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Ameliorative effects of ginger and α -lipoic acid on oxidative stress and inflammation in senile female rats

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Oxidative stress is recognized as an important environmental factor in aging. The reactive oxygen species and related free radicals are normally produced both intra and extracellular and air-breathing organisms cannot avoid the risk of oxidative stress. Moreover, recent studies have advanced the notion of chronic inflammation as a major risk factor underlying aging and age-related diseases. In the present study, the evaluation of the protective effects of ginger and α -lipoic acid (ALA) supplementation on senile female rats is evaluated during inflammation. The results showed a significant increase in lipid peroxidation but a significant reduction in the reduced glutathione level (GSH), the activities of superoxide dismutase (SOD), catalase (CAT) and cytochrome P450 (CytoP450) in hepatic aged female rats. In addition, this study revealed a significant increase in the inflammatory mediators interleukin-1(IL-1), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- α) as well as the activity of cyclooxygenase enzyme (COX-2). Furthermore, there was a significant decrease in serum nitric oxide (NO) in aged female rats. Ginger and ALA were effective in minimizing aged-related oxidative burden through decreasing lipid peroxidation, increasing GSH content and promoting antioxidant enzymes. Moreover, the compounds under investigation reduced the levels of the pro-inflammatory cytokines IL-1, IL-6 and TNF- α , in addition to inhibiting the activity of COX-2. The levels of serum NO was also increased by the treatments.

Key words: Ginger- α -lipoic acid- oxidative stress- inflammatory mediators' factors- cyclooxygenase-2.

INTRODUCTION

Modern science has made tremendous attempts to understand the phenomenon of the aging process. Several theories have been postulated, but at present, the most popular and widely tested one is the free radical theory of aging. This theory proposes that aging occurs as a consequence of the deleterious effects of radicals produced during the course of cellular metabolism. According to this theory, the primary cause which initiates the processes leading to the aging of an organism and its ensuing death is the uncontrolled production of free radicals. The free radicals have a direct influence on the genetic and molecular mechanisms that determine the life span of the organisms. Reactive oxygen species can attack vital cell components like polyunsaturated fatty acids, proteins and nucleic acids (Arivazhagan et al., 2002). Moreover, free radicals-induced cellular stress response mechanism is a major contributing factor to cell and tissue decline with age. Susceptibility versus resistance to exogenous and endogenous stresses is

now recognized as a key determinant of successful aging (Shay and Hagen, 2009). Today many botanicals natural products are used in therapy of different disease (Ogungbe and Lawal, 2006). Ginger is an example of botanicals which is gaining popularity amongst modern physicians. The underground rhizomes of ginger are the medicinally useful part (Ahmed et al., 2008). Among the pharmacological effects demonstrated are anti-platelets, antioxidant, anti-tumor, anti-rhino viral, anti-hepatotoxicity, anti-arthritis and anti-inflammatory effects (Lantz et al., 2007; El-Sharaky et al., 2009). The strong antioxidant action of ginger has been proposed as one of the major possible mechanisms for the protective actions of the plant against toxicity and lethality of radiation (Jagetia et al., 2003; Haksar et al., 2006). Ginger counteracts the toxic effects of carbon tetrachloride and cisplatin (Amin and Hamza, 2006; Yemitan and Izegebu, 2006), and is effective as an antiulcer agent (Siddaraju and Dharmesh, 2007).

Recently, it has been shown that ginger has strong anti-inflammatory and anti-apoptotic actions (Kim et al., 2007). It is well known that T-helper (Th)-lymphocytes play a key role in the regulation of immune and inflammatory reactions through the release of cytokines (Ahui et al., 2008). The anti-inflammatory properties of ginger have been known for a long time (Grzanna et al., 2005; Ali et al., 2008). Kiuchiet al. (1982) reported, for the first time, that the anti-inflammatory action was the result of its inhibitory effects on prostaglandins synthesis. Further, Kiuchiet al. (1992) demonstrated gingerols (the major bioactive compound present in ginger) were more potent inhibitors of leukotrienes synthesis than prostaglandins synthesis *in vitro*. More recently, it has been shown that ginger as a whole or some of its constituents are effective against cytokines synthesized and secreted at sites of inflammations (Grzanna et al., 2005). α -Lipoic acid (ALA), a disulphide derivative of octonic acid, and its reduced form dihydrolipoic acid (DHLA) are natural compounds widely distributed in plants and animals. They are synthesized through a reaction catalyzed by lipoic acid synthase within the mitochondria (Wollin and Jones, 2003). The therapeutic actions of ALA are based on unique antioxidant/ALA/DHLA system. Thus DHLA is able to reduce not only reactive oxygen species (ROS) but also oxidized forms of other antioxidants. ALA regenerates other antioxidants and for this reason it is called an antioxidant of antioxidants (Bilska et al., 2008). Therefore, dietary lipoic acid is effective in attenuating oxidative stress induced by drugs (Amudha et al., 2006) and aging (Arivazhagan et al., 2002).

Previous studies showed that ALA acted as a potent antioxidant by inhibiting lipid peroxidation and revitalizing antioxidants in the liver and kidney of aged rats (Arivazhagan et al., 2000). Unfortunately, the level of ALA has been found to decrease gradually by aging (Lykkesfeldt et al., 1998).

ALA is also used as modulator in several liver disorders such as alcohol induced liver damage, mushroom poisoning, metal intoxication and chloroform poisoning (Basamante et al., 1998). ALA elevates the hepatic GSH levels due to the presence of thiol groups (Packer et al., 1995). Free thiols represent essential precursors or intermediates in GSH synthesis and degrading pathways as well as in the metabolism of several agents used in medical treatments (Lilling and Holmgren, 2007).

In the light of these studies, the present work was designed to scrutinize the hepatoprotective and antioxidant potentials of ginger and ALA against aging oxidative stress in senile female rat liver.

MATERIAL AND METHODS

Ginger was purchased from MEPACO (Arab Company for Pharmaceuticals and Medicinal Plants), Egypt and thiotacidalpha

lipoic acid (thiotacid) was purchased from EVA Company, Egypt. All other chemicals and solvents used were of the highest purity and analytical grade. Adult female albino rats weighing approximately 130 to 150 g (3 to 4 months old) and senile (24 months old) weighing 280 to 300 g were used. Rats were maintained in plastic cages (five per cage at $24 \pm 2^\circ\text{C}$ and 40 ± 10 humidity) and housed for ten days prior to the initiation of the experiments, for adaptation to laboratory conditions. Animals were fed with commercial standard rat-pellet and tap water was provided *ad libitum*. Handling and usage of animals agreed strictly with the regulations and guidelines set by the research Ethics Committee of the Faculty of Science, Ain Shams University.

The animals were divided into four groups each of five rats as follows: The first was the control adult rats and received orally (by means of stomach tube) 0.5% carboxymethyl cellulose (CMC) sodium salt (0.1 ml/ 100 g body weight), the second was the control aged rats and received the same amount of CMC. The third group, aged rats administered ginger at a dose of 250 mg/kg body weight dissolved in CMC vehicle. The fourth group was aged rats administered ALA (65 mg/kg body weight CMC). All groups received the different treatments for four consecutive weeks. The doses were calculated from the human therapeutic dose (Regan-Shaw et al., 2007).

At the end of the experimental period, all the animals were sacrificed by cervical decapitation. Liver tissues were immediately excised and rinsed in ice cold physiological saline. Blood was collected and serum was separated for the assessment of total nitric oxide (NO) using commercial ELISA kits specific for rat assays (Assay Designs, Inc- Germany), the levels of rat interleukin-1 and interleukin-6 (IL-1 and IL-6) and tumor necrosis factor (TNF- α) were determined using enzyme immunoassay (EIA) techniques (IBL Gesellschaft, Hamburg, Germany). The activity of cyclooxygenase2 (COX-2) was assayed by ELISA (sandwich) using commercial kits (IBL-Hamburg Co., Germany). Liver tissue was removed, cleared of blood, and rinsed in cold saline and used for the biochemical analyses. The levels of reduced glutathione (GSH) were determined according to Ellman (1959), the activity of cytochrome P450 (CytoP450) in S-9 fractions according to the method modified by McLean and Day (1974), superoxide dismutase (SOD) activity according to the method of Oyanagui (1984) and catalase (CAT) activity by the method of Sinha (1972). The levels of lipid peroxidation (LP) were determined by measuring the content of the thiobarbituric acid reactive substances (TBARS) in the tissue homogenates following the procedure of Hogberg et al. (1974).

Statistical analysis

The results were presented as the mean \pm standard error (SE) for five animals in each group. Statistically significant differences between groups were calculated using one-way analysis of variance (ANOVA) followed by Snedecor and Cochran (1982). The criterion for significance was set at $p < 0.05$.

RESULTS

Effect of ginger and α -lipoic acid on endogenous antioxidant defense system

Table 1 depicts the effect of ginger and lipoic acid on reduced glutathione content (GSH) and lipid peroxidation (LP) of adult and aged female rats. The activities of superoxide dismutase (SOD), catalase (CAT) and cyto-

Table 1. Effect of ginger and α -lipoic acid on oxi-redox system in liver senile female rats.

Parameter	Treatment	Adult female rats	Senile female rats	Senile female rats treated with ginger	Senile female rats treated with α -lipoic acid
GSH (mg/g tissue)	Mean	20.54 ^A	14.11 ^B	16.56 ^C	18.89 ^D
	\pm SE	\pm 0.22	\pm 0.32	\pm 0.29	\pm 0.28
	% of change		(-31.30)	(-19.38)	(-8.03)
SOD (U/mg tissue)	Mean	7.61 ^A	5.13 ^B	5.98 ^C	6.61 ^C
	\pm SE	\pm 0.21	\pm 0.18	\pm 0.64	\pm 0.11
	% of change		(-32.59)	(-21.42)	(-13.27)
CAT (u/mg tissue)	Mean	61.63 ^A	40.62 ^B	47.56 ^C	52.69 ^D
	\pm SE	\pm 0.17	\pm 0.32	\pm 0.59	\pm 0.73
	% of change		(-34.09)	(-22.83)	(-14.51)
CYTO P450 (P mol/ 100 mg)	Mean	69.39 ^A	44.54 ^B	52.53 ^C	56.31 ^D
	\pm SE	\pm 0.34	\pm 0.17	\pm 0.35	\pm 0.31
	% of change		(-35.81)	(-24.30)	(-18.85)
LP (nmol/ 100 mg tissue)	Mean	0.35 ^A	0.64 ^B	0.56 ^C	0.44 ^D
	\pm SE	\pm 0.01	\pm 0.02	\pm 0.01	\pm 0.01
	% of change		82.86	60.00	25.71

Values are expressed as mean \pm SE. A, B, C, D values with different superscripts within the same row are significantly different at P<0.05.

chrome P450 in liver tissues are also represented. A significant decrease in GSH level was found in all groups (P<0.05) as compared with the corresponding control adult values. This effect was paralleled by elevation in the level of LP (P<0.05) in the liver of all groups as compared with normal young control (Table 1). The activities of the endogenous antioxidant enzymes SOD, CAT and hepatic microsomal cytochrome P450 were significantly reduced in the liver tissue (percentage of change -32.59, -34.09 and -35.81, respectively, P<0.05) in aged group and (-21.42/-13.13.27, -22.83/-14.51, and -24.30/-18.85, respectively, P<0.05) in aged groups treated with both ginger and α -lipoic acid as compared with normal control young group. In addition, the hepatic LP content exhibited a negative correlation with the GSH content and with the SOD, CAT and CytoP450 activities (Figure 1).

Effect of ginger and α -lipoic acid on pro-inflammatory mediators in aged female rats

Table 2 illustrates the serum levels of total nitric oxide (NO), the pro-inflammatory cytokines tumor necrosis factor alpha (TNF- α), interukin-1(IL-1), interleukin-6(IL-6) and the activity of cyclooxygenase-2 (COX-2) of adult control in comparison with aged group and with aged groups treated with ginger or lipoic acid. A significant decrease in NO⁺ level was found in all groups (percent of

change -35.03, P<0.05 in aged group, and -23.53,-18.29 in aged groups treated with ginger and alpha lipoic acid, respectively, P<0.05) as compared with the corresponding control adult values. On the other hand, the indicators of the immune response, IL-1, IL-6 and TNF- α , were significantly higher, percent of change was 55.38, 83.48 and 60.97, respectively, P<0.05 in aged group while the percent of change was 38.98, 57.45 and 31.26, respectively in group administrated with ginger (P<0.05) and 25.54, 39.79 and 16.89 respectively in group administrated with α -lipoic acid (P<0.05) as compared with those of the normal adult control group. Furthermore, there was a significant increase in the serum activity of COX-2 in all groups (42.59%) in aged group (35.10 and 29.27%) in ginger and α -lipoic acid, respectively (P<0.05) in comparison with the adult animals. In addition, Figure 2 shows a positive correlation between the TNF- α and IL-1, IL-6 levels on one hand and the activity of COX-2 on the other hand, while there was a negative correlation between the TNF- α and NO in aged rats.

DISCUSSION

The free radical theory of aging supports the notion that the physiological decline that accompanies aging is likely

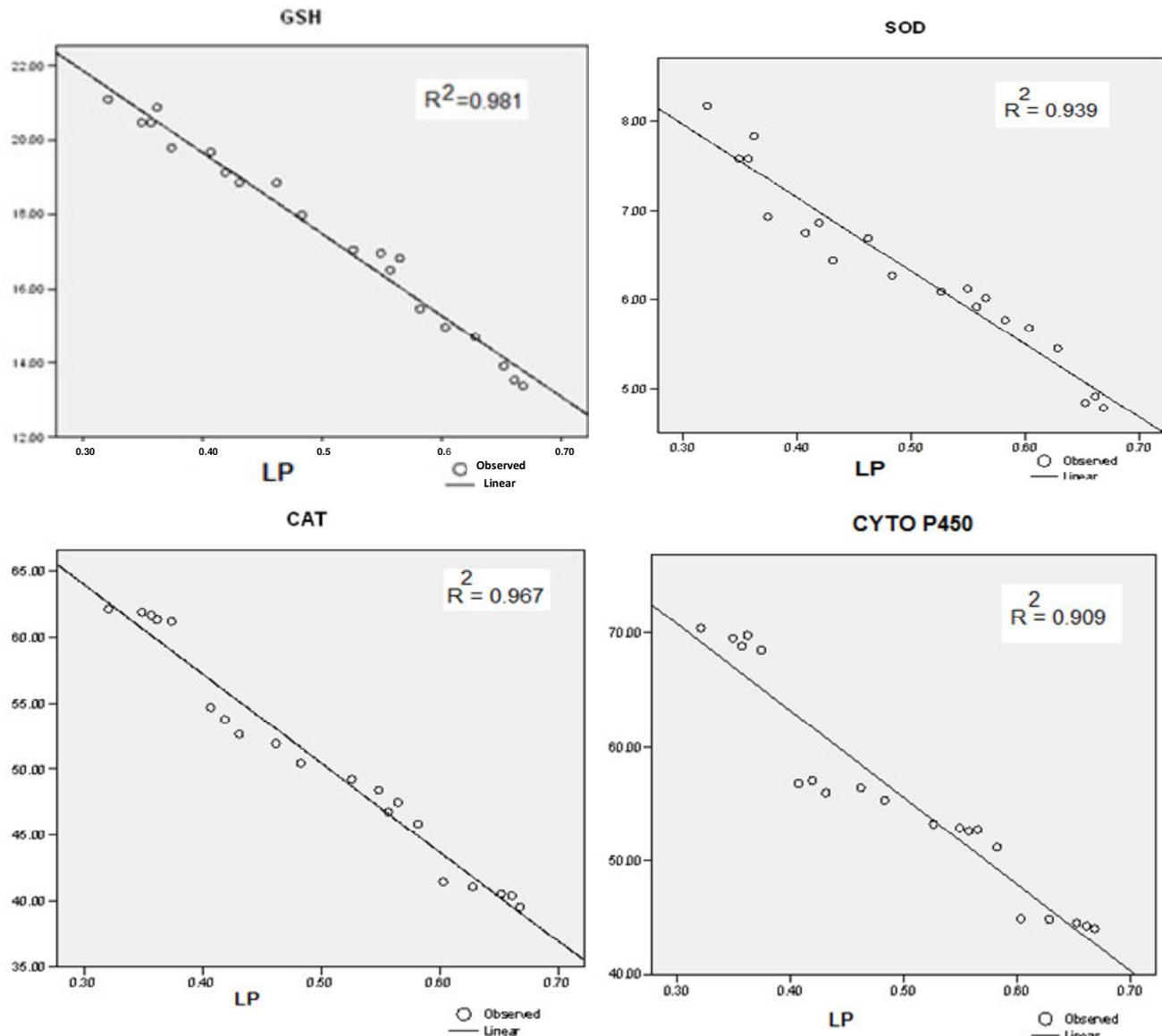


Figure 1. Simple correlation between the hepatic lipid peroxidation content (LP) and GSH level, and activities of antioxidant enzymes (SOD, CAT, CYTO P450) in the liver of female senile rats.

due to accumulative oxidative damage to cells and molecules (Castillo et al., 2006). Several authors believe that the reactive species, oxygen (ROS) and nitrogen (RNS) are the primary causal factor underlying aging – associated declines in physiological function (Gonzalo-Calvo et al., 2010; Castillo et al., 2006; Kregel and Zhang, 2006). An impairment in mitochondrial function with age has been also reported (Van Remmen and Richardson, 2001), which may be a major factor underlying the increase in the rate of ROS production in mitochondria as well as in the reduction of the energy supply in old cells. The results of this work support the importance of oxidative stress in the aging process and emphasize the role of lipid peroxidation-induced damage.

After LP initiation by free radicals, it becomes a self-perpetuating chain reaction which could induce the peroxidation of bivotal lipid molecules of the cell, reduce membrane fluidity and alter closely situated proteins. These perpetuations would result in deterioration of the functions of cell membrane and other biological membranes inside the cell (VanRemmen and Richardson, 2001). In the present study, lipid peroxidation has been found to significantly increase in old female rats. Endogenous antioxidant defenses include a network of compartmentalized antioxidant enzymes that are usually distributed within the cytoplasm and among various organelles in cells. Several ubiquitous primary antioxidant enzymes such as SOD, CAT and different

Table 2. Effect of ginger and α -lipoic acid on pro- inflammatory mediators in the serum of senile female rats.

Parameter	Treatment	Adult female rats	Senile female rats	Senile female rats treated with ginger	Senile female rats treated with α -lipoic acid
	IL-1 (Pg/ml)	Mean \pm SE % of change	3.72 ^A \pm 0.02	5.78 ^B \pm 0.04 55.38	5.17 ^C \pm 0.04 38.98
IL-6 (Pg/ml)	Mean \pm SE % of change	10.53 ^A \pm 0.04	19.32 ^B \pm 0.17 83.48	16.58 ^C \pm 0.24 57.45	14.72 ^D \pm 0.28 39.79
TNF- α (Pg/ml)	Mean \pm SE % of change	5.15 ^A \pm 0.03	8.29 ^B \pm 0.05 60.97	6.76 ^C \pm 0.86 31.26	6.02 ^D \pm 0.09 16.89
TNO (N mol /L)	Mean \pm SE % of change	58.35 ^A \pm 0.34	37.91 ^B \pm 0.38 -35.03	44.62 ^C \pm 0.27 -23.53	47.68 ^D \pm 0.29 -18.29
COX-2 (Ng/ml)	Mean \pm SE % of change	61.59 ^A \pm 0.73	87.82 ^B \pm 0.45 42.59	83.21 ^C \pm 0.49 35.10	79.62 ^D \pm 0.79 29.27

Values are expressed as mean \pm SE. A, B, C, D values with different superscripts within the same row are significantly different at $P < 0.05$.

forms of peroxides work in a complex series of integrated reaction to convert ROS to more stable molecules. Moreover, small molecular-weight antioxidants such as GSH can also function as direct scavengers (Kreged and Zhang, 2006). These finding are in accordance with the present study where hepatocytes from senile intact female rats showed alterations in some parameters related to oxidative stress. A significant decrease in GSH level and activities of SOD, CAT, and cytochrome P450 in old female rats were found in comparison with the adult group.

On the other hand, an increase in pro-inflammatory enzymes and molecules with aging has been reported (Chung et al., 2001). One mediator molecule involved in the inflammatory responses is nitric oxide (Boveris et al., 2002). In the present study, serum total nitric oxide was less in aged female rats compared with adult female animals. It is convenient to point out that total nitric oxide, in addition to its role as inflammatory mediator, can also act as a free radical either directly or through generation of peroxynitrites through its interaction with superoxide anion (O_2^-) (Szabo, 2003). Furthermore, an excess of nitric oxide and peroxynitrites can negatively affect mitochondrial function and phosphatidylcholine (PC) synthesis (Sastre et al., 2000). Moreover, lysosomes and lysosomal enzymes are the main factors playing a vital role in tissue injury and repair, inflammation, phagocytosis, intracellular degeneration, cancer and

rheumatoid arthritis. It was further postulated that the lysosomal enzymes are released in inflammation diseases to stimulate the synthesis of prostaglandins. Extracellular release of such enzymes may be crucial to pathogenesis of tissue injury and inflammation (Rasool et al., 2006; Sabina et al., 2010) it is likely that a reduction in the release of such enzymes would prove beneficial. In adjuvant-induced arthritis, the glycohydrolases released from invading macrophages, neutrophils, and from tissue cells such as synoviocytes and chondrocytes, initiates the synthesis of inflammatory mediators (thromboxanes, prostaglandins, and leukotrienes) which plays an important role in the rheumatoid process (Rasool et al., 2006). As shown by the results, our data indicated that aging process was found to cause significant increase in IL-1, IL-6, TNF- α and COX-2 along with a significant decline in NO level in female senile rats as compared with the adult ones. Kim et al. (2010) reported that the expression of the pro- inflammatory genes (COX-2 and NO) increased significantly in aged rats. In addition, Chung et al. (2009) reported that accumulated data strongly suggested that continuous (chronic) upregulation of pro-inflammatory mediators (TNF- α , IL-1, IL-6, COX-2, iNO) is induced during the aging process. This is likely to be due to an age-related redox imbalance (the activation of redox-sensitive transcription of nuclear factor kappa B, during aging) that activates many pro-inflammatory signaling pathways, including the mitogen- activated pro-

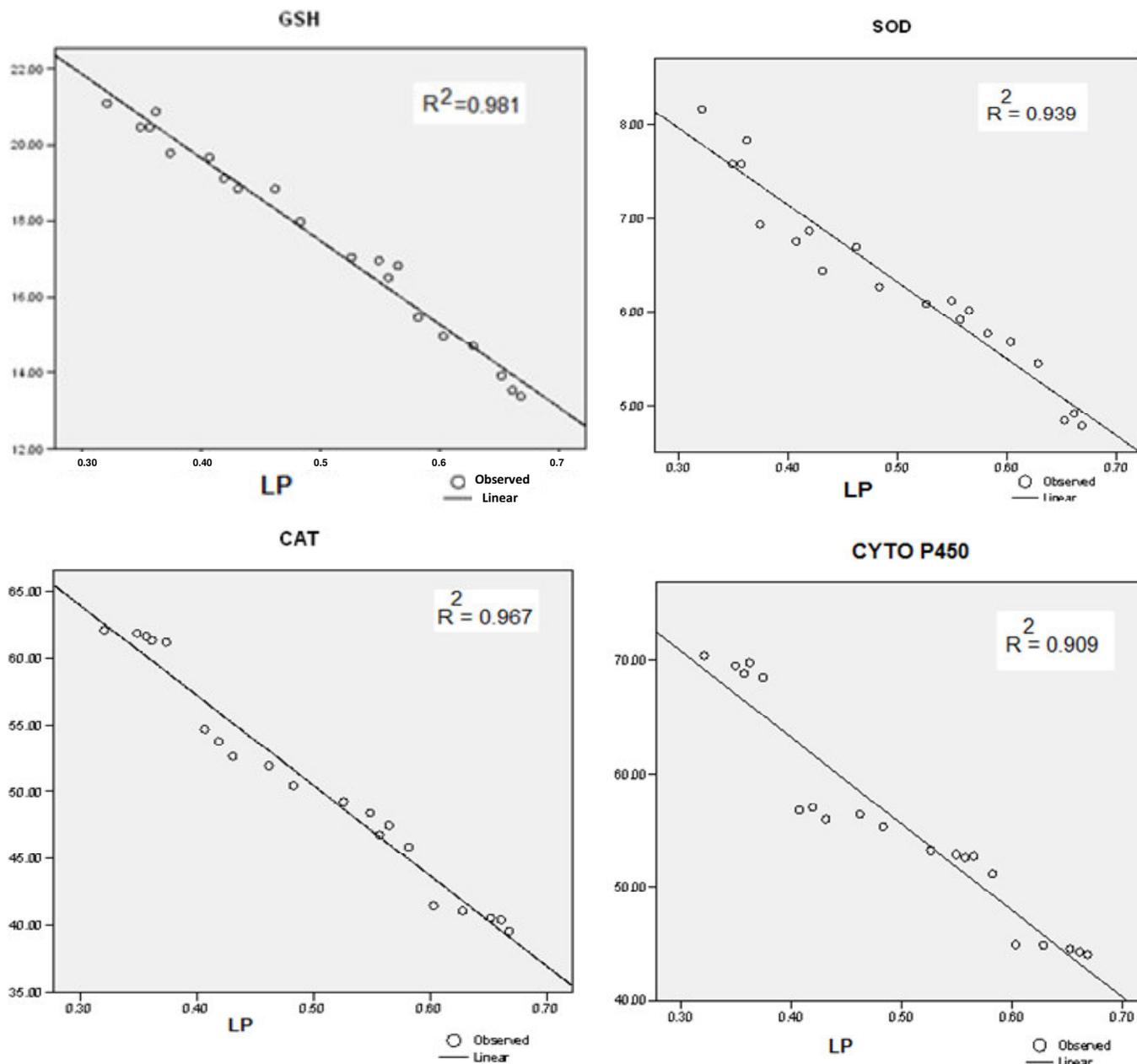


Figure 2. Simple correlation between serum tumor necrosis factor – alpha and IL1, IL6, NO and COX-2 in the serum of senile female rats.

tein kinase (MAPK) pathways and the NF- κ B signaling pathways. Moreover, Kim et al. (2000) reported that the up-regulation of NF- κ B activity is accompanied by increased ROS production during aging. Furthermore, Gonzalo-Calvo et al. (2010) showed that TNF- α was significantly increased in the aged population, implying that aging is accompanied by a gradual increase in this inflammatory biomarker which is triggered by oxidative stress induced by decrease in antioxidant defenses in the elderly population. The change in antioxidant system due

to aging process might be due to the changes of the cellular structure and function which result in redox deregulation during aging. The redox deregulation implicated with NF- κ B activation in central to inflammation process during aging because of the sensitivity of NF- κ B to oxidative stress (Chung et al., 2001). It is possible to suggest that the anti-inflammatory properties of ginger are crucial in its antioxidant effects. Ginger was reported to suppress inflammatory actions of macrophages and the release of monocyte chemo-attractant protein-1 from

adipocytes (Woo et al., 2007). Furthermore, Kim et al. (2010) indicated that the beneficial efficacy of short-term ginger supplementation at the molecular levels is through its ability to blunt age-related oxidative stress and suppress age-related inflammatory actions via NF-KB activation.

On the other hand, xanthine oxidase is a source of oxygen free radicals. In the reperfusion phase (that is, reoxygenation), xanthine oxidase reacts with molecular oxygen, thereby releasing superoxide free radicals (Dugasani et al., 2010). Based on the report (Cos et al., 1998) that the phenolic compounds inhibited xanthine oxidase and/ or scavenged superoxide, the inhibition of superoxide production by ginger extract in this study might have resulted from the combined effects of scavenging superoxide and inhibition xanthine oxidase activity.

Furthermore, in addition to cytokines, metabolites of arachidonic acid also participate in the inflammatory process. Products such as PGE₂ are representative of one of the pathways that initiate polymorphonuclear leukocytes recruitment and change in vascular tone and blood flow. Increased production of prostaglandins during an inflammatory response is achieved by induction of cyclooxygenase 2. COX-2 is not normally present, but is inducible in certain cells in response to inflammation stimuli and control of cell growth (Dewick, 2002). Thus, compounds that inhibit the activity of COX-2 are crucial for anti-inflammation. In the present study, ginger extract showed a remarkable ability to inhibit Cox-2 activity. Lantz et al. (2007) demonstrated that organic extracts of ginger and compounds found in ginger are highly effective at inhibiting lipo-polysaccharides induced production of prostaglandin E₂. These compounds appear to not only inhibit COX-2 enzyme activity, but are also able to alter COX-2 mRNA levels, suggesting at least two sites of action. Moreover, ginger was found to reduce thromboxane-B₂ and prostaglandin-E₂ production in rats (Thomson et al., 2002).

In the present work, the decrease in hepatic lysosomal cytochrome P450 enzyme activities was observed in aged rats after ginger treatment. Moreover, Arkene et al. (2006) suggested that this herb can inhibit the release of lysosomal enzymes by its stabilizing action and showed that ginger caused significant reduction in total leucocytes migration as well as in lymphocytes and monocytes/macrophages migration from the blood into the synovial cavity. These inflammatory cells are the major contributors to the initiation and maintenance of inflammation response (Weyand, 2000). A recent study revealed that 6-gingerol isolated from ginger plays a vital role in suppressing ROS and RNS generation, and inhibits the expression of inducible pro-inflammatory genes in macrophages (Pan et al., 2008). These results are supported in the present study by the depleted TNF- α level, IL-1 and IL-6 in ginger treated group when compared

to the senile rats. Moreover, Aktan et al. (2006) reported that ZTX42 (a closely related gingerol analog) suppressed NO production in murine macrophages by partially inhibiting inducible nitric oxide synthase (iNOS) enzymatic activity and reducing iNOS protein production. This effect was through attenuation of NF-Kappa B-mediated iNOS gene expression, which provides a possible mechanism for the anti-inflammatory activity reported for this class of compounds. It has been also shown that ginger and its constituents are effective at the sites of inflammation by suppression of pro-inflammatory cytokines, and chemokines produced by chondrocytes, synoviocytes and leucocytes (Phan et al., 2005). In this regard, Tripathi et al. (2009) demonstrated that whole ginger extract has a global inhibitory effect on macrophages function *in vitro* and that this accounts for its reputed anti-inflammatory effect *in vivo*. They also hypothesized that the active constituent in ginger, 6-gingerol, is an effective anti-inflammatory substance because of its inhibition of macrophages activation, more specifically by its inhibition of pro-inflammatory cytokines and antigen presentation by lipopolysaccharide-activated macrophages. One advantage of 6-gingerol is the fact that it does not affect the antigen presenting cells functions and this may be useful to reduce inflammation without interfering with antigen presenting function of macrophages. Antigen presentation by the macrophages is an integral step in the initiation of the adaptive immunity by lymphocytes. Furthermore, 6-gingerol has been shown to suppress the production of pro-inflammatory cytokines (TNF- α , IL-1 and IL-12) from macrophages (Williams et al., 2007). Recently, Kim et al. (2010) showed that zingerone, a major component found in ginger root, had not only antioxidant effects by constitutive suppression of ROS, but also anti-inflammatory effects through interference with NF-kB activation in aged rats. In addition, zingerone treatment suppressed gene activation of pro-inflammatory enzymes, COX-2 and iNO, which were upregulated with aging through NF-kB activation and I κ B kinase/mitogen-activated protein kinase signaling pathway. These findings strongly indicate that zingerone treatment exerts a beneficial efficacy by suppressing both oxidative stress and age-related inflammation through the modulation of several key pro-inflammatory genes and transcription factors. When the female senile rats were treated with ALA in the present study, there was a significant reduction in LP level and a significant elevation in the GSH level and the activities of SOD, CAT and cytochrome P450 as compared with the senile female rats. In this regard, it was demonstrated that ALA prevented inflammation induced LP production in the plasma, liver and brain. The reduction in the level of LP was ascribed to the potent free radical scavenging capacity of ALA. As thiols play a pivotal role in protecting cells against LP, it was postulated that the dithiol nature

of DHLA is responsible for the suppression of LP levels in specify the nature of the experiment (Jesudason et al., 2008). Moreover, Lapenna et al. (2003) reported that DHLA, at therapeutical concentrations, can counteract 15-lipoxygenase-dependant lipid peroxidation. The authors showed that its effect stems primarily from the reduction of the active ferric 15-lipoxygenase form to the inactive ferrous state after DHLA-enzyme hydrophobic interaction. Other possible interventions of DHLA include scavenging of fatty acid peroxy radicals formed during lipoperoxidative processes and inhibition of 15-lipoxygenase oxidative activity.

CAT activity is the key component of the enzymatic antioxidant defense system and the inhibition of protective mechanism of defense system leads to free radicals-induced cellular damage (Zaidi and Banu, 2004). The observed decrease in the activity of CAT in aged rats is consistent with earlier studies. It was reported that ALA increases the glucose uptake in vitro and this enhanced cellular glucose uptake which serves as the fuel for both the pentose phosphate shunt and oxidative phosphorylation, thus bringing up the cellular levels of NADPH and NADH, thereby enhancing the activity of CAT under stressful conditions (Arivazhagan et al., 2000). SOD is an enzyme extensively used as biochemical indicator of pathological states associated with oxidative stress. This antioxidant eliminates superoxide anions thereby preventing free radical chain reaction. Decreased SOD activity may result from a suppression of SOD synthesis due to a genetic defect, leak of SOD out of cell due to increased production of oxygen radical causing cell membrane damage and inactivation of SOD by increased intracellular H_2O_2 level (Suzuki et al., 1991). Increased superoxide anions may be one of the factors responsible for decreased activation of CAT (Meister and Anderson, 1983). The restoration of SOD and CAT activities in the liver with ALA treatment as the present results indicate that in liver tissue this rescue agent can protect the cells against maintaining the functions of these enzymes (Jesudason et al., 2008). It has been shown that the diminution in the concentration of GSH level in senile female rats leads to fast accumulation of lipid peroxide in cells and the GPx which is coupled with GSH, together with CAT functions as a major cellular reducer of hydrogen peroxide and subsequent lipid peroxidation (Arteel and Sies, 2001). Studies have also shown that ALA indirectly influences the activities of SOD, increases intercellular GSH content that might also activate the GSH-dependent enzymes, GPx and increases the activity of CAT (Shanmugarajan et al., 2008). Moreover, Kokilavani et al. (2005) showed that ALA increases the levels of these enzymes by directly reacting with various reactive oxygen species and nullify the oxidation processes in lipids and inter cellular components. More recently, Shay and Hagen (2009) demonstrated that Akt is a highly regulated

serine/threonine kinase involved in stress response and cell survival. Stress response pathways must cope with increasing chronic stress susceptibility with age and an age-related lesion in Akt activity via loss of phosphorylation on Ser473. In hepatocytes from old rats, the basal phosphor-Ser473Akt was 30% lower when compared to the adult animals. Treatment with physiologically relevant doses of ALA provided a 30% increase in phosphor-Ser-473; thus, ALA appears to induce a compensation for the constitutive decline in Akt activity, thereby maintaining this enzyme's critical function which otherwise declines with age. Some emerging evidence strongly supports the notion that the molecular inflammatory process plays a central role in the aging process and age-related diseases (Chung et al., 2006). COX-derived reactive species generation as well as gene expressions of IL-1, IL-6, TNF- α , COX-2 and iNO are enhanced during aging (Chung et al., 2006; Kim et al., 2007). COX activity and the production of thromboxaneA2 and prostaglandins are also increased during aging. The present work is consistent with these findings. The treatment with ALA significantly inhibited IL-1, IL-6, TNF- α , COX-2 and increased NO hepatocytes in aged female rats. In this regard, it was reported that ALA significantly inhibited IL-1 induced osteoclast formation in cocultures of mouse osteoblasts and bone marrow cells. In addition, ALA inhibited COX-2 activity, PGE2 production, and sustained receptor activator of NF-kappaB ligand (RANKL) expression, thereby inhibiting osteoclast formation and bone loss in inflammatory conditions associated with the aging process (Ha et al., 2006). In conclusion, ginger and α -lipoic acid have shown a significant ameliorative value in counteracting age-induced oxidative stress in liver of female aged rats via scavenging free radicals as well as enhancing the antioxidant system and can efficiently reduce systemic inflammation.

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