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Beneficial effect of hesperetin on cadmium induced oxidative stress in rats: an *in vivo* and *in vitro* study

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Abstract. – *Objectives:* **Cadmium (Cd) is ubiquitous in the environment and exposure through food and water as well as occupational sources can contribute to a well-defined spectrum of disease. The present study was undertaken to evaluate the role of hesperetin (Hp) in alleviating the Cd induced biochemical changes in rats.**

Materials and Methods: **During the experiment, male Wistar rats were injected with Cd 83 mg/kg day) subcutaneously alone or with oral administration of Hp 840 mg/kg day) for 21 days.**

Results: **In Cd treated rats the levels of plasma lipid peroxidation (LPO) markers: thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LOOH) were significantly increased while the levels of plasma non-enzymatic antioxidants: reduced glutathione (GSH), vitamins C and E were significantly decreased in Cd administered rats. Administration of Hp along with Cd significantly decreased the level of LPO markers with elevation of non-enzymatic antioxidants in plasma. *In vitro* studies on the effect of Hp on scavenging 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+}$), superoxide anion ($O^{\bullet -}$), hydroxyl ($OH^{\bullet}$) radicals and reducing power also confirmed the free radical scavenging and antioxidant activity of Hp. In addition to that, ascorbic acid, butylated hydroxyl toluene was used as the reference antioxidant radical scavenger compounds. Thus, the observed effects are due to the free radical scavenging and antioxidant potential of Hp. Interestingly, among the different concentrations, tested 50 µM of Hp showed the highest antioxidant and free radical scavenging activities when compared to other concentrations.**

Conclusions: **The result of these findings provides further evidence to the neutraceutical and pharmaceutical potentials of Hp.**

Key Words:

Cadmium, Hesperetin, Oxidative stress, Antioxidant.

Introduction

Cadmium (Cd) is a very toxic heavy metal and an important environmental pollutant, which causes poisoning in various tissues of humans and animals[1]. After entering into the organism through food or water, cadmium binds to albumin and erythrocytes in the blood and then is transferred into tissues and organs, where it is bound to proteins of low molecular mass producing metallothioneins (Cd-MT) by the induction of metallothionein mRNA synthesis[2]. In these Cd-MT complexes generate reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ($O_2(^{-})$ and hydroxyl radical (OH^{-}), nitric oxide (NO^{-})[3] and can reduce the level of the main antioxidant compounds in the cells by inactivating enzymes and other antioxidant molecules[4]. As a result, lipid peroxidation (LPO) is generated[5,6].

Most *in vivo* and *in vitro* studies have focused mainly on acute effects of Cd intoxication on the redox status of the liver. Animals and animal cells were exposed to high doses of Cd to assess oxidative damage, up regulation of genes encoding heat-shock proteins or genes in response to DNA damage and activities of the most important antioxidant enzymes[7,8]. Furthermore, an increase in lipid peroxidation and a decrease in GSH levels were often observed in the plasma[9] liver and the kidney[10,11].

Flavonoids are naturally occurring diphenylpropanoids that are ubiquitous in plant foods and important components of the human diet[12]. Flavonoids are ubiquitous compounds, occurring in various plants such as tea, herbs, citrus fruits and red wine and many of them have been shown to be strong free radical scavengers and antioxidants.

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Hesperetin (Hp) (5,7,3¢-trihydroxy-4¢-methoxyl flavonone), one of the most abundant flavonoids found in citrus fruits[13], can act as a potential antioxidant[14,15], can increase the ocular blood flow and minimize ischemic injury to the retina[16], can decrease vascular permeability[17,18] and act as a neuroprotectant[19,15], anti-inflammatory agent[20,21] and anticancer agent[22]. Hp, an aglycon of hesperidin, is actually a bioactive molecule. The *in vitro* studies suggest that Hp is a powerful radical scavenger that promotes cellular antioxidant defense related enzyme activity[23,24]. To our knowledge, there are no reports available in the literature on the effect of Hp on Cd induced toxicity in experimental animals.

In the light of above information, the present study was carried out to investigate the protective effect of Hp on Cd induced toxicity in rats by studying the biochemical alterations in plasma and also evaluated the possible antioxidant effects of Hp on various *in vitro* antioxidant assays including 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\bullet}$) free radical scavenging, total antioxidant activity by 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+}$) assay, superoxide, hydroxyl radicals scavenging activities and reducing power.

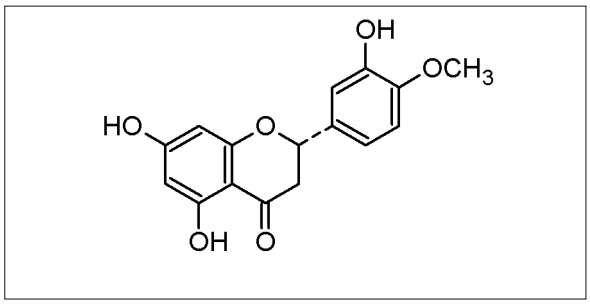

Figure 1. Chemical structure of hesperetin.

Materials and Methods

Animals

Male albino Wistar rats, body weight of 200-220 g bred in Central Animal House, Rajah Muthiah Medical College, Annamalai University were used in this study. The animals were housed six per polypropylene cage and were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India, and approved by the Institutional Ethical Committee (Vide. No. 643, 2009), Annamalai University. The animals were fed on a pellet diet (Lipton India Ltd., Mumbai, India) and water *ad libitum.*

Chemicals

Hesperetin (Figure 1) cadmium chloride, 2-thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), reduced glutathione (GSH), 2,2′-dipyridyl, xylenol orange, 2,4-dinitrophenylhydrazine (DNPH), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), DPPH, ABTS and butylated hydroxytoulene were obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were obtained from S.D. (Fine chemicals, Mumbai, India) and were of analytical grade.

Treatments

The animals were randomly divided into four groups of six rats in each group. Cd dissolved in isotonic saline, Hp powder was suspended in 0.1% carboxymethyl cellulose (CMC) and used in this study.

Group 1: Control rats treated subcutaneously (sc) with isotonic saline and orally with CMC.
Group 2: Rats received Hp (40 mg/kg/day) suspended in CMC administered orally for 21 days using intragastric tube.
Group 3: Rats sc received Cd as cadmium chloride (3 mg/kg/day)[9] in isotonic saline for 21 days.
Group 4: Rats sc received Cd as cadmium chloride (3 mg/kg/day) followed by oral administration of Hp (40 mg/kg/day) in CMC for 21 days.

At the end of experimental period, animals in different groups were sacrificed by cervical decapitation under pentobarbitone sodium (60 mg/kg body weight) anesthesia. Blood was collected in a tube, heparinised for plasma. Plasma was separated by centrifugation and used for various biochemical estimations.

Biochemical Assays

Estimation of Lipid Peroxidation (LPO)

Lipid peroxidation in plasma was estimated spectrophotometrically (Elico-Sl177, Elico Ltd. Hyderabad, Andra Pradesh, India) by measuring thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LOOH) by the method

of Niehiaus and Samuelsson[25] and Jiang et al.[26], respectively. In brief, plasma (0.1 ml) was treated with 2 ml of TBA-trichloroacetic acid (TCA)-HCl reagent (0.37% TBA, 0.25N HCl and 15% TCA, 1:1:1 ratio) placed in a water bath for 15 min and cooled and centrifuged at room temperature, clear supernatant was measured at 535 nm against a reagent blank.

A 0.1 ml aliquot of plasma was treated with 0.9 ml of Fox reagent (88 mg of butylated hydroxytoluene, 7.6 mg of xylenol orange and 0.8 mg of ammonium iron sulfate were added to 90 ml methanol and 10 ml of 250 mM sulfuric acid) and incubated at 37°C for 30 min. The colour that developed was read at 560 nm in a spectrophotometer (Elico-Sl177, Elico Ltd. Hyderabad, Andra Pradesh, India).

Determination of Plasma Non-Enzymatic Antioxidants

Ascorbic acid (vitamin C) concentration was measured by Omaye et al.[27] method. To 0.5 ml of plasma, 1.5 ml of 6% TCA was added and centrifuged (3500 × g, 20 min). To 0.5 ml of supernatant, 0.5 ml of DNPH reagent (2% DNPH and 4% thiourea in 9N sulfuric acid) was added and incubated for 3 h at room temperature. After incubation, 2.5 ml of 85% sulfuric acid was added and colour developed was read at 530 nm after 30 min.

Plasma vitamin E was estimated by the method of Desai[28]. Vitamin E was extracted from plasma by addition of 1.6 ml ethanol and 2.0 ml petroleum ether to 0.5 ml plasma and centrifuged. The supernatant was separated and evaporated on air. To the residue, 0.2 ml of 0.2% 2,2-dipyridyl, 0.2 ml of 0.5% ferric chloride was added and kept in dark for 5 min. An intense red coloured layer obtained on addition of 4 ml butanol was read at 520 nm.

Reduced glutathione (GSH) was determined by the method of Ellman[29]. One millilitre of supernatant was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5,5′-dithiobisnitro benzoic acid in 100 ml of 0.1% sodium citrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm in a spectrophotometer (Elico-Sl177).

Free Radical Scavenging Activity

The ability to scavenging the stable free radical, DPPH was measured as a decrease in absorbance at 517 nm by the method of Mensor et al[30]. To a methanolic solution of DPPH (90.25 mmoles), an equal volume of Hp (10-50 µMoles) dissolved in distilled water was added and made up to 1.0 ml with methanolic DPPH. An equal amount of methanol was added to the control. After 20 min, the absorbance was recorded at 517 nm in a Spectrophotometer (Elico-Sl177).

Total Antioxidant Activity Assay

Total antioxidant potential of Hp was determined by the ABTS assay, as described by Miller et al.[31] The reaction mixture contained ABTS (0.002 M), Hp (10-50 µMoles) and buffer in a total volume of 3.5 ml. The absorbance was measured at 734 nm in a Spectrophotometer (Elico-Sl177).

Superoxide Anion Scavenging Activity

Superoxide anion scavenging activity of Hp was determined by the method of Nishmiki et al.[32] with modification.

One ml of NBT (100 µMoles of NBT in 100 mM phosphate buffer, pH 7.4), 1 ml of NADH solution (14.68 µMoles of NADH in 100 mmoles phosphate buffer, pH 7.4) and varying volumes of Hp (10-50 µMoles) were mixed well. The reaction was started by the addition of 100 µMoles of PMS (60 µMoles) 100 mmoles of phosphate buffer, pH 7.4). The reaction mixture was incubated at 30ºC for 15 min. The absorbance was measured at 560 nm in a spectrophotometer (Elico-Sl177). Incubation without Hp was used as blank. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging.

Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity was determined by the method of Halliwell et al.[33] The following reagents were added in the order stated below.

The incubation mixture in a total volume of 1 ml contained 0.1 ml of 100 mmoles of potassium dihydrogen phosphate- KOH buffer, varying volumes of Hp (10-50 µMoles), 0.2 ml of 500 mmoles of ferric chloride, 0.1 ml of 1 mmoles of ascorbic acid, 0.1 ml of 10 mmoles of H_2O_2 and 0.2 ml of 2-deoxy ribose. The contents were mixed thoroughly and incubated at room temperature for 60 min. Then added, 1 ml of 1% TBA (1 gm in 100 ml of 0.05 N sodium hydroxide) and 1 ml of 28% TCA. All the tubes were kept in a boiling water bath for 30 min. The absorbance was read in a spectrophotometer at 532 nm (Elico-Sl177) with reagent blank containing distilled

water in a place of Hp. The percentage scavenging activity was determined. Decreased absorbance of the reaction mixture indicated increased hydroxyl radical scavenging activity.

Reducing Power

The reducing power was determined according to the Oyaizu[34] method. Different concentrations Hp were prepared in methanol mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min and 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). The absorbance was measured at 700 nm in a spectrophotometer (Elico-Sl177). Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a standard.

Statistical Analysis

The data for various biochemical parameters were analysed using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). Values were considered statistically significant when $p<0.05$.

Results

Table I shows the changes in plasma LPO indices (TBARS and LOOH) in control and experimental rats. In rats treated with Cd, the levels of plasma TBARS and LOOH were significantly increased ($p<0.05$). Administration of Hp significant decrease in Cd induced LPO.

Table II shows the changes in plasma non-enzymic antioxidant levels in control and experimental rats. In rats treated with Cd, the concentrations of non-enzymic antioxidants namely vitamins C, E and GSH were significantly decreased ($p<0.05$). Administration of Hp significantly increased the depleted levels of non-enzymic antioxidants.

Figure 2 shows the percentage free radical scavenging effect of Hp *in vitro* by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH•) assay. The hydrogen atom or electron donation abilities of the compounds were measured from the bleaching of the purple-colored methanol solution of 2,2′-diphenyl-1-picrylhydrazyl (DPPH). Hp scavenges DPPH radical in a dose dependent manner (10, 20, 30, 40 and 50 µM). The percentage scavenging activity of Hp on DPPH radical increases with increasing concentration. At the concentration of Hp (50 µM), the maximum scavenging (53.7%) effect compared with ascorbic acid was observed.

Figure 3 shows the superoxide radicals scavenging effect of Hp *in vitro*. Hp scavenges superoxide radicals scavenging activities in a dose dependent manner (10, 20, 30, 40 and 50 µM). The percentage scavenging activity of Hp on superoxide radicals scavenging activities increases with increasing concentrations. At the concentration of Hp (50 µM), the maximum scavenging (71.7%) effect compared with ascorbic acid was observed.

Figure 4 shows the total antioxidant scavenging effect of Hp *in vitro* by using 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical assay. Hp scavenges 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical in a dose dependent manner (10, 20, 30, 40 and 50 µM). The percentage scavenging activity of Hp on 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical increases with increasing concentration.

Table I. Changes in the levels of plasma lipid peroxidation and lipid hydroperoxides of control and experimental rats.

Groups	TBARS (mM/dl)	Hydroperoxide (× 10^{-5} mM/dl)
Control	0.13 ± 0.01^a	9.89 ± 0.61^a
Normal + Hesperetin (40 g/kg)	0.12 ± 0.01^a	9.58 ± 0.54^a
Normal + Cadmium (3 mg/kg)	0.26 ± 0.05^b	15.07 ± 0.26^b
Cadmium + Hesperetin (40 mg/kg)	0.17 ± 0.03^c	12.19 ± 0.58^c

Values are given as means ± S.D. from six rats in each group; Values not sharing a common letter (a-c) differ significantly at $p<0.05$ (DMRT).

Table II. Changes in the activities of plasma non-enzymatic antioxidant status of control and experimental rats.

Groups	Vitamin C (mg/dl)	GSH (mg/dl)	Vitamin E (mg/dl)
Control	1.65 ± 0.08[a]	1.29 ± 0.03[a]	20.31 ± 1.18[a]
Normal + Hesperetin (40 g/kg)	1.71 ± 0.09[a]	1.34 ± 0.24[a]	21.61 ± 1.41[a]
Normal + Cadmium (3 mg/kg)	1.39 ± 0.04[b]	0.77 ± 0.19[b]	14.26 ± 1.16[b]
Cadmium + Hesperetin (40 mg/kg)	1.51 ± 0.03[c]	1.08 ± 0.04[c]	17.92 ± 1.15[c]

Values are given as means ± S.D. from six rats in each group; Values not sharing a common letter (a-c) differ significantly at $p<0.05$ (DMRT).

At the concentration of Hp (50 µM), the maximum scavenging (75.3%) effect compared with BHT was observed.

Figure 5 shows the hydroxyl radicals scavenging effect of Hp on *in vitro*. Hp scavenges hydroxyl radicals scavenging activities in a dose dependent manner (10, 20, 30, 40 and 50 µM). The percentage scavenging activity of Hp on hydroxyl radicals scavenging activities increases with increasing concentration. At the concentration of Hp (50 µM), the maximum scavenging (39.3%) effect compared with ascorbic acid was observed.

Figure 6 shows the reducing power of Hp and the standard compound ascorbic acid increasing steadily with increasing concentration. At the concentration of Hp (50 µM), the maximum scavenging (0.049) effect compared with ascorbic acid was observed.

Discussion

Cadmium (Cd) is one of the main environmental and occupational pollutants in industrialized countries and induces a wide array of toxicological effects, biochemical dysfunctions in various organ systems posing a serious threat to health[35,36]. Cd induces oxidative damage by producing Reactive Oxygen Species[37-39] and decreasing the biological activities of some antioxidant enzymes, such as vitamins C, E, GSH, superoxide dismutase, catalase and glutathione peroxidase[11,40,41] which play an important role in antioxidation and elimination of free radicals.

Cd has also been reported to cause damage to lipids and by that to generate LPO[42,43]. Cd induced damage is associated with increased lipid peroxidation[44,45]. Cell membranes are phospho-

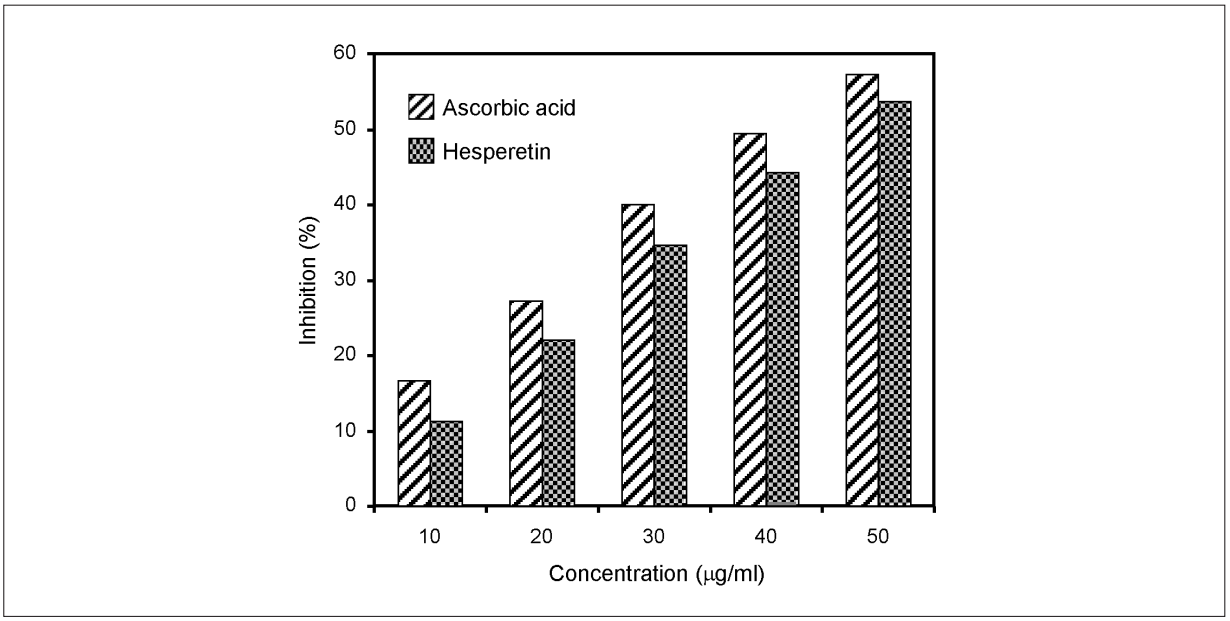

Figure 2. *In vitro* scavenging effects of hesperetin on ascorbic acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH).

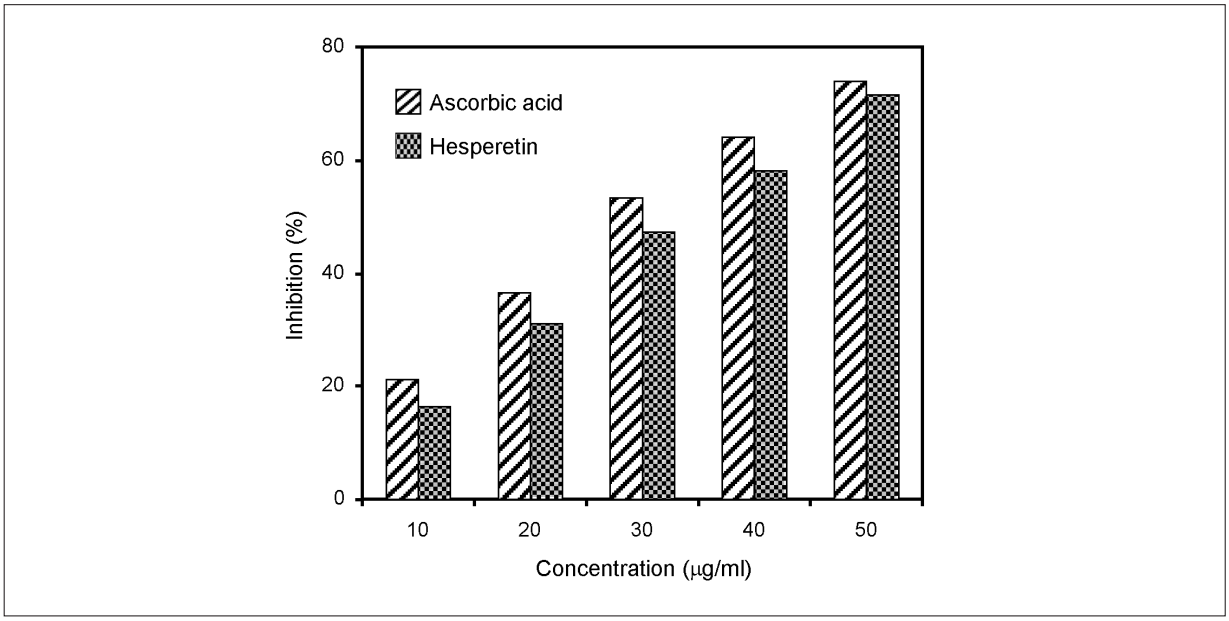

Figure 3. *In vitro* scavenging effects of hesperetin on ascorbic acid and superoxide radicals.

lipid bilayers with extrinsic proteins and are the direct target of lipid peroxidation which leads to a number of deleterious effects such as increased membrane rigidity, osmotic fragility, cell membrane destruction and cell damage[46]. The observed increase in the level of plasma TBARS and LOOH in Cd toxicity is generally thought to be the consequence of an increased production and liberation of tissue lipid peroxides into circulation due to the pathological changes in tissues. Ognjanovic et al.[47] observed that the treatment with Cd increased the lipid peroxide concentration in blood. Increase in lipid peroxidation in plasma and tissues have been implicated in cadmium-induced organ damage and dysfunction[9,48]. Treatment with Hp significantly reverted the Cd

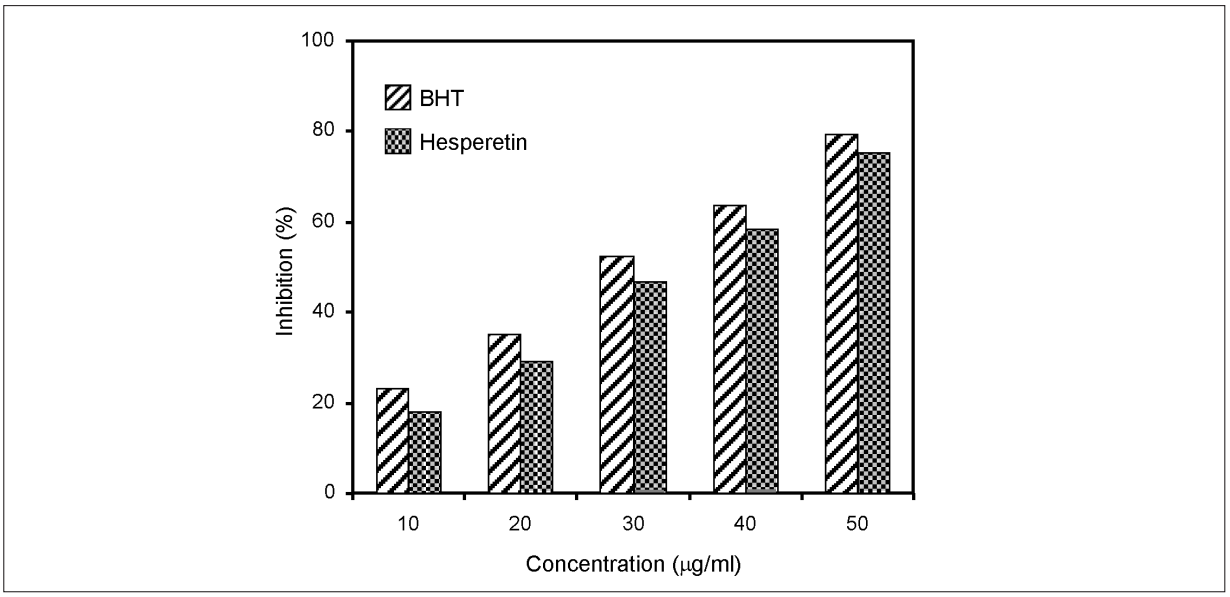

Figure 4. *In vitro* scavenging effects of hesperetin on BHT and 2,2´-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radicals.

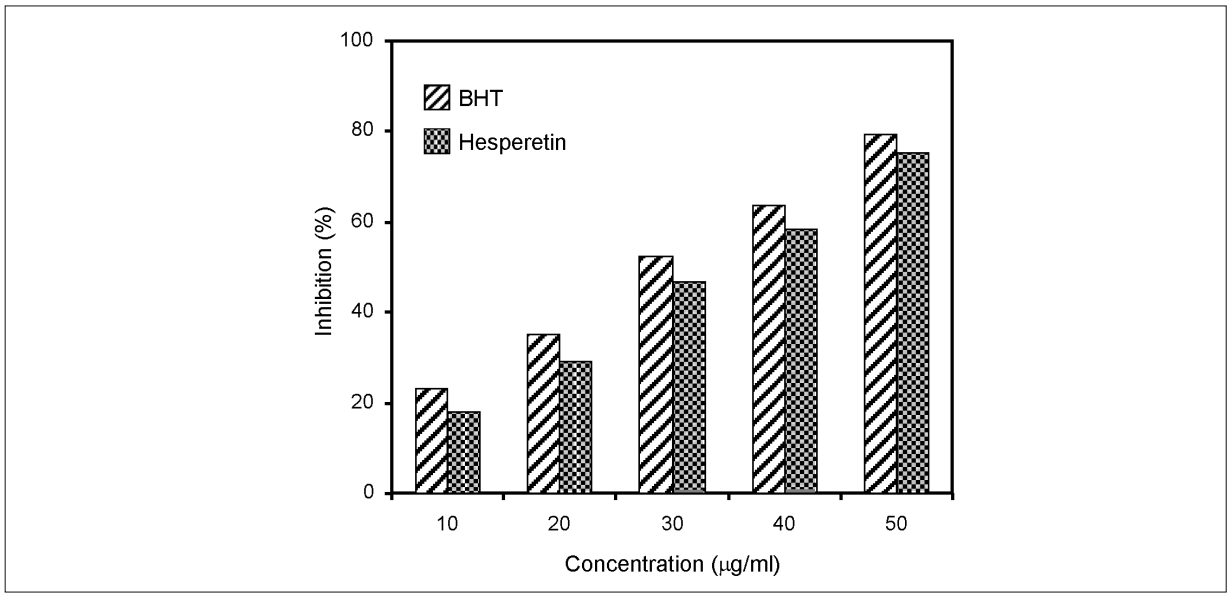

Figure 5. *In vitro* scavenging effects of hesperetin on ascorbic acid and hydroxide radicals.

induced peroxidative damage in plasma which is evidenced from the lowered levels of TBARS and LOOH. This may be due to the antioxidative effect of Hp[22].

Vitamin C is the primary preventive antioxidant in the cells and body fluids, scavenges the free radicals and serves as metabolic markers of Cd toxicity[49]. Vitamin E is a lipophilic antioxidant, which plays a critical role in detoxifying the Cd toxicity[50]. A decreased level of vitamins C and E during Cd intoxication leads to increased susceptibility of the tissues to free radical damage. GSH is known to play a major role in the regulation of intracellular levels of reactive oxygen species by direct reaction, scavenging, or via the GSH peroxidase/GSH system.

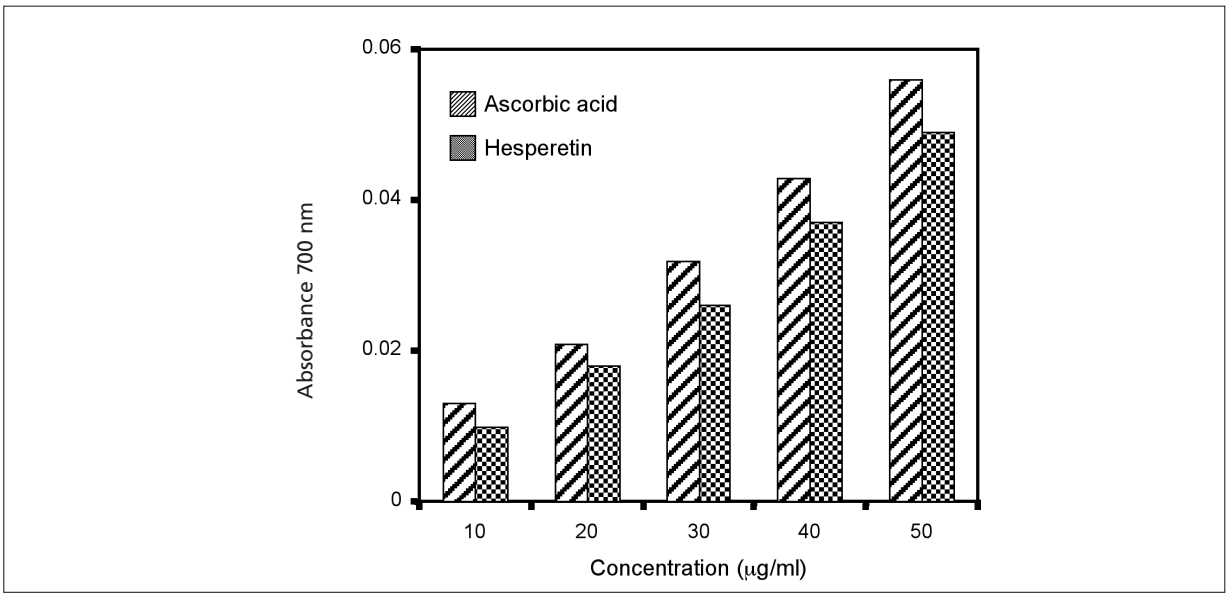

Figure 6. *In vitro* scavenging effects of hesperetin on ascorbic acid and reducing power.

Thus, GSH is a major cellular antioxidant and protects cells against oxidative damage. The present study indicates that the Cd will reduce the levels of non-enzymatic antioxidants in plasma. Renugadevi and Milton Prabu[11] have reported the depressed level of reduced glutathione, vitamin C and vitamin E in Cd-intoxicated rats which is in consonance with our findings. Treatment with Hp in Cd-intoxicated rats protects the depletion of non-enzymatic antioxidants via its metal-chelating and antioxidant property[51] and may minimize the usage of these antioxidants, thus restoring their levels.

Free radicals have been implicated in a number of diseases including asthma, cancer, cardiovascular disease, cataract, diabetes, gastrointestinal inflammatory diseases, liver diseases, muscular degeneration and other inflammatory processes[52]. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. In this study, free radical scavenging activity of Hp was determined using DPPH$^{\bullet}$ method. Research reports have shown that DPPH$^{\bullet}$ is widely used to evaluate the free radical scavenging effects of various antioxidant substances, and polyhydroxy aromatic compounds[53]. In the present investigation, Hp scavenges DPPH$^{\bullet}$ radical dose dependently. The highest percentage scavenging effect of Hp (53.7%) on 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical was observed at the concentration of 50 μM Hp. *In vitro* study on DPPH$^{\bullet}$ clearly revealed the antioxidant property of Hp.

Superoxide anion is a precursor to active free radicals that have potential of reacting with biological macromolecules and, thereby, inducing tissue damage. Yen and Duh[54] have reported that antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radicals. It is well known that superoxide anions damage biomolecules directly or indirectly by forming H_2O_2, OH, peroxy nitrite or singlet oxygen during aging and pathological events such as ischemic reperfusion injury. Superoxide has also been observed to directly initiate lipid peroxidation[54]. In this study, we will use different concentration of Hp. The highest percentage scavenging effect of Hp (71.7%) on superoxide anion radicals was observed at the concentration of 50 μM Hp.

Phenolic compounds have more ability to quench ABTS radical and that effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl group's substitution[55]. ABTS is a relatively stable free radical which involves in the direct generation of ABTS radical monocation without any involvement of intermediary cation.

In this study, the total antioxidant activity of Hp was investigated *in vitro* by 2,2′-azinobis- (3-ethyl-benzothiazoline-6-sulfonic acid) cation which forms the basis of one of the spectrophotometric methods that have been applied to the total antioxidant activities of solutions of pure substances. In this investigation, Hp scavenges 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical dose dependently. The highest percentage scavenging effect of Hp (75.3%) on 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical was observed at the concentration of 50 μM Hp.

Hydroxyl radical (OH$^{\bullet}$) is chiefly responsible for lipid peroxidation, which impairs the normal function of cell membranes, motility and permeability. In this study, we will used different concentration of Hp. The highest percentage scavenging effect of Hp (39.3%) on hydroxyl radicals was observed at the concentration of 50 μM Hp. *In vitro* study on superoxide anion clearly revealed the antioxidant property of Hp. In this investigation, Hp *in vitro* exhibits 89.2% of hydroxyl radical scavenging activity at the concentration of 50 μM Hp.

Increased absorbance with the increased concentrations of the reaction mixture indicated the increased reducing power. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[56]. In this study, we used different concentration of Hp. The highest percentage scavenging effect of Hp (0.049) on hydroxyl radicals was observed at the concentration of 50 μM Hp. *In vitro* study on the reducing power of Hp clearly revealed its high antioxidant property. DPPH and ABTS^{+} assays have also been widely used to test the free radical scavenging ability of various antioxidant polyphenols commonly found in foods[57], plant extracts[58], beverages[59] and flavonoids[60].

Conclusion

According to data obtained, Hp significantly reduced lipid peroxidation (TBARS and LOOH) in plasma and increases the non-enzymatic antioxidant levels (vitamin C, E and GSH) mainly due to the antioxidant properties of Hp. Further

the *in vitro* researches have also shown that the Hp at a dose of 50 μM exhibits highest free radical scavenging and antioxidant effects which has been proved by the methods of DPPH•, total antioxidant activity, superoxide anion scavenging activity, hydroxyl radical scavenging activity and reducing power.

References

1) Stohs SJ, Bagchi D, Hassoun E, Bagchi M. Oxidative mechanisms in the toxicity of chromium and cadmium ions. J Environ Pathol Toxicol Oncol 2000; 19: 201-213.

2) George SG, Todd K, Wright J. Regulation of metallothione in inteleosts of induction of MT mRNA and protein by cadmium in hepatic and extra hepatic tissues of a marine flat fish, the turbot (Scophthalmus maximus). Comp Biochem Physiol 1996; C113: 109-115.

3) Hassoun EA, Stohs SJ. Cadmium-induced production of superoxide anion and nitric oxide, DNA single strand breaks and lactate dehydrogenase leakage in J774A.1 cell cultures. Toxicology 1996; 112: 219-226.

4) Filipic M, Fatur T, Vudrag M. Molecular mechanisms of cadmium induced mutagenicity. Hum Exp Toxicol 2006; 25: 67-77.

5) Sarkar S, Yadav P, Trivedi R, Bansal AK, Bhatnagar D. Cadmium-induced lipid peroxidation and the status of the antioxidant system in rat tissues. J Trace Elem Med Biol 1995; 9: 144-149.

6) Rikans LE, Yamano T. Mechanisms of cadmium-mediated acute hepatotoxicity. J Biochem Mol Toxicol 2000; 14: 110-117.

7) Boujelben M, Ghorbel F, Vincent C, Makni-Ayadi F, Guermazi F, Croute F, El-Feki A. Lipid peroxidation and HSP72/73 expression in rat following cadmium chloride administration: interactions of magnesium supplementation. Exp Toxicol Pathol 2006; 57: 437-443.

8) Liu J, Kadiiska MB, Corton JC, Qu W, Waalkes MP, Mason RP, Liu Y, Klaassen CD. Acute cadmium exposure induces stress-related gene expression in wild-type and metallothionein-I/II-null mice. Free Radical Biol Med 2002; 32: 525-535.

9) Pari L, Mrugavel P. Role of diallyl tetrasulfide in ameliorating the cadmium induced biochemical changes in rats. Environ Toxicol Pharmacol 2005; 20: 493-500.

10) El-Maraghy SA, Gad MZ, Fahim AT, Hamdy MA. Effect of cadmium and aluminum intake on the antioxidant status and lipid peroxidation in rat tissues. J Biochem Mol Toxicol 2001; 15: 207-214.

11) Renugadevi J, Prabu SM. Cadmium- induced hepatotoxicity in rats and the protective effect of naringenin. Exp Toxicol Pathol 2010;62:171-181.

12) Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. Lancet 1993; 342: 1007-1011.

13) Gil-Izquierdo A, Gil MI, Ferreres F, Tomas-Barberan FA. *In vitro* availability of flavonoids and other phenolics in orange juice. J Agric Food Chem 2001; 49: 1035-1041.

14) Choi EJ. Antioxidative effects of hesperetin against 7,12-dimethylbenz (a) anthracene-induced oxidative stress in mice. Life Sci 2008; 82: 1059-1064.

15) Hwang SL, Yen GC. Neuroprotective effects of the citrus flavonones against H_2O_2-induced cytotoxicity in PC12 cells. J Agric Food Chem 2008; 56: 859-864.

16) Chiou GC, Xu XR. Effects of some natural flavonoids on retinal function recovery after ischemic insult in the rat. J Ocul Pharmacol Ther 2004; 20: 107-113.

17) Paysant J, Sansilvestri-Morel P, Bouskela E, Verbeuren TJ. Different flavonoids present in the micronized purified flavonoid fraction (Daflon 500 mg) contribute to its anti-hyperpermeability effect in the hamster cheek pouch microcirculation. Int Angiol 2008; 27: 81-85.

18) Garg A, Garg S, Zaneveld LJ, Singla AK. Chemistry and pharmacology of the citrus bioflavonoid hesperidin. Phytother Res 2001; 15: 655-669.

19) Choi EJ, Ahn WS. Neuroprotective effects of chronic hesperetin administration in mice. Arch Pharm Res 2008; 31: 1457-1462.

20) Hirata A, Murakami Y, Shoji M, Kadoma Y, Fujisawa S. Kinetics of radical scavenging activity of hesperetin and hesperidin and their inhibitory activity on COX-2 expression. Anticancer Res 2005; 25: 3367-3374.

21) Galati EM, Monforte MT, Kirjavainen S, Forestieri AM, Trovato A, Tripodo MM. Biological effects of hesperidin, a citrus flavonoid. (Note I): anti inflammatory and analgesic activity. Farmaco 1994; 40: 709-712.

22) Aranganathan S, Nalini N. Efficacy of the potential chemopreventive agent, hesperetin (citrus flavonone) on 1,2-dimethyl hydrazine induced colon carcinogenesis. Food Chem Toxicol 2009; 47: 2594-2600.

23) Kim JY, Jung KJ, Choi JS, Chong HY. Hesperetin: potent antioxidant against peroxynitrite. Free Radic Res 2004; 38: 761-769.

24) Pollard SE, Whiteman M, Spencer JP. Modulation of peroxynitrite-induced fibroblast injury by hesperetin: a role for intracellular scavenging and modulation of ERK signaling. Biochem Biophys Res Commun 2006; 347: 916-923.

25) Niehaus WG, Samuelsson D. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem 1968; 6: 126-130.

26) Jiang ZY, Hunt JV, Wolff SD. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal Biochem 1992; 202: 384-389.

27) Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Method Enzymol 1979; 62: 1-11.

28) Desai ID. Vitamin E analysis method for animal tissues. Method Enzymol 1984; 105: 138-143.

29) Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys 1959; 82: 70-77.

30) Mensor LL, Menezes FS, Leitao GG, Reis AS, Dos santos TC, Coube CS, Leitao SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 2001; 15: 127-130.

31) Miller NJ, Castelluccio C, Tijburg L, Rice-Evans C. The antioxidant properties of the flavins and their gallate esters-radical scavengers or metal chelators. FEBS Lett 1996; 392: 40-44.

32) Nishimiki M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. Biochem Biophys Res Comm 1972; 46: 849-853.

33) Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose methods; a simple assay for determination of rate constants for reaction of hydroxyl radicals. Anal Biochem 1989; 165: 215-219.

34) Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jap J Nutr 1986; 44: 307-315.

35) World Health Organisation, Environmental Health Criteria 134, Cadmium. International Programme on Chemical Safety (IPCS), WHO, Geneva, 1992.

36) Satarug JR, Baker S, Urbenjapol M, Haswell-Elkins PE, Reilly DJ, Williams MR, Moore A. Global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol Lett 2003; 137: 65-83.

37) Hassoun EA, Stohs SJ. Cadmium induced production of superoxide anion and nitric oxide, DNA single strand breaks and lactate dehydrogenase leakage in J774A1 cell cultures. Toxicology 1996; 112: 219-226.

38) Chen L, Liu L, Huang S. Cadmium activates the mitogen-activated protein kinase (MAPK) pathway via induction of reactive oxygen species and inhibition of protein phosphatases 2A and 5. Free Radic Biol Med 2008; 45: 1035-1044.

39) Liu J, Qian SY, Guo Q, Jiang J, Waalkes MP, Mason RP, Kadiiska MB. Cadmium generates reactive oxygen- and carbon-centered radical species in rats: insights from in vivo spin-trapping studies. Free Radic Biol Med 2008; 45: 475-481.

40) Ikediobi CO, Badisa VL, Ayuk-Takem LT, Latinwo LM, West J. Response of antioxidant enzymes and redox metabolites to cadmium-induced oxidative stress in CRL-1439 normal rat liver cells. Int J Mol Med 2004; 14: 87-92.

41) Uchida M, Teranishi H, Aoshima K, Katoh T, Kasuya M, Inadera H. Reduction of erythrocyte catalase and superoxide dismutase activities in male inhabitants of a cadmium-polluted area in Jinzu river basin, Japan Toxicol Lett 2004; 151: 451-457.

42) Sarkar S, Yadav P, Trivedi R, Bansal AK, Bhatnagar D. Cadmium-induced lipid peroxidation and the status of the antioxidant system in rat tissues. J Trace Elem Med Biol 1995; 9: 144-149.

43) Rikans LE, Yamano T. Mechanisms of cadmium-mediated acute hepato-toxicity. J Biochem Mol Toxicol 2000; 14: 110-117.

44) Jin T, Nordberg M, Frech W, Dumont X, Bernard A, Ye TT, Kong Q, Wang Z, Li P, Lundstrom NG, Li Y, Nordberg GF. Cadmium biomonitoring and renal dysfunction among a population environmentally exposed to cadmium from smelting in China. Biometals 2002; 15: 397-410.

45) Shimada T, Takamure Y, Shimada A, Yasutake A, Waalkes MP, Imamura Y. Strain differences of cadmium- induced hepatotoxicity in Wistar-Imamichi and Fischer 344 rats: involvement of cadmium accumulation. Toxicology 2004; 203: 189-197.

46) El-Sharaky AS, Mohammed GB, Kandeel KM, Wahby MM. An electrochemical approach to characterization of metallothione: direct evidence of interaction of cadmium with cysteine residue. In: Proceedings of the XXV-International Conference on Co-ordination Chemistry, Nanjing, China, July 26-31, 1987.

47) Ognjanovic BI, Pavlovic SZ, Maletic SD, Zikic RV, Stajn AS, Radojicic ZS, Saicic ZS, Petrovic VM. Protective influence of vitamin E on antioxidant defense system in the blood of rats treated with cadmium. Physiol Res 2003; 52: 563-570.

48) Kelley C, Sargent DE, Uno JK. Cadmium therapeutic agents. Curr Pharm Des 1999; 5: 229-240.

49) Pavlovic SZ, Onjanvoic BI, Stajn AS, Zikic RV, Saicic ZS, Petrovic VM. The effects of coenzyme Q10 on blood ascorbic acid, vitamin E and lipid peroxide in chronic cadmium intoxication. J Environ Pathol Toxicol Oncol 2001; 20: 133-144.

50) Mates M. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology 2000; 153: 83-104.

51) Cai YZ, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional. Life Sci 2004; 74: 2157-2184.

52) Wilson RL. Free radicals and tissue damage, mechanistic evidence from radiation studies. Biochemical mechanism of liver injury. Academic Press. New York 1998; p. 123.

53) Nishizawa M, Kohno M, Nishimura M, Kitagawa A, Niwano Y. Non-reductive scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) by peroxyradical: a useful method for quantitative analysis of peroxyradical. Chem Pharm Bull 2005; 53: 714-716.

54) Yen GC, Duh PD. Scavenging effect of methanolic extract of peanut hulls on free radical and active oxygen species. J Agric Food Chem 1994; 42: 629-632.

55) Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J Agric Food Chem 1998; 46: 1887-1892.

56) Meir S, Kanner J, Aldri B, Hadas SP. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J Agric Food Chem 1995; 43: 1813-1815.

57) Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 1999; 40: 945-948.

58) Chen Y, Wang M, Rosen RT, Ho CT. 2,2-Diphenyl-1-picrylhydrazyl radical scavenging active components from polygonum multiflorum thumb. J Agric Food Chem 1999; 47: 2226-2228.

59) Sanchez-Moreno C, Larrauri JA, Saura-Calixto F. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenol constituents. Food Res Int 1999; 32: 407-412.

60) Rajadurai M, Stanely Mainzen Prince P. Preventive effect of naringin on isoproterenol-induced cardiotoxicity in Wistar rats: an in vivo and in vitro study Toxicology 2007; 232: 216-225.