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Response of Wistar Rats to Low Levels of Dietary *Allium cepa*, *Allium sativum* and Sodium Selenite

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Abstract: *Allium cepa* and *Allium sativum* were fed to male Wistar rats at 2 or 6% of standard diet for 4 weeks. Sodium selenite was also fed to rats at 1 ppm and 3 ppm for a similar period. Two percent *A. cepa* and 2% *A. sativum* or 1 ppm sodium selenite were not toxic to rats. Impairment of growth and hepatonephropathy were observed in the rats fed diets containing 6% *A. cepa* and 6% *A. sativum* or 3 ppm sodium selenite. These changes were correlated with alterations in serum aspartate aminotransferase (AST) alanine aminotransferase, (ALT) and alkaline phosphatase (ALP) activities and total protein, albumin, cholesterol and urea concentrations and hematology.

Key words : *Allium cepa*, *Allium sativum*, selenite, toxicity

Introduction

Allium cepa, Onion, (Liliaceae) is used in folk medicine of many countries including Sudan as antispasmodic, carminative, diuretic, expectorant, antiseptic, stomachic and antihelmenthic and for the treatment of skin diseases and diabetes mellitus by virtue of it's contents of organic sulphur compounds (Fenwick and Hanley, 1985; Abdel Gadir, 2005). Onion and Onion extracts have been shown to decrease blood lipid levels, increase fibrinolysis, decrease platelet aggregation and lower blood pressure in several clinical studies (Yin and Cheng, 1998).

Allium sativum, Garlic, a member of the family (Liliaceae) contains allicin, a hypotensive diallyl disulphide oxide and is used as an antidiabetic agent and for it's bacteriostatic action (Oliver-Bever and Zahnd, 1979; Reuter, 1995).

Selenium is an essential microelement in humans and animals (Thomson, 2004) and is incorporated into proteins to make selenoproteins, which are important antioxidant enzymes that protect cells against the effects of free radicals that contribute to the development of some chronic diseases such as cancer and heart disease (Hodgson and Levi, 1997; Combs and Gray, 1998). Other selenoproteins help regulate thyroid function and play a role in the immune system (Corvilain *et al.*, 1993).

There is paucity of information of the effects of low levels of dietary *A. cepa*, *A. sativum* and sodium selenite on animals. The present study was planned to investigate the effects of these substances on the growth and pathological, biochemical and hematological characteristics of Wistar rats.

Materials and Methods

Experimental Design

Fifty six clinically healthy male Wistar rats were housed within the premises of the Institute of Medicinal and Aromatic plants, National Centre for Research, Khartoum, with feed and water provided

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ad libitum. The rats were divided at random into seven groups of eight rats each. Rats in group 1 were the controls and fed untreated diet. *A. cepa* (Onion) and *A. sativum* (Garlic), were purchased from a local market, separately ground and mixed with the control diet. Groups 2 and 3 were fed diets containing 2% (w/w) and 6% (w/w) of Onion, respectively. Groups 4 and 5 were fed diets containing 2% (w/w) and 6% (w/w) of Garlic, respectively and groups 6 and 7 were fed diets containing 1 and 3 ppm of sodium selenite, respectively. All rats were fed the designated experimental diets for 4 weeks.

Body weight and body weight gain were measured weekly for each group. The rats from each group were killed under diethyl ether anaesthesia. Blood samples were collected from each of the killed rats for serochemical analysis and hematology.

Rats from each group were killed at week 4 to identify gross lesions and specimens of the liver, intestines, kidneys, spleen and heart were immediately fixed in 10% neutral buffered formalin and processed for histopathology.

Serobiochemical Parameters

Serum samples were analysed for the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and concentrations of total protein, albumin, globulin, bilirubin, cholesterol and urea by commercial kits (Linear Chemicals, Barcelona, Spain).

Hematological Parameters

Hemoglobin (Hb) concentration, Red Blood Cell (RBC) and White Blood Cell (WBC) counts, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were determined by standard methods (Schalm *et al.*, 1975).

Statistical Analysis

The significance of differences between means was compared using Duncan's multiple range test after ANOVA for one-way classified data (Snedecor and Cochran, 1989).

Results

Effect on Growth

The effects of treatment with diets consisting of 2 and 6% *A. cepa*, 2 and 6% *A. sativum* and 1 and 3 ppm sodium selenite on body weight and body weight gain of the rats are shown in Table 1. The rats on diet containing 2% *A. sativum* (group 4) showed no significant changes in growth over the 4-week feeding period. The rats fed a diet containing 6% *A. cepa* (group 3), 6% *A. sativum* (group 5) and 3 ppm sodium selenite (group 7) had lower ($p < 0.05$) growth than control (group 1) and rats on 2% *A. cepa* (group 2) and 1 ppm sodium selenite diets (group 6) but none of the rats died during the feeding period.

Pathological Changes

Neither gross lesions nor microscopic changes were seen in the vital organs of the control (group 1) or of the rats fed 2% *A. cepa* (group 2), 2% *A. sativum* (group 4) or 1 ppm sodium selenite (group 6). In the rats on the 6% *A. sativum* diet (group 5), there was fatty cytoplasmic vacuolation and individual-cell necrosis of the centrilobular hepatocytes and degeneration of the epithelial cells of the renal convoluted tubules. No significant lesions in other organs or tissues were observed. Degenerative changes were observed in the liver and kidneys of the rats fed a diet containing 6% *A. cepa* (group 3). Necrotic foci and aggregates of lymphocytes were observed. No significant lesions were seen in the heart, spleen and intestines of rats in group 3. In group 7, the liver revealed fatty cytoplasmic

Table 1: Changes in growth of rats fed *A. cepa*, *A. sativum* and sodium selenite for 4 weeks

Treatment groups	Body weight (g)	Body weight gain (g)
Control (normal diet)	100.6±4.9 ^a	35.0±2 ^a
2% <i>A. cepa</i>	98.7±3.6 ^a	34.6±2.3 ^a
6% <i>A. cepa</i>	84.5±1.4 ^b	20.0±1.2 ^b
2% <i>A. sativum</i>	98.7±3.6 ^a	34.6±2.3 ^a
6% <i>A. sativum</i>	84.5±1.4 ^b	20.0±1.2 ^b
1 ppm Sodium selenite	98.7±3.6 ^a	34.6±2.3 ^a
3 ppm Sodium selenite	84.5±1.4 ^b	20.0±1.2 ^b

Values are means±SE, Means within columns not sharing common letter(s) are significantly different (p<0.05)

Table 2: Serobiochemical and haematological changes in rats fed *A. cepa*, *A. sativum* and sodium selenite for 4 weeks

Parameters	Treatment groups						
	1 Control normal diet	2 Onion 2%	3 Onion 6%	4 Garlic 2%	5 Garlic 6%	6 Selenite 1 ppm	7 Selenite 3 ppm
AST (iu)	10.0±1.5 ^b	21.0±2.2 ^a	17.0±1.8 ^a	21.0±1.6 ^a	17.0±0.9 ^a	21.0±2.2 ^a	21.0±1.4 ^a
ALT (iu)	5.0±0.4 ^c	20.0±1.9 ^b	30.0±2.5 ^a	20.0±1.9 ^b	30.0±2.2 ^a	19.0±1.8 ^b	25.0±2.3 ^a
ALP (iu)	393.8±6 ^c	46.5±2.4 ^d	146.6±4.8 ^c	191.6±4.9 ^b	167.7±2.7 ^b	173.2±4.3 ^b	186.0±5.9 ^b
Total protein (g dL ⁻¹)	9.3±0.7 ^a	8.2±1.2 ^a	9.3±0.8 ^a	8.8±0.6 ^a	7.8±0.8 ^a	8.04±1.2 ^a	9.4±0.6 ^a
Albumin (g dL ⁻¹)	3.4±0.2 ^a	3.4±0.8 ^a	2.0±0.4 ^b	2.9±0.6 ^{ab}	1.7±0.5 ^c	3.01±0.8 ^a	2.4±0.2 ^b
Globulin (g dL ⁻¹)	5.9±0.4 ^{ab}	4.8±0.9 ^b	7.3±0.7 ^a	5.9±0.5 ^{ab}	6.1±0.8 ^{ab}	5.03±0.9 ^{ab}	7.0±0.6 ^a
Cholesterol (mg dL ⁻¹)	78.4±3.1 ^b	114.7±3.2 ^a	58.1±1.9 ^c	75.4±1.9 ^b	99.3±3.5 ^a	89.98±3.2 ^a	92.03±2.2 ^a
Urea (mg dL ⁻¹)	8.5±1.9 ^b	10.2±1.9 ^b	13.2±1.1 ^b	4.9±0.9 ^c	61.3±1.1 ^{ab}	21.1±1.3 ^b	85.2±2.2 ^a
Hb (g dL ⁻¹)	9.2±0.9 ^b	12.6±0.9 ^a	12.8±0.9 ^a	9.0±0.8 ^b	11.97±0.6 ^a	12.6±0.9 ^a	12.3±0.8 ^a
RBC (×10 ⁶ mm)	5.4±0.5 ^a	3.6±0.7 ^b	3.7±1.2 ^b	5.2±0.3 ^a	3.6±0.3 ^b	3.9±0.8 ^b	2.9±0.08 ^c
PCV (%)	29±0.3 ^{ab}	24.6±1.3 ^b	25.5±1 ^b	27.0±0.6 ^{ab}	23.5±0.4 ^b	38.0±1.2 ^a	37.0±1.2 ^a
MCV (m ³)	53.7±2.1 ^c	68.3±2.3 ^b	68.9±2.2 ^b	51.9±3.1 ^c	65.3±1.5 ^b	97.4±2.3 ^{ab}	127.6±3.2 ^a
MCH (pg)	17.03±1.3 ^c	35.0±2.3 ^{ab}	34.6±2.8 ^{ab}	17.3±1.3 ^c	32.3±2.1 ^{ab}	32.3±1.9 ^{ab}	42.4±3.4 ^a
MCHC (%)	31.7±1.7 ^b	51.2±3.3 ^a	50.2±3.5 ^a	33.3±1.4 ^b	50.8±2.2 ^a	33.2±1.7 ^b	33.2±1.7 ^b
WBC (×10 ³ mm)	6.15±1.2 ^a	5.9±0.7 ^a	5.1±0.6 ^a	4.5±0.9 ^{ab}	3.9±0.7 ^b	4.7±0.6 ^{ab}	4.2±0.5 ^{ab}

Values are means±SE, Means within rows with no common letter(s) are significantly different (p<0.05)

vacuolation and necrosis of the centrilobular hepatocytes. The fatty vacuoles coalesced and the cells of the renal proximal convoluted tubules were degenerated or necrotic and some of the glomerular tufts were packed or segmented.

Serobiochemical and Hematological Changes

In the rats on the 2% (group 2) and 6% *A. cepa* diets (group 3) and in the rats on 2% (group 4) and 6% *A. sativum* diets (group 5) or on the 1 ppm (group 6) and 3 ppm sodium selenite diets (group 7), there were significant increases in the activities of serum ALT and AST and decreases in the activity of ALP (Table 2). The concentration of cholesterol in groups 2, 5, 6 and 7 and that of urea in groups 5 and 7 were higher (p<0.05) than that in control and other groups. Albumin level decreased in groups 3, 5 and 7 and that of globulin was higher (p<0.05) in groups 3 and 7 than control and other groups (Table 2).

The values of Hb, MCV and MCH in groups 2-7 were higher (p<0.05) and those of RBC in groups 2-7 were lower (p<0.05), PCV in groups 2, 3 and 5 and WBC in groups 4-7 were lower (p<0.05) than other groups. The values of MCHC in groups 4, 6 and 7 did not change.

Discussion

As expected, there were no differences in mean body weight gains between the groups of rats for the 2 week pretrial period. This may be explained by the feeding of identical diets to each group and the useful randomized assignment for examination. There are currently no reports on the open literature of the effects on the growth of Wistar rats of dietary *A. cepa* or *A. sativum*. The results of the present

study indicate that feeding rats with *A. cepa* and *A. sativum* at 2% of the normal diet for 4 weeks is not toxic as evidenced by the absence of mortality, of clinical changes, of growth impairment and of lesions in the vital organs. The incorporation of *A. cepa* and *A. sativum* in the normal diet at 2 and 6% was chosen for several reasons. For rats, the two dietary levels represent non toxic concentrations of some plants and are exemplified by *Thymus vulgaris* (Haroun *et al.*, 2002). On the other hand, levels of 2 and 5% of dietary *Jatropha curcas* and *Ricinus communis* have been found to be toxic to rodents and chickens (Adam, 1974; El-Badwi *et al.*, 1992).

In the rats fed a diet consisting of 6% *A. cepa* and 6% *A. sativum*, the damage to vital organs could explain the depression in growth. The mechanism whereby the plant constituents injured body tissues can not be derived from the present study but the injury to these organs probably contributed to the elevated serum AST and ALT activities and cholesterol and urea concentrations and the decreased albumin concentration and ALP activity. The increase in MCV without significant effects on MCHC indicates macrocytic normochromic anaemia.

The rats fed a diet containing 3 ppm of sodium selenite had a significantly lower body weight gain than the rats fed dietary selenite at 1 ppm for 4 weeks. The growth retardation in rats by dietary selenite at 3 ppm suggests a possible inhibitory effect on protein metabolism. The mechanism whereby sodium selenite exerts its effects on rats can not be stated on the basis of the present study. It appears that selenite affects the physiological functions. The liver and kidneys are the main organs affected by selenite. In the present study, the hepatic changes comprised focal necrosis and fatty cytoplasmic vacuolation of the centrilobular hepatocytes, congestion, hemorrhage and lymphocytic accumulation. The renal lesions consisted of scattered lymphocytic infiltrations in the cortex, congestion, hemorrhage and degeneration or necrosis of the epithelial cells of the convoluted tubules. Severe hepatorenal lesions were described in Hibro chicks fed dietary selenite at 3 ppm (Dafalla and Adam, 1986). These authors found that the presence of focal myocardial degeneration and of hemorrhage on the thigh, breast and internal organs suggest some affinity of selenite for the cardiovascular system. This might be due to the greater degree of vascularity in the thigh compared to the breast (Dafalla *et al.*, 1986).

The species of animal plays an important role in exhibiting a chemical's effect. In addition to studying different species, investigations into the isolation and characterization of the active constituents of *A. cepa* and *A. sativum* to elucidate their modes of actions are necessary. The results of interaction of the two plants and of sodium selenite with potassium bromate need to be published.

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