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Histopathology and biochemical assessment of excess high dose of methionine on liver, heart and kidney tissues in rabbit

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ABSTRACT

This study focuses on the histopathology and biochemical effects of excess high dose of methionine in rabbits. Two groups of 30 rabbits were tested by some experiments: the first group(control group) was given a commercial diet; and the second group(care group) was also given an experimental diet containing 1.2% methionine by weight in the diet for 3 months. In all of the rabbit species mentioned above blood was obtained via samples by their marginal ear veins throughout 10,20,30,40,50,60,70,80 and 90 days, respectively. On necropsy, the liver, kidney and heart samples of 5 cases out of 10 rabbits of the first group and 10 cases out of 20 of the second group were taken at the end of the second and third month. In addition, pathologic lesion on the liver and kidney after two months was observed, so that cases were developed in a three month period. Eventually, the biochemical parameters confirmed the histopathology results; deity excess methionine causes a toxic effect on liver, kidney and heart tissues.

Key Word: Excess Methionine, Histopathology Effect, Liver, Kidney, Heart, Rabbits

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INTRODUCTION

Nowadays, despite the progress made in various fields, Feed and Metabolic diseases are still one of the most important social and economic issues that is seen as a strategic aspect. Since a great deal of importance is placed on the metabolic diseases effects, they are associated with feeding. Furthermore, they consist of some terms, directly and indirectly: the directly they cause several diseases; and indirectly, stages caused a number of economically losses, so any study on these diseases is worthwhile[5].

Apart from the above, amino acids are one of the important elements in the diet of animals[4]; therefore, evaluation of the side effects of abuse of amino acids for prevention of some metabolic disorders will be useful.

Methionine is a sulfur containing amino acid essential for maintaining proper growth and development in mammals, in addition, its supplementation in domestic animals-like chicks, contributes to better production efficacy[1,2,5].

However, supplementation of too much methionine causes various toxic changes including suppression of feed intake and growth[6]. Methionine toxicity is more pronounced than the toxicity of other amino acids, because some optimal intake levels of methionine are narrower than others[1].Ingestion of excess methionine causes depression in food intake and growth and also tissue abnormalities if the ingestion is prolonged[9].

The sulfur amino acids have gained renewed interest in recent years largely because of their relation to homocysteine and glutathione[8].

In the present study, we aimed to detect histopathological and biochemical effects of the intake of excess high dose of methionine in rabbits.

MATERIALS AND METHODS

30 healthy adult male New Zeland white (NZW) rabbits (ortycotolagus cunigulus) , weighing 2500-300 g, were provided from the animal laboratory of Tabriz University of Medical Sciences. The experimental animals were randomly divided in two groups (10 in the first group[control group] and 20 in the second group[care group]), then the animals were accommodated individually in stainless metabolic cages under controlled temperature (21-23^{0}C) in a 12 hour light/dark cycle with free access to water, labium and pellet food diets. The first group was given rabbit commercial diet contenting alfalfa meal, cornmeal, barley, wheat, soybean meal and minerals as well as vitamins. The other group was given an experimental diet containing 1.2 percent methionine by weight in the diet for 3 months.

Blood sampling:
Blood samples were collected from the marginal ear veins of rabbits, then whole blood was collected aseptically using sterile 2ml syringe with 25 gauge needles and poured into tubes without anticoagulant. The blood was centrifuged at 3000 g for 10 minutes for room temperature and sera was harvested using disposable micro tubes (Eppendorf,Germany). Serum was analyzed on the same day. This process was repeated in 10 day intervals over a three month period.

Biochemical Parameters:
The serum concentration of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Creatinine(Crt) and Blood Urea Nitrogen (BUN) were determined by biochemical automatic analyzer (AUtolab®,AMS®;Rome, Italy), using commercial kits (Pars Azmoon, Iran).

Histopathological Assays:
On necropsy, the liver, kidney and heart tissue samples from 5 out of 10 animals of the first group and 10 out of 20 animals of the second group were preserved in 10% neutral buffered formalin solution for histological examination at the end of the second and third month. Formalin fixed tissues were processed by the standard paraffin wax technique, and sections of 5µm thickness were cut and stained with hematoxyline and eosin (H&E).

Serum homocysteine level:
The serum homocysteine level were determined by Enzyme Immunoassay (EIA) using commercial kits (Axis®-Homocystein EIA, United Kingdom).

Statistical analysis:
All data are expressed as mean ± Standard Error (SE) of the mean. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncans Test; a value of $P<0.05$ was considered statistically significant.

RESULTS

Serum biochemical parameters results are presented in table.1. Analysis of biochemical data reveal that after the first month of the experimental period, AST, ALT, BUN and Crt serum levels in the second groups were significantly increased ($P<0.05$) in comparison with those of the first group. This increase continued in the second and third month of the experimental period, which agreed with the histopathologic results.

In histopathology,the samples were taken after the second month of the experimental period in kidney tissue; tubular degeneration and interstitial nephritis were observed. In hepatic tissue, focal hepatitis, peri portal hepatitis and infiltration of mononuclear inflammatory cells in peri portal area were observed. In heart tissue, mild cell swelling was observed.
In histopathology,the samples were taken after the third month of the experimental period in the kidney tissue, cell swelling (Fig.2) with tubular degeneration was observed. In hepatic tissue mild cell swelling with infiltration of mononuclear inflammatory cells in the peri portal area(Fig.1,3) and hepatic fatty degeneration were observed .In heart tissue, mild cell swelling in the muscle cells and mild infiltration of mononuclear inflammatory cells in the muscle cells were observed(Fig. 4,5).

There are conclusions of serum hemocysteine level in the second group in table.1. As it is shown serum hemocysteine level increases by time which this increasing meaning ($P<0.05$) in comparison with those of the first group.

DISCUSSION

In the present study, we aimed to detect histopathological and biochemical effects of the intake of excess high dose of methionine in some rabbits over a three month period, in which the biochemical finding would be in agreement with those studies which performed.

In a clinical plasma examination, AST and ALT activities in plasma represent biomarkers for liver function[28]. Alterations of AST and ALT activities are liver- specific and have been used as a tool to study varying cell viability and changes in cell membrane permeability[21]. Our study demonstrates that in excess high dosage methionine loaded rabbits there was a significantly increased activity of plasma ALT and AST ($P<0.05$ and $P<0.05$, respectively). Based on these observations, alterations in plasma AST and ALT activities (Table.1) reflect adverse effects of excess methionine on hepatic function, histopathologic results confirm biochemical outcome. On the other hand, results of our studies show that AST and ALT activities in plasma of rats were significantly elevated by excess methionine, indicating excess methionine- related injury to the liver[21,28].

Moreover, Blood Urea Nitrogen (BUN) is one of the traditional blood indices of glomerular filtration. Simply stated, most of the urea produced in the body is excreted in the urine by filtration across the glomerulus. Therefore, reduction in the glomerular filtration rate (GFR) results in increases in the BUN concentration. Creatinine (Crt) is removed from the body almost entirely by renal excretion through glomerular filtration[19]. Furthermore, Crt is typically increased by pathologic processes that cause decreased GFR, so increasing BUN and Crt generally indicate kidney disorders and are used as biomarkers for kidney function[29]. This research demonstrates that in excess high dosage methionine loaded rabbits there was a significantly increased activity of plasma BUN and Crt ($P<0.05$ and $P<0.05$, respectively). As per these observations, alterations in plasma BUN and Crt activities (Table.1) reflect adverse effects of excess methionine on kidney function that histopathologic results confirm biochemical outcome. Results of previous studies show that BUN and Crt activities in plasma of rats were significantly elevated by excess methionine[19], indicating excess methionine- related injury to the kidney.

Biochemistry results revealed by our study are in accordance with a previous report by Toue et al (2006)[1].

On the effects of the excess methionine on the heart, there are some studies that indicate methionine and homocysteine caused cardiovascular diseases [14,15,16,17].

Our research indicated that excess methionine over a period of three months has a toxic effect on heart muscle cells, was the same noticed by Mayer et al (2003) and by David Sw et al (2002).

Pathologic and biochemistry results indicated that excess high dose of methionine has a toxic effect on the tissues. There are different views regarding the mechanisms of methionine toxicity[6]. However, methionine itself does not seem to be directly responsible for its toxicity, because no adverse symptoms are observed in patients with extremely high plasma levels of methionine[22]. Thus, it appears that metabolites in the normal catabolic pathways or abnormal metabolites arising from the overloading of certain metabolic pathways might be responsible for toxicity[1,4].

Ingestion of excess methionine causes depressions in food intake and growth and also tissue abnormalities if the ingestion is prolonged[32]. Recent evidence, mostly from in vitro studies has prompted Benevenga and associates to propose an alternate mathionine-catabolic pathway in which methionine was believed to be transaminated and then decarboxylated. Benevenga had suggested earlier that a product or products of this pathway might be responsible for the toxicity of methionine[23]. This hypothesis stressed the relative importance of the methyl group of methionine in causing the toxicity[32]. The observation that glycine was more effective than serine in alleviating methionine toxicity was attributed to the role of glycine in accepting the methyl group of methionine in a reaction catalyzed by glycine methyl transferase[32,23].
From observation mainly with chicks Baker and associates, however ,emphasized the importance of the homocysteine moiety of methionine in causing the toxicity .This view was evolved from the observations with chicks and rats that homocysteine and methionine were equally toxic when ingested in excess[24].

Thus, it appears that Homocysteine might be a toxic metabolite as methionine is a precursor of homocysteine and there is significant amount of sulfur amino acid intermediate in the methylation and transsulfuration pathways[4].
Toxicities of homocysteine, both in vivo and in vitro, have been reported previously[25,31] ,and methionine intake has been shown to raise plasma homocysteine concentrations[26] .

Because hemocysteine is one of the most important candidates to be toxic methionine[1], we have measured the serum hemocysteine rate in different periods and as it is shown in table.1. Hemocysteine rate has been increased by time in second group that has been fed by excess high dosage of methionine diet. As it is mentioned above toxicities of hemocysteine, both in vivo and in vitro have been proved[25,31]. So blood hemocysteine level increasing may cause toxic effects on tissues which has been achieved by histopathology and biochemical results.

Also other metabolites that were produced by extra methionine metabolism can cause toxic effect. Some of these metabolites which can be effective in methionine toxicity are mentioned down:

3-Methylthiopropionate(3MTP), one of the metabolites in the transamination pathway, is also a candidate because its addition to the diet causes similar hematological changes in rate to those in animals fed excessive methionine[27] and because downstream metabolites of 3MTP, such as methanethiol.hydrogen sulfide(H_2S), and formate, are highly toxic[28]. Another candidate is s-adenosylmethionine (SAM), one of the most important methyl donors for 1-carbon metabolism: the hepatic content of SAM is increased by methionine load, and the increase is prevented by glycine supplementation, which is known to alleviate methionine toxicity[29]. Although there are several candidates for the toxic metabolite, the biochemical mechanisms underlying methionine toxicity remain controversial, and biomarkers for toxicity have yet to be elucidated[1].

Table 1 . Levels of serum AST,ALT,BUN,Crt and Homocysteine concentration of rabbits fed with excess high dose of methionine over a Time period

Parameters	Frist group (control group)	Second group(care group)							
		$10^{th}day$	$20^{th}day$	$30^{th}day$	$40^{th}day$	$50^{th}day$	$60^{th}day$	$70^{th}day$	$80^{th}day$	$90^{th}day$
AST[IUL^{-1}]	36/80±7/65[a]	38/20±6/17[a]	62/50±17/73[a]	79/30±16/18[b]	81/30±31/40[c]	86/50±15/37[d]	84/80±11/19[e]	82/60±4/30[f]	84/00±4/30[g]	93/6±12/37[h]
ALT[IUL^{-1}]	32/50±6/88[a]	33/60±5/85[a]	36/90±6/15[a]	58/30±11/90[a]	74/30±32/70[b]	67/90±12/27[c]	75/50±12/51[d]	88±25/40[e]	89/8±13/60[f]	105/80±29/60[g]
BUN[$mg\ dl^{-1}$]	9/17±1/54[a]	13/80±1/89[b]	16/57±1/62[c]	17/60±2/31[d]	16/33±1/28[e]	19/11±1/59[f]	20/71±5/71[g]	20/32±2/99[h]	21/5±5/06[j]	20/10±3/31[k]
Crt[$mg\ dl^{-1}$]	0/66±0/08[a]	1/14±0/26[b]	1/65±0/46[c]	1/56±0/55[d]	1/60±0/73[e]	1/48±0/47[f]	1/82±0/40[g]	1/82±0/19[h]	2/08±0/20[j]	1/88±0/32[k]
Homocysteine [$umol\ L^{-1}$]	0/234±0/11[a]	0/48±0/20[a]	1/25±0/30[a]	2/51±0/54[b]	4/47±0/80[c]	5/63±0/60[d]	7/09±0/85[e]	9/34±1/39[f]	12/43±1/69[g]	14/38±1/33[h]

Data are expressed as mean ±Se.
Different symbols[a,b,c,d,e,f,g,h,j and k] show statistical significance between groups in each row.
AST:Aspartate Amino-Transferase; ALT: Alanine Amino-Transferase; BUN: Blood Urea Nitrogen; Crt: Creatinine.

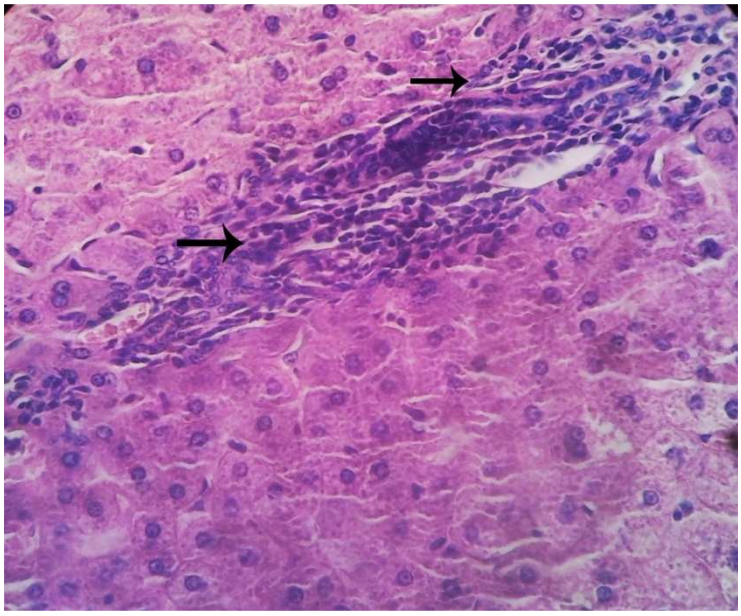

Fig 1.Liver. Second group after third month. Infiltration of mononuclear Inflammatory cells in periportal area (arrows).H&E.(400x)

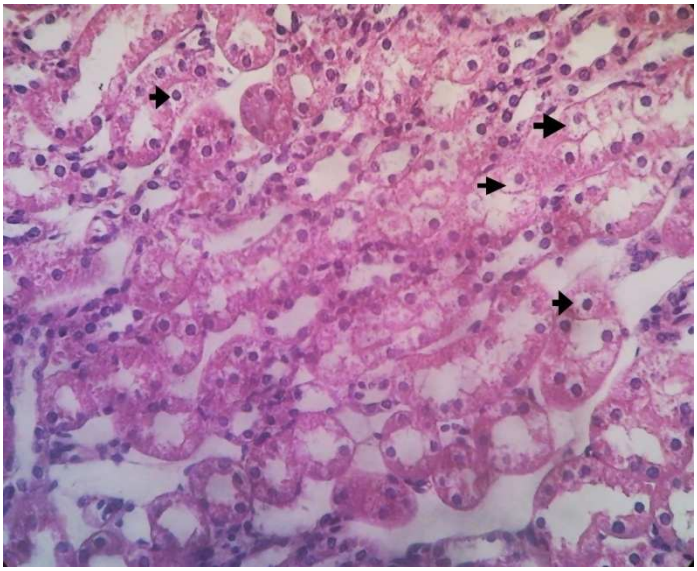

Fig2.Kidney.Second group after third month .cell swelling in tubules (arrows) .H&E.(400x)

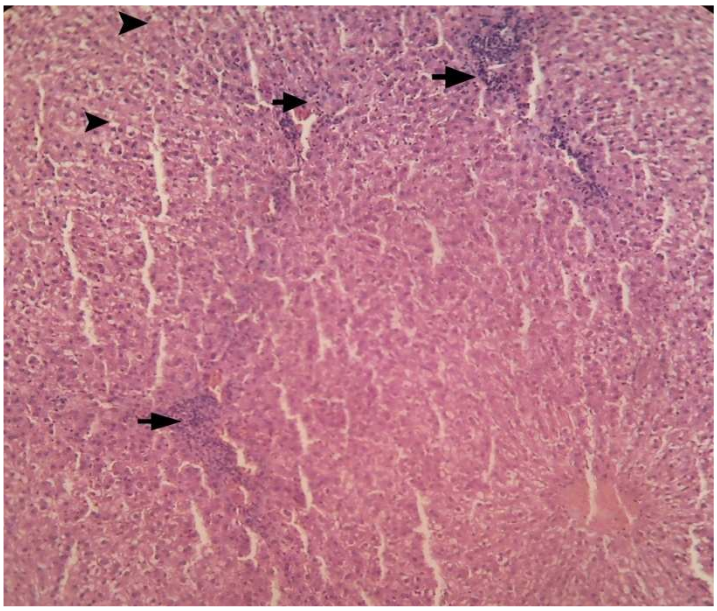

Fig3.Liver. Second group after third month. Infiltration of mononuclear Inflammatory cells (arrows) and cells welling(arrowheads).H&E.(100x)

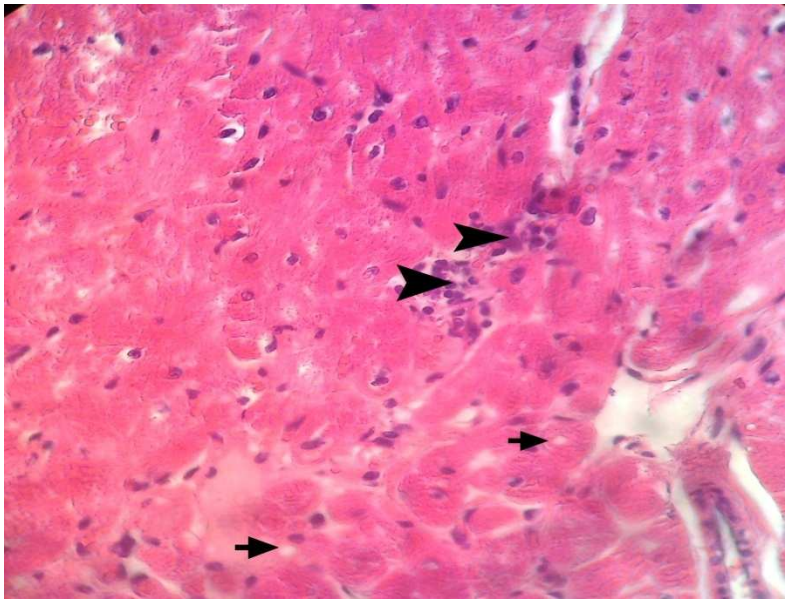

Fig4.Heart. Second group after third month. Infiltration of mononuclear inflammatory cells (arrowheads) and cell swelling(arrows) between muscle cells.H&E.(400x)

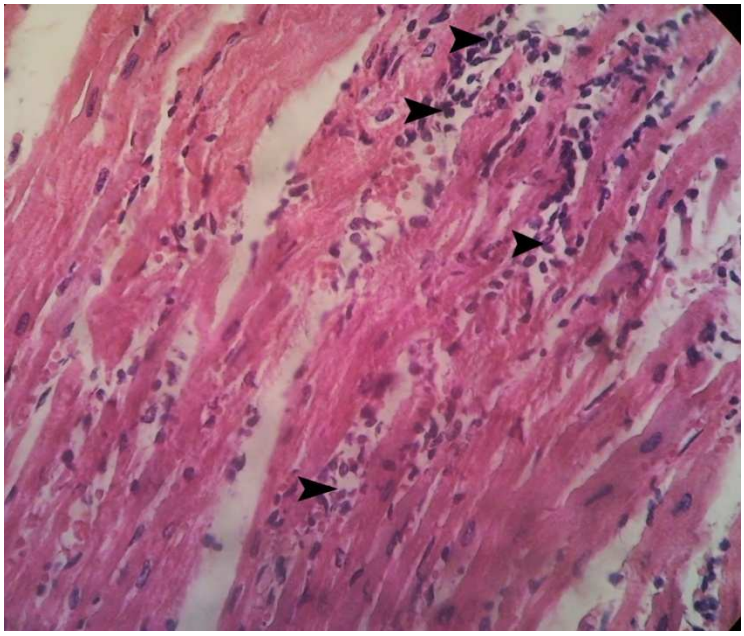

Fig5 Heart. Second group after third month. Infiltration of mononuclear Inflammatory cells (arrowheads) between degeneration muscle cells

CONCLUSION

Pathologic and biochemistry results revealed that excess high dose of methionine has a toxic effect on the tissues ., In other words, optimal intake levels of methionine are narrower than the other amino acids so we should be more cautious when using methionine in diets.

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