American-Eurasian J. Agric. & Environ. Sci., 13 (9): 1246-1251, 2013
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2013.13.09.11035

Lemon Juice Elevated Level of Reduced Glutathione and Improved Lipid Profile in Wistar Rats

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Abstract: The beneficial uses of lemon juice have been reported from ages. Such uses are however more of speculations than investigation. In this study, the actions of lemon juice on antioxidant status and lipid profile in wistar rats were investigated. Thirty rats were randomized into two groups of fifteen rats each. The first group (control) received distilled water and the second group was given lemon (10% in water), daily for five weeks in addition to the rat chow diet. Serum total protein, reduced glutathione (GSH), catalase (CAT), superoxide (SOD) levels were determined as antioxidant status, while total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were measured as the lipid profile. The resultof the antioxidant status showed significant increase ($p<0.05$) in the GSH levels of the lemon treated group (0.63±0.01 µg/ml/mg protein) relative to the control (0.53±0.03 µg/ml/mg protein) after 5 week of lemon intake. The lipid profile improved as shown by the significant reduction of total cholesterol, LDL-cholesterol and significant increase in HDL cholesterol. The elevation of serum GSH could be responsible in part for the reported antioxidant effects of lemon juice. It is possible that one or more components in the administered lemon juice stimulated the production of GSH or the reduction of GSSG to GSH.

Key words: Lemon juice · Glutathione · Oxidative stress · Antioxidant · Hypolipidemia

INTRODUCTION

Lemon is from a small evergreen tree (*Citrus limón*). Its juice is characterized by the presence of flavanones, flavonoids, glycosy and flavones glucoside [1, 2]. In addition, the juice is about 5-6% citric acid (approximately 0.3 M), which gives lemons a sour taste and pH of 2 – 3. Lemon juice is also rich in ascorbic acids (Vitamin C), citric acid, essential oil, pectin and minerals, sugars, polysaccharides, carotenoid (pigment) and bitter limonoid [3]. The presence of these phytophenolic compounds makes lemon juice an inexpensive, readily available source of antioxidantsIn addition to vitamin C, lemon contains other vitamins like vitamin A, B_1, B_2 and B_3. It is also a good source of potassium and calcium [4]. Lemon is low in calories, containing 27 Kcal per 100 g [5]. These components and more make lemon juice a functional food of immense therapeutic use.

Lemon juice has been used for so many years as a therapeutic agent. It is well known for its antiseptic properties and as an antidote for various poisons. It has been implicated for treatment of several diseases such as meniere's disease, scurvy, common cold and prevention of kidney stones [6]. These therapeutic activities might not be unconnected with the presence of vitamins, flavonoids and other phenolic compounds in lemon juice. It has been reported that vitamin C, even in small amounts, can protect molecules such as proteins, lipids (fats), carbohydrates and nucleic acids (DNA and RNA), from damage by free radicals and reactive oxygen species that are generated during normal metabolism as well as through exposure to toxins [7]. Flavonoids, on their parts, have been reported to protect the gastric mucosa from ulceration, to prevent the formation of peptic ulcers and inhibit lipid peroxidation [8]. These polyphenolic compounds are also effective in many

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health-related properties, such as anticancer, antiviral and anti-inflammatory activities [9]. Many studies have also shown that increased dietary intake of natural phenolics correlates with reduced oxidative stress and longer life expectancy [10]. This study thus aimed at determining the effect of lemon juice on the antioxidant status markers in Wistar rats.

MATERIALS AND METHODS

Animals and Chemicals: The study protocol was approved by the relevant Institutional Scientific Ethic Committee. Thirty male rats of the Wistar strain with an average weight of 209.6±7.23g were purchased from Covenant Farms, Ibadan. They were acclimatized to a well ventilated animal room at 25±2°C under controlled light cycles at the Redeemer's University Animal House facility, Mowe, Ogun State, Nigeria and fed with standard commercial chow and clean water *ad libitum* for at least a week prior to the experiment.

The lemons were purchased at Oyingbo market, Lagos State, Nigeria. Other reagents were of analytical grade and are used as supplied unless otherwise stated.

Physiochemical Characterization of Lemon Juice: The following characterizations were carried out on and lemon juice: moisture content, electrical conductivity, total acidity, relative density, glucose and fructose concentrations. Moisture content was determined by refratometric method [11], electrical conductivity using conductivity meter (Schott Instrument, Germany), total acidity was determined by titration to pH 8.30 with 0.1M sodium hydroxide as described by Lord *et al*. [12], relative density, glucose and fructose concentrations were determined using Benedict methods. Cu^{2+} and Zn^{2+} contents were carried out using of atomic absorption spectroscopy (AAS-7000, Shimadzu, Japan). Vitamin C content was based on the decolorization of dichlorophenolindophenol [13].

Experimental Design: The animals were randomly distributed into 2 groups of 15 rats each. The experimental group was given 10% lemon juice along with the normal chow for a period of 5 weeks. The other group (control) was given distilled water in lieu of the juice. Three rats were sacrificed from each group weekly.

Sample Collection: Rats were sacrificed after cessation of treatment and an overnight fasting. Blood was collected from the inferior vena cava of heart of the animals into plain universal tubes and was allowed to stand for 1 hour. Serum was prepared by centrifugation at 4000 g for 10 min in a Centrifuge (HeraeusLabofuge 300 model). The clear supernatant was used for the estimation of the serum enzymes and other parameters.

Biochemical Analysis: Catalase (CAT) level was determined according to the method of Ashru and Sinha [14]. In this method, dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, perchromic acid is formed which is an unstable intermediate. The chromic acid produced is then measured colorimetrically at 570 nm.The level of superoxide dismutase (SOD) was determined by adopting the method described by Misra and Fridovich [15]. The procedure is premised on the ability of SOD to inhibit the autoxidation of epinephrine at pH 10.2. Protein concentration in serum was estimated by the protocol of biuret reaction as described by Gornal *et al*. [16] with slight modification.

Reduced glutathione level (GSH) was carried out using the method described by Ellman [17]. The reduced form of glutathione is the bulk of cellular non-protein, sulfhydryl groups. This method is based on the development of a relatively stable yellow colour when 5'5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction with the reduced glutathione, 2-nitro-5-thiobenzoic acid posses a molar absorption at 412 nm. Therefore, reduced GSH is proportional to the absorbance at 412 nm. Total Cholesterol, triglyceride and high density lipoprotein-Cholesterol were measured by spectrophotometry in serum using Randox commercial kits.

Data Analysis: Data were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's post-test for analysis of biochemical data. Statistical analyses were performed using SPSS statistical version 8 software package. Values were considered statistically significant at $P<0.05$.

All values are expressed as mean±standard error of mean (SEM) of three observations.

RESULTS

The biochemical mechanism of the therapeutic effect of lemon juice is poorly understood. As a preliminary step towards unraveling the mechanism of actions of this functional food, its effects on serum oxidative stress marker enzymes (CAT and SOD), GSH and lipid profile were reported.

Table 1: Characterization of the lemon juice

Parameters	Lemon
Relative density	1.05
Electrical conductivity (mS/cm)	6.29
Acidity (%/mass)	3.12
Moisture content(g/100g)	21.3
vitamin C (mg/ml)	4.0
Fructose (g/100g)	0.09
Glucose (g/100g)	0.10
Copper (p.p.m)	0.198
Zinc (p.p.m)	0.372
Colour	yellow

Physio-Chemical Analyses of the Lemon Juice: The result of the physio-chemical analyses of the lemon juice used in the experiment is presented in Table 1. The lemon juice was yellow in colour with a relative density of 1.05 and an electrical conductivity of 6.29 mS/cm; it was slightly acidic 3.12%/g, with a moisture content of 21.3, a fructose and glucose concentration of 0.09 g and 0.1 g, respectively and vitamin C concentration of 4 mg/ml. It had a relatively minute copper and zinc level of 0.198 and 0.372 ppm, respectively.

Effect of lemon on CAT, SOD, GSH and Total Protein: The results of lemon juice on the levels of catalase activity (CAT), superoxide dismutase (SOD) and reduced glutathione levels are presented in Table 2. The group that received lemon juice showed no significant ($p > 0.05$) differences in both the activities of CAT and SOD when compared with the control, however there were significant increase ($p<0.05$) in serum GSH levels. A correlation was observed between SOD and CAT levels ($r = 0.770$) and also between the SOD and GSH levels ($r =0.764$) of the control. There was also correlation between the SOD and CAT levels of the group treated with lemon ($r=1.00$), however SOD and GSH levels had an inverse relationship ($r = -0.54$). The result for protein levels are also presented in Table 2, lemon treatment showed no significant ($p > 0.05$) differences relative to the control at the end of the five weeks of the experiment.

Effect of Lemon Juice on Serum Lipid: At the end of the fifth week, it was observed that oral lemon juice administration resulted in significant decrease in serum total cholesterol, triglyceride (TG) and LDL-cholesterol levels when compared with the control group with a commensurate significant increase in the HDL-cholesterol (Table 3). An increase, but not significant, was also recorded in the total protein over the same period.

Table 2: Effect of Lemon juice on the levels of Catalase Activity CAT, Superoxide Dismutase Activity SOD, Reduced Glutathione Level GSH and Total Protein

Parameters	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5
CAT (µmol/ml/mg protein)	Control	8.22±0.82	2.68±0.39	7.83±0.71	1.89±0.21	2.49±0.28
	Lemon	5.64±0.42	3.37±0.24	6.00±1.12	2.70±0.50	3.03±0.60
SOD(U/mins/mg protein)	Control	0.44±0.04	0.19±0.02	0.05±0.01	0.17±0.01	0.11±0.01
	lemon	0.35±0.01	0.23±0.03	1.40±0.5	0.22±0.02	0.07±0.01
GSH (µg/ml/mg protein)	Control	0.70±0.07	0.49±0.04	0.31±0.03	0.29±0.01	0.35±0.03
	Lemon	0.71±0.13	0.47±0.02	0.45±0.02*	0.54±0.08*	0.63±0.01*
Total Protein (mg/ml)	Control	28.90±4.31	53.13±1.78	63.65±8.15	63.97±1.77	50.00±1.87
	Lemon	48.45±3.86	50.72±1.02	60.37±1.15	59.37±1.03	56.77±2.02

Values were expressed as mean±S.E.M.

*Group treated with lemon differed significantly ($p < 0.05$) relative to the control.

Table 3: Effect of lemon on total cholesterol, HDL- cholesterol, LDL- cholesterol and triglyceride in the serum of rats

	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5
Total Cholesterol (mg/dl)	Control	71.34±8.34	57.52±1.99	59.31±2.32	79.69±1.39	58.01±2.44
	Lemon	71.61±3.16	62.67±2.98	57.97±2.72	53.22±2.4*	42.99±0.53*
Triglyceride (mg/dl)	Control	69.42±1.80	71.94±1.64	74.05±3.39	90.23±4.56	90.35±2.54
	Lemon	74.15±4.43	65.92±1.97	49.36±2.95*	47.68±0.17*	45.65±3.38*
HDL (mg/dl)	Control	14.50±1.13	10.60±0.35	11.94±0.43	11.71±1.33	12.25±0.73
	Lemon	17.81±0.47*	15.36±0.29*	17.64±0.78*	18.95±0.41*	17.1±0.55*
LDL (mg/dl)	Control	42.94±7.15	32.54±1.44	32.54±1.25	49.93±1.01	27.70±2.47
	Lemon	39.29±1.96	34.12±2.65	30.46±1.50	24.70±2.36*	16.75±0.58*

Values are mean±SD of three rats in each group.

* Comparison against control; significance at $P < 0.05$

DISCUSSION

The biochemical mechanism of the therapeutic effect of lemon juice is currently poorly understood. As a preliminary step towards unveiling the mechanism of actions of these functional foods, their effects on serum GSH activities of common oxidative stress marker enzymes (CAT and SOD) were investigated. The significant increase in serum GSH of the lemon group coupled with non significant depletion of the total protein suggested that the activation of the GSH synthetic pathway does not occur as a consequence of an increased production of free radicals [18]. It could also be inferred that one or more components of lemon probably have some biochemical effect on GSH production or play a role in the reduction process of GSSG to GSH. Molecular evidence has been provided of the ability of some phenolic compounds to activate c-glutamylcysteine synthetase, the rate-limiting enzyme in GSH synthesis [19]. Abraham and Singh [20] suggested that the increase in GSH concentration induced by plant bioactive secondary metabolites contributes to the chemo-prevention against environmental carcinogens. Glutathione, an important intracellular free radicals scavenger and co-substrate for many important enzymes, plays a prominent role in the degradation of H_2O_2, the molecule itself undergoing oxidation from its reduced state GSH to an oxidized state GSSG [21].

The roles of SOD and CAT are certainly important in the regulation of oxidative stress. The slight decreased in the activity of SOD, experienced in this study, might be from the suppression of superoxide anions generation by lemon juice or its conversion to H_2O. Enhanced activity of antioxidant enzymes has been reported as an adaptive mechanism to protect cells against the toxic radicals [22]. Since SOD converts superoxide anions to hydrogen peroxide (H_2O_2), an enhanced SOD activity might lead to increased turnover of H_2O_2 [23] which normally could also cause elevated activities of CAT. Normally, H_2O_2 is further metabolized to H_2O and O_2 by CAT which has been reported to be highly susceptible to increased superoxide anions [24]. Data obtained in this study revealed no significant changes in the activities of serum SOD and CAT in lemon treated group relative to those of the control rats. Studies have demonstrated that flavonoids are important components of lemon offering good protective properties because they can effectively inhibit lipid peroxidation and scavenge free radicals such superoxide's and H_2O_2 [25-27].

The significant reduction in the serum total cholesterol, triglycerides and low-density lipoprotein levels, while the high-density lipoprotein levels was increased in the studied groups are in agreement with those obtained by Gorinstein [28] which reported the hypolipidemic effect of citrus juice generally. Also, Monforte *et al*. [29] reported similar trend using citrus flavonoids. The reduction in serum cholesterol, triglycerides, low-density lipoprotein levels may be due to its antioxidant effect since previous studies suggested that antioxidant (vitamin C) administration in rats improves endothelial function of coronary and peripheral vessels [30]. It is widely accepted that elevations in cholesterol and LDL plasma levels are major factors for coronary heart disease. High flavonoid intake from fruits and vegetables has been associated with decreased risk for the development of kidney and cardiovascular disease [31].

CONCLUSION

This study revealed that administration of lemon to Wistar rats increased serum GSH levels suggesting that this could be one means by which lemon juice exerts its antioxidant effect. On the other hand the insignificant effects observed on the levels of SOD and CAT in lemon treated animals may be an indication that these antioxidant enzymes may not be central to the chemo-preventive and theraupeutic properties of lemon. However more investigations are required in this direction to elucidate further, the probable mechanism of actions of this ancient remedy.

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