

Role of folic acid in reducing the renal damage induced by amino acid methionine in male rats

Zena Muhammad Hamad

Assistant lecturer/College of basic education-University of Wassit

دور حامض الفوليك في التقليل من التلف الكلوي المستحدث بالحمض الاميني الميثيونين في ذكور الجرذان

زينة محمد حمد/كلية التربية الأساسية /جامعة واسط

الخلاصة

يعد الميثيونين من الأحماض الامينية الأساسية . عندما يتعرق المسار الايضي للميثيونين ، سيؤدي إلى ارتفاع مستوى الهوموستين ، وهذه الزيادة مسببه المرض . حامض الفوليك فعال في خفض مستوى الهوموستين بالدم. تم استخدام 30 من ذكور الجرذان البالغة وقسمت عشوائيا إلى ثلاثة مجاميع متساوية وعوملت كالتالي لمدة ٤٢ يوم: أعطيت المجموعة الأولى (T_1) الماء العادي وعدت كمجموعة سيطرة ؛ المجموعة الثانية (T_2) فقد جرعت بالميثيونين؛ بينما جرعت حيوانات المجموعة الثالثة الميثيونين بالإضافة إلى حامض الفوليك. تم جمع عينات الدم في الأيام ٢١، ٤٢ من التجربة لدراسة المؤشرات التالية : تركيز الكرياتنين ونتروجين يوريا الدم وحامض البوليك فضلا عن اخذ مقاطع نسيجية من الكلية لغرض دراسة التغيرات النسيجية المرضية. أظهرت النتائج حدوث زيادة معنوية ($P < 0.05$) في تركيز الكرياتنين ونتروجين يوريا الدم وحامض البوليك في اليوم ٢١ و ٤٢ من التجربة لمجموعة الميثيونين بالمقارنة مع مجموعة السيطرة. من جهة أخرى أظهرت المجموعة المعاملة بحامض الفوليك بالإضافة إلى الميثيونين وجود انخفاض معنوي ($P > 0.05$) في تراكيز الكرياتنين ونتروجين يوريا الدم وحامض البوليك في اليوم ٤٢ من التجربة . بينت المقاطع النسيجية للحيوانات المعاملة بالميثيونين إلى حدوث تغيرات تنكسية في الخلايا المبطنة للنبيبات الكلوية بينما الحيوانات المعاملة بحامض الفوليك أظهرت انكفاء التغيرات المرضية الكلوية التي سببها الميثيونين. يبدو من نتائج هذه الدراسة أن الميثيونين احدث إجهاد تأكسدي وتغير في بعض المعايير الحيوية المتعلقة بأمراض الكلية . و يمارس حامض الفوليك التأثير الوقائي ضد أضرار الميثيونين

Abstract

Methionine is an essential amino acid. When the metabolic pathway of methionine are interrupted, Homocysteine (Hcy) level will be elevated ,and this elevation is pathogenic. Folic acid can effectively reduce blood Hcy levels. The thirty adult male rats were randomly divided in to three equal groups and were treated daily as follows for 42 days. first (T_1) served as control group. Rats of the second group (T_2) were intubated orally 100 mg / kg B.W. of methionine . rats of the third group (T_3) were intubated same doses of methionine plus 5mg of folic acid supplementation . Fasting blood samples were collected at 21, and 42 days of experiment to study the following parameters: Serum creatinine (SC)

concentration, Blood urea nitrogen (BUN) concentration and Serum uric acid (SUA) concentration . Furthermore; section of kidney was assessed for histopathological studies. The resulted revealed significant increase ($P < 0.05$) of methionine group in SC , BUN and SUA concentrations in 12 , 42 day of treatment as comparing to control (T1). On the other hand that animals treated with folic acid plus methionine (T3) showed significant decline in SC , BUN and SUA concentrations in 42 day of treatment . Histological section of rat kidney received methionine showed monocyte cells around collected renal tubules ,while folic acid supplementation in groups T3 caused regression of renal damage induced by methionine.

It seems that methionine was effective in induction of oxidative stress and change in some biological markers related to kidney disease. Also it seems that folic acid exert protective actions against the damaging effect of methionine.

Introduction

Methionine is an essential amino acid with sulfur group. It performed many metabolic functions including: protein synthesis, transmethylation, and provision of homocysteine for remethylation reaction (1). Homocysteine (HCY) is exclusively formed as an intermediary product of methionine metabolism (2). Methionine overload is one of many factors responsible for causing disturbance in homocysteine metabolism resulting in accumulation of homocysteine with subsequent development of hyperhomocysteinemia (3). Several polymorphism in the gene coding for the enzymes involved in methionine metabolism such as cystathione- beta- synthase (CBS), methylene tetrahydrofolate reductase (MTHFR) or enzymes involved in methyl B12 synthesis and HCY methylation, are associated with elevated homocysteine levels (4). Furthermore, vitamin status(B6 & B12) folic acid, fish oil, insulin and melatonin which are involved in homocysteine metabolism. Particularly folate is the strongest nutritional and pharmacological determinant of plasma homocysteine concentrations (5,6).

The healthy kidney seems to has a high capability to filter ,reabsorb and metabolize free Hcy fraction. Homocysteine has molecular mass of 135 Dalton, which is well within the filtration range of normal glomeruli .As with other amino acid ,there is abundant evidence that filtered Hcy is avidly reabsorbed and only minimal amount is excreted in the urine . (7)In addition to the filtered load , Hcy uptake may also occur on the basolateral tubular cell surface, which means that normal kidney play a primordial role in Hcy handling .This occurs mainly in the distal tubule cells, possibly to provide metabolic substrates to cell which luminal amino acid delivery is decreased(8).The kidney remove 70% of the daily Hcy load mainly through transsulfuration pathway(9) .Accordingly strong inverse relationship between Hcy levels and renal function ,even in the range of minimally reduced GFR were observed (10).

Observations in many clinical and epidemiologic studies have suggested that hyperhomocysteinemia is an independent risk factor for a various diseased condition including coronary artery disease, congestive heart failure (11). It may also be relevant for dementia and Alzheimer's disease (12).In addition to type II

diabetes (13). It has been suggested that reactive oxygen species play an important role in the development of tissue damage by hyperhomocysteinemia (14).

Folate is a generic term for a B-vitamin containing the basic chemical structure composed of a pteridine residue and a P-aminobenzoylglutamate residue (15). Folic acid is the stable synthetic analog of the parent structure of the folate family. Human cannot synthesize folate must rely on dietary source (16). Folate decrease due to a genetic defect or malnutrition is associated with HHcy. The active metabolite of folic acid, 5-methyltetrahydrofolate, facilitates remethylation of Hcy to methionine by donating a methyl group to Hcy with vitamin B12 as a cofactor (17).

Because of the protective effects exerted by administration of folic acid, and because of the cell culture and some in vivo studies showing that methionine overload lead to considerable toxic effect mediated by ROS formation, we can suggested that the toxic effect of methionine overload could be treated by folic acid

Material and methods

Thirty adult male Albino Wister rats with a body weight (175-250 gm) were used in this investigation. Their ages were ranged between (2.5 - 3) months. were randomly divided into three groups (10 rats/ group) and were treated daily for 42 days as follows:-Group (T1) served as control, Group (T2) The rats in this group were orally intubated (by gavage's needle) methionine (100 mg /kg B.W) (18). Group (T3) Animals in this group were administered orally methionine (100mg/kg B.W) plus 5 mg of folic acid diet (19). Fasting blood samples were collected at 21 and 42 days of experiment. Blood were drawn via cardiac puncture technique from anesthetized rats and the serum was used for the assay of, Serum creatinine (SC) concentration, Blood urea nitrogen (BUN) concentration and Serum uric acid (SUA) concentration. After death the kidney preserved in 10% neutral formalin buffer solution. Haematoxylin and eosin were used for staining and later the microscopic slides of the kidney cell were photographed.

Statistical analysis

Data was performed on the basis of two way analysis of variance (ANOVA) using significant level of ($P < 0.05$). Specific group differences were determined using least significant differences (L.S.D) (20).

Results

Serum creatinine concentration significantly increase ($p < 0.05$) in methionine treated groups (T1) at day 21 & 42 of the experiment comparing to folic acid treated group (T3) and control. At the end of the experimental (day 42) highest significant reduction ($P < 0.05$) in serum creatinine concentration were observed after folic acid supplementation in group T3 comparing to T2 group. Within the time, significant elevation ($P < 0.05$) in serum creatinine concentration in T2 treated group was observed at the end the experiment comparing to the pretreated period table (1) show the result.

Table (1) serum creatinine concentrations (mg/dl) in rats orally administered by methionine and folic acid compared with control group

Groups Days	Control (T1)	Methionine treated (100 mg/kg B.W) (T2)	Methionine +folic acid (T3)
21	0.70±0.07 A a	1.32±0.09 B b	0.95±0.01 DC b
42	0.69±0.01 AC a	0.99±0.03 BC c	0.86±0.02 C ab

Capital letters denote between groups differences, $P < 0.05$ v.s control.

Small letters denote within group differences, $P < 0.05$.

Results of table (2) indicated that methionine overload is more deleterious to the biomarkers of kidney function (BUN concentration) caused significant elevation ($P < 0.05$) in serum concentration at day 21 & 42 of the experiment comparing to the control and folic acid group. However, at the end of the experiment, folic acid caused significant decrease ($P < 0.05$) in mean value of serum blood urea nitrogen concentration in T3 group comparing to T2 group. Within groups, significant increment ($P < 0.05$) was observed during the 21 day of treatment till the end of the experiment in methionine treated (T2) group comparing to the pretreated period.

Table (2) serum blood urea nitrogen concentrations (mg/dl) in rats orally administered by methionine and folic acid compared with control group

Groups Days	Control (T1)	Methionine treated(100 mg/kg B.W (T2)	Methionine +folic acid (T3)
21	19.64±0.6 A a	45.2±1.1 B b	40.20±1.5 C b
42	20.4±0.8 A a	52.40±1.9 B c	25.0±1.4 D c

Depending on the results clarified in table (3), showed a significant increase ($P < 0.05$) in serum uric acid concentration in methionine treated group (T2) at the 21 & 42 days of experiment as compared to control and folic acid group. On the other hand, lack of significant differences ($P > 0.05$) were observed in T3 along the treated period as compared to control group. Lowest reduction in SUA value was observed at the end of the experiment in T3 with mean value of (4.0±0.29). Within the time the concentration of serum uric acid in two treated groups T2, T3 increased significantly ($P < 0.05$) compared to the pretreated period.

Table (3) serum uric acid concentrations (mg/dl) in rats orally administered by methionine and folic acid compared with control group

Groups Days	Control (T1)	Methionine treated (100 mg/kgB.W (T2)	Methionine +folic acid (T3)
21	3.89±0.14 A a	4.50 ±0.19 BC b	4.18±0.14 AC b
42	3.79±0.13 A a	4.71±0.14 B b	4.0±0.29 A b

Histopathological examination

The histological structure of kidney of untreated rats (control) were shown in figure(1). Histological changes of rat kidney received methionine including, degeneration in the epithelial cell lining renal tubules (figure 2) While histological section of rat kidney intubated methionine plus folic acid supplementation showed mild accumulation of monocytic cell in kidney parenchyma (figure3).

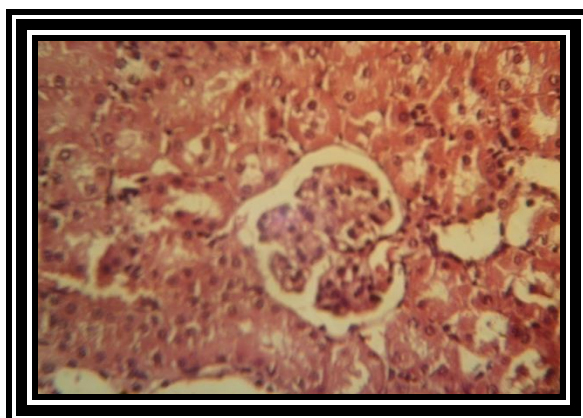
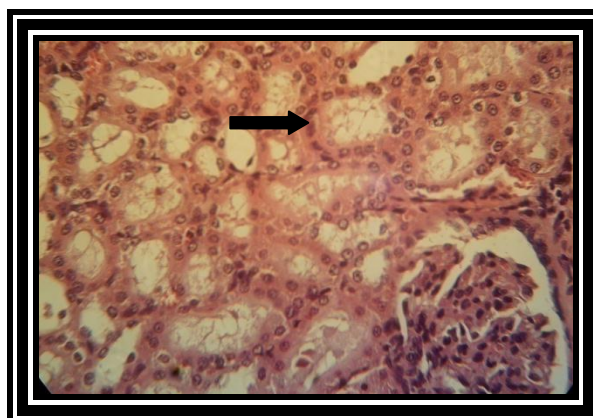


Figure (1) Histological section in kidney of control group. Show normal histology of kidney section (40x H & E).



Figure(2) Histological section in kidney of methionine treated rat. Note degeneration in epithelial cell lining the renal tubule(➡) (40x H & E).

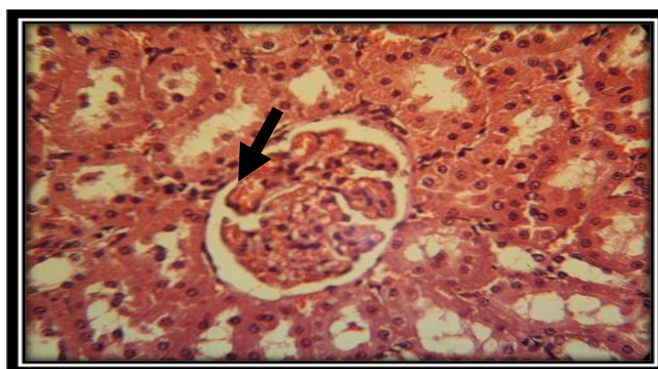


Figure (3) Histological section in kidney of methionine + folic acid treated rat. Note : Regression of almost all damaged tissue with exception of mild accumulation of monocytic cells in kidney parenchyma (➡) (40x H & E)

Discussion

The present study showed that daily oral intubation of methionine overload for 6 weeks caused significant elevation in serum creatinine, serum blood urea nitrogen and serum uric acid concentrations (tables 1,2, 3). The elevation of blood urea nitrogen is a good indicator for kidney disorders specially as it relates to glomerular function (21). Besides, (22), indicated that high levels of uric acid has been correlated with gout, hypertension, renal damage, and hyperhomocysteinemia (23). The association of methionine overload with renal damage in this study is significant in light of proposed role of elevated Hcy after methionine overload as risk factor for renal disease (24), where the proposed hyperhomocysteinemia may lead to overproduction and release of reactive oxygen species (ROS) from glomerulus, renal damage, impairment of (GFR) glomerular filtration rate, and significant increase in creatinine clearance, serum blood urea nitrogen and creatinine concentrations (25, 26). Because Hcy and adenosine are produced via hydrolysis to S-adenosyl homocysteine, we can hypothesize that hHcy following methionine overload may produce its pathogenic effects by suppression of plasma or tissue adenosine concentration (9). The excess of adenosine would react with methionine forming S-adenosyl methionine then degraded to form uric acid as its end product leading to hyperuricemia (27). Besides, enhanced protein catabolism and accelerated amino acid deamination for gluconeogenesis is possible an acceptable postulate to interpret the elevated levels of urea (28). The result of the present study revealed that methionine overload caused renal injury manifested by significant degeneration in the epithelial cell lining renal tubules (figure 2). This results were documented by some authors (27). Where remarkable glomerular injury and progression of glomerulosclerosis were observed after methionine overload. manifested histologically by increased matrix formation and aggregated proteoglycans with fibrous deposition in glomeruli (29). Some investigators have found that hHCY induced through different approaches renal damage in both man and animals (9,30). It was recently reported that treatment with high methionine diet or methionine overload in drinking water, as done in the present study, exhibited glomerular dysfunction and sclerosis and these renal damage may be due to HHcy associated with suppression of adenosine level which are related to enhanced sclerotic changes in glomeruli (31).

Folic acid supplementation exert protective actions against damaging effect methionine on renal system causing significant decrease in kidney biomarkers (SC, BUN, SUA). Such increase in kidney function biomarkers after folic acid supplementation is correlated with previous reports (32). Folic acid may attributed to its renoprotective effect. First of all, folic acid has protective effect against high glucose induced renal cellular damage. Secondly, attenuated renal dysfunction as indicated by suppression of BUN, & SC (33). Folic acid may counteract the mechanism of oxidative toxicity against various oxidative challenges (34), besides folic acid may protect the kidney against deleterious effect of oxidized LDL on glomerulus there by improving GFR through suppression of BUN, SC, and SUA concentrations. Meanwhile, as significant increase in uric acid is associated with impaired GFR, we can suppose that folic acid supplementation in the experiment may lead to improvement of glomerular filtration rate and subsequent depression in

uric acid concentration.(35). Functional differences due to folic acid supplementation observed in this study were also confirmed histologically , caused regression of renal lesion caused by methionine (Figures 3).The renoprotective effect of folic acid was documented(36) and renal protection to different levels of glomerular injury by antioxidants could be suggested (37).

Reference

1. **Ball, R. O.; Courtney-Martin, G. and Pencharz, P.B.(2006).**The in vivo sparing of methionine by cysteine in sulfur amino acid requirements in animal models and adult humans .J. Nutr., 136 (6):1682S-1693S.
2. **Dever,J.T and Elfarra,A.A.(2006).**In v.ivo metabolism of L. methionine in mice :evidence for stereoselective formation of methionine-d-sulfoxide an quantitation of other major metabolites .Drug Meta.Dispos.,34(12):2036-2043.
3. **Lijfering, W.M.;Veeger ,N.J.G.M.;Brouwer,J.L.P. and Van der Merer,J. (2007).**The risk of venous and arterial thrombosis in hyperhomocysteinemic subjects may be a result of elevated factor VIII levels.Haematol.,92:1703-1706.
4. **Gupta,S.; Wang, L.; Hua, X.; Krijt, J.; Koich, V. and Kruger,W.D. (2008)** . Cystathionine β -synthase p.S466 Lmutationcauses hyperhomocysteinemia in mice. Human Mutation ., 29(8): 1048-1054.
5. **Halsted , C.H.; Wong , D.H ;Peerson , J.M.; Warden,C.H. ;Refsum , H.; Smith , A.D.;Nygard , O.K.;Ueland , P.M.;Vollset , S. E. and Tell , G.S (2007)** . Relations of glutamate carboxy peptidase II (GCP II) Polymorphisms to folate and homocysteine concentrations and to scores of cognition, anxiety, and depression in ahomogeneous Norwegian population :the Hordaland Homocysteine study . Am . J. Clin . Nutr .,86:514-521.
6. **Westphal, S. ; Radding, A.; Luley,C. and Dierkes, J.(2003)** . Antihypertensive treatment and homocysteine concentrations .Metabolism., 52(3):261-3.
7. **House, A.A. ;Wells, G.A. ;Donnelly, J.G.;Nadler, S.P. and Hebert, P. C. (2000).**Randomized trial of high-flux vs.low-flux haemodialysis :Effect on homocysteine and lipids . Nephrol Dial transplant 15 : 1029-1034
8. **Friedman, A.N.; Bostom, AG.; Selhub, J.;Levey, A.S. and Rosenberg, I.H. (2001).**The kidney and homocysteine metabolism. J. Am. Soc. Nephrol., 12:2181-9.
9. **Li, N., Chen, L.; Muh, R.W. and Li, P.-L.(2006).** Hyperhomocy - steinemia associated with decreased renal transulfuration activity in dahl rats .Hypertension .,47 (6):1094-1100.
10. **Fridman, A.N., Rosenberg ,I.H., Selhub ,J., Levey ,A.S. and Bostom,A .(2002).** Hyperhomocysteinemia in Renal transplant patients .Transplantation., 2:308-313.

11. **Rowan, E. N. ; Dickinson , H. O. ; Stephenns , S. ;Ballard , C. ; Kalaria , R. and Anne Kenny , R. (2007) .** Homocysteine abnd post –stroke cognitive decline . Age Ageing., 36 :339-343.
12. **Chertkow , H. (2008) .**Diagnosis and treatment of dementia. Introducing a series based on the third canadian consensus con ference on the diagnosis and treatment of dementia .CMAJ., 178:316-321.
13. **Masuda, Y.; Kubo, A.; Kokaze, A.; Yoshida, M.; Fukuhara,N.; and Takaashima,Y.(2008).**Factors associated with serum total homocys -teine level in type 2 diabetes .Environmental Health Preventive Medicine 13(3).148-155.
14. **Kerkeni, M.; Addad, f.; Farhat, M.; Miled, A.;Trivin ,F. and Maaroufi, K.(2008).**Hyperhomocysteinemia and parameters of anti oxidative defence in Tunisian patients with coronary heart disease. Ann. Clin . Biochem.,45:193-198.
15. **Lucock ,M.(2000).** Folic acid: nutritional biochemistry, molecular biology ,and role in disease processes .Mol. genet metab .,71:121-38
16. **Steinberg,S.E.(1984).**Mechanism of folate homeostasis.Am J physiol., 246:G319-324
17. **Symons ,J.D. Mullick, A.E. Ensunsa, J.L. Ma, A.A and Rutledge, J.C.(2002).**Hyperhomocysteinemia evoked by folate depletion :effect on coronary and carotid arterial function .Arterioscler Thromb vasc Boil .,22:772-780
18. **Seshadri, N. and Robinson, K . (2000).** Homocysteine , B vitamin and coronary artery disease .Med. Clin. North. Am., 84(1):215-37
19. **Doshi S.N., McDowell I.F.,Moat S.J.,Lang D.,Newcombe R.G., Kredan M.B.,Lewis M.j., Goodfellow J.(2001).**Folate improves endothelial function in coronary artery disease:an effect mediated by reduction of intracellular superoxide. Arterioscler Thromb vasic Biol 21:1196-202
20. **Steel,R.G. and Torries, J.H.(1980).**Principles and Procedures of Statisticas. Abiometrical approach, 2nd edition.McGraw- Hill Book Co.New York,USA.
21. **Bishop, L.M.; Fody, P.E. and Schoe, H. L. (2005).** Clinical chemistary principles procedures correlations.5th ed.Lippincoh Williams and wilkins, Philadelphia, Hong Kong.Pp.220-253.
22. **Kutzing, M.K. and Firestein, B.L.(2008).**Altered uric acid levels and disease states. JPET., 324:1-7.
23. **Rosolowsky,E.T.; Ficociello, L.H.;Maselli,N.J.;Niewczas,M.A.; Binns, A.L.; Roshan, B.;Warram,J.H. and Krolewski ,A.S.(2008).**High-normal serum uric acid is associated with impaired glomerular filtration rate nonproteinuric patients with type 1 diabetes .Clin. J. Am. Soc. Nephrol.,3(3):706-713.
24. **Rensma , P. L. ; Apperloo , A.J. and De Jong , P.E. (2003).**Why does elevated plasma homocysteine result in severe microvascular injurys but not glomerular damage ? Circulation., 107:e77.
25. **Mallat,S.G. and Anoun,M.(2002).**Hyperhomocysteinemia and its role in chronic renal failure. Sudia J. Kidney Dis. Transplantation.,13:336-343.

26. **Bostom, A.G.(2000).** Homocysteine: “expensive creatinine” or important modifiable risk factor for arteriosclerotic outcomes in renal transplant recipients. *JAM soc Nephrol.*, (1: 149- 15).
27. **Diez, N.; Perez, R.; Hurrtado.; V. and Santidrian.; S.(2005).** Hyperhomocysteinaemia induced by dietary folate restriction causes kidney oxidative stress in rats. *British .Journal of Nutrition* .94:204-210.
28. **Thrall,M.A.(2004).**Laboratory evaluation of the liver in:veterinary hematology and clinical chemistry.Lippincott Williams and wilkins com.,Philadelphia.
29. **Li,N.; Chen,Y.-F.; Zou,A.-P.(2002)** .Implications of hyperhomocysteinemia in glomerulosclerosis in hypertension . *Hypertension* .,39:443-448.
30. **Dani, C.; Pasquali,M.A.B.; Oliveira,M.R.; Umezu,F.M.; Salvador, M .; Henriques,J.A.P. and Moreira, J.C.F. (2008).** Hepatoprotective agent in wister rats. *J. Med. Food.*,11(1): 127-132.
31. **Chen,Y.-F.;Li,P .-L.and Zou,A.- P.(2002).** Effect of hyperhomocysteinemia on plasma or tissue adenosine levels and function renal . *Circulation.* ,106: 1276 - 1281.
32. **Thambyrajah,J.;Landray,M.J.;McGlynn,F.J.;Jones,H.J.;Wheeler,D.C. and Townend,J.N.(2000).**Does folic acid decrease plasma homocysteine and improve endothelial function in patients with predialysis renal failure?.*Circulation.*,102:871-5.
33. **Bostom , A.G. Shemin, D.; Bagley, P. ;and Selhub, J.(2000).** Controlled comparison of L-5-Methyltetrahydrofolate versus folic acid for the treatment of hyperhomocysteinemia in hemodialysis patients. *Circulation* ., 101: 2829.
34. **Vermeulen , E.G.J. ; Stehouwer, C.D. A.;and Twisk, J.W. R.(2000).** Effect of homocysteine lowering treatment with folic acid plus vitamin B₆ on progression of subclinical atherosclerosis; a randomized, placebo controlled trial . *Lancet*; 355: 517-22.
35. **Mehta , K.N.; Chag, M.C.; Parikh, K.H. and Shah, U.G. (2005).** Effect of folate treatment on homocysteinemia in patients : A prospective study. *Indian J Pharm*; vol 37: 13-17.
36. **Pastore, A.; De Angelis, S.; CasCiani, S.; Ruggia, R.; Di Giovamberardino, G .; Cortese, C.; Federici, G. and Dessi, M.(2006).** Effects of folic acid before and after vitamin B₁₂ on plasma homocysteine concentration in hemodialysis patients with known MTHFR genotypes . *Clin. Chem.*, 52(1): 145-148
37. **Kitamura,M. and Ishikawa,Y.(1999).**Oxidant-induced apoptosis of glomerular cells: intracellular signalling and its intervention by bioflavonoid. *Kidney Int.*,56:1223-1229.

Recived (9/6 /2010)

Accepted (24/8/2010)

