THE POSSIBLE PROTECTIVE ROLE OF BEES HONEY AGAINST HAZARD EFFECTS OF SOME SYNTHETIC FOOD ADDITIVES ON THE KIDNEY FUNCTIONS OF MALE RATS

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ABSTRACT

Recently the use of synthetic food additives was increased and the levels of human exposure to such agents are very broad, thus feeding over long periods may continually possess potential hazards to the human health. Also, protection against the adverse biological action of these widely used preservatives deserves a great attention. Therefore the aim of this work was to evaluate the protective role of bees honey against the kidney dysfunctions induced by food additives (sodium nitrite as a food preservative and sunset yellow as a food colorants) in male albino rats. The results of this study revealed that the oral administration of both sodium nitrite (10 mg/Kg body weight) and sunset yellow (0.6% w/w in diet) for 4 weeks induced renal dysfunction as reflected by a significant elevation in serum levels of uric acid, urea and creatinine, which was accompanied with a significant reduction in their urinary levels as well as in creatinine clearance. This results is apparently linked to the presently observed increase in potassium and decrease in sodium and calcium levels in both serum and urine. The study also showed a significant decrease in total protein and albumin levels as well as an increase in renal protein carbonyl content in rats administered both sodium nitrite and sunset yellow. Furthermore, enzymes activity represented in alkaline phosphatase (ALP), gamma-glutamyl transferase (γGT) and Na^+/K^+ ATPase showed marked increase in both ALP and γGT and decrease in Na^+/K^+ ATPase activity, while the number of the nephrons was non-significantly changed after the administration of these food additives. On the other hand, the daily intake of natural bees honey at a dose of 2.5g/Kg body weight resulted in sufficient amelioration against the hazard effects of food additives as indicated by the observed improvement in all tested biochemical parameters of kidney functions. Virtually, strict regulations have been set for the use of synthetic food additives to avoid introducing materials injurious to health.

Key words: **Synthetic food additives – Kidney functions – Bees honey – Rats.**

INTRODUCTION

It has been noticed that people especially children at the age of nursery usually used food containing colorant and additives with great amounts which attracts their attention. Synthetic compounds should be either omitted completely or highly restricted to lower levels due to its side effects (Yamagishi *et al.,* 2006).

Food additives are common in our life and plays an important role in human being's life. Nitrate and nitrite are ubiquitous in nature and their presence are essential for the fertility of the soil and used as international food additives added to the original food or mixture of foods for specific purposes (Kigore and Li, 1980). The toxicological effects of nitrites in different mammalian species are well documented and include carcinogenesis, hepatotoxicity, nephrotoxicity, impairment of reproductive function, endocrine disturbance, growth retardation, methaemoglobinaemia and impairment of certain defence mechanisms (Choi, 1985 ; Jahries *et al.*, 1986 ; Ismail *et al.,* 2003).

Synthetic food coloring additives are products from modern organic chemistry, which have been added to the list of additives used by the food industries. However, the available literature regarding their toxicity or metabolism revealed that little work has been published in the last decade (El-Saadany, 1991). Colors as an additive pigment or other substances synthesized, extracted, isolated or otherwise derived from vegetables or other sources (Shaker *et al.,* 1989). Coloring additives are vital constituents of food. They are probably the first characteristics perceived by the senses and are indispensable to the modern day consumer as a means for the rapid identification and affiants acceptance of food (Babu and Shenolikar, 1995). Colors have attracted more attention than any other food additives because they are the most obvious cosmetics and partially

because people have made connection between some artificial dyes and adverse reactions to food (Marmian, 1984). However, such levels of intake is not normally encountered. One of the common dyes is called sunset yellow. It is used as a food coloring in foods including dairy products, snake food, jams and dry drink powders. In addition to food, it is also used in aqueous drug solutions, tablets, capsules, tooth pastes, hair rinses and cosmetics (Helal and Abdel-Rahman, 2005).

The wide use of these food additives in food technology elevates the importance of studying their effects on mammals. The question of primary protection of these widely used additives also deserves a great attention. Among the protective agents are bees honey. It is a highly nutritious material as it is considered a balanced food source (Ezz El-Arab *et al.,* 2006). Honey is a natural product with very complex chemical composition. It is composed primarily of fructose and glucose but also contains 4 to 5% fructooligosaccharides which serve as prebiotic agents. It contains more than 180 substances, including amino acids, vitamins, minerals and enzymes (Lopez-Garcia *et al.*, 1999 ; Merken and Beecher, 2000). Although very well known as a food, honey is not well recognized as a medicine, yet it is one of the oldest medicines known and has continued to be used as such through the ages (Jones, 2001). For a long time, it has been observed that honey has an antioxidant, anti-inflammatory and anti-tumor; possesses a considerable hydroxyl radicals scavenging activity and prevents the depletion of the antioxidant enzymes. Moreover, it is said to normalize kidney functions and protect the liver from intoxication (Gribel and Pashinski, 1990 ; Bariliak *et al.,* 1996).

Therefore, the present work was designed to evaluate the role of bees honey against the adverse effects on the kidney functions induced by fed intake of both sodium nitrite and sunset yellow in male albino rats.

MATERIALS AND METHODS

Materials:

The substances used in this study were sodium nitrite ($NaNO_2$) as a food preservative and sunset yellow as a one of food colorants, both of them were provided by a local commercial producer company in a powder form. While the natural bees honey was obtained from herbs market in Egypt.

Experimental animals:

Twenty four adult male albino rats (*Rattus rattus*) weighed 150-170 g were used in this study. The animals were maintained under normal condition and fed on a basal diet composed of 60% ground corn meal, 15% grounded beans, 10% wheat bran, 10% corn oil, 3% casein, 1% mineral mixture and 1% vitamins mixture and supplied with water ad libitum. All experiments were carried out in accordance with protocols approved by the local experimental animal ethics committee.

Rats were randomized into four groups, six rats each as follows:

Group 1: This group of animals consisted of healthy normal adult male rats served as untreated control group.

Group 2: The second group of animals were received daily 2.5g/ Kg body weight of natural bees honey in an aqueous solution form (25%) by gastric tube (Yamada *et al.,* 1999) for 4 weeks.

Group 3: The third group of rats were given orally sodium nitrite at a dose of 10 mg/ Kg body weight (Helal and Abdel-Rahman, 2005) and sunset yellow at a dose of 0.6% w/w in a diet (Tanaka, 1996) for 4 weeks.

Group 4: The last fourth group of rats were given both sodium nitrite and sunset yellow in the same dose and route of administration as in third group, in addition to oral supplementation with bees honey in the same dose of the second group for 4 weeks.

At the end of the experimental duration, animals of the four groups were housed individually in metabolic cages. Twenty four hour (24-hr) urine sample were collected, centrifuged, labeled and stored at -20 ºC for future analysis (Baverstock, 1976). At the end of the metabolic studies, the experimental animals were sacrificed after being fasted for 12 hours. Blood samples were collected and the sera were used for the various biochemical analysis. The animals were rapidly dissected and the kidneys were removed out and the left kidney homogenates were prepared in ice cold physiological saline solution and kept at -20ºC for further biochemical analysis. While, the right kidney was used for enumeration of the nephrons following the method of Nelson (1922).

Biochemical methods:

Biochemical indices of kidney functions such as uric acid, urea and creatinine levels were measured using Stanbio Kits (Stanbio Laboratory, INC. 2930 East Houstion Street San Antonio, Texas, USA). Total protein was assayed by Biuret reaction according to Doumas (1975). Albumin was estimated by the method of Doumas and Giggs (1972). Enzymatic activities of ALP and γGT were estimated by the methods of Teitz (1976) and Young (1990) respectively. While renal Na^+/K^+ ATPase enzyme activity was estimated according to Bonting (1970). Sodium, potassium and calcium concentrations were determined by the method of Zettner and Seligson (1964) by using flame photometer (Jenway PFP7).The product of protein carbonyl was determined in the kidney homogenate as described by Smith *et al.* (1991).

Statistical analysis:

Data were statistically analyzed using analysis of variance (ANOVA) followed by Student's *t-test* as described by Snedecor and Cochran (1982). The collected data were expressed as means ± standard error (SE) of six rats and the percent of changes were calculated in relation to both

control and $NaNo_2$+Sunset rats group. The level of significance was expressed as P>0.05 for non-significantly different, while P<0.05 was significantly different.

RESULTS

Concerning the effect of food additives on the kidney functions, the recorded data in table 1 revealed a significant elevation in serum uric acid, urea and creatinine levels in the third group which represents sodium nitrite and sunset yellow treated group as compared with control group. Regarding urine analysis, the data showed significant decrease in urinary uric acid, urea and creatinine levels as well as creatinine clearance. Also, significant reduction was observed in serum total protein and albumin contents in rats received both sodium nitrite and sunset yellow. At the same time protein oxidation was observed as indicated by an increase in renal protein carbonyl content in the same rats. Concerning the number of nephrons, it is clear that there was no obvious significant changes in the number of the nephrons in rats treated with both sodium nitrite and sunset yellow. However, the administration of bees honey during supplementation of used food additives was found to counteract the observed changes in the above mentioned biochemical parameters when compared with sodium nitrite and sunset yellow treated group, indicating the positive action of bees honey on kidney functions.

In table 2 a significant increases in ALP and γGT enzymes activity were recorded in sodium nitrite and sunset yellow treated group. However, Na^+/K^+ ATP ase activity followed an opposite direction where it was significantly inhibited after sodium nitrite and sunset yellow as compared to control group. Meanwhile, the administration of bees honey recorded a significant noticeable improvement in the changes of these enzymes induced by sodium nitrite and sunset yellow.

Also, ionic content of serum Na^+, K^+ and Ca^{++} were represented in table 3. The observation revealed a significant decrease in Na^+ and Ca^{++}, these decreases concomitant with significant increases in K^+ content after sodium nitrite and sunset yellow treatment. Urinary Na^+, K^+ and Ca^{++} concentration followed a similar trend to that observed in serum in the same rats group. On the other hand, the daily administration of bees honey along with food additives caused a significant amelioration in the extent of disturbances of these elements if compared to their corresponding sodium nitrite and sunset yellow treated group. Concerning ANOVA analysis of the investigated parameters it was revealed that the general effect between groups was significant throughout the experiment with the exception of number of nephrons whereas it was non significantly different.

DISSCUSSION

Nowadays, food additives are considered to be one of the difficult problems in the food industry. All food additives, whether actually in use or being proposed for use, should be subjected to appropriate toxicological testing and evaluation?. The natural additives are safer and appreciated the addition of food preservation and coloring.

Nitrate and nitrites are environmental pollutants present in food and water and it is suggested that they may be contribute to the etiology of kidney diseases and problems related to immunity in animals (Goger and Sawant, 1992). It is known that nitrate and nitrite may react in the gastrointestinal tract synthesizing the harmful dimethynitrosamine substances which had adverse effects on animal and human organs specially kidney (Anthony *et al.,* 1994). Generally, kidney is the most sensitive organ to physiological changes in case of toxicology, therefore, kidney functions are greatly affected. In this study we try to evaluate the side effects of these food additives on kidney functions. In addition to evaluate the role of bees honey in alleviating the adverse effects induced by these synthetic compounds.

Effect of sodium nitrite and sunset yellow:

The present data showed that co-administration of sodium nitrite and sunset yellow to rats leads to kidney functional changes as detected by a significant elevation in serum uric acid, urea, creatinine levels which was accompanied with an inhibition in their urinary levels as well as creatinine clearance. The observed disturbance in these parameters is clinical manifestations of kidney dysfunction (Gavin, 1995). These results are consistent with previous investigations showing that nitrite caused impairment of kidney functions (Anthony *et al.,* 1994; Ahmed and Mannaa, 2000). The adverse effects of nitrite on kidney might be due to nitric oxide (NO) formations which causes kidney dysfunctions (Ismail *et al.,* 2003) or could be attributed to oxidation of important iron-containing enzymes such as the cytochromes responsible for cellular respiration and other oxidation reduction processes (Wood, 1980) where oxidation of haemoglobin to methaemoglobin induced cell hypoxia and cell injury (Atef *et al.,* 1991). Furthermore the observed increase in serum uric acid, urea and creatinine as well as decreased calculated creatinine clearance indicates renal dysfunction which may be due to changes in the threshold of tubular reabsorption, renal blood flow and glomerular filtration rate (GFR) (Zurovsky and Haber, 1995).

In addition to the reported effects of sodium nitrite and sunset yellow intoxications, the present results in agreement with Helal and Abdel-Rahman (2005) showed highly significant decrease in serum total protein and albumin concentrations. This reduction might be due to that the secondary and tertiary amines which supplied from the toxic N-nitroso compounds (Swann, 1975; Lih *et al.,* 1997), therefore the amino acids required to protein synthesis in the liver will decrease. Supporting evidence was supplied by the suggestion of Harper *et al.* (1979) who reported that dietary protein serves as the source of amino acids utilized for synthesis of plasma protein. Also it is clear that sodium nitrite decreased serum protein and albumin mainly through its effect on the liver through inhibiting oxidative

phosphorylation process and hence the availability of the energy source of protein synthesis (Anthomy *et al.*, 1994) and other metabolic process or through the necrotic changes especially of the plasma membrane (Guler *et al.*, 1994). At the same time, the nitrite effects on the reabsorption in the kidney tubules and impaired absorption of digested food material can not be ignored. Rodriguez-Morona and Tarazona (1994) indicated that uronyl nitrate decreases proximal tubular reabsorption which results in the activation of glomerular feed-back and lowers nephron filtration rate.

Other investigated studies indicated that sodium nitrite and sunset yellow supplementation is associated with oxidative stress, as reflected by increased protein carbonyl level. Such observation is comparable with those of Squillace *et al.* (2002), who noted that an increase in oxidative stress is closely associated with the widespread use of nitrates and nitrites in drinking water. Protein oxidation serves as a useful biological marker for assessing oxidative stress (Bruning and Bolt, 2000 ; Cummings *et al.*, 2001). So the observed increment of protein carbonyl in the present study is more likely to explain the reduction in the serum total protein and albumin concentration (Schanaider *et al.*, 2005). An increase in carbonyl content and protein oxidation may occur as the consequence of attack by free radicals (Levine *et al.*, 1994; Qujeq *et al.*, 2005). Carbonyl groups may be introduced into proteins by primary reactions such as metal-catalyzed oxidations, radiation-mediated oxidation, and oxidation by ozone or nitrogen oxides or in secondary reactions where proteins are oxidized by reactive species generated by the oxidation of other molecules (Levine *et al.*, 1994). Therefore, the increase in content of carbonyl groups would indicate drastic oxidative modification leading to drastic functional impairments.

In the obtained results, there was a marked increase in serum ALP and γGT enzymes activity in rats receive sodium nitrite and sunset yellow. This may correlate with the damage of liver cell membranes and hence liver dysfunction which may be due to in part to the effect of nitric oxide (NO) free radical production induced by nitrites (Ahmed and Mannaa, 2000; Ismail *et al.*, 2003). This result was in agreement with the finding of Dudka *et al.* (1995) as well as Helal and Abdel-Rahman (2005) who found that nitrite toxicity resulted in an increase in the activity of these enzymes.

In addition, it has been reported that, ATPase is an integral membrane protein enzyme, concerned with the immediate release of energy used for several specific physiological activities (Reddy *et al.*, 1992). The inhibition of Na^+/K^+ ATPase activity indicate an overall disruption in the energy metabolism (Nakao *et al.*, 1974) in addition to the membrane damage that correlated with production of hydroxyl radicals (Shen and Sangiah, 1995). Our findings are in agreement with Moriyama and Nelson (1987) which postulated that nitrite in a partly diffusible anion that can cross the plasma membrane and significantly affect membrane enzymes; ATPase activity inhibit the energy metabolism of the cells and damage cell membrane integrity (Kurimotom *et al.*, 1984).

The other evidence for kidney dysfunction by sodium nitrite and sunset yellow supplementation was provided by electrolytes disturbance as manifested by a marked elevation of serum K+ and reduction in both Na^+ and Ca^{++} levels. This observation was similarly recorded by Helal and Abdel-Rahman (2005) who recorded higher values of minerals in rats treated with sodium nitrite and sunset yellow. The importance of serum ionic Na^+ and K^+ is correlated with their involvement in many vital activities of cells and tissues, where they are actively transported through cell membranes, beside their role in muscle contraction. The present results could be correlated with cell membrane damage which lead to disturbances in Na^+ and K^+ pumping and disorders in membrane permeability (Ganong, 1999 ; El-Missiry *et al.*, 2001).The data obtained through the present study concerning the changes in the tested electrolytes goes parallel with the findings of an inhibition of Na^+/K^+ ATPase activity, where this enzyme is responsible for the active transport of sodium and potassium across cell membrane and indicating that it the enzymatic equivalent of the Na^+, K^+ pump (Keller, 1986).

The application of bees honey as a protective agent along with food additives:

Inspection of the data obtained in the present study displayed occurrence of a considerable protection by bees honey. The beneficial protective effects of bees honey have been described by several authors (Shambaugh *et al.*, 1990; El-Khayat and Ahmed, 2000; Ezz El-Arab *et al.*, 2006). They found that there was a direct link between the honey consumption and the level of polyphenolic antioxidants in the plasma. These findings further strengthen existing evidence that suggests that honey in the diet can provide people with protective antioxidant compounds.

The data of the present work showed that oral supplementation of bees honey induced significant amelioration in the observed abnormalities resulted from sodium nitrite and sunset yellow as achieved by a marked improvement in the examined biochemical parameters indicating kidney functions. These observations may be attributed to the antioxidant properties of honey which contain zink and selenium (Jamoussi *et al.*, 1996), in addition to many forms of flavonoid compounds (Merken and Beecher, 2000). These compounds were known for their hydrogen donating antioxidant activities as well as their ability to form complexes with divalent transition metal cations (Soler *et al.*, 1995). Thus, this highly antioxidant capacity of bees honey made it able to scavenge the free radicals, reducing the level of nitric oxide and consequently decrease the level of lipid peroxidation as well as prevent protein oxidation as reflected by the observed reduction in protein carbonyl content. Also, zink in honey plays an important role in the development of normal cellular immunity (Singh *et al.*, 1992). The pronounced

increase in serum contents of total protein and albumin with concomitant decrease in the activities of serum ALP and γGT were detected after oral administration of bees honey to rats received both sodium nitrite and sunset yellow. In support of the aforementioned results, El-Khayat and Ahmed (2000) indicated a marked hepatoprotection induced by bees honey. On contrast, the elevation in the activity of Na^+/K^+ATPase enzyme after the administration of the rats with honey may be due to the stability of the cell mechanism. Also the neutralization of the ions levels were achieved by clear improvement in the observed imbalances in these electrolytes when administration of bees honey to rats. The obtained results may be attributed to alterations of the cell membrane permeability due to the effect of bees honey in the face of an oxidative damage to cell membrane preventing the propagation of nitric oxide and lipid peroxidation (Rosenblat *et al.,* 1997 ; Frankel *et al*., 1998).

It is concluded that, kidney functions are affected clearly by co-administration of sodium nitrite and sunset yellow as indicating by the observed perturbation in the investigated physiological parameters. However, bees honey is expected to serve as a protective agent against these adverse effects. So we must avoid food stuffs containing these substances as much as possible because it has many adverse effects and harmful to health., in addition to eating a balanced diet which can provide people with protective antioxidant compounds to modulate the noteworthy effect of the update take away highly synthetic food additives.

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