

Imbalanced Dietary Mineral Intake Induced Cellular Inflammation in Murine Liver and Kidney

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Abstract.- Balanced diet provides an adequate intake of energy and nutrients for maintenance of the body and hence good health. Acidic and basic diets could harm both the hepatic and renal tissues. This study was designed to investigate the effect of excess intake of dietary minerals on hepatic and renal tissues of mice. Animals were divided into three groups and fed for 8 weeks. The first group was fed on a standard diet, the second group fed on an acidic diet and the third group fed on a basic diet. Hepatic and renal tissues were damaged by the imbalanced diet. This was evidenced by (i) Increased inflammatory cells in the liver and kidney, (ii) vacuolization of some hepatic and renal cells, and (iii) increased leukocytes count. Our data indicated that imbalanced diet affects the health and induced a pronounced inflammation and marked hepatic and renal tissues damage.

Keywords: Imbalanced diet, minerals, inflammation, liver, kidney, mice

INTRODUCTION

One of the famous methods of sex selection is the consumption of particular foods. It is reported that the ratio of the minerals sodium, potassium, calcium and magnesium are important in determination of baby gender (Meikle and Thornton, 1995; Vahidi and Sheikhha, 2007; Noorlander *et al.*, 2010). However, nutrition is vital in the growth and development of the fetus. A balanced, nutritious diet is an important aspect of a healthy pregnancy. Eating a healthy diet, balancing carbohydrates, fat, and proteins, and eating a variety of fruits and vegetables, usually ensures good nutrition (Chandrabu *et al.*, 2013; Pedersen *et al.*, 2013). In addition, imbalanced diets play important roles in development and progression of liver and kidney diseases (Toshimitsu *et al.*, 2007).

The liver is the largest metabolic organ in the body and is actively involved in many metabolic functions (Munding and Tannapfel, 2011; Amer *et al.*, 2013). It performs a number of important and complex biological functions that are essential for survival. Hepatic damage is associated with

distortion of these metabolic functions (Wolf, 1999; Smathers *et al.*, 2011). Also, the kidneys are vital where they keep the body's fluids, electrolytes, and organic solutes in a healthy balance. A well-planned diet can replace lost protein and ensure efficient utilization of ingested proteins through provision of adequate calories. Dietary changes can also help control hypertension, edema, and hyperlipidemia, and slow the progression of renal disease (Mahan and Arlin, 1992).

Even though research continues to provide information on the interactions between sodium, potassium, magnesium and calcium, little effort has been directed toward determining the effect of these elements on the physiology of organs, excretion and tissue distribution of one another. Therefore, the aim of the present study was to evaluate the effect of excess dietary minerals intake on hepatic and renal tissues of mice.

MATERIALS AND METHODS

Animals and diet

Ten weeks old female adult Swiss albino mice were obtained from the animal facilities of King Saud University (Riyadh) were fed on the experimental diets and water *ad libitum* for 8 weeks. The experiments were performed and approved by State authorities and followed Saudi Arabian rules for animal protection.

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0030-9923/2014/0002-0517 \$ 8.00/0

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Table I.- Histopathological liver score induced by the diet.

Microscopic observations	Groups		
	Standard diet	Acidic diet	Basic diet
Histological activity index ^a	2	9-11	9-11
Inflammatory cellular infiltration	0	+	+++
Sinusoid dilatation	0	+	+
Cytoplasmic vacuolization	0	+++	+
Binucleated cells	0	+	+
Cell swelling	0	++	+
Hyperplasia of Kupffer cells	0	++	+

^a Modified according to Ishak *et al.* (1995). Score: 1-3, minimal; 4-8, mild; 9-12, moderate; 13-18, severe. 0, absent; +, mild; ++, moderate, and +++, severe.

The diet was obtained from Arasco (Riyadh, Saudi Arabia). It is based on the principle of making the diet acidic or basic (Vahidi and Sheikha, 2007). The acidic diet contained 20 % extra calcium, magnesium and phosphorus than the normal diet. Basic diet contained 20 % extra sodium, potassium and Iodine.

Experimental design

Thirty mice were allocated into 3 groups of 10 mice per group. Animals were fed on the corresponding diet for 8 weeks. The first group was fed on a standard diet, the second group was fed on an acidic diet and the third group was fed on a basic diet. Animals were sacrificed after 8 weeks.

Pieces from liver and kidney were freshly removed, fixed in 10% neutral buffered formalin and then embedded in paraffin. Sections were cut and stained with hematoxylin and eosin. Histological damages were scored as follows: 0, absent; +, mild; ++, moderate; and +++, severe. Also, modified quantitative Ishak scoring system (Ishak *et al.*, 1995) was used for the liver; scores of 1-3 were assigned to cases of minimal liver damage, scores of 4-8 to mild, scores of 9-12 to moderate and scores of 13-18 to severe cases.

Alanine aminotransferase

Colorimetric determination of blood plasma alanine aminotransferase (ALT) was carried out by measuring the amount of pyruvate produced by forming 2, 4-dinitrophenylhydrazine. The color was measured at 546 nm according to Bergmeyer (1985).

Total leukocytes count

Complete blood was withdrawn from mice hearts into heparinized tubes. Total leukocytes count was analyzed using Vet abc TM Animal Blood Counter (HoribaABX, Montpellier, France) using a specified kit (Horiba ABX, France).

Statistical analysis

One-way ANOVA was carried out, and the statistical comparisons among the groups were performed with Duncan's test using a statistical package program (Sigma Plot version 11.0). All *P* values are two-tailed and $P \leq 0.05$ was considered as significant for all statistical analysis in this study.

RESULTS

Mice fed a standard diet for 8 weeks appeared with normal architecture of normal central vein and surrounding hepatocytes and sinusoids lined with Kupffer cells (Fig. 1, Table I). The liver of mice fed on both of acidic and basic diets has undergone some moderate pathological changes such as inflammatory infiltrations, hepatocytic vacuolations, sinusoid dilatations, and edematous hepatocytes in comparison to livers of control mice (Fig. 1, Table I). All these alterations are considered in the histological liver activity index according to Ishak *et al.* (1995), which can be categorized as 9-11 for the liver of mice fed on either acidic or basic diet (Table I). Both of the acidic and basic diets induced a significant increase in the total leukocytes counts (Fig. 2) but there is no significant difference between the leukocytes counts in the mice groups fed on acidic or basic diets (Fig. 2).

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Table II.- Histopathological kidney score induced by the diet.

Microscopic observations	Groups		
	Standard diet	Acidic diet	Basic diet
Tubular vacuolization	0	+	++
Hydropic degeneration change	0	++	+
Glomerular damage	0	++	++
Inflammatory cellular infiltration	0	++	++

0, absent; +, mild; ++, moderate; +++, severe

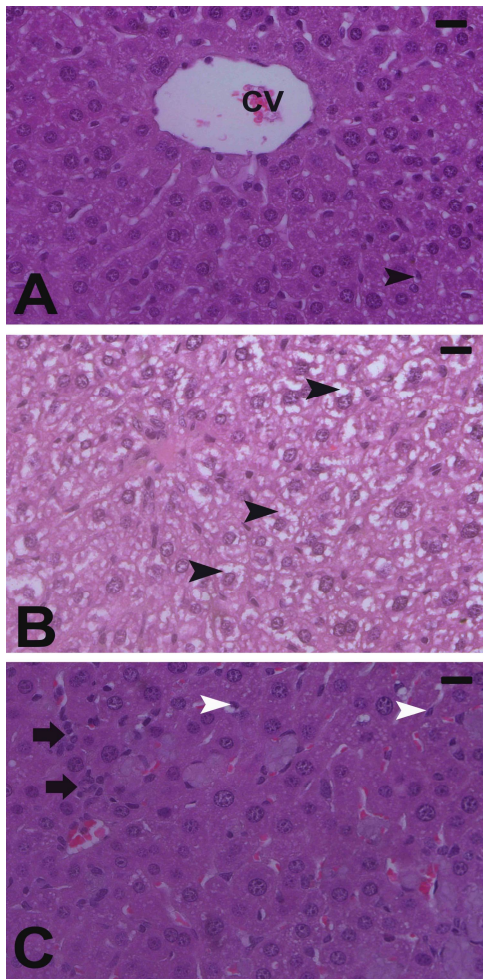


Fig. 1. Histological changes in hepatic tissue of mice. A, Control liver with central vein (CV) and surrounding hepatocytes, sinusoids lined with Kupffer cells. B, Liver sections of mice fed acidic diet. C, Liver sections of mice fed basic diet. Inflammatory cellular infiltrations (arrow), hepatocytic vacuolations (black arrow head) and prominent Kupffer cells (white arrow heads). Sections were stained with hematoxylin-eosin. Scale bar = 50 μ m.

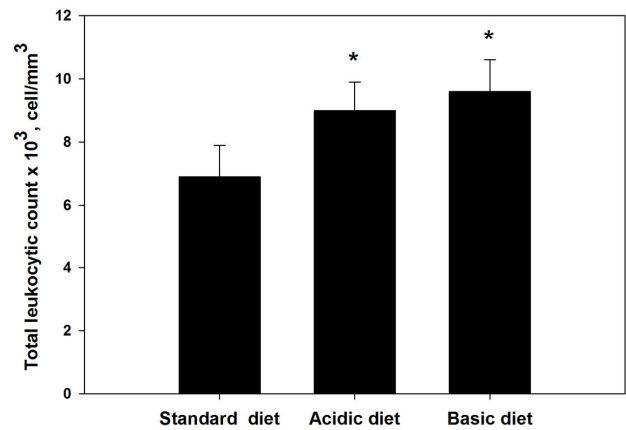


Fig. 2. Diet induced changes in the leukocytic count. * Significant change at $P \leq 0.05$ with respect to control mice fed a standard diet.

Figure 3 showed that acidic and basic diets induced a significant increase in ALT enzyme in blood plasma of mice. However, ALT level was much more elevated in mice fed the basic diet (Fig.3). Both of acidic and basic diets induced marked alterations in renal tissues of mice (Fig. 4, Table II) when compared to the control group (Fig.4A). These changes were in the form of tubular dilatation, vacuolar and cloudy epithelial cells lining, interstitial inflammatory cells and appearance of some cellular debris. Glomeruli of mice fed the acidic diet appeared congested and the kidney tubules were edematous (Fig. 4B) while more of that of mice fed basic diet appeared shrunken the urinary space is widened (Fig. 4C).

DISCUSSION

Increased attention has been paid in recent years to the role of nutrition in the sex selection.

The diets nutritional content is questionable and contains multiple warnings. The diet may influence the conditions within the reproductive tract and the outer barrier surrounding the ovum (Chandraju *et al.*, 2013). Also, the selected diet (acidic or basic) could harm hepatic and renal tissues. Several

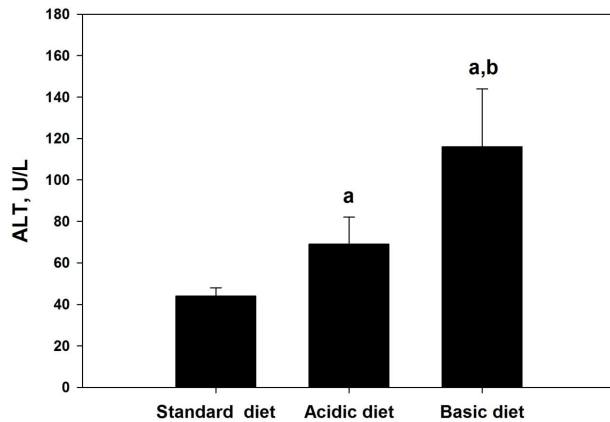


Fig. 3. Blood plasma alanine aminotransferase level in mice fed on different diets. Values are means±SD. a, significant change at $P \leq 0.05$ with respect to control mice fed a standard diet; b: significant change at $P \leq 0.05$ between mice fed acidic and basic diets.

nutritional imbalances including electrolyte and trace element deficiencies may, in turn, cause or increase inflammation by mechanisms such as increased tissue oxidative stress (Ianco *et al.*, 2013). Such major changes might be related to the consumption of essential vitamins, minerals, antioxidants or fibers, which could thus potentially contribute to pouch inflammation.

Acidic and basic diets cause an inflammatory response in the hepatic tissue of mice due to the abundance of leucocytes, in general, and lymphocytes, in particular as a prominent response of body tissues facing any injurious impacts (Nwaopara *et al.*, 2007). Also, the cytoplasmic vacuolation which is mainly a consequence of considerable disturbances in lipid inclusions and fat metabolism occurring under pathological cases were prominent in hepatocytes of mice fed on acidic diet (Zhang and Wang, 1984). Also, there was a significant increase activity of ALT indicating a change in the liver function.

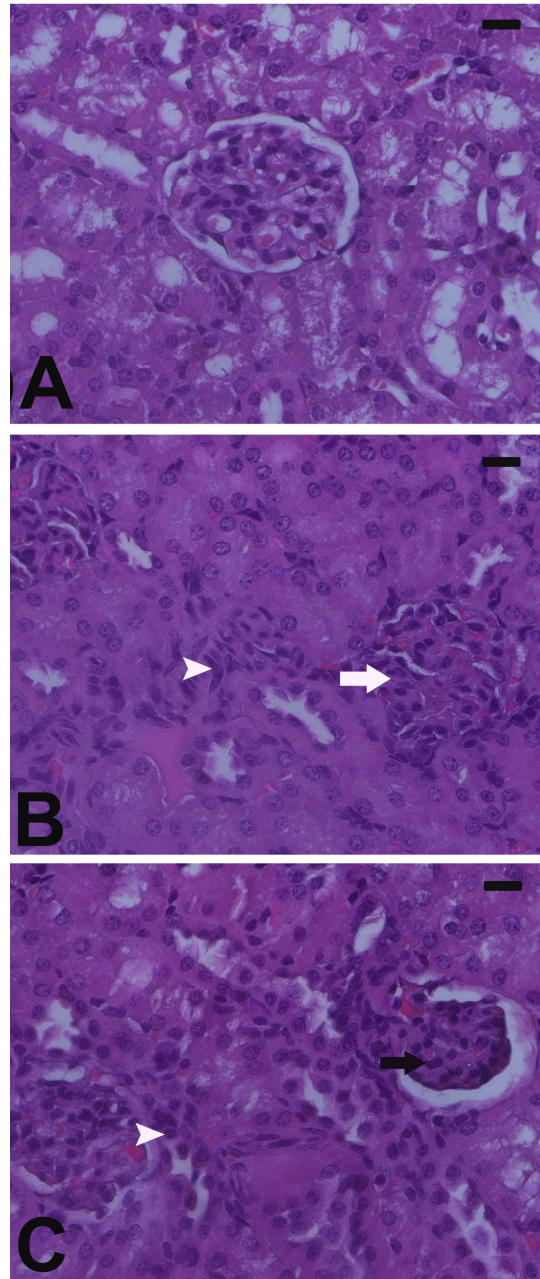


Fig. 4. Histological changes in the renal tissue of mice. A, control kidney with normal architecture. B, kidney section from mice fed basic diet. The glomeruli appeared congested (White arrow) and some inflammatory cells were prominent (White arrow head). C, kidney sections of mice fed basic diet. Glomeruli appeared shrunken (black arrow) and inflamed cells are prominent. Sections were stained with hematoxylin-eosin. Scale bar = 50 μm .

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Metabolic products from the minerals in the diet were concentrated in the blood that led to capillary constrictions and then to a decrease in glomerular filtration of that metabolic products which minimizes its effect and protects the tubular cells. This may affect the shrinkage of the glomeruli. edema was observed in the kidney tubules. This leads to decreased blood osmotic pressure, with subsequent decreased drainage of tissue fluids, which explains the edema and congestion observed in the different tissues (Ebaid *et al.*, 2007).

Collectively, imbalanced diet induced a pronounced inflammation and marked hepatic and renal tissue damage. Further studies are required to understand the mechanism of the induced tissue injury.

ACKNOWLEDGEMENT

The project was supported by King Saud University, Deanship of Scientific Research, College of Science, Research Center.

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(Received 23 January 2014, revised 26 February 2014)