Biosciences, Biotechnology Research Asia Vol. **6**(2), 659-664 (2009)

Effect of *Achyranthes aspera* (Linn) seeds on redox and oxidative status in plasma and selected tissues of rats fed with high doses of fructose

T. MALARVILI* and N. GOMATHI

Department of Biochemistry, J.J. College of Arts and Science, Pudukkottai - 622 422 (India).

(Received: August 01, 2009; Accepted: September 11, 2009)

ABSTRACT

Oxidative stress plays an important role as a mediator of damage produced by fructose. The present study reports the effects of *A. aspera (Linn)* on the liver, kidney lipid peroxidation and antioxidant defence system of rats that are fed with high fructose diet. Male Wistar rats of body weight 100-120g were divided into four groups containing six rats each. The rats were divided into four groups as Group I (control), Group II (Fructose fed rats), Group III (Fructose fed treated with *A. aspera*) and Group IV (control treated with *A. aspera*). Control animals received the control diet containing starch while fructose-fed animals received a fructose-enriched diet (> 60% of total calories). Fructose fed rats showed increased lipid peroxidation (TBARS and conjugated dienes) and impaired antioxidant status (SOD, CAT, Cyt 450, NADPH, XO, GSH, GPX). *A. aspera* is well documented for the presence of phytoactive constituents. Reduction in rate of lipid peroxidation and enhancement in free radical scavenging activity of the herbal seed powder is due to presence of phytoactive constituents.

Key words: HFF: High fructose fed rats, *Achyranthes aspera.*

INTRODUCTION

Feeding rats with high fructose diet (> 60% of total calories) affects both glucose and lipid metabolism which result in a cluster of metabolic abnormalities such as glucose intolerance, hypertension[1]. Fructose feeding is also reported to facilitate oxidative damage and has deleterious effects both due to reduction in antioxidant defence and enhanced free radical production[2]). Oxidative damage inflicted by Reactive oxygen species (ROS) can arise both from increased production of free radicals and from reduction of antioxidants defenses[3]. Free radicals may react with lipid and carbohydrate moieties of lipoproteins and glycoproteins forming products such as hydroxy nonenal[4]. Under some circumstances, they are reported to play an important role in destruction of cell membranes, proteins and nucleic acids, Free radicals first react with lipid component of cell membrane inducing lipid peroxidation and its increased membrane permeability leading to cell lysis or death. Thus antioxidant status reflects the dynamic balance between the antioxidant defense and pro-oxidant conditions and has been suggested as a useful tool in estimating the risk of oxidative damage. The methanolic whole plant extract of the *A. aspera* was already reported to possesses hypolipidemic and antioxidant potential activity in high fat diet fed rabbits[5]. *A. aspera* extract also decreased the hepatic lipid peroxidation, without increasing the antioxidant enzymes, suggesting the direct free radical scavenging action of the plant extract, as observed in other plants[6]. In the present paper, we report the effects of *A. aspera* seeds on oxidant-antioxidant balance in Liver and kidney of fructose fed rats.

MATERIAL AND METHODS

Animals

Healthy female adult albino rats (Wistar strain) 6-7 weeks old, weighing 100-120g was procured from “Sri Venkateswara Enterprises”, Bangalore, India. They were housed in clean sterile polypropylene cages with proper aeration and lighting (12 ± 1 hr day / night rhythm) throughout the experimental period. During the course of the

experiments, the temperature was maintained between 27ºC ± 2ºC. The animals were fed with commercially available pelleted rat feed (Gold-Mohur, M/S Hindustan Lever Ltd, Mumbai, India) during the acclimatization period and water *ad libitum*. The usage and handling of experimental rats was done by following the rules and regulations given by the Institutional Ethics Committee.

Chemicals

Fructose, bovine serum albumin, glucose-6-phosphate, γ-glutamyl paranitroaniline, nicotinamide adenine dinucleotide (NAD^+, NADH) nicotinamide adenine dinucleotide phosphate ($NADP^+$, NADPH), reduced glutathione, oxidized glutathione, adenosine triphosphate (ATP), adenosine monophosphate (AMP) thiobarbituric acid (TBA) and 1,2,4-aminonapthol sulphonic acid were obtained from Sigma Chemical Company, ST. Louis, MO, USA. All other chemicals and reagents used were of highest purity and of analytical grade marketed by Glaxo Laboratories, Mumbai, SD Fine Chemicals, Mumbai and Sisco Research Laboratories, Pvt. Ltd., India.

Experimental Design

The rats were divided into four groups, each group consisting of six animals.

Group I : Normal control rats.

Group II : Fructose fed rats (>60% fructose for 10weeks).

Group III : Fructose fed rats treated with the crude powder of *A. aspera* seeds (100mg kg^{-1} body weight) twice daily for a period of 3 weeks.

Group IV : Control rats treated with the crude powder of *A. aspera* seeds (100mg kg^{-1} body weight) for a period of three weeks.

Collection of Samples

At the end of the three weeks the rats were fasted overnight and killed by cervical decapitation under mild ether anesthesia. Blood was collected in heparin rinsed tubes to separate the plasma. Blood collected in another set of test tubes without anticoagulant was used to separate the serum. Liver, kidney was perfused *in situ* with cold 0.15M NaCl at 37ºC.

Analytical method

The level of lipid peroxidation in liver, kidney was studied by measuring the Thiobarbituric acid-reactive substances (TBARS) in the liver, kidney homogenate by the method of Niehaus and Samuelsson (7), Conjugated dienes was measured by the method of Rao and Recknagel[8], cytochrome P_{450} was measured by the method of Omura and Sato[9]. NADPH oxidase (NADPH; EC 1.6.99.1) was measured by the method of Styne-Parve and Benert[10], Xanthine oxidase (XO; EC.1.1.3.22) was measured by the method of Stripe and Della corte[11]. Reduced glutathione (GSH) levels were determined by the method of Ellman[12] , Superoxide dismutase (SOD; EC 1.15.1.1) was assayed by the method of Kakkar *et al.*, [13] Catalase (CAT; EC 1.11.1.6) was estimated by that of Sinha[14], and Glutathione peroxidase (GPx; EC 1.11.1.9) by that of Rotruck *et al.,*[15].

Statistical analysis

Values or mean ± SD for six rats in the each group and statistically significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by Tukey's test for multiple comparison values of $p<0.05$ was considered to be significant. Statistical Package for Social Studies (SPSS) 7.5 version was used for this analysis.

RESULTS

Table 1 & 2, shows the level of lipid peroxidation as TBARS, Conjugated dienes in liver, kidney of control and experimental rats. In the homogenates of liver, kidney the level of lipid peroxides was found significantly ($p<0.001$) increased in group II high fructose fed rats when compared with group I control rats. After administration of *A. aspera* a significant decrease in the level of lipid peroxidation TBARS, Conjugated dienes ($p<0.001$) in liver, kidney of the group III and IV rats were observed when compared with group II high fructose fed rats.

Table 1 and 2 depicts the activity of cytochrome P_{450} in liver, kidney of the control and experimental rats. Rats fed with high fructose diet showed a significant ($p<0.001$) increase in the activity of Cyt P_{450} in liver, kidney when compared

with Group I control rats. Quite interestingly, administration of *A. aspera* to group III, IV rats had significantly ($p<0.001$) decreased the activity of Cyt P_{450} when compared with group II high fructose fed rats.

Table 1 and 2, Shows the activities of NADPH(O), Xanthine oxidase (XO), Reduced glutathione (GSH) in liver, kidney of control and experimental rats. The activities of NADPH(O), XO, GSH in liver, kidney homogenate of group II high fructose fed rats showed a significant ($p<0.001$) increase when compared with group I control rats. On the other hand, group III, IV rats, which received the oral administration of *A. aspera* showed a significant ($p<0.001$) decrease in the activities of liver, kidney NADPH(O), XO, GSH when compared with group II high fructose diet fed rats.

Activity of superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) in the liver, kidney and Reduced glutathione (GSH plasma), of control and experimental rats are given in table 1 and 2. In high fructose fed, group II rats, a significant ($p<0.001$) decrease in the activity of SOD, CAT, GPx, in the homogenates of liver, kidney

Table 1: Activities of redox and enzymatic antoioxidants in liver of control and experimental animals

Groups	I (Normal Control)	II (HFF Control)	III (HFF + AAS)	IV (control + AAS)
TBARS(µmol/mg protein)	1.402 ± 0.139	2.407 ± 0.231^{a***}	1.549±0.152 ^{b***}	1.486± 0.139 ^{b***}
CD (A 233/215)	0.67 ± 0.07	0.86 ± 0.08 ^{a***}	0.68±0.06 ^{b***}	0.69±0.06 ^{b***}
NADPH (O)(mmol/min/mg protein)	3.265 ± 0.31	6.86 ± 0.68 ^{a***}	3.21±0.31 ^{b***}	3.15±0.31 ^{b***}
XO (mmol/min/mg protein)	1.67 ± 0.17	3.86 ± 0.38 ^{a***}	1.68±0.16 ^{b***}	1.69±0.16 ^{b***}
Cyt P_{450} (mmol/mg protein)	0.156 ± 0.02	0.42 ± 0.04 ^{a***}	0.16±0.01 ^{b***}	0.17±0.01 ^{b***}
SOD (Units/mg protein)	3.94 ± 0.39	2.47 ± 0.236 ^{a***}	3.89 ± 0.37 ^{b**}	3.85 ± 0.37 ^{b***}
CAT (µmol/min/mg protein)	54.7 ± 5.48	36.23 ± 3.61 ^{a***}	53.99 ± 5.39 ^{b***}	53.52 ±5.42 ^{b***}
GSH (mg/gm protein)	0.480 ± 0.020	0.093±0.003 ^{a***}	0.426±0.034 ^{b***}	0.438 ± 0.041 ^{b***}
GSH Plasma (mg/dL)	43.01 ± 4.21	22.36 ± 2.16 ^{a***}	43.52 ± 4.23 ^{b***}	44.72 ± 4.46 ^{b***}
GPx (µmol/min/mg protein)	6.19 ± 0.61	4.71 ± 0.46 ^{a***}	6.10 ± 0.61 ^{b***}	6.03 ± 0.59 ^{b***}

Values are expressed as mean ±SD for six animals in each group.
Superscript letters represent $p<0.05$ (Tukey's Test).
One Unit/mg protein is 50% inhibition in the reduction of NBT min^{-1} mg $protein^{-1}$
^{a}As compared with Group I, ^{b}As compared with Group II.$^{***}p<0.001$.

Table 2: Activities of redox and enzymatic antoioxidants in kidney of control and experimental animals

Groups	I (Normal Control)	II (HFF Control)	III (HFF + AAS)	IV (control + AAS)
TTBARS(µmol/mg protein)	1.687 ± 0.091	2.439±0.236 ^{a***}	1.761±0.176 ^{b***}	1.723 ± 0.165 ^{b***}
CD (A 233/215)	0.64 ± 0.06	0.83 ± 0.08 ^{a***}	0.65±0.06 ^{b***}	0.68±0.06 ^{b***}
NADPH (O)(mmol/min/mg protein)	3.66 ± 0.35	7.12 ± 0.69 ^{a***}	3.57±0.346 ^{b***}	3.52±0.35 ^{b***}
XO (mmol/min/mg protein)	1.72 ± 0.17	3.99 ± 0.39 ^{a***}	1.71±0.17 ^{b***}	1.7±0.16 ^{b***}
Cyt P_{450} (mmol/mg protein)	0.183 ± 0.02	0.45 ± 0.04 ^{a***}	0.175±0.01 ^{b***}	0.16±0.01 ^{b***}
SOD (Units/mg protein)	4.06 ± 0.40	3.08 ± 0.31 ^{a***}	4.02 ± 0.41 ^{b***}	4.01 ± 0.39 ^{b***}
CAT (µmol/min/mg protein)	55.74 ± 5.48	37.33 ± 3.66 ^{a***}	54.85 ± 5.39 ^{b***}	53.92 ±5.65 ^{b***}
GSH(mg/gm protein)	0.436 ± 0.028	0.106±0.003 ^{a***}	0.376±0.028 ^{b***}	0.421 ± 0.036 ^{b***}
GSH Plasma (mg/dL)	48.01 ± 4.72	22.36 ± 1.96 ^{a***}	47.52 ± 4.63 ^{b***}	46.72 ± 4.58 ^{b***}
GPx (µmol/min/mg protein)	5.18 ± 0.56	4.27 ± 0.421 ^{a***}	5.12 ± 0.49 ^{b***}	5.09 ± 0.501 ^{b***}

Values are expressed as mean ±SD for six animals in each group.
Superscript letters represent $p<0.05$ (Tukey's Test).
One Unit/mg protein is 50% inhibition in the reduction of NBT min^{-1} mg $protein^{-1}$
^{a}As compared with Group I, ^{b}As compared with Group II.$^{***}p<0.001$.

and GSH (plasma) were observed when compared with group I rats maintained as control. Oral administration of the *A. aspera* respectively to the group III, IV rats showed a significant ($p<0.001$) increase in the activity of SOD, CAT, GPx, in the liver, kidney homogenates and GSH(plasma), when compared with group II high fructose fed rats.

DISCUSSION

Fructose feeding can induce free radical formation by down regulation of hexose monophosphate pathway shunt enzymes that generate reduced environment in the form of NADPH and NADH(16). Previous studies in male rats have shown that a short term consumption of a sucrose-based diet negatively affects the balance between free radical production and antioxidant defense in male rats, leading to increased lipid susceptibility to peroxidation[17]. Higher plasma levels of TBARS and conjugated dienes and higher urinary TBARS excretion were found in the sucrose group than in the starch group, suggesting increased production of these substances from *in vivo* lipid peroxidation[18]. Enhanced lipid peroxidation in fructose-fed rats could be associated with high circulating glucose, which enhances free radical production from glucose autoxidation and protein glycation. Prolonged exposure of rats to hyperglycemic condition reduces the activities of SOD and other antioxidant enzymes. Inactivation of Cu, Zn- SOD by glycation of specific lysine residues has been reported by Oda *et al.,*[19] Reactive oxygen species (ROS) can themselves reduce the activity of antioxidant enzymes such as CAT and GPx (20). In the present study a significant increase in the activity of Cyt P_{450} observed in the liver, kidney of high fructose diet fed rats could be well correlated with the increased generation of oxygen free radicals. Cyt P_{450} is a versatile enzyme, catalyzes the entry of one atom of O_2 to R-OH and one atom to water. This dual fate of the oxygen atom accounts for the basis of lipid peroxidation due to oxygen free radicals generated by Cyt P_{450} [21].

An interesting finding by Marino Janiszewski *et al.,*[22] suggested that reactive thiol groups at or close to the catalytic sites of NAD(P)H Oxidoreductase(s) are essential for enzymatic activity. Increase in the levels of TBARS and hydroperoxides were observed in the liver, kidney of fructose-fed rats. Fructose feeding can induce free radical formation by a number of mechanisms. It causes down regulation of the key enzymes of the hexose monophosphate pathway, namely glucose-6- phosphate dehydrogenase and 6-phosphogluconate dehydrogenase that generate a reduced environment in the form of NADPH and NADH[23]. Impaired regeneration of NADPH could result in an increased oxidative state of the cell. The effects of regulatory thiols on NADPH-oxidase activity together influence the superoxide production. In diseased conditions, large amounts of XO and XD are believed to be released into circulation to produce more amounts of ROMs[24]. Recently Szold *et al.*,[25] have also reported that XO is the principal mediator of remote tissue injury (lung, heart, liver, and kidney). XO generates ROMs as by-product while catalyzing their reaction[26].

The decreased activities of Cyt P_{450}, NADPH-oxidase and XO observed in *A. aspera* treated rats of the present study indicate that the protective effect of these plant products against the reactive oxygen species by inhibiting the superoxides generated by the Cyt P_{450}, NADPH-oxidase and XO. It is also reported that flavonoid quercetin is not only free radical scavenger, but also proved for its metal chelation and inhibition of enzymes like NADPH oxidase in human neutrophils[27]. Thus the inhibition of these superoxide generating enzymes by the flavonoids found in the seeds of *A. aspera* strongly suggest the potential inhibition of peroxidative reactions caused due to free radical production during the high fructose feeding in experimental rats. Flavonoids are proved inhibitors of enhanced production of MDA and conjugated dienes and are able to inhibit lipid peroxidation.

Oxidative stress mediated free radicals cause increased exhaustion/inactivation of enzymatic antioxidants such as SOD and CAT. It is reported that the inactivation of SOD and CAT is possibly by glycation and inactivation due to hypertriglyceridemia[28].A concomitant decrease in the activities of both SOD and CAT in the liver, kidney with a corresponding decrease in the levels of plasma copper, zinc and manganese of HFF rats was observed in the present study is known to be

inactivated during oxidative stress. Copper, Zinc or Manganese are the potent metal cofactors responsible for the activity of SOD. It is reported that decreased level of copper is due to decreased bioavailability on fructose feeding to rats[17].The antioxidant enzymes SOD and CAT found to be inhibited in liver, kidney tissues of the HFF rats in the present study were found increased many folds in *A. aspera* administrated rats. Moreover these herbal powders may serve as an effective free radical scavenger and/or neutralizes the free radicals and induce the activities of SOD and CAT, which is due to the phytoactive constituents (polyphenols) of *A. aspera* seeds.

The diminishing effect of a high fructose diet on hepatic GPX activity was particularly apparent in the combination of the groups. Such result is in accordance with those reported by other authors, and may originate from fructose dependent pathology and/or lower glutathione (GSH) availability, as this phenomenon often accompanies conditions of increased oxidative stress[29]. GSH, being a potent free radical scavenger, is also a cofactor of GPX and plays the essential role in the antioxidant defence of the body. The decrease in the activity of hepatic GPX might also be due to increased turnover of the enzyme. Higher GPX activity in the heart of animals fed extra amounts of buckwheat suggests the cardioprotective effect of the added nutrient on the oxidative stress, regardless of the mechanisms involved. However, recently Girard *et al.* [30] found a significant increase in heart GPX activity in rats fed diet enriched with fructose and they concluded that Frcutose s diet may increase the total antioxidant status of animals. Polyphenols are antioxidants found in high amount in plants, found to be get into cells to exert this protective effects[31].

Flavonoids have been reported not only to inhibit lipid peroxidation, and the activity of enzyme system such as cyclooxygenases and lipooxygenases[32], but also effective free radical scavenger and metal chelators[33] by inhibiting the enzymes like NADPH oxidase and metal (iron and calcium) chelating activity[27]. *A. aspera* is well documented for the presence of phytoactive constituents. Reduction in rate of lipid peroxidation and enhancement in free radical scavenging activity of these herbal seed powders are due to presence of active constituents.

REFERENCES

1. Dai, S., Todd, M.E., S. Lee and J.H, McNeill. Fructose loading induces cardiovascular and metabolic changes in nondiabetic and diabetic rats. *Can J Physiol Pharmacol*., **72**: 771-781 (1994).
2. Busserolles, J., Zimowska, W., Rock, E., Y. Rayssiguier and A. Mazur, Rats fed a high sucrose diet have altered heart antioxidant enzyme activity and gene expression. Life Sci., **71:** 1303-1312 (2002).
3. Parlik alikemets, K., R. Teesalu and M. Zilmer, Oxidative stress and hyperinsulinaemia in essential hypertension: different factors of increase risk. *J. hyper*., **14**: 407-410 (1996).
4. Spragg, R.G., DNA strand break formation following exposure of bovine pulmonary artery and endothelial cells to reactive oxygen products; *Am. J. Respir. Cell. Mol. Biol*., **4**: 4 (1991).
5. Muthu, A.K., Sepathupathy, S., R. Manavalan and P.K. Karar, Antioxidant potential of methanolic extract of *Dolichos biflorus* Linn in high fat diet fed rabbits. *Ind J. Pharmacol*., **44**: 131-132 (2006).
6. Adegoke, G O., Vijay, K.M., K. Sambaiah and B. R, Lokesh, Inhibitory effect of *Garcinia kola* on lipidperoxidation in liver homogenate. *Ind. J. Expt. Biol.,* **36**: 907-910 (1998).
7. Nichans, W.G. and B. Samuelson, Formation of malonldialdehyde from phospholipids arachidonate doing microsomal lipid peroxidation. *Eur J Biochem*., **6**: 126-130 (1968).
8. Rao, K.S., and RO. Recknagel, Early onset of lipid peroxidation in rat liver after carbon tetrachloride administration. *Exp Mol pathol.,* **9**: 271-278 (1968).
9. Omura, T and R. Sato, The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem*., **239**: 2370-2378 (1964).
10. Steyn-Parve, E.P and H. Benert, On the mechanism of dehydrogenation of fatty acyl derivatives of coenzyme A. *J. Biol. Chem*., **233**: 843-852 (1958).

11. Stripe, F and E. Della Corte, The regulation of rat liver xanthine oxidase; *J. Biol. Chem*, **244**: 3855 (1969).
12. Ellman, G.L., Tissue sulphydryl groups. *Arch Biochem Biophys*., **82**: 70-77 (1959).
13. Kakkar, P., B. Das and P.N. Viswanathan, A modifiedspectrophotometric assay of superoxide dismutase. *Ind J Biochem Biophys*.,**21**: 130-132 (1984).
14. Sinha, A.K, Colorimetric assay of catalase. *Anal Biochem*., **47**: 389-394 (1972).
15. Rotruck, J.T., Pope, A.L., Gantler, H.E., Swanson, A.B., D.G. Hafeman and W.G. Hoekstra, Selenium: biochemical role as a component of glutathione peroxidase. *Sci*., **179**: 588-590 (1973).
16. Giardino, I., D. Edelstein and M. Brownlee, BCL-2 expression antioxidants prevent hyperglycemia-induced formation of intracellular advanced glycation end products in bovine endothelial cells. *J Clin Invest.,* **97**: 1422-1428 (1996).
17. Busserolles, J., Rock, E., Gueux, E., Mazur., A., P. Grolier and Y. Rayssiguier, Short-term consumption of a high sucrose diet has a pro-oxidant effect in rats. *Br J Nutr.,* **87**: 337-342 (2002).
18. Busserolles, J., Gueux, E., Rock, E., A. Mazur and Y. Rayssiguier, Substituting honey for refined carbohydrates protects rats from hypertriglyceridemic and prooxidative effects of fructose. *J Nutr*., **11**: 3379-3382 (2002).
19. Oda, A., Bannai, C., Yamoka, T., Katori, T., T. Matsushima and K. Yamashita, Inactivation of Cu-Zn. SOD by in vitro glycosylation in erythrocytes of diabetic patients. *Horm Metab Res*., **26**: 1-4 (1994).
20. Datta, K., S. Sinha and., P, Chattopadhyay, Reactive oxygen species in health and diseases. *Nat Med J Ind*., **13**: 304-310 (2000).
21. Murray, R.K, Metabolism of Xenobiotics, In: Harper's Biochemistry. Murray, R.K., Granner, D.K., P.A. Mayes and V.W. Rodwell(Eds.), 25th Edtion. Prentice Hall International, Appleton & Lange, Stamford, Connecticut. 780-786 (2000).
22. Mariano Janiszewski, Marcelo De Pedro, A., Robert, C.H., Scheffer, Jan-Thies, H., Van Asseldonk, Liliete, C., Souza, Protasio, L., DA. Luz, Ohara Augusto and Francisco RM. Laurindo, Inhibition of vascular NADH/ NADPH Oxidase Activity by thiol Reagents: Lack of correlation with cellular Glutathione Redox Status. *Free Radic. Bio. Med*., **29**(9): 889-899 (2000).
23. Srividhya, S. and C.V. Anuradha, Metformin improves liver antioxidant potential in rats fed a high-fructose diet. *Asia Pacific J Clin Nutr.,* **11**(4): 319-322 (2002).
24. Yokkoyama, Y., Beckman, J.S., Beckman, T.K., Wheat, J.K., Cash, T.G., B.A. Freeman and D.A. Parks, DA. Circulating xanthine oxidase: potential mediator of ischemic injury. *Am. J. Physiol*., **258**: G564 (1990).
25. Szold, O., Weinbroum, A.A., Ben-Abraham, R., Englender, T.E., D. Ovaida and P. Sorkine. Xanthine oxidase and tumour necrosis factor alpha: possible mediators of remote tissue injury after viper envenomation; *Isr. Med. Assoc. J*., **2**: 816 (2000).
26. Ali, S., Diwakar, G., Pawa, S., Siddiqui, M.R., Abdin, M.Z., J.F. Ahmad and S.K, Jain, Xantine oxidase derived reactive oxygen metabolites contributes to liver necrosis protection by 4-hydroxypyrazolo. *Biochim. Biophys. Acta*., **1536**: 21 (2001).
27. Meyer, JW and M.E. Schmitt, FEBS *Lett*., 472: 1-14 (2000).
28. Hunt, J and S. Wolff, Oxidative glycation and free radical production: A causual mechanism of diabetic complications. *Free Radi. Res. Commun*., **72**: 115 (1991).
29. Armutcu, F., Coskun, O., Gurel, A., Kanter, M., Can, M., F. Ucar and M.Unalacak, Thymosin alpha 1 attenuates lipid peroxidation and improves fructose-induced steatohepatitis in rats. *Clin Biochem*., **38**: 540-547 (2005).
30. Girard, A., Madani, S., Boustani, E.S., J. Belleville and J. Prost, Changes in lipid metabolism and antioxidant defense status in spontaneously hypertensive rats and Wistar rats fed a diet enriched with fructose and saturated fatty acids. *Nutr*., **21**: 240-248 (2005).
31. Cooper, R., New scientific findings on traditional Chinese medicines and health benefits of green tea. *Phytomed*., **7**(Suppl.II): 60 (2000).
32. Middleton, E. Jr and Kandaswami, The impact of plant flavonoids on mammalian biology: Implications for immunity inflammation and cancer. In: The Flavonoids: Advances in Research Since 1986. J.B.Harborne (Ed.), Chapman and Hall, London.,619-652 (1993).
33. Hanasaki, Y., S. Ogawa and S. Fukui, *et al*., The correlation between active oxygen scavengers and antioxidative effect of flavonoids. *Free Rad. Biol. Med.* **16**: 845-850 (1994).