

Full Length Research Paper

Effect of aspartame on some oxidative stress parameters in liver and kidney of rats

Iman, M. Mourad

Department of Zoology, Faculty of Science, Cairo University, Egypt. E-mail: yaramorad@yahoo.com

Accepted 11 May, 2011

Aspartame is one of the most widely used artificial sweeteners in over 90 countries worldwide. It is a highly intensity sweetener added to a large variety of food, most commonly found in low calorie beverages, desserts and table top sweeteners added to tea or coffee. The present study examined whether the daily oral administration of ASP (40 mg/kg) for 2, 4 and 6 weeks induce oxidative stress in the liver and kidney of male albino rats. Lipid peroxidation (LPO), glutathione reduced (GSH) levels as well as the activities of superoxide dismutase (SOD), glutathione-S-transferase (GST) and catalase (CAT) enzymes were determined. A significant increase in LPO levels was obtained in the liver tissue after 4 and 6 weeks of ASP administration while there was a significant decrease in LPO level after 2 weeks followed by a significant increase in the renal tissue at the end of the 6 weeks. SOD activity significantly decreased in the liver tissue after 2, 4 and 6 weeks of treatment. Also, there was a significant decrease in SOD activity after 2 and 4 weeks in the renal tissue. CAT activity significantly decreased in the liver tissue after 2 and 4 weeks of ASP administration. Regarding to GSH content, there was a significant decrease in the liver tissue after 2, 4 and 6 weeks which was accompanied by a significant increase in GST activity after 4 and 6 weeks of ASP administration. In conclusion, ASP may induce an oxidative stress in the liver and kidney of male albino rats.

Key words: Aspartame, oxidative stress, liver, kidney.

INTRODUCTION

Aspartame (L- aspartyl- L-phenylalanine methyl ester) is one of the most widely used artificial sweeteners in over 90 countries worldwide in over 6000 products (Magnuson et al., 2007). It is a highly intensity sweetener added to a large variety of food, most commonly found in low calorie beverages, desserts and table top sweeteners added to tea or coffee (Butchko and Stargel, 2001; Oyama et al., 2002).

Its peptide nature, aspartyl-phenylalanine methyl ester facilitates its intestinal hydrolysis and absorption of innocuous amino acids together with small amounts of free methanol (Burgert et al., 1991; Lipton et al., 1991). Phenylalanine and aspartic acid are both amino acids which are found in natural proteins and under normal circumstances are beneficial, if not essential, for health (Woodrow, 1984). It has been reported that aspartame consumption was linked with neurological and behavioral disturbances (Humphries et al., 2008), but most of the results yielded negative or inconclusive correlations (Spiers et al., 1998; Magnuson et al., 2007).

On the other hand, a relatively small amount of aspartame can significantly increase methanol levels (Davoli, 1986). Accidental or suicidal ingestion can cause severe metabolic acidosis and clinical disturbances such as blindness, serious neurologic sequelae and death (Kuteifan et al., 1998; Liu et al., 1998). Methanol is being increasingly recognized as a substance that damages the liver cells where it is oxidized to formaldehyde and latter to formate (Trocho et al., 1998). These processes are accompanied by elevation of NADH level and the formation of superoxide anion which may be involved in lipid peroxidation (Poli, 1993; Parthasarathy et al., 2006). Also, methanol intoxication is associated with mitochondrial damage and increased microsomal proliferation resulting in increased production of oxygen radicals (Liesivuori and Savolainen, 1991; Castro et al., 2002). These factors together with the excess of formaldehyde, formed during acute methanol intoxication cause significant increase in lipid peroxidation (Parthasarathy et al., 2006).

Oxidative stress is a state where oxidative forces exceed the antioxidant systems due to loss of the balance between them. It occurs when the generation of reactive oxygen species (ROS) in a system exceeds the body's ability to neutralize and eliminate them thus creating an imbalance or overabundance of free radicals (Naito and Yoshikawa, 2002). So, the present study was designed to investigate the lipid peroxidation and antioxidant status in liver and kidney of male albino rats after oral administration of 40 mg/ kg body weight of aspartame.

MATERIALS AND METHODS

Animals

Adult male Wistar albino rats weighing 120 to 180 g were used as experimental animals. The animals were obtained from animal house of the National Research Center (Cairo, Egypt). They were maintained on stock diet and kept under fixed appropriate conditions of housing and handling. All experiments were carried out in accordance with the research protocols established by the Animal Care Committee of the National Research Center (Cairo, Egypt), which followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Chemicals

Pure aspartame (ASP) powder was purchased from ADWIA Co., Cairo, Egypt. Phosphate buffer and reagent kits were purchased from Bio-diagnostic Company, Giza, Egypt.

Experimental design

The animals were divided into 4 groups. Animals of group (1) served as control and were administered orally distilled water at each time segment of the experiment. Animals of groups (2), (3) and (4) were daily administered orally ASP (40 mg/kg) (JECFA, 1980) dissolved in distilled water for 2, 4 and 6 weeks, respectively.

Handling of tissue samples

At the end of the experimental periods (2, 4 and 6 weeks) the animals were sacrificed after being fasted. The liver and kidney of each animal was quickly removed and rapidly weighed and frozen until analyzed. The liver is homogenized in 10 ml and kidney in 5 ml of ice cold phosphate buffer (50 mM pH 7.4, 0.1% tritonX and 0.5 mM EDTA). The homogenates were centrifuged at 1753 g for 15 min at 4°C using a high speed cooling centrifuge (Type 3K-30, Sigma, Osterode-am-Harz, Germany). The clear supernatants were separated and used for analysis.

Determination of lipid peroxidation, glutathione reduced levels:

Lipid peroxidation (LPO) levels were determined by using Biodiagnostic kit. No. MD 25 29 which is based on the spectrophotometric method of Ohkawa et al. (1979), in which the malondialdehyde (MDA) release served as the index of LPO. MDA

was determined by measuring the thiobarbituric acid reactive species. The absorbance of the resultant pink product was measured at 534 nm in a helios alpha thermospectronic (UVA 111615, Cambridge, UK). The assay of glutathione reduced levels was performed using Biodiagnostic kit No. GR 25 11 which is based on the spectrophotometric method of Beutler et al. (1963). It depends on the reduction of 5,5'-dithiobis 2-nitrobenzoic acid with glutathione to produce a yellow color whose absorbance is measured at 405 nm.

Determination of enzyme activities

Superoxide dismutase activity was assayed by using Biodiagnostic kit No. SD 25 21. This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitro blue tetrazolium dye (Nishikimi et al., 1972). The change in absorbance at 560 nm. Over 5 min. was measured. Glutathione-S-transferase activity was determined according to the method of Habig et al. (1974). 0.4 ml potassium phosphate buffer (50 mmol/l; pH 6.5), 0.1 ml of supernatant, 1.2 ml water and 0.1 ml CDNB (1-chloro-2, 4 dinitrobenzene, 30 mmol/l) were added and incubated in a water bath at 37°C for 10 min. After incubation, 0.1 ml of reduced glutathione (30 mmol/l) was added. The change in absorbance was measured at 340 nm at one min interval. Catalase activity was measured using Biodiagnostic Kit No. CA 25 17 which is based on the spectrophotometric method described by Aebi (1984). Catalase reacts with a known quantity of hydrogen peroxide and the reaction is stopped after 1 min with catalase inhibitor. In the presence of peroxidase, the remaining hydrogen peroxide reacts with 3,5-Dichloro-2- hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the sample. The absorbance was measured at 510 nm.

Statistical analysis

The data were expressed as means \pm standard error of the mean (S.E.M.) All variables were tested for normal distribution using t test ($P < 0.05$) and one way analysis of variance (ANOVA). If the groups showed significant differences, Tukey's multiple comparison test was performed.

RESULTS

The present study showed the effect of oral administration of 40 mg/kg body weight of ASP on liver and kidney of male albino rats at different three time periods. The data represented that LPO level increased significantly in the liver tissue after four and six weeks of ASP administration when compared to the control group. Also, there was a significant ($P < 0.05$) increase in LPO level after four and six weeks comparing with two weeks of ASP administration. On the other hand, LPO level in the renal tissue showed a significant decrease after two weeks followed by a significant increase in LPO level at the end of six weeks of ASP administration as compared to the control. Furthermore, there was a significant increase in LPO level after the 4th and 6th weeks compared with the 2nd week.

Regarding to SOD activity, Table 1 indicated that there was a significant decrease in SOD activity in the liver

Table 1. Effect of oral administration of aspartame (40mg/kg) on some oxidative stress parameters in the liver of male albino rats at different time periods.

Parameter	Control	Time period		
		2 weeks	4 weeks	6 weeks
MDA(nmol/g tissue)	59.38±0.65	53.08±1.36	70.34±1.53 ^a	76.84±2.51 ^a
SOD(U/mg tissue)	2.90±0.11	2.31±0.22 [*]	2.34±0.07 [*]	2.27±0.10 [*]
CAT(U/g tissue)	9.26±0.78	5.31±0.25 [*]	5.93±0.87 [*]	7.39±0.65
GST(U/g tissue)	14.10±0.71	24.02±1.39 [*]	24.69±1.44 [*]	25.27±1.28 [*]
GSH(mg/g tissue)	97.75±2.16	100.57±3.04	73.59±5.96 ^a	64.49±5.26 ^a

Data are expressed as Mean ± S.E, number of animals in each group is five, P> 0.05 non significant, * P < 0.05 significant, the symbol represent statistical significance; * all treated period with the control, ^a 4 and 6 weeks with 2 week; ^b 6 week with 4 week.

Table 2. Effect of oral administration of aspartame (40mg/kg) on some oxidative stress parameters in the kidney of male albino rats at different time periods.

Parameter	Control	Time period		
		2 week	4 week	6 week
MDA(nmol/g tissue)	56.04±0.60	47.48±0.96 [*]	62.06±1.56 ^a	63.04±2.75 ^a
SOD(U/mg tissue)	3.58±0.05	2.60±0.22 [*]	3.07±0.05 [*]	3.24±0.09 ^a
CAT(U/g tissue)	8.54±0.67	8.47±0.93	8.07±0.42	7.74±0.47
GST(U/g tissue)	24.45±0.32	21.40±0.93	23.31±0.88	23.71±1.08
GSH(mg/g tissue)	92.96±2.25	95.47±1.64	92.62±5.85	75.57±1.19 ^{ab}

Data are expressed as Mean ± S.E, number of animals in each group is five, P> 0.05 non significant, * P < 0.05 significant, the symbol represent statistical significance; *all treated period with the control, ^a 4 and 6 weeks with 2 week; ^b 6 week with 4 week.

tissue after two, four and six weeks of ASP administration compared with the control group and there was a significant decrease in the renal SOD activity after two and four weeks of treatment as indicated in Table 2. However, the renal SOD activity significantly increased at the end of the 6th week in comparison to the 2nd week of ASP treatment. The results also showed that there was a significant decrease in CAT activity in the liver after two and four weeks of ASP treatment compared with control rats. On the other hand, there was a non significant decrease in the CAT activity in the renal tissue after the different three times periods of ASP administration when compared with the control group.

The data in Table 1 represented that the oral administration of ASP caused a significant increase in GST activity in the liver tissue after two, four and six weeks of treatment compared with the control animals. However, there was a non significant effect of ASP on GST activity in the renal tissue as regarded in Table 2. Comparing with the control group, GSH content significantly decreased in the liver tissue after the 4th and 6th weeks of treatment. Moreover, there was a significant (P< 0.05) decrease in the 4th and 6th week when compared with the 2nd week. In the renal tissue, GSH content was significantly decreased at the end of the 6th week of

treatment when compared with control and two weeks.

DISCUSSION

ASP has been approved as a sweetener for liquid carbonated beverages. It has had a wide acceptance as an additive in many dry food applications. However, its consumption was associated with many complaints include visual impairment, ear buzzing, high SGOT, loss of equilibrium, severe muscle aches, pancreatitis, episodes of high blood pressure and depression (Woodrow, 1984).

It was suggested that ASP was metabolized in the gastrointestinal tract into aspartic acid, phenylalanine and methanol. On weight basis, metabolism of ASP generates approximately 50% phenylalanine, 40% aspartic acid and 10% methanol (Karim and Burns, 1996; Stegink and Filer, 1996). Aspartic acid is mostly eliminated through the lungs in the form of CO₂. In humans, plasma level of aspartic acid does not increase significantly following oral administration of aspartame at a dose of 34 mg/ kg (Stegink, 1984). However, some of the phenylalanine formed in the intestine following ingestion of ASP is excreted in the form of CO₂, most of it

is incorporated into the pool of amino acids and contributes to protein synthesis (Stegink and Filer, 1996; Trefz and Bickel, 1996). Methanol is primarily metabolized by oxidation to formaldehyde and then to formate, these processes are accompanied by the formation of superoxide anion and hydrogen peroxide (Parthasarathy et al., 2006).

The present study represented that, the oral administration of ASP (40 mg/ kg) led to a significant elevation in LPO level in the liver tissue after four and six weeks of treatment. Also, there was a significant increase in LPO level in the renal tissue at the end of the six week of treatment. LPO is an auto catalytic mechanism leading to oxidative destruction of cellular membranes (Cheese- man, 1993). It is initiated by the abstraction of a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane (Bergendi et al., 1999). These latter compounds then decompose to form a wide variety of products in particular malonaldehyde (MDA) (Zeyuan et al., 1998). LPO in cellular membranes damages polyunsaturated fatty acids tending to reduce membrane fluidity which is essential for proper functioning of the cell. So, the increase in MDA level observed in this study, which is an index of LPO, indicated liver and kidney cell membrane damage after ASP administration. This is in accordance with Parthasarathy et al. (2006) who investigated that methanol administration significantly increased MDA level in the lymphoid organs; also, Zararsiz et al. (2007) recorded a significant increase in MDA level in the kidney of rats after treatment with formaldehyde.

The present work also showed that the increase in MDA level was accompanied by a concomitant decrease in the activities of antioxidant enzymes, SOD and CAT in the liver after the 2nd, 4th and 6th week and in the renal tissue SOD activity was significantly decreased after the 2nd and 4th week of ASP administration. SOD constitutes an important link in the biological defense mechanism through dismutation of endogenous cytotoxic superoxide radicals to H₂O₂ and O₂ which are deleterious to polyunsaturated fatty acids and proteins (Fridovich, 1975). Also, in the presence of inadequate CAT activity to degrade H₂O₂, more H₂O₂ could be converted to toxic hydroxyl radicals that may contribute to oxidative stress due to methanol metabolite from ASP. This is in agreement with Parthasarathy et al. (2006) who recorded that methanol administration caused a decrease in the enzymatic antioxidant (SOD and CAT) in the lymphoid organs. Furthermore, the decrease in SOD and CAT activities may be due to the formation of formaldehyde from the methanol. This is in accordance with Gulec et al. (2006) who indicated that formaldehyde exposure led to a decrease in SOD and CAT activities in the liver tissue compared to the control. Also, Chang and Xu (2006) recorded a decrease in SOD activity and there was a dose- response relationship between formaldehyde concentration and SOD activity. Zararsiz et al. (2007) demonstrated that the renal tissue activity of

SOD was significantly decreased in rats treated with formaldehyde when compared with the control animals.

Glutathione-S-transferases constitute a family of enzymes involving in the detoxification of xenobiotics, signalling cascades and serving as ligandins or/and catalyzing the conjugation of various chemicals and drugs (Tuna et al., 2010). The present study also indicated that there was a significant increase in GST activity in the liver tissue after the different three time periods of treatment. This increase could be a response of the organ to the oxidative stress induced by the methanol metabolized from ASP and possibly the induction of enzyme synthesis by ASP. However, this increase in GST activity was accompanied by a depletion in GSH content in the liver tissue after four and six weeks of treatment. Since glutathione is an endogenous substance that protect cell suffering from oxidative stress, GSH can function as antioxidant by catalyzing the reduction of H₂O₂ to water (Abuja and Albertini, 2001); it can react chemically with singlet oxygen, superoxides and hydroxyl radicals (Singh et al., 2003; Hashimoto et al., 2008). It also be able to attack electrophilic centers and thus protect proteins, lipids and nucleic acids from the attack of electrophilic compounds which are capable of reacting with their SH- groups (Hayes et al.,1991; Ahluwalia et al., 1996). So the decrease in cellular glutathione content increases cell vulnerability to oxidative stress (Meister and Anderson, 1983; Oyama et al., 2002). The decrease in GSH activity observed in the present study seems to have been caused by methanol, because methanol metabolism depends upon GSH (Pankow and Jagielki, 1993). In addition, a decrease in GSH content would also be caused by its rapid reaction with the highly reactive compound, formaldehyde, which is generated during methanol metabolism forming nucleophilic adducts and/ or LPO products (Shrzylewska and Farbisewski, 1996; Parthasarathy et al.,2006). Therefore, the present study concluded that ASP administration at a dose level of 40 mg/kg may induce an oxidative stress in the liver and kidney of male albino rats and the liver tissue is more sensitive and rapidly affected than renal tissue.

REFERENCES

- Abuja PM, Albertini R (2001). Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. *Clin. Chim. Acta*, 306: 1-17.
- Aebi H (1984). Catalase *in vitro*. *Methods Enzymol.*, 105: 121-126.
- Ahluwalia P, Tewari K, Choudhary P (1996). Studies on the effects of monosodium glutamate (MSG) on oxidative stress in erythrocytes of adult male mice. *Toxicol. Lett.*, 84: 161-165.
- Bergendi L, Benes L, Durackova Z, Ferencik (1999). *Chemistry, Physiology and Pathology of free radicals. Life Sci.*, 65: 1865- 1874.
- Beutler E, Duron O, Kelly BM (1963). Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.*, 61:882-888.
- Burgert SL, Andersen DW, Stegink KD, Takeuchi H, Schedl HP (1991). *Metabolism*, 40: 612- 618.
- Butchko HH, Stargel WW (2001). Aspartame: scientific evaluation in the post marketing period. *Regul. Toxicol. Pharmacol.*, 34(3): 221-233.

- Castro GD, Costantini MH, Delgado de layno AM, Castro A (2002). Rat liver microsomal and nuclear activation of methanol to hydroxyl methyl free radicals. *Toxicol. Lett.*, 129(3): 227- 236.
- Chang JR, Xu DQ (2006). Effects of formaldehyde on the activity of superoxide dismutase and glutathione peroxidase and the concentration of malondialdehyde. *Wei Sheng Yan Jiu. Sept.*, 35(5): 653- 655.
- Cheese-man KH (1993). Mechanisms and effects of lipid peroxidation. *Mol. Aspects Med.* 14(3): 191-197.
- Davoli E (1986). Serum methanol concentrations in rats and in men after a single dose of aspartame. *Food Chem. Toxicol.*, 24: 187- 189.
- Fridovich I (1975). Superoxide dismutase. *Ann. Rev. Biochem.*, 44: 147-159.
- Gulec M, Gurel A, Armutcu F (2006). Vitamin E protects against oxidative damage caused by a formaldehyde in the liver and plasma of rats. *Mol. Cell Biochem.*, 290(1-2): 61-67.
- Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, 249: 7130-7139.
- Hashimoto K, Takasaki W, Yamoto T, Manabe SI, Tsuda S (2008). Effect of glutathione (GSH) depletion on DNA damage and blood chemistry in aged and young rats. *J. Toxicol. Sci.*, 33 (4): 421 – 429.
- Hayes PC, Bouchier IAD, Beckett GJ (1991). Glutathione – S – transferase in humans in health and disease. *Gut*, 32: 813- 818.
- Humphries P, Pretorius E, Naude H (2008). Direct and indirect cellular effects of aspartame on the brain. *Eur. J. Clin. Nutr.*, 62: 451- 462.
- JECFA (1980). Toxicological evaluation of certain food additives: aspartame, WHO food additives series No 15, Report series, p. 653.
- Karim A, Burns T (1996). Metabolism and pharmacokinetics of radio labeled aspartame in normal subjects. In: Tschanz C, Butchko HH, Stargel WW, Kotsonis FN(eds) *The clinical evaluation of a food additive. Assessment of aspartame.* Boca Raton, New York, London, Tokyo: CRC, pp. 57-66.
- Kuteifan K, Oesterle H, Tajahmady T, Gutbub AM, Laplatte G (1998). Necrosis and haemorrhage of the putamen in methanol poisoning shown on MRI. *Neuroradiology*, 40: 158- 160.
- Liesivuori J, Savolainen H (1991). Methanol and formic acid toxicity: biochemical mechanism. *Pharmacol. Toxicol.*, 69: 157- 163.
- Lipton WE, Li YN, Younoszai MK, Stegink LD (1991). Metabolism. 40: 1337-1345.
- Liu JJ, Daya MR, Carrasquillo O, Kales NS (1998). Prognostic factors on patients with methanol poisoning. *J. Toxicol. Clin. Toxicol.* 36: 175-181.
- Magnuson BA, Burdock GA, Doull J, Kroes RM, Marsh GM, Pariza MW, Spencer PS, Waddell WJ, Walker R, Williams GM (2007). Aspartame: a safety evaluation based on current use levels, regulations, and toxicological and epidemiological studies. *Crit. Rev. Toxicol.*, 37(8): 629-727.
- Meister A, Anderson S (1983). Glutathione . *Ann. Rev. Biochem.*, 52: 711-760.
- Naito Y, Yoshikawa T (2002). What is oxidative stress? *JMAJ*, 45(7): 271-276.
- Nishikimi M, Appaji N, Yagi K (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Biophys. Res. Commun.*, 46: 849-854.
- Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, 95: 351-358.
- Oyama Y, Sakai H, Arata T, Okano Y, Akaike N, Sakai K, Noda K (2002). Cytotoxic effects of methanol, formaldehyde and formate on dissociated rat thymocytes: a possibility of aspartame toxicity. *Cell Biol. Toxicol.*, 18(1): 43-50.
- Pankow D, Jagielki S (1993). Effect of methanol on modifications of hepatic glutathione concentration on the metabolism of dichloromethane to carbon monoxide in rats. *Hum. Exp. Toxicol.*, 12: 227-231.
- Parthasarathy JN, Ramasundaram SK, Sundaramahalingam M, Pathinasamy SD (2006). Methanol induced oxidative stress in rat lymphoid organs. *J. Occup. Health*, 48: 20-27.
- Poli G (1993). Liver damage due to free radicals. *Br. Med. Bull.*, 49: 604- 609.
- Shrzylewska E, Farbisewski R (1996). Diminished antioxidant defense potential of liver, erythrocyte and serum from rats with subacute methanol intoxication. *Vet. Hum. Toxicol.*, 38: 429- 433.
- Singh P, Mann KA, Mangat HK, Kaur G (2003). Prolonged glutamate excitotoxicity: Effects of mitochondrial antioxidants and antioxidant enzymes. *Mol. Cell Biochem.*, 243 (1-2): 139-145.
- Spiers PA, Sabounjian L, Reiner A, Myers DK, Wurtman J, Schomer DL (1998). Aspartame: neuropsychologic and neurophysiologic evaluation of acute and chronic effects. *Am. J. Clin. Nutr.*, 68(3): 531- 537.
- Stegink LD (1984). Aspartame metabolism in humans: acute dosing studies. In: Stegink L, Filer, L(edts) *Aspartame: Physiology and biochemistry*, New- York, Marcel Dekker, pp. 509-553.
- Stegink LD, Filer LJ (1996). Effects of aspartame ingestion on plasma aspartate, phenylalanine and methanol concentrations in normal adults. In: Tschanz C, Butchko HH, Stargel WW, Kotsonis FN (eds). *The clinical evaluation of a food additive. Assessment of aspartame.* Boca Raton, New York, London, Tokyo: CRC Press, pp. 67-86.
- Trefz FK, Bickel H (1996). Tolerance in PKU heterozygotes. In: Tschanz C, Butchko HH, Starge, WW, Kotsonis FN (eds). *The clinical evaluation of a food additive. Assessment of aspartame*, pp. 149-160.
- Trocho C, Pardo R, Rafecas I, Virgili J, Remesar X, Fernandez- Lopez JA, Alemany M (1998). Formaldehyde derived from dietary aspartame binds to tissue components in vivo. *Life Sci.*, 63(5): 337-349.
- Tuna G, Erkmen GK, Dalmizrak O, Dogan A, Ogus IH, Ozer N.(2010). Inhibition characteristics of hypericin on rat small intestine glutathione- S- transferase. *Chem. Biol. Interact*, 188(1): 59-65.
- Woodrow C (1984). Monte and methanol. *J. Appl. Nutr.*, 36(1): 1-15.
- Zararsiz I, Sarsilmaz M, Tas U, Kus I, Meydan S, Ozan E (2007). Protective effect of melatonin against formaldehyde- induced kidney damage in rats. *Toxicol. Ind. Health*, 23(10): 573-579.
- Zeyuan D, Bingyin T, Xiaolin L, Jinming H, Yifeng C (1998). Effect of green tea and black tea on the blood glucose, the blood triglycerides and antioxidant in aged rats. *J. Agric. Food Chem.*, 46(10): 3875-3878.