The Prophylactic Role of Garlic Oil against deleterious Effects of sodium nitrite(Na No2) in Male Mice

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Abstract

In this study was investigated the Prophylactic role of garlic oil against sodium nitrite $NaNO_2$-induced alteration in physiology and biochemical parameters and oxidative status in male mice. $NaNO_2$ sodium nitrite, a food color fixative and preservative, contributes to carcinogenesis forty 40 male mice were randomly divided into four equal groups (n= 10 as follow: 1- Control group was received standard diet without any treatment. 2-Garlic oil-treated group, was received standard diet, and given orally with garlic oil at a dose of 3 ml/kg body weight for a period of 1months, group 3- $NaNO_2$ treated group, was received standard diet and given orally sodium nitrite at dose of 40 mg/kg body weight, group 4- $NaNO_2$ garlic oil-treated group, was handled as animals group 3 in addition to garlic oil. The results recorded a significant increase ($P<0.05$) in serum levels of glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphates (ALP) activity, serum bilirubin, urea and creatinine concentration as well as hepatic AST and ALT activity, Besides significant decrease ($P<0.05$) was recorded in liver ALP activity, glycogen content, and renal urea and creatinine levels. In addition a significant increase ($P<0.05$) in lipid peroxidation, and a decrease in glutathione content and catalase activity were observed in the liver and the kidney. While garlic oil supplementation showed a remarkable induced alteration in blood matters above in male mice. The data indicate that garlic is a phyto antioxidant with powerful prophylactic properties against $NaNo_2$ chemically-induced oxidative stress.

الدور الوقائي لزيت الثوم بشكل غذاء إضافي ضد تأثير نتريت الصوديوم و بشكل مادة حافظة في ذكور الفئران

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الخلاصة

هذه الدراسة كانت قد بحثت في الدور الوقائي لزيت الثوم ضد التغيرات الناتجة من تأثير نتريت الصوديوم في المعايير الفسلجية والكيموحيوية وحالات الأكسدة في ذكور الفئران، نتريت الصوديوم كمادة حافظة ومثبت للون الطعام تساهم في تكوين التسرطن. تم استخدام 40 من ذكور الفئران كانت قد قسمت إلى أربع مجاميع أساسية بواقع 10 ذكور لكل مجموعة وكما يلي: مجموعة السيطرة كانت قد تلقت الغذاء القياسي بدون معاملة لمدة شهرا والمجموعة الثانية: مجموعة المعاملة كانت قد تلقت الغذاء القياسي المجهز بزيت الثوم بجرعة 3 ملغم من وزن الجسم لمدة شهر واحدا والمجموعة الثالثة مجموعة نتريت الصوديوم كانت قد تلقت الغذاء القياسي مع نتريت الصوديوم المجهز فمويا بجرعة 40 ملغم/ كغم من وزن الجسم ولمدة شهرا واحدا والمجموعة الرابعة

مجموعة المعاملة بنتريت الصوديوم وزيت الثوم والمجهزة في الطعام بنفس الجرع في المجموعة الثانية والثالثة لمجموعة نتريت الصوديوم وزيت الثوم ولمدة شهرا واحد. سجلت النتائج زيادة معنوية على مستوى 5% في مستوى الكلوكوز في المصل والأنزيمات الناقلة للامين AST,ALT وأنزيم الفوسفاتيز القاعدي والبيلروبين واليوريا والكرياتنين إضافة إلى الأنزيمات الناقلة الكبدية AST,ALT كما سجل انخفاض معنوي في فعالية الفوسفاتيز القاعدي في الكبد ومحتوى الكلاكوجين ومستوى اليوريا الكلوية والكرياتنين اضافة إلى زيادة معنوية على مستوى 5% في الدهون الموكسدة ولوحظ انخفاض محتوى الكلوثاثيون وفعالية الكاتيلز في الكبد. أوضح تجهيز زيت الثوم حدوث تغيرات في المعايير أعلاه وأوضحت نتائج الدراسة الحالية بان الثوم مضاد قوي للأكسدة مع خواص كيميائية ضد الإجهاد التاكسدي الناتج من نتريت الصوديوم.

Introduction

Garlic (Allium sativum L.) cloves is well known for its medicinal benefits especially in helping to prevent cancer and cardiovascular diseases (1). Alliins, (S-alk(en) yl-**1**-cysteine sulfoxides) are sources of major active compounds in alliums plants Allicin (diallythiosulfinate) is the main biologically active component of freshly crushed garlic (2). Garlic activity was compared with dietary curcumin and capsaicin (3). The chemo preventive effect of S-allylcysteine (SAC), a water-soluble garlic constituent, against gastric carcinogenesis induced in male mice by N-methyl-N‡nitro-N-nitroso- guanidine (MNNG) and saturated sodium chloride (S-NaCl) was studied (4). The modulatory effects of garlic on hepatic and blood oxidant–antioxidant status may play a key role in preventing cancer development at extra hepatic sites (5). The anti atherogenic effects of egg yolk-enriched garlic powder, which has been used as a traditional health-promoting food in southern Japan since ancient times, on LDL oxidation and oxidant stress- induced cell injury models were reported (6). Garlic was studied in different forms of extracts: aqueous, ethanol, dried powders (7,8). It was shown that aqueous garlic extract alleviates ischemia– reperfusion-induced oxidative hepatic injury in mice (9). In vitro and in vivo experiments conducted mainly with cultures of rat hepatocytes showed the inhibitory effects of garlic on important enzymes in the biosynthesis of cholesterol and fatty acids. The garlic anti atherogenic effects decreased lipid plaques in the arteries of hypocholesterolemic animals and decreased accumulation of cholesterol in the walls of their blood vessels. The reports were based on garlic extracts and their frozen fractions on cholesterol plasma levels and vascular reactivity in cholesterol fed rats (10) Similar studies were conducted on alloxan-induced diabetic rats. Natural and synthetic food additives approved day the U.S. and drug administration are commonly used to maintain or improve safety, the nutrient value and the taste and texture of food (11). 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 tables and is routinely used as a color fixative and preservative for meats and fish (12). The hazardous effect of $NaNo_2$ derives from the reaction of nitrites with amines to produces nitrosamines. With amides to produces nitrosamides. The toxic effects of nitrates and nitrites are well documented in mammalians, including impairment of reproductive function (13). Hepatotoxicity and methaemogobenemia(14). disregulation of inflammatory responses and tissue injury (15). Growth retardation (16). And endocrine disturbances (17) instance, highly carcinogenesis N- nitroso-compounds are produced when nitrite reacts with secondary amines and N-alkyl amides under acidic conditions in vitro (21) and in vivo (22). Other studies have demonstrated that treatment with NaNo2 in combination with phenolic compounds (23) or ascorbic acid (24). Strongly enhanced fore stomach carcinogenesis in

a mice two –stage carcinogenesis model. Garlic is known for its antibacterial (27) anticarcinogenic (28) hypolipidemic (29) hypoglycemic (30), anti fungal (31) anti-atherosclerotic (32), and antioxidant properties (33). In this study, we investigated the role of garlic oil in preventing NaNo2-induced changes in the biochemical parameters associated with the oxidative stress in male mice.

Materials and Methods

- **Chemicals:** $NaNo_2$ was applied as a freshly prepared solution and given by gavages at a dose of 60 mg/kg B., (34).garlic oil was given by gavages at a dose of 3ml/kg (35).
- **Animals:** Male mice weighing about 50-60 g were used in this study. The animals were kept under good ventilation and received a balanced diet and water throughout the experimental period. Male mice were equally divided into four groups at (10 for each), group 1: as control recived, standard diet without any treatment, group 2: garlic oil-treated group, received standard diet, was given orally with garlic oil at a dose of 3ml/kg b.w for a period of 1month And, 3: $NaNo_2$ treated group received standard diet supplemented orally with $NaNo_2$ at a dose of 50 mg/kg B.w 4: group $NaNo_2$ +garlic oil treated group, received standard diet and were supplemented orally with similar doses of $NaNo_2$ and garlic oil as group 3 .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 650 nm for 30min 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 measurement hepatic AST and ALT activity, liver ALP activity, glycogen content parameters and anti oxidant substance (malondialdehyde) MDA, catalase CAT, glutathione GSH in kidney tissue.
- **Biochemical analysis:** Serum glucose level was determined using the Bio merieux reagent kits (35). Liver glycogen content was determined according to the method described by (36). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined according to the method described by (37).whereas alkaline phosphatase (ALP) activity was estimated by Belfield method (38). Total protein, bilirubin, urea and creatinine levels were determined using Diamond Diagnostic Kit as previously reported (39). The product of lipid peroxidation, thiobarbituric acid reactive substances (TBARS), was determined in serum as previously described (40). Glutathione (GSH) content was estimated by the method of (41). And the activity of catalase was determined by the method of (42).
- **Statistical analysis:** The data was analyzed using the Statistical, one way analysis of variance by SPSS.

Results

A number of biochemical parameters were examined in the serum collected from each group and the results are summarized in table 1. While mice 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 mice treated with $NaNO_2$ for a period of one months. The total protein content significantly decreased in serum (Table 1). However, supplementation of $NaNO_2$ intoxicated mice 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 mices, a statistically significant decrease 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 (Table2). The activity of hepatic ALP was significantly increased in the $NaNO_2$-treated mice (Table 2). However, administration of garlic oil to the $NaNO_2$-intoxicated mice 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 NaNO2-intoxicated mice (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 mice groups. Results are presented as means ± SE (n=10). Serum glucose, Total protein, bilirubine, urea, creatinine concentration, serum alanine aminotransferase ALT, aspartate aminotransferase AST; alkaline phosphatase ALP activity in mice with $NaNo_2$, garlic or both for 1 month

Parameters	Group 1 (Control)	Group 2 (Garlic oil)	Group 3 (NaNo2)	Group 4 ($NaNO_2$+Garlic oil)
Glucose mg/dl	99.2±1.2 A	97.2±0.9 A	100.2±0.2 A	99.7±1.1 A
AST U/ml	82.4±0.4 A	83.3±0.1 A	84.3±0.5 A	83.5±0.3 A
ALTU/ml	19.6±0.4 A	18.4±0.6 A	20.8±0.4 A	19.9±0.2 A
ALP U/100ml	20.8±0.8 A	19.2±0.7 A	20.9±0.2 A	19.7±1.2 A
Total Protein g/dl	5.6±0.2 A	6.0±0.5 A	5.3±0.1 A	5.8±0.4 A
Bilirubin mg/dl	0.18±0.001 A	0.15±0.002 B	0.23±0.005 C	0.20±0.008 C
Urea mg/dl	30.8±0.3A	29.8±0.6 A	32.4±0.9 A	32.0±0.1 A
Creatinine mg/dl	1.2±0.2 A	1.4±0.5 A	1. 6±0.1 A	1.2±0.8 A

Table (2) Renal biochemical parameters in different mice groups. Results are presented as means ± SE (n=10). measurements Serum glucose, Total protein, bilirubine, urea, creatinine concentration , serum alanine aminotransferase ALT, aspartate aminotransferase AST; alkaline phosphatase ALP activity in mice with $NaNo_2$, garlic or both for 1 month

Parameters	Group 1 (Control)	Group 2 (Garlic oil)	Group 3 ($NaNO_2$)	Group 4 ($NaNO_2$+Garlic oil)
GLYCOGEN MG/100G	30.6±0.6 A	32.3±0.2 A	28.0±1.0 B	28.8±1.2 B
AST U/MG	10.4±0.5 A	10.8±0.8 A	10.0±0.1 A	10.7±0.9 A
ALT U/MG	1.9±0.06 A	2.6±0.01 B	1.7±0.08 A	2.2±0.05 B
TOTAL PROTEIN G/100G	30.5±0.3 A	30.2±0.1 A	29.6±0.5 A	28.8±0.9 B
UREA MG/100G	33.4±0.4 A	33.9±0.8 A	30.5±1.2 B	31.5±1.2 B
CREATININE MG/G	1.3±-0.01 A	1.0±0.04 A	0.9±0.02 A	1.5±0.02 A
TOTAL PROTEIN G/100G	40.1±0.8 A	40.8±0.4 A	39.6±0.9 A	39.0±0.2 A
ALP U/G	100.2±1.0 A	105.4±1.0 B	108.1±1.5 C	110.2±1.8 C

Table (3) Hepatic, renal biochemical parameters in different mice groups. Results are presented as means ± SE (n=10). measurements thiobarburic acid reactive Substance (TBARS), glutathione (GSH), catalase (CAT), malondialdehyde (MDA)

Parameters	Group 1 (Control)	Group 2 (Garlic oil)	Group 3 ($NaNO_2$)	Group 4 ($NaNO_2$+Garlic oil)
TBARS NMOL/Gin liver	99.3±1.2 A	95.0±2.4 B	109.2±2.0 C	102.2±2.5 B
GSH MG/Gin liver	0.22±0.1 A	0.25±0.1 B	0.20±0.3 A	0.28±0.4 C
CAT KU/MG in liver	0.12±0.05 A	0.15±0.02 B	0.10±0.02 A	0.09±0.01 C
MDA NMOL/G in kidney	150.4±1.5 A	144.4±1.7 B	164.0±2.3 C	160.2±2.2 D
GSH mg/G in kidney	0.50±0.05 A	0.55±0.02 B	0.48±0.02 A	0.58±0.06 C
CAT KU/MG in kidney	0.10±0.02 A	0.13±0.01 B	0.9±0.05 A	0.10±0.01 A

Discussion

The $NaNO_2$ 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, an excessive production of reactive oxygen species (ROS) above the body's antioxidant capacity, has been implicated in the development of many path physiological conditions including hypertension, (38,2). Our results clearly showed that there was a significant increase in serum glucose concentration and a decrease in liver glycogen content of $NaNO_2$-treated mice. The findings suggest nitrite-stimulation of gluconeogensis (39,8), and glucose shift from tissue to blood or an impairment of glucose mobilization. Furthermore, nitroso-compounds can alter the anti-oxidant system causing disturbance in the metabolic processes leading to their capacity to scavenge these hyperglycemia (40). However, serum glucose and liver glycogen levels were ameliorated upon garlic oil supplementation. The hypoglycemic effect of garlic oil and its organ sulfur compounds might be due to their ability to enhance insulin secretion (41, 42). These results are in agreement with our earlier observation that the hypertensive effect of garlic in mice (43,41). The results also indicate an inhibitory effect $NaNO_2$ 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 $NaNO_2$ which can lead to a blockade in protein synthesis, fast breakdown, increased rate of free amino acids, and decreased protein turnover (44). In addition, nitrite interactions results into nitric oxide release, which can inhibit total protein synthesis (45). However, the increase in bilirubin concentration as well as the activity of AST, ALT and ALP enzymes in the serum of $NaNO_2$-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 (46). 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 (47,5). In response to $NaNO_2$ 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 (48). 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, $NaNO_2$-inhibited glutathione content and catalase enzyme activity in the liver and the kidney may be attributed to the observed induction of lipid peroxidation (49). However, garlic improved the antioxidant mechanism due to the ability of Diallyl disulfide and Diallyl tri sulfide present in garlic oil in modulating the oxidative stress and detoxifying enzyme system (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.

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