

Ameliorating Effects of Licorice and Vitamin C on Side Effects from Consumption of Overheated Toasted Bread in Rats

Soheir Ahamed Al-Masri

Department of Nutrition and Food Sciences, Faculty of Food Sciences and Agriculture,
King Saud University, Riyadh, Saud Arabia

Abstract: Fifty rats were randomly classified into normal group and 4 groups (consumed overheated roasted breads in basal diet) which were positive, licorice, vitamin C and licorice with vitamin C groups. The experimental period was 60 days. Results clearly revealed that positive control rat group which consumed overheated roasted breads in diet showed a significant decrease in body weight gain, food intake and feed efficiency ratio (FER) compared to normal group. It also showed a significant increase in alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and free radicals (ALT, AST, ALP and FR, respectively) and malondialdehyde (MDA) in blood, kidney and liver. Moreover, it showed a significant decrease in superoxide dismutase (SOD) in serum kidney and liver; glutathione-peroxidase (GSP) in blood and kidney and glutathione S-transferase (GST) in liver and kidney compared to normal rat group. Administration of licorice, vitamin C and licorice with vitamin C to rats could improve growth performance and also increase in blood GSP and SOD but decrease levels of AST, ALT, ALP, FR and MDA compared to positive control rat group. Licorice, vitamin C and licorice with vitamin C rat groups showed normal values of food intake; liver SOD, catalase, GST& MDA; and kidney SOD, GSP, GST & MDA compared to positive control rat group. This study concluded that licorice with vitamin C could improve growth performance and increase antioxidant enzymes. Therefore, this study recommends to increased dietary intake of natural antioxidant as vitamin C and licorice to consumers who prefer to eat overheated roasted bread.

Key words: Antioxidants • Licorice • Overheated roasted bread • Rat

INTRODUCTION

Cereal grains and legumes plays an important role in supplying the nutrients, as well as over 70% of the daily energy requirements, of over two-thirds of the world's population [1]. Bread, the most common form of cereal intake in many countries has been designated the Staff of life and rightly so, since it contains more nutrients per weight than meat, milk, potatoes, fruits and vegetables. Breads and cereals give most of the carbohydrate, B vitamins, fiber and minerals, such as zinc and magnesium [2]. Bread is a staple food prepared by baking dough of flour and water and served in different forms at any meal of the day. Toast is bread that has been browned by exposure to radiant heat. This browning is the result of a Maillard reaction which is a chemical reaction between amino acid and reducing sugar; usually requiring the

addition of heat to makes it firmer and more palatable [3]. Bread becomes stale when the starches crystallize and warming the bread returns them to their soft gel state, making the bread taste and feels fresh. Any extra cooking can destroy the most fragile vitamins. More importantly, the overheated bread can increase the higher the concentration of carcinogens, especially acrylamides and polycyclic aromatic hydrocarbons (PAHs). Acrylamides are created when the carbohydrates are heated [4]. There is evidence that consumption of burned baked foods which contain 40 micrograms of acrylamides a day increased risk of cancer. Some polycyclic aromatic hydrocarbons (PAHs), particularly those with a high molecular weight, have been classified as probably carcinogens to humans by the International Agency for Research on Cancer. Like other thermally processed foodstuffs, toasted bread can contain these carcinogenic

Corresponding Author: Soheir Ahamed Al-Masri, Department of Nutrition and Food Sciences,
Faculty of Food Sciences and Agriculture, King Saud University, Riyadh, Saud Arabia.

hemicals, not only due to a contamination at source but also during toasting [5]. It is well known that PAHs and acrylamide increase reactive oxygen species (ROS) which are over-produced under various stressful conditions, which promotes the development of chronic diseases. Therefore, it is important to identify phytochemicals with antioxidant activity that could be used to prevent and treat ROS-associated chronic diseases. Polyphenols, which are abundant in plants, fruits and vegetables, have drawn much attraction due to their protective effects against cancer, cardiovascular decline and cognitive function and memory impairment [6-8]. *Glycyrrhiza glabra* (Licorice) originated in the Mediterranean and Middle East. It is sometimes known as the grandfather of herbs. As licorice has been consumed by humans for several thousand years and is known to be safe for human consumption except inducing hypertension. Licorice has been widely used as a flavoring and sweetening agent in tobacco products, chewing gum, candy, toothpaste and beverages and it has long been prescribed as a treatment in Oriental herbal medicine [9]. Vitamin C is potent antioxidants that can scavenge various reactive oxygen and nitrogen radicals. The critical role of vitamin C in ameliorating the adverse effects of reactive oxygen and nitrogen radicals has been well established. In addition, numerous epidemiological studies strongly support the protective role of vitamin C in decreasing the incidence of chronic diseases like atherosclerosis where oxidative stress caused by excessive oxygen or nitrogen radicals may play a causal role [10].

The present study was undertaken to evaluate the antioxidant effect of licorice and vitamin C in lowering risk of cancer induced by consumption of overheated toasted bread.

MATERIALS AND METHODS

Material: Licorice (*Glycyrrhiza glabra*) was purchased from a local herb shop in Riyadh. Bio Merieux Kits were purchased from Alkan Co. for Chemicals and Biodiagnostics. Vitamin C was purchased from Sigma Chemical Co. in Riyadh at dose 200 mg/kg body weight in 5.0 ml of the vehicle by stomach tube all over period of the experiment. Fifty white male albino rats (Sprague dawley strain), weighing between (200±5g) provided from experimental animals center in Medicine collage of King Saud University in Riyadh. Breads were purchased from a local bakery in Riyadh. The basal diet consisted of protein (13%), fat (4%), salt mixture (3.5%), vitamin mixture (1%), cholin (0.2%), cellulose (5%) and the remainder was starch according to Reeves *et al.* [11].

Methods: The licorice root was grinded to give a fine powder. 200g of the licorice powder was suspended in 3L of distilled water for 2 hours then filtrate to remove cellulose fibers. Licorice extract was administered orally through stomach tube at dose 200 mg/kg b.w) according to previous study of Hai *et al.* [12]. Breads were toasted at toaster till become deep brown black in color then grinded to fine powder. The overheated roasted bread was added to basal diet as 5% in diet. Experimental rats were divided randomly into five groups of 10 rats. One group (n=10) of rats served as a normal control and four groups were fed overheated roasted bread all over the period of the experiment (60 days). One group of them served as a control positive while the rest of rats were classified into three treated groups which were licorice, vitamin C and licorice with vitamin C. Food intake was monitored daily and the growth of animals was monitored weekly by recording body weight. Feed efficiency ratio (FER) was calculated according to Chapman *et al.* [13]. The animals were sacrificed after 60 days under light ether anesthesia. Blood, liver, kidneys were collected for biochemical assays. Briefly, serum alanine and aspartate aminotransferase (ALT&AST) activity and alkaline phosphatase (ALP) were estimated according to Kaplan [14], respectively. Blood malondialdehyde (MDA), free radical (FR), superoxide dismutase (SOD) and glutathione peroxidase (GSP) were estimated according to Draper and Hadly [15], Borg [16], Misra and Fridovich [17] and Pagila and Valentine [18], respectively. Kidneys and liver superoxide dismutase (SOD), glutathione S-transferase (GST), malondialdehyde (MDA) and were estimated according to the methods of Misra and Fridovich [17], Ellman [19] and Sinha [20], respectively. Kidneys GPX and liver catalase enzymes were estimated according to the methods of Rotruck *et al.* [21] and Xu *et al.* [22], respectively.

Statistical Analysis: Statistical analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests at a level of $P < 0.05$ [23].

RESULTS

Data in Table 1 showed that positive control rat group which consumed overheated roasted breads in diet showed a significant decrease in body weight gain, food intake and FER compared to normal group at $p < 0.001, 0.05$ and 0.001 , respectively. Administration of licorice, vitamin C and licorice with vitamin C to rats could improve growth performance as increase in weight gain

Table 1: Mean± SD of weight gain, food intake and FER in experimental rat groups.

Variables	Groups				
	Normal	Positive control	Licorice	Vitamin C	Licorice with vitamin C
Weight gain(g)	87.66±5.41a	49.41±3.21c***	71.44±5.11b*	75.71±6.14b*	73.22±5.27b*
Food intake (g/w)	23.71±2.60a	18.31±1.51b*	21.21±2.14a	22.41±2.11a	22.11±2.10a
FER	0.061±0.004a	0.044±0.001c***	0.056±0.002b*	0.056±0.003b*	0.055±0.002b*

Significant with control (-ve) group * P < 0.05 ** P < 0.01 *** P < 0.001

Mean values in each raw having different superscript (a, b, d, c.....) are significantly different at P < 0.05

Table 2: Mean ± SD of serum AST, ALT and ALP of the experimental rat groups.

Variables	Groups				
	Normal	Positive control	Licorice	Vitamin C	Licorice with vitamin C
AST(μ /ml)	32.71±3.61c	65.71±7.84a***	40.21±4.27b*	41.31±5.11b*	39.21±4.14bc
ALT(μ /ml)	41.11±4.14c	73.11±8.21a***	50.11±6.17b*	47.21±5.61b*	48.71±5.20b*
ALP(μ /ml)	42.77±4.16c	108.71±11.11a***	61.77±8.22b**	60.21±6.14b**	59.21±7.08b**

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c, d) are significant

Table 3: Mean ± SD of blood FR, GSP, SOD and MDA of the experimental rat groups.

Variables	Groups				
	Normal	Positive control	Licorice	Vitamin C	Licorice with vitamin C
FR	11357.81± 301.71d	33521.50±631.44a***	20174.31±503.22b**	16894.20±411.27bc*	14732.10±336.31cd
GSP (mmol/l)	9.71±1.33a	4.10±0.66c***	6.81±1.01b*	7.03±1.15ab	7.31±1.14ab
SOD (mmol/l)	28.55±3.11a	14.60±1.20d***	19.71±1.55bc**	23.11±2.44ab	21.47±2.35b*
MDA (mmol/l)	3.99±0.35b	8.65±1.14a***	4.67±0.66b	4.55±0.58b	4.24±0.50b

Significant with control (-ve) group * P < 0.05 ** P < 0.01 *** P < 0.001

Mean values in each raw having different superscript (a, b, d, c.....) are significantly different at P < 0.05

and FER compared to positive control rat group. There were no significant changes in weight gain, food intake and FER among rats administered licorice, vitamin C and licorice with vitamin C. Results in Table 2 showed that rats of positive control group had significant ($p<0.001$) increase in serum level of AST, ALT and ALP compared to normal rat group. Oral administration of licorice and vitamin C showed a significant increase ($p<0.05$) in AST & ALT and ($p<0.01$) ALP compared to normal rat group but showed a significant decrease compared to positive control rat group. Better results were appeared in licorice with vitamin C rat group as recorded normal value of AST and a significant increase in values of ALT and ALP at $p<0.05$ & 0.01 in comparing to normal rat group and also showed a significant decrease compared to positive control rat group. There were no significant changes in serum level AST, ALT and ALP among rats given licorice, vitamin C and licorice with vitamin C.

Rats of positive control showed a significant ($p<0.001$) increase in blood levels of free radicals (FR) and MDA; and a significant ($p<0.001$) decrease in GSP and SOD compared to normal rat group. Rat group which administered licorice showed a significant ($p<0.01$)

increase in serum level of free radicals (FR) and a significant ($p<0.05$ & 0.001) decrease in GSP and SOD compared to normal rat group. Vitamin C rat group showed a significant ($p<0.05$) increase in blood level of free radicals (FR) and normal values of GSP, SOD and MDA while licorice with vitamin C rat group showed a significant ($p<0.05$) decrease in SOD and the values of FR, GSP and MDA were within normal values compared to normal rat group as shown in Table 3. Results in Table 4 showed that rats of positive control showed a significant ($p<0.001$) decrease in kidney levels of SOD, GSP and GST and a significant ($p<0.001$) increase in MDA compared to normal rat group. Rat groups which administered licorice, vitamin C and licorice with vitamin C showed a non significant difference in kidney levels of SOD, GSP, GST and MDA except low level of GST in licorice rat group compared to normal rat group. On the other side, they had a significant increase in kidney levels of SOD, GSP, GST and a significant decrease in kidney MDA compared to positive rat group. Results in Table 5 showed that rats of positive control showed a significant ($p<0.001$) decrease in liver SOD, catalase and GST and a significant ($p<0.001$) increase in MDA compared to normal rat group.

Table 4: Mean \pm SD of kidney SOD, GSP, GST and MDA of the experimental rat groups.

Variables	Groups				
	Normal	Positive control	Licorice	Vitamin C	Licorice with vitamin C
SOD(μ /mg)	110.17 \pm 16.17a	55.14 \pm 6.19c***	91.41 \pm 10.22ab	97.24 \pm 11.14a	95.33 \pm 10.59ab
GSP(μ /mg)	99.71 \pm 8.88a	40.31 \pm 5.11b***	94.01 \pm 9.66a	103.21 \pm 14.22a	101.14 \pm 13.18a
GST(μ /mg)	3.24 \pm 0.16a	1.49 \pm 0.15c***	2.58 \pm 0.37b*	2.96 \pm 0.42ab	3.71 \pm 0.33a
MDA(μ /mg protein)	7.11 \pm 0.80bc	13.66 \pm 1.22a***	9.20 \pm 1.10b	8.44 \pm 1.08b	8.11 \pm 1.41b

Significant with control (-ve) group * P < 0.05 ** P < 0.01 *** P < 0.001

Mean values in each raw having different superscript (a, b, d, c.....) are significantly different at P < 0.05

Table 5: Mean \pm SD of liver SOD, catalase, GST and MDA of the experimental rat groups.

Variables	Groups				
	Normal	Positive control	Licorice	Vitamin C	Licorice with vitamin C
SOD(μ /mg)	71.14 \pm 6.98a	39.77 \pm 3.24c***	69.31 \pm 5.99ab	72.11 \pm 7.14a	73.41 \pm 6.90a
Catalase(μ /mg)	66.71 \pm 7.10a	32.16 \pm 3.21c***	55.99 \pm 6.14ab	61.41 \pm 7.15a	60.88 \pm 6.09a
GST(μ /mg)	3.11 \pm 0.31a	1.14 \pm 0.52b***	3.07 \pm 0.77a	3.22 \pm 0.86a	3.50 \pm 0.70a
MDA(μ /mg protein)	10.21 \pm 1.16bc	15.96 \pm 2.11a***	12.16 \pm 1.13b	10.66 \pm 1.02bc	11.41 \pm 1.18bc

Significant with control (-ve) group * P < 0.05 ** P < 0.01 *** P < 0.001

Mean values in each raw having different superscript (a, b, d, c.....) are significantly different at P < 0.05

Rat groups which administered licorice, vitamin C and licorice with vitamin C showed a non significant difference in levels of liver SOD, catalase, GST and MDA compared to normal rat group.

DISCUSSION

Oxidative stress is a harmful condition that occurs when there is an excess production of oxygen free radicals. Over heated roasted bread produce acrylamide and PAHs which act xenobiotics enhance free radicals. Body weight is frequently the most sensitive indicator of the adverse effects of xenobiotics, so it is considered as a determined parameter of toxicity testing. Administration of vitamin C and licorice increased body weight gain compared to control group, in agreement with previous researchers. Licorice root is used for flavouring, confectionery applications and medicinal purposes for centuries. Licorice Root contains a number of healthy compounds such as flavonoids, volatile oils, plant sterols, coumarins, glycosides, asparagine, chalcones, glycyrrhizic acid and anethole [9]. Besides tasting delicious, licorice is an excellent source of iron. Licorice extract contains the natural sweetener glycyrrhizin, said to be fifty times sweeter than sucrose [24]. Vitamin C or L-ascorbic acid or L-ascorbate is an essential nutrient for humans and certain other animal species. In living organisms ascorbate acts as an antioxidant by protecting the body against oxidative stress and cofactor in at least eight enzymatic [25].

The reduction of AST and ALT activities by vitamin C and licorice as an antioxidant is an indication of repair of tissue damage. This is in agreement with those reported by Rekka *et al.* [26], who found that serum transaminases returned to normal activities with the healing of tissue parenchyma and regeneration of hepatocytes and renal tissues. Vitamin induced suppression of increased ALT and AST activities. Thus, administration of this vitamin C revealed protective activity against the toxic metabolites. Licorice extract could significantly reduce the elevated levels of AST, ALT and MDA and increased the reduced levels of SOD and GSP by CCl₄ toxicity [27]. Glycyrrhizin (10.5 mg/kg) suppressed the increases in AST and ALT and protein, cell infiltration and the degeneration of hepatocytes in the liver of treated mice [28].

It is known that, free radicals are highly reactive molecules that are produced internally by in human organism. The internal anti-free radical system consists of enzymatic and non-enzymatic mechanisms including superoxide dismutase, catalase, carotenoids, polyphenols and anthocyanines among others. If the quantity of free radicals produced by the human body is superior to the physiological and biological processes, the end result is oxidative stress causing cellular damage. Antioxidants protect against cellular damage stress by either preventing the uncontrolled formation of free radicals or directly scavenging them or inhibiting their disruptive reaction with sensitive biological sites [29]. The obtained results revealed better antioxidant activities of licorice and vitamin C resulting from chemical constituents.

Licorice is a widely used Oriental herbal medicine from which the potent antioxidant like phenylflavonoids dehydroglyasperin C, dehydroglyasperin D and isoangustone A are derived. It has also been reported that phenylflavonoids dehydroglyasperin C plays an important role in cancer prevention by inducing detoxifying enzymes. Licorice root contains triterpenesaponins, flavonoids, isoflavonoids and chalcones, coumarins, stilbenoids, as well as miscellaneous compounds as biologically active components [30, 31]. Vitamin C acts as a potent water soluble antioxidant by scavenging reactive oxygen species and reactive nitrogen species. Vitamin C is an excellent source of electron and thus donates electron to free radicals such as hydroxyl radical and superoxide radical and quenches their reactivity. In addition to scavenging action vitamin C can regenerate other small molecule antioxidants such as α -tocopherol, glutathione, urate from their respective radical species. Moreover, the level of liver lipid peroxidation is even reduced, confirming the protective effect of vitamin C supplementation [32, 33]. Based on the obtained results, licorice extract and vitamin C may play an important role in medicine by scavenging free radicals and stimulating activities of antioxidant enzymes in rats consumed over heated roasted bread that can lower the incidence of cancer.

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