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Biochemical Studies on Effect of Sodium Nitrite and Monosodium Glutamate (MSG) on Male Rats

KEYWORDS: Food Additives, Monosodium Glutamate, Sodium Nitrites, MDA

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ABSTRACT *Preservatives consider one of the most important food additives that used in most food industries like meat, fish, oil and others. The aim of the present study is to determine the effect of two `widely used preservatives, sodium nitrite and monosodium glutamate (MSG) on male rats by different doses. The experiment was carried out on 50 adult male rats weighing 150-200 gm. They have been divided into 5 groups,10 rats in each group (control group, second and third group were maintained on ration contains sodium nitrite at concentration 0.2% and 0.4% of the diet respectively, fourth and fifth group were maintained on ration contains MSG at concentration 0.04mg/kg and 0.08 mg/kg of the diet respectively). Blood and tissue samples were collected after 6weeks from dietary manipulation. The biochemical results of sodium nitrite administrated rats showed significant increase in urea, creatinine, and MDA levels as well as glucose, ALT and AST levels. In addition, there were decrease in testosterone hormone, GSH, body weight and hemoglobin concentration. The biochemical results of MSG treated rats showed increase in body weight, urea, creatinine levels as well as MDA and glucose levels. Moreover, increase activity of ALT and AST were seen.*

INTRODUCTION

Food additives are substances intentionally added to food that changes its characteristics, to maintain and improve safety (preservatives), to improve or maintain nutrient value and to improve taste, texture and appearance. **(U.S Food and Drug Adminstration and International Food Information Council, 2004)**.

Sodium nitrite is an inorganic salt with wide spread applications in food industry as color fixative and preservative in meat and fish and also it inhibits the growth of *Clostridium botulinum,*. It is consumed in the manufacturing of azo dyes, nitroso compounds and other organic compounds **(EFSA, 2009)**. Furthermore, sodium nitrite has many medical applications; it is used as a vasodilator a bronchial dilator, an intestinal relaxant (Hunter et al, 2004). post hemorrhagic cerebral vasospasm **(Pluta et al.,2005)** and in myocardial infarction (Webb et al,2004) . In veterinary medicine, it is used as an antiseptic by topical application to the teats of dairy cows after milking in order to prevent mastitis **(EFSA, 2009)**.

The toxic effects of nitrates and nitrites are well documented in mammalians including impairment of reproductive function, hepatotoxicity, dysregulation of inflammatory responses and tissue injury, growth and retardation and endocrine disturbance. It inhibits a number of anti-tumor cytotoxic effector cell types as natural killer cells against pathogens and tumor cells **(Abuharfeil et al,2001) .** Sodium nitrite exerts its effect by generation of free, radicals that impair oxidant / antioxidant balance **(Naik et al,2006).**

Reactive nitrogen species produced by exposure to nitrate is considered one of the most important causes of carcinogenesis through its reaction with body tissues and triggering lipid peroxidation, DNA lesions, enzyme inactivation and damage of different organs **(El-Wakf *et al.*, 2009).**

Monosodium glutamate (MSG) is commonly consumed as a flavor enhancer or food additive **(Moore, 2003; Alao et al, 2010)** improves the palatability of meals and thus influences the appetite center positively with its resultant increase in body weight **(Gobatto *et al.*, 2002).** MSG is the sodium salt of the non-essential amino acid-glutamic acid **(NHIC, 2008)**. MSG contains 78% of glutamic acid, 22% of sodium and water (**Samuels, 1999)**.It is known to have some adverse effects in humans and experimental animals. It is metabolized in liver and eliminated through the kidney **(Schwerine et al, 1950).** Glutamic acid is transformed into alanine in intestinal mucosa and lactate in liver **(Bhattacharya et al., 2011).** Chronic administration of MSG (4mg/k and above) induced oxidative stress in experimental animals.MSG causes retinal degeneration, endocrine disorder, addiction, stroke, epilepsy, brain trauma, neuropathic pain, schizophrenia, anxiety, depression, Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis **(Adrienne, 1999; Eweka and Adjene, 2007)**. Subsequently it was documented that MSG produces oxygen derived free radicals **(Singh and Ahluwalia, 2003).** It is reported that MSG causes changes in the liver parenchyma of mice around central vein, dilated sinusoids, inflammatory cells and nuclei were pyknotic **(Bhattacharya et al, 2011).**

AIM OF THE WORK

In the present investigation, we planned to study the biochemical harmful effect of MSG (mono sodium glutamate) as flavoring agent and sodium nitrite as antimicrobial , which commonly used in the food industry on male rats.

MATERIAL AND METHODS

1) Animal:-

The present study was carried out on a total number of (50) adult male Spraque Dawely rats, weighing 150-200g. The animals were obtained from Animal Health Research Center in Dokki.

Diet & Management of rats:

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They were kept for 4 weeks for acclimatization at the Animal House of Faculty of Veterinary Medicine (Suez Canal University). Before the beginning of the experiment they were housed in separate metal cages under controlled environmental and nutritional conditions (20°C and 55-60% relative humidity). The animals had free access to water and food.

2) Drugs and chemicals:-

- Preservatives used:-

1-Sodium nitrite: obtained from S.D. fine – chem . Ltd .It is white tiny powder to be mixed in rats ration used as antimicrobial & antioxidants agent.

2-Monosodium glutamate (MSG): obtained from loba company (India).white colored substance used as flavor enhancer.

3-Expermintal Design:-

Rats were divided into "5" groups according to the following table:-

Main groups	No. of animals/ group	Preservatives used	Concentration of preservative	Duration of experiment
C	10	Basal diet	_	For six weeks
LN	10	Low dose of sodium nitrite	O.2% of the diet	
HN	10	High dose of sodium nitrite	O.4% of the diet	
LM	10	Low dose of MSG	0.04 mg/ kg	
HM	10	High dose of MSG	0.08 mg/ kg	

4-Sampling:

-Blood samples:-

Blood samples were collected at 6 week from the beginning of the experiment from the medial canthus of the eye using micro hematocrit tubes, Two blood sample were taken from each rat in each group. The first blood sample were taken into clean and dry screw capped centrifuge tube and left to clot at room temperature , then centrifuged 3000 r.p.m for 15 min to separate clear serum samples for determination of the different biochemical parameters such as :creatinine, Urea, ALT(Alanine –aminotransferase),AST(Aspartate – aminotransferase), cholesterol, triglyceride, testosterone hormone, glucose level, sMDA (malondialdeyde) , serum GSH (reduced glutathione). The second blood sample were taken with EDTA tubes used for determination of Hb.

II: METHODS

1-Hemoglobin: was estimated spectro-photometrically according to **(Van Kampen and Zijlstra, 1961).**

2- Serum creatinine: was estimated spectophotometrically according to **(Schirmeister, 1964).**

3- Serum urea: was determined calorimetrically according to the method described by **(Fawcott and scott, 1960).**

4- Serum ALT and AST: were determined spectrophotometrically According to **(Retman and Frankle, 1957).**

5- GSH : was determined by enzymatic colorimetric method by using readymade kits provided by Biodiagnostic according to **(Beutler et al., 1963)**

6- Total cholesterol (T.C). was determined by **(Richmond, 1973 and Allain et al., 1974)**

7- Triacylglycerol (TAG):- was determined by **(Fossati and Prencipe, 1982).**

8-Serum glucose: was determined according to **(Young, 2001)**

9- Serum testosterone hormone: was determined by The electrochemmiluminescence immunoassay according to method described by **(Arlt, 2006)**. Use on Elecsys and Cobas immunoassay analyzers.

Statistical analysis

All data were subjected to statistical analysis according to ***(Snedector and Cochran, 1982)*** by using computer programs, **SPSS** for analysis of data and **COSTAT** for determination of LSD.

RESULTS

Table (1):show that high doses of sodium nitrite and MSG make significant elevation in ALT,AST, Urea, Creatinine and MDA while significant decrease in levels of GSH.

Table (1): Effect of different doses of sodium nitrite and mono sodium glutamate for 6 weeks on the following parameters.

Parameters / **Groups**	ALT **(IU/L)**	AST **(IU/L)**	Urea **(mg/dl)**	Creatinine **(mg/dl)**	GSH **(mg/dl)**	MDA **(nmol/ml)**
Control	$63^{b}\pm1.20$	$171.50^{b}\pm0.87$	$29.25^{b}\pm0.750$	$0.425^{a}\pm0.03$	$22.87^{a}\pm0.47$	$2.75^{c}\pm0.16$
N1	$76^{ab}\pm4.62$	$183.50^{a}\pm8.38$	$31^{b}\pm1.35$	$0.375^{a}\pm0.03$	$17.50^{b}\pm0.51$	$3.04^{c}\pm0.012$
N2	$79.50^{ab}\pm6.06$	$215^{a}\pm9.83$	$31.25^{b}\pm2.93$	$0.450^{a}\pm0.05$	$10.08^{c}\pm0.70$	$5.34^{b}\pm0.35$
M1	$90.25^{a}\pm9.49$	$235.75^{a}\pm15.343$	$41^{a}\pm2.79$	$0.525^{a}\pm0.08$	$17.10^{b}\pm0.71$	$3.26^{c}\pm0.18$
M2	$74.00^{ab}\pm.1.15$	$241.^{a}\pm11.365$	$38.50^{a}\pm0.65$	$0.500^{a}\pm0.04$	$8.88^{c}\pm0.67$	$6.82^{a}\pm0.28$

Values are means ± SE

Means carrying different superscripts considered significant (P < 0.05).

Table (2):show that high doses of sodium nitrite and MSG make significant elevation in TC,TG, Glucose, while significant decrease in levels of testosterone.sodium nitrite is causing significant reduction in Hb and body weight while MSG causing

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significant increase in body weight with no effect on Hb.

Table (2) : effect of different doses of sodium nitrite and mono sodium glutamate for 6 weeks on the following parameters.

Parameters / Groups	T.C (mg/dl)	T.G (mg/dl)	Glucose (mg/dl)	Testosterone ng/ml))	HB (mg/dl)	Body weight (gm)
Control	76.50^{a}±0.50	118.50^{a}±11.41	95.33^{c}±2.93	0.756^{a}±0.24	14.26^{a}±0.19	233.33^{bc}±10.38
N1	87.50^{a}±6.28	120.75^{a}±13.27	113.50^{b}±7.98	0.430^{b}±0.16	11.12^{b}±0.44	207.50^{c}±8.54
N2	82.75^{a}±8.39	124.75^{a}±25.79	133.17^{a}±2.52	0.427^{b}±0.04	10.46^{b}±0.97	154.17^{d}±16.19
M1	85^{a}±2.78	142^{a}±14.05	119.67^{ab}±.95	0.275^{c}±0.005	13.60^{a}±0.44	235.50^{ab}±10.80
M2	82.25^{a}±4.151	196.25^{a}±36.15	123.83^{ab}±7.10	0.395^{b}±0.18	13.06^{a}±.0.37	270.50^{a}±3.45

Values are means ± SE
Means carrying different superscripts considered significant (P < 0.05).

DISCUSSION

I: Sodium nitrite

Sodium nitrite is fundamental component of the global nitrogen cycle and is found throughout the environment **(Abuharfeil et al., 2001).**In this experiment it was obvious that the hair of male rats was very fragile and easily removed .this change may be attributed to aneamia that may cause hair loss or destruction of vitamin A caused by sodium nitrite .Vitamin A was responsible for maintenance of epithelial cells of all organs including skin **(Brady, 2007).** Otherwise this may be occur due to effect of sodium nitrite on thyroid function in the rats causing primary hypothyrodism which cause thin brittle hair and hair loss **(Kostogrys etal.,(2006).**

About the body weight of sodium nitrite treated groups there were highly significant decrease in the body weight of male rats given 0.2% ,0.4% in there diet when compared with the control group in spite of decrease food intake .This agreed with that given by **(Abuharfeil et al., 2001) and (Kostogrys etal.,(2006)** who reported that there were significant change in the body weight between treated rats and mice with the control animal. This may be due to the interference of sodium nitrite with thyroid function in rats by preventing thyroidal iodine uptake lead to primary hypothyroidism (thyroxin is the main metabolic hormone).

Regarding to the effect of sodium nitrite on hemoglobin there were highly significant decrease in Hb concentration in treated rats at 6 week of experiment . These results were agreed with that of **(Haymond et al.,(2005) and McKenzie,(2010).**this result may be due to Methemoglobinemia is the critical health effect from exposure to nitrates and nitrites. Depending on the percentage of total MetHb, the clinical presentation may be one of oxygen deprivation with cyanosis, cardiac dysrhythmias and circulatory failure, and **Skold et al.,(2011)**.Otherwise due to due to slowing down or obstructing the NAD and NADP reduction processes in the erythrocytes by nitrite ions causing disturbance in the cell respiration ,lysis of red cells and break down of haemoglobin to bilirubin of liver**(Agata Wawrzyniak,2000).**Or due to vitamin A deficiency caused by sodium nitrite treatment, which has adverse effect on Hb synthesis**(Dreyfuss et al .,(2000).**these result disagreed with **(Van dijk et al.,(1983)** mentioned that there was no anemia in cows.

With regard to the serum biochemical constituent in relation to liver function of sodium nitrite treated groups, there were highly significant increase in ALT, AST levels . These results agreed with that observed by **(Abdul-Ameer and Abed,(2012) ; Abdel-Reheim et al ., (2014)**).

In this investigation for the biochemical analysis of serum in relation to kidney function recorded significant increase in the level of urea and creatinine these results are in agreement with **(Piacenza et al., 2009, Vinodini et al., 2010)**. **El-Demerdash et al., (2005)** observed that food preservatives caused changes in kidney convoluted tubules cell lining as well as in Bowman's corpuscles.and these result dis agreed with**(Duncan et al., 1994)**who reported may be attributed to hepatic insufficiency (liver respo- nsible for conversion of ammonia into urea) that indicated by elevated ALT and AST.

Concerning to malondialdehyde shows highly significant increase in serum MDA . These result agreed with **(choi et al .,2000)** they mentioned that nitrosamine and other free radical produced from (sodium nitrite reaction with secondary amine) increase lipid peroxidation. 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 findings are consistent with elevated lipid peroxide, malondialdehyde **(Kalaivanam et al., 2006).**

The decreased GSH content in the present study may be attributed to the increased LPO rather than reduced synthesis (Kalaivanam et al., 2006), this also in accordance with (El sheikh and Khalil, 2011). DNA damage was induced by radicals formed in the reaction mixtures of phenol and nitrite. Some researchers have reported that combined treatment of antioxidants and NaNO2 generates reactive oxygen species (ROS) in vitro **(Kuroiwa et al., 2007)).** 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 (GSHpx.) as a component of the enzyme system containing GSH oxidase and reductase **(Hong and Lee, 2009)**. **Amin et al.(2010)** observed a decreased level of GSH , this is due to the most hepatic reduced glutathione (GSH) is converted to its oxidized form (GSSG) by the enzyme glutathione reductase to protect the cells from damage by the toxic materials and free radicals and this explain why GSH is depleted as a result of their toxicity.

Moreover, significant hypercholesterolemia was recorded in sodium nitrite administered group compared to control group. This elevation may be due to mobilization of free fatty acids from the adipose tissue the blood stream and increase level of acetyl CoA, leading to increase in the synthesis of cholesterol due to peroxidation of cell membrane

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lipids **(Helal et al.,2000).**

Compared to control group, a significant increase in the serum glucose level was observed in sodium nitrite intoxicated rats. This finding suggests nitrite- stimulation of gluconeogenesis and glucose shift 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 The obtained increases in the percentage of chromosomal aberrations results go in parallel with those reported on the in hyperglycemic effect of sodium nitrite in rats **(Anil et al., 2005).**

II: MONO SODIUM GLUTAMATE

The present investigation revealed that body weight increased in rats treated with MSGas compared with the normal ones and this result in harmony with **(Moore, (2003) and (Manal and Nawal, 2012)** concluded that monosodium glutamate is described and listed on food labels as a "Flavouring" or "Hydrolysed vegetable protein". Through its stimulation of the Orosensory receptors and improving the palatability of meals, monosodium glutamate influences the appetite positively and induces weight gain also agree with **(Iwase et al., 2000)** who weight gain was found to be significantly greater in MSG-treated animals, and that this might be independent of an increase in appetite .

There is an increase in total cholesterol and triglyceride as compared with normal ones and this result agreement with **(Schummer *et al.,* 2008)**shown the elevated serum cholesterol concentration indicate hyper lipidemia and obesity so body weight increased also agree with **(Mariyamma *et al.* (2009))** reported hyperlipidaemia with significantly elevated levels of serum Triacylglycerol and cholesterol in monosodium glutamate treated rats and proposed that a shift in glucose metabolism towards lipogenesis might account for the hyperlipidaemia.

Declearing to transaminases enzymes, there was highly significant increase in ALT, AST and this result in harmony with **(Al-Mamary 2002)** who showed the transaminases are abundant in the liver and are released into the blood stream following hepatocellular damage, making them sensitive marker of liver damage. The marked increase in the plasma ALT and AST activities observed in the monosodium glutamate fed rats might be indicative of liver damage. Plasma levels of transaminases were used as an indicator of damage to the liver structural integrity because these enzymes are cytoplasmic in location and are released into the circulating blood only after structural damage **(Janbaz and Gilani 2000; Hagar 2004)**. The metabolism of most amino acids and their derivatives occur to a significant extent in the liver **(Mayes & Bender 2003)** and essentially involves deamination to produce ammonium ion that could be toxic unless made less toxic via the reactions of the urea cycle. The sodium moiety in monosodium glutamate could easily dissociate to yield free glutamate. Thus, the possible ammonium ion overload that may occur with glutamate or monosodium glutamate intake could damage the liver, consequently releasing the transaminases; hence it observed elevation in the plasma. The result is similar to **Onyema et al. (2006) and Egbuonu et al. (2009)** who reported that monosodium glutamate increased the serum transaminases in male albino rats due to possible ammonium ion overload resulting from an increase level of glutamate. Also, **Mariyamma et al. (2009)** reported increase in plasma transaminases due to oxidative stress which induces alteration in the membrane integrity, thus changing the membrane permeability resulting in leakage of intracellular enzymes.

Our results demonstrated that the daily intake of MSG and exhibited an increase in serum creatinine, urea when compared with the control group, these results are in agreement with **(Khadiga et al., (2009)** observed that there is an elevation in kidney functions parameters after administration of MSG and lead to alterations in kidney functions, these impairments could also be attributed to the changes in the threshold of tubular reabsorption, renal blood flow and glomerular filtration rate (GFR) **(El-sheikh and Khalil,2011).**

Regarding to the effect of MSG on s MDA there were highly significant increase in treated rats at 6 week of experiment. These results were agreed with that of **(Vinodiniet al., 2010)** who reported Lipid peroxidation could also be increased due to the increases in the blood glutamate and glutamine which are reported to favour lipogenesis .In liver glutamine degradation yields glutamate which then undergoes oxidative deamination to produce ammonium ions, α-ketoglutarate and NADH. Hence the increased level of glutamine could also initiate lipid peroxidation by changing the redox potential of the cell.

The present investigation revealed that oral administration of MSG suppressed the activity of glutathione (GSH) and this result agreement with **(Natalie et al.,2010)** showed that MSG (250 and 500 mg/kg) produced significant elevation of MDA levels accompanied by depletion of GSH concentrations in mouse brain, which suggested increased oxidative stress. Oxidative stress occurs when reactive oxygen species (ROS) accumulate in cells, either from excessive production or insufficient degradation, resulting in cellular damage.

About the glucose level with MSGtreated rats there were an increase in glucose level . this result agreement with **(Macho et al., 2000; Mourtzakis and Graham, 2002; Chevassus et al., 2002)** who reported that MSG alters the regulatory mechanisms that affects fat metabolism **(Tsang, 2008)**; MSG inhibits ketone secretion, resulting in an obese rat with a propensity for creating adipose tissue (fat) **(Nakai et al., 1986; Vice et al., 2005);** The weakening potential of fats on insulin action **(Guyton and Hall, 2006)** and the attendant increase in FBG as shown by the results of this study. Moreover, obesity is a major factor for a number of co-morbidities such as noninsulin-dependent diabetes mellitus **(Haslam and James, 2005).** The present investigation on hemoglobin concentration showed that there was significant decrease in hemoglobin concentration as compared with control ones. And this result dis agreement with Laura et al, (2004) and Baker et al, (2005) which showed that MSG does not alter Hb.

Also there were significant decrease in testosterone hormone as compared with control ones. This result agreement with **(Burde et al., 1971; Bodnár et al., 2001)** The lowered serum testosterone levels in MSG-treated rats in this study may have resulted from disruption of the hypothalamic-pituitary-testes regulatory axis that controls testosterone production by testicular Leydig cells. This proposition is supported by the reports of previous authors who stated that administration of monosodium glutamate destroyed neurons of the hypothalamus in rats and mice. Such neuronal losses in the hypothalamus can result in disruption of the hypothalamic-pituitary-testes regulatory axis

and also agree with**(Ochiogu et al .,(2015)** showed that on Days 14 and 28 of MSG administration the mean serum luteinising hormone, testosterone and cholesterol levels of the treated groups were significantly ($P < 0.05$) lower than those of the control group.

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