

Study the Efficacy of Folic Acid Supplementation on Hepatotoxicity Induced by Hyperhomocysteinemia in Male Rats

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ABSTRACT

Increased serum homocysteine (Hcy) can induce liver diseases and can play a role in hepatic disorders. The purpose of the present study therefore was to investigate the relationship between serum hyperhomocysteinemia (HHcy) induced by methionine administration , folic acid and liver functions . The present study was designed to induce hyperhomocysteinemia (HHcy) in male rats. Also, to evaluate, the effect of (HHcy) as a risk factor for liver disorder and folic acid supplementation on serum levels of Hcy, alanine transaminase (AST), aspartate transaminase (AST), lactic dehydrogenase (LDH) , albumin ,globulin ,and albumin /globulin ratio and total protein . In this work 50 male albino rats were used and divided into five groups .The first served as control ., the second and third group received two different dose of L-methionine ., the fourth and fifth group received fortified diet with folic acid powder plus L-methionine . The results showed that homocysteine levels in rats received low and high dose of methionine were higher than in the control group , and increased activity of serum AST with low and high dose of methionine , while LDH significantly decreased in high dose treated rats compared to control group . Serum level of proteins , albumin and globulin significantly decreased in rats received low and high dose of methionine compared with control group . In rats supplemented with folic acid we found an increased activity of AST in low dose of methionine treated rats , while serum ALT activity increased accompanied with decreased LDH activity in low and high dose of methionine compared to both methionine treated groups .Serum level of proteins , albumin and globulin significantly decreased in rats received low dose of methionine and supplemented with folic acid when compared with low dose treated rats, while rats treated with folic acid and high dose of methionine a significant increase in total proteins , albumin , compared to high methionine treated groups . A positive correlation was seen between the activities of transferases in serum of HHcy rats . It can be concluded that hyperhomocysteinemia may be an additional risk factor for liver cirrhosis and dietary supplementation with folic acid blocking the activity of homocysteine and may be considered as a therapeutic possibility in patients with hepatic disorders and . Also, plasma Hcy and folic acid measurement may be useful in the evaluation of liver cirrhotic patients .

KEYWORDS: : Homocysteine , folic acid , methionine ,liver cirrhosis ,hepatic disorders .

INTRODUCTION

Although hepatocytes are rarely replicate in the normal adult liver , they are able to reenter the cycle and proliferate after liver damage caused by ischemia , chemical compounds or hepatitis(Remkova and Remko , 2009). Impaired liver regeneration can be an important clinical complication of the pathogenesis of liver failure , cirrhosis severe steatosis , and liver cancer (Shinohara *et al.* , 2010) .

Homocysteine is formed as an intermediate in sulfur amino acid metabolism . Elevated levels of circulating homocysteine , a condition known as hyperhomocysteinemia , are correlated with hepatic fibrosis (Woo *et al.* , 2006, Park *et al.* , 2008 and Einollahi *et al.* , 2011) One of the mechanisms underlying homocysteine –

mediated organ dysfunction results from induction of cell cycle arrest , apoptosis , and cell injury (Hirashima *et al.* ,(2010); Koz , *et al.*,(2010) and Jia , *et al.*,(2011)) . Liver plays a central role in homocysteine metabolism . Impaired liver function has been associated with elevated plasma levels of homocysteine . (Robert , *et a.*,l (2005). Homocysteine has been shown to enhance hepatic lipid metabolism via transcription factor , sterol regulatory elements- binding protein -1(Alam , *et al.* ,(2009) and (Ji, C. and Kaplowitz, N. (2003) In mice fed with methionine promotes oxidative stress , leading to liver injury (Park , *et al.* , (2008) and Armada , *et al.* , (2012) . Elevated homocysteine levels are defined as hyperhomocysteinemia (HHcy) , a disorder that is associated with hepatic fibrosis . Recent studies have shown that HHcy promotes hepatic injury by increasing oxidative stress(Hamelet , *et al.*,(2007) . Although homocysteine induces cell cycle arrest in a variety of different cell types , it is not known whether HHcy has a definitive role in hepatocyte proliferation . Our results demonstrated that rats with HHcy exhibitad an impairment in liver, as measured by liver enzymes activity and proteins . Also , our results indicated that folic acid administration mediated inhibitory effect of homocysteine on liver function . These findings provide evidence that impairment of liver by HHcy may result in delayed recovery from liver injury induced by homocysteine itself .

Materials and Methods :-

Chemicals

1-L-Methionine : supplied by Sigma – Aldrich Company . The chosen dose was 1 g/kg b wt(Papandreou , *et al.*,2010)

2- Folate : supplied by Sigma –Aldrich Company . The chosen dose was 19 g/ kg diet (Givvimani,*et al.* , 2011)

Animal groups

The study was conducted on 50 male albino rats, weighing ~ 200 g. Rats were maintained on commercial rat chow and water *ad libitum* and allowed to adapt to the prevailing environment for two weeks prior to the beginning of the experiment in the laboratory.

The animals were divided into equal five group as follows: The first group (I): served as control group.

The second group (II) received L- methionine in a dose of 1g/kg b wt dissolved in drinking water to induce hyperhomocysteinemia(HHcy)] .

*The third group(III) received L- methionine in doses 2g/kg dissolved in drinking water to induce hyperhomocysteinemia (Papandreou , *et al.*,(2010).

*The fourth group(IV) received L- methionine in doses 1g/kg dissolved in drinking water and supplemented with folic acid in a dose 19g/kg diet ((Givvimani, *et al.*, 2011)

* The fifth group (V) received L- methionine in doses 2g/kg dissolved in drinking water and supplemented with folic acid in a dose 19g/kg diet (Givvimani, *et al.*,2011).

Sample preparation

Blood samples were collected at the end of experimental period (8 weeks) for biochemical analysis .

Blood samples were obtained from retro-orbital sinus of an over night fasted rats under light ether anesthesia according [Blasco, *et al.*, (2005)], then centrifuged. The separated sera were analyzed for estimation of, homocysteine (Bosy-Westphal , *et al.*,(2003), ALT ,AST and LDH activity , total proteins , albumin , globulin and albumin /globulin ratio.

Statistical Analysis :-

The data were analyzed using SPSS program version 16 .The analysis of covariance (one way ANOVA) was used to detect the differences in the mean between the treated groups and the control and between supplemented group with folate and methionine treated groups . The mean differences is significant at P < 0.05 .

Results :

The results showed that homocysteine levels in rats received low and high dose of methionine (group II&III)were higher than in the control group (group I) (Table 1andFigure 1) , and had increased activity of serum AST with low and high dose of methionine , while LDH significantly decreased in high dose treated rats compared to control group (table 2 &figure 2). Serum level of proteins , albumin and globulin significantly decreased in rats received low and high dose of methionine compared with control group(table 3 &figure 3) . In rats supplemented with folic acid we found an increased activity of AST in low dose of methionine treated rats , while serum ALT activity increased accompanied with decreased LDH activity in low and high dose of methionine compared to both methionine treated groups .Serum level of proteins , albumin and globulin significantly decreased in rats received low dose of methionine and supplemented with folic acid when compared with low dose treated rats, while rats treated with folic acid and high dose of methionine a significant increase in total proteins , albumin , compared to high methionine treated groups . A positive correlation was seen between the activities of transferases in serum of HHcy rats .

Discussion:

Hyperhomocysteinemia is a disorder of methionine metabolism, in which a liver plays a role: it may be frequently due to nutrient deficiencies, particularly low folate status (Remkova and Remko, 2009).

In the present study methionine in both doses caused a significant increase in serum Hcy and activities of AST , ALT with significant decrease in the activity of LDH compared with control group. Plasma concentrations of homocysteine is controlled by two metabolic pathways : the remethylation and transsulfuration pathways. Remethylation of

homocysteine requires the enzymes 5 , 10 - methylenetetrahydrofolate reductase (5,10-MTHFR) and methionine synthase . transsulfuration is dependent on cyathionine B-synthase (CBS) enzyme activity. Deficiencies in any of these enzymes and/or increased substrate for HCY metabolism elevates in metabolized intracellular HCY, which is exported from the cell into plasma (Symons, *et al.* , 2006 and Selicharoval et al 2013). The marked significant increase in AST, ALT were consistent with the finding of (Remkova. and Remko, 2009) who reported that, hyperhomocysteinemia causes oxidative stress is considered the main cause of hepatotoxicity which leading to impairment of liver metabolism and local changes in vessel integrity . Although Hcy is produced in every cell as an intermediate of the methionine cycle , the liver contributes the major portion found in circulation , and fatty liver is a common finding in homocysteinemic patients (DiBello, *et al.*, 2010).

The study of Di Bello *et al.*, (2010) showed that HHcy, whether caused by a genetic mutation or diet , alters the abundance of several liver proteins, involved in Hcy/ methionine metabolism and antioxidant defense.

The result of Shinohara *et al.*, (2010) reported that high methionine diet induced less steatosis and ALT increase with HHcy in rats than in mice which is due to endoplasmic reticulum stress and liver injury.

Recent studies have shown that elevated Hcy levels are a disorder that is associated with hepatic fibrosis and promotes hepatic injury by increasing oxidative stress (Liu , *et al.*, 2010). Hyperhomocysteinemia stimulated hepatic 3-hydroxyl. 3 methylglutaryl coenzyme A (HMG-coA) reductase leading to hepatic lipid accumulation and liver injury(Wu , *et al.*,2011). HHcy unleashes mediators of inflammation such as Nf kappa B, IL-1beta,IL-6, and IL-8, increases production of intracellular superoxide anion causing oxidative stress and induces endoplasmic reticulum (ER) stress which can explain many processes of Hcy promoted cell injury such as apoptosis , fat accumulation and inflammation, and ER stress may be involved in liver diseases such as viral hepatitis (Ji and Kaplowitz , 2004)

In agreement with the present study Park, *et al.*, (2008), who found that HHcy induced by methionine supplementation promotes oxidative stress and nuclear factor kappa B activation in liver of mice when fed a 2% methionine and low folate diet for 12 weeks , with normal hepatic function while hepatic triglyceride concentration was lowered by methionine feeding.

In contrast with the present data the findings of (Einollahi, *et al.*, 2011) they revealed no significant correlation between Hcy concentration and ALT or AST concentration.

ALT is a more proper marker of hepatic damage than AST as the activity of ALT resides mainly in the liver , while AST indicates intra and extra hepatic injury (Micle, *et al.* , 2012). The mechanism of HHcy induced acceleration of lipid peroxidation leading to organelle membrane dysfunction and subsequent cell injury and death. Also HHcy catalyzed generation of reactive oxygen species which is responsible for initiating the peroxidative reaction .

On the other hand , significant reduction in the activity of serum LDH in the present study after methionine

administration may be due to ischemic hepatitis (Hirashima, *et al.*, 2010) induced by HHcy.

The decrease in LDH concentration could explain the mechanisms of HHcy induced toxicity by stimulating the release of reactive intermediate species from macrophages (Koz, *et al.*, 2010).

Findings from the present study indicate that HHcy with low independently impair vascular function. Mechanisms responsible for these observations are that HHcy increase oxidative stress in general and vascular O₂ in particular.

As regard to the marked decrease in protein, albumin and globulin in rats subjected to large dose of methionine may due to decreased synthesis of albumin and globulin in advanced liver disease. Also, several studies urged that, oxidative stress was an additional process that account for hepato-cellular damage and releasing inflammatory mediators (Jia, *et al.*, 2011). Hyperhomocysteinemia, whether caused by a genetic mutation or diet, alters the abundance of several liver proteins involved in homocysteine / methionine metabolism and antioxidant defence (Devika Rani, *et al.*, 2008).

In the present study supplementation of folic acid to methionine treated rats we found an increased activity of AST in low dose of methionine treated rats, while serum ALT activity increased accompanied with decreased LDH activity in low and high dose of methionine compared to both methionine treated groups. Serum level of proteins, albumin and globulin significantly decreased in rats received low dose of methionine and supplemented with folic acid when compared with low dose treated rats, while rats treated with folic acid and high dose of methionine a significant increase in total proteins, albumin, compared to high methionine treated groups. A positive correlation was seen between the activities of transferases in serum of HHcy rats. These findings indicate that the elevated level of plasma Hcy may be indicative of much broader and deeper alterations in intracellular methylation dysfunction, and suggest that dietary enrichment with folic acid is essential for the metabolism of Hcy, especially in adult animals. (pogribny *et al.*, 2005).

The study of Papandreou *et al.*, (2010) clouded that folate supplementation may reduce tHcy and total cholesterol levels in H Hcy children by modulating the increased cholesterol synthesis in the liver. The findings of (Givvimani *et al.*, 2011) support the notation that metabolic derangement in H Hcy causes the chronic decline or dysfunction in vascular density of liver. Moderate HHcy in common in liver damage suggesting that, although folate deficiencies may have a contribute any role, liver impairment, through changes in methionine metabolism, is the most important mechanism for elevated plasma Hcy found in there patients. (Blasco *et al.*, 2005 and Korinek *et al.*, 2013). The study of Armada *et al.*, (2001) reported that about 8.7. of total Hcy is bound to albumin and tHcy is reduced with folic acid supplementation, and tHcy directly correlate with albumin levels.

In contradiction basal HHcy is seen in 50% of liver cirrhosis and after liver transplantation and Hcy concentration do not change significantly after folate supplementation but postprandial Hcy metabolism improves (Bosy-West phal, *et al.*, 2003). Also, Matte, *et al.*, (2009) showed a decrease

antioxidant defenses and increased lipid peroxidation in liver will amino transferase activities were not altered by Hcy. consistent profile of liver injury elicited by Hcy which could contribute to explain the mechanism involved in human liver diseases associated to Hcy.

Hcy is a sulfur containing amino acid produced during metabolisms of methionine. elevated Hcy impairs vascular function, including impairment of endothelial function, production of Reactive oxygen species (Ros) and consequent oxidation of low density lipids. Folic acid required for remethylation of Hcy to methionine, are the most important dietary determinants of hcyemia and daily supplementation lowers plasma Hcy levels. Homocysteine levels are significantly increased in liver transplant recipients, therefore a specific treatment with folate for patients after liver transplantation might reduce the risk of complications resulting from HHcy (Akoglu, *et al.*, 2008).

Conclusion

On conclusion, these results suggest that hyperhomocysteinemia can cause liver injury and supplementation of folic acid offers a hepatoprotective effect.

Recommendation

The finding of the present research showed a correlation between homocysteine levels, folic acid which gives information that hyperhomocysteinemia probably due to vitamin deficiency. Folic acid, required for remethylation of homocysteine to methionine, are the most important dietary determinants of homocysteine and daily supplementation typically lowers plasma homocysteine levels and decreasing plasma Hcy levels through diet may be paralleled by a reduction in liver diseases.

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Table (1) Serum homocysteine levels (mg/dl) of male rats in the control and different treated groups.

Group Parameter	Homocysteine		
	Mean ± S.E	(Ta) Significant Test	(Tb) Significant Test
I- Control	11.736 ±.870	-----	-----
II- Treated with 1g/kg .B.W. Methionine.	42.653 ± 5.075	* .001 263.4 %	-----
III Treated with 2g/kg .B.W. Methionine.	54.838 ± 6.838	* .001 367.2 %	-----
IV- Treated with 1g/kg .B.W. methionine and supplemented with folic acid.	42.001 ± 2.781	* .001 257.8 %	.912 1.5 %
V- Treated with 2g/kg .B.W. methionine and supplemented with folic acid.	29.536 ± 2.106	* .001 151.6 %	* .001 46.1 %

The mean difference is significant at the 0.05 level

(Ta): significant as compared with normal control group.

(Tb): significant as compared with the same dose of methionine treated group.

Table (2) Serum enzyme activity (AST , ALT , and LDH) in male rats in control and treated groups.

Groups Parameter	AST			ALT			LDH		
	Mean ± S.E	(Ta) Significant Test	(Tb) Significant Test	Mean ± S.E	(Ta) Significant Test	(Tb) Significant Test	Mean ± S.E	(Ta) Significant Test	(Tb) Significant Test
I- Control	71.78 ±2.31	-----	-----	29.94 ±.1.94	-----	-----	554.5 5 ±54.9 3	-----	-----
II- Treated	93.75		-----	32.80	.350	-----	324.2	.106	-----

with 1g/kg .B.W. methionine.	±3.07	* .031 263.4 %		±2.16			0 ± 28.49		
III- Treated with 2g/kg .B.W. methionine.	99.45 ±6.91	* .008	-----	28.58 ±87	.655	-----	271.1 2 ± 23.84	* .01	-----
IV- Treated with 1g/kg .B.W. methionine and supplemented with folic acid.	108.28 ±, 11.76	* .001	.146	43.30 ±2.01	* .001	* .001	177.5 6 ± 19.53	* .001	* .001
V- Treated with 2g/kg .B.W. methionine and supplemented with folic acid.	84.46 ±.6.14	..202	.1	49.74 ± 3.10	* .001	* .001	106.7 0 ± 74.79	* .001	* .001

The mean difference is significant at p< 0.05

(Ta): significant as compared with normal control group.

(Tb): significant as compared with the same dose of methionine treated group.

Table (3) Serum proteins, albumin, globulin levels (mg/dl) of male rats in the control and different treated groups.

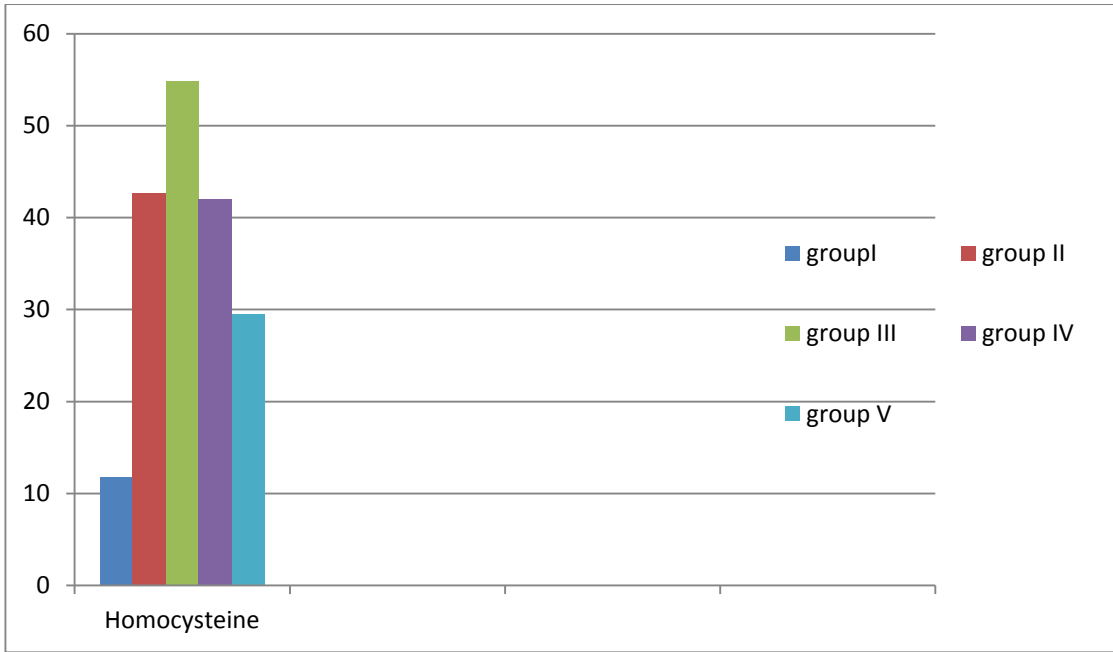
Groups Parameters	Proteins			Albumin			Globulin			A/G ratio		
	Mean ± S.E	(Ta) Signi- ficant Test	(Tb)) Sig- nifi- cant Tes- t	Mean ± S.E	(Ta)) Sig- nifi- cant Tes- t	(Tb)) Sig- nifi- cant Tes- t	Mean ± S.E	(Ta) Signi- ficant Test	(Tb)) Sig- nifi- cant Tes- t	Mean ± S.E	(Ta)) Sig- nifi- cant Tes- t	(Tb)) Sig- nifi- cant Tes- t

I- Control rats	6.29± .154	-----	---- --	3.97± .086	---- -		2.28 ± .202	-----		1.78 ± .198	---- --	----- -
II- Rats treated with 1g/kg. methionine.	6.01± .170	.241	---- --	3.70± .071	.05 7	---- -	2.31 ± .171	.894	-----	1.69 ± .196	.75 2	----- -
III- Rats treated with 2g/kg. methionine.	4.86± .155	* .001	---- --	3.13± .120	* .001	---- -	±1.7 93 .133	* .043	-----	±1.8 20 .171	.87 4	----- -
IV- Rats treated with 1g/kg. methionine & folic acid.	4.50± .148	* .001	* .001	3.01± .091	* .001	* .001	1.37 ± .112	* .001	* .001	2.28 ± .190	* .085	* .044
V- Rats treated with 2g/kg. methionine & folic acid.	5.78± .187	* .033	* .000	3.71± .107	* .066	* .001	2.07 ± .190	.360	.251	1.95 ± .238	.542	.651

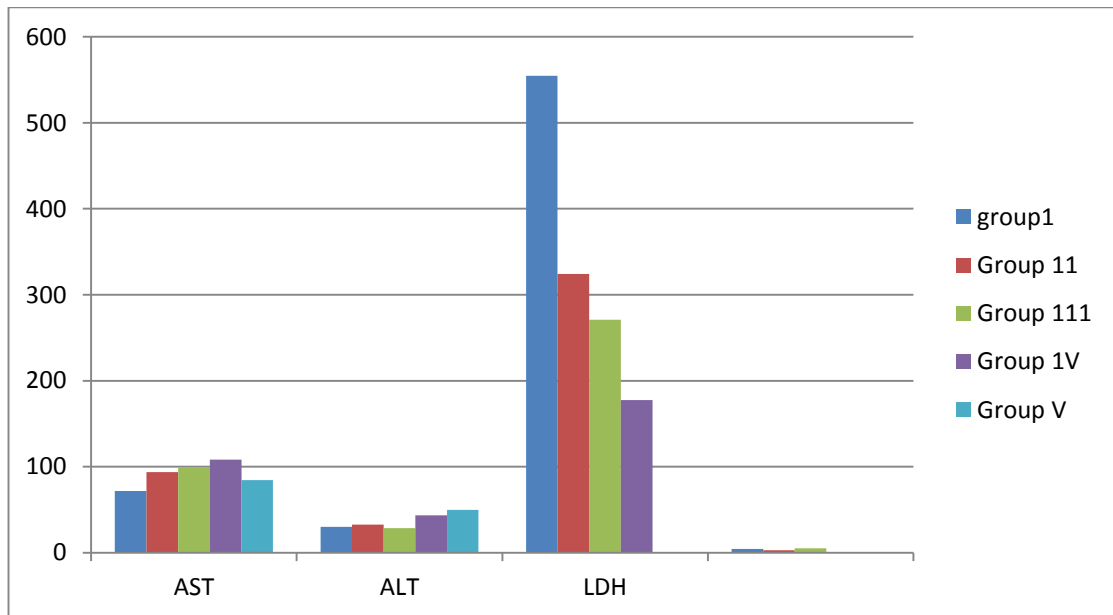
The mean difference is significant at the 0.05 level

(Ta): significant as compared with normal control group.

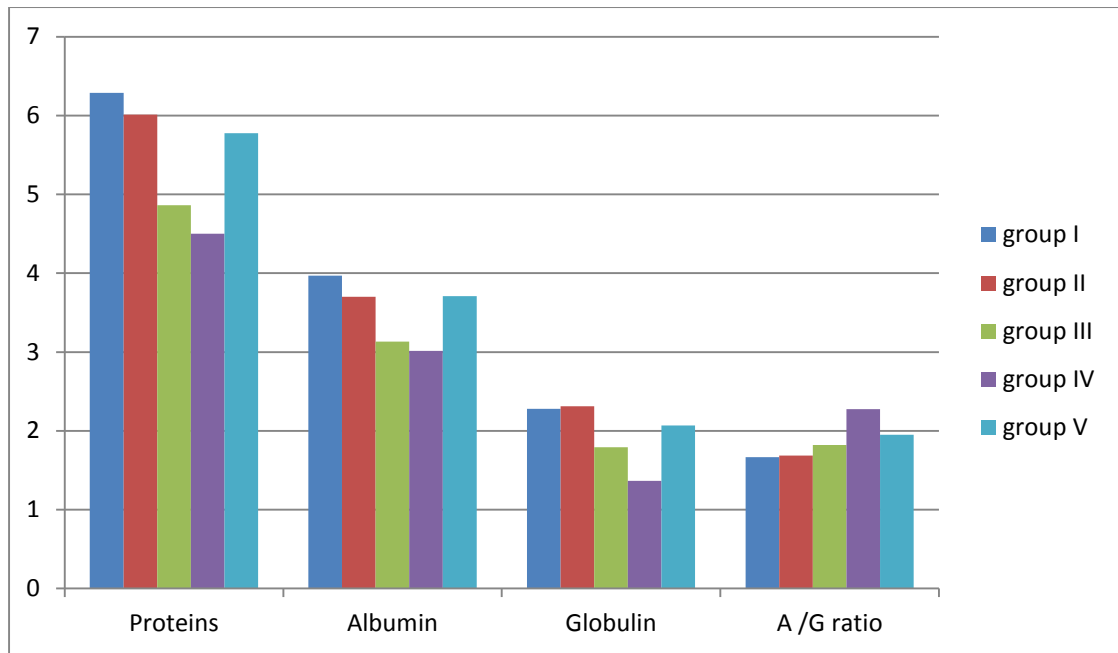
(Tb): significant as compared with the same dose of methionine treated group



Figure(1) Serum homocysteine levels(mg/dl) of male rats in the control and different treated groups



Figure(2) SerumAST , ALT ,LDH activity (mg/dl) of male rats in the control and different treated groups.



Figure(3) Serum proteins , albumin ,globulin and A/G ratio levels(mg/dl) of male rats in the control and different treated groups