# Biochemical Studies On The Effect Of Sodium Nitrite And/Or Glutathione Treatment On Male Rats

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## Abstract

**Introduction:** Using food preservatives as sodium nitrite are increased in industrial food productions. Teratogenic, mutagenic and carcinogenic effects have been related to using of some food preservatives.

**Purpose:** To study the effect of sodium nitrite (food additives) and treated with glutathione (nature antitoxic) on rats.

**Material And Methods:** Certain parameters were measured as percentage of body weight change**,** body temperature, heart rates, Red & white blood cells count (RBCs & WBCs), hemoglobin (Hb) level, hematocrite (Hct) value, serum total lipids, serum cholesterol, serum total protein, serum albumin, serum glucose, serum alanine transaminase and aspartate transaminase (ALT & AST) activity and serum total cholinesterase. The organs, body weight were detected. Organs were prepared for biochemical analysis.

**Results:** Body weight, respiration rate, hepatosomatic index, RBCs & WBCs count, Hb, Hct, serum total lipids, protein, albumin, A/G ratio, liver and muscle total lipids and cholesterol were significantly reduced while serum cholesterol, kidney total lipids and cholesterol, serum ALT & AST was significantly increased. Supplementation of sodium nitrite to rats had no effect on serum glucose level or cholinesterase activity

**Conclusion:** Due to the hazardous effect of food additives as sodium nitrite, it is recommended that the use of sodium nitrite as food additives must be limited and gluathione has the ability to prevent its toxic effect.

**Key Words:** Glutathione, Rats, Sodium nitrite

## Introduction

Food additives are substances intentionally added to food. They may be natural or synthetic (Harris, 1986). The principal classes of food additives are coloring agents, preservatives, flavors, emulsifiers and stabilizers (Lindsay, 1985). One of the principal preservatives is the nitrite, which used in the form of salts or free acids (HMSO[1], 1987). The use of sodium nitrite as a preservative is common in cooked meat and sausages. Because of the use of more than one type of such food, the percentage of nitrite content of the daily food consumption may be higher than the admissible level (Bilczuk *et al*., 1991). Apparently very little nitrites are formed by endogenous synthesis and most, if not all are of dietary origin (Bartholomew and Hill, 1984).

Nitrites are used as human food additives mainly for production of specific flavor and for preservation of meat products. Several organic nitrites and nitrates have been used clinically but the only inorganic nitrites of therapeutic are sodium nitrite (Heibashy and Abd El-Moneim, 1999). Nitrites and nitrates are environmental pollutants present in food and water and it is suggested that they may contribute to the etiology of liver and kidney diseases and problems related of immunity in domestic fowls (Ibrahim *et al.*, 1999). Glutathione (GSH) may act as free radicl acceptor to counteract oxidant damage. Favilli *et al.* (1997) have noticed

[1] **H M S O: Her majesty's stationary office, London**

that dietary GSH can be absorbed intact from the intestinal lumen and the orally administered GSH increases plasma GSH concentration. GSH plays an important role in protecting cells against oxidative stress and toxic agents by virtue of being substrate for glutathione peroxidase (El-Missiry *et al.*, 1995). Almar *et al.* (1998) indicated that the glutathione system constitutes a sensitive biochemical indicator of chemical pollution. Acetylcholinesterase inhibition is one of the most important negative effects and the interaction with the enzyme results in acute cholinergic poisoning (Wills 1972; and Howard & Janice 1989).

## Aim Of The Work

The study aimed to study the effect of sodium nitrite (food additives) and glutathione (antioxidants) as protective agent on rat's lipid profile, kidney and liver function, and some enzymes.

## Material And Methods

The present study was carried out on forty immature growing male albino rats of about one month age. The weight range was from 40-50 g. The animals were kept in clean cages. Food and water were added ad-libitum. The Experimental animals were divided into four groups (10 rats/group) as follows: Group 1(G 1): serve as control group; Group 2 (G 2): supplemented with glutathione (6.42 mg/kg BW); Group 3 (G 3): supplemented with sodium nitrite (30 mg/kg BW); Group 4 (G 4): supplemented with sodium nitrite and glutathione. These treatments were administrated orally by stomach tube for six months. All animals groups were weighted before beginning and monthly during the experiment period. Half of each group was decapitated after 3 month, while the other half were left till the end of 6 months. At the end of the experiment, animals were decapitated and blood samples were taken in dry clean centrifuge tube. Serum was separated and kept at –20 °C until analysis. At the same time, blood samples for hematological analysis were taken in heparinized capillary tube. The organs (brain, liver, kidney, heart and skeletal muscle) were removed and cleared in isotonic saline solution then weighted. A piece of each organ were weighted and put in appropriate amount of 30 % KOH for total protein and in conc. $H_2SO_4$ for total lipid determination. Other pieces of organ were weight and put in saline solution then homogenized for biochemical analysis.

**Measured Parameters**

Certain parameters were measured. Percentage of body weight change was recorded monthly. Heart rates and respiration rate were recorded according to the method of Soliman e*t al.* (1973); Body temperature was recorded according to the method of MaCaffery *et al.* (1979). Red & white blood cells were counted according to Mitruka *et al.* (1977). Hemoglobin was determined according to Van Kampen and Zijlstra (1961). Hematocrite value was determined according to Rodak (1995). Serum total lipids were determined according to Knight *et al.* (1972). Serum cholesterol was determined according to Martinek *et al.* (1970). Serum total protein was determined according to Doumas (1975). Serum albumin was determined according to Doumas *et al.* (1971). Serum glucose was measured according to Trinder (1969). Serum alanine transaminase and aspartate transaminase (ALT & AST) activity were detected according to Reitman & Frankel (1957). Serum total cholinesterase was recorded according to Gorun *et al.* (1978).

**Statistical Analysis**

Data are expressed as Mean ±SE. Data were assessed by t-test (Armitage 1974; and Lenter *et al.*, 1982). P-values < 0.05 were considered statistically significant.

## Result

Data of table (1) shows insignificant change in body weight, heart beat rate, rectal temperature, respiration rate in rats treated with glutathione alone or in combination with sodium nitrite in comparison with the control group. Also data of table (1) revealed that rats treated with sodium nitrite alone showed highly significant decrease in body weight (P<

0.01), respiration rate (P< 0.01) when compared with the control group during the experimental period.

Data of table (2) shows insignificant changes in the percentage of organ /body weight ratio of male albino rats treated with glutathione or in combination with sodium nitrite for three and six months in brain, kidney and heart. On the other hand, a highly significant decrease (P < 0.01) was recorded in hepatosomatic index in rats treated with sodium nitrite alone or in combination with glutathione after 3 & 6 months when compared with the control group.

Data of table (3) shows insignificant change in the red blood corpuscles (RBCs) count, hemoglobin (Hb) concentration hematocrite (Hct) value, MCV and MCH of rats treated with glutathione alone or in combination with sodium nitrite for three and six months. Also table (3) revealed that a significant decrease in RBCs count, WBCs count, Hb and Hct, group treated with sodium nitrite alone after 3 & 6 months in comparison with the control group except MCV and MCH where they showed a significant increase (P < 0.05) after 3 & 6 months.

Table (4) shows that total lipids, total cholesterol, total protein, albumin, globulin levels and A/G ratio of rats treated with glutathione alone or in combination with sodium nitrite revealed insignificant difference after 3 & 6 months of treatment while rats treated with dual treatment (sodium nitrite and glutathione) showed that cholesterol level were significantly increased (P<0.01). On the other hand, a significant decrease (P < 0.01) in total lipid, total protein, albumin and globulin level was observed in rats treated with sodium nitrite after 3 & 6 months of treatment in comparison with the control group, while a significant increase (P < 0.01) in total cholesterol level in comparison with the control group was recorded. No significant was observed in A/G ratio except rats treated with Na nitrite for 3 months where a significant decrease was observed (P<0.05).

Table (5) shows that rats supplemented with glutathione showed insignificant difference in total lipid content in brain, liver, muscle, kidney and heart tissues after 3 & 6 months of treatment in comparison with the control group. On the other hand, rats treated with sodium nitrite revealed a significant decrease (P< 0.05 & 0.01) in total lipid content in musle, and liver tissues respectively, while total lipid content in kidney were significantly increased. Rats treated with dual treatment (sodium nitrite & glutathione) showed a significant decrease (P< 0.05) in total lipids of liver tissue after three and six months.

Table (6) shows that rats treated with sodium nitrite and/or glutathione revealed insignificant change in total cholesterol in brain, liver, kidney, muscle and heart tissues after three and six months in comparison with the control group while rats treated with sodium nitrite for six months showed a significant decrease (P< 0.01) in total cholesterol in heart tissue.

Data of table (7) shows that rats treated with sodium nitrite and/or antioxidants (glutathione) revealed insignificant change in total protein in brain, liver, kidney, muscle and heart tissues throughout the experiment periods in comparison with the control group.

Data of table (8) revealed that rats treated with glutathione alone or sodium nitrite and glutathione showed insignificant change in serum glucose level after 3 & 6 months. Serum activity of AST, ALT of rats treated with glutathione alone or in combination with sodium nitrite revealed insignificant difference after 3 & 6 months. On the other hand, rats treated with sodium nitrite showed a significant increase (P< 0.01) in AST activity after 3 & 6 months when compared with the control group, while highly significant increase (P< 0.01) in ALT activity were observed after 6 months only. Obtained data (in table 8) revealed insignificant difference in cholinesterase activity in all treated groups after 3 & 6 months.

From table (9) It is clear that rats treated with glutathione alone or in combination with sodium nitrite or treated with sodium nitrite alone revealed insignificant change in AST, ALT activity in brain, liver, kidney, muscle, and heart tissues after 3 & 6 months when compared with the control group except AST, ALT activity of liver rats treated with sodium nitrite alone where it showed a significant increase (P< 0.05; 0.01 respectively).

**Table (1): Percentage of body weight changes, heart beat, rectal temperature and respiration rate of male albino rats treated with food additives and antioxidants for three and six months.**

| | | Third month | | | | Six month | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH |
| Body Weight | Mean ±S.E | 23.56 2.83 | 18.20 2.12 | 13.92** 0.95 | 24.71 1.68 | 22.11 1.27 | 17.25 2.15 | 12.80** 1.07 | 18.05 1.94 |
| Heart Beat | Mean ±S.E | 315.00 4.01 | 313.00 2.60 | 314.00 3.71 | 317.00 3.35 | 312.00 3.74 | 318.00 3.74 | 306.00 6.78 | 318.00 3.74 |
| Rectal Temperature | Mean ±S.E | 37.35 0.04 | 37.46 0.06 | 37.34 0.06 | 37.37 0.05 | 37.26 0.05 | 37.32 0.06 | 37.24 0.05 | 37.31 0.07 |
| Respiration Rate | Mean ±S.E | 110.60 1.82 | 111.20 1.09 | 101.3** 1.53 | 112.10 2.10 | 109.40 2.79 | 110.40 1.44 | 99.00** 0.89 | 109.0 2.17 |

* = P< 0.05 significant. ** = P < 0.01 highly significant

**Table (2): Mean values of weight of different organs of male albino rats treated with food additives and antioxidants for three and six months.**

| | | Third month | | | | Six month | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH |
| Brain | Mean | 0.95 | 0.80 | 1.03 | 0.82 | 0.59 | 0.73 | 0.57 | 0.61 |
| | ±S.E | 0.06 | 0.03 | 0.09 | 0.04 | 0.01 | 0.09 | 0.03 | 0.02 |
| Liver | Mean | 3.43 | 3.18 | 3.86* | 2.43** | 3.01 | 3.04 | 3.30** | 2.41** |
| | ±S.E | 0.10 | 0.08 | 0,14 | 0.13 | 0.08 | 0.18 | 0.04 | 0.05 |
| Kidney | Mean | 0.62 | 0.61 | 0.54 | 0.52 | 0.65 | 0.67 | 0.64 | 0.60 |
| | ±S.E | 0.02 | 0.03 | 0.05 | 0.04 | 0.04 | 0.05 | 0.06 | 0.01 |
| Heart | Mean | 0.34 | 0.27 | 0.35 | 0.29 | 0.29 | 0.32 | 0.33 | 0.31 |
| | ±S.E | 0.03 | 0.01 | 0.03 | 0.01 | 0.01 | 0.02 | 0.03 | 0.01 |

* = P< 0.05 significant. ** = P < 0.01 highly significant

**Table (3): Mean values of Haemogram (R.B.Cs., W.B.Cs., Hb, Hct, MCV , MCH and MCHC) of male albino rats treated with food additives and antioxidants for three and six months.**

| | | Third month | | | | Six month | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH |
| RBCs | Mean | 7.88 | 7.56 | 5.48** | 7.62 | 7.50 | 6.52 | 4.78** | 6.52 |
| | ±S.E | 0.12 | 0.20 | 0.13 | 4.14 | 0.41 | 0.13 | 0.14 | 0.16 |
| WBCs | Mean | 11.56 | 9.64 | 7.46** | 10.04 | 12.32 | 11.62 | 8.22** | 10.60* |
| | ± S.E | 0.88 | 0.45 | 0.44 | 0.17 | 0.40 | 0.37 | 0.35 | 0.46 |
| Hb (g/dl) | Mean | 15.18 | 16.00 | 13.06** | 14.76 | 14.38 | 14.16 | 12.08** | 13.64 |
| | ±S.E | 0.59 | 0.19 | 0.16 | 0.34 | 0.38 | 0.48 | 0.51 | 0.41 |
| Hct | Mean | 47.06 | 49.44 | 38.34** | 50.16 | 45.76 | 42.56 | 37.14** | 43.96 |
| | ±S.E | 1.93 | 2.04 | 1.70 | p.so | 1.37 | 1.01 | 1.39 | 2.29 |
| MCV | Mean | 59.72 | 65.40 | 69.96** | 65.83 | 61.01 | 65.28 | 77.70** | 67.42 |
| | ±S.E | 2.57 | 2.43 | 1.86 | 11.27 | 2.18 | 2.73 | 4.65 | 4.07 |
| MCH | Mean | 19.26 | 21.16 | 23.83** | 19.37 | 19.17 | 21.72 | 25.27** | 20.92 |
| | ±S.E | 0.89 | 0.59 | 0.53 | 0.94 | 1.41 | 0.85 | 0.71 | 0.68 |

* = P< 0.05 significant. ** = P < 0.01 highly significant

**Table (4): Serum total lipids, total cholesterol, total protein, albumin, globulin and A/G ratio of different organs of male albino rats treated with food additives and antioxidants for three and six months.**

| | | Third month | | | | Six month | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH |
| Total Lipids (mg/dl) | Mean | 352.32 | 331.80 | 278.3** | 294.88 | 357.52 | 352.66 | 257.84 | 344.84 |
| | ±S.E | 8.21 | 11.98 | 6.61 | 16.80 | 8.05 | 6.17 | 7.14 | 9.45 |
| Total Cholesterol (mg/dl) | Mean | 123.24 | 124.24 | 151.8** | 132.1* | 114.34 | 1 12.98 | 135.6** | 135.06* |
| | ±S.E | 2.01 | 2.22 | 6.00 | 2.21 | 4.91 | 3.58 | 3.30 | 6.09 |
| Total Protein (g/dl) | Mean | 6.90 | 6.98 | 5.82** | 6.68 | 6.76 | 6.36 | 5.70** | 6.40 |
| | ±S.E | 0.07 | 0.15 | 0.06 | 0.20 | 0.10 | 0.17 | C.07 | 0.12 |
| Albumin (g/dl) | Mean | 4.50 | 4.66 | 3.36** | 3.96 | 4.43 | 4.58 | 3.52** | 4.51 |
| | ±S.E | 0.08 | 0.21 | 0.11 | 0.29 | 0.07 | 0.15 | 0.13 | 0.16 |
| Globulin (g/dl) | Mean | 2.40 | 2.32 | 2.46 | 2.72 | 2.33 | 1.78 | 2.18 | 1.89 |
| | ±S.E | 0.14 | 0.09 | 0.06 | 0.16 | 0.04 | 0.16 | 0.13 | 0.32 |
| A/G ratio | Mean | 1.88 | 2.01 | 1.37* | 1.46 | 1.90 | 2.57 | 1.61 | 2.39 |
| | ±S.E | 0.14 | 0.16 | 0.08 | 0.21 | 0.02 | 0.31 | 0.17 | 0.29 |

* = P< 0.05 significant. ** = P < 0.01 highly significant

**Table (5): Tissue total lipids (mg/g tissue) of different organs of male albino rats treated with food additives and antioxidants for three and six months.**

| | | Third month | | | | Six month | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH |
| Brain | Mean | 79.48 | 82.94 | 73.32 | 86.90 | 59.44 | 61.50 | 53.60 | 57.54 |
| | ±S.E | 5.63 | 2.29 | 3.43 | 3.78 | 4.28 | 1.38 | 3.16 | 3.80 |
| Liver | Mean | 91.76 | 93.24 | 73.2** | 75.98* | 86.08 | 76.66 | 63.3** | 68.32* |
| | ±S.E | 3.51 | 4.88 | 3.55 | 3.32 | 4.29 | 2.28 | 3.57 | 3.87 |
| Kidney | Mean | 55.92 | 59.20 | 75.3** | 54.34 | 62.82 | 57.12 | 82.1** | 55.42 |
| | ±S.E | 1.90 | 2.75 | 4.18 | 1.70 | 3.61 | 3.20 | 3.76 | 4.29 |
| Muscle | Mean | 51.08 | 62.24 | 33.5** | 61.80 | 40.98 | 41.80 | 32.6** | 38.76 |
| | ±S.E | 6.74 | 3.02 | 3.20 | 2.26 | 2.30 | 3.70 | 1.90 | 1.29 |
| Heart | Mean | 58.60 | 62.68 | 58.78 | 69.66 | 53.58 | 51.02 | 42.42 | 52.38 |
| | ±S.E | 2.61 | 2.99 | 2.35 1 | 4.44 | 6.50 | 4.73 | 4.42 | 4.24 |

* = P< 0.05 significant. ** = P < 0.01 highly significan

**Table (6): Tissue total cholesterol (mg/g tissue) of different organs of male albino rats treated with food additives and antioxidants for three and six months.**

| | | Third month | | | | Six month | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH |
| Brain | Mean | 66.42 | 73.89 | 71.12 | 77.93 | 80.54 | 75.66 | 70,71 | 82.46 |
| | ±S.E | 3.24 | 4.83 | 3.92 | 4.92 | 3.89 | 2.87 | 2.82 | 3.87 |
| Liver | Mean | 55.47 | 57.73 | 52.70 | 56.23 | 59.39 | 58.08 | 62.39 | 57.80 |
| | ±S.E | 2.77 | 2.99 | 3.92 | 2.24 | 3.69 | 2.88 | 3.93 | 2.53 |
| Kidney | Mean | 61.33 | 67.43 | 53.25 | 56.23 | 61.56 | 64.45 | 62.27 | 59.34 |
| | ±S.E | 2.72 | 2.94 | 2.4>2 | 3.91 | 2.46 | 2.53 | 3.96 | 2.40 |
| Muscle | Mean | 55.50 | 59.14 | 49.35 | 56.98 | 55.97 | 63.57 | 54.85 | 60.05 |
| | ±S.E | 3.79 | 4.99 | 2.92 | 2.61 | 4.83 | 3.40 | 2.43 | 2.88 |
| Heart | Mean | 64.20 | 64.94 | 55.45 | 62.58 | 68.08 | 52.70 | 55.1** | 61.82 |
| | ±S.E | 3.11 | 3.46 | 2.92 | 3.22 | 2.49 | 2.25 | 3.06 | 3.55 |

* = P< 0.05 significant. ** = P < 0.01 highly significant

**Table (7): Tissue protein (mg/g tissue) of different organs of male albino rats treated with food additives and antioxidants for three and six months.**

| | | Third month | | | | Six month | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH |
| **Brain** | **Mean** | **43.36** | **38.52** | **39.82** | **37.56** | **44.06** | **39.68** | **38.32** | **40.48** |
| | **±S.E** | **1.80** | **2.43** | **2.53** | **3.32** | **2.75** | **2.48** | **3.05** | **2.83** |
| **Liver** | **Mean** | **40.06** | **31.86** | **32.32 i** | **34.50** | **49.74** | **48.20** | **42.84** | **42.80** |
| | **±S.E** | **3.26** | **5.02** | **2.18** | **1.70** | **2.81** | **4.20** | **1.81** | **2.47** |
| **Kidney** | **Mean** | **26.04** | **30.02** | **30.12** | **28.50** | **44.22** | **42.14** | **40.40** | **37.94** |
| | **±S.E** | **3.77** | **1.63** | **2.44** | **1.90** | **4.44** | **2.31** | **3.37** | **2,12** |
| **Muscle** | **Mean** | **32.62** | **25.60** | **28.46** | **39.14** | **42.76** | **51.70** | **47.06** | **52.20** |
| | **±S.E** | **3.14** | **1.18** | **1.38** | **2.71** | **4.30** | **2.02** | **1.97** | **3.56** |
| **Heart** | **Mean** | **33.40** | **36.26** | **23.20** | **27.66** | **38.60** | **45.98** | **44.74** | **38.08** |
| | **±S.E** | **4.44** | **1.36** | **1.44** | **3.01** | **2.21** | **3.51** | **3.32** | **2.15** |

**Table (8): Serum glucose, AST, ALT and cholinesterase of different organs of male albino rats treated with food additives and antioxidants for three and six months.**

| | | Third month | | | | Six month | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH |
| **Glucose Mg/dl** | **Mean** | 99.38 | 95.60 | 87.36 | 97.58 | 96.34 | 95.52 | 82.34 | 91.16 |
| | **±S.E** | 6.34 | 5.41 | 9.00 | 4.33 | 4.16 | 4.10 | 5.26 | 5.70 |
| **AST IU/L** | **Mean** | 27.94 | 30.94 | 55.3** | 25.66 | 30.98 | 29.36 | 39.3** | 29.62 |
| | **±S.E** | 1.55 | 1.64 | 4.11 | 2.52 | 0.40 | 1.07 | 2.01 | 0.97 |
| **ALT IU/L** | **Mean** | 24.50 | 26.78 | 38.36 | 21.16 | 24.64 | 25.74 | 31.8** | 25.88 |
| | **±S.E** | 1.50 | 0.81 | 1.36 | 2.69 | 1.19 | 1.72 | 1.31 | 0.74 |
| **Cholinesterase IU/ml** | **Mean** | 11.70 | 12.16 | 10.22 | 13.06 | 10.50 | 11.82 | 8.86 | 11.44 |
| | **±S.E** | 0.89 | 0.17 | 0.57 | 0.89 | 1.05 | 0.38 | 0.77 | 0.43 |

* = P< 0.05 significant. ** = P < 0.01 highly significant

**Table (9): Tissue AST, ALT (u/g tissue) of different organs of male albino rats treated with food additives and antioxidants for three and six months.**

| | | Third month | | | | Six month | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH | Control | Glutathione (GSH) 6.42 mg/kg | Na. nitrite 30 mg/kg | Na. nitrite +GSH |
| **AST** | | | | | | | | | |
| **Brain** | **Mean** | 17.84 | 22.98 | 24.56 | 23.68 | 22.30 | 25.26 | 25.52 | 24.60 |
| | **±S.E** | 1.92 | 2.20 | 1,04 | 0.91 | 1.88 | 1.13 | 1.24 | 1.06 |
| **Liver** | **Mean** | 20.22 | 18.22 | 31.0** | 23.08 | 21.72 | 18.30 | 34.02 | 22.12 |
| | **±S.E** | 2.49 | 1.75' | 1.54 | 2.00 | 2.61 | 1.71 | 2.17 | 1.86 |
| **Kidney** | **Mean** | 20.60 | 19.92 | 23.24 | 24.90 | 21.12 | 22.34 | 24.06 | 21.92 |
| | **±S.E** | 2.27 | 2.68 | 2.55 | 3.19 | 1.38 | 1.85 | 0.99 | 1.91 |
| **Muscle** | **Mean** | 18.62 | 19.64 | 21.56 | 23.40 | 18.54 | 19.92 | 22.10 | 18.20 |
| | **±S.E** | 1.64 | 1.75 | 3.68 | 2.12 | 1.68 | 1.84 | 1.88 | 1.76 |
| **Heart** | **Mean** | 16.52 | 18.28 | 22.78 | 21.52 | 22.88 | 19.86 | 23.70 | 19.76 |
| | **±S.E** | 1.82 | 2.13 | 3.62 | 3.33 | 2.23 | 1.87 | 2.17 | 1.68 |
| **ALT** | | | | | | | | | |
| **Brain** | **Mean** | 16.14 | 18.48 | 16.04 | 15.30 | 15.94 | 16.48 | 16.64 | 16.14 |
| | **±S.E** | 1.36 | 1.56 | 1.47 | 1.26 | 0.90 | 1.11 | 1.69 | 1.24 |
| **Liver** | **Mean** | 14.40 | 16.84 | 25.54** | 16.62 | 15.44 | 16.20 | 21.80** | 14.24 |
| | **±S.E** | 1.48 | 2.00 | 2.62 | 1.59 | 1.12 | 1.34 | 1.04 | 0.45 |
| **Kidney** | **Mean** | 15.22 | 19.20 | 17.16 | 16.14 | 16.66 | 16.18 | 17.04 | 17.00 |
| | **±S.E** | 1.77 | 1.82 | 3.34 | 1.49 | 1.13 | 1.34 | 1.45 | 1.43 |
| **Muscle** | **Mean** | 13.70 | 15.20 | 21.56 | 13.66 | 19.98 | 17.24 | 19.72 | 17.44 |
| | **±S.E** | 1.02 | 1.79 | 0.97 | 1.68 | 2.55 | 1.19 | 2.99 | 1.29 |
| **Heart** | **Mean** | 16.20 | 19.30 | 24.04 | 21.2? | 17.84 | 18.54 | 20.02 | 15.88 |
| | **±S.E** | 1.33 | 1.43 | 1.85 | 1.78 | 1.52 | 1.47 | 2.84 | 0.94 |

* = P< 0.05 significant. ** = P < 0.01 highly significant

## Discussion

In the present work, rats treated with sodium nitrite showed highly significant decrease in body weight and less weight gain than the control group throughout the experiment periods, and this reduction may be due to the reduction of food utilization (Grant and Butler, 1989) or vitamin C deficiency (Uchida *et al.*, 1990), or may also be due to increased level of sodium nitrite in the body leading to increased catabolic process in the body. The results of this study are in agreement with Grant and Butler (1989); and Porter *et al.* (1993). In general the reduction in body weight may be attributed to the decrease in food intake, the disturbance in hormonal balance and direct cytotoxic effect of sodium nitrite treatment.

The present study revealed insignificant change in rats heart beat rate in any treated groups till the end of the experiment. The stability of body temperature may be due to the high ability of rats to adjust their body temperature (Helal *et al.*, 1997).

Data of the present study showed a significant increase in hepatosomatic index in groups treated with sodium nitrite due to the toxic effect of sodium nitrite on liver components as nucleic acids, glycogen, fat and protein which accounts for liver growth (Ravinder *et al.*, 1989 and Dikshith *et al.*, 1991).

Data of the present study showed a highly significant decrease in RBCs count, Hb concentration and Hct ratio in groups treated with sodium nitrite. The decrease may be attributed to microcytic and /or hypochromic anemia possibly as a consequence of the toxic effect of sodium nitrite on bone marrow, spleen and liver (Mason *et al.*, 1974; Abu El-Zahab *et al.*, 1997). Hassan *et al.* (1988) reported that this reduction might be due to sodium nitrite administration, which accompanied by a remarkable increase of methemoglobin (met Hb) level. It is known that the nitrite converts the ferrous ion of Hb to ferric ion. Beaupre and Schiffman (1994); and Nyakas *et al.* (1994) found that sodium nitrite increase met Hb, but had no effect on RBCs hemolysis. Vorhess *et al.* (1984) reported that sodium nitrite in drinking water of pregnant mice produce maternal anemia and increase offspring mortality. Nitrite is readily absorbed from the digestive tract and diffuses into the red blood cells where it oxidizes the ferrous ion of the oxyhemoglobin (oxy Hb) molecules to the ferric state forming met Hb (Ibrahim *et al.*, 1999).

Results of the present work indicate that administration of sodium nitrite revealed highly significant decrease in white blood cells of rats after 3 & 6 months, but rats treated with sodium nitrite combined with glutathione showed a significant decrease after 6 months only. Our results are in agreement with Tan *et al.* (1992) and Soheir *et al.* (1996). The reduction in WBCs count lower the defense mechanism (immune system) which play an important role in attacking and interacting with foreign antigens and initiating a primary immune response. The decrease in WBCs count after treatment with sodium nitrite may be due to the failure of the hematopoietic tissues to produce new WBCs (Tan *et al.*, 1992). Results of the present work indicate that MCH and MCV of rats treated with sodium nitrite showed a significant increase throughout the experiment period. This result disagrees with Wang and Davidson (1983).

Result of the present work indicates that administration of sodium nitrite revealed highly significant decrease in serum total lipids of rats after 3 months only. Also a significant decrease in total lipid of liver of rats treated with sodium nitrite alone or in combination with glutathione was recorded. These decreases in total lipids may be due to lipolysis, via stimulation of hormone sensitive lipase (Abd El-Dayem, 2002).

Data of the present work revealed a highly significant increase in serum total cholesterol levels in rats treated with sodium nitrite alone or in combination with glutathione. This elevation may be attributed to the blockage of liver bile ducts, causing reduction or cessation of its secretion to the duodenum. Consequently, it appeared in the serum causing cholestasis (Gomaa (1995); and Helal *et al.*, (1997).

Also the elevation may be due to the mobilization of free fatty acids from the adipose tissue to the blood stream and increase level of acetyl CoA, leading to increase in the synthesis of cholesterol or due to peroxidation of cell membrane lipids (Standberg 1977). The results are in agreement with the finding of Hassan *et al.*, (1995); and Helal *et al.*, (1997).

The present result indicated a highly significant decrease in serum total proteins, and albumin levels throughout the experiment period in sodium nitrite treated groups. This reduction may be due to substantially of protein synthesis by the liver. This depression may be due to an alteration in the intracellular protein synthesis mechanism and that the oxidative enzyme change were probably secondary in altering proteins. These results were in agreement with Hurkat (1977); Shakoori *et al.* (1988). Also Mekkawy *et al.* (1988) and Amr *et al.* (1994) reported that the decreases of protein might be due to reduction of serum globulin level supports with the disturbances on the immunoglobulin production, these was accompanied by a decrease of body weight gain and this may be as a result of toxicity especially on the muscle. The decrease in serum albumin level may be due to loss of protein from the alimentary tract, or due to decreased albumin formation in the liver. These results were in harmony with that obtained by Said *et al.* (1992), concluded that the decrease in serum albumin level was due to trap of protein from the alimentary tract or due to hepatic necrosis as a result of sodium nitrite administration to male albino rats. Rats treated with sodium nitrite showed significant decrease in globulin level after 3 months' treatment, which may be due to the disturbance on immunoglobulin production. Eremin and Yocharina (1981) reported that sodium nitrite blocks protein synthesis while fast breakdown occurs. This leads to an increase of free amino acids and to a decrease of protein turnover (Yanni *et al.*, 1991).

The present data showed insignificant decrease in serum glucose level of rats treated with sodium nitrite.

Liver has a vital physiological role in case of toxicity-liver eliminates the toxicants, after their metabolization and degradation. This process in turn disturbs the integrity of cell membrane resulting in an increase of the enzyme level in blood (Begum and Vijayaraghvan, 1995). The present results showed that rats treated with sodium nitrite revealed a highly significant elevation in serum AST, ALT activity. Elevation of transferase activity in blood has been considered as an indicator of tissue damage. However, other factors are considered for this process such as alteration in permeability of cell membrane, increasing the synthesis of the enzyme or decreasing the rate of degradation of the enzyme (Dinman *et al.*, 1963; Luckens and Phelps 1969). Ignatov (1976) recorded that the elevation in serum AST is due to the degradation and necrosis of liver cells, which is accompanied by a damage of cell wall, cytolysis and so pouring a considerable amount of these mitochondrial enzymes into the blood stream. However the adverse effect of nitrite on liver could be attributed to oxidation of important iron containing enzymes such as cytochromes responsible for cellular respiration and other oxidation-reduction process. Helal *et al.* (1997) recorded a significant increase of serum AST & ALT activity in rats treated with sodium nitrite. Also Helal *et al.* (1997) suggested that the observed stimulation of ALT activity was due to sodium nitrite interaction with the enzyme molecule rather than with the tissues.

Generally the activities of ALT & AST are considerably increased following the administration of various hepatotoxic compounds, which lead to acute hepatocellular damage or extrahepatic obstructions by being considered as highly sensitive liver markers (Abu El-Zahab *et al.*, 1997). Saleh (1986) related the elevation of ALT & AST activities to the damage of the liver cell, while Abu El-Zahab *et al.* (1997) report that coloring agents induce liver tissue damage and cause a significant increase of ALT, AST & alkaline phosphatase activities in rats serum.

Data of the present work showed that rats treated with sodium nitrite and/or glutathione showed insignificant decrease in serum cholinesterase activity after 3 & 6 months of treatment which are in agreement with Abd El-Rahiem *et al.* (1999).

## Conclusion

Due to the hazardous effect food additives as sodium nitrite, it is recommended that the use of sodium nitrite as food additives must be limited and use of gluathione as antioxidant to prevent the toxic effect.

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# دراسات كيميائية حيوية على تأثير معاملة نتيريت الصوديوم و الجلوتاثيون كعامل حماية على صغار ذكور الفئران

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**المقدمة:**

إن استخدام المواد الحافظة كنتيريت الصوديوم فى الصناعات الغذائية أخذ فى الزيادة. يعزى بعض التأثيرات الوراثية والسرطانية الى استخدام بعض هذه المواد الحافظة. لذا هدفت هذه الدراسة لتوضيح تأثير نيتريت الصوديوم (مضافات غذائية) و الجلوتاثيون (مضاد للأكسدة طبيعي) على الفئران.

**المواد والطرق:**

تم قياس بعض المتغيرات مثل نسبة التغير فى وزن الجسم، درجة حرارة الجسم، معدل ضربات القلب/دقيقة، عدد كرات الدم الحمراء والبيضاء، الهيموجلوبين، الهيماتوكريت، الدهون الكلية، الكوليسترول، البروتين الكلى، الأليبومين، الجلوكوز، آلانين ترانس اميينيز، اسبرتات تراس امينييز، الكولين استيريز.

**النتيجة:**

وجد نقص ملحوظ فى معظم المتغيرات مثل وزن الجسم، معدل التنفس، نسبة وزن الكبد/ وزن الجسم، عدد كرات الدم الحمراء، البيضاء والكوليسترول واللبيدات الكلية فى الكبد والعضلات، بينما لوحظ ارتفاع واضح فى كوليسترول الدم، كوليسترول والدهون الكلية فى الكلى،و الآلانين ترانس امينيز، اسبرتات ترانس امينيز.

لوحظ عدم تأثر مستوى السكر (الجلوكوز)، الكولين استيريز فى الدم عند إضافة نيتريت الصوديوم الى غذاء الفئران.

**الاستنتاج:**

نتيجة للتأثيرات العشوائية لمضافات الأغذية مثل نيتيريت الصوديوم، فإنه يوصى بتحديد استخدام نيتريت الصوديوم مع استخدام الجلوتاثيون كمادة مضادة للأكسدة لمنع التأثير الضار لمضافات الأغذية.