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The Protective Effect of Radish (*Raphanus sativus*) Seeds Against the Oxidative Stress Induced by Sodium Nitrite in Male Rabbits (*Oryctolagus cuniculus*)

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Abstract:

The aim of this study is to investigate the protective effect of Radish (*Raphanus sativus*) seed alcoholic extract 70% against oxidative stress induced by sodium nitrite NaNO_2 . Twenty five adult male rabbits were divided into five groups of (five rabbits in each group) and treated daily for 30 days. Group T1: intubated orally 20 mg/kg NaNO_2 , Group T2: intubated orally 20 mg/kg NaNO_2 + 50 mg/kg of alcoholic extract from *Raphanus sativus* seeds, Group T3: intubated orally 20 mg/kg NaNO_2 + 100 mg/kg of alcoholic extract from *Raphanus sativus* seeds, Group T4: intubated orally 20 mg/kg NaNO_2 + 200 mg/kg of alcoholic extract from *Raphanus sativus* seed as well as Group C: control intubated orally distilled water. In comparison with normal rabbits there were significantly increase in the level of Methemoglobin (MetHb%), lipid peroxidation indicator malondialdehyde (MDA), and significantly decrease in the level of glutathione (GSH) in the rabbits intubated NaNO_2 . In rabbits intubated NaNO_2 + 50,100,200 mg/kg of alcoholic extract it was found there were significant decreased in the level of MetHb%, MDA, and increase in the level of GSH. In conclusion according to results obtained from this study approved that alcoholic extract of *Raphanus sativus* seeds in dose (50,100,200 mg/kg) have protective effect against oxidative stress induced by sodium nitrite in male rabbits.

Key words: Radish seed, Sodium nitrite, Methemoglobin (MetHb), malondialdehyde (MDA), glutathione (GSH).

Introduction:

Food Additives are used for maintaining the high quality of foods. Food preservatives are the additives that are used to inhibit the growth of bacteria, molds and yeasts in the food. Sodium nitrite, an inorganic salt is used as a preservative and a color fixative in meats and fish it stabilizes the color of preserved fish and meats and also it inhibits the growth of *Clostridium botulinum*[1].

Sodium nitrite with both healthful and harmful effects, acts as vasodilator, Bronchial dilator Intestinal relaxant [2], Antidote for cyanide poisoning [3] resp. The toxic effects of nitrites in various mammalian species involved hepatotoxicity, nephrotoxicity, deterioration of reproductive function, endocrine disturbance, growth retardation, dysregulation of inflammatory responses and cause

tissue injury[4]. Sodium nitrite exerts its effect by increase production of free radicals that cause imbalance pro-oxidant / anti-oxidant system . The main biological effects of nitrite in human include the oxidation of normal haemoglobin (Hb) to methaemoglobin (metHb). The latter is unable to transport oxygen to the tissues. Increase the level of metHb cause cyanosis and, at higher concentrations, asphyxia. Nitrite methemoglobinemia is a process that responsible for free radical generation. Free radicals are reactive molecules these have one or more unpaired electrons and are naturally produced in human body by natural biological processes or may be resulting from an outside source (such as tobacco smoke, toxins, or pollutants). These free radicals are toxic compounds that can attack and damage biomolecules, including proteins, nuclei acids and lipids. These damages cause different diseases including atherosclerosis, arthritis, neurodegenerative disorders and cancer etc [5]. It has been reported that Nitrite has been enhance lipid peroxidation (LPO) and cause change in antioxidant enzyme activity. Previous studies suggest that intracellular reactive oxygen species (ROS) production is a possible mechanism underlying nitrite toxicity. These reactive oxygen species (ROS) induce lipid peroxidation (LPO), a chain process which affects unsaturated fatty acids mainly localized in cell membranes leading to generation of malondialdehyde. These damages can be neutralized with antioxidant systems such as glutathione, glutathione reductase, etc. and nutritional antioxidants from diet such as Ascorbic acid (vitamin C), Tocopherols and tocotrienols (vitamin E) carotenoids etc. Glutathione is one of the major studied antioxidants. it is present in all the cells. GSH act as

antioxidant and detoxification of ROS. If the natural antioxidants are unable to scavenger the ROS, when free radicals cause damage to the molecules, a condition known as oxidative stress occurs where in cellular function can be affected and cells may be damaged[6]. Risks associated with food preservatives urged for study the protective effect of Radish seed against the hazardous effect of sodium nitrite. Radish has been used as medicinal foods for several disease, involving liver dysfunction and poor Digestion[7]. There are some studies which showed that the radishes or radish extracts have biological activities including antioxidant, Antimutagenic, and antiproliferative effects [8]. Previous study Explained that *R. sativus* extract have a protective effect against sodium nitrite that lead to elevated blood MetHb% and MDA and lowering GSH level , white *R.sativus* seed extract revealed that this extract have antioxidant effect by increasing GSH level and lowering MetHb and MDA level [9].

Materials and Methods:

Animals and experimental design

In this study, 25 adult male rabbits were kept in conditioned room (22-25°C) with providing proper ventilation. Rabbits were left for two weeks for adaptation with the experimental conditions. Animals treated daily for four weeks. They were randomly divided into five groups, (5 rabbits/group). Group C (control): administered distilled water orally, Group T1: Animals in this group were administered 20 mg/kg b.w of sodium nitrite orally by gavage needle, Group T2: Animals in this group were administered 20 mg/kg b.w of sodium nitrite plus 50 mg/kg b.w of ethanol extract from (*Raphinus sativus*) orally by gavage needle,.

Group T3: Animals in this group were administered 20 mg/kg b.w of sodium nitrite plus 100 mg/kg b.w ethanol extract from (*Raphinus sativus*) orally by gavage needle. Group T4: Animals in this group were administered 20 mg/kg b.w of sodium nitrite plus 200 mg/kg b.w ethanol extract from (*Raphinus sativus*) orally by gavage needle. Fasting Blood samples were collected from animals by cardiac puncture technique and MetHb, MDA, GSH parameters were then measured.

Plant materials

The plant materials (seeds) were obtained from commercial sources from Baghdad and then deposited to be identified and authenticated at the National Herbarium of Iraq Botany directorate in Abu-Ghraib. The plant materials were extracted according to [10].

Phytochemical analysis: There were two tests for detection of one active compound.

Detection of Alkaloids by Mayer's test:- Appeared of white or creamy precipitate indicate the presence of alkaloids. Detection of Alkaloids by wagner's test:- A reddish or brown precipitate appeared indicate the presence of alkaloids.

Detection of Saponins by Foam test:- 1 cm layer of foam appeared indicate the presence of saponins. Detection of Saponins by Mercuric chloride test:- Appearance of white precipitate indicate the presence of saponins.

Detection of Tannins by Ferric chloride test:- A dark green color appeared indicate the presence of Tannins. Detection of Tannins by Lead acetates test:- Bulky red precipitate indicate the presence of Tannins.

Detection of Terpenoids by Chloroform-sulphuric acid test :- A reddish brown colour indicate the presence of Terpenoids. Detection of Terpenoids by Anace-aldehyde test:- A

brown precipitate indicate the presence of Terpenoids.

Detection of Flavonoids by Magnesium test :- Appearance of pink to crimson colour indicate the presence of flavonoids. Detection of Flavonoids by Sulphuric acid test:- A greenish yellow precipitate appeared indicate the presence of flavonoids [11].

Determination of Methemoglobin (MetHb)

MetHb level was determined according to the method of [12]. Add 0.1ml of blood test tube containing 3.9 ml of distilled water and mixed well. Add 4 ml of phosphate buffer saline PBS and mixed well. Add (1.5ml) of PBS and (1.5ml) of distilled water to blank cuvet... this is C1. Then 3ml of hemolysate blood was added to test tube C2, C3. Then, add (0.1ml) of $K_3Fe(CN)_6$ to one test tube and mixed three times and left for two minutes. Measuring the absorbance at wavelength 630 nm of each C2, C3 with use C1 as a blank. The result recorded as A2a, A3a. Then add (0.1ml) of KCN to test tube C2 and C3. Mixed three times and left for 5 minutes, the absorbance measured again at the same wave length with use C1 as blank, the result recorded as A2b, A3b.

Methemoglobin (percent of total pigment) = $\{(A2a - A2b) / (A3a - A3b)\} \times 100$

Determination of Malondialdehyde (MDA)

Determination of MDA according to the method of [13]. One hundred fifty μ L of serum sample was poured in a test tube and 1ml of 17.5% TCA was added, then one milliliter of 0.6% TBA was added. Tubes were mixed well by vortex, incubated in boiling water bath for 15 minutes, and then allowed to cool, then one milliliter of 70% TCA was added, The mixture was left to stand at room temperature for 20

minutes then the tubes were centrifuged at 2000 Xg for 15 minutes, and the supernatant was taken out for measuring spectrophotometrically (at 532 nm).

Malondialdehyde (MDA) concentration

$$= (\text{Absorbance} / L \times \epsilon) \times D$$

L: Light bath (cm), ϵ : Extinction coefficient $1.56 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$ D: Dilution factor = (volume used in Ref. (ml) / 0.15) = (1+1+1+0.15) / 0.15 = 21

Determination of Glutathione (GSH)

Determination of Glutathione (GSH) according to the method of [14]. Duplicates of each standard and sample test tubes were prepared. Solutions were mixed as in the following:

Reagents	Sample (μL)	Reagent blank (μL)	Standard (μL)
Serum	100	-	-
Standard	-	-	100
DW	800	900	800
TCA	100	100	100

Tubes were mixed in a vortex mixer intermittently for 10-15 min, and centrifuged for 15 min at 3000 xg, then pipette into test tubes.

Reagents	Sample (μL)	Reagent blank (μL)	Standard (μL)
Supernatant	400	400	400
Tris-EDTA	800	800	800
DTNB reagent	20	20	20

Tubes were mixed in a vortex mixer, the spectrophotometer was adjusted with reagent blank to read zero absorbance (A) at 412 nm and the absorbance of standards and sample was read with in 3 min of the addition of the DTNB reagent, The concentration of serum GSH is obtained from the calibration curve in μM :-

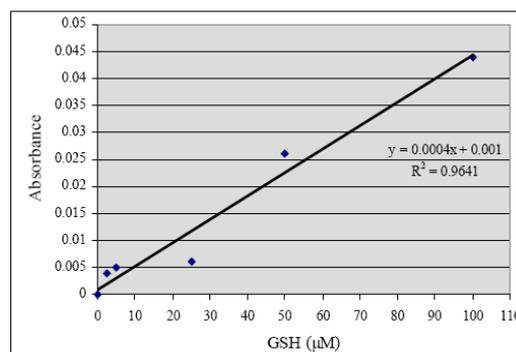


Fig. (1): calibration curve for serum glutathione (GSH) concentration (mmol/L)

Results:

The results of phytochemical analysis in table {1} explained that *Raphanus sativus* seed contains some of active ingredients that include:

Table (1): Phytochemical analysis of *Raphanus sativus* seed

Active ingredients	Reagents or Tests
Alkaloids	Mayer's Reagent (+), Wagner's reagent (+)
saponins	foam test (+), Mercuric chloride test (+)
Tannins	Ferric chloride test (+), Lead acetates test (+)
flavonoids	Magnesium test (+), Sulphuric acid test (+)
Terpenoids	Chloroform, H_2SO_4 (+), Anace-aldehyde reagent (+)

The results of current study explained methemoglobin (MetHb%) level in table {2} that there was a significant increase ($P \leq 0.05$) in MetHb% level in animals treated with sodium nitrite compared with control(C), and there was a significant decrease in animals treated with sodium nitrite and *Raphanus sativus* seed extract (50,100,200 mg/kg) compared animal group treated with sodium nitrite and normalize the level of MetHb% in T_2 , T_3 , T_4 as compared with control.

Table (2): The Effect of 70% *Raphanus sativus* seed extract in Methemoglobin (MetHb%) in control and sodium nitrite treated rabbits

Stage	Treatment					LSD value
	Cont.	T1	T2	T3	T4	
Pre	12.22 ± 0.35 _a	11.5 ± 0.39 _a	11.66 ± 0.42 _a	11.86 ± 0.55 _a	12.18 ± 0.38 _a	6.83 NS
Post	11.74 ± 0.41 _b	40.92 ± 1.8 _a	8.17 ± 0.26 _b	10.76 ± 0.63 _b	12.40 ± 0.41 _b	13.59 *
LSD value	5.77 NS	12.68 *	6.84 NS	5.72 NS	5.09 NS	----

* (P<0.05), NS: Non-significant.

Values expressed as mean ± SE n=5 each group; C: control group; T₁: animals received 20 mg/kg B.W sodium nitrite orally; T₂: animals received 20 mg/kg B.W sodium nitrite + 50 mg/kg B.W *Raphanus sativus* seed extract; T₃: animals received 20 mg/kg B.W sodium nitrite + 100 mg/kg B.W *Raphanus sativus* seed extract; T₄: animals received 20 mg/kg B.W sodium nitrite + 200 mg/kg B.W *Raphanus sativus* seed extract. Small letters denote differences between groups, P≤0.05 Vs control.

The results of table {3} show that there was a significant increase

(P<0.05) in serum level of malondialdehyde (MDA) in animal treated with sodium nitrite compared with control(C), and non-significant increase in animals treated with sodium nitrite and *Raphanus sativus* seed extract(50,100,200 mg/kg) compared with control, but there was a significant decrease in the level of MDA in animals treated with sodium nitrite and *Raphanus sativus* seed extract(50,100,200 mg/kg) compared with animal group treated with sodium nitrite.

Table (3): The Effect of 70% *Raphanus sativus* seed extract on serum malondialdehyde (MDA) concentration (mmol/L) in adult male rabbits

Parameter	Treatment					LSD value
	Cont.	T1	T2	T3	T4	
MDA	0.133 ± 0.005 _b	0.516 ± 0.029 _a	0.264 ± 0.007 _b	0.228 ± 0.003 _b	0.158 ± 0.003 _b	0.218 *

* (P<0.05).

Values expressed as mean ± SE n=5 each group.

The results of table {4} show that there was a significant decrease in serum level of glutathione (GSH) in animal treated with sodium nitrite compared with control, and there was a significant decrease in animals treated with sodium nitrite and *Raphanus sativus* (50,100,200 mg/kg) as compared with control. also there was non significant increase in serum

level of GSH in animals treated with sodium nitrite and *Raphanus sativus* (50,100 mg/kg) compared sodium nitrite animals group, and there was a significant increase in serum level of GSH in animals treated with sodium nitrite and *Raphanus sativus* (200 mg/kg) compared with animal group treated with sodium nitrite.

Table (4): The Effect of 70% *Raphanus sativus* seed extract on serum glutathione (GSH) concentration (mmol/L) in adult male rabbits

Parameter	Treatment					LSD value
	Cont.	T1	T2	T3	T4	
GSH	4.446 ± 0.26 _a	2.234 ± 0.13 _c	2.756 ± 0.17 _{b,c}	2.856 ± 0.09 _{b,c}	3.51 ± 0.14 _b	0.872 *

* (P<0.05).

Values expressed as mean ± SE n=5 each group.

Discussion:

The results of the present study pointed to significant change in MetHb level in group treated with sodium nitrite. This change manifested by an increase in MetHb level in agreement with [15]. The main toxic effect of sodium nitrite, It acts as pro-oxidant induces a primary extensive methemoglobin formation as a result of production of several free radical species such as hydroxyl, superoxide anion, peroxy nitrite and nitrogen oxide radicals which are implicated in promoting oxidation of Hb by nitrite. Nitrite reacts with oxygenated Hb to form metHb and nitrate (NO_3^-), and it reacts with deoxygenated Hb to form metHb and nitric oxide (NO) leading to methemoglobinemia. Nitrite is readily absorbed from the digestive tract and diffuses into the red blood cells where it oxidizes the ferrous ion (Fe^{+2}) of the oxyhemoglobin (oxy Hb) molecules to the ferric state (Fe^{+3}) forming met Hb. Elevation of MetHb level lead to reduce of oxygen transport, And when MetHb levels increase can be life threatening [16]. The results of current study showed that significant decrease glutathione (GSH) level and Elevation of malondialdehyde (MDA) level indicate for lipid peroxidation in group treated with sodium nitrite, These results agreed with those reported by [17], [18], [19]. The high MDA level in serum may reflect the oxidative stress exerted different tissues as it has been reported that oxidant/antioxidant status may reflect the extracellular response to the external agents or the tissue status. These results indicated elevated lipid peroxide, Malondialdehyde. The elevated amounts of MDA may be due to generation of reactive oxygen species (ROS) and also attributed to the depletion of liver GSH resulting in tissue degeneration and elevated lipid

peroxidation products in target organs. NaNO_2 inhibited glutathione activity in the plasma may be attributed to the observed induction of lipid peroxidation (LPO) and may be explained according to their function as a free radical scavenger, which suppress the formation of the reactive oxygen species (ROS) and/or oppose their action [20]. The decreased GSH content in the present study may be attributed to the increased LPO rather than reduced synthesis [21]. Glutathione (GSH) is a major non-enzymatic antioxidant molecule that is involved in the second line of defense against free radical damage in the body. GSH donates an electron in the reduction of peroxides catalyzed by Glutathione peroxidase (GSHpx) as a component of the enzyme system containing GSH oxidase and reductase. Some studies observed a decreased level of GSH, this is due to the most hepatic reduced glutathione (GSH) is converted to its oxidized form Glutathione disulfide (GSSG) by the enzyme glutathione reductase that protect the cells from damage caused by free radicals. Previous studies suggested that the decrease in GSH level may be due to the consumption of antioxidants and the increased production of ROS due to the toxic action of nitrosamine and free radicals generated by sodium nitrite [22].

According to The results of the current study which showed that there was a significant decrease in level of MetHb and MDA in animals treated with sodium nitrite and *Raphanus sativus* seed extract (50,100,200 mg/kg) compared animal group treated with sodium nitrite only, and there is significant increase in serum level of GSH in animals treated with sodium nitrite and *Raphanus sativus* (200 mg/kg) compared with animal group treated with sodium nitrite. These

results agreed with [23] explained that the radish fed group decrease free radicals production. This was indicated by the lowest the level of malondialdehyde and the reactive nitrogen species, specifically the powerful oxidant molecule peroxynitrite (ONOO⁻). Radish seeds were found to contain alkaloid such as flavonoids, saponins, coumarins and anthocyanins. The anthocyanins are important dietary antioxidants that have many physiological functions. They protect living cells from harmful effect of oxidative stress resulting in the prevention of diseases. Oil of Raphani Semen was revealed to have significant antioxidative activity in the ferric reducing *antioxidant* power assay [24]. *R. sativus* is one of the major sources of dietary flavonoids which are powerful antioxidants against free radicals and are described as free-radical scavengers [25].

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التأثير الواقي لبذور الفجل (*Raphanus sativus*) ضد الاجهاد التاكسدي
المستحدث بواسطة نترتيت الصوديوم في ذكور الارانب (*Oryctolagus*
(*cuniculus*)

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الخلاصة :

أن الهدف الرئيسي لهذه الدراسة هي معرفة التأثير الواقي لبذور نبات الفجل على الاجهاد التاكسدي المستحدث بواسطة نترتيت الصوديوم. استعمل في الدراسة الحالية 25 ارنب ذكر ، قسمت الى خمس مجاميع كل مجموعة مؤلفة من 5 حيوانات. المجموعة الاولى (مجموعة السيطرة C) جرعت فمويا بجرعة من الماء المقطر مساوية لحجم الجرعة المعطاة لمجاميع المعالجة. المجموعة الثانية T₁ جرعت فمويا ب 20 ملغم/كغم من مادة نترتيت الصوديوم ولمدة 4 اسابيع . المجموعة الثالثة T₂ جرعت فمويا ب 20 ملغم/كغم من مادة نترتيت الصوديوم واعطيت المستخلص الكحولي لبذور نبات الفجل وجرعة 50 ملغم/كغم. المجموعة الرابعة T₃ جرعت فمويا ب 20 ملغم/كغم من مادة نترتيت الصوديوم واعطيت المستخلص الكحولي لبذور نبات الفجل وجرعة 100 ملغم/كغم. المجموعة الخامسة T₄ جرعت فمويا ب 20 ملغم/كغم من مادة نترتيت الصوديوم واعطيت المستخلص الكحولي لبذور نبات الفجل وجرعة 200 ملغم/كغم. اوضحت نتائج هذه الدراسة حصول ارتفاع معنوي في نسبة الميتيهموكلوبين وناتج اكسدة الدهون وانخفاض معنوي لمحتوى الكلوتاثيون في الحيوانات المعاملة بمادة نترتيت الصوديوم فقط، وكذلك حصول انخفاض معنوي في نسبة الميتيهموكلوبين وناتج اكسدة الدهون وحصول ارتفاع في محتوى الكلوتاثيون في الحيوانات المعاملة بمادة نترتيت الصوديوم والمستخلص الكحولي 200,100,50 ملغم/كغم . اعتمادا على نتائج هذه الدراسة نستنتج ان المستخلص الكحولي لبذور نبات الفجل 50 و 100 و 200 ملغم/كغم يمتلك الفعالية كمضاد للاجهاد التاكسدي المستحدث بمادة نترتيت الصوديوم.

الكلمات المفتاحية : بذور نبات الفجل، نترتيت الصوديوم، ميتيهموغلوبيين، ماونداي الديهايد، كلوتاثيون.