

Research Paper

***In vivo* evidence of hepato- and reno-protective effect of garlic oil against sodium nitrite-induced oxidative stress**

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Abstract

Sodium nitrite (NaNO_2), a food color fixative and preservative, contributes to carcinogenesis. We investigated the protective role of garlic oil against NaNO_2 -induced abnormalities in metabolic biochemical parameters and oxidative status in male albino rats. NaNO_2 treatment for a period of three months induced a significant increase in serum levels of glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, urea and creatinine as well as hepatic AST and ALT. However, significant decrease was recorded in liver ALP activity, glycogen content, and renal urea and creatinine levels. In parallel, a significant increase in lipid peroxidation, and a decrease in glutathione content and catalase activity were observed in the liver and the kidney. However, garlic oil supplementation showed a remarkable amelioration of these abnormalities. Our data indicate that garlic is a phytoantioxidant with powerful chemopreventive properties against chemically-induced oxidative stress.

Key words: Food additives; Sodium nitrite ; Oxidative stress; Garlic oil; Liver; glycogen; Alanine aminotransferase; Aspartate aminotransferase; Alkaline phosphatase

Introduction

Natural and synthetic food additives approved by the U.S. Food and Drug Administration are commonly used to maintain or improve safety, the nutrient value, and the taste and texture of food [1]. Although many of the 3,000 these additives enhance our food supply, others are the subject of fierce controversy. The discovery that children at the age of nursery consume food containing great amounts of additives prompted the scientific community to oversee this issue. Sodium nitrite (NaNO_2) is present in vege-

tables and is routinely used as a color fixative and preservative for meats and fish [2]. The hazardous effect of NaNO_2 derives from the reaction of nitrites with amines to produce nitrosamines, and with amides to produce nitrosamides. The toxic effects of nitrates and nitrites are well documented in mammals, including impairment of reproductive function [3], hepatotoxicity and methaemoglobinemia [4], dysregulation of inflammatory responses and tissue injury [5], growth retardation [6], and endocrine dis-

turbance [7]. The wide use of nitrates as preservatives in food technology elevates the importance of studying their effects.

Although NaNO_2 is generally accepted as a weak carcinogen [8, 9], Wistar rats exposed to 0.3% NaNO_2 in their drinking water for at least one year developed squamous papillomas of the forestomach [10]. Using F344 rat in a multiorgan carcinogenesis model, 0.3% NaNO_2 given in the drinking water for 28 weeks increased the incidence of forestomach neoplasm in the post-initiation period [11]. Thus, it cannot be precluded that NaNO_2 has very weak carcinogenic potential, particularly in the squamous epithelium of the forestomach. Moreover, in combination with other chemicals, NaNO_2 has also been shown to form carcinogens or to enhance carcinogenesis. For instance, highly carcinogenic N-nitroso-compounds are produced when nitrite reacts with secondary amines and N-alkyl amides under acidic conditions *in vitro* [8, 12] and *in vivo* [13]. Other studies have demonstrated that treatment with NaNO_2 in combination with phenolic compounds [11, 14] or ascorbic acid [15] strongly enhanced forestomach carcinogenesis in a rat two-stage carcinogenesis model.

Recent trends in controlling and treating diseases tend to favor natural antioxidant compounds rather than synthetic ones [16]. The human diet, which contains large number of natural compounds, is essential in protecting the body against the development of diseases, and the garlic *Allium sativum* is one of the well known plant with remarkable anti-carcinogenic properties is [16, 17]. Garlic, is a commonly worldwide used food, and its medical properties have been well recognized since the ancient times. Garlic is known for its antibacterial [18], anticarcinogenic [19], hypolipidemic [20], hypoglycemic [21], antifungal [22], and anti-atherosclerotic [23] properties, and an antioxidant against free radicals [24, 25].

Here, we investigated the role of garlic oil in preventing NaNO_2 -induced abnormalities in the biochemical parameters associated with the oxidative stress in male albino rats.

Materials and methods

Chemicals

NaNO_2 (Sigma Aldrich, St Louis, MO) was applied as a freshly prepared solution and given by gavages at a dose of 80 mg/kg body weight as previously described [26]. Garlic oil was purchased from El-Captain Company (Cairo, Egypt). Garlic oil was

given by gavages at a dose of 5ml/kg as described [27].

Animals

Male Albino Rats (*Rattus rattus*) weighing about 100-120 g were used in this study. The animals were kept under good ventilation and received a balanced diet and water *ad libitum* throughout the experimental period. Rats were divided into four main groups (n= 6) as follow: 1) Control group received, standard diet without any treatment; 2) Garlic oil-treated group, received standard diet, which was supplemented orally with garlic oil at a dose of 5 ml/kg body weight for a period of 3 months; 3) NaNO_2 -treated group, received standard diet supplemented orally with sodium nitrite at dose of 80 mg/kg body weight for a period of 3 months; and 4) NaNO_2 +garlic oil-treated group, received standard diet and were supplemented orally with similar doses of NaNO_2 and garlic oil as group 3 for similar period of 3 months.

At the end of the experimental period, overnight fasted animals were sacrificed by cervical dislocation, and blood samples were collected in centrifuge tubes. Serum was separated from coagulant blood by centrifugation at 860g for 20 min, and then quickly frozen at -20°C for biochemical analysis. Small pieces of liver and kidney tissues were separated, weighed, homogenized in ice cold water and stored at -20°C for subsequent measurements.

Biochemical analysis

Serum glucose level was determined using the Biomerieux reagent kits [28]. Liver glycogen content was determined according to the method described by Van-Handle [29]. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined according to the method described by Reitman and Frankel [30], whereas alkaline phosphatase (ALP) activity was estimated by Bel-field method [31]. Total protein, bilirubin, urea and creatinine levels were determined using Diamond Diagnostic Kit as previously reported [32-33]. The product of lipid peroxidation, thiobarbituric acid reactive substances (TBARS), was determined as previously described [34]. Glutathione (GSH) content was estimated by the method of Prins and Loose [35], and the activity of catalase was determined by the method of Aebi [36].

Statistical analysis

The data was analyzed using the Statistical Package for Social Science program (S.P.S.S. 11). For comparison between different experimental rat

groups, one way analysis of variance (ANOVA) was used followed by Tukey's test. The results were expressed as means + SE and % of change, and $P < 0.05$ was considered to be statistically significant.

Results

A number of biochemical parameters were examined in the serum collected from each group and the results are summarized in table 1. While rats fed on standard diet supplemented with garlic oil did not show any significant changes in the majority of the parameters examined, a significant increase in serum levels of glucose, bilirubin, urea and creatinine as well as the activity of the AST, ALT and ALP enzymes were observed in rats treated with NaNO_2 for a period of three months. The total protein content significantly decreased in serum (Table 1). However, supplementation of NaNO_2 -intoxicated rats with garlic oil ameliorated the nitrite adverse effects as evidenced by a significant increase of serum total protein content, and a decrease of serum glucose, bilirubin, urea and creatinine levels, as well as the activity of AST,

ALT and ALP enzymes (Table 1). The same parameters were examined in liver and kidney tissues, and the data are shown in table 2. In the NaNO_2 -treated rats, a statistically significant inhibition of hepatic glycogen and total protein contents, and the activity of the enzymes ALT and AST as well as the levels of renal urea, creatinine and total protein content (Table 2). The activity of hepatic ALP was significantly increased in the NaNO_2 -treated rats (Table 2). However, administration of garlic oil to the NaNO_2 -intoxicated rats significantly restored these parameters in the liver and kidney organs (Table 2).

Further, we assessed oxidative stress parameters and antioxidant activity in the liver and the kidney and the results are summarized in table 3. The data indicate that TBARS concentration increased significantly, while GSH content, as well as catalase activity were decreased in both organs of NaNO_2 -intoxicated rats (Table 3). However, combination of garlic oil with NaNO_2 reduced TBARS concentration and restored the levels of GSH as well as the activity of catalase (Table 3).

Table 1. Serum biochemical parameters in different rat groups. Results are presented as means \pm SE (n=5) and % of change. AST: aspartate aminotransferase ; ALT: alanine aminotransferase; ALP: alkaline phosphatase

a: Compared to control group; b: Compared to NaNO_2 treated rats group

* % of change compared with control group.

** % of change compared with NaNO_2 treated rats group.

Parameters	Animal Groups				ANOVA	
	Control	Garlic Oil (GO)	NaNO_2	NaNO_2 +GO	F	P
Glucose (mg/dl)	102.4 \pm 1.5	93.5 \pm 2.5 ^a -8.7*	207.3 \pm 1.5 ^a +102.3*	105.9 \pm 1.1 ^b +3.4*&-48.9**	934.1	P<0.05
AST (U/ml)	88.8 \pm 1.6	88.6 \pm 1.5 -0.2*	138.2 \pm 2.1 ^a +55.6*	115.1 \pm 0.8 ^{a&b} +29.6*&-16.7**	224.8	P<0.05
ALT (U/ml)	22 \pm 0.84	20.8 \pm 0.64 -5.5*	32.2 \pm 1.1 ^a +46.4*	26 \pm 0.63 ^{a&b} +18.2*&-19.3**	38.4	P<0.05
ALP (K.Arm.U/100ml)	21.5 \pm 0.8	19.9 \pm 0.8 -7.4*	27.8 \pm 0.7 ^a +29.3*	22.5 \pm 0.2 ^b +4.7*&-19.1**	70.8	P<0.05
Bilirubin (mg/dl)	0.29 \pm 0.006	0.28 \pm 0.002 -3.4*	0.47 \pm 0.005 ^a +58*	0.34 \pm 0.005 ^{a&b} +15.4*&-27**	425.4	P<0.05
Total protein (g/dl)	6.8 \pm 0.17	7.1 \pm 0.45 +4.4*	4.9 \pm 0.16 ^a -27.9*	6.7 \pm 0.19 ^b -1.5*&+36.7**	3.5	P<0.05
Urea (mg/dl)	37.9 \pm 0.45	36.5 \pm 0.52 -3.7*	49.8 \pm 0.93 ^a +31.4*	38.1 \pm 0.47 ^b +0.53*&-23.5**	97.4	P<0.05
Creatinine (mg/dl)	1.52 \pm 0.11	1.48 \pm 0.08 -2.6*	1.98 \pm 0.14 ^a +35.5*	1.22 \pm 0.04 ^b -20*&-38.4**	12.0	P<0.05

Table 2. Hepatic and renal biochemical parameters in different rat groups. Results are presented as means ± SE (n=5) and % of change. AST: aspartate aminotransferase ; ALT: alanine aminotransferase; ALP: alkaline phosphatase

a: Compared to control group; b: Compared to NaNO₂ treated rats group
 * % of change compared with control group.
 ** % of change compared with NaNO₂ treated rats group.

Parameters	Animal Groups				ANOVA		
	Control	Garlic Oil (GO)	NaNO ₂	NaNO ₂ +GO	F	P	
Liver	Glycogen (mg/100g)	36.7±1.6	42.4±1.5 ^a +13.6*	12±0.5 ^a -67.9*	19±1.5 ^{a&b} -49.2* & +58.3**	115.4	P<0.05
	AST (U/mg)	11.4±0.20	11.7±0.10 +2.6*	10.1±0.21 ^a -11.4*	11.6±0.24 ^b +1.8* & +14.9**	98.2	P<0.05
	ALT (U/mg)	2.7±0.09	2.9±0.13 +7.4*	2.1±0.05 ^a -22.2*	2.6±0.05 ^b -3.7* & +23.8**	110.9	P<0.05
	ALP (K.Arm.U/g)	170.4±2.1	168.2±1.0 -1.3*	271.2±2.1 ^a +59.2*	233.4±1.7 ^{a&b} +37* & -13.9**	1633.1	P<0.05
	Total protein (g/100g)	34.5±0.8	33.4±0.6 -3.2*	31.2±0.5 ^a -9.6*	29.6±0.6 ^a -14.2* & -5.1**	13.1	P<0.05
Kidney	Urea (mg/100g)	34.3±14.4	35.5±12.6 +3.5*	24.5±13.9 ^a -28.6*	27.1±13.3 ^{a&b} -21* & +10.7**	1770.5	P<0.05
	Creatinine (mg/g)	1.02± 0.01	1.04±0.02 +2.0*	0.95±0.02 ^a -6.9*	1.13±0.01 ^{a&b} +10.8* & 18.9**	25.1	P<0.05
	Total protein (g/100g)	42.4±1.5	42.6±2.2 +0.5*	21.1±1.4 ^a -50.2*	39±1.2 ^b -8.0* & +84.8**	40.8	P<0.05

Table 3. Hepatic and renal oxidative stress and anti-oxidant parameters in different rat groups. Results are presented as means ± SE (n=5) and % of change. TBARS: Thio-barbituric acid reactive substance; GSH: Glutathione; CAT: Catalase; MDA: malondialdehyde

a: Compared to control group; b: Compared to NaNO₂ treated rats group
 * % of change compared with control group.
 ** % of change compared with NaNO₂ treated rats group.

Parameters	Animal Groups				ANOVA		
	Control	Garlic Oil (GO)	NaNO ₂	NaNO ₂ +GO	F	P	
Liver	TBARS (nmol/g)	102.5±1.3	70.4±2.2 ^a -31.3*	253.6±2.9 ^a +147.4*	148.4±1.3 ^{a&b} +44.8* & - 41.5**	1580.5	P<0.05
	GSH (mg/g)	0.65±0.02	0.69±0.01 +6.2*	0.52±0.01 ^a -20*	0.66±0.01 ^b +1.5* & +27**	35.8	P<0.05
	CAT (KU/mg)	0.17±0.01	0.19±0.01 +11.8*	0.05±0.01 ^a -70.6*	0.08±0.01 ^{a&b} -52.9* & +60**	66.3	P<0.05
	MDA (nmol/g)	176.6±1.4	139.7±2 ^a -20.9*	234±7.6 ^a +32.5*	203.1±0.6 ^{a&b} +15* & -13.2**	99.8	P<0.05
Kidney	GSH (mg/g)	0.76±0.01	0.77±0.02 +1.3*	0.63±0.02 ^a -17.1*	0.75±0.01 ^b -1.3* & +19**	20.5	P<0.05
	CAT (KU/mg)	0.14±0.01	0.15±0.01 +7*	0.06±0.01 ^a -57*	0.10±0.01 ^{a&b} -28.6* & +66.7**	23.7	P<0.05

Discussion

The NaNO₂ and other additives may react with amines of the foods in the stomach and produce nitrosamines and free radicals. Such products may increase lipid peroxidation, which can be harmful to different organs including liver and kidney [37]. On the other hand, these free radicals, known to cause oxidative stress, can be prevented or reduced by dietary natural antioxidants through their capacity to scavenge these products [38]. The present study was undertaken to determine whether garlic oil can prevent and/or reduce NaNO₂-induced oxidative stress by examining different biochemical parameters of oxidative damage in the serum, the liver and the kidney of male rats.

Our results clearly showed that there was a significant increase in serum glucose concentration and a decrease in liver glycogen content of NaNO₂-treated rats. The findings suggest nitrite-stimulation of gluconeogenesis [39], and glucose shift from tissue to blood or an impairment of glucose mobilization. Furthermore, nitroso-compounds can alter the antioxidant system causing disturbance in the metabolic processes leading to hyperglycemia [40]. However, serum glucose and liver glycogen levels were ameliorated upon garlic oil supplementation. The hypoglycemic effect of garlic oil and its organo-sulfur compounds might be due to their ability to enhance insulin secretion [41, 42].

Our results also indicate an inhibitory effect NaNO₂ on the biosynthesis of protein, which was restored by garlic oil supplementation. These data suggest a stimulation of the thyroid and the adrenal glands by NaNO₂ which can lead to a blockade in protein synthesis, fast breakdown, increased rate of free amino acids, and decreased protein turnover [43]. In addition, nitrite interactions results into nitric oxide release, which can inhibit total protein synthesis [44]. However, the increase in bilirubin concentration as well as the activity of AST, ALT and ALP enzymes in the serum of NaNO₂-treated rats could be attributed to the toxic effect of nitroso-compounds, formed in the acidic environment of the stomach, in causing severe hepatic necrosis [45]. These abnormalities were prevented by supplementation of garlic oil, perhaps due to its role in stabilizing the cell membrane and protect the liver from free radical-mediated liver cell toxicity [46].

In response to NaNO₂ treatment, urea and creatinine were increased in the serum but decreased in the kidney, suggesting an impairment of kidney functions. These effects could also be attributed to the

changes in the threshold of tubular re-absorption, renal blood flow and glomerular filtration rate [47]. Garlic oil showed a clear improvement in kidney functions, perhaps due to the antioxidant properties of garlic in scavenging free radicals leading to reduced levels of nitric oxide and lipid peroxidation. Moreover, NaNO₂-inhibited glutathione content and catalase enzyme activity in the liver and the kidney may be attributed to the observed induction of lipid peroxidation [48]. However, garlic improved the antioxidant mechanism due to the ability of Diallyl disulfide and Diallyl trisulfide present in garlic oil in modulating the oxidative stress and detoxifying enzyme system [49, 50, 51].

In conclusion, from the results achieved it can be concluded that the administration of garlic has an extremely beneficial role in overcoming the occurred adverse effects of chronic ingestion of sodium nitrite, which is probably through its excellent antioxidant properties and highly nutritional values.

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Conflict of Interest

The authors have declared that no conflict of interest exists.

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