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Effect of chitosan-Cu complex on biological disturbance induced by dibutyl nitrosamine (DBNA)

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ABSTRACT

The present work was to elucidate the effectiveness of orally administered chitosan-Cu complex (200 mg/kg b.wt.) as a prophylactic agent against some harmful disturbances in mice orally treated freshly prepared dibutyl nitrosamine (DBNA) (NaNO₂ + DBA, 2000 ppm & 1000 ppm respectively) for 12 weeks. DBNA resulted in dramatic decrease in T₃ and T₄ levels, accompanied by decline in serum total protein, albumin and globulin concentration. In addition total leukocytes and neutrophils remarkably decline, parallel with exceed in lymphocytes and serum total lipid. Also, the total body weight negatively affected. However, most of these noteworthy effects of DBNA were suppressed by chitosan-Cu complex pretreatment indicating its protective role against various metabolic alterations caused by DBNA.

Key words: Chitosan, DBNA, Thyroxin, Metabolism.

INTRODUCTION

Nitrosamine is found in many food stuffs especially beer, fish byproducts and in meat and cheese products preserved with nitrite picking salts; they can be also formed by frying or smoking [Khan and Malhotra 2011], in addition to rubber workers, breathing asbestos or exposure to radiation, Straif *et al* [2000].

Toxicological effect of nitrosamines include hepatotoxicity growth retardation, impairment of reproductive functions and endocrine disturbances, Proksch [2001].

The wide environmental distribution of nitrites and secondary amines has been show to result in an increased risk of endogenous formation of N-nitroso compounds, Choi *et al* [2002].

The administration of 4-hydroxybutylnitrosamine to male mice yielded a high incidence of large palpable urinary bladder cancer, Lubet *et al* [2005].

Chitosan is the most important derivative of chitin which is the structural element of the exoskeleton of crustaceans, [Sanjiv *et al* 2011]. Chitosan, the linear polymer of d-glucosamine in β -(1-4) linkage has been recommended as a suitable functional material because of its biocompatibility, biodegradability, non-toxicity, adsorption properties and regulation cell activation, Pae *et al* [2001] and Yoon *et al* [2005]. Due to its antimicrobial activity chitosan have many pharmaceutical application and generally used as natural preservative for the safety of food [Sharma *et al* 2010] and [kalyan *et al* 2010].

Chitosan was reported by Yan *et al* [2006] to inhibit malondialdehyde formation and hence reduce lipid peroxidation.

The purpose of this study was to investigate the effectiveness of chitosan-Cu complex on some metabolic abnormalities caused by DBNA in male mice.

MATERIALS AND METHODS

Thirty, four weeks old male mice were purchased from eye-bank in Giza, Egypt, they were adapted for one week at the air conditioned animal house at Mansoura University, fed a chow diet for rodent. Food and drinking water were available *ad libitum* for 6 weeks. The animals were maintained in accordance with the guidelines for the care and use of the laboratory. They are classified into 4 groups, (a) control group(n=6), (b) DBNA treated group (n=8) (NaNO₂ 2000 + DBA 1000 ppm respectively) in drinking water for 6 weeks. (c) chitosan-Cu complex orally treated group (n=8) (200 mg/kg) orally for 6 weeks. (d) Chitosan-Cu complex followed by DBNA treatment (n=8), by the same route and time as in groups (c) and (b) respectively. Chitosan-Cu complex Mwt=9000D_a, deacetylation 62% was prepared in physics Dept., Faculty of Science Mansoura University, through fund from research box in Mansoura University. Body weight in each group was recorded every week for calculation of body weight changes.

Blood samples were collected in heparinized tubes for estimation of WBCs and detection of differential count in a blood smear stained with lishman's stain [Brown, 1973].

Other blood samples were collected in nonheparinized tubes, left to clots allowed to centrifuge where sera were collected for determination of T₃, T₄ using ¹²⁵I, radio-immune assay [Larsen, 1981], total protein (Cannon & Henry 1974),total lipids [Fring *et al* 1972].

Statistical analysis: All data were expressed as mean \pm SE. The data analysis was performed by ANOVA test, Tukey test, a P value of <0.05 was considered significant. The comparison was carried out with control or with DBNA treated data.

RESULTS

As shown in the figure and the table, DBNA treatment results in a significant decline in most estimated parameters especially T₃ level (-35%) except for the increase in total lipids, total cholesterol, lymphocytic and monocytic counts. Administration of chitosan-Cu complex nearly did not apparently affect the estimated parameters. Chitosan-Cu complex treatment pre DBNA administration nearly rended the estimated parameters towards normal values.

DISCUSSION

With increment detection and internally synthesis of nitrosamine in foods it is of importance to study how to protect peoples effectively from it. Chitosan and its derivatives have received most attention and are currently used in many fields. Low molecular weight chitosan is especially important medicine foods, cosmetics and agriculture because of the special physiological activity such as antitumor, antifungal, enhancing immunity, lowering cholesterol levels, water retention [Singh & Rary 2000]. The present work showed a reduction in T_3 and T_4 level in DBNA treated mice, these reduction can be explained by the reduced amino acids levels due to liver damage by DBNA, Anthony *et al* [1994], endocrine disorders [Proksch, 2001] and/or enzymes needed for thyroxin synthesis or liberation from thyroglobin, a point which need further investigation. The suggested ameliorative role of chitosan may be through ameliorating the hypothalamic pituitary axis which regulates the secretion of TRH, Edrees *et al* [1995].

Regarding leukocytes, a decrease were showed in its number after DBNA administration which may be due to the fatal blood disorders methemoglobinemia, a views which in accordance with Terblanche, [1995]. The data obtained revealed that DBNA showed a remarkable decrease in the counts of neutrophils, this is belived to be due to depression of haematopoietic function, where Lijinsky & Reuber [1984] found that nitrosamine and nitrosomethylanine given to rats induced depression in hepatopoietic properties.

The production of reactive oxygen species by neutrophils predominantly comes from the activation of NADPH oxidase to generate O_2^- , Clark *et al* [1990], and Kadri-Hassani *et al* [1995].

As neutrophils produce reactive oxygen species (ROS) which contribute to the brain pathogenesis and signal the inflammatory response, Liou *et al* [2003].

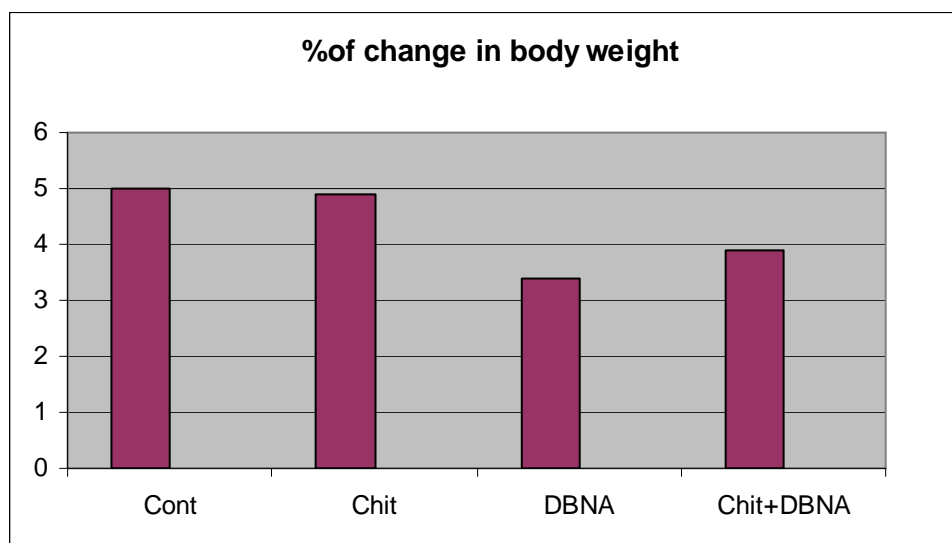
A recovery of leucopenia was noted with chitosan-Cu complex treatment pre DBNA that may be attributed to the role of chitosan in protecting bone marrow with its antioxidant properties, Jeon *et al* [2001].

Obtained decline in serum total protein and that of albumin in DBNA treated animals may be due to the destruction of liver architecture and necrosis caused by DBNA Mclean *et al* [1965]. In addition, the inability of hepatic cell to synthesis protein must be taken into consideration as reported by El-Nagar [2006]. Protein synthesis may be also blocked through the inactivation and degradation of the isoenzyme CYP_{2E1} [Vittozzi and Nastainczyk, 1987].

Regarding amelioration in body weight in chitosan -Cu complex treatment pre DBNA administration, it seems that chitosan interact with cell membrane enhancing peptide and protein uptake, Poropratto *et al* [2005].

The effect of chitosan-Cu complex in normalization of serum protein level, in agreement with Serag *et al* [2009] and may be through the antioxidant property leading to free radical scavenging activity by the effect of H_2O_2 in breakdown of chitosan at the weak points to get low molecular weight chitosan that possess good O_2^- scavenging activity because its geometry (square-planar), favours reaction of O_2^- reacting with Cu^{2+} giving normal liver function and hence protein synthesis, Yin *et al* [2004]. The attenuation caused by chitosan-Cu complex may be also due to its capability in exerting significant cytoprotection in necrotic damage induced by DBNA, Jeon *et al* [2003].

The obtained hyperlipidemia in DBNA treated mice are in agreement with Beaupre and Schiffman [1994] and may be attributed to protein loss in urine that decrease plasma osmotic pressure enhancing hepatic synthesis of lipoprotein causing a secondary hyperlipidemia., a view which in agreement with Samuels *et al* [2002].



Biochemical and hematological parameters in control and different treated groups.

groups Parameters	Control	Chitosan	DBNA	DBNA + Chitosan	ANOVA
T ₃ (ng/dl)	119.6±1.3	114±1.2	80.8 ^{ab} ±1.4	100.8 ^{abc} ± 2.7	p≤ 0.05 S
T ₄ (ng/dl)	5.9±2.09	5.7 ±0.15	4.3 ^{ab} ±0.12	5.0±0.15 ^{abc}	p≤ 0.05 S
Total Protein (g/dl)	8.3±0.9	8.0 ±0.8	5.2 ^{ab} ±0.09	6.3 ^{abc} ±0.1	p≤ 0.05 S
Albumin(g/dl)	5.0±0.09	4.9±0.07	3.4 ^{ab} ±0.09	3.9 ^{abc} ±0.1	p≤ 0.05 S
Globulin (g/dl)	3.3±0.09	3.1±0.06	1.8 ^{ab} ±0.09	2.3 ^{abc} ±0.1	p≤ 0.05 S
Total Lipid (mg/dl)	343±5.5	331.±5	402 ^{ab} ±2.6	355^{bc} ±3.6^{bc}	p≤ 0.05 S
Total Cholesterol (mg/dl)	90±2.6	88 ±0.6	170 ^{ab} ±5.2	130 ^{abc} ±2.2	p≤ 0.05 S
WBCs count (10 ³ /μL)	9.2±0.9	9.6±0.8	4.9 ^{ab} ±0.6	5.9 ^{abc} ±0.7	p≤ 0.05 S
Neutrophil%	47 ±2.3	45 ±3.1	20^{ab} ±1.3	22^{ab} ±2.1	p≤ 0.05 S
Lymphocytes%	40 ±3.3	40 ±2.3	60^{ab} ±5.1	58^{ab} ±4.2	p≤ 0.05 S
Monocytes%	8 ±0.4	10 ±0.9	15^{ab} ±0.5	12^{ac} ±0.3	p≤ 0.05 S
Basophils%	2 ±0.04	1 ±0.07	1^a ±0.09	5^{abc} ±0.03	p≤ 0.05 S
Eosinophils% ^o	3 ±0.01	4 ±0.06	4 ±0.07	3 ±0.03	p≤ 0.05 S

Data are expressed as means ± SE of 6 animals at least.

ANOVA: p = probability

Tukey test: a = significant difference as compared to control group.

b = significant difference as compared to Chitosan group.

c = significant difference as compared to DBNA group.

The improvement caused in mice administered chitosan-Cu complex pre DBNA treatment agree with Yao and Chiang [2002] and may be explained by inhibition of dietary fat digestion Kanauchi *et al* [1995] and/or decrease of plasma VLDLc and LDLc which increase HDLc level as reported by Chiang *et al* [2000]. The antilipidemic property of chitosan through regulating total cholesterol reported by Xeng *et al* [2008] must be taken into consideration.

The ability of chitosan in reducing lipid absorption in the intestine by binding with fatty acids and bile acids and increasing their excretion may also lead to this hypolipidemic effect, Chee *et al* [2005].

This study suggested that chitosan-Cu complex with low molecular weight possess good O₂⁻ scavenging activity and improve protein and lipid metabolism as well as thyroid abnormalities.

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