

## EFFECT OF PAPAS SEEDS TO HIGH DOSES OF NITRATE AND NITRITE IN IMMUNOGLUBIN PRODUCTION AND DETOXIF ENZYMES ACTIVITIES.

\* Mubarak. A.E    \*\*Nehad. R. EL-Tahan    \*Mona .E.M.Naga  
\*\*\*Hanan .S.Abdul Salam

\*Dept, Home Economics , Faculty of Specific Education Menofia University.

\*\*Dept, Nutrition and Food Science , Faculty of Home Economics, Menofia University.

\*\*\* Researcher in Facultya of Specific Education, Home Economics Dept, Menofia University.

### Abstract:

The present work is conducted to study the effect of papas seeds on reducing the toxic effect of high doses of nitrate and nitrite .Papis added to high doses of nitrate and nitrite in rats diet as 20%. Potassium nitrate used at 0.6 and sodium nitrite used at 0.2% for two months of feeding. Nutritional, immunological parameters and detoxifying enzyme activities were estimated. The results indicated that present of nitrate and nitrite in rats diet affected a significant decrease in feed intake, body weight gain and feed efficiency ratio when compared with control group. It can be observed that values of IgG and IgM production are significantly decreased as a result of adding nitrate and nitrite. Nitrate and nitrite lowered the activities of hepatic catalase and glutathione peroxidase , whereas it drastically increased Gamma glutamyl transpeptidase activity in kidney. Addition of papas seeds to high doses of nitrate and nitrite affected increased feed intake, body weight and feed efficiency ratio. A significant increase of IgG and IgM were observed when compared with control group. Feeding of diet containing papas seeds at level 20% has variable effects on the different antioxidant/bio-transformation enzymes. It can be concluded that presence of papas seeds in the diet that containing nitrate and nitrite salts is very important. Present study recommends the use of nitrate and nitrite in food products especially meat production must be monitored.

Key words : nitrate and nitrite salts – papas seeds- IgG- IgM- detoxifying enzymes.

## Introduction:

Nitrate and nitrite salts are used in food industry especially meat products to improve the color, taste and control undesirable gas and flavor production by anaerobic bacteria (Merino *et al.*,2000). Nitrate and nitrite are added due to their protective effect against toxic bacteria as *clostridium botulinum* Hassan (1998). This bacterocidal effect is referred to compound derived from it during food processing (Merino *et al.*,2000). The antibody production and cell proliferation of B cells are significantly influenced by addition of nitrite (Suketa, 2002). Viral diseases, acute respiratory diseases and skin diseases increased between children who living on drinking water with high content of nitrates Takagi and Suketa (1997). IgG, IgA and IgM are decreased in these children while spontaneous blastogenesis of lymphocytes are increased (Kolyuk, 2003). High doses of nitrate and nitrite in food products decreased the food intake, body weight , vitamin A in liver and utilization of  $\beta$ -carotene ( Majcherzak *et al.*, 1991). NAS, 1995 calculated the dose of nitrate per day which effect on the human health, this dose nearly was 317 mg. Pregnant women who living in region with high content of nitrate and nitrite may lead to effect on their fetus (Till *et al.*, 2005). Chronic uses of nitrate at low level in food products may cause mental depression and headache Barawska *et al.* (1998) Nitrites may increase methemoglobinemia, anemia, the risk of goiter and carcinogenic nitrosamines in human Bruning-fann and. Meat products supply 98% of nitrites while the vegetable products supply 94-98% of nitrates especially potato and cabbage which contained around 32% and 24% respectively (Wawrzyniak *et al.*, 2006 and Sebecic *et al.*, 2008). Papas fruit is a rich source of nutrients such as provitamin A carotenoids, vitamin C, B vitamins, lycopene, dietary minerals and dietary fiber. Papas skin, pulp and seeds also contain a variety of phytochemicals, including natural phenols. Danielone is a phytoalexin found in the papas fruit. This compound showed high antifungal activity against *Colletotrichum gloeosporioides*, a pathogenic fungus of papas (Echeverri *et al.*, 1997).

The ripe fruit of the papas is usually eaten raw, without skin or seeds. The unripe green fruit can be eaten cooked, usually in curries, salads, and stews. Green papas is used in Southeast Asian cooking, both raw and cooked (Oderinde *et al.*, 2002). In Thai cuisine, papas is used to make Thai salads such as *som tam* and Thai curries such as *kaengsom* when still not fully ripe. In Indonesian cuisine, the unripe green fruits and young leaves are boiled for use as part of *lalab* salad, while the flower buds are sautéed and stir-fried with chillies and green tomatoes as Minahasan papas flower vegetable dish. Papas have a

relatively high amount of pectin, which can be used to make jellies. The smell of ripe, fresh papas flesh can strike some people as unpleasant. Papas is marketed in tablet form to remedy digestive problems. Papain is also applied topically in countries where it grows for the treatment of cuts, rashes, stings and burns. Papain ointment is commonly made from fermented papas flesh, seeds and is applied as a gel-like paste. Harrison Ford was treated for a ruptured discincurred during filming of Indiana Jones and the Temple of Doom by papain injections. Women in India, Bangladesh, Pakistan, Sri Lanka, and other countries have long used green papas as an herbal medicine for contraception and abortion. Enslaved women in the West Indies were noted for consuming papas to prevent pregnancies and thus preventing their children from being born into slavery. Ripe papas is not teratogenic and will not cause miscarriage in small amounts. Phytochemicals in papas may suppress the effects of progesterone(Titanji *et al.*, 2008).

## Material and methods

### Materials:

Potassium nitrate and sodium nitrite were obtained from EL-Naser Pharmaceutical Chemical Company, Cairo, Egypt. Papas seeds were obtained from local market, Shiben El-Kom, Menofia Governorate. Normal albino rats, their weighted  $120\pm 10$  g were obtained from International Research Center, Dokky, Cairo, Egypt.

### Methods

#### Experimental animal design

A total of 30 male rats, weighing  $120\pm 10$  g were housed in cages under hygiene conditions in the biological laboratory of Research, Institute of Ophthalmology Medical Analysis Dep., Giza, Egypt. Rats were divided into 5 groups, each group contained 6 rats as following:

Group (1) fed on basal diet as a control group.

Group (2) fed on basal diet containing 6 g potassium nitrate / kg diet.

Group (3) fed on basal diet containing 6 g nitrate potassium/kg diet and 20% papas seeds.

Group (4) fed basal diet containing 2g sodium nitrite /kg diet.

Group (5) fed basal diet containing 2g sodium nitrite /kg diet and 20% papas seeds.

## Preparation of diet

The basal diet consisted of protein 12%, fat 10%, vitamin mixture (1%), salt mixture (4%), fiber (cellulose) (5%), and the remaining was starch choline chloride (0.2%) according to (Reeves *et al.*, 1993; Hegested, 1941 and Campbell, 1963).

## Nutritional parameters

Body weight and feed intake were measured once a week of the experimental period (2 months). Feed efficiency ratio was calculated according to Hsu *et al.* (1978). At the end of the experiment, rats were anaesthetized and scarified. Blood samples were collected from portal veins. Organs such as liver and kidney were rapidly excised and part of the organs was stored frozen in liquid nitrogen container for assay of glutathione (GSH) and anti-oxidant/detoxifying enzymes.

## Analytical methods

Blood samples were centrifuged and serum was separated to estimate some biochemical parameters. Detection of IgG and IgM by direct ELISA, according to Engvall and. Liver glutathione content was determined by the method of Ellman (1985). Liver catalase (E.C.1.11.1.6) activity was assayed according to the method of Cohen *et al.* (1970). Gluathione reductase (E.C.1.6.4.2, GSSGR) and glutathione peroxidase (E.C.11.1.9, GSHP) levels in liver were determined by the method of Weiss *et al.* (1980). Hepatic glutathione-S-transferase (E.C.2.5.1.1.8, GST) activity was determined by the procedure of Habig *et al.* (1992).  $\gamma$ -glutamyl transpep-tidase (E.C.3.3.2, GGT) in kidney was estimated by the method of Meister *et al.* (1981) .

## Histopathological study

On the last day of the experiment, rats were anesthetized then liver and kidney were removed and kept in 10% formaldehyde. Dehydration and clearing of the tissues were formed automatically. The prepared 5-micron thickness sections were stained with hematoxilin and eosin (H/E) and Gomeri aldehyde-fuchsin (GAF) according to Gomeri (1980)

## Statistical analysis

Data obtained from this study was analyzed statistically for standard error and significant differences between treatment means were determined using Duncan's multiple range tests (1955).

## Results

### Nutritional parameters

Effects of potassium nitrate and sodium nitrite with and without papas seeds on nutritional parameters were studied. The parameters included feed intake, body weight gain and feed efficiency ratio.

From data in Table (1), it could be observed both of the nitrate and nitrite caused high decrease of feed intake, body weight gain and feed efficiency ratio, compared with control group. Addition of papas seeds to nitrate and nitrite in the diet led to increase feed intake, body weight gain and feed efficiency ratio of rats.

The highest values of feed intake and body weight gain is observed in control group followed by groups 3 and 5 which fed on the basal diet containing 6 g nitrate/kg diet and 20% papas seeds and the group fed on basal diet containing 2g/kg diet and 20% papas seeds. The lowest value of feed intake and body weight are noticed in group which fed on basal diet containing 2g nitrite /kg diet. Feed efficiency ratio is changed and improved as a result of papas seeds addition.

**Table (1): Effects of nitrite and nitrate salts with and without papas seeds on feed intake, weight gain and feed efficiency ( Mean $\pm$  SE).**

Parameters Groups	Feed intake	Body weight gain	Feed efficiency ratio
Control group (1)	11.71 <sup>a</sup> $\pm$ 0.29	43.64 <sup>a</sup> $\pm$ 0.91	0.089 <sup>a</sup> $\pm$ 0.001
Basal diet + 6 g nitrate / kg diet group (2)	2.83 <sup>c</sup> $\pm$ 0.18	4.61 <sup>c</sup> $\pm$ 0.03	0.038 <sup>c</sup> $\pm$ 0.001
Basal diet + 6 g nitrate / kg diet + 20% papas seeds group (3)	7.7 <sup>b</sup> $\pm$ 0.23	17.54 <sup>b</sup> $\pm$ 1.31	0.054 <sup>b</sup> $\pm$ 0.001
Basal diet + 2 g nitrite / kg diet group (4)	2.69 <sup>c</sup> $\pm$ 0.04	1.83 <sup>f</sup> $\pm$ 0.78	0.016 <sup>d</sup> $\pm$ 0.001
Basal diet + 2 g nitrite / kg diet + 20% papas seeds group (5)	8.32 <sup>b</sup> $\pm$ 0.23	18.03 <sup>b</sup> $\pm$ 0.21	0.052 <sup>b</sup> $\pm$ 0.001

Means in the same column with different letters are significantly different ( $P \leq 0.05$ ).

### Biochemical parameters

#### Immunoglobulins production

Effect of adding papas seeds at the level of 20% on the IgG and IgM production was studied . IgG and IgM of control group were

higher than that of all the tested groups . Adding papas seeds to rats diet which feed on nitrite and nitrate compounds led to significant increase values of immunoglobulins production more than rats received papas seeds free diets containing nitrite and nitrate compounds. The decreasing of IgG and IgM were increased with diet which containing nitrite and nitrate. The lowest IgG and IgM values were observed in group of diet containing 2 g nitrite. The relative values were increased by adding papas seeds and higher than control group( more than 100%)

**Table (2): Effects of nitrite and nitrate salts with and without papas seeds on Immunoglobulins production ( Mean± SE).**

Parameters Groups	IgG mg/l	Relative value (%)	IgM mg/l	Relative value (%)
Control group (1)	900.05 <sup>c</sup> ± 0.05	100	78.2 <sup>c</sup> ± 0.005	100
Basal diet + 6 g nitrate / kg diet group (2)	748 <sup>d</sup> ± 2.01	83.11	61.65 <sup>d</sup> ± 0.65	78.9
Basal diet + 6 g nitrate / kg diet + 20% papas seeds group (3)	1010.66 <sup>a</sup> ± 6.27	112.2	108.16 <sup>a</sup> ± 2.5	138.5
Basal diet + 2 g nitrite / kg diet group (4)	970.66 <sup>b</sup> ± 25.16	107.7	109.33 <sup>a</sup> ± 3.5	139.7
Basal diet + 2 g nitrite / kg diet + 20% papas seeds group (5)	1006.2 <sup>a</sup> ± 20.27	111.7	98.96 <sup>b</sup> ± 0.5	126.6

Means in the same column with different litters are significantly different ( $P \leq 0.05$ ).

### Glutathione and bio-transformation enzymes in liver and kidney:

Glutathione (GSH) level in control group was the highest recorded value in groups 2 and 4 which fed on basal diet containing nitrate and nitrite (Table 3). In groups 3 and 5, the values of the glutathione were increased with adding papas seeds when compared with the groups of papas seds- free diet.

Catalase activity in liver of rats fed on control diet with nitrite was drastically reduced to less than half of the value obtained of control animals followed by group fed on nitrate. Values of Catalase activity in liver for groups fed on basal diet containing 6 g nitrate/kg diet and 20% papas seeds and the group fed on basal diet containing 2g/kg diet and 20% papas seeds were higher than rat groups fed on papas seeds-free diet.

Glutathione peroxidase (GSHP) activity liver was significantly reduced ( $P<0.001$ ) in groups were fed on the basal diet with nitrite and nitrate. While, it was increased in control, nitrite and nitrate containing diet groups supplemented with papas seeds. The activity of glutathione reductase (GSSGR) was increased in rats fed papas seedscontaining diet, compared with those of diet with nitrite and nitrate. Glutathione-S-transferase (GST) activity was found to increase in papas seedsdiet group (3) when compared to the control.

Gamma glutamyl transpeptidase (GGT) activity in kidney was found to be elevated in nitrite and nitrate treatment. Thus, nitrite and nitrate treatment to control rats is associated with increasing GGT level nearly four folds; whereas nitrite and nitrate treatment to papas seedsdiet group increased the values only one or two fold indicating effect of papas seedsabsorption.

**Table (3): Level of glutathione and antioxidant and detoxifying enzymes in liver and kidney of rats fed control or nitrate and nitrite salts diets with or without treatment of papas seeds**

Diets	GSH*	Catalase $\times 10^{4\#}$	GSHP**	GSSGR+	GST++	GGT $^{\$}$
Control group (1)	2.62±0.23	37.14±0.19	0.38±0.14	0.53±1.34	24.21±1.02	0.58±1.63
Basal diet + 6 g nitrate / kg diet group (2)	2.41±0.26	*20.68±2.05	*0.29±0.43	0.49±1.82	21.79±3.71	**2.02±0.38
Basal diet + 6 g nitrate / kg diet + 20%Papas seeds group (3)	2.54±0.85	34.07±3.11	0.34±1.03	0.54±2.08	25.38±2.72 <sup>a</sup>	0.67±0.54
Basal diet + 2 g nitrite / kg diet group (4)	**2.01±1.47	**18.68±1.25	*0.22±1.47	**0.34±1.63	16.79±3.01	**2.37±1.36
Basal diet + 2 g nitrite / kg diet + 20% Papas seeds group (5)	2.49±0.49	30.04±2.12	0.32±0.45	0.51±0.97	23.78±1.20	**1.55±1.09

\* moles/g liver, #  $\Delta$  of 0.1/min/g/g liver; \*\* mM NADP formed/min/g liver.

+ mM NADP formed/min/g liver; ++ M conjugate formed/min/g liver.

$\$$   $\mu$  moles/min/g kidney; Values are Mean  $\pm$  SD for 6 rats.

\* high significant  $p<0.001$

\*\* very significant  $p<0.001$

#### Histopathological study

No change in normal histological pattern of liver (Photo1) and kidney ( photo 6) . Tissues were noted changing under both of nitrate and nitrite ( photo 2 and 4). Appearances of intercellular spaces and increase in the number of cytoplasmic vacuoles and eosinophilic granules were noted in the liver cells of nitrate and nitrite treated rats. The nuclei showed diffused chormation bodies under the experimental conditions. After supplementation of papas seedsat the level 20% (photos 3 and 5), the intercellular spaces disappeared and the cytoplasmic vacuoles and eosinophilic granules became less abundant, but the nuclei still showed diffused chromatin bodies. In case of kidney tissues under nitrate and nitrite toxic (groups 7 and 9) , there was an increase in the space between glomerulus and the capsule in the nephrons. Occasional hemorrhages in the kidney tissues were also noted under these conditions. There were an increase in the eosinophilic granules. Supplementation with papas seeds(Photos8 and 10) to the toxic groups of rats could reverse these defects significantly.

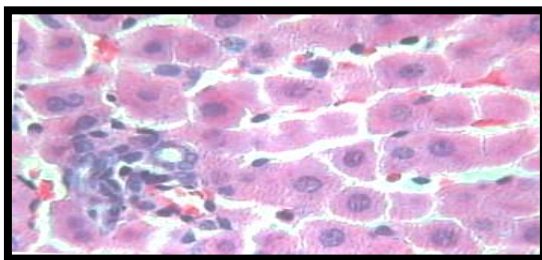


Photo (1) : Liver of rat (Group 1) fed on basal diet showing normal histological pattern of liver.

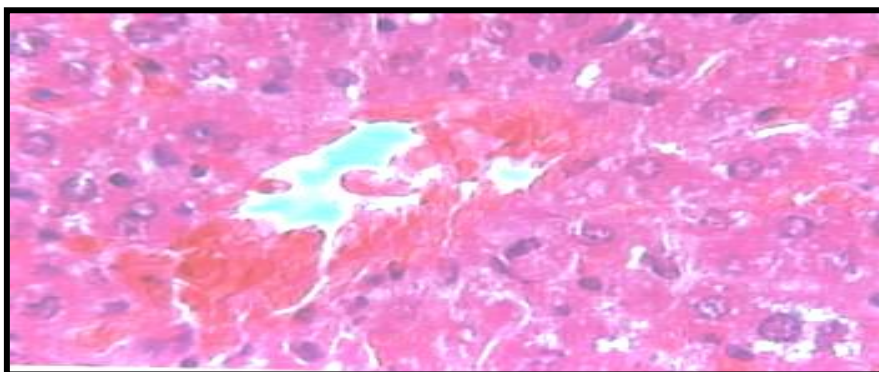


Photo (2) : Liver of rat (Group 2) fed on basal diet with 6 g potassium nitrate/kg diet showing spaces and increase in the number of cytoplasmic vacuoles



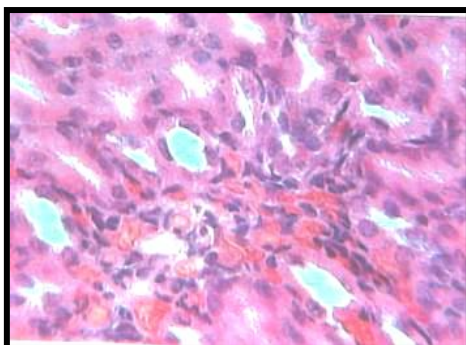


Photo (3) : Liver of rat (Group 3) fed on basal diet with 6 g potassium nitrate/kg diet and 20% Papas seeds showing less cytoplasmic vacuoles and eosinophilic granules.

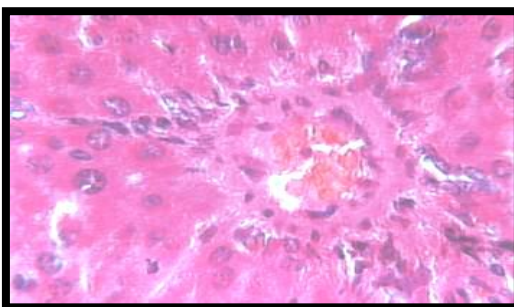


Photo (4) : Liver of rat (Group 4) fed on basal diet with 2 g nitrite / kg diet showing increase in the number of cytoplasmic vacuoles and eosinophilic granules.

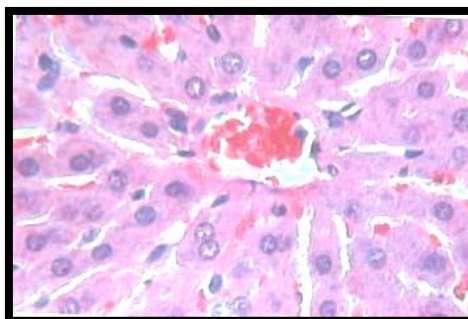


Photo (5) : Liver of rat (Group 5) fed on basal diet with 2 g nitrite / kg diet and 20% Papas seeds showing the intercellular spaces disappeared and less the cytoplasmic vacuoles.

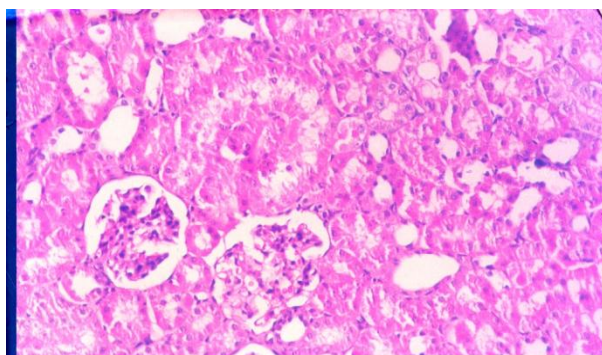


Photo (6) : Kidney of rat (Group 1) fed on basal diet showing normal histological pattern of kidney.

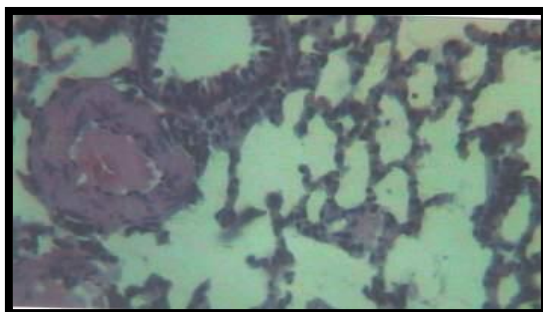


Photo (7): Kidney of rat (Group 2) fed on basal diet with 6 g potassium nitrate/kg diet showing an increase in the space between glomerulus.

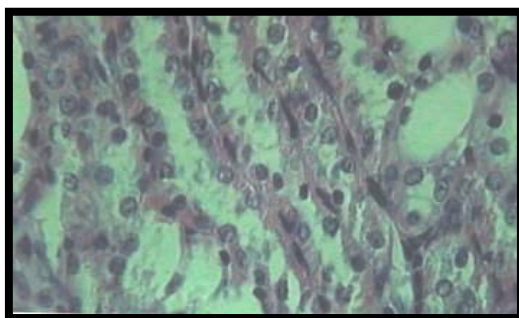


Photo (8): Kidney of rat (Group 3) fed on basal diet with 6 g potassium nitrate/kg diet and 20% Papas seeds showing the decrease in the eosinophilic granules.

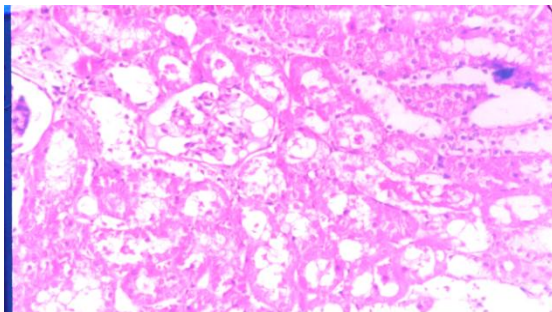


Photo (9): Kidney of rat (Group 4) fed on basal diet with 2 g nitrite / kg diet showing an increase in the space between glomerulus.

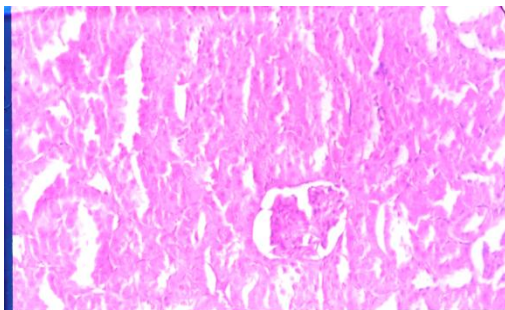


Photo (10): Kidney of rat (Group 5) fed on basal diet with 2 g nitrite / kg diet and 20%Papas seeds showing the space between glomerulus was narrow.

## Discussions

Nitrate and nitrite can be an indicator of more serious pollution problems. Nitrate is an inorganic compound that can be a natural or manmade contaminant in drinking water. Nitrate and nitrite salts are used in food industry especially meat products to improve the color, taste and control undesirable gas. So, This study was carried out to study the effect of some Fruits seeds (papas seeds) to High Doses of Nitrate and Nitrite on Immunoglobulin Production and Detoxifying Enzymes Activities.

Nitrate and nitrite ions are both highly soluble in water. These ions are considered as a part of the diet and also produced from nitric oxide. When taken with food, they are readily absorbed from the small

intestine **Walker (1998)**. Nitrate salt is partially converted to nitrite by oral bacteria and by stomach acids, helping to reduce gastrointestinal tract infection (**Combs, 2000**). Nitrate is in high concentration in drinking water and meat products which induced methemoglobinemia in infinite. It has been implicated in the formation of methemoglobin and carcinogenic nitrosamine in humans (**Kapor, 2004**). The papas is one of the oldest known edible fruits. This fruit is one of the species mentioned in the Bible and Koran and is often associated with fertility. It is native to Persia and perhaps some surrounding areas and cultivated in ancient Egypt, Greece and Italy. Papas seeds spreads into Asia (Turkmenistan, Afghanistan, India, China, etc.), North Africa and Mediterranean Europe including Turkey. The domestication process took place independently in various regions (**Salaheddin and Kader, 1984 and Sarkhosh et al., 2006**).

**Sabbar et al. (2010)** studied the chemical composition of papas. The results revealed that papas seeds riches in fiber, calcium , vitamin C and phosphorus, 100g of papas seeds produced 65 Caloric value .

Potential health problems associated with a high intake of vegetables and meat products which contain salt of nitrate and nitrite have been linked to decreased energy intakes, weight gain and the weight loss epidemic as indicated by **Govoni et al. (2008)** .

Similar results obtained by **Wawrzyniak et al. (2006)** published a systematic review of 30 studies conducted in the United States and Europe from 1966 through 2005 examining the relationship between nitrite, nitrate and weight gain. They concluded that greater consumption of these materials is associated with weight loss and underweight in both children and adults, with findings of 23 of the 30 studies supporting this conclusion.

Meanwhile, **Jung et al. (2009)** found that the rising consumption of vegetables fertilizer and meat additives provides a rising intake of nitrite and nitrate which can contribute to weight loss and underweight.

Also, study done by **Webb et al. (2004)** increased nitrite consumption would decrease total energy intake by decreased appetite and decreased fat intake. **Aravind et al. (2013)** who found that papas seeds increased weight gain to contained many biological active compounds including chymopapain and papain which is the ingredient that aids digestive system and a good supply of vitamin A and C that are highly essential for maintaining a good health.

Immunoglobins are specific groups of glucoprotein and produced by one type of immune cells. This helps to prevent and control

infections (**Gordon, 2000**). Rats fed on diet containing nitrate and nitrite at high dose have had lower values of IgG and IgM when compared with the control group. It can be observed that there is a significant increase in IgG and IgM values in rats that received papas seeds (groups 3 and 5) when compared with groups (2 and 4) which fed on the same diet without selenite sodium. These results are completely agreement with **Kolb et al. (2004)** who stated that antibody production of all B cell hybridomena is significantly suppressed by addition of nitrite. Also IgG and IgM were decreased at 42.3 and 59.6% in children who living in a region having a high content of nitrate in drinking water. **Kolb et al. (1997)** reported that nitrosamine formation can reliably be prevented by papas seeds. These results agree with Vincent (2010) who reported that serum IgM showed a gradual increase with the decrease of supplement level of antioxidant as vitamin C and E concentration then, IgM decreased with decreasing the supplement level.

The purpose of this study was to examine the modulation of the detoxifying enzymes in rats fed on diet with nitrate and nitrite in high dose in rats fed on the same diet with addition of selenite sodium. It can be observed that, addition of nitrite and nitrate to the diet of rats resulted in a significant decrease in glutathione, Catalase activity in the liver, Glutathione peroxidase (GSHP) and increase in Gamma glutamyl transpeptidase (GGT) in the kidney of groups ( 2 and 4) when compared with control group. These changes may be attributed to the harmful effect of nitrite nitrosamine formation and the damage in liver cells. Presence of papas seeds in food prevents nitrosamine and the decreasing of important enzymes in liver and kidney which prevent the man from cancer (**Wawrzyniak et al., 2006**).

GSH represents an important defense mechanism in protecting cells against oxygen free radicals. Its presence in excess in vivo could scavenge the electrophilic moieties produced by xenobiotics by conjugation to less toxic products (**Linder, 1995**). The present study revealed that addition of nitrate and nitrite lead to decrease the hepatic GSH but adding papas seeds to the same diet elevated the hepatic GSH. The increase of GSH by papas seeds diet could be described as the result of modulation of several enzyme systems **Brown (2007)**. The major responsibility for cellular protection against oxygen mediated toxicity rests on glutathione peroxidase/ reductase redox cycle enzymes, catalase and superoxide dismutase (**Reed, 1998**). There was a significant increase in the GSSGR activity on papas seeds diet as a result of increasing in GSH on papas seeds diet. The absence of decrease in hepatic GSH level on nitrate and nitrite treatment was also possible due to the reaction of the reactive intermediates of the

carcinogen with GSH being at slow rates insufficient to deplete the pool of glutathione in the liver.

Enhancement of GST activity has been shown to increase the ability for detoxification of some carcinogens (Stavroc, 2001). Presence of an inhibitory effect on this enzyme as well as catalase by continuous feeding of papas seedsdiet after nitrate and nitrite treatment shows a protective effect of selenite sodium. Feeding of higher levels of papas seedsor food rich in papas seedshas been reported by some workers to result in enhanced GST activity in rats (DeGroot *et al.*, 2001).

Gamma glutamyl transpeptidase in cells can be considered a marker enzyme of preneoplastic lesions. WHO has recognized elevated GGT in liver cells (determined by employing a histochemical technique as GGT positive foci) as a valid in vivo test of preneoplasia in short-term animal experimentation in animals such as rat (WHO, 1990). In our study, nitrate and nitrite treatment showed a more than two fold increase in GGT level in kidney indicating possible preneoplastic changes. The elevated level of GGT is attributed to the exposure of rats to the chemical carcinogen, nitrate and nitrite. On the other hand, feeding on diet containing the nitrate and nitrite with a high level of papas seedslead to a significant decline in GGT levels, which is attributed to the papas seedscontent of the diet. Thus, incorporation of papas seeds with nitrate and nitrite in the diet elicited a beneficial effect.. So, the administration of high level papas seeds is effective in practically reversing some of the alterations produced by nitrate and nitrite either at the enzymatic and histological properties of the tested liver and kidney.

## Conclusion

The present study, concluded that high dose intake of papas seeds 100 mg / kg bw result in decrease the toxic effect of nitrate and nitrite compounds on the nutritional parameters (BWG, FI and FER), Immunoglobulins production ( IgG and IgM) and Hepatic glutathione and bio-transformation enzymes in liver and kidney. So, it should be added high level of papas seeds 100 mg/kg body weight to food as meat products which had high content of nitrite and nitrate compounds.

## References

- Borawska, M.; Markiewicz, R. and Witkowska, A. (1998): Nitrate and nitrite content in daily hospital diets during the winter season-comparison of analytical and calculation methods. *European J. of Clinical Nutri.*, 52 (7): 489-93.
- Brown, K.M. (2007): Papas seeds, selenoproteins and bio-transformation enzymes in liver and kidney. *Public Health Nutr.*, 6: 456-505.
- Bruning-Fann, C.S. and Kaneene, J.B. (1993): The effect analytical and t of nitrate, nitrite and N-nitroso compounds on human health: A review. *Vet. Hum. Toxicol.*,35:521-38.
- Campbell, J. A. (1963): Methodology of Protein Evaluation. RAG Nutr. Document R.10 Led., 37. June Meeting, New York.
- Cohen, G.; Dembiec, C. and Marens, J. (1970): Measurement of catalase activity in tissue extracts. *Anal Biochem.*, 34: 30-38.
- Combs, G.F. (2000): Food system-based approaches to improving micronutrient nutrition; the case for papas seeds. *Biofactors*. 12: 39-43.
- De Groot, A.P.; Willems, M.I. and De Vos, R.H. (2001): Effects of high levels of papas seeds in the diets of rat. *Food Chem. Toxicol.*, 29: 828-837.
- Duncan, D.B. (1955): Multiple range and multiple F-test. *Biometries*, 11:1-47.
- Ellman, G.L. (1985): Liver glutathione: A colorimetric method or determining low concentrations of mercaptans. *Arch Biochem Biophys.*, 74: 443-450.
- Engvall, E. and Perlmann, P. (1971): Enzyme-Liked immuno. Sorbent (ELISA): Qualitative assay of Immunoglobulins. *Immuno Chem.*, (8): 817. Hegsted, A. (1941): Salt mixture. *J. Biol. Chem.*, 138: 459.
- Echeverri, S.; Nwabueze, T. U. and Nwabueze, J. C. (1997): Growth, nutrient utilization and biomass production of *Saccharomyces cerevisiae* from papas waste. *J. Agric. Biotech. Environ.*, 3:32-37.
- Gomeri, G. (1980): Aldehyde-fuchsin, A new staining for elastic tissue. *Am J Path.*,17:395-406.
- Goldhaber, S.B. (2003): Trace element risk assessment: essentiality vs. toxicity. *Regulatory toxicology and Pharmacology*. 38:232-42.
- Gordon, M.W. (2000): Contemporary Nutrition : Water and minerals, Nutrients; The heart of Nutrition, Part 2 Fourth Edition. P.284.
- Govoni, M.; Jansson, E. A.; Weitzberg, E. Lundberg, J. O. (2008): The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouth. *Oxide*, 19:333-337.
- Habig, W.B.; Pabst, M.J. and Jokoby, W.B. (1992): Glutathione S-transferases. *J. Biochem.*, 249: 7130-7139.
- Hassan, H.H. (1998): The intake of Nitrite with meat products and its biological effects. M.Sc. Thesis, Faculty of Home Economics, Menofiya Univ.
- Hegsted, A. (1941): Salt mixture. *J. Biol. Chem.*, 138: 459.

- Hsu,H.W.; Sutton,N.E.;Banjo,M.O.; Satterlee,L.D. and Kendrick, J.G.(1978): The C.PER and T.PER assays for protein quality. J.FD. Technol.,12:69.
- Jung, C.; Gonon, A.T.; Sjoquist, P.O.; Lundberg, J.O. and Pernow, J. Arginase (2009) : Inhibition media tescardio protection during ischaemia-reperfusion. Cardiovasc. Res., 85:147-154.
- Kapor,A.(2004): Nitrite removal from drinking water, review . J. Environ. Eng. Assoc.,124:378-384.
- Kolb, E.;Houg,M. and Vetter,A.(2004): Potential nitrosamines formation and its prevention during biological denitrification of red beet Juice. Food and Chemical Toxicology, 40(2):219-24
- Kolb, E.; Houg, M.; Janzowski, C. and Eisenbrand, G. (1997): Toxicity of hyper does of antioxidants. Food and Chemical Toxicology, 35 (2):218-225.
- Kolb, E.;Houg,M. and Vetter,A.(2004): Potential nitrosamines formation and its prevention during biological denitrification of red beet Juice. Food and Chemical Toxicology, 40(2):219-24
- Kolyuk, A.S.(2003): Immune system of children living in high content of nitrites region. Gringiena. Sanitary,15 (4): 20-23.
- Linder, M.C.(1995): Nutrition and Cancer Prevention. In: Linder, M.C. (Ed)., Nutritional Biochemistry and Metabolism With Chemical Applications. Elsevier, New York., 347-368.
- Majcherzak, D.; Gronowska, A. and Senger, A. ( 1991): Nitrates and nitrites and utilization of  $\beta$ -carotene. Effect of different amounts of nitrates and nitrites in the diet on utilization of  $\beta$ -carotene in the rats.Nut.ABS. and Reviews, 61(6).
- Meister, A.; Tate, S.S. and Griffith, O. W. (1981):  $\gamma$ -Glutamy1 transpeptidase. Methods in Enzymology., 77: 237-253.
- Merino,L.; Edberg,U.;Fuchs,G and Amam,P. (2000): Liquid chromatographic determination of residual nitrite and nitrate in food. J. of Aoca. International,83(2):365-375.
- NAS.(1995): Nitrate and nitrite in Drinking Water. Nat.Acad. of Sci.,USA.
- Oderinde, P H., Toma, R. B., Munson, S. T., and D'Appolonia, B, (2002): Sensory evaluation of breads containing various levels of papas seeds. American Journal, 59.605-611.
- Reed, D.J. (1998): Glutathione and vitamin E in Protection Against Mutagens and Carcinogens. In: Taylor II. And Scanalan RA (Eds). Food Toxicology: A Perspective on the Relative Risks. Marcel Dekker Inc., New York, P169-203.
- Reeves,P.G.;Nielsen,F.H. and Fahmy,G.G.(1993): AIN-93.Purified diets for laboratory rodents: Final report of the American Institute of nutrition adhoc wriling committee on the reformation of the AIN-76 ARodent diet.J.Nutrition, 123:1939-151.
- Sebecic, D. Vendri, S.A. and Dragsogevic, M. (2008): The effect of nitrite and nitrate in vegetabals and meat on human health. Nahrung, 43(4): 284-287.
- Sharon,M.(1997): Complet Nutrition" How To Live in Total Health". Prion Books Limited, 32/34 Gordon House Road,London NW5,ILP.



- Suketa,H(2002): Effect of food additives on cell growth and antibody production. Chem. Pharm. Bull Tokyo, 45: 459-64.
- Stavroc, B. (2001): Antimutagens and anticarcinogens in food. Food Chem. Toxicol., 32 : 79-90.
- Sarkhosh, A.; Zamani, Z.; Fatahi, R. and Ebadi, A. (2006): RAPD markers reveal polymorphism among some Iranian orange genotypes. Scientia.Horticulturae., 111: 24-29.
- Salaheddin, M. E. and Kader, A. A. (1984): Post-harvest physiology and storage behavior of citrus fruits. Scientia.Horticulturae., 24: 287-298.
- Sabbar, S. D.; Mir, N.A.; Hajera T. and Mazharuddin, K. (2010): Studies on Antibacterial and antifungal activity of orange peels . American Eurasian J. Agric. & Environ. Sci., 9 (3): 273- 281.
- Takagi, K.andSuketa, Y. (1997): Effect of nitrite on cell growth and antibody production in mouse splenic B cells stimulated with lipopolysaccharide and B cell hybridomas. Chem. Pharm. Bull Tokyo. 40(2): 419-22.
- Till, H.P.;Falke,H.E. and Willems, M.I.(2005): Evaluation of the toxicity of the potassium nitrite from drinking water in rats. Food Chem.Toxicol.,53(10): 451-459.
- Titanji, R. B.; D'Appolonia, B.; Dintzis, F. R., and Tabekhia, M.M., (2008).Biological and chemical properties of papas wastes as a source of dietary fiber in bread. J. Food Sci., 44: 14O3.
- Vincent, D.; Govoni, M. and Kamiya, S. (2010): Dietary nitrate in Japanese traditional foods lowers diastolic blood pressure in healthy volunteers. Nitric Oxide, 22:136–140.
- Walker,R(1998): The metabolism of dietary nitrites and nitrates. Biochem. Soc.Trans.,24:780-785.
- Wawrzyniak,A.;Gronowska-Senger,A. and Goacute-reka,K.(2006): The evaluation of nitrite and nitrate food intake among households . Roczniki Panstwowego Zakladu Higieny., 50 (31):269-287.
- Weiss, C.; Maker, H.S and Lehrer, G.M. (1980): Sensitive fluorometric assays for glutathione peroxidase and reductase. Anal.Biochem., 106: 512-516.
- WHO. (1990): International Programme on Chemical Safety. Environmental Health Criteria 109. WHO,Geneva.

## تأثير بذور الباباز للحد من الجرعات العالية من النترات والنترت على

## المواد المناعية ونشاط الإنزيمات المضادة للأكسدة

\*عادل السيد مبارك \*\* نهاد رشاد الطحان \* منى إبراهيم محمد

\*\*\* حنان سعيد عبدالسلام

\* قسم الإقتصاد المنزلي( التغذية وعلوم الأطعمة ) - كلية التربية النوعية - جامعة المنوفية .

\*\* قسم التغذية وعلوم الأطعمة - كلية الاقتصاد المنزلي - جامعة المنوفية .

\*\*\* باحثه بقسم الإقتصاد المنزلي( التغذية وعلوم الأطعمة ) - كلية التربية النوعية - جامعة المنوفية.

## المستخلص :

يهدف هذا البحث إلى معرفة مدى تأثير بذور الباباز على الحد من التأثير السام للجرعات العالية من النترات والنترت. وقد أضيفت بذور الباباز إلى الوجبة بنسبة ٢٠% و نترات البوتاسيوم بنسبة ٠.٦% اما نترت الصوديوم قد أضيف بنسبة ٠.٢% لمدة شهرين من التغذية. وقد تم تقدير القياسات الغذائية والمناعية ونشاط الإنزيمات المزيلة للسموم. وقد أشارت النتائج إلى أن وجود هذه النسبة من النترات والنترت في وجبة الفئران أدى إلى انخفاض كبير في المأخوذ من الطعام، الزيادة في وزن الجسم ومدى كفاءة الوجبة بالمقارنة مع المجموعة الضابطة. وقد أوضحت النتائج أن قيم المواد المناعية (الاميوجلوبيين) قد انخفضت بشكل ملحوظ نتيجة لإضافة النترات والنترت. ووجود النترات والنترت خفض أنشطة الكاتالاز والجلوتاثيون الكبدية ، في حين أنها أدت إلى زيادة بشكل كبير جما جلوتاميل ترانس ببتيديز في الكلى. وقد أدى إضافة بذور الباباز لجرعات عالية من النترات والنترت إلى زيادة المأخوذ من الطعام، الزيادة في وزن الجسم ومدى كفاءة الوجبة . وقد لوحظ زيادة معنوية في المواد المناعية (الاميوجلوبيين) بالمقارنة مع المجموعات الضابطة الموجبة. لذلك نجد أن التغذية على بذور الباباز في الوجبة بنسبة ٢٠% له تأثيرات متنوعة على مختلف الإنزيمات المضادة للأكسدة والناقلة. ونستنتج من هذا أن وجود بذور الباباز في الوجبة التي تحتوي على أملاح النترات والنترت مهم جدا. لذا نوصي في هذه الدراسة باستخدام النترات والنترت في المنتجات الغذائية وخاصة منتجات اللحم..

الكلمات التوضيحية : أملاح النترات والنترت - بذور الباباز - المواد المناعية- الإنزيمات المزيلة للسموم .