Vol 5 Issue 3 Sept 2015

ISSN No :2231-5063

International Multidisciplinary Research Journal

Golden Research Thoughts

Welcome to GRT

RNI MAHMUL/2011/38595 **ISSN No.2231-5063**

Golden Research Thoughts Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial board.Readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.

International Advisory Board

Editorial Board

Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.aygrt.isrj.org

Golden Research Thoughts
ISSN 2231-5063

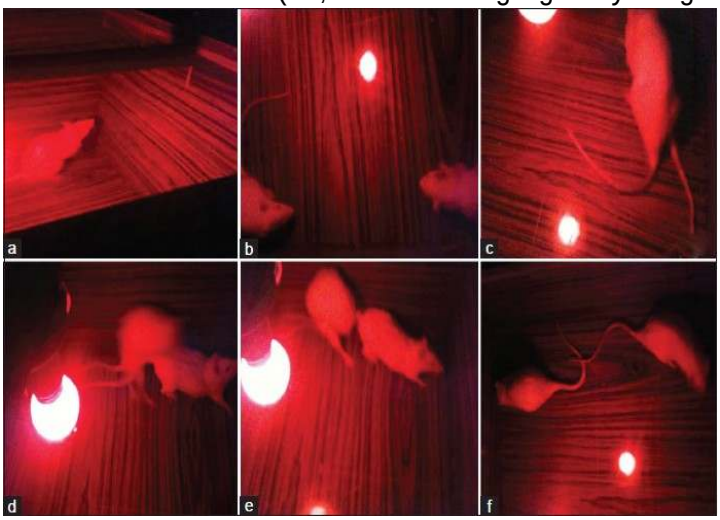
Impact Factor : 3.4052(UIF)
Volume - 5 | Issue - 3 | Sept - 2015

EFFECT OF ELLAGIC ACID ON CIRCULATORY ANTIOXIDANTS AND LIPIDS DURING IRON-INDUCED TOXICITY IN EXPERIMENTAL RATS

S.Palani[1], D.Santhakumari[2], S.Balachandar[3] and S.Ambika[4]
[1]Asst.professor, Department of Biochemistry, Shanmuga Industries Arts & Science College, Tiruvannamalai, Tamilnadu, India.
[2]Principal, Indira Gandhi Jayanthi womens, arts and Science College, Kilgudalur, Tindivanam. Tamilnadu, India.
[3]Asst.professor, Department of Chemistry, Shanmuga Industries Arts & Science College, Tiruvannamalai, Tamilnadu, India.
[4]Asst.professor, Department of MCA, Shanmuga Industries Arts & Science College, Tiruvannamalai, Tamilnadu, India.

ABSTRACT

Ellagic acid (EA) is a naturally occurring polyphenolic compound that exhibits antioxidative, anti-inflammatory, anti-hyperlipidaemic and anticarcinogenic activities in a wide range of assays both in vitro and in vivo. It occurs in various foods such as strawberries, grapes, walnuts, etc. The aim of this study was to assess the effect of ellagic acid on Iron-induced changes in the circulatory antioxidative status, micronutrients and lipid levels in a dose-dependent fashion. Male albino Wistar rats weighing 180–210 g were used to assess the effects of EA against Iron-induced damage. Three different concentrations of EA (15, 30 and 60 mg/kg body weight) were tested against Iron via intraperitonial administration. At the end of the experimental duration of 10 days, we evaluated endogenous antioxidants: both enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic (vitamin C and E, and reduced glutathione) status, micronutrients, lipids: cholesterol, triglycerides, free fatty acids and phospholipids in the circulation. EA significantly inhibits Iron-induced toxicity by restoring antioxidant status, modulating micronutrients and attenuating the lipid levels in the circulation. The greatest inhibitory effect was observed with 60 mg/kg body weight of EA in all the biochemical assessments and could be developed as a potential for Iron in the near future.



KEYWORDS : *Iron, antioxidants, Ellagic acid, lipids*

INTRODUCTION :

The use of medicinal plants is getting popularized in developing and developed countries for the treatment of various ailments [1–3]. Presently, the active constituents of these plant sources are extracted, purified and tested for their activities. Results are promising that phyto- chemicals are well established to exert their benefits in the prevention and therapy of many diseases including cancer,

atheroscelerosis and Feism, partially based on their ability to quench reactive oxygen species (ROS) and thereby protecting critical cellular targets (i.e. DNA, proteins, lipids) from oxidative insult [4–6]. Ellagic acid (EA) (Figure 1) one of the phytochemicals, is of particular interest from a dietary viewpoint as it has been reported to have antiviral properties [7] and to provide protection against cancers of the colon [8], lung and oesophagus [9]. It is a polyphenolic compound, widely distributed in many fruits and vegetables, such as strawberries, black currants, grapes, walnuts and raspberries. Indeed, EA exerts a potent scavenging action on both superoxide and hydroxyl anion in vitro [10]. In recent years, chronic iron overload, there is evidence that excess iron induces cellular injury and functional abnormalities in hepatocytes by the process of lipid peroxidation in lysosomal, mitochondrial and microsomal membranes. Lipid peroxidation is a likely outcome of oxidative stress in biological system, and its measurement is often used as a method of assessing the degree of oxidative damage.

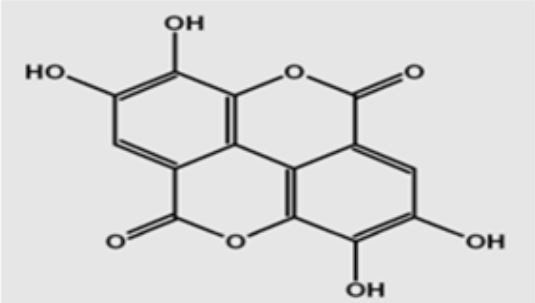


Figure-1 Structure of ellagic acid (4,4',5',5,6,6'-hexahydroxydiphenic acid 2,6,2',6'-dilactone).

In this study, we have investicated the effects of dietary Fe in the rat on endogenous antioxidant status and several parameters of lipid peroxidation in plasma and liver. Lipid peroxidation was assessed by measuring lipid hydroperoxides, thiobarbituric-acid-reactive substances (TBARS). Elevated circulatory lipid profile was reported to be a major risk factor for cardiovascular diseases [11]. In such conditions, external supply of antioxidants is essential to countervail the deleterious consequences of Fe-induced oxidative stress and hyperlipidaemia. Because EA was found to be an effective antioxidant, we hypothesize that administration of EA might decrease the intensity of toxicity during chronic Fe consumption.

EXPERIMENTAL PROCEDURES

Maintenance of animals

Thirty male albino wister rats 180-210g were bred in the central Animal House, Rajan muthiah medical college, Annamalai University. The animals were housed in polypropylene cages (47x34x18 cm) in an air conditioned room with controlled temperature (25±2^{0}c) and automatic lighting (alternating 12h period of light and dark). The animal had access to standard pellet diet (Pranaw Agro Industries Ltd, Bangalore, India) and water ad libitum. This study was approved (Vide No, 523, 2008) by the Animal ethical committee, Annamalai University.

CHEMICALS

Ellagic acid was supplied by sigma chemicals company, St Louis MO, USA. Ferrous sulphate ($FeSO_4 .7H_2O$) was obtained from Qualigen Fine chemicals (Bombay, India). All Other chemicals and biochemicals were of analytical grade obtained from local forms. The organic solvents were distilled before use.

EXPERIMENTAL DESIGN

The animals were randomly divided into six groups of six rats in each. Group I: Control rats orally treated with dimethyl sulphoxide (0.2%); Group II: Control rats received EA (60 mg/kg body weight) dissolved in dimethyl sulphoxide (0.2%) using intra gastric intubation; Group III: Rats received iron as iron sulfate (30 mg/kg body weight) intraperitoneally in isotonic saline. Group IV: Rats received Fe (30 mg/kg body weight) followed by oral administration of EA (15 mg/kg body weight) in dimethyl sulphoxide (0.2%); Group V: Rats received Fe (30 mg/kg body weight) followed by oral administration of EA (30 mg/kg body weight) in dimethyl sulphoxide (0.2%); Group VI: Rats received Fe (30 mg/kg body weight) followed by oral administration of EA (60 mg/kg body weight) in dimethyl sulphoxide (0.2%) for 10 days. At the end of experiments the rats were made to fast overnight and killed by cervical dislocation after anaesthetizing them with intramuscular injection of ketamine hydrochloride (30mg/kg body weight).

Preparation of plasma

Blood was collected in a heparinized tube and plasma was separated by centrifugation at 1000g for 10 min for the estimation of biochemical parameters.

Preparation of hemolysate

After centrifugation, the buffy coat was removed and the packed cells were washed thrice with physiological saline. A known volume of erythrocyte was lysed with hypotonic phosphate buffer, PH 7.4, The haemolysate was separated by centrifugation at 2500 g for 15 min at 2^0c.

Estimation of total protein

The total protein present in the plasma was determined after trichloroacetic acid precipitation by the method of Lowry et al. [12].

Estimation of marker enzymes

To assess the membrane damage, activities of liver marker enzymes; aspartate transaminase (AST) and alanine transaminase (ALT) were assayed by the method of Reitman and Frankel [13], gamma-glutamyl transferase(GGT) by the method of Fiala etal [14] and alkaline phosphatase (ALP) by the method of King and Armstrong [15] were assayed.

A volume of 0.5 ml of buffered (for AST: aspartate α-keto glutarate and for ALT: alanine a-keto glutarate) substrate was incubated at 37^0c for 60 min. To this 0.5 ml dinitro phenyl hydrazine (DNPH), a colour reagent was added, mixed well and allowed to stand at room temp. for 20 min. after 20 min.5.0ml of 0.4N sodium hydroxide was added, mixed well and allowed to stand at room temperature for 10 min. a set of pyruvic acid standard was also treated in this same manner. Absorbance was read at 505 nm against water blank. The activity of AST was expressed as IU/plasma.

Alkaline phosphatase activity was assayed using disodium phenyl phosphate as the substrate. After pre incubation of buffer (0.1 M bicarbonate buffer PH 10) with the substrate for a min, 0.2ml of serum was added and incubated for 15min. at 37^0c. The liberated phenols from substrate react with folinphenol reagent (1ml). The suspensions were centrifuged and supernatant was collected. To this supernatant 2 ml of 10 % sodium bicarbonate was added and the colour developed was read at 680 nm after 10 min.

Gamma glutamyl transferase was analysed by adding 2 ml of buffer (Tris HCL 120mm, $mgcl_2$ 12mm glucyl glycine 90mm, PH 7.8) to 0.2ml substrate (L-?-glutamyl-Þ-nitro anilide 48 mm in 150 mm

HCL), warmed to 37^{0}c. to this 0.1ml serum was added, mixed and incubated at 37^{0}c. the reaction was then stopped by adding 2 ml of glacial acetic acid and the absorbance was read at 450nm.

Estmation of endogenous antioxidants

Reduced glutathione (GSH) was determined by the method of Ellman (16). To the 0.5ml plasma 10% of trichloro acetic acid (TCA) was added and centrifuged. After centrifugation, 1.0 ml of supernatant was treated with 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer. The absorbance was read at 412nm.

Vitamin E was estimated by the Baker and Frank method [17]. To 0.5 ml of plasma, 1.5 ml of ethanol and 2.0 ml of petroleum ether were added and centrifuged. The supernatant was evaporated to dryness at 80^{0}C. to this,0.2 ml of 2,2' dipyridyl solution and 0.2 ml of ferric chloride solution were added. It was mixed well and kept in dark for 5 min. to this 4ml of butanol was added.the intense red colour developed was read at 520nm.

Vitamin C was estimated by the Roe and Kuether method [18]. To 0.5 ml of 6% TCA was added and allowed to stand for 5 min and centrifuged. The supernatant was collected and 0.3g of nacid washed norit was added, shaken vigorously and filtered. To 2ml of the filtrate,0.5 ml DNPH was added,stoppered and placed in a water bath at 37^{0}C for exactly 3h. after the incubation, the tubes were mixed well and allowed to stand at room temperature for 30 min. the colour developed was read at 540 nm.

Superoxide dismutase (SOD) was assayed utilizing the technique of Kakkar et al. [19]. To 0.5 mL of plasma, ethanol and chloroform mixture was added and centrifuged. To the supernatant, assay mixture [sodium pyrophosphate buffer (0.025 M, pH 8.3), phenazine methosulphate, nitroblue tetrazolium and nicotinamide adenine dinucleotide: reduced (NADH)] was added and incubated at 30 _C for 90 s. The reaction was stopped by the addition of glacial acetic acid and mixed with n-butanol. The intensity of the chromogen present in the butanol was measured at 560 nm.

Catalase (CAT) was assayed colorimetrically at 620 nm and expressed as lmol of H2O2 consumed/min/mg protein as described by Sinha [20]. The reaction mixture contained phosphate buffer (0.01 M, pH 7.0), plasma and 2 M H2O2 and the reaction was stopped by the addition of dichromate-acetic acid reagents (5% potassium dichromate and glacial acetic acid were mixed in a ratio of 1 : 3).

Glutathione peroxidase (GPx) was assayed by the method of Rotruck et al. [21]. To 0.2 mL of plasma, 0.2 mL of phosphate buffer (0.4 M, pH 7.0), 0.2 mL EDTA, and 0.1 mL sodium azide, were added. To this mixture, 0.2 mL of glutathione, followed by 0.1 mL of hydrogen peroxide was added. The contents were mixed well and incubated at 37 _C for 10 min along with a control tube containing all reagents but no enzyme. After 10 min, the reaction was arrested by the addition of 0.4 mL of 10% TCA. The tubes were centrifuged and the supernatant was assayed for glutathione content by using Ellman's reagent. The activity of GPx was expressed as lmole of GSH utilized/min/mg protein.

Estimation of cholesterol

Plasma cholesterol was estimated by the method of Allain et al. [22] using a reagent kit. To 0.01 mL of plasma and/or standard, 1 mL of the cholesterol reagent was added, mixed and kept at 37 _C for 10 min. The colour developed was read at 505 nm against reagent blank colorimetrically. Plasma cholesterol values were expressed as mg/dL plasma.

Estimation of triglycerides

Plasma triglycerides (TG) were estimated by the method of Neagele et al. [23] using a reagent kit. To 5 lL each of plasma and standard, 500 lL of TG reagent was added, mixed well and incubated for 10 min at 37 _C. The absorbance was measured at 546 nm against reagent blank within 60 min. Absorbance of the quinoneimine dye measured at 546 nm was directly proportional to triglyceride concentration. The concentration of TG was expressed as mg/dL plasma.

Estimation of free fatty acids

Free fatty acids (FFA) were estimated by the method of Falholt et al. [24]. A volume of 0.1 mL lipid extract was evaporated to dryness. To this 1.0 mL phosphate buffer, 6.0 mL extraction solvent and 2.5 mL copper reagent were added. All the tubes were shaken vigorously for 90 s and were kept aside for 15 min. It was centrifuged and 3.0 mL of the upper layer was transferred to another tube containing 0.5 mL diphenylcarbazide solution and mixed carefully. The absorbance was read at 550 nm after 15 min. A volume of 1.0 mL phosphate buffer was treated as blank. The amount of FFA was expressed as mg/dL of plasma.

Estimation of phospholipids

Phospholipids were estimated by the method of Zilversmit and Davis [25]. An aliquot of the lipid extract was pipetted into a Kjeldhal flask and evaporated to dryness. A volume of 1.0 mL of 5 N H_2SO_4 was added and digested in a digestion rack till the appearance of light brown colour. Two to three drops of concentrated nitric acid was added and the digestion was continued until it become colourless. The Kjeldhal flask was cooled, 1.0 mL double-distilled water was added and heated in a boiling water bath for about 5 min. Then 1.0 mL of 2.5% ammonium molybdate and 0.1 mL of 1-amino-2-naphthol-4-sulphonic acid (ANSA) were added. The volume was then make upto 10 mL with distilled water. A series of standards containing 8–40 lg of phosphorus was treated similarly. The absorbance was measured at 660 nm within 10 min using a reagent blank. The values were expressed as mg/dL of plasma.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) and the groups were compared by Duncan's multiple range test (DMRT) using SPSS Software Package, version 11.0. Results were expressed as mean ± SD for six rats in each group. A value of P £ 0.05 was considered to be statistically significant.

RESULTS

Liver marker enzymes

Table I shows the activities of hepatic marker enzymes in the plasma of both control and experimental rats. The activities of GGT, AST, ALT and ALP were significantly increased in group III animals when compared with control animals. EA (group V to VI) co-administration with Fe decreased the activities of liver marker enzymes in the circulation. The decrease was more significant in the 60 mg/kg body weight treated group of EA when compared with 15 and 30 mg/kg body weight treatments.

Table 1. Changes in the activities of plasma AST, ALT, ALP, LDH and Bilirubin in control and experimental rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)
Control	70.35 – 3.44[a]	27.36– 2.05[a]	81.19 – 6.07[a]	0.50 – 0.05[a]
Normal + EA (60mg/kg)	71.36 – 3.48[a]	28.28 – 2.61[a]	81.61 – 6.02[a]	0.50 – 0.05[a]
Normal + Iron (30 mg/kg)	130.90 – 5.17[b]	85.63 – 3.14[b]	176.05 – 9.29[b]	1.25 – 0.07[b]
Iron (30 mg/kg) + EA (15 mg/kg)	119.46 – 5.93[c]	45.75 – 2.96[c]	143.20 – 8.63[c]	0.78 – 0.06[c]
Iron (30 mg/kg) + EA (30 mg/kg)	93.24– 5.51[d]	33.42 –2.74[d]	131.61 – 7.87[d]	0.80 – 0.06[d]
Iron (30 mg/kg) + EA (60 mg/kg)	76.01 – 3.77[e]	30.80 – 2.69[e]	98.54 – 8.57[e]	0.57 – 0.06[ad]

EA - Ellagic acid; Iron. Values are mean ± SD for 6 rats in each group. a-e In each columns, means with different superscript letter differ significantly at p<0.05 (DMRT).

Table 2. Changes in the levels of vitamin C, vitamin E and GSH in plasma of control and experimental rats

Groups	Vitamin C (mg/dl)	Vitamin E (mg/dl)	GSH (mg/dl)
Control	1.89 – 0.09[a]	1.37 – 0.08[a]	21.93 – 1.20[a]
Normal + EA (60 mg/kg)	1.97 – 0.11[a]	1.41 – 0.12[a]	22.08 – 1.56[a]
Normal + Iron (30 mg/kg)	1.57 – 0.07[b]	0.92 – 0.06[b]	17.16 – 1.09[b]
Iron (30 mg/kg) + EA (60 mg/kg)	1.73 – 0.08[c]	1.24 – 0.10[c]	20.04 – 1.01[c]

EA - Ellagic acid;Iron; Values are mean ± SD for 6 rats in each group.
a-c In each columns, means with different superscript letter differ significantly at p<0.05 (DMRT).

Table 3. Changes in the activities of SOD, CAT, GST and GPX in plasma of control and experimental rats

Groups	SOD (Units[#]/mg protein)	CAT (Units[#]/mg protein)	GPX (Units[#]/mg protein)
Control	6.54 – 0.53[a]	72.51 – 5.01[a]	5.04 – 0.43[a]
Normal + EA (60 mg/kg)	7.31 – 0.55[a]	76.41– 5.24[a]	5.29 – 0.46[a]
Normal + Iron (30 mg/kg)	4.43 – 0.15[b]	37.01 – 3.53[b]	2.62 – 0.24[b]
Iron (30 mg/kg) + EA (60 mg/kg)	5.70 – 0.22[c]	56.06 – 4.32[c]	4.03 – 0.30[c]

EA - Ellagic acid; Iron. Values are mean ± SD for 6 rats in each group.
[a-c] In each columns, means with different superscript letter differ significantly at p<0.05 (DMRT).
[#] Units of enzyme activities are expresses as:
SOD - One unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in one minute.
CAT - μ moles of hydrogen peroxide consumed / minute.
GST - μ moles of CDNB-GSH conjugate formed / minute.
GPX - μ moles of glutathione consumed / minute.

Table 4. Changes in the levels of plasma cholesterol, triglycerides, free fatty acids and phospholipids in control and experimental rats

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Free fatty acids (mg/dl)	Phospholipids (mg/dl)
Control	1.25 – 0.31[a]	0.18 – 0.25[a]	1.12 – 0.28[a]	1.45 – 0.72[a]
Normal + EA (60 mg/kg)	1.26 – 0.29[a]	0.19 – 0.26[a]	1.14 – 0.20[a]	1.39 – 0.41[a]
Normal + Iron (30 mg/kg)	1.58 – 0.24[b]	0.56 – 0.68[b]	1.45 – 0.32[b]	1.57 – 0.72[b]
Iron (30 mg/kg) + EA (60 mg/kg)	1.28 – 0.27[c]	0.21 – 0.28[c]	1.14 – 0.28[c]	1.47 – 0.87[c]

EA - Ellagic acid; Iron
Values are mean ± SD for 6 rats in each group. [a-c] In each columns, means with different superscript letter differ significantly at p<0.05 (DMRT).

ENDOGENOUS ANTIOXIDANTS

The influence of EA on the endogenous antioxidants of Fe-fed rats is shown in Tables II and III. The levels of non-enzymatic antioxidants: vitamin C and E and GSH, and activities of enzymatic antioxidants, viz. SOD, CAT and GPx were significantly depleted in the Fe groups which were positively modulated by EA treatment. Better positive modulation was observed on treatment with 60 mg/kg body weight of EA when compared with the other two doses (15 and 30 mg/kg body weight).

Plasma lipids

Iron administration significantly increased the levels of lipids including cholesterol, TG, fatty acids and phospholipids in the circulation (Table IV). Co-administration of EA along with Fe significantly decreased the lipid levels when compared with the Fe-fed group.

DISCUSSION

Involvement of ROS and alterations in cellular metabolism are the important mechanisms, which play a vital role during Fe induced toxicity. Therefore, use of dietary antioxidants is an important preventive method to minimize the pathological and toxic effects associated with oxidative stress [26,27]. Plasma GGT, AST, ALT and ALP are the sensitive markers for drug-induced liver damage. GGT is a glycoprotein present in the hepatocyte plasma membrane. Serum GGT activity was used as a marker for excessive Fe consumption in clinical practice [28]. AST and ALT are cytosolic enzymes whereas ALP is

an ectoenzyme of the hepatocyte plasma membrane. The elevated activities of these marker enzymes in plasma are indicative of cellular leakage and loss of the functional integrity of cell membranes in the liver [29,30]. Consistent with an earlier report [31], we observed increased activities of liver marker enzymes in circulation during Fe administration. Treatment with EA at different doses (15, 30 and 60 mg/kg body weight) effectively decreased the activities of the liver marker enzymes (GGT, AST, ALT and ALP) in plasma when compared with the Fe-induced group. As the polyphenols are known antioxidants [32], they reduce the concentration of oxidation products by scavenging free radicals. EA being a phenolic compound might have exerted antioxidant- sparing action by scavenging free radicals.

Furthermore, studies have shown that two lactone groups of EA (Figure 1) can act as both hydrogen bond donor and acceptor [33], which might also involve free radical scavenging potential. Thus, EA quenches free radicals and inhibits peroxidation of lipids thereby preserving the structural integrity of hepatocellular membrane by the liver cell membrane-stabilizing action and consequently prevents the leakage of liver markers into the circulation. It has also been reported that oral administration of EA decreases the activities of liver marker enzymes during CCl4-induced toxicity [34]. As the liver is the primary organ for many metabolic processes, damage to the liver causes weight loss. Induction of xenobiotic detoxifying enzymes is an important mechanism by which plant phenols are capable of competing with steps in xenobiotic activation [35]. EA has also been reported to influence xenobiotics metabolism via activation of phase II enzymes [36]. Because of its antioxidant property, EA has been expected to interact with metabolites of Fe and neutralize their toxic effects on liver and thus helps in improving body weight. Vitamin E is a major lipophilic antioxidant and vitamin C is an essential water-soluble antioxidant that play vital role in preventing oxidative stress [37]. GSH is an important cellular reductant involved in the protection of cells against free radicals, peroxides and other toxic compounds [38]. GPx and CAT acts as preventive antioxidants and SOD is a chain-breaking antioxidant, which plays a vital role in the protection of cells against the deleterious effects of lipid peroxidation [39]. The decreased antioxidant status in Fe-administered rats in our study may be because of the increased utilization of these antioxidants to counteract the ROS generated by Fe. Co-administration of EA resulted in normalization of the antioxidants in the circulation. EA effectively scavenges O2 •-, hydroxyl radical, peroxy radical and peroxynitrite [40], decreases activities of cytochrome P450 particularly cytochrome P450 2E1 (CYP2E1), which plays a vital role in the generation of oxidative stress during Fe-induced toxicity. Hence, EA co-administration might have decreased the utility of antioxidants by (i) reacting with reactive oxygen species and (ii) affecting the metabolic pathways of Fe.

Further, reports suggest that EA besides acting as an antioxidant and also enhances the GSH dependent protection [41]. Thus, EA has been expected to restore the activities of antioxidants in circulation.

The results showed increased levels of cholesterol, TG, FFA and phospholipids in the Fe-administered group. Administration of EA effectively reduced the level of lipids in the circulation. EA effectively prevents lipid peroxidation [42], which paralleled the inhibition of membrane damage and prevents release of FFA from the membrane. As FFA is the substrate for other lipids, its decrease may reflect on the level of other lipids. In addition, many polyphenolic compounds possess hypolipidaemic activity and have been shown to increase the fecal fat excretion and low-density lipoprotein (LDL) receptor activity [43]. EA being a polyphenolic compound might possess the same hypolipidaemic activity. EA has been reported to affect the cellular lipid metabolism during benzoyl peroxide-induced toxicity [44]. Furthermore, studies have shown that EA has some protective effects against LDL oxidation [45]. Yu et al. [46] have reported that EA supplementation effectively reduced the elevations of plasma cholesterol in hyperlipidaemic rabbits. It can be speculated that EA might have decreased the

activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, which is a rate-limiting enzyme involved in the cholesterol metabolism or enhanced the rate of lipid degradative process and increased the hepatic bile acids and fecal neutral sterol and thus decreased the level of other lipids.

In this study, all the three doses of EA did not produce same effects. The lower dose of EA (15 mg/kg body weight) was not effective, because its concentration might not have been enough to quench all free radicals generated. The high dose of EA (30 mg/kg body weight) was not as effective as the medium dose (60 mg/kg body weight) because at higher concentration EA might react with some other ligands in the system and thus might not be completely available for quenching free radicals. Based upon the results, we found that EA (60 mg/kg body weight) was most effective in preventing the toxic effects of Fe.

CONCLUSION

To summarize, the findings of the present study indicate that co-administration of EA with Fe decreases Fe-mediated damage by decreasing the liver marker enzymes, improving the antioxidant status and attenuating the lipid levels in the circulation. Among the three doses of EA, 60 mg/kg body weight was found to be more effective in modulating Fe-induced toxicity. However, the mechanisms and probable mode of action of EA need to be studied in greater detail.

REFERENCES

1.Naika G.H., Priyadarsinia K.I., Naika D.B., Gangabhagirathib R., Mohan H. Studies on the aqueous extract of Terminalia chebula as a potent antioxidant and a probable radioprotector. Phytomedicine (2004) 11 530–538.

2.Yesilada E., Ustun O., Sezik E., Takaishi Y., Ono Y., Honda G. Inhibitory effects of Turkish folk remedies on inflammatory cytokines: interleukin-1a, interleukin-1b and tumor necrosis factor _. J. Ethanopharmacol. (1997) 58 59–73.

3.Rachana T., Shailesh S.D., Girish B.M. Mechanism(s) of turmeric-mediated protective effects against benzo(a)pyrenederived DNA adducts. Cancer Lett. (2002) 175 79–88.

4.Rukkumani R., Aruna K., Varma P.S., Menon V.P. Comparative effects of curcumin and an analog of curcumin and PUFA induced oxidative stress. J. Pharm. Pharm. Sci. (2004) 7 274–283.

5.Murakami A., Nakamura Y., Koshimizu K. et al. FA15, a hydrophobic derivative of ferulic acid, suppresses inflammatory responses and skin tumor promotion: comparision with ferulic

6.acid. Cancer Lett. (2002) 180 121–129.

7.Susanne U., Mertens-Talcot T., Percival S.S. Ellagic acid and quercetin interact synergistically with resveratrol in the induction of apoptosis and cause transient cell cycle arrest in human leukemia cells. Cancer Lett. (2005) 218 141–151.

8.Corthout J., Pieters L.A., Claeys M., Berghe V., Vlietinck A.J. Antiviral ellagitannins from Spondia mombin. Phytochemistry (1991) 30 1129–1130.

9.Rao C.V., Tokumo K., Rigotty J., Zang E., Kelloff G., Reddy B.S. Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, difluoromethlornithine, 16b-fluor-5-androsten-17-one and ellagic acid individually and in combination. Cancer Res. (1991) 51 4528–4534.

10.Stoner G.D., Morse M.A. Isocyanates and plant polyphenols as inhibitors of lung and esophageal cancer. Cancer Lett. (1997) 114 113–119.

11.Iino T., Tashima K., Umeda M. et al. Effect of ellagic acid on gastric damage induced in ischemic rat stomachs following ammonia or reperfusion. Life Sci. (2002) 70 1139–1150.

12.Kilaru S., Frangos S.G., Chen A.H. et al. Nicotine: a review of its role in atherosclerosis. J. Am. Coll.

Surg. (2001) 193 538–546.
13.Lowry O.H., Rosebrough M.J., Farr L., Randall R.J. Protein measurement with the folin-phenol reagent. J. Biol. Chem. (1951) 93 464–478.
14.Reitman S., Frankel A.S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. (1957) 28 53–56.
15.Fiala S., Fiala A.E., Dixon B. Gamma glutamyl transpeptidase in chemically induced rat hepatomas and spontaneous mouse hepatomas. J. Natl Cancer Inst. (1972) 48 1393–1409.
16.King E.J. Armstrong A.R. Calcium, phosphorus and phosphatases, in: Varley H. (Ed), Practical Clinical Biochemistry, CBS Publishers, New Delhi, 1988, pp. 458.
17.Ellman G.L. Tissue sulphydryl groups. Arch. Biochem. Biophy. (1959) 82 70–77.
18.Baker H., Frank O., DeAngelis B., Friengold S. Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. Nutr. Res. Inst. (1980) 21 531–536.
19.Roe K.M., Kuether C.A. Detection of ascorbic acid in whole blood and urine through 2,4-DNPH derivative of dehydro ascorbic acid. J. Biol. Chem. (1943) 147 399–407.
20.Kakkar P., Das B., Viswanathan P.N. A modified spectrophotometric assay of superoxide dismutase (SOD). Indian J. Biochem. Biophys. (1984) 21 130–132.
21.Sinha K.A. Colorimetric assay of catalase. Anal. Biochem. (1972) 47 389–394.
22.Rotruck J.T., Pope A.L., Ganther H.E., Swanson A.B., Hafeman D.G., Hoekstra W.G. Selenium: biochemical rates as a component of glutathione peroxidase. Science (1973) 179 588–590.
23.Taylor A., Bryant T.N. Comparison of procedures for determination of copper and zinc in serum by atomic absorption spectroscopy. Clin. Chim. Acta (1981) 110 83–90.
24.Allain E.C., Poon L.S., Chan C.S., Richmond W., Fu F.C. Enzymatic determination of total serum cholesterol. Clin. Chem.(1974) 20 470–475.
25.Neagele U. Single step colorimetric determination of triglycerides. Clin. Chem. Biochem. (1984) 22 165–167.
26.Falholt K., Falholt W., Lund B. An easy colorimetric method for routine determination of free fatty acids in plasma. Clin. Chim. Acta (1973) 46 105.
27.Zilversmit D.B., Davis A.K. Micro determination of plasma phospholipids by trichloroacetic acid precipitation. J. Lab. Clin.Med. (1950) 35 155.
28.Steinmetz K.A., Potter J.D. Vegetables, fruit and cancer. I. Epidemiology. Cancer Causes Control (1991) 2 427–442.
29.Ames B.N., Shigeng M.K., Hagen T.M. Oxidants, antioxidants and the degenerative diseases of aging. Proc. Natl. Acad. Sci. (1993) 90 7915–7922.
30.Teschke R., Brand A., Strohmeyer G. Induction of hepatic microsomal gamma-glutamyl transferase activity following chronic Fe consumption. Biochem. Biophys. Res. Commun.
31.(1977) 75 718–724.
32.Drotman R.B., Lacohorn G.T. Serum enzymes as indicators of chemical-induced liver damage. Drug Chem. Toxicol. (1978) 1 163–171.
33.Rao R.R. Mechanism of drug induced hepato toxicity. Indian J. Pharmacol. (1973) 5 313–318.
34.Varma P.S., Aruna K., Rukkumani R., Menon V.P. Fe and thermally oxidized PUFA induced oxidative stress: role of N-acetyl cysteine. Italian J. Biochem. (2004) 53 10–15.
35.Lee J., Koo N., Min D.B. Reactive oxygen species, aging, and antioxidative nutraceuticals. Compr. Rev. Food. Sci. Food Saf. (2004) 3 21–33.
36.Bala I., Bhardwaj V., Hariharan S., Ravikumar M.N.V. Analytical methods for assay of ellagic acid and its solubility studies. J. Pharm. Biomed. Anal. (2006) 40 206–210.

37.Thresiamma K.C., Kuttan R. Inhibition of liver fibrosis by ellagic acid. Indian J. Physiol. Pharmacol. (1996) 40 363–366.
38.Suresh V.P., Aruna K., Rukkumani R., Viswanathan P., Venugopal P.M. Protective effect of n-acetyl cysteine treatment on Fe- and heated sunflower oil-induced hyperlipidaemia. Alc. Res. (2004) 9 266–272.
39.Manson M.M., Bal H.W., Barrett M.C. et al. Mechanism of action of dietary chemoprotective agents in rat liver: induction of phase I and II drug metabolizing enzymes and aflatoxin B1 metabolism. Carcinogenesis (1997) 18 1729–1738.
40.Ahn D., Putt D., Kresty L., Stoner G.D., Fromm D., Hollenberg P.F. The effects of dietary ellagic acid on rat hepatic and esophageal mucosal cytochromes P450 and phase II enzymes. Carcinogenesis (1996) 17 821–828.
41.Ray G., Husain S.A. Oxidants, antioxidants and carcinogenesis. Indian J. Exp. Biol. (2002) 40 1213–1232.
42.Gerster H. b-carotene, vitamin E and vitamin C in different stages of experimental carcinogenesis. Eur. J. Clin Nutr. (1995) 49 155–168.
43.Dinkova-Kostova A.T. Protection against cancer by plant phenyl propenoids: induction of mammalian anticarcinogenic enzymes. Mini Rev. Med. Chem. (2002) 2 595–610.
44.Murakami M., Asagoe K., Dekigai H., Kusaka S., Saita H., Kita T. Products of neutrophil metabolism increase ammonia induced gastric mucosal damage. Dig. Dis. Sci. (1995) 40 268– 273.
45.Khanduja K.L., Gandhi R.K., Pathania V., Syal N. Prevention of N-nitrosodiethylamine-induced lung tumorigenesis by ellagic acid and quercetin in mice. Food Chem. Toxicol. (1999) 37 313–318.
46.Singh K., Khanna A.K., Chander R. Hepatoprotective activity of ellagic acid carbon tetrachloride induced hepatotoxicity in rats. Indian J. Exp. Biol. (1999) 37 1025–1026.

Publish Research Article
International Level Multidisciplinary Research Journal
For All Subjects

Dear Sir/Mam,

We invite unpublished Research Paper,Summary of Research Project,Theses,Books and Book Review for publication,you will be pleased to know that our journals are

Associated and Indexed,India

- International Scientific Journal Consortium
- OPEN J-GATE

Associated and Indexed,USA

- EBSCO
- Index Copernicus
- Publication Index
- Academic Journal Database
- Contemporary Research Index
- Academic Paper Databse
- Digital Journals Database
- Current Index to Scholarly Journals
- Elite Scientific Journal Archive
- Directory Of Academic Resources
- Scholar Journal Index
- Recent Science Index
- Scientific Resources Database
- Directory Of Research Journal Indexing

Golden Research Thoughts
258/34 Raviwar Peth Solapur-413005,Maharashtra
Contact-9595359435
E-Mail-ayisrj@yahoo.in/ayisrj2011@gmail.com
Website : www.aygrt.isrj.org