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Biochemical studies on albino rats after administration of nitrosamine and the therapeutic actions of vitamin C, honey bee or crushed citrus seeds

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Abstract: The current investigation was carried out to study the effect of administration of dibutyl nitrosamine (DBNA) precursors namely: dibutylamine (DBA) and sodium nitrite for eight weeks on some biochemical blood indices of albino rats and the therapeutic action of vitamin C (150 mg/L), honey bee (100 mg/L) or crushed citrus seeds (100 g/Kg diet) against toxicity induced by DBNA. Nitrosamine administration elevated the concentrations of serum glucose**,** triglycerides, total cholesterol and nonprotein nitrogenous constituents. Activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were increased also significantly following DBNA treatment to rats for eight weeks, but total protein were decreased. However, honey bee, Vitamin C and crushed citrus seeds were able to modulate the affected values of the previous biochemical parameters, approximately, near to the control values. Our findings suggested that honey bee, Vitamin C and crushed citrus seeds suppresses DBNA induced hepato-renalo-carcinogenesis, maybe, by modulating the antioxidant defense status.
Key words: Nitrosamine, liver and kidney functions, glucose, nonprotein nitrogenous constituents, lipids, albino rats, natural products.

دراسة بيوكيميائية علي الفئران البيضاء بعد إعطائها النيتروزأمين والدور العلاجي لفيثامين ج أو عسل النحل أو مسحوق بذور الحمضيات

الملخص : البحث الحالي يهدف إلى دراسة تأثير إعطاء ثنائي بيوتيل نيتروزأمين DBNA لمدة ثمانية أسابيع علي بعض قياسات الدم البيوكيميائية للفئران البيضاء والدور العلاجي لفيثامين ج (150 ملجرام / لتر) أو عسل النحل (100ملجرام / لتر) أو بذور الحمضيات المطحونة (100ملجرام / كجم) ضد التسمم بالنيتروزأمين

أدى إعطاء النيتروزأمين إلى ارتفاع في تركيز الجلوكوز والدهون الثلاثية والكولسترول الكلي والمكونات النيتروجينية غير البروتينية، بينما انخفضت البروتينات الكلية في مصل دم الفئران المعاملة. كذلك ازداد نشاط الانزيمات الناقلة لمجموعة الامين AST , ALT والفوسفاتيز القلوي. أدى إعطاء عسل النحل وفيثامين ج وبذور الحمضيات المطحونة إلى التقليل المؤثر للأعراض السابقة الذكر حيث تعتبر من المواد المضادة للأكسدة

الكلمات المفتاحية: نيتروزأمين – وظائف الكبد والكلية – الجلوكوز – المكونات النيتروجينية غير البروتينية – الدهون – فئران بيضاء – المنتجات الطبيعية

Introduction

Nitrosamines, one of the most important environmental carcinogen, has been suggested to cause the generation of reactive oxygen species (ROS) resulting in oxidative stress , alter the antioxidant defense system in the tissues and cellular injury, which may be one of the factors in the etiology of cancer [1-4].On the other hand, Several investigations have provided convincing evidence that N-nitrosamines cause a wide range of tumors in all animal species and induce cancer in a variety of rodent organs, especially the liver and esophagus [5].

Thirunavukkarasu and Sakthisekaran [6] reported that animals treated with N-nitrosodiethylamine (NDEA) resulted in a significant increased levels of hexose, hexosamine and sialic acid in serum, whole liver tissue (control), hepatoma and surrounding liver tissue of control and experimental animals.

Mimata, *et al*.[7] observed that Plasma membranes (PMs) had longer chains composed of more unsaturated fatty in treated rats by butyl-N-(4-hydroxybutyl)nitrosamine solution than the normal ones. On the other hand, there were high amounts of arachidonic acid in rat bladder epithelium, and it accounted for more than fifty percent of phosphatidylethanolamine. PMs of precancerous epithelium had more arachidonic acid than those of the normal epithelium. However, oxidative stress caused by reactive oxygen species generated after administration of NDEA has been reported in membrane lipid peroxidation, and has been associated with various stages of tumor formation process [8]. On the other hand, Mittal, *et al.,* [4] noticed a substantial and significant increase in lipid peroxidation in all the studied tissues after the administration of NDEA.

Mittal, *et al.* [4] reported that NDEA administration caused a substantial liver damage as evidenced by nearly 5- and 10-fold increase in the activities of AST and ALT enzymes in treated animals, respectively. However, Omotosho *et al.,* [9] noticed that the obtained values for total protein, albumin and creatinine in male patients having urinary dimethylinitrosamine were not significantly different from those of healthy men.

Several natural and synthetic antioxidants were shown to have anticancer effects [10]. The studies of Sies, [11] and Bansal, *et al* [2-3] demonstrated that the pre-treatment with vitamin E prior to the administration of NDEA was effective in counteracting and modulating oxidative stress in rat erythrocytes in a time-dependent manner.

In the study of Al-Ali and El-Alfy,[12] all animals treated with (DBNA), honey, *N. sativum* and *L. termis* seeds induced chromosomal aberrations. However, animals treated with honey did not produce polyploidy. The maximum chromosome aberrations were obtained from the animals

treated with DBNA and in the group of animal fed *L. termis* in addition to DBNA, respectively. The lowest percentage of chromosomal aberrations was obtained from the group of animals treated with DBNA in addition to honey.

Orsolic et al., [13] investigated the effect of propolis and related polyphenolic compounds of propolis (caffeic acid, caffeic acid phenethyl ester and quercetin), honey, royal jelly and bee venom on tumour growth, metastasizing ability and induction of apoptosis and necrosis in murine tumour models (mammary carcinoma and colon carcinoma). Their findings clearly demonstrated that honey bee products given orally or systemically might have an important role in the control of tumour growth and tumour metastasizing ability.

The present investigation was aimed to study some biochemical blood indices of albino rats treated with (DBNA) precursors namely: (DBA) and sodium nitrite and the therapeutic action of vitamin C, honey bee or crushed citrus seeds against toxicity induced by DBNA.

Materials and Methods

Experimental Animals and dosing

Thirty adult male albino rats were used in the present study, weighing 100-120 gm. They were purchased from the breeding unit of Biology Department, Faculty of Science, the Islamic University of Gaza. Rats were left for one week before experimentation to adapt to laboratory conditions. They were kept in plastic cages with wire mesh covers. Animals were divided into five groups, as follows:

1- The first group served as control. Where commercial balanced diet and water were continuously and regularly supplied *ad .libitum* to animals all over the experimental period.
2- The second group was administered 1000 ppm dibutylamine and 2000 ppm nitrite as sodium salt dissolved in the drinking water for eight weeks, according to Galea *et al*.,[14].
3- The third group was drunk dibutylamine-sodium nitrite mixture plus vitamin C (150 mg/L) [15] for eight weeks.
4- The forth group was drunk dibutylamine-sodium nitrite mixture plus honey bee (100 mg/L) as mentioned by Al-Ali and El-Alfy [12] for eight weeks.
5- The fifth group was drunk dibutylamine-sodium nitrite mixture and received dried crushed citrus seeds mixed in the diet at (100 g/Kg diet) all over the experimental period.

Dibutylamine and sodium nitrite were Analytical grade and were purchased from Sigma Chemical Company. Honey bee was obtained from known source in the local market.

Blood sampling and processing

At the end of the experiment, animals were decapitated and 5 ml of blood was collected into centrifuge tubes without any anticoagulant. The centrifuge tubes were left for about 15 min. to allow blood coagulation. Then, clear serum samples were separated by centrifugation at 3000 r.p.m. for 20 min. and then kept in the refrigerator for different biochemical assays. However, determination of enzyme activities were carried out on fresh serum samples.

Measurement of biochemical blood indices

Serum glucose, triglycerides and total cholesterol were determined using the methods described by Trinder [16] , Fossati and Prencipe [17] and Allain, *et al.*, [18] respectively. Serum urea measurement was based upon the cleavage of urea with urease (Berthelot's reaction) according to Fawcett and Scott [19]. Serum uric acid was determined following the method described by Fossatti *et al.*, [20]. Serum creatinine was measured without protein precipitation according to Bartels *et al.*, [21]. Serum total protein was determined according to the Biuret reaction as designated by Armstrong and Carr [22]. The activities of serum AST and ALT were determined according to the method of Reitman and Frankel [23]. The measurement of serum ALP activity was based on Bessey et al., [24] method.

Data analysis

Data were computer analyzed using SPSS version 11.0 for windows (Statistical Package for the Social Sciences Inc, Chicago, Illinois). Means were compared by independent-samples t-test. Percentage change was also calculated.

Results

The mean values of albino rats serum glucose, triglycerides and total cholesterol as affected by dibutylamine nitrosamine (DBNA) with\without the chemoprevention of honey bee, vitamin C or crushed citrus seeds were summarized in table 1. Daily drinking of DBNA for eight weeks increased serum glucose level by 27.1% compared to the control level. However, the treatments of intoxicated rats by honey bee, vitamin C and crushed citrus seeds reduced the increment rate to 21.6, 11.2 and 5.9% compared to the control level. Mean values of serum triglycerides at the end of the experimental period were 85.6, 103.4, 120.2, 98.5 and 90.8 mg/dl in the treatments of the control, DBNA, DBNA +honey bee, DBNA + Vitamin C

and DBNA + crushed citrus seeds, respectively. On the other hand, mean values of serum Cholesterol were 180.3, 220.3, 198.5, 189.6 and 186.1, respectively.

Table (1): Glucose, triglycerides and cholesterol concentrations in albino rats after administration of nitrosamine and the therapeutic action of vitamin C, honey bee or crushed citrus seeds for 8 weeks

Parameters	Experimental groups				
	Control	**Nitrosamine (DBNA)**	**DBNA +honey bee**	**DBNA +Vit. C**	**DBNA +citrus seeds**
Glucose (mg/dl)	90.5 ±0.3	115.0 ±1.8	110.0±1.7	100.6 ±2.0	95.8 ±2.19
% change		27 .1 %	21. 6 %	11.2%	5 .9%
P value		<0.01	<0.01	<0.01	<0.05
Triglycerides(mg/dl)	85.6±0.1	103.4±2.15	102.2±3.7	98.5±2.6	90.8±2.16
% change		20.8%	19.4%	15.1%	6.1%
P value		<0.01	<0.01	<0.01	>0.05
Cholesterol (mg/dl)	180.3±0.2	220.3±3.6	198.5±2.8	189.6±4.1	186.1±2.4
% change		22.2%	10.1%	5.2%	3.2%
P value		<0.01	<0.01	>0.05	>0.05

All values expressed as mean ± SE

* Non significant differences at $p > 0.05$

** Significant differences at $p < 0.05$

*** Highly significant differences at $p < 0.01$

Protein and nonprotein nitrogenous constituents concentration in albino rats serum after oral administration of nitrosamine and the therapeutic action of vitamin C, honey bee or crushed citrus seeds were tabulated in table 2.

In general, oral administration of DBNA increased urea, Uric acid and creatinine compared to control level. The effect of DBNA was more pronounced on Uric acid. However, honey bee, Vitamin C and crushed citrus seeds were able to lowered the elevated values. Total protein values were decreased exhibiting percentage decreases of 19.2, 9.0, 11.9 and 10.3% in DBNA, DBNA +honey bee, DBNA + Vitamin C and DBNA + crushed citrus seeds, respectively, compared to control levels.

Activities of serum AST, ALT and ALP were increased significantly following DBNA treatment to rats for eight weeks (Table 3). However, these activities were reduced after the treatment by honey bee, Vitamin C and crushed citrus seeds when compared to DBNA treated rats alone. However, the AST, ALT and ALP enzyme activities remained high in all treatment

groups as compared to control group. Honey bee were effective in reducing the elevation of ALT but Vitamin C was effective on AST and ALP.

Table (2): Total protein and nonprotein nitrogenous constituents concentration in albino rats after administration of nitrosamine and the therapeutic action of vitamin C, honey bee or crushed citrus seeds for 8 weeks.

Parameters	Experimental groups				
	Control	**Nitrosamine (DBNA)**	**DBNA +honey bee**	**DBNA +Vit. C**	**DBNA +citrus seeds**
Urea (mg/dl)	29.5±0.1	40.1± 1.9	33.5 ±1.7	35.2± 0.7	30.6± 0.9
% change		36.1%	13.7 %	19.4%	3.8%
P value		<0.01	<0.01	<0.01	>0.05
Uric acid (mg/dl)	3.50±0.20	6.80± 0.26	5.70 ±0.24	4.50± 0.23	4.1± 0.13
% change		94.3%	62.9%	28.6%	17.1%
P value		<0.01	<0.01	<0.01	<0.05
Creatinine (mg/dl)	0.85±0.03	1.50± 0.01	1.30 ±0.02	1.25± 0.01	1.19 ±0.01
% change		76.5%	53.0%	47.1%	40.0%
P value		<0.01	<0.01	<0.01	<0.01
Total Protein (mg/dl)	6.81±0.10	5.50±0.50	6.20±0.4	6.00±0.38	6.11±0.31
% change		-19.2%	-9.0%	-11.9%	-10.3%
P value		<0.05	>0.05	>0.05	>0.05

All values expressed as mean ± SE

* Non significant differences at $p > 0.05$

** Significant differences at $p < 0.05$

*** Highly significant differences at $p < 0.01$

Discussion

Dibutylamine-sodium nitrite mixture fed to albino rats, in drinking water, for eight weeks produced harmful changes in the studied biochemical blood parameters. However, most of these changes showed signs of improvements with the treatments with honey bee, Vitamin C and crushed citrus seeds compared to DBNA treated rats alone. Nitroso-compounds have been shown ,in experiment conducted on animals, to be one of the most potent biochemical carcinogens. They interact with cellular macromolecules such as DNA, RNA and protein. These interactions cause both biochemical and physical alterations of these macromolecules [25].

Table (3): Serum AST, ALT and ALP activities in albino rats after administration of nitrosamine and the therapeutic action of vitamin C, honey bee or crushed citrus seeds for 8 weeks.

Parameters	Experimental groups				
	Control	**Nitrosamine (DBNA)**	**DBNA +honey bee**	**DBNA +Vit. C**	**DBNA +citrus seeds**
ALT (IU/ml)	26.5±0.2	43.0± 0.4	30.2± 1.3	35.3± 1.8	32.9± 0.1
% change		62.3%	14.0%	33.2%	24.2%
P value		<0.01	<0.05	<0.01	<0.01
AST(IU/ml)	31.9±0.4	46.1 ±2.1	40.0 ±3.0	34.9 ±3.1	37.8 ±1.2
% change		44.5%	25.3%	9.31%	18.3%
P value		<0.01	<0.05	>0.05	<0.05
ALP (IU/ml)	81.4±0.3	100.2 ±4.1	90.0± 2.8	86.5 ±3.1	89.9± 2.2
% change		23.1%	10.6%	6.3%	10.5%
P value		<0.01	<0.05	>0.05	<0.05

All values expressed as mean ± SE

* Non significant differences at $p > 0.05$

** Significant differences at $p < 0.05$

*** Highly significant differences at $p < 0.01$

Data revealed a general increase in serum glucose levels in albino rats in response to DBNA oral administration were in agreement with that observed by Thirunavukkarasu and Sakthisekaran [6]. DBNA may, indirectly, play a specific role in carbohydrate metabolism may bee by enhancing gluconeogenesis and glucose mobilization to the blood [26-28], Albino rats drunk DBNA plus honey bees, Vitamin C or crushed citrus seeds showed a decrease in serum glucose level. Honey bees, Vitamin C or crushed citrus seeds might be potentiate insulin effects by increasing insulin secretion [29].

Concerning lipid metabolism, results demonstrated that triglycerides and total cholesterol levels were increased in response to DBNA oral administration to the rats. The possible explanation of these observed increments may be reside in direct or indirect action of DBNA on lipid metabolism or lipid peroxidation [26].

The significant decreased levels of total protein in the DBNA intoxicated rats is concomitant with that observed by Thirunavukkarasu and Sakthisekaran [6]. The decrease of total protein cold be attributed to an increase in amino acids deamination.

The elevation of blood Urea is a good indicator for kidney disorders. Urea is the principal end product of protein catabolism. Enhanced protein catabolism and accelerated amino acid deamination for gluconeogenesis is

possible an acceptable postulate to interpret the elevated levels of urea [28]. The presence of some toxic compounds might increase blood urea and decrease plasma protein [30]. Uric acid is the end product of the catabolism of tissue nucleic acid, i.e. purine bases metabolism [31]. The increments in uric acid concentrations might be due to degradation of purines or to an increase of Uric acid levels by either overproduction or inability of excretion [31]. The decrease of uric accompanied with the treatment by honey bee, Vitamin C or crushed citrus seeds agreed with that reported by Pevicharova *et al.*,[32].

Creatinine is the last variable of nonprotein nitrogenous blood constituents. It appears in the serum in amounts proportional to the body's muscles mass and is more readily exerted by the kidneys than urea and uric acid [33]. Elevated creatinine concentration is associated with abnormal renal function, especially as it relates to glomerular function [28].

Serum transaminases (AST & ALT) and ALP exhibited a general increase in DBNA treated rats compared to the control. The observed elevation in serum AST, ALT and ALP activities in response to DBNA administration is in agreement with previous studies of Pevicharova *et al.*,[32] and Bansal *et al.*, [3] who found that, activities of AST, ALT and ALP were increased significantly following other N-nitroso compounds treatment to rats. The liver enzymes are normally found in circulation in small amounts because of hepatic growth and repair. As a liver specific enzyme ALT only significantly elevated in hepatobiliary disease. Increase in AST levels, however, can occur in connection with damages of heart or skeletal muscle as well as of liver parenchyma [34-35]. Consequently, elevated activities of ALT and AST observed in the current study in response to DBNA administration could be a common sign of impaired liver function. On the other hand, Alkaline phosphatase belongs to a group of enzymes catalyze the hydrolysis of phosphomonoesters at alkaline pH. ALP present in cell surface in most human tissues. The highest concentration are found in the intestine, liver bone, spleen and kidney [34, 36]. The specific location of the enzyme within both sinusoidal and bile canalicular membranes accounts for the more predominant elevations in certain disorders [28] as observed in the present study with DBNA administration. Impaired secretion of hepatic ALP of liver cell origin. Acute cell necrosis liberate ALP in the circulation and serum enzyme level is elevated. However, the activities of these enzymes were reduced after the treatment by honey bee, Vitamin C and crushed citrus seeds when compared to DBNA treated rats alone. The DBNA induced oxidative stress has lowered, however, the cellular injury may still persist as indicated by increased AST, ALT and ALP activities.

Considerable attention has been focused upon dietary items which inhibit the carcinogenic process. Zakhary, *et al.,* [37] have studied the protective effect of soybean and vitamin C against the carcinogenicity induced in the liver of albino mice by dibutyl nitrosamine (DBNA) precursors namely: dibutylamine and sodium nitrite. They reported that mild autoclaved soybeans as well as vitamin C, showed a significant prophylactic effect against the action of DBNA precursors as induced by the improvement of the changes of the biochemical parameters. Soybeans have been suggested as possible anticancer agents for their contents of Saponins [38]. The study of Sundaresan, and Subramanian [39] showed that the intoxication of rats by N-Nitrosodiethylamine was accompanied by a significant decrease in the levels of β carotene, ascorbic acid, vitamin E, reduced glutathione (GSH), glutathione peroxidase, superoxide dismutase and catalase. On the other hand, Rywotycki [40] revealed that the added sodium ascorbate caused a decrease in nitrosamine contamination level in meat.

Our findings suggested that honey bee, Vitamin C and crushed citrus seeds suppresses DBNA induced hepato-urino-carcinogenesis, maybe, by modulating the antioxidant defense status of the animals in response to the antioxidant action of their contents of flavinoids and polyphenolic compounds.

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