDA level was significantly increased in the group 4 and 3 of hypertension cases as compared to that in the control group ($p<0.05$) but there was no significant difference in the SOD and GPx levels between the hypertension group treated with ACE inhibitor G2 group and the control group G1 ($p>0.05$), thus indicating an increase in the oxidative stress levels in the hypertensive subjects (G3 and G4) as compared to those in the normotensive individuals. On the other hand among the hypertensive group, there was a negative correlation between the antioxidant enzymes and the mean systolic blood pressure. The MDA levels showed a positive correlation with the systolic blood pressure. This showed that there was a positive correlation between oxidative stress and the levels of systolic blood pressurein hypertension groups G3 and G4.

The values obtained in this study are in close agreement with those reported by Aydin (**22**) .Excessive lipid peroxidation occurring in hypertension can be attributed to hypercholesterolemia which promotes the formation of free radicals.Thus, lipid alterations observed may promote oxidative stress, leading to endothelial dysfunction in hypertension. Highly significant reduction in antioxidants was observed in patients with hypertension in this study. The total antioxidative serum capacity is not a simple sum of the activities of the various antioxidative substances but the cooperation of the antioxidants in human serum that provides greater protection against attacks by free radicals. Decreased total antioxidant capacity (TAC) is indicative of a disturbance in the antioxidant system

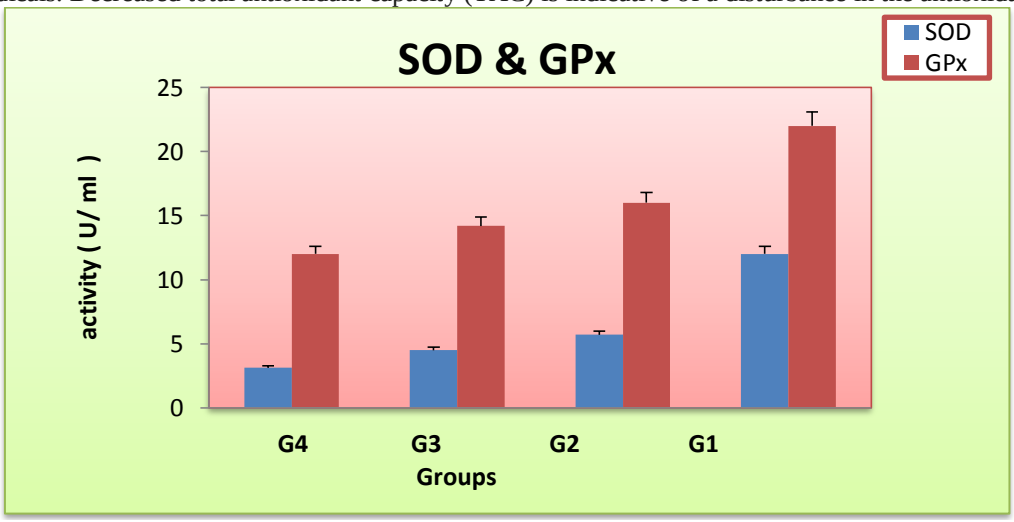


Figure 1: Activity of enzymatic antioxidants (SOD) and (GPx) in hypertension patients groups under study , and healthy subjects (Mean ± SD).

Lipid profile in hypertension:

In this study, serum TC, TG, and LDL-C concentrations were significantly higher in hypertensive patients than in normotensive subjects in the order (G4>G3>G2> G1) as shown in figure(2).This was consistent with earlier observations in parts of the world. This was unlike the findings of Lepira and Akintunde, (**23,24)** .They reported that the TC, TG, and LDL-C of newly diagnosed hypertensive patients did not differ significantly from that of control subjects, though the newly diagnosed hypertensive tended to have a higher level of LDL-C, TG, TC. High levels of serum cholesterol were known to increase the risk of developing macrovascular indicate a progressive increase in CHD risk as the serum TC exceeds 5.0 mmol/L such as coronary heart disease (CHD) and stroke. (**25**).

The exact pathogenetic mechanisms underlying the CVD risk mediated by dyslipidemia were not fully elucidated, but high levels of serum cholesterol were known to increase the risk of developing macrovascular complications such as coronary heart disease (CHD) and stroke **(26)**.Epidemiological studies indicate a progressive increase in CHD risk as the serum TC exceeds 220 mg/dl. It is thus generally recognized and recommended that treatment of hypertension should, in addition to lowering blood pressure, target correction of dyslipidemia (as well as other CVD risk factors) if present, to reduce overall CVD risk and increase the cost-effectiveness of therapy.

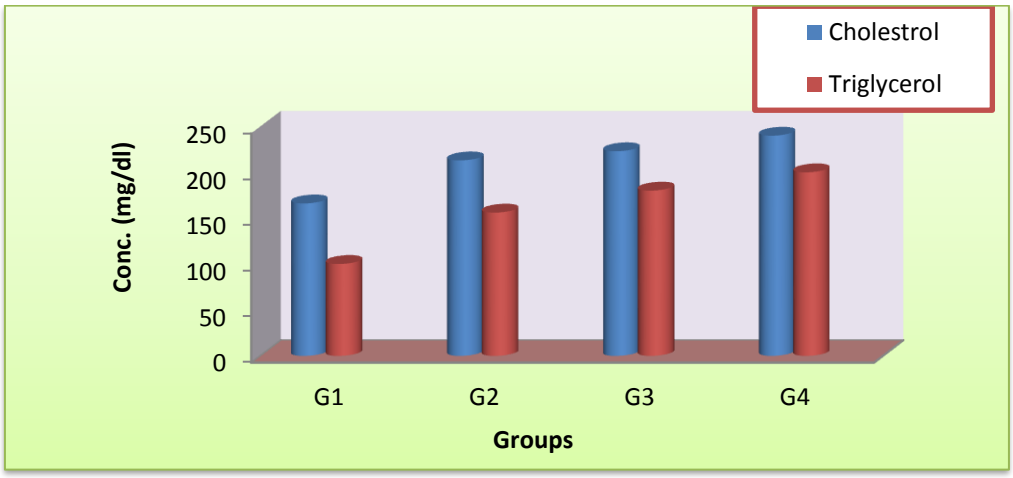


Figure (2): Serum levels of cholesterol and triglyceride in different hypertension cases and healthy normotensive subjects.

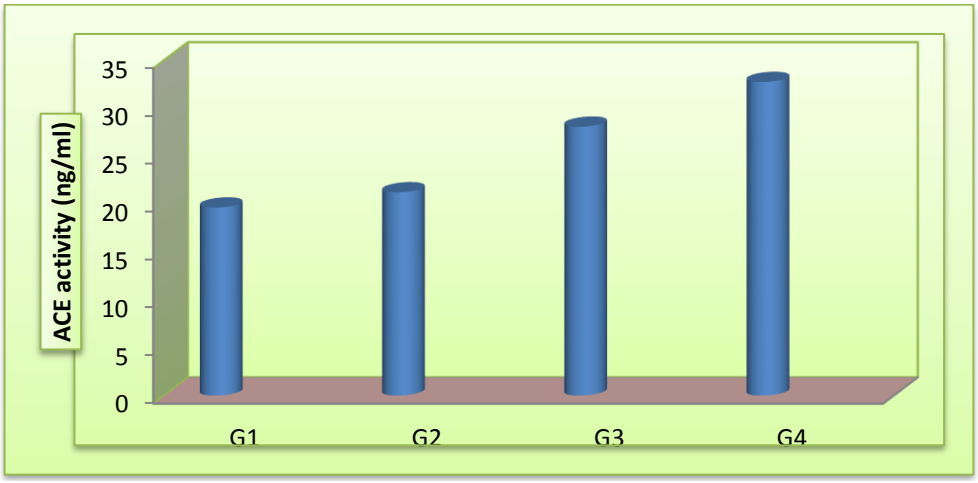
HDL-C can result in endothelial damage and trigger an increase in BP. The exact mechanism by which a low HDL-C increases CVD risk has however not been fully elucidated, though experimental studies suggest a direct role for HDL-C in promoting cholesterol efflux (this is called reverse cholesterol transport) from foam cells in the atherosclerotic plaque depots in blood vessels to the liver for excretion. HDL-C also exhibits potent anti-inflammatory and antioxidant effects that inhibit the atherogenic process(**22,27**).It has additionally been shown that a low HDL-C level correlates with the presence of other atherogenic risk factor(some of which were emerging risk factors not considered separately during prevalence). According to Pavithran (**28**) alteration in lipid metabolism including a decrease in HDLC can result in endothelial damage and trigger an increase in blood pressure which may partially account for its strong predictive power for CHD.

This study had shown that lipid abnormalities were highly prevalent among newly diagnosed hypertensive patients in Iraqi population. Efforts should therefore be intensified to fully evaluate Iraqi patients with hypertension from a lipid and lipoprotein standpoint, and any abnormalities detected were to be taken into consideration during therapy of this group of high-risk patients.

Angiontensin converting enzyme in hypertension:

Serum ACE-activity was studied in 75 elderly patients with uncomplicated hypertension. The possible importance of an increase in ACE for the pathogenesis of hypertension was evaluated by comparing the ACE levels to the blood pressure lowering effect of Captopril as in group G2. The mean total ACE activity in the hypertensive subjects without treatment (G4 group) was significantly increased($P < 0.001$) to 32.74 ± 8.19 ng/ml compared with 19.61 ± 3.97 ng/ml in normotensive subjects (G1 group) ,while the mean ACE activity was

slightly but significantly decreased in the hypertensive patients treated with Captopril when compared to hypertension group without treatment as showed in figure(3). On the other hand mean ACE activity in hypertension patients intake ginger extract as treatment from hypertension was also significantly different compared with healthy control . ACE activity was decreased in blood following Captopril. It is concluded that the increase in ACE activity in hypertension was of pathophysiological or clinical significance. Owing to the pathogenesis of hypertension related to the activity of RAAS, there might indeed be a role of ACE polymorphism in hypertension. Future studies should be more refined to study ACE gene aberrations in the context of age, gender, environmental and geographic factors, and importantly, to the duration of hypertension. ultimately, in life, it boils down to survival.



Figure(3): levels of ACE activity in hypertension patients and normotensive control.

On the basis of the aforementioned results, we can conclude that ginger was an effective herbal remedy for the risk factors (hypertension and hyperlipidemia) of IHD and if taken preventively, it will be very effective in reducing the chances of developing these risk factors. So ginger has the potential to provide not only the cheaper and natural alternative but also the effective preventive remedy for the risk factors (hypertension and hyperlipidemia) of ischemic heart disease (IHD) to develop and therefore reducing the chances of developing various cardiovascular disorders with significantly lower side effects.

Proposed mechanisms for this effect of ginger were could be as follows: ● It inhibits the hydroxymethylglutaryl Co A (HMG-Co A) reductase which was a rate limiting enzyme for cholesterol biosynthesis (like that of statins),as well as it promotes excretion and impairs absorption of cholesterol, and finally might be increase the activity of 7-alpha hydroxylase, the rate limiting enzyme in the catabolic conversion of cholesterol to bile acids in liver. Despite the aforementioned studies on mechanism of lowering the cholesterol levels by ginger more studies are needed for the confirmation, that is, whether only one or more of the aforementioned proposed mechanisms are associated with decrease in lipid levels. Furthermore, the treatment of ginger extract could significantly prevent the depletion of antioxidant concentration and antioxidant enzymes activities. In addition, Ajith reported that the presence of polyphenols and flavonoids in ginger extract might be responsible for the antioxidant activities and the reduction of hypertension (8).

On the other hand, injection ginger extract decreased cholesterol level due to elevated the activity the hepatic cholesterol 7-alpha-hydroxylase which is a rate-limiting enzyme in the biosynthesis of the bile acids and stimulate the conversion of cholesterol to bile acids leading to the execration cholesterol from the body (**26**). In this study ginger extract caused reduction in the levels of plasma cholesterol, LDL, but HDL statistically increased, this finding are in agreement with previous studies suggest that ginger extract produced significantly decrease in serum cholesterol and increased HDL-cholesterol levels(**27**). Furthermore the extract of ginger reduced plasma cholesterol and inhibited LDL oxidation in mice(**29**). This may explained that ginger contain monoterpens and shogaols compounds interfered with cholesterol biosynthesis in liver homogenates of hypercholesterollaemic mice causing its reduction(**30**). The reduction of cellular

cholesterol biosynthesis is associated with increased activity of the LDL receptor, which leads to enhanced removal of LDL from plasma, resulting in reduced plasma cholesterol concentration(**31)**.

The present study has demonstrated significant hypotensive effects of ginger as compared to placebo and showed comparable effects with that of Captroil. Ginger could be a good addition in combination therapy for hypertension. Comprehensive clinical trials of longer duration, using standardized ginger preparations are desirable to confirm the findings of present study.

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