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BENEFICIAL EFFECT OF ELLAGIC ACID AGAINST IRON INDUCED OXIDATIVE DAMAGE IN THE LIVER OF RATS

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Short Profile

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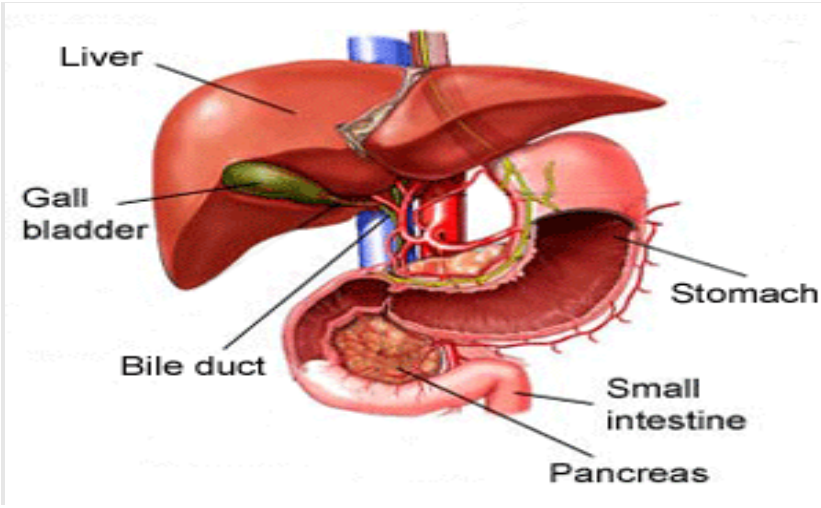

ABSTRACT:

Male wistar rats injected i.p with 30 mg Fe^{2+} / kg (Ferrous sulfate) body weight for 10 days. Show hepatic damage as measured by an increase in renal markers namely aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT), bilirubin, urea, cretinine and a significance decrease in creatinine clearance. Plasma thiobarbituric acid – reactive substance and hydroperoxide were significantly elevated where as the levels of non enzymatic antioxidants (reduced glutathione, vitamin E and vitamin C) were significantly decreased in iron treated rats. Administrations of Ellagic acid (EA) at 60mg / kg body weight significantly decreased the levels of antioxidants were significantly increased in circulation of iron – fed rats. All these results were accompanied by histological observations in liver. The results demonstrate that ellagic acid has a beneficial effect in the iron – induced hepatic damage by lowering lipid peroxidation.

KEYWORDS
Iron, Ellagic acid, hepatic damage, biochemical changes.

INTRODUCTION :

Iron is an essential constituent of the body, being found in functional form in haemoglobin, myoglobin, the cytochromes, enzyme with iron sulphur complexes and other iron dependent enzymes. At the same time, excess iron in the body is associated with toxic effects and poses health problems[1]. The toxic effects of iron overloading leads to chronic liver disease impaired cardiac function diabetes mellitus endocrinopathies, skin pigmentation and orthopathy[2, 3]. Hepatotoxicity is the most common finding in patient with iron overloading. The massive deposition of iron in hepatic parenchymal cells eventually produces fibrosis and ultimately results in cirrhosis[4].

One of the mechanisms by which iron induces the toxicity is by increasing oxidative stress and in vitro and lipid peroxidation. The role of iron in invivo and invitro lipid peroxidation has been well studied5. The ability of iron to catalyze the formation of reactive oxygen species, including the hydroxyl radical (OH), has been extensively reviewed [5,6,7]. Liver damage by iron toxicity can also be assessed by leakage of enzymes such as Aspartate transaminase (AST), Alanine transaminases (ALT), alkaline phosphatase (ALP), and Gama glutamyl transaminase (GGT), Lactate dehydrogenase (LDH)[8,9]

The use of medicinal plants is getting popularized in developing and developed countries for the treatment of various ailments[10,11] . Presently, the active constituents of these plant sources are extracted, purified and tested for their activities. Results are promising that phytochemicals are well established to exert their benefits in the prevention and therapy of many diseases. Phenolic phytochemicals possesses phenolic ring and hydroxyl substituents and its ability to quench free radicals and prevent cellular damage, can function as effective antioxidants. Several studies have shown that plant – derived polyphenolic antioxidants exhibit anti-inflammatory, antimutagenic, anticarcinogenic, antiviral, and antioxidant activities[12]. Ellagic acid (EA) is one of the polyphenolic compounds found in a wide variety of fruits and nuts[13] including blackberries, blueberries, raspberries, strawberries and walnuts[14]. EA (Figure-1) contains four hydroxyl groups and two lactone groups in which hydroxyl group is known to increase antioxidant activity in lipid peroxidation and protects cells from oxidative damage[15]. Recent reports have shown that oral administration of EA exerts a protective influence against cyclosporine A. induced toxicity[16].

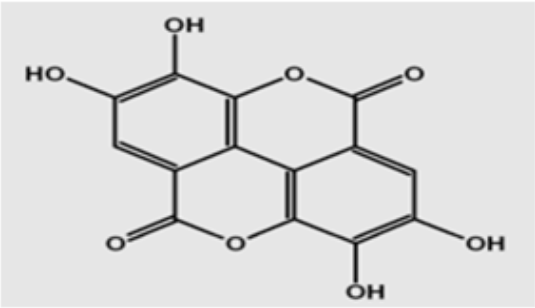

Figure-1. Structure of Ellagic acid.

In this study was carried out to investigate the beneficial effect of ellagic acid on iron induced biochemical changes in the liver.

MATERIALS AND METHODS:

Adult male albino wistar rats (8 Weeks old), weighing 180-210g bred in the central Animal House, Rajan muthiah medical college, Annamalai University. The animals were housed in plastic cages and maintained in a room with a 12-h day night cycle, temperature of 26±4°c and humidity of 50-75%. 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%) for 10 days.
Group II	:	Control rats received EA (60 mg/kg body weight) dissolved in dimethyl sulphoxide (0.2%) for 10 days using intra gastric intubation.
Group III	:	Rats received iron as iron sulfate (30 mg/kg body weight) intraperitoneally in isotonic saline for 10 days
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%) for 10 days.
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%) for 10 days.
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 the experiments, animals in different groups were killed by decapitation. Blood was collected in two different tubes, one heparinized for plasma, and the other not heparinized for serum separation. Serum and plasma were separated by centrifugation and used for various biochemical estimations.

BIOCHEMICAL DETERMINATION

The activities of serum aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) were assayed spectrophotometrically according to the standard procedures using commercially available diagnostic kits (Sigma diagnostics (1) Pvt Ltd, Baroda, India). Gamma- glutamyl transferase (GGT) activity was determined by using γ- glutamyl – p –nitroanilide as the substrate[17]. Serum bilirubin level was estimated by the method of malloy and Evelyn[18]. The levels of LPO was assessed by measuring the levels of thiobarbituric acid – reactive substances (TBARS) and lipid hydroperoxide in the plasma following the procedure of Nichans and samuelson19,20. Respectively Reduced glutothione (GSH)[21] ascorbic acid[22] and a – tocophenol[23] were estimated in the plasma according to the procedures described previously.

HISTOPATHOLOGICAL STUDIES

The liver samples fixed for 48h in 10% formalin were dehydrated by passing successfully in different mixture of ethyl alcohol and water, cleaned in xylene and embedded in paraffin. Section of liver (5-6 m thick) were prepared and then stained with hematoxylin and eosin dye (H&E) and mounted in neutral deparaffinated xylene (DPX) medium for microscopic observations.

STATISTICAL ANALYSIS

Data were analyzed by one–way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using a statistically software package (SPSS for windows, v. Chicago, IT, USA). Result is presented as mean ± SD. P-values < 0.05 were considered to be statistically significant.

RESULTS

The activities of AST, ALT, ALP, GGT and the levels of bilirubin, urea and creatinine were significantly ($p<0.05$) increased, whereas the levels of creatinine clearance was significantly ($p<0.05$) decreased in iron groups when compared with control rats. However, the rats administered iron and ellagic acid showed significantly ($p<0.05$) decreased activities of serum AST, ALT, GGT, bilirubin, urea and creatinine, with restoration of creatinine clearance in a dose dependent manner in comparison to the iron alone group. EA at 60 mg / body weight was more effective when compared with two other doses (15 and 30 mg / body weight). Hence, EA at 60 mg / body weight was used for further biochemical studies.

In table 2, the levels of LPO (measured by the levels of TBARS and lipid hydroperoxides) was increased significantly ($p<0.05$) and the levels of non enzymatic antioxidants (vitamin C, vitamin E and reduced glutathione) were significantly decreased ($p<0.05$) in the iron treated rats. Administration of EA significantly decreased the lipid peroxidation products and significantly increased the levels of non enzymatic antioxidants compared to the iron alone group.

Table 1. Changes in the activities of serum hepatic markers in control and experimental rats

Groups	Control	Normal +EA (60 mg/kg)	Normal + Fe (30 mg/kg)	Fe (30 mg/kg) +EA (15 mg/ kg)	Fe (30 mg/kg) +EA (30 mg/ kg)	Fe (30 mg/kg) +EA (60 mg/ kg)
AST (IU/l)	58.62 – 4.44[a]	59.13 – 4.48[a]	86.73 – 6.17[b]	79.46 – 5.93[c]	73.24 – 5.51[d]	66.01 – 4.77[e]
ALT (IU/l)	29.36 – 2.05[a]	30.28 – 2.61[a]	45.63 – 3.14[b]	41.75– 2.96[c]	37.42 – 2.74[d]	33.80 – 2.69[e]
ALP (IU/l)	92.19 – 8.07[a]	91.61 – 8.02[a]	146.05 – 11.49[b]	133.20 – 10.83[c]	121.61 – 9.87[d]	104.54 –9.57[e]
LDH (IU/l)	109.60 –8.51[a]	109.47 – 8.53[a]	161.30 – 14.03[b]	148.51 – 11.69[c]	135.97 – 10.52[d]	123.37 –9.21[e]
GGT (IU/l)	0.70 – 0.05[a]	0.70 – 0.05[a]	0.95 – 0.07[b]	0.88 – 0.06[c]	0.81 – 0.06[d]	0.76 – 0.06[e]
Bilirubin (mg/dl)	0.76 – 0.06[a]	0.74– 0.06[a]	1.27 – 0.11[b]	1.08 – 0.09[c]	0.98 – 0.08[d]	0.86 –0.07[e]

Fe –Iron; EA –Ellagic acid. Values are given as mean ? S.D from 6 rats in each group. Values not sharing common superscripts letters (a-e) differ significantly at p<0.05 (DMRT).

Table 2. Changes in the levels of lipid peroxidation and nonenzymatic antioxidant status in liver experimental rats

Parameters	Control	Normal + EA (60mg/kg)	Normal + Fe (30mg/kg)	Fe (30mg/kg) + EA (60mg/kg)
TBARS (mM / g tissue)	9.10 – 0.59[a]	8.69 – 0.52[a]	17.42 – 1.30[b]	10.48 – 0.72[c]
Hydroperoxides (mM / g tissue)	0.85 – 0.06[a]	0.83 – 0.05[a]	1.33 – 0.09[b]	0.97 – 0.06[c]
Vitamin C (mmole / mg tissue)	1.61 – 0.07[a]	1.64 – 0.07[a]	1.16 – 0.05[b]	1.47 – 0.06[c]
Vitamin E (mmole / mg tissue)	0.80 – 0.06[a]	0.85 – 0.07[a]	0.51 – 0.04[b]	0.64 – 0.05[c]
GSH (mg/ g tissue)	5.21 – 0.20[a]	5.30 – 0.29[a]	3.84 – 0.16[b]	4.53 – 0.22[c]

Fe –Iron; EA –Ellagic acid.. Values are given as mean ? S.D from 6 rats in each group. Values not sharing common superscripts letters (a-c) differ significantly at p<0.05 (DMRT).

Table 3. Changes in the activities of liver antioxidant enzymes in control and experimental rats.

Parameters	Control	Normal + EA (60mg/kg)	Normal + Fe (30mg/kg)	Fe (30mg/kg) + EA (60mg/kg)
SOD (units#/ mg protein)	8.74 – 0.53 [a]	9.31 – 0.75 [a]	6.43 – 0.30 [b]	7.70 – 0.42 [c]
CAT (units#/ mg protein)	92.51 – 6.01 [a]	96.41 – 5.34 [a]	57.01 – 3.73 [b]	76.06 – 4.52 [c]
GPX (units#/ mg protein)	8.04 – 0.43 [a]	8.29 – 0.46 [a]	5.62 – 0.24 [b]	7.03 – 0.30 [c]
GST (units#/ mg protein)	8.99 – 0.43 [a]	9.11 – 0.38 [a]	6.91 – 0.25 [b]	8.09 – 0.32 [c]

Fe –Iron; EA –Ellagic acid. Values are given as mean ± S.D from 6 rats in each group. Values not sharing common superscripts letters (a-c) 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.

GPx - μg of glutathione consumed / minute.

GST- μ moles of CDNB-GSH conjugate formation / minute.

Histopathological studies showed that iron administration induces the pathological changes in liver including focal hepatic necrosis with inflammatory cell cirrhosis (fig. 3). The liver was almost of normal appearance with mild changes in hepatocytes of rats treated with ellagic acid and iron (fig.4). Control (fig.2) and control rats treated with EA showed normal liver histology without any alterations (fig.5).

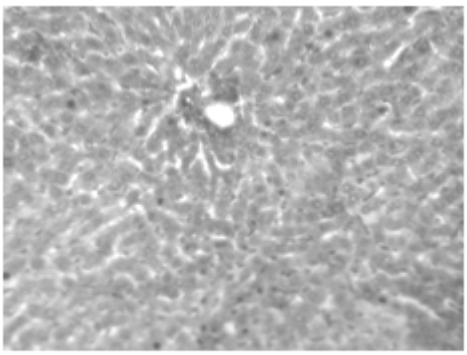

Figure 2: Control rat liver H&E x 20: Liver showing normal architecture

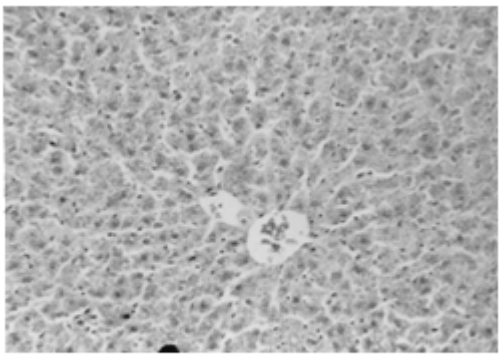

Figure 3 Normal + EA (60 mg/kg) treated rat liver H&E x 20: Normal appearance of liver

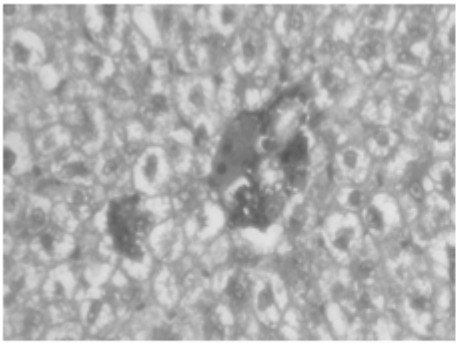

Figure. 4 Normal + Fe (30 mg/kg) treated rat liver H&E x 20: feathery degeneration

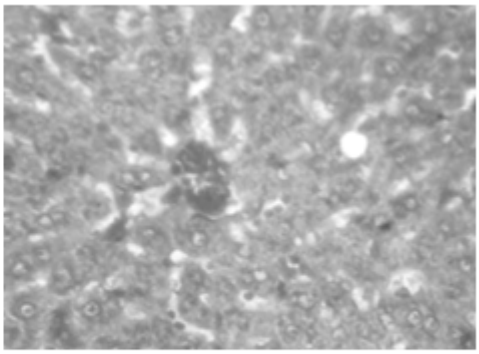

Figure. 5 Fe+ EA (60mg/kg) treated rats liver H&E x 20: Normal hepatocytes with mild portal inflammation

DISCUSSION:

The serum enzymes are very important adjuncts to clinical diagnosis of disease and tissue damage. AST, ALT, ALP, LDH and GGT have been used as sensitive indicators of liver damage [8,9,24]. Higher activities of these enzymes in blood have been found in response to oxidative stress. The i.p. injection of iron significantly elevated the hepatic lipid peroxides and serum ALP, ALT, AST, LDH and GGT. Administration of EA significantly decrease the activities of ALP, ALT, AST, LDH and GGT levels, suggesting that they ofter production by preserving the structural integrity of the hepatocellular membrane against CSA[12]. In this context, a recent report showed the protective effects of EA on liver and heart toxicity induced by cisplatin25 and the productive effects of EA on cyclosporine-A induced oxidative damage in the liver rats[16].

Iron overloading in rats is an excellent model to study the in vivo lipid peroxidation. Jacob[26] presented the direct evidences to indicate that the lipid peroxidation of membranes is the major damaging factor in iron toxicity[27] reported that feeding 2.5% iron (carboxyl iron) in the diets induced higher levels of conjucated dienes in hepatic microsomes of rats. Evidences for iron-induced lipid peroxidation in hepatic mitochondria and microsomes have been found in studies using rats with chronic dietary iron overload28. Iron plays on important role in lipid peroxidation. The ability of iron to accelerate lipid peroxidation is well-established[29,5]. The primary mechanism for this acceleration is believed to be the iron-catalyzed decomposition of lipid peroxides. The results of the present study demonstrated that excess iron introduced by i.p. injection induced oxidative stress by increasing lipid peroxide levels in liver as well as in serum.

$$ROOH + Fe^{2+} \longrightarrow Fe^{3+} + Ro^{o} + {}^{-}OH$$

$$ROOH + Fe^{3+} \longrightarrow Fe^{2+} + Roo^{o} + H^{+}$$

Oral administration of EA for 10 days. EA offered production through attenuation of lipid peroxidation and production of free radical derivatives as evident from decreased level of lipid peroxidation markers[30]. The antioxidant activities of EA shows that it scavenges the free radicals (superoxide anion, hydroxyl radical and peroxy radical) and quenches the lipid peroxidation markers[31]. Thus, EA offers production to cells from lipid peroxidation against oxidative stress by scavenging free

radicals.

Iron was associated with significant changes of the oxidant status in plasma and liver. Vitamin C concentrations in plasma iron rats were significantly decreased compared with control animals. Vitamin C deficiency as assessed by levels of leucocyte ascorbic acid, has been reported in patients with idiopathic haemochromatosis[32] out observations that dietary iron led to a marked decreased in both plasma and hepatic α-tocopherol levels agree with previous data[33,34]. Although a-tocopherol was the only lipid soluble antioxidant detectable in rat plasma. Iron also decreased in GSH levels.

Administration of EA (60 mg / kg body weight) significantly increased the levels of non-enzymatic antioxidants in plasma. Which show protection of cell membrane damage based on the above results, it could be concluded that EA is a hepato-stimulant and exerts a significant hepatoprotection against iron induced toxicity.

In conclusion, this study demonstrates that EA can effectively lower the hepatic injury caused by iron over loading as monitored by lipid peroxides, sensitive serum enzymes levels and non enzymatic antioxidants.

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