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Protective Effect of Turmeric, *Ginkgo biloba*, Silymarin Separately or in Combination, on Iron-Induced Oxidative Stress and Lipid Peroxidation in Rats

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Abstract: The present study is designed to investigate the protection conferred by dietary supplementation of turmeric, *Ginkgo biloba* extract, silymarin separately or in combination in comparison to vitamin E against iron-induced oxidative stress. We, also, studied the effect of these agents on the activities of cytosolic and (m) EH hydrolase as well as GST. Results revealed that exposing the rats to iron overload 6 h before decapitation caused a significant rise in hepatic and plasma TBARS by 1200 and 166%, respectively. This was associated with depletion in blood lysate GSH (59%) and significant induction of hepatic and erythrocyte SOD; hepatic CAT (186.6%); GST (118%), (s)EH (122.8%) and (m)EH (295%) as compared to normal control group. Pretreatment with turmeric caused a significant repletion of blood lysate GSH levels and a significant rise in hepatic SOD. Also, it counteracted the rise in plasma TBARS levels, erythrocyte SOD and (s)EH caused by iron. In comparison to iron-loaded rats, pretreatment with EGb caused a significant rise in liver cytosol SOD, blood CAT and (s)EH, while silymarin caused a significant reduction in hepatic TBARS, SOD and CAT with significant increase in blood GSH and CAT. Combination group counteracted the effect of iron on blood GSH and SOD; hepatic CAT and GST. Accordingly, we suggest that turmeric, silymarin, ginkgo might be useful herbal remedies to suppress oxidative damage caused by iron overload and emphasize the additive effect of the dietary antioxidants.

Key words: *Curcuma longa*, *Ginkgo biloba*, silymarin, glutathione s-transferase, epoxide hydrolase, antioxidant parameters

INTRODUCTION

Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), are products of normal cellular metabolism. They are well recognized for playing a dual role as both deleterious and beneficial species (Valko *et al*., 2006). The harmful effect of free radicals causing potential biological damage is termed oxidative stress and nitrosative stress (Ridnour *et al*., 2005). Defense mechanisms against free radical-induced oxidative stress involve: (i) preventative mechanisms (ii) repair mechanisms, (iii) physical defenses and (iv) antioxidant defenses. Enzymatic antioxidant defenses include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Non-enzymatic antioxidants are represented by ascorbic acid, α-tocopherol, glutathione, carotenoids, flavonoids and other antioxidants (Valko *et al*., 2007). Under normal physiological conditions, a homeostatic balance exists between the formation of reactive oxidizing/oxygen species and their removal by endogenous antioxidant scavenging compounds (Gutteridge and Mitchell, 1999). Oxidative stress occurs in biological systems when there is an overproduction of ROS/RNS on one side and a deficiency of enzymatic and non-enzymatic antioxidants on the other side (Valko *et al*., 2007).

Many studies reported that transition metals play a key role in the initiation and propagation of free radical-induced peroxidative damage. In case of iron overload, the natural storage and transport proteins such as ferritin and transferrin become saturated and overwhelmed and then the iron spills over into other tissues and organs. At the same time, oxidative stress arises because it has the ability to catalyze the Fenton or Haber-Weiss reaction, producing highly reactive oxygen radicals. Tissues that become subject to oxidative stress witness steady state levels of ROS-mediated damage to all biomacromolecules (polynucleotides, proteins, lipids and sugars) that can lead to a critical failure of biological functions and ultimately cell death (Sayre *et al*., 2005).

Indeed, oxidative damage happens in all our tissues all the time, even under 21% O_2; there is always a basal level of oxidative damage to DNA, lipids and proteins (Halliwell and Gutteridge, 2006). Hence, it is of paramount importance to search for exogenous preventative agents in the form of antioxidants to help, sustain and spare endogenous antioxidants. It has been reported that some naturally occurring agents are good antioxidants. More

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recently, the ability of antioxidant nutrients to affect cell response and gene expression has been reported *in vitro*, providing a novel and different mechanistic perspective underlying its biological activity. Among these agents are turmeric (CL), *Ginkgo biloba* (EGb) and silymarin (SL).

Curcuma longa, commonly known as turmeric, has been used as a spice and coloring agent in foods. Current traditional Indian medicine claims the use of *Curcuma longa* L. (*Zingiberaceae*) powder against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis. Curcumin (CMN) is a major component in curcuma/turmeric, being responsible for its biological action including anti-inflammatory, anti-carcinogenic and antiproliferative (Aggarwal *et al*., 2003), anti-human immunodeficiency virus (Taher *et al*., 2003), anti-bacterial (Pal and Pal, 2000) and nematocidal activities. Various *in vitro* and *in vivo* studies have established the anti-oxidant properties of curcumin (Iqbal *et al*., 2003).

Ginkgo biloba leaf extract, EGb761, is standardized to contain 24% flavonoids and 6% terpene lactones (ginkgolides and bilobalide). Now this drug has received great attention and much used in Europe for the treatment of a variety of pathological conditions such as peripheral arterial disease and organic brain syndromes (Watanabe *et al*., 2001). We were prompted to incorporate *Ginkgo biloba* in our study to provide more insight into its biological activities in liver and blood.

Silymarin is a standardized extract from the black shiny seeds of milk thistle plant [*S. marianum* (L.), Gaertner Family Asteraceae] which is also known as Mary thistle. It is mainly composed of silibinin or (silybin) with a small amounts of other silibinin stereoisomers, namely isosilybin, dihydrosilybin, silydianin and silychristin. Nowadays, silymarin is used in supportive therapy namely of human chronic liver diseases (Wellington and Jarvis, 2001).

Epoxide intermediates are formed enzymatically in cells and tissues during the oxidative metabolism of both endogenous and xenobiotic substances; they have been implicated as critical initiators of diverse cellular damage, including protein and RNA adduction as well as genetic mutation. The epoxides can be metabolically transformed in mammals by conjugation with glutathione, a reaction often catalyzed by glutathione S-epoxide transferases (GSTs), or hydrated by epoxide hydrolases to 1,2-diols (Misawa *et al*., 1998). The enzymatic hydration is essentially irreversible and produces metabolites of lower reactivity that can readily be conjugated and excreted. Therefore, the action of EHs is generally regarded as detoxifying. GSTs protect cells against numerous oxidant generating compounds and drugs, including pollutants, quinones, hydroperoxides and other end products of oxidative metabolism. Diminished GST activity could enhance cellular sensitivity to chemical carcinogens (Jaitovitch-Groisman *et al*., 2000). Hence, we were incited to examine the effect of these dietary antioxidants on these detoxifying enzymes; glutathione S-transferase (GST) and Epoxide Hydrolase (EH).

GSH (reduced) is utilized in the liver principally as a conjugation agent, a cofactor for GSH-peroxidase in the metabolism of organic peroxides and as a direct free radical scavenger. Depletion of GSH leads to cell death and hence measurement of GSH status is considered a vital component in the evaluation of the protective effect of natural substances. Brown *et al*. (2003) demonstrated that the induction of antioxidant defenses by iron loading appears to be an important factor limiting oxidative damage. Accordingly, we investigated the effect of these natural substances against lipid peroxidation induced by iron overload and their effect on endogenous enzymatic and non-enzymatic antioxidants.

MATERIALS AND METHODS

Animals: Adult male Sprague-Dawley rats, weighing 60-70 g were used. Animals were housed in metal cages with soft wood chips for bedding, at room temperature with alternating 12 h light and dark cycles. They were allowed free access to water and specific Semi-Synthetic Diet (SSD) corresponding to group type. Food intake and growth of the animals were monitored at regular intervals. Animals were provided by the experimental animal unit affiliated with Cairo University. All the research work conducted at the research laboratory, Biochemistry Department. Cairo University-Egypt.

Diet: The composition of the SSD is summarized in Table 1. The experimental diet (1% turmeric), (1% silymarin), (1% *Ginkgo biloba*) and their combination was prepared by mixing the SSD thoroughly with turmeric,

Table 1: Standard diet composition

Ingredients	Amount/100 g
Casein	20.0
Cellulose	5.0
Sucrose	40.0
Starch	20.0
Choline chloride	0.2
Methionine	0.3
Ground nut oil	10.0
Mineralmix+.	3.5
Vitamin mix+.	1.0

+: AIN 76 vitamin and mineral mix

silymarin, EGb761 and their combination (each suspended in groundnut oil), respectively. 0.03 g % Vitamin E (VE) was incorporated as a standard antioxidant into VE group diet. SSD pellets prepared in lab and dried in appropriate temperature. Fresh feed was provided daily.

Plants: The rhizome of *Curcuma longa* Linn. plant was purchased from commercial herbal store and identified in the Pharmacognosy Department of the Faculty of Pharmacy, Cairo University, then finely grinded and powdered. Silymarin powder was gratefully donated by Sedico Pharmaceutical Co. (6th October city-Egypt). *Ginkgo biloba* powder was purchased from UC Medicine, NY, USA.

Chemicals: All chemicals and standards used in this study were analytical grade reagents.

Experimental design: In the present study, rats were divided randomly into seven groups of 8-10 rats as follows: the first group was served as negative control (N) and at the end of the 10th week, it was ip injected with saline (1 mL kg^{-1} b.wt.) 6 h before decapitation, The second group was used for induction of lipid peroxidation, was fed plain SSD and considered iron-induced control (FE). Another five groups were used to estimate the protective effects of the studied agents against iron-induced lipid peroxidation. Group VE: was fed SSD supplemented with 0.03 g % vitamin E. Group CL: was fed SSD supplemented with 1% turmeric. Group EGb: was fed SSD supplemented with 1% EGb. Group SL: was fed SSD supplemented with 1% Silymarin. Group CS was fed SSD supplemented with combination of (1% turmeric, 1% silymarin, 1% *Ginkgo biloba*). At the end of the 10th week, 6 h before decapitation, all groups except the negative control are ip injected with ferrous sulfate in a dose of 30 mg mL^{-1} saline kg^{-1} b.wt. (Reddy and Lokesh, 1994).

Biochemical analysis: The animals of all groups were decapitated; the separated liver was rinsed with ice-cold saline, dried by blotting between filter papers. Tissue homogenates and supernatants were prepared from liver for assessment of the following: the basal and incubated lipid peroxidation levels in liver homogenates was measured as thiobarbituric acid reactive substances (TBARS) following the method described by Mihara and Uchiyama, (1978). GSH was measured according to Beutler *et al.* (1963). SOD was measured as described by Beauchamp and Fridovitch (1971). CAT activity was assayed according to the method of Aebi (1984) by following the decomposition of H_2O_2 at 240 nm. Epoxide Hydrolase (EH) was measured via continuous spectrophotometric assay, according to method described by Hasegawa *et al.* (1982), by monitoring the decrease in absorbance at 229 nm due to loss of Trans-Stilbene Oxide (TSO). GST activities in liver cytosol was estimated by measuring the change in absorbance per unit time produced by the addition of the sample to a mixture containing the substrate CDNB and glutathione (Habig *et al.*, 1974).

Statistical analysis: The data were subject to one-way ANOVA. Tukey's multiple comparison test was performed to evaluate the significance of difference in mean between various treatment groups, using SPSS version 12. Values were presented as means±SE.

RESULTS

Effect of *Curcuma longa*, silymarin, *Ginkgo biloba* extract separately or in combination and vitamin E feeding on oxidative stress parameters such as (TBARS, GSH, SOD, CAT) and detoxifying enzymes [GST, (s) EH and (m)EH] of iron -loaded rats are shown in Table 2 and Fig. 1-4. It was evident from our results that iron overload induced lipid peroxidation in the form of significant rise in hepatic TBARS by 1200% and plasma TBARS by 166% coupled with depletion in blood lysate GSH by ≈ 59% as compared to normal control. This was associated with a significant rise in liver cytosol and erythrocyte SOD activities by 141 and 156%, respectively, as well as hepatic CAT activity by 186.6%; liver cytosol GST (118%); (s) EH (122.8%) and (m) EH (295%).

From Table 2 and Fig. 1, pretreatment with turmeric to iron-overloaded rats caused a significant reduction in plasma TBARS levels to normal levels associated with a significant increase of blood lysate GSH levels (23%) towards normal values and induction of liver cytosol SOD

Table 2: Effect of curcuma longa, silymarin, *Ginkgo biloba* extract separately or in combination and Vitamin E feeding on hepatic and plasma TBARS levels in iron -loaded rats

Group parameter	NC	FE	VE	CL	EGb	SL	CS
Hepatic TBARS (nmol mg^{-1} protein)	0.24±0.029	2.88±0.26^{a}	0.40±0.02^{ab}	2.24±0.19^{a}	2.47±0.72^{a}	1.51±0.09^{ab}	2.26±0.15^{a}
Plasma TBARS (nmol mL^{-1} plasma)	1.48±0.19	2.47±0.23^{a}	1.20±0.18^{ab}	1.60±0.19^{b}	1.79±0.29	2.08±0.24	2.16±0.26

Results expressed as means for groups of 10 rats±SE; (a) Significantly different from the corresponding normal control at $p<0.05$; (b) Significantly different from the corresponding iron-loaded control at $p<0.05$

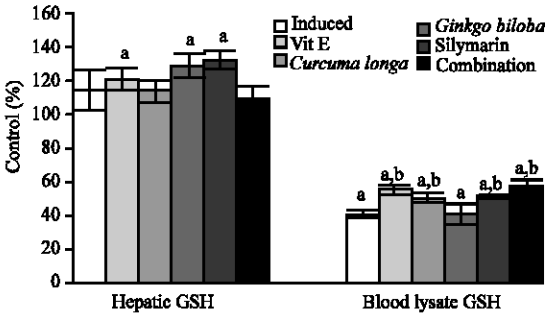

Fig. 1: Hepatic and blood lysate GSH levels in iron-induced oxidative stressed rats (FE), pretreated with *Curcuma longa* (CL), *Ginkgo biloba* extract (EGb), silymarin (SL), separately or in combination (CS) and vitamin E (VE). Results are expressed as percentage of normal control data±SE (n = 8-10). (a) indicates significant difference from the normal control and (b) indicates significant difference from the iron-induced control

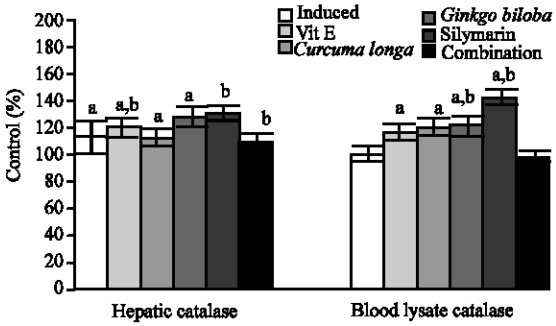

Fig. 3: Hepatic and blood lysate CAT activities in iron-induced oxidative stressed rats (FE), pretreated with *Curcuma longa* (CL), *Ginkgo biloba* extract (EGb), silymarin (SL), separately or in combination (CS) and vitamin E (VE). Results are expressed as percentage of normal control data±SE (n= 8-10). (a) indicates significant difference from the normal control and (b) indicates significant difference from the iron-induced control

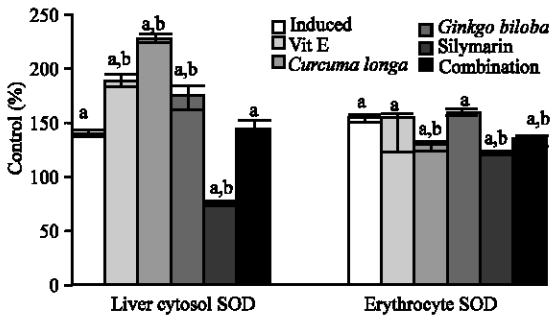

Fig. 2: Liver cytosol and erythrocyte SOD activities in iron-induced oxidative stressed rats (FE), pretreated with *Curcuma longa* (CL), *Ginkgo biloba* extract (EGb), silymarin (SL), separately or in combination (CS) and vitamin E (VE). Results are expressed as percentage of normal control data± SE (n = 8-10). (a) indicates significant difference from the normal control and (b) indicates significant difference from the iron-induced control

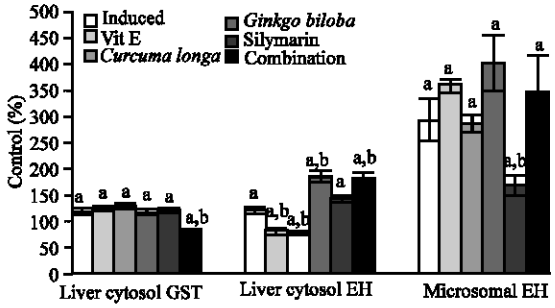

Fig. 4: Liver cytosol GST, (s) EH and (m) EH activities in iron-induced oxidative stressed rats (FE), pretreated with *Curcuma longa* (CL), *Ginkgo biloba* extract (EGb), silymarin (SL), separately or in combination (CS) and vitamin E (VE). Results are expressed as percentage of normal control data±SE (n = 8-10). (a) indicates significant difference from the normal control and (b) indicates significant difference from the iron-induced control

activities while erythrocyte SOD level was significantly decreased as compared to iron-loaded group (Fig. 2). Also, it significantly ameliorated the rise in (s) EH activity caused by iron-overload (34%) (Fig. 4). Nevertheless, hepatic TBARS; GSH levels; hepatic and blood lysate CAT and (m) EH activities were unchanged by turmeric as compared to iron-induced control. It was obvious that the effect of CL was more pronounced on plasma TBARS (35%) than liver tissue (22%). Also, it was noted that the lowest TBARS levels produced and the highest liver cytosol SOD activity among all groups was shown in CL group followed by VE group. Furthermore, the most prominent rise in liver cytosol GST was shown in CL group (1.28 fold) as compared to normal control (Fig. 4). Therefore, a parallel effect on most of the parameters in CL and vit E groups was observed.

Pretreatment with EGb to iron-overloaded rats failed to cause a significant change in hepatic or plasma TBARS; hepatic and blood GSH levels as compared to iron-loaded group (Table 2, Fig. 1). However, a slight but

not significant decrease in plasma TBARS levels (27%) was observed as compared to normal control. This was associated with a significant rise in liver cytosol SOD (123%) and blood lysate CAT (122%), (s) EH (52%) and a slight increase (36%) in (m) EH activities as compared to iron-induced control. It should be highlighted that EGb group has the highest (s) EH and (m) EH activities among all groups.

Pretreatment with silymarin to iron-overloaded rats produced a significant decrease in hepatic TBARS levels (48%) associated with modest decrease in plasma TBARS levels (16%), a significant increase in blood lysate GSH (25%), blood lysate CAT activities (42%) and a small induction of (s) EH by 17% with a remarkable inhibition of (m) EH by 42.5% as compared to iron-loaded group (Fig. 4). Additionally, it significantly prevented the rise in liver cytosol, erythrocyte SOD and hepatic CAT activities caused by iron-overload by 46, 28 and 38.3%, respectively (Fig. 2, 3). A significant increase in hepatic GSH levels up to 1.3 fold the normal control was evident. In our study, the major effect on hepatic TBARS and blood lysate GSH levels was revealed by Vit E and silymarin treatment. It is worth mentioned that only SL and CS group that were able to counteract the rise in hepatic CAT caused by iron overload to normal values.

It should be emphasized that the CS fed group showed the most prominent repletion of blood GSH levels (41%) and counteracted the rise in erythrocyte SOD (Fig. 2), hepatic CAT and GST activities caused by iron overload to a point lower than normal control values (Fig. 3 and 4); this was coupled with a significant rise in (s) EH (49.6%) and (m) EH activities (18%) as compared to iron loaded rats (Fig. 4). However, this group failed to show significant changes neither in hepatic and plasma TBARS nor hepatic GSH levels as compared to iron-loaded group (Table 2, Fig. 1). It seems that the combined lowering effect of CL and SL prevail the slight effect of EGb on erythrocyte SOD. Hence, our results emphasize the additive effect of the dietary antioxidants.

DISCUSSION

The present investigation showed that the supplementation of dietary antioxidants significantly ameliorated the changes in biochemical parameters in iron-loaded rats. The most changes were pronounced in iron loaded rats treated with silymarin and *Curcuma longa* groups. Generally, it is clear from our data that iron-overload caused significant changes in oxidative stress parameters and detoxifying enzymes. Fletcher *et al.* (1989), Fischer *et al.* (2002), Desmots *et al.* (2002), Cockell *et al.* (2005), Bagnyukova *et al.* (2006), Okazaki *et al.* (2005) and Bhatt *et al.* (2005) previously reported that hepatic TBARS levels, liver SOD, goldfish liver goldpish liver CAT and liver GST activities were significantly increased whereas hepatic GSH levels were unchanged and blood lysate GSH were depleted, in iron-loaded rats. Moreover, some studies have shown a positive correlation between lipid peroxidation and EH which, might suggest that induction of lipid peroxidation may enhance EH (Hur *et al.*, 2003; Cheol *et al.*, 2004).

In the particular case of TBARS, one of the potential sources of plasma TBARS is peripheral blood cells (Akbulut *et al.*, 2003). However, the fact that serum TBARS do not correlate with TBARS of any tissue could be due to the marker being non-diffusible from tissue and therefore, serum TBARS would only indicate the oxidative stress localized in serum which may explain the small rise in plasma TBARS as compared to liver TBARS in iron loaded rats.

The drop in blood lysate GSH caused by iron overload, indicate that the GSH-redox cycle is operating at an accelerated rate following iron injection ip, as enhanced generations of ROS are expected to consume the endogenous tissue antioxidants such as GSH (reduced), thereby depleting its concentration within the tissue. On the other hand, Brown *et al.* (2003) showed major alterations in the thiol pools leading to up-regulation of glutathione synthesis, suggesting an enhanced capacity for GSH synthesis in iron-loaded livers. Therefore, the fact that steady-state levels of GSH are increased only modestly in iron-loaded livers likely reflects increased utilization of GSH, as would be predicted under circumstances of iron-induced oxidative stress parallel to attempts to replenish the loss of GSH. Accordingly, the unchanged hepatic GSH levels could be as a result of the two opposing forces of utilization and synthesis. The discrepancy between the effect of iron on hepatic an blood lysate GSH levels might be because GSH appear to originate from liver, therefore, it takes a great deal of oxidative insult to deplete hepatic GSH. In addition, Fletcher *et al.* (1989) explained the controversy of GSH in literature and suggested that the cellular site of iron deposition as well as the hepatic iron concentration is important in determining iron-induced liver injury.

The significant rise in liver cytosol and blood SOD activities in iron loaded group may be explained on the basis that a major adaptive response of rats to oxidative stress is the induction of antioxidant enzymes such as SOD and CAT. Hence, the significant increase in SOD activity with iron overload may represent a partial compensatory response to induce SOD biosynthesis in various tissues (Zelko *et al.*, 2002).

Since SOD dismutates the $O_2^{\bullet -}$ into hydrogen peroxide and molecular oxygen and CAT breaks down H_2O_2 into water and O_2. Therefore, it is expected that an

increase in dismutation activity would be followed by a rise in CAT activity to breakdown the formed H_2O_2 (Argüelles *et al*., 2004). Levels of lipid peroxidation products had a positive correlation to activities of CAT in the liver, indicating possible up-regulation of the enzyme by these products (Bagnyukova *et al*., 2006). The effect of iron on hepatic and blood lysate CAT activity could be explained on the basis that catalase is an iron-containing antioxidant enzyme. As a heme protein, high catalase sensitivity to iron overload could be related to destruction of the heme by iron-induced peroxidation (Galleano and Puntarulo, 1992). However, the outcome of iron-overload varies depending on the length of overload exposure. The other possible outcome is up regulation of enzyme synthesis as a feed back mechanism to heme-containing enzyme loss. Present results have shown that acute exposure to iron caused an up regulation in liver CAT synthesis, predominantly. Therefore, the unchanged blood lysate CAT reflects that equilibrium between these two pathways (degradation and synthesis) in blood lysate CAT may exist.

Prochaska and Talalay (1988) studies suggested that GST induction occurs in response to electrophilic stress caused by xenobiotics and/or their electrophilic metabolites. The induction of GSTs in response to electrophiles may be viewed as the need of the cells to protect their environment from toxic electrophilic compounds which can interact with cellular components including DNA and proteins. Since lipid peroxidation also results in electrophilic stress, it is predicted that lipid peroxidation should cause induction of GSTs. It is proposed that iron-induced GST and (m) EH elevation is established at the gene-transcriptional level (Garcon *et al*., 2004).

Reddy and Lokesh (1994), Aggarwal *et al*. (2003), Koo *et al*. (2004), Okazaki *et al*. (2005), Eyble *et al*. (2006), El-Ashmawy *et al*. (2006) and Jiao *et al*. (2006) reported a significant decrease in TBARS levels, unchanged hepatic GSH levels and CAT activities and rise in kidney GSH, hepatic SOD and GST activities caused by turmeric. Argüelles *et al*. (2004) postulated that turmeric chelate Fe^{2+} needed for Fenton reaction for generating $OH^{\bullet}$ radicals. Also, Thonnesen and Greenhill (1992) reported that turmeric is a good reducing agent. It was shown to reduce Fe^{3+} to Fe^{2+} and this alters the Fe^{2+} to Fe^{3+}, which may result in inhibition of lipid peroxidation. Furthermore, CMN was shown to inhibit the oxidation of Fe^{2+} by H_2O_2 and also scavenge O_2^- and $^{\bullet}OH$ radicals (Reddy and Lokesh, 1994).

The rise in blood lysate GSH by turmeric could be explained by the increase in GSH synthesis using the highly available glutamate, caused by iron overload, as a precursor (Abd El-Azim and Ibrahim, 2002). Moreover, turmeric has a strong anti-oxidant capacity and ability to conjugate with GSH (Aggarwal *et al*., 2003). A proposed mechanism for curcuma antioxidant properties is by maintaining the activities of antioxidant enzymes (SOD, Catalase and glutathione peroxidase) (Reddy and Locesh, 1994). Furthermore, Mishra *et al*. (2004) showed that CMN reacts with superoxide radicals. The complete regeneration of CMN at low superoxide concentration suggested that it is very effective as a catalytic agent like SOD, which enhances dismutation of superoxide. Therefore, the SOD like activity of turmeric is actually sparing hepatic SOD (Mishra *et al*., 2004). The effect of turmeric on blood lysate SOD activities may be explained in view of the rise in blood GSH, caused by turmeric. An enhanced removal of ROS by GSH would lead to minimal blood lysate SOD up regulation in turmeric pretreated group.

The effects of CMN on GST and its expression are complex and may involve competitive inhibition as well as indirect enzyme induction. Although orally administered CMN has been shown to increase GST activity towards CDNB in mouse liver, the magnitude of this effect is quite small (Susan and Rao, 1992). Because GSTs consists of several catalytically distinct isoenzymes, each isoenzyme expression is differentially regulated perhaps by the oxidant or pro-oxidant environment within the cell (Hayes and Pulford, 1995). It has been suggested that GST play a role in the defense mechanisms against lipid peroxidation through their selenium-independent GSH peroxidase activity displayed primarily by the α-class GSTs mammalian tissues (Awasthi *et al*., 1994). Additionally, Jiao *et al*. (2006) have reported that CMN increased mRNA levels of ferritin and GST α in cultured liver cells. Therefore, it is clear that CMN effect on GST is at the transcription level and may contribute to its role against lipid peroxidation.

The significant drop in (s) EH activity caused by turmeric pretreatment is comparable to vitamin E effect. This drop might be due to that turmeric inhibits lipid peroxidation and consequently, it prevents the rise in (s) EH induced by iron overload.

Coskun *et al*. (2005) reported that EGb 761 increased hepatic GSH concentration, SOD and CAT activities in mouse and rat. Rimbach *et al*. (2001) suggested that the rise in GSH levels by ginkgo most likely through transcriptional up regulation of γ-GCS gene expression. The augmentation of cellular GSH is an ubiquitous effect of EGb761 and seems to be independent of changes in the cell oxidant status. The antioxidant effect of EGb may be explained by its SOD-like activity (Duke and Salin, 1985) and iron chelating properties. Additionally, EGb 761 not only interacts directly with reactive oxygen species, but it could interfere with their generation. Moreover, EGb 761

inhibits the activity of xanthine oxidase, a cellular enzymatic source of superoxide and hydrogen peroxide (McCord, 1985). As a result, sparing CAT enzyme would be anticipated. It has been noted by Hur *et al.* (2003) and Cheol *et al.* (2004) that antioxidants are inducers of epoxide hydratase, which may explain that EGb group effect on EH activities.

Song *et al.* (2006) and Soto *et al.* (2003) reported that silymarin caused a drop in hepatic TBARS levels, SOD and CAT activities in rats; while Preetha *et al.* (2006) reported an increase in hepatic GSH. The reported data herein indicates that silymarin hepatoprotective effect is at least partly, attributed to inhibition of hepatic TBARS levels (Pietrangelo *et al.*, 2002) which is possibly related to the ability to act as chain breaking antioxidant presumably as an oxygen free radical scavenger (Rastogi *et al.*, 2001). It is obvious that silymarin could not affect cellular GSH levels by direct transport capacity for intact GSH donation, as most cells do not have the direct transport capacity for intact GSH. Therefore, GSH must be synthesized intracellularly and silymarin might influence this process. Furthermore, several recent studies reported that flavonoids could improve of γ-GCS function by up regulating of γ-GCS gene expression (Moskaug *et al.*, 2005; Alidoost *et al.*, 2006). Pharmacological studies in patients with liver cirrhosis have demonstrated the ability of silymarin to increase levels of GSH and GSH peroxidase activities. Another postulated mechanism for the effect of silymarin on GSH is that, it is known to significantly protect the activity of GSH reductase in the liver tissue and this could help replenish GSH.

The effect of silymarin on liver cytosol and erythrocyte SOD (Fig. 2) and hepatic CAT (Fig. 3) could be explained by its iron chelating properties. Moreover, glutathione system, constitute the more important defense mechanisms against damage by free radicals. Since, SL increase the availability of the antioxidant GSH; therefore, iron overload and the consequent oxidative mediators will, preferably, be handled by the glutathione peroxidase rather than the SOD and CAT. As a result, fewer up regulation of these antioxidants (SOD and CAT) would be expected. It is clear that silymarin showed more antioxidant effect on liver rather than blood. This could possibly because it is highly concentrated in liver.

The apparent difference in catalase response between hepatic and blood tissue in these groups was explained by Arguelles *et al.* (2004) that the measurement of antioxidant capacity (AOC) remains troublesome because changes in AOC of serum do not necessarily reflect changes in AOC in other tissues, a high value of AOC in serum doesn't necessarily mean that all tissues are under greater protection against attack by reactive oxygen species. In addition, it is clear from our results that the three antioxidants caused a rise in blood lysate CAT activities. It has been shown that human erythrocyte CAT contains tightly bound NADPH which is effective in preventing and reversing inactivation of the enzyme (Kirkman *et al.*, 1987). Lexis *et al.* (2006) reported that the antioxidant supplementation indirectly caused an increased availability of erythrocyte NADPH leading to increased CAT activity.

It is obvious from present results that the effect of SL pretreatment on hepatic CAT and (m) EH is parallel and unique among the studied plants. Guenthner *et al.* (1989) concluded that catalase and (s) EH contain some similar immunogenic epitopes and they surmised that similarities between subunits of these two enzymes may lead to their partial co purification. Also, functional similarities between the two enzymes were demonstrated, as several compounds that inhibit catalase were also shown to inhibit (s)EH activity in the same concentration range and rank order.

The effect of CS group on EH could be explained by the combined effect of exogenous antioxidants (Ginkgo and silymarin) that increased (s) EH activities, individually which surmounted the negative effect of curcuma on (s) EH.

CONCLUSION

There is growing evidence that implicates iron in several diseases, including cancer, ischemia, Parkinson's disease and rheumatoid arthritis. This is presumably, via iron-induced lipid peroxidation and cellular organelle damage. The finding that dietary antioxidant administration significantly ameliorates the effect of iron overload may suggest their benefit in pathological sequence of iron overload-linked disease. In conclusion, the present study suggests that a diet containing even low levels of different natural occurring compounds is effective in modulation of oxidative stress and lipid peroxidation induced by iron overload.

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REFERENCES

Abd El-Azim, S.A. and S.S. Ibrahim, 2002. Neurochemical changes in rats suspected to iron-induced oxidative stress: Prophylactic effect of silymarin, CMN and *Ginkgo biloba* extract compared to vitamin E. Egypt. J. Biochem., 20: 373-392.

Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol., 105: 121-126.

Aggarwal, B.B., A. Kumar and A.C. Bharti, 2003. Anticancer potential of CMN: Preclinical and clinical studies. Anticancer Res., 23: 363-398.

Akbulut, H., K.G. Akbulut, F. Icli and Buyukcelik, 2003. Daily variations of plasma malondialdehyde levels in patients with early breast cancer. Cancer Detect. Prev., 27: 122-126.

Alidoost, F., M. Gharagozloo, B. Bagherpour, A. Jafarian, S.E. Sajjadi, H. Hourfar and B. Moayedi, 2006. Effect of silymarin on the proliferation and glathione levels of peripheral blood mononuclear cells from β-thalassemia major patients. Int. Immunopharmacol., 6: 1305-1310.

Arguelles, S., S. Garcia, M. Maldonado and A. Machado, A. Ayala, 2004. Do the serum oxidative stress biomarkers provide a reasonable index of the general oxidative stress status. Biochem. Biophys. Acta, 1674: 251-259.

Awasthi, Y.C., R. Sharma and S.S. Singhal, 1994. Human glutathione S-transferases. Int. J. Biochem., 26: 295-308.

Bagnyukova, T.V., O.I. Chahrak and V.I. Lushchak, 2006. Coordinated response of goldfish antioxidant defenses to environmental stress. Aquat. Toxicol., 78: 231-325.

Beauchamp, C. and I. Fridovich, 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem., 44: 276-287.

Beutler, E., O. Duron and B.M. Kelly, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.

Bhatt, P., D. Swarup, R.C. Patra, A.K. Pattanaik and R. Ranjan, 2005. Enhanced erythrocytic lipid peroxides level in rabbits after repeated parental administration of iron. Indian J. Exp. Biol., 43: 854-858.

Brown, K.E., P.A. Dennery, L.A. Ridnour, C.J. Fimmel, E.D. Kladney and R.M. Brunt, 2003. Effect of iron overload and dietary fat on indices of oxidative stress and hepatic fibrinogenesis in rats. Liver Int., 23: 232-242.

Cheol Park, J., S. Chul Kim, J. Moon Hur, S.H. Choi, K. Yeon Lee and J. Won Choi, 2004. Anti-hepatotoxic effects of *Rosa rugosa* root and its compound, rosamultin, in rats intoxicated with bromobenzene. J. Med. Food, 7: 436-441.

Cockell, K.A., A.T. Wotherspoon, B. Belonje, M.E. Fritz, R. Madere, N. Hidiroglou, L.J. Plouffe, W.M. Ratnayake and S. Kubow, 2005. Limited effects of combined dietary copper deficiency/iron overload on oxidative stress parameters in rat liver and plasma. J. Nutr. Biochem., 16: 750-756.

Coskun, O., F. Armutcu, M. Kanter and G.M. Kuzey, 2005. Protection of endotoxin-induced oxidative renal tissue damage of rats by vitamin E or/and EGb 761 treatment. J. Applied Toxicol., 25: 8-12.

Desmots, F., M. Rissel, C. Pigeon, P. Loyer, O. Loreal and A. Guillouzo, 2002. Differential effects of iron overload on GST isoform expression in mouse liver and kidney and correlation between GSTA4 induction and overproduction of free radicals. Free Radic. Biol. Med., 32: 93-101.

Duke, M.V. and M. Salin, 1985. Purification and characterization of an iron-containing superoxide dismutase from Eukaryote, *Ginkgo biloba*. Arch. Biochem. Biophys., 243: 305-314.

El-Ashmawy, I.M., K.M. Ashry, A.F. El-Nahas and O.M. Salama, 2006. Protection by turmeric and myrrh against liver oxidative damage and genotoxicity induced by lead acetate in mice. Basic Clin. Pharmacol. Toxicol., 98: 32-37.

Eyble, V., D. Kotyzova and J. Koutensky, 2006. Comparative study of natural antioxidants-curcumin, resveratrol and melatonin-in cadmium-induced oxidative damage in mice. Toxicology, 225: 150-156.

Fischer, J.G., H.P. Glauert, T. Yin, M.L. Sweeney-Reeves, N. Larmonier and M.C. Black, 2002. Moderate iron overload enhances lipid peroxidation in livers of rats, but does not affect NF-kappaB activation induced by the peroxisome proliferator, Wy-14,643. J. Nutr., 132: 2525-2531.

Fletcher, L.M., F.D. Roberts, M.G. Irving, L.W. Powell and J.W. Halliday, 1989. Effects of iron loading on free radical scavenging enzymes and lipid peroxidation in rat liver. Gastroenterology, 97: 1011-1018.

Galleano, M. and S. Puntarulo, 1992. Hepatic chemiluminescence and lipid peroxidation in mild iron overload. Toxicol., 76: 27.

Garcon, G., P. Gosset and F. Zerimech, 2004. Effect of Fe_2O_3 on the capacity of benzo (a) pyrene to induce polycyclic aromatic hydrocarbon-metabolizing enzymes in the respiratory tract of Sprague-dawley rats. Toxicol. Lett., 150: 179-189.

Guenthner, T.M., M. Qato, R. Whalen and S. Glomb, 1989. Similarities between catalase and cytosolic epoxide hydrolase. Drug Metab. Rev., 20: 733-748.

Gutteridge, J.M.C. and J. Mitchell, 1999. Redox imbalance in the critically ill. Br. Med. Bull., 55: 49-75.

Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferase: The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Halliwell and Gutteridge, 2006. Free Radicals in Biology and Medicine. 4th Edn. Clarendon Press, Oxford.

Hasegawa, L.S. and B.D. Hammock, 1982. Spectrophotometric assay for mammalian cytosolic epoxide hydrolase using trans-stilbene oxide as the substrate. Biochem. Pharmacol., 31: 1979-1984.

Hayes, J.D. and D. Pullford, 1995. The glutathione S-transferase supergene family: Regulation of GST and the isoenzymes to cancer chemoprotection and drug resistance. Crit. Rev. Biochem. Mol. Biol., 30: 445-600.

Hur, J.M., J.G. Park, K.H. Yang, J.C. Park, J.R. Park, S.S. Chun, J.S. Choi and J.W. Choi, 2003. Effect of methanol extract of zanthoxylum piperitum leaves and its compound, protocatechuic acid, on hepatic drug metabolizing enzymes and lipid peroxidation. Biosci. Biotechnol. Biochem., 67: 945-950.

Iqbal, M., S.D. Sharma, Y. Okazaki, M. Fujisawa and S. Okada, 2003. Dietary supplementation of CMN enhances antioxidant and phase II metabolizing enzymes in ddY male mice: Possible role in protection against chemical carcinogenesis and toxicity. Pharmacol. Toxicol., 92: 33-38.

Jaitovitch-Groisman, I., N. Fotouhi-Ardakani, R.L. Schecter, A. Woo, M.A. Alaoui-Jamali and G. Batist, 2000. Modulation of glutathione S-transferase alpha by hepatitis B virus and the chemopreventive drug oltipraz. J. Biol. Chem., 275: 33395-33403.

Jiao, Y., J. Wilkinson, E. Christine Pietsch, J.L. Buss, W. Wang, R. Planalp, F.M. Torti and S.V. Torti, 2006. Iron chelation in the biological activity of CMN. Free Radic. Biol. Med., 40: 1152-1160.

Kirkman, H.N., S. Galiano and G.F. Gaetani, 1987. The function of catalase-bound NADPH. Biol. Chem., 262: 660-666.

Koo, B.S., W.C. Lee, K.H. Chung, J.H. Ko and C.H. Kim, 2004. A water extract of *Curcuma longa* L. (Zingiberaceae) rescues PC12 cell death caused by pyrogallol or hypoxia/reoxygenation and attenuates hydrogen peroxide induced injury in PC12 cells. Life Sci., 75: 2363-2375.

Lexis, A., R.G. Fassett and J.S. Commbes, 2006. α-tocopherol and α-lipoic acid enhance the erythrocyte antioxidant defence in Cyclosporin A-treated rats. Basic Clin. Pharmacol. Toxicol., 98: 68-73.

McCord, J.M., 1985. Oxygen-derived free radicals in postischemic tissue injury. N. Engl. J. Med., 312: 159-163.

Mihara, M. and M. Uchiyama, 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem., 86: 271-278.

Misawa, E., C.K. Chan Kwo Chion, I.V. Archer, M.P. Woodland, N.Y. Zhou, S.F. Carter, D.A. Widdowson and D.J. Leak, 1998. Characterization of a catabolic epoxide hydrolase from a *Corynebacterium* sp. Eur. J. Biochem., 253: 173-183

Mishra, B., K.I. Priyadarsini, M.K. Bhide, R.M. Kadamand H. Mohan, 2004. Reactions of superoxide radicals with CMN; Probable mechanism by optical spectroscopy and EPR. Free Radic. Res., 38: 355-362.

Moskaug, J.O., H. Carlsen, M.C. Myhrstad and R. Blomhoff, 2005. Polyphenols and glutathione synthesis regulation. Am. J. Clin. Nutr., 81: 277S-283S.

Okazaki, Y., M. Iqbal and S. Okada, 2005. Suppressive effects of dietary CMN on the increased activity of renal ornithine decarboxylase in mice treated with a renal carcinogen, ferric nitrilotriacetate. Biochim. Biophys. Acta, 1740: 357-366.

Pal, A. and A.K. Pal, 2000. Studies on the genotoxicity of turmeric extracts in bacterial system. Int. J. Antimicrob. Agents, 16: 415-417.

Pietrangelo, A., G. Montosi, C. Garuti, M. Contri, F. Giovannini, D. Ceccarelli and A. Masini, 2002. Iron-induced oxidant stress I nonparenchymal liver cells: Mitochondrial derangement and fibrosis in acutely iron-dosed gerbils and its prevention by silybin. J. Bioenerg. Biomembr., 34: 67-79.

Preetha, S.P., M. Kanniappan, E. Selvakumar, M. Nagaraj and P. Varalakshmi, 2006. Lupeol ameliorates aflatoxin B1-induced peroxidative hepatic damage in rats. Comp. Biochem. Physiol. C. Toxicol. Pharmacol., 143: 333.

Prochaska, H.J. and P. Talalay, 1988. Regulatory mechanisms of monofunctional and bifunctional anticarcinogenic enzyme inducers in murine liver. Cancer Res., 48: 4776-4782.

Rastogi, R., A.K. Srivastava and A.K. Rastogi, 2001. Long term effect of aflatoxin B_1 on lipid peroxidation in rat liver and kidney: Effect of picroliv and silymarin. Phytother. Res., 15: 307-310.

Reddy, A.C. and B.R. Lokesh, 1994. Effect of dietary turmeric (*Curcuma longa*) on iron-induced lipid peroxidation in the rat liver. Food Chem. Toxicol., 32: 279-283.

Ridnour, L.A., J.S. Isenberg, M.G. Espey, D.D. Thomas, D.D. Roberts and D.A. Wink, 2005. Nitric oxide regulates angiogenesis through a functional switch involving thrombosondin-1. Proc. Natl. Acad. Sci. USA., 102: 13147-13152.

Rimbach, G., K. Gohil and S. Matsugo, H. Moini, C. Saliou, F. Virgili, S. Weber and P. Lester, 2001. Induction of GSH synthesis in human keratinocytes by *Ginkgo biloba* extract (Egb761). Biofactors, 15: 39-52.

Sayre, L.M., P.I. Moreira, M.A. Smith and G. Perry, 2005. Metal ions and oxidative protein modification in neurological disease. Annal Ist Super Sanita, 41: 143-164.

Song, Z., I. Deaciuc, M. Song, D.Y. Lee, Y. Liu, X. Ji and C. McClain, 2006. Silymarin protects against acute ethanol-induced hepatotoxicity in mice. Alcohol Clin. Exp. Res., 30: 407-413.

Soto, C., R. Recoba, H. Barron, C. Alvarez and L. Favari, 2003. Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. Compar. Biochem. Physiol. Part C, 136: 205-212.

Susan, M. and M.N. Rao, 1992. Induction of glutathione S-transferase activity by curcumin in mice. Arzneimittelforschung, 42: 962-964.

Taher, M.M., G. Lanimering, C. Hershey and K. Valerie, 2003. Curcumin inhibits ultraviolet light induced human immunodeficiency virus gene expression. Mol. Cell. Biochem., 254: 289-297.

Thonnesen, H.H. and J.V. Greenhill, 1992. Studies on curcumin and curcuminoids. XXII. Curcumin as a reducing agent and radical scavenger. Int. J. Pharm., 87: 79-87.

Valko, M., C.J. Rhodes, J. Monco. M. Izakovic and M. Mazur, 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact., 160: 1-40.

Valko, M., D. Leibfritz, J. Moncol, M. Cronin, M. Mazur and J. Telser, 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., 39: 44-84.

Watanabe, C.M.H., S. Wolffram, P. Ader, G. Rimbach, L. Paker, J.J. Maguire, P.G. Schultz and K. Gohil, 2001. The *in vivo* neuromodulatory effect of the herbal medicine *Ginkgo biloba*. Biochemistry, 98: 6577-6580.

Wellington, K. and B. Jarvis, 2001. Silymarin: A review of its clinical properties in the management of hepatic disorders. BioDrugs, 15: 465-489.

Zelko, I.N., T.J. Mariani and R.J. Folz, 2002. Superoxide dismutase multigene family: A comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2) and EC-SOD (SOD3) gene structures, evolution and expression. Free Radic. Biol. Med., 33: 337-349.