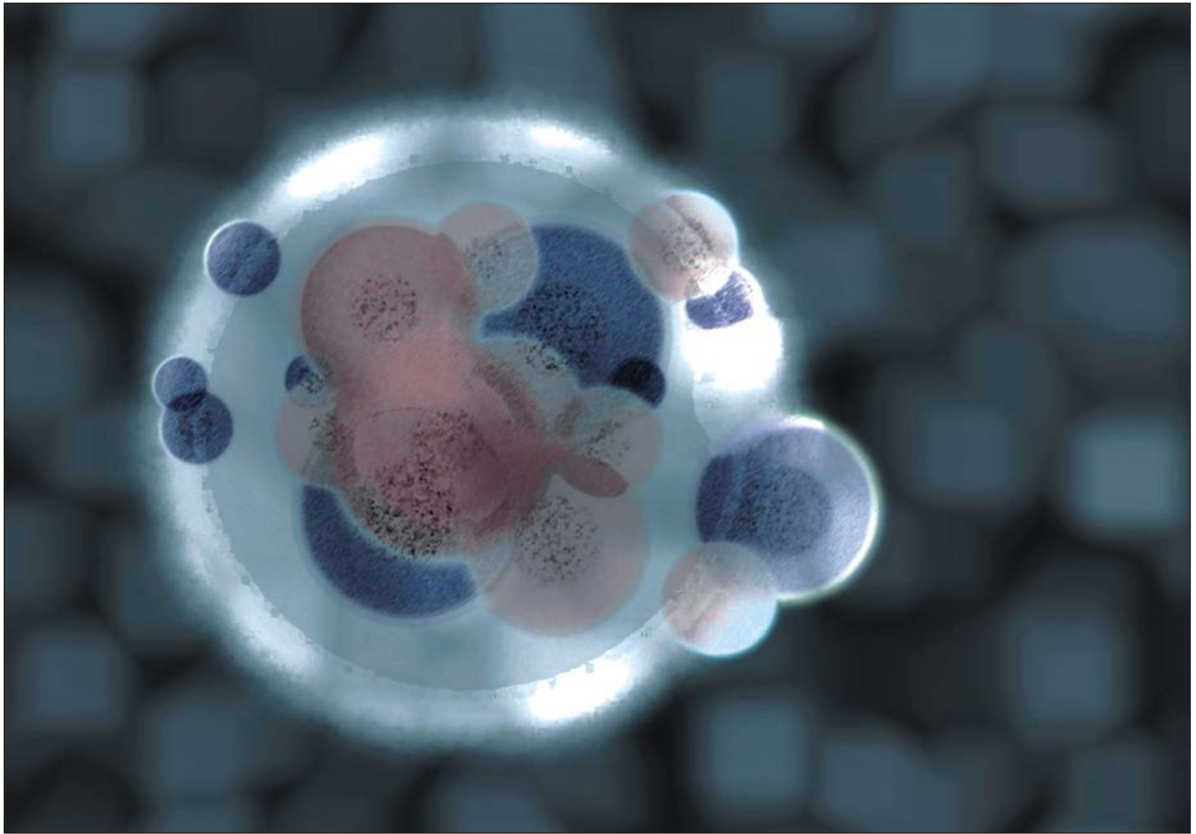

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In vitro Protective Effect of Mangiferin Against Glycated Protein-Iron Chelate Induced Toxicity in Human Umbilical Vein Endothelial Cells

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Abstract: The aim of the present study was to evaluate the protective effect of mangiferin on Human Umbilical Vein Endothelial Cells (HUVEC) against glycated protein-iron chelate induced toxicity. HUVEC incubated in glycated protein either alone or combined with iron chelate showed a significant ($p<0.001$) elevation of Lipid Peroxidation (LPO) accompanied by depletion of Superoxide Dismutase (SOD), catalase, Glutathione Peroxidase (GPx) and Glutathione Reductase (GR), in addition to increased microsomal cytochrome c reductase and decreased glutathione S-Transferase (GST). Treatment of HUVEC with mangiferin at a concentration of 5 and 10 μg significantly decreased the level of LPO and altered antioxidants, cytochrome c reductase and GST levels to near normal in a dose dependent manner. Present results suggest that mangiferin have protective effect against glycated protein-iron chelate induced toxicity.

Key words: Mangiferin, glycated protein-iron chelate, lipid peroxidation, antioxidant enzymes, xenobiotic enzymes

INTRODUCTION

Complications of Diabetes Mellitus (DM) are associated with vascular diseases including atherosclerosis. One primary risk factor causing diabetic complications is long-term exposure to hyperglycemia (Greene *et al*., 1987; Merimee, 1990; Ross and Agius, 1992). Four mechanisms relating to hyperglycemia have been suggested namely sorbitol pathway, diacylglycerol protein kinase pathway, non-enzymatic glycation and alteration of redox potential (King *et al*., 1994). The chronic maintenance of high glucose levels in the blood leads to a drastic modification of the structure, function and properties of many biomolecules. It is due to a multistep non-enzymatic reaction between the glucose molecule and the α-or ε-amino-groups of the proteins, called glycation. The products formed during the early stage of the nonenzymatic glycation, named Amadori-products, undergo complex rearrangements and are converted further into advanced Glycation End-products (AGEs). It has been shown that the later are the major cause for the clinical complications in diabetes mellitus patients such as nephropathy, retinopathy, neuropathy and atherosclerosis (Wu, 1993a, b).

Reactive oxygen species are involved in many pathological events (Halliwell and Gutteridge, 1990; Davies and Dean, 1997; Yagi, 1998; Keaney Jr, 2000). The toxicity produced by iron in biological systems generally described to the enhanced production of powerful oxidants capable of initiating and propagating lipid peroxidation processes, oxidizing proteins and damaging DNA (Halliwell and Gutteridge, 1984). Several workers in their studies have demonstrated the ability of iron chelates or complexes to catalyze the formation of reactive oxygen species and stimulate lipid peroxidation. Aust (1989) has reviewed the relationship between metal ions, oxygen radicals and tissue damage. The role of iron in the initiation of lipid peroxidation has also been reviewed by Minotti and Aust (1987) and Alleman *et al.* (1985). These investigators have presented evidence that lipid peroxidation requires both Fe (III) and Fe (II), probably as a dioxygen-iron complex. Iron is capable of catalyzing redox reactions between oxygen and biological macromolecules that would not occur if catalytically active iron were not present. Iron complexed with Adenosine 5'-Diphosphate (ADP), histidine, Ethylene Diamine Tetraacetic Acids (EDTA), citrate and other chelators has been shown to facilitate the formation of

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reactive oxygen species and enhance production of lipid peroxidation (Ryan and Aust, 1992). Evidence indicates that chelated iron acts as a catalyst for the Fenton reaction, facilitating the conversion of superoxide anion and hydrogen peroxide to hydroxyl radical, a species frequently proposed to initiate lipid peroxidation.

The level of glycated protein is known to increase in diabetic patients and the Fructosamine Value (FV) of serum is regarded as an indicator of blood glucose level. Yagi *et al.* (1985) have reported that chinoform-ferric iron chelate caused lipid peroxidation in cultured neural retinal cells and Nishigaki *et al.* (1998) have shown that glycated protein-iron chelate provokes lipid peroxidation in endothelial cells, these facts explain at least in part the mechanism of atherogenesis in diabetic patients.

Many dietary antioxidants and some non-nutrient based antioxidants from plants such as sulphur containing compounds in garlic, phyto-estrogens in soy, green tea, anthocyanins in redberries, lycopene in tomatoes, red and white wines from grape seeds are increasingly being recognized as potential health promoters through the reduction of risk of Cardio Vascular Disease (CVD) and atherosclerosis (Walker, 1996). Mangiferin is a pharmacologically active phytochemical and natural polyphenolic antioxidant present in the bark, fruits, roots and leaves of *Mangifera indica* Linn (Family: Anacardiaceae) (Sanchez *et al.*, 2000). The chemical name of mangiferin is 2-β-D-glucopyranosyl-1, 3 ,6, 7-tetrahydroxy xanthone (Muruganandan *et al.*, 2002), generally called as C-glucosyl xanthone (Scartezzini and Speroni, 2000). It is a reputed medicinal constituent recommended in the Indian system of medicine for the treatment of immunodeficiency diseases such as arthritis, diabetes, hepatitis, cardiac and mental disorders (Sanchez *et al.*, 2000). It has a strong antioxidant activity in the biological peroxidation system, which might result from the action of scavenging free radicals, e.g., OH and O_2 associated with initiation of lipid peroxidation rather than terminating radical chain reaction in lipid peroxidation (Leiro *et al.*, 2003). It has been reported to possesses antidiabetic, antioxidant, antiproliferative, immunomodulatory, cardiotonic and diuretic properties (Andreu *et al.*, 2005).

The aim of the present study was to investigate the protective effect of mangiferin against glycated protein-iron chelate induced toxicity in human umbilical vein endothelial cells *in vitro*.

MATERIALS AND METHODS

Cells and chemicals: Human umbilical vein endothelial cells and endothelial medium (EG2) were purchased from Kurabo (Japan). Mangiferin and Cytochrome c reductase were purchased from Sigma (St. Louis, USA). Catalase and glutathione S-transferase assay kit was obtained from Cayman chemical company (USA). Glutathione peroxide and glutathione reductase assay kit was purchased from Oxi research (USA) and Trevigen Inc (USA), respectively. Superoxide dismutase was obtained from Dojindo laboratories (Japan). Protein assay kit was obtained from Bio-RAD, USA. Cell culture flask was from Nunc, Denmark. All other chemicals were of analytical grade and water was purified through Millipore Milli Q system (Millipore Co., USA).

Serum glycation: For glycation of fetal bovine serum (FBS), 20 mL of FBS was incubated with 50 mM glucose in 1 mL of 67 mM phosphate buffer (pH 7.4) at 37°C for 15 days under sterile conditions. After incubation, the mixture was dialysed against the same buffer at 4°C. The dialysate was used as glycated FBS after concentration by using aquacide II (Calbiochem-Novabiochem Corp., USA). The extent of glycation of FBS was assayed with a commercial kit (Nippon Roche, Japan) and expressed as Fructosamine Value (FV). The FV of GFBS prepared were 12 mM and that of original FBS was 0.20 mM.

Cell culture and experiments: HUVEC were grown in endothelial medium-2 (EG2), supplemented with 10% (v/v) FBS, gentamicin sulphate (50 mg mL^{-1})/amphotericine-B (50 μg mL^{-1}) in addition to human recombinant fibroblast growth factor-B (HFGF-B; 5 μg mL^{-1}), human recombinant epidermal growth factor (hEGF; 10 μg mL^{-1}), hydrocortisone (1 mg mL^{-1}) and heparin (10 mg mL^{-1}). Cells were grown in 25 cm^2 flasks. The cultures were maintained in a humidified atmosphere containing 5% CO_2 at 37°C, until reaching 80% confluence and used for experiments between passage 3 and 4.

The experimental design was formed by 10 groups with 5 flasks in each group. Group I was treated with 15% of FBS. The group II, III and IV were treated with GFBS (70 μM), FBS+$FeCl_3$ (20 μM) and GFBS+$FeCl_3$, respectively. Other 6 groups were treated similar to group II, III, IV and to them mangiferin was added at doses of 5 and 10 μg, respectively. After 24 h incubation, cells were collected from all the experimental groups by detaching from the flasks using cell scraper-M (Sumitomo bakelite Co., Japan) with phosphate buffer and centrifuged at 1000 x g for 10 min at 4°C. The cell pellet were suspended with cold phosphate buffer and homogenized by using sonicator (Misonix incorporated, USA). The mixture was again centrifuged at 10,000 x g for 15 min at 4°C. A portion of supernatant was used for lipid peroxidation and antioxidants enzymes activities. Another portion of the

supernatant was subjected to centrifugation at 100,000×g for 60 min at 4°C for microsomal separation and was used to assay of cytochrome C reductase and glutathione S-transferase.

Protein assay: Protein content was quantified by means of the BIO-RAD reagent protein assay kit, following the method of Lowry *et al.* (1951).

Lipid peroxidation assay: Lipid peroxidation was determined by using the assay described in Kyowa kit (Japan) manual. In brief, the LPO assay is based on the reaction of a chromogenic reagent, N-methylcarbamoyl-3,7-dimethyl amino-10H-phenothaizine with ascorbic acid oxidase and cumene hydroperoxide at 30°C. The stable chromophore with maximal absorbance at 675 nm and LPO activity were expressed as nmol MDA/mL/mg protein.

Superoxide dismutase (SOD) activity: SOD enzymatic activity was determined using the assay described in Dojindo laboratories SOD kit manual. In brief, cell lysates were treated with Water Soluble Tetrazolium salt (WST) and SOD activity (% inhibition) was determined by measuring the absorbance at 450 nm using microplate reader.

Catalase (CAT) activity: CAT activity was determined using Cayman chemical company kit. After addition of 10 mM H_2O_2, cell lysates were incubated for 20 min and mixed with chromogen substrate followed by addition of stopping reagent. Developed color for 10 min incubation was detected at 540 nm using microplate reader (ImmunoMini NJ). The rate of absorbance was converted to nmol min^{-1} mL^{-1} of enzyme activity, determined from the linear regression of standard curve using CAT.

Glutathione peroxide (GPx) activity: Glutathione peroxide activity was determined using the assay described in Oxi research GPx-340 kit. After addition of 350 μL of NADPH to cell lysates, the reaction was initiated by the addition of tert-butyl hydroperoxide. The changes in absorbance were recorded at 340 nm using spectrophotometer (Jenway, UK).

Glutathione reductase (GR) activity: GR activity was determined by using the method described in Trevigen kit. The cell lysates were treated with oxidized glutathione and changes were recorded at 340 nm after the addition of Nicotinamide Adenine Dinucleotide Phosphate (NADPH). The glutathione reductase activity was expressed in terms of the oxidation of NADPH mU mL^{-1}.

Cytochrome c reductase assay: It is a colorimetric assay using Sigma kit and all reagents required for performing the assay are included within the assay kit. The assay was performed according to the manufacture's instructions and cell lysate was treated with cytochrome c reductase in presence of NADPH. The reduction of cytochrome c was measured at 550 nm.

Glutathione S-Transferase (GST) assay: GST assay was done using Cayman chemical company kit. This assay is based on measuring the Conjugation of 1-chloro-2,4-Dinitrobenzene (CDNB) with reduced glutathione in the microsomal fraction. The conjugation is accompanied by an increased absorbance was measured at 340 nm. The GST activity was expressed as $nmol^{-1}$ min mL^{-1}.

Statistical analysis: The results were expressed as mean±SD and the statistical significance was analyzed by one way ANOVA at $p<0.05$ and $p<0.01$ followed by Tukey multiple comparison test using the GraphPad InStat, USA.

RESULTS

Lipid peroxidation: The lipid peroxide levels were measured as MDA concentration and expressed as nmol mL^{-1} mg^{-1} protein (Fig. 1). Treatment of HUVEC cells with GFBS-iron chelate elevated lipid peroxidation levels significantly when compared with FBS treatment group ($p<0.001$). The treatment with mangiferin significantly ($p<0.001$) reduced the GFBS-iron chelate induced lipid peroxidation.

Antioxidant enzymes: The GFBS-iron chelate significantly reduced the SOD, CAT, GPx and GR levels in HUVEC

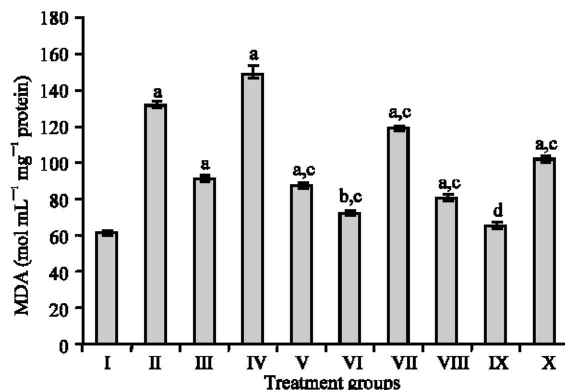

Fig. 1: Effect of mangiferin on lipid peroxidation in HUVEC against glycated protein-iron chelate induced toxicity; Average of 5 determinations; Values are expressed as mean±SEM; [a]$p<0.001$; [b]$p<0.05$ vs FBS; [c]$p<0.001$; [d]$p<0.05$ vs GFBS

Table 1: Effect of mangiferin on antioxidant levels in HUVEC against glycated protein-iron chelate induced toxicity

Treatments	SOD (% inhibition)	Catalase (nmol min^{-1} mL^{-1})	Gpx (mU mL^{-1})	GR (mU mL^{-1})
FBS	94.48±1.94	23.78±1.15	0.92±0.007	53.27±2.90
GFBS	83.06±3.81[b]	16.36±1.08[a]	0.70±0.006[a]	40.00±1.46[a]
FBS + $FeCl_3$	87.96±2.42	18.85±0.97	0.75±0.005[a]	47.25±1.77
GFBS + $FeCl_3$	78.44±2.36[a]	12.38±0.52[a]	0.68±0.002[a]	35.42±1.26[a]
GFBS+ Mangiferin (5 μg)	87.65±1.42	17.43±0.97[b]	0.78±0.002[b]	47.00±2.00
FBS + $FeCl_3$+ Mangiferin (5 μg)	93.27±1.79[f]	19.78±0.88	0.88±0.007[d]	49.33±2.51[f]
GFBS + $FeCl_3$ + Mangiferin (5 μg)	82.00±1.65[b]	16.67±1.24[a]	0.84±0.002[e]	43.25±1.64[c]
GFBS+Mangiferin (10 μg)	88.36±1.27	19.18±1.19	0.74±0.001[a]	50.32±1.52[f]
FBS + $FeCl_3$+ Mangiferin (10 μg)	92.08±1.15	21.65±1.16[f]	0.97±0.003[d]	51.65±1.29[e]
GFBS + $FeCl_3$ + Mangiferin (10 μg)	80.54±1.27[a]	18.76±1.20[c]	0.94±0.002[d]	49.33±1.57[f]

Average of 5 determinations; Values are expressed as mean±SEM; [a]$p<0.001$; [b]$p<0.01$; [c]$p<0.05$ vs FBS; [d]$p<0.001$; [e]$p<0.01$; [f]$p<0.05$ vs GFBS

Table 2: Effect of Mangiferin on cytochrome C reductase and GST levels in HUVEC against glycated protein-iron chelate induced toxicity

Treatment	Cyt C reductase (Unit mL^{-1})	GST (nmol min^{-1} mL^{-1})
FBS	10.89±0.35	8.61±0.75
GFBS	28.66±0.98[a]	3.97±0.15[a]
FBS + $FeCl_3$	17.66±1.32[a]	4.63±0.16[a]
GFBS + $FeCl_3$	34.76±1.08[a]	3.30±0.10[a]
GFBS+ Mangiferin (5 μg)	18.00±0.76[a,c]	5.96±0.64
FBS + $FeCl_3$+ Mangiferin (5 μg)	13.60±0.56[c]	7.95±0.36[c]
GFBS + $FeCl_3$ + Mangiferin (5 μg)	21.45±1.08[a,c]	6.08±0.78
GFBS+ Mangiferin (10 μg)	14.33±0.82[c]	7.86±0.26[c]
FBS + $FeCl_3$+ Mangiferin (10 μg)	11.80±1.02[c]	8.92±0.67[c]
GFBS + $FeCl_3$ + Mangiferin (10 μg)	17.24±0.94[a,c]	6.95±0.97[d]

Average of 5 determinations; Values are expressed as mean±SEM; [a]$p<0.001$; [b]$p<0.05$ vs FBS; [c]$p<0.001$; [d]$p<0.05$ vs GFBS

when compared to FBS control group. Treatment with 5 and 10 μg of mangiferin significantly altered the SOD, CAT, GPx and GR levels to near normal when compared to GFBS-iron treated group (Table 1).

Cytochrome c reductase: Cytochrome c plays an important role in cell death. It is released from the mitochondria in response to apoptotic signals (Skulachev, 1998). In the present study, the protective effect of mangiferin on glycated protein-iron chelate induced toxicity in HUVEC was examined, by using cytochrome c reductase kit. As shown in Table 2, cytochrome c reductase activity was inhibited by mangiferin in a concentration-dependent manner.

Glutathione S-transferase: Introduction of GFBS-iron chelate into HUVEC significantly ($p<0.001$) reduced GST levels when compared with FBS treated group. Treatment of HUVEC with mangiferin, marginally increased the microsomal GST levels when compared with GFBS-iron chelate treated group (Table 2).

DISCUSSION

Recently, much attention has focused on the protective biochemical function of naturally occurring antioxidants in biological systems and on the mechanisms of their action. Polyphenolic compounds, which are widely distributed in plants, were considered to play an important role as dietary antioxidants for the prevention of oxidative damage in living system.

In the present study, we demonstrated that glycated protein with $FeCl_3$ increased intercellular reactive oxygen species formation and subsequent lipid peroxidation in endothelial cells. Glycated proteins, which elevate the blood glucose in diabetic patients, cause direct attenuation of endothelial function. Diabetic patients exhibit an oxidative stress status that is an imbalance between reactive oxygen species and antioxidant defenses, in favor of the first ones. This oxidative stress, together with formation of Advanced Glycation End products (AGEs), is involved in diabetic complications. It could thus be of great interest to propose antioxidant therapeutics as complementary treatment in these patients.

Two mechanisms have been proposed to explain how hyperglyecemia causes increased ROS formation. One mechanism involves the transition metal catalyzed autooxidation of protein-bound Amadori products which yields superoxide and hydroxyl radicals and highly reactive dicarbonyl compounds. The other mechanism involves the transition metal-catalysed autoxidation of free sugars, which also yields dicarbonyl compounds and superoxide and hydroxyl radicals. Autoxidation of protein-bound Amadori products appears to be the most important source of free radicals under near physiological conditions (Hunt *et al*., 1993).

Lipid peroxidation is regarded as one of the basic mechanisms of cellular damage caused by ROS. Free radical mediated lipid peroxidation leads to accumulation of lipid peroxidation products such as malondialdehyde, hydrogen peroxide and also hydroxyl radicals, which in turn propagate lipid peroxidation and cause serious damage to the membrane and changes in intracellular enzymes, resulting in loss of cell function and cell death (Pompella *et al*., 1991; Kehrer, 1993). Growing body of evidence has shown that excessive LPO may be involved

in atherosclerosis (Nagy and Spiteller, 2005). Previous studies shown increased level of lipid peroxidation in glycated protein-iron chelate induced toxicity (Nishigaki *et al.*, 1998). However, this investigation indicate an increase in the level of lipid peroxidation was found in GFBS-iron chelate induced toxicity and treatment of HUVEC with mangiferin significantly ($p<0.001$) reduced lipid peroxidation. Hence it is likely that mangiferin acts by antioxidant effect.

Antioxidant enzymes that scavenge intermediates of oxygen reduction provide the primary defence against cytotoxic oxygen radical (Gopalakrishnan *et al.*, 1996). It is well known that SOD, CAT and GPx play an important role as protective enzymes against lipid peroxidation in tissues (Deepa and Varalakshmi, 2003). Several investigators reported that the reduced activities of SOD, CAT and GPx in atherogenesis conditions (Mann *et al.*, 2007; Siow *et al.*, 1998). The present study indicates a reduction in the activities of SOD, CAT and GPx in GFBS-iron induced toxicity. On mangiferin treatment, the activities of these enzymes inclined towards normal.

Glutathione a tripeptide and an essential bio-factor synthesized in all living cells, is an abundant and ubiquitous antioxidant. It functions mainly as an effective intracellular reductant (Rahman and MacNee, 1999). Glutathione S-transferase, is one of the major mechanism of protection against peroxide-induced damage (Prohaska, 1980). GST, the major phase II detoxification enzyme, favors elimination of polar end products of cytochrome P_{450} mediated phase I reactions through conjugation with reduced glutathione (Coles and Kadlubar, 2003). It protects cells from free radical-mediated damage caused by drugs and ionizing radiation. It forms an important substrate for GPx, GST and several other enzymes, which are involved in free radical scavenging. The mangiferin inhibited the GFBS-iron chelate induced decrease in glutathione reduction and GST levels in HUVEC.

Xenobiotic enzymes like cytochrome c reductase, are toxic due to their redox-cycling capability. Thus synergistically interact with iron chelate to increase the catalytic effectiveness of iron in generating reactive oxygen intermediates. Microsomes isolated from HUVEC when incubated with GFBS-iron show an elevated production of superoxide and H_2O_2. In the presence of iron, there is an increased production of reactive oxygen intermediates, which can cause lipid peroxidation, chemiluminescence, oxidation of OH^- chemical scavengers and inactivation of metabolic enzymes. Increased oxygen radical production by microsomes after incubation with the toxicant is due to inefficient coupling between the reductase and cytochrome; increased content of enzymes such as cytochrome c reductase, which comprise the mixed-function oxidase system and increased oxidase activity of the iron chelate-inducible cytochrome isoenzyme. Treatment of HUVEC with 5 and 10 μg of mangiferin significantly decreases microsomal cytochrome c reductase levels when compared with the GFBS-iron treated group ($p<0.001$).

In conclusion, the present study has demonstrated that the mangiferin has protective effect against glycated protein-iron chelate induced toxicity *in vitro*. The enhanced level of antioxidant enzymes and reduced amount of lipid peroxides are suggested to be the major mechanism of mangiferin in preventing development of vascular complication induced by glycated protein and iron.

REFERENCES

Alleman, M.A., J.F. Koster, J.H.P. Wilson, A. Edixhoven-Bosdijk, R.G. Slee, M.J. Kross and H.G.V. Eijk, 1985. The involvement of iron and lipid peroxidation in the pathogenesis of HCB-induced porphyria. Biochem. Pharmacol., 34: 161-166.

Andreu, G.P., R. Delgado, J.A. Velho, C. Curti and A.E. Vercesi, 2005. Iron complexing activity of mangiferin, a naturally occurring glucosy lxanthone, inhibits mitochondrial lipid peroxidation induced by Fe(2+)-citrate. Eur. J. Pharmacol., 513: 47-55.

Aust, S.D., 1989. Metal ions, oxygen radicals and tissue damage. Bibl. Nutr. Diem., 43: 266-277.

Coles, B.F. and F.F. Kadlubar, 2003. Detoxification of electrophilic compounds by glutathione S-transferase catalysis: Determinants of individual response to chemical carcinogens and chemotherapeutic drugs? Biofactors, 17: 115-130.

Davies, M.J. and R.T. Dean, 1997. The Pathology of Protein Oxidation, Radical Mediated Protein Oxidation. Oxford Science Publications, Oxford, pp: 203-237.

Deepa, P.R. and P. Varalakshmi, 2003. Salubrious effect of low molecular weight heparin on atherogenic diet-induced cardiac, hepatic and renal lipid peroxidation and collapse of antioxidant defences. Mol. Cell Biochem., 254: 111-116.

Gopalakrishnan R., A. Murugesan and D. Sakthisekaran, 1996. Protective role of vitamin E and acetazolamide in cisplatin-induced changes in lipid peroxidation and antioxidant enzyme levels in albino rats. J. Clin. Biochem. Nutr., 20: 203-210.

Greene, D.A., S.A. Lattimer and A.A.F. Sima, 1987. Sorbitol phosphoinositides and sodium-potassium-ATPase in the pathogenesis of diabetic complication. N. Eng. J. Med., 316: 599-606.

Halliwell, B. and J.M.C. Gutteridge, 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J., 219: 1-14.

Halliwell, B.J. and M.C. Gutteridge, 1990. Role of Free Radicals and Catalytic Metal Ions in Human Disease: An Overview. In: Methods in Enzymology. Packer, L. and A.N. Glazer (Eds.), Vol. 186, Academic Press, New York, pp: 1-85.

Hunt, J.V., M.A. Bottoms and M.J. Mitchinson, 1993. Oxidative alterations in the experimental glycation model of diabetes mellitus are due to protein-glucose adduct oxidation. Biochem. J., 291: 529-535.

Keaney, Jr. K.F., 2000. Oxidative Stress and Vascular Disease, Kluwer, Boston.

Kehrer, J.P., 1993. Free radicals as mediators of tissue injury and disease. Crit. Rev. Toxicol., 23: 21-48.

King, G.L., T. Shiba, J. Oliver, T. Inoguchi and S.E. Brursell, 1994. Cellular and molecular abnormalities in the vascular endothelium of diabetes mellitus. Annu. Rev. Med., 45: 179-188.

Leiro, J.M., E. Alvarez, J.A. Arranz, I.G. Siso and F. Orallo, 2003. *In vitro* effects of mangiferin on superoxide concentrations and expression of the inducible nitric oxide synthase, tumour necrosis factor-alpha and transforming growth factor-beta genes. Biochem. Pharmacol., 65: 1361-1371.

Lowry, O.H., N.T. Rosenbrough and A.L. Farr, 1951. Protein measurement with folin-phenol reagent. J. Biol. Chem., 173: 265-275.

Mann, G.E., J. Niehueser-Saran, A. Watson, L. Gao, T. Ishii, P. De Winter and R.C. Siow, 2007. Nrf2/ARE regulated antioxidant gene expression in endothelial and smooth muscle cells in oxidative stress: Implications for atherosclerosis and preeclampsia. Sheng Li Xue Bao, 59: 117-127.

Merimee, T.J., 1990. Diabetic retinopathy: A synthesis of perspectives. Eng. J. Med., 322: 978-983.

Minotti, G. and S.D. Aust, 1987. The role of iron in the initiation of lipid peroxidation. Chem. Phys. Lipids, 44: 191-208.

Muruganandan, S., S. Gupta, M. Kataria, J. Lal and P.K. Gupta, 2002. Mangiferin protects the streptozotocin-induced oxidative damage to cardiac and renal tissues in rats. Toxicology, 176: 165-173.

Nagy, L. and Gerhard Spiteller, 2005. Atherosclerosis and Lipid Peroxidation. Mol. Nutr. Food Res., 49: 989-991.

Nishigaki, I., A. Tanimoto, S. Yasuyuki and K. Yagi, 1998. Glycated protein-iron chelate increases lipid peroxide level in cultured aortic endothelial and smooth muscle cells. Biochem. Mol. Biol. Int., 45: 519-526.

Pompella, A., A. Romani, A. Benedetti and M. Comporti, 1991. Loss of membrane protein thiols and lipid peroxidation in allyl alcohol hepatotoxicity. Biochem. Pharmacol., 41: 1255-1259.

Prohaska, J.R., 1980. The glutathione peroxidase activity of glutathione S-transferases. Biochim. Biophys. Acta., 611: 87-98.

Rahman, I. and W. MacNee, 1999. Lung glutathione and oxidative stress: Implications in cigarette smoke-induced airway disease. Am. J. Physiol., 277: L1067-L1088.

Ross, R. and L. Agius, 1992. The process of atherogenesis-cellular and molecular interaction: From experimental animal models to humans. Diabetologia, 35:34-40.

Ryan, T.P. and S.D. Aust, 1992. The role of iron in oxygen-mediated toxicities. Crit. Rev. Toxicol., 22: 119-141.

Sanchez, G.M., L. Re, A. Giuliani, A.J. Nunez-Selles, G.P. Davison and O.S. Leon-Fernandez, 2000. Protective effects of *Mangifera indica* L. extract, mangiferin and selected antioxidants against TPA-induced biomoleculesoxidation and peritoneal macrophage activation in mice. Pharmacol. Res., 42: 565-573.

Scartezzini, P. and E. Speroni, 2000. Review on some plants of Indian traditionalmedicine with antioxidant activity. J. Ethnopharmacol., 71: 23-43.

Siow, R.C., H. Sato, D.S. Leake, J.D. Pearson, S. Bannai and G.E. Mann, 1998. Vitamin C protects human arterial smooth muscle cells against atherogenic lipoproteins: Effects of antioxidant vitamins C and E on oxidized LDL-induced adaptive increases in cystine transport and glutathione. Arterioscler Thromb Vasc. Biol., 18: 1662-70.

Skulachev, V.P., 1998. Cytochrome c in the apoptosis and antioxidant cascade. FEBS Let., 423: 275-299.

Walker, A.F., 1996. Of hearts and herbs. Biologist, 43: 177-180.

Wu, J.T., 1993a. Advanced glycation end-products: A new disease marker for diabetes and aging. J. Clin. Lab. Anal., 7: 252-255.

Wu, J.T., 1993b. Review of diabetes: Identification of markers for early detection, glycemic control and monitoring clinical complications. J. Clin. Lab. Anal., 7: 293-300.

Yagi, K., K. Ohtsuka and N. Ohishi, 1985. Lipid peroxidation caused by chinoform-ferric chelate in cultured neural retinal cells. Experientia, 41: 1561-1563.

Yagi, K., 1998. Pathophysiology of Lipid Peroxides and Related Free Radicals, Japan Scientific Societies Press, Tokyo.