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## PROTECTIVE EFFECT OF FOLIC ACID ON OXIDATIVE STRESS INDUCED BY METHIONINE OVERLOAD IN MALE MICE

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### ABSTRACT

Methionine is a basic sulfur that contains important amino acid and is necessary for many vital activities necessary in the body, Methionine is considered a preventive agent for many liver damages, But in the case of excessive iron administration, it has a negative effect. One hundred and fifty (150) mice model selected for present study is Albino pulp-c and their ages between (12-16) a week, One hundred fifty (150) adult mice were randomly divided into 5 groups (thirty each group) and treated for six weeks. Group I animals were incubated orally tap water, serving as control, Group II animals were incubated orally L-methionine, Group III animals were incubated orally folic acid ,Group IV animals were incubated methionine plus folic acid, and after the end of the experiment, the following criteria were measured GSH, MDA, AST, ALP, ALT, albumin concentration and globulin concentration. methionine overload administration leads to a decrease in concentration of GSH in G2 and an increase in concentration of GSH in G3 and G4, and an increase in concentration of MDA in G2 and decrease in concentration of MDA in G3 and G4, and a significant decrease in globulin concentration in G2 compared to G1 and non significant difference between G3,G4 and G1, Also, the albumin concentration in this experiment was found to have a significant increase in G3 compared to G1, and also there was a significant decrease in G2, and there was non significant difference between G4 and G1, a significant increase in activity of AST in G2 compared to G1 and a significant decrease in activity AST in G4 and G3, compared to G1, a significant increase in G2 in the ALT activity compared to G1, and a significant decrease in activity in G3 compared with G1 and there was non significant difference in activity ALT between G1 and G4, Also showed a significant increase in activity ALP in G2 compared to G1 and a significant decrease in activity ALP in G3 and non significant difference in activity ALP in G4 compared to G1, methionine overload may cause oxidative stress in most cells of the body resulting in the release of free radicals in the cells of the tissues.

**Keywords :** L-Methionine, folic acid, oxidative stress

### Introduction

Methionine is defined as basic sulfur that contains important amino acid and is necessary for many vital activities necessary in the body, Methionine is considered a preventive agent for many liver damages, The daily amount of methionine that the body needs are 13 mg/ kg or one gram per day for adult humans ,The amino acid methionine is naturally present in animal-derived proteins such as beef, fish, poultry, eggs, and cheese, It is also present in juices, vegetables and sunflower seeds, crab meals, blood meals, gluten meals and tormented foods, as well as in fruit and casein and rice, which have been established as new sources of methionine or can be developed industrially in pharmaceutical preparation and nutritional supplements (Salman,2014)

Studies have also shown that methionine is important for the body, but also that high levels of methionine in the food cause damage to the erythrocyte membrane and also trigger delays in tissue growth and injury, steatosis, hepatitis, hypertriglyceridemia and hepatotoxicity (Balkan *et al.*, 2004).

methionine one of the factors triggering a disorder of homocysteine metabolism and the accumulation of homocysteine in large amounts triggered by a case of hyperhomocysteinemia (HHcy) and other pathological conditions ,the risk factor for the digestive system, neurological diseases, strokes, arteriosclerosis, coronary artery inflammation and myocardial infarction involves congestive heart failure and coronary artery disease as well as Alzheimer's disease and type II diabetes and homocysteine (Micovic *et al.*, 2016).

As the homocysteine levels increase, cell damage is caused by high free radicals and oxidative stress and therefore cell apoptosis, because oxidative stress is the main cause of apoptosis (Mangiagalli *et al.*, 2004).

The increase in hyperhomocystinemia leads to a decrease in the glomerular filtration rate, kidney damage and increased concentration of creatine and urea in the blood and increased production and release of glomerulus reactive oxygen species, resulting in damage and weakness of the urinary system (Al-Hashmy and Khudiar, 2009).

Methionine overload works on the degeneration of the epithelial cells lining the renal tubes and severe vacuolar degeneration of the renal tubular epithelial lining cells and atrophy of glomerular tufts and infiltration of mononuclear cells between renal tubules. Pomegranate seed oil has been found to act to reduce oxidative stress and scavenge free radicals and reduce fat peroxide from excessive methionine processing, and to reduce methionine damage to the urinary system (Salman, 2014)

Excessive homocysteine in the blood will be followed by people with kidney failure, which results in high levels of antioxidants and lipid peroxidation, this condition can be corrected if folic acid is given as a treatment (Jordao Júnior *et al.*, 2009).

Folic acid is an important vitamin cofactor needed to remethylate Hcys to methionine in the metabolism of Hcys (Vijayakumar *et al.*, 2017)

It was found that consuming vitamin B12 with B6 and B9 decreases the effect of hyperhomocysteinemics due to methionine deficiency and that using such vitamins together is better than using folic acid alone, and that vitamin B9 is better than B6 or B12 when used separately to decrease Hhcy (Al-Beer *et al.*, 2013).

Folic acid decreases blood homocysteine levels and reduces the risk of heart disease and is also essential for cell proliferation in the fetus and works to reduce homocysteine levels through vitamin B12 work in combination with vitamin B9 to turn homocysteine into methionine (Kerkeni *et al.*, 2006).

### Materials and Methods

One hundred and fifty (150) mice (20-40g) were used in the current study at different intervals were taken from the Cancer Research Center in Baghdad, Iraq. model selected for present study is Albino pulp-c and their ages between (12-16) a week and the animals were placed in the animal house of the College of Veterinary Medicine/University of Karbala in special plastic cages and equipped with a metal mesh cover. The cages were spread with soft sawdust, which was replaced daily, as was the care of cleaning the cages and sterilizing them from time to time, and provided the animals with the appropriate conditions. In terms of temperature around (25 ± 5 °C) and ventilation and The light system was 14/10 hrs light/dark cycle with a relative humidity of 50±5%. They were kept for 2 weeks for adaptation with standard experimental condition.

One hundred fifty (150) adult mice were randomly divided into 5 groups (thirty each group) and treated as follows for six weeks.

Group I animals were intubated orally tap water, serving as control, group II animals were incubated orally 100mg/kg B.W of L-methionine, group III animals were intubated orally 0.07 mg/kg BW of folic acid, group IV animals were incubated 100 mg/kg BW of methionine plus 0.07mg/kg B.W of folic acid (Al-Bazii, 2009).

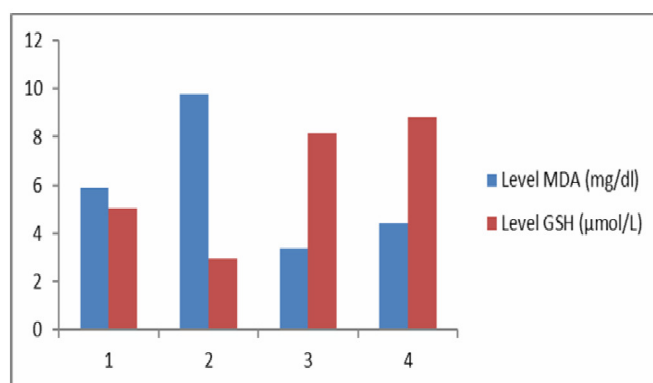
Malondialdehyde was estimated and Reduced glutathione, albumin concentration and globulin concentration, Alanine Aminotransferase (ALT), Alkaline phosphatase (ALP) and Aspartate Aminotransferase (AST) Depending on the method (Al-Bazii, 2009).

### Results

The laboratory results of blood samples taken from mice after the end of the experiment period proved that giving methionine and folic acid for six weeks at a high dose has an effect on some physiological parameters.

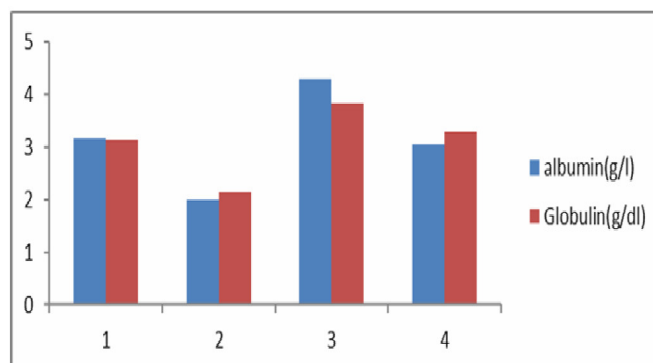
The results showed the administration of folic acid in excess doses of G3 led to a significant increase ( $P < 0.01$ ) in concentration of GSH and in G4 (methionine-folic acid) comparison with G1, and the presence of a significant decrease ( $P < 0.01$ ) in G2 compared to G1, as in Figure (1), which shows concentration of GSH in this trial

The result also showed concentration of MDA there was a significant ( $P < 0.01$ ) increase in G2 treated with methionine, and a significant decrease ( $P < 0.01$ ) in G3 and G4 when compared to G1 in concentration of MDA, as in Figure (1)



**Fig. 1 :** Effect of daily oral incubation of methionine and folic acid for six weeks on Glutathione (GSH) concentration ( $\mu\text{mol/l}$ ) and Malondialdehyde (MDA) concentration ( $\mu\text{mol/d l}$ ) in male mice.

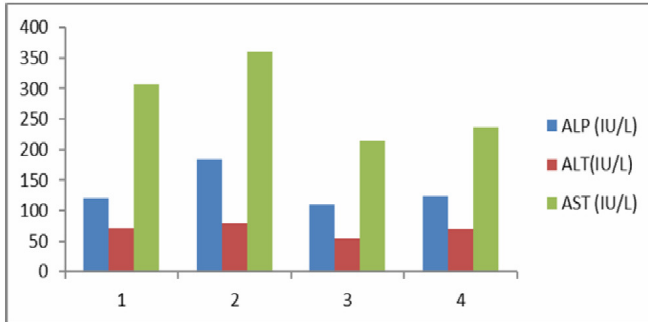
The results showed a significant decrease ( $P < 0.01$ ) in concentration of globulin concentration in G2 compared to G1 and there was non significant difference between G3, G4 and G1, Also, the albumin concentration in this experiment was found to have a significant increase in G3 compared to G1, and also there was a significant decrease in G2, and there was non significant difference between G4 and G1, as in Figure (2).



**Fig. 2 :** Effect of daily oral intubation of methionine and folic acid for six weeks on Globulin (g/l) and albumin concentration (g/dl) in male mice

The results of a AST test showed a significant increase ( $P < 0.01$ ) in activity of AST in G2 compared to G1 and there is also showed a significant decrease in activity AST in G4 and G3, compared to G1, and When performing a ALT test, showed was a significant increase ( $P < 0.01$ ) in G2 in the

ALT activity compared to G1, and there was also showed a significant decrease ( $P < 0.01$ ) in activity in G3 compared with G1 and there was non significant difference in activity ALT between G1 and G4, Also showed a significant increase ( $P < 0.01$ ) in activity ALP in G2 compared to G1 and there was a significant decrease ( $P < 0.01$ ) in activity ALP in G3 and non significant difference in activity ALP in G4 compared to G1, as shown in the figure (3).



**Fig. 3 :** Effect of daily oral intubation of methionine and folic acid for six weeks on Aspartate transaminase (AST) activity (IU/L), Alanine transaminase (ALT) activity (IU/L) and Alkaline Phosphatase (ALP) activity (IU/L) in male mice

### Discussion

Studying the effect of methionine overload on the antioxidant status of the male mice in the present study, showed a significant decrease in serum GSH concentration in methionine treated group. The result of the present study was Agreement with (Hsu *et al.*, 2015; Cacciapuoti, 2019). Such changes may be attributed to Hyperhomocysteinemia HHcy induced after methionine overload.

Many authors recognized that methionine overload caused HHcy through disturbing remethylation pathway, preventing normal conversion of SMTH to methionine and subsequent stress of transulfuration pathway. Such HHcy will lead to formation of homocysteine S-S mixed disulfide conjugates which inhibit the superoxide radical scavenging activity of metallothionein (Barbato *et al.*, 2007)

Low serum levels of glutathione (GSH) and total antioxidant capacity are considered to be indicators of oxidative stress, a metabolic condition that involves high level of homocysteine (HCY). Under oxidative stress, the cellular antioxidants capacity is not counterbalancing the oxidative damage induced by various insults including, free radicals and environmental toxins (Waly *et al.*, 2011)

The subsequent increase in serum MDA concentration in the current study may be attributed to high sensitivity of mice to free radicals production by Hyperhomocysteinemia. An elevation in generation of lipid peroxidation (MDA level) was postulated to cause a gradual cell injury by free radicals liberating lipoxygenase enzymes which oxidized unsaturated membrane fatty acids and subsequent production of MDA, overwhelming endogenous scavenging system including GSH resulting in oxidative stress (Kim *et al.*, 2015).

The increase of MDA level may be due to an increase in the production of free radicals more than ability of the scavenging system to remove them increased serum MDA and decrease GSH levels. This findings is in agreement with many laboratory studies which indicated alteration in the antioxidants status of different tissue as a results of an increase in lipid peroxidation of these tissue after induction oxidative stress (Hsu *et al.*, 2015)

There is a strong correlation between increase GSH levels and decrease in lipid peroxidation (MDA production) after folic acid intervention, these biomarkers of oxidative stress are interrelated and they assure that folic acid induced suppression of oxidative stress of affected mice. Moreover, GSH regarded as a major cellular antioxidant so it is elevation after folic acid incubation augmented cellular protection against free radicals damage (Villa *et al.*, 2004), induced by methionine overload.

The current study indicated that incubation of methionine to adult male mice for six weeks caused a significant decrease in serum albumin and globulin concentrations (Liu *et al.*, 2013)

Methionine overload and subsequent Hyperhomocysteinemia markedly suppressed voluntary food intake, and micronutrient deficiency that lead to growth retardation (Jolin *et al.*, 2005). As there was positive correlation between body weight and total protein concentration, we can postulate that HHcy occurred after methionine overload may lead to decrease in total serum protein.

Protein molecules are essential target for free radicals attack, both intracellularly and extracellularly (Au-Yeung *et al.*, 2004), where under conditions of severe oxidative stress, free radicals generation at inappropriate sites could lead to protein modification. Proteins are also modified indirectly with reactive carbonyl compounds formed by autoxidation of carbohydrate and lipid, with eventual formation of advanced glycation and lipid peroxidation (Sans *et al.*, 2006). The consequence of such damage may impair enzymatic activity, modified membrane and cellular function.

Hypoalbuminemia has been reported in hyperhomocysteinemic patients, which serve to reduce the overall antioxidant protection against disease development (Rondal, 2002) including albumin, therefore the hypohomocysteinemic effect of folic acid, may participate in elevation of serum albumin level (Stadman, 1998). Besides, folic acid supplementation may suppress the formation of protein content of carbonyl (Solini *et al.*, 2006) leading to reduction in protein glycation and peroxidation end product, alleviate the damage in cellular function leading to maintenances of protein content of liver cells and extracellular fluid (Mimic-Oka *et al.*, 2001).

Transaminases (ALT and AST) are two closely related enzymes of clinical significance, particularly in the assessment of liver function (Tiikinen *et al.*, 2004). In this study a significant elevation in both transaminase enzymes in animals treated with methionine for six weeks were observed, indicating occurrence of liver disorder. The result of the present study was Agreement with (Kim *et al.*, 2015).

Homocysteine metabolism and its synthesis occur in the liver as the liver plays a significant and essential role in Hcy metabolism, the liver is important in metabolism as it produces an important enzyme in Met and Hcy metabolism, and any defect that occurs in the liver tissue or enzymes that are responsible for the metabolism of Met or Hcy (García-Tevijano *et al.*, 2001).

Methionine is beneficial for the liver, but for dietary excess methionine, liver damage due to methionine overload leads to increased levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (

ALP) in blood circulation due to cell death and injury, studies have shown that overdose of methionine results in multifocal granulomatous lesion characterized by mononuclear aggregations in the blood vessel and parenchyma in the liver and hepatocytic necrosis (Al-Shammry and Al-Okaily, 2009).

The researchers found that HHcy causes several liver disorders, including hepatic compromise, cirrhosis of the liver, fat accumulation, and necrosis of the liver. Signs of necrosis include high levels of AST and ALT, high levels of inflammatory cells and collagen, where they accumulate in the tissues, and high levels of lipid peroxidation (Matte *et al.*, 2009).

Methionine over load may caused oxidative stress in the most of the body cells causing release of free radicals in tissue cells leading to damaging the cell membrane and central portal liver cells with subsequent release of AST and ALT enzymes (Kim *et al.*, 2015).

The present study revealed that administration of folic acid effectively improved liver function by return to normal levels AST and ALT enzymes activities. The observed restorations in the activity of previous enzymes after folic acid incubation are in agreement with previous studies (Al-Bazii, 2009).

Similarly (Ekaidem *et al.*, 2007). Reported that serum ALT and AST activities were return to normal activity by folic acid therapy. It has been found that folic acid supplementation quickly and efficiently normalized hepatic function and reversed the sustained elevation in aminotransferase activities in patients with rheumatoid arthritis and liver cirrhosis (Keech *et al.*, 2005).

### References

- Al-Bazii, W.G. (2009). Protective And Therapeutic Role Of Olive Oil And Folic Acid In Some Biochemical Parameters And Histological Changes In Male Rabbits Exposed To Methionine Over Load. PH. D. Thesis, College of veterinary medicine, University of Baghdad .
- Al-Ber, A. A.; Al-Shalah, H. H. and Abdul-Latif, A. R. (2013). The Role of Some B Vitamins in Methionine-Induced Hyperhomocysteinemia in Male Rabbits. *Karbala Journal of Medicine*, 6(1), 1539-1545.
- Al-Hashmy, R.A. and Khudiar, K.K. (2009). Protective role of alcoholic extract of black currant (*Vitis vinifera* L.) on renal function of adult male rats exposed to methionine overload and hydrogen peroxide. *Iraqi Journal of Veterinary Sciences*, 23(3).
- Al-Shammry, Z.M.M. and Al-Okaily, B. (2009). Effect of alcoholic extract of black currant (*Vitis vinifera* L.) on hepatic function and damage induced by hydrogen peroxide and methionine overload in rats. *Al- Anbar Journal of Veterinary Sciences*, 2(2): 19-34.
- Au-Yeung, K.; Woo, C.; Sang, F.; Yip, J.; Siow, Y. and O, K. (2004). Hyperhomocysteinemia activates nuclear factor-Kappa B in endothelial cells via oxidative stress. *Circ Res.*, 94: 28-36.
- Balkan, J.; Dogru-Abbasoglu, S.; Cevikbas, U.; AYKAÇ-TOKERİ, G. and Uysal, M. (2004). Methionine supplementation did not augment oxidative stress, atherosclerotic changes and hepatotoxicity induced by high cholesterol diet in C57BL/6J mice. *Journal of nutritional science and vitaminology*, 50(4): 258- 264.
- Barbato, J.C.; Catanescu, O.; Murray, K.; Di Bello, P.M. and Jacobsen, D.W. (2007). Targeting of metallothionein by L- homocysteine: a novel mechanism for disruption of zinc and redox homeostasis *Arterioscler. Thromb. Vasc., Biol.*, 27:49.
- Cacciapuoti, F. (2019). N-Acetyl-Cysteine supplementation lowers high homocysteine plasma levels and increases Glutathione synthesis in the trans-sulfuration pathway. *Italian Journal of Medicine*, 13(4): 234-240.
- Ekaidem, I.S.; Akpanabiatu, M.I.; Uboh, F.E. and Eka, O.U. (2007). Effect of folic acid and vitamin B 12 administration on phenytoin induced toxicity in rats. *Indian Journal of Clinical Biochemistry*, 22(2): 36-40.
- García-Tevijano, E.R.; Berasain, C.; Rodríguez, J.A.; Corrales, F.J.; Arias, R.; Martín-Duce, A. and Avila, M.A. (2001). Hyperhomocysteinemia in liver cirrhosis: mechanisms and role in vascular and hepatic fibrosis. *Hypertension*, 38(5): 1217-1221.
- Hsu, C.C.; Cheng, C.H.; Hsu, C.L.; Lee, W.J.; Huang, S.C. and Huang, Y.C. (2015). Role of vitamin B6 status on antioxidant defenses, glutathione, and related enzyme activities in mice with homocysteine-induced oxidative stress. *Food & Nutrition Research*, 59(1): 25702.
- Jolin, W.J.; Aafje, S.; Gertjan, S.; Kok, F.J.; Struys, E.A.; Jakobs, C. and Hendriks, F.J. (2005). Kinetics of homocysteine metabolism after moderate alcohol consumption. *Alcohol Clin. Exp Res.*, 29: 739-45.
- Jordao Júnior, A.A.; Domenici, F.A.; Lataro, R.C.; Portari, G.V. and Vannucchi, H. (2009). Effect of methionine load on homocysteine levels, lipid peroxidation and DNA damage in rat receiving ethanol. *Brazilian Journal of Pharmaceutical Sciences*, 45(4): 709-714.
- Keech, A.; Simes, R. and Barter, P. (2005). Study investigators, effects long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the field study) randomized controlled trial. *Lancet*, 366: 1849-1861.
- Kerkeni, M.; Addad, F. and Xhauffert, M. (2006). Hyperhomocysteinemia, methylenetetrahydrofolate reductase polymorphism and risk of coronary artery disease. *Ann. Clin. Bio.*, 43:200-6.
- Kim, S.Y., Kim, H. and Min, H. (2015). Effects of excessive dietary methionine on oxidative stress and dyslipidemia in chronic ethanol-treated rats. *Nutrition Research and Practice*, 9(2): 144-149.
- Liu, H.H.; Shih, T.S.; Huang, H.R.; Huang, S.C.; Lee, L.H. and Huang, Y.C. (2013). Plasma homocysteine is associated with increased oxidative stress and antioxidant enzyme activity in welders. *The Scientific World Journal*, 2013.
- Mangiagalli, A.; Samuele, A.; Armentero, M.T.; Bazzini, E.; Nappi, G. and Blandini, F. (2004). Effects of homocysteine on apoptosis-related proteins and antioxidant systems in isolated human lymphocytes. *European journal of biochemistry*, 271(9): 1671-1676.
- Matte, C.; Stefanello, F.M.; Mackedanz, V.; Pederzoli, C.D.; Lamers, M.L.; Dutra-Filho, C.S. and Wyse, A.T. (2009). Homocysteine induces oxidative stress, inflammatory infiltration, fibrosis and reduces glycogen/glycoprotein content in liver of rats. *International Journal of Developmental Neuroscience*, 27(4): 337-344.
- Micovic, Z.; Stamenkovic, A.; Nikolic, T.; Stojanovic, M.; Scepanovic, L.; Hadzibegovic, A. and Jakovljevic, B.

- (2016). The effects of subchronic methionine overload administered alone or simultaneously with L-cysteine or N-acetyl-L-cysteine on body weight, homocysteine levels and biochemical parameters in the blood of male wistar rats. *Serbian Journal of Experimental and Clinical Research*, 17(3): 215-224.
- Mimic-Oka, J.; Simic, T.; Pljesa, M.; Stupar, N. and Turkovic, S. (2001). Oxidative modifications of plasma proteins in deferent stages of chronic renal failure. *Sci. Med. Bio.*, 8(5): 1-5.
- Rondal, J. (2002). Orchestrating the unfolded protein response in health and disease. *J. Clin. Invest.*, 110: 1389-1398.
- Salman, A.D. (2014). The Protective role of Pomegranate seed oil (Pometon) on kidney (functional and structural) damage induced by Methionine overload in adult female Rabbits. *The Iraqi Journal of Veterinary Medicine* (ISSN-P:1609-5693 ISSN-E: 2410- 7409), 38(2): 66-73.
- Sans, A.; Caro, P.; Ayala, V.; Portero- Otin, M and pamplana, R. (2006). Methionine restriction decrease mitochondrial oxygen radical generation and leak as well as oxidative damage to mitochondrial DNA and proteins *FASEB. J.*, 20(8): 1064- 1073.
- Solini, A.; Sanline, E. and Ferrannim, G. (2006). Effect of solvent lesion folic acid supplementation on insulin sensitivity and inflammatory markers in over weight subjects. *Int. J.*, 30(8): 1197-1202.
- Stadman, E. (1998). Reactive oxygen mediated protein oxidation in aging and disease. *Drug/Metab. Rev.*, 30: 225-243.
- Tiikkinen, N.; Hakkinen, A.; Karsheninnikova, E.; Nyman, T. Makimattia, S. and Ki- Jarvinen, H. (2004). Effect of rasiglitazone and metformin on liver fat content hepatic insulin resistance clearance and gene expression adipose tissue in patients with type 2 diabetes , *Diabetes.*, 53: 2169-2176.
- Vijayakumar, A.; Kim, E.K.; Kim, H.; Choi, Y.J.; Huh, K.B. and Chang, N. (2017). Effects of folic acid supplementation on serum homocysteine levels, lipid profiles, and vascular parameter in post-menopausal Korean women with type 2 diabetes mellitus. *Nutrition research and practice*, 11(4): 327- 333.
- Villa, P.; Perri, C.; Suriano, R.; Cueinelli, S.; Panunzi, S.; Ranieri, M.; Mele, C. and Lanzona, A. (2004). Folic acid supplementation in healthy menopausal women. Effect on Homocysteineand glycolipid metabolism. *J. of clin. Endocrinol. and Metabolism.*, 90(8): 4622- 4629.
- Waly, M.I.; Al-Farsi, Y.M.; Al-Sharbati, M.M.; Ali, A.; Al-Khalili, M.M.; Essa, M.M. and Al-Shafae, M.S. (2011). Low serum levels of glutathione, homocysteine and total antioxidant capacity are associated with increased risk of autism in Oman.