

Protective Role of Either Ginger or Lipoic Acid in Senile Female Rats

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Abstract Aging associated with neurodegenerative disorders. The dysregulation of brain lipid metabolism might contribute to the mechanisms of aging and Alzheimer's disease. The study aimed to evaluate the change in free fatty acids and free calcium ion contents, as well as the total antioxidant activity in senile female rat's brain. Moreover, the possible protective effect of either ginger or alpha lipoic acid. The experimental groups (n=6/group) administered orally for four weeks and classified into: 1- Control adult female rats group and 2- Senile female group (both groups) received one ml/100 g b.wt. 0.5 g/100 ml CMC. 3- Senile ginger group received 250 mg/kg ginger. 4- Senile lipoic treated group administered 65 mg/kg lipoic acid. The results suggested protective effect of ginger and alpha lipoic acid in senile female rats through modulation of, saturated, unsaturated fatty acids and the total antioxidant capacity in cortex and hippocampus. In addition, the cortical free calcium ions content that could protect the senile rats from ageing diseases.

Keywords Ginger, Alpha Lipoic Acid, Cortex, Hippocampus, Free Fatty Acids, Total Antioxidant Capacity

1. Introduction

The central nervous system is particularly vulnerable to oxidative injury. It contains high concentrations of readily oxidizable poly-unsaturated fatty acids and has a high rate of oxygen consumption per unit volume. Also possesses relatively low levels of antioxidant defense system (Koenig and Meyerhoff, 2003; Freitas, 2009). In the central nervous system, lipids are one of the main targets of reactive oxygen species, because their cell membranes are rich in polyunsaturated fatty acids (Cini and Moretti, 1995). Studies suggesting that dysregulation of brain lipid metabolism might contribute to the mechanisms of aging and Alzheimer's disease (AD); lipid metabolism has not evaluated extensively in aging brain (Snigdha et al. 2012). Epidemiological studies implicate that the high-fat diet confers a significant risk for development of AD and the degree of saturation of fatty acids is critical in determining the risk for AD (Mattson et al., 2002). The saturated free fatty acids (FFAs), palmitic and stearic acids, caused increased amyloidogenesis and tau hyperphosphorylation in primary rat cortical neurons. These FFA-induced effects observed in neurons mediated by astroglial FFA metabolism (Patil et al., 2007). Fatty acid is required for both the structure and function of every cell in the body and forms an

important component of cell membranes. It undergoes changes during the process of injury, repair and cell growth (Cameron and Cotter 1997; Murugan and Pari, 2007). These FFA-induced effects observed in neurons mediated by astroglial FFA metabolism (Patil et al., 2007). Fatty acid is required for both the structure and function of every cell in the body and forms an important component of cell membranes. It undergoes changes during the process of injury, repair and cell growth (Cameron and Cotter 1997; Murugan and Pari, 2007).

Deficiencies in polyunsaturated essential fatty acids (PUFA) implicated in mood disorders (Sublette et al., 2009). Linoleic acid, eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) are especially important during human brain development. Maternal deficiency of omega-3-fatty acids related to deficits in neurogenesis, neurotransmitter metabolism, and altered learning and visual function in animals (Shahidi et al., 2005; Innis, 2008). Dietary omega-3-fatty acids are certainly involved in the prevention of some neuropsychiatric disorders, particularly depression, as well as in dementia, notably Alzheimer's disease (Calon and Cole, 2007). Alpha linoleic acid and DHA is essential for nervous tissue growth and function (Bourre, 2004).

Ginger (*Zingiber officinale*) used in the Indian traditional system of medicine for digestive disorders, common cold, and rheumatism (Sharma and Singh, 2012). The nonvolatile pungent compounds namely gingerols, shogaols, paradols, and zingerone) are some of the extensively studied phytochemicals. They account for the antioxidant,

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anti-inflammatory, antiemetic, and gastroprotective activities (Kubra and Jaganmohanrao, 2012). The nonvolatile pungent compounds (namely gingerols, shogaols, paradols, and zingerone) are some of the extensively studied phytochemicals and account for the antioxidant, anti-inflammatory, antiemetic, and gastro protective activities (Kubra and Jaganmohanrao, 2012).

Alpha lipoic acid (ALA, DL-6, 8-dithiooctanoic acid) is a naturally occurring compound synthesized in small quantities by most plants and animals (Perera et al., 2011). Considered as endogenous thiol antioxidant that quench ROS, regenerate glutathione (GSH). It chelate metals such as iron, copper, mercury, and cadmium, which known to mediate free-radical damage in biological systems (Akpınar et al., 2007; Applegate et al., 2008).

The study aimed to declare the possible protective effect of ginger or alpha lipoic acid on senile female rats as antioxidants through studying the change in metabolic brain free fatty acids in cortex and hippocampus.

2. Material and Methods

Twenty-four female rats, 18 senile (30 months old) and 6 adults (6 months) obtained from the National Organization for Drug Control and Research (NODCAR). Animals housed plastic cages each cage contained six rats. Animals were maintained under controlled temperature of $25 \pm 2^\circ\text{C}$ and 12 hours light/12 hours dark cycle throughout the experiment.

The animals allowed to food, as commercial pelleted diet and water were available *ad libitum*.

Chemicals: Ginger purchased from Arab Co. For Pharmaceuticals & Medicinal Plants MEPACO- EGYPT as tablet contains treated ginger (*Zingiber officinale*) 400mg. Lipoic acid supplied as tablets containing 300 mg manufactured by EVA Company, Egypt. Saturated fatty acids (SFAs), lauric (C12:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0) and arachidic (C20:0). Unsaturated fatty acids (USFAs), oleic (C18:1 ω -9), linoleic (C18:2 ω -6), *cis*-5.8.11.14.17-eicosapentaenoic (C20:5 ω -3) (EPA) and *cis*-4.7.10.13.16.19-docosahexaenoic acid (DHA) (C22:6 ω -3). All FAs purchased from Sigma-Aldrich Co. (St. Louis, USA). All other chemicals and solvents used were of the High Performance Liquid Chromatography and analytical grade.

Animal Grouping: The animals divided into four main groups; each one contained six rats as follows: 1- control normal adult female rats (C) received 1 ml/100 g.b.wt. Carboxymethylcellulose sodium salt (CMC) orally. 2-Control senile female rats (S) received CMC as group 1. 3-Senile ginger group (S+G) received 250 mg/kg in CMC. 4-Senile lipoic acid group (S+L) received 65 mg/kg lipoic acid in CMC. Treatments extended for four weeks with daily oral administration. Doses calculated as equivalent to the human recommended dose according to Reagan-Shaw *et al.* (2008).

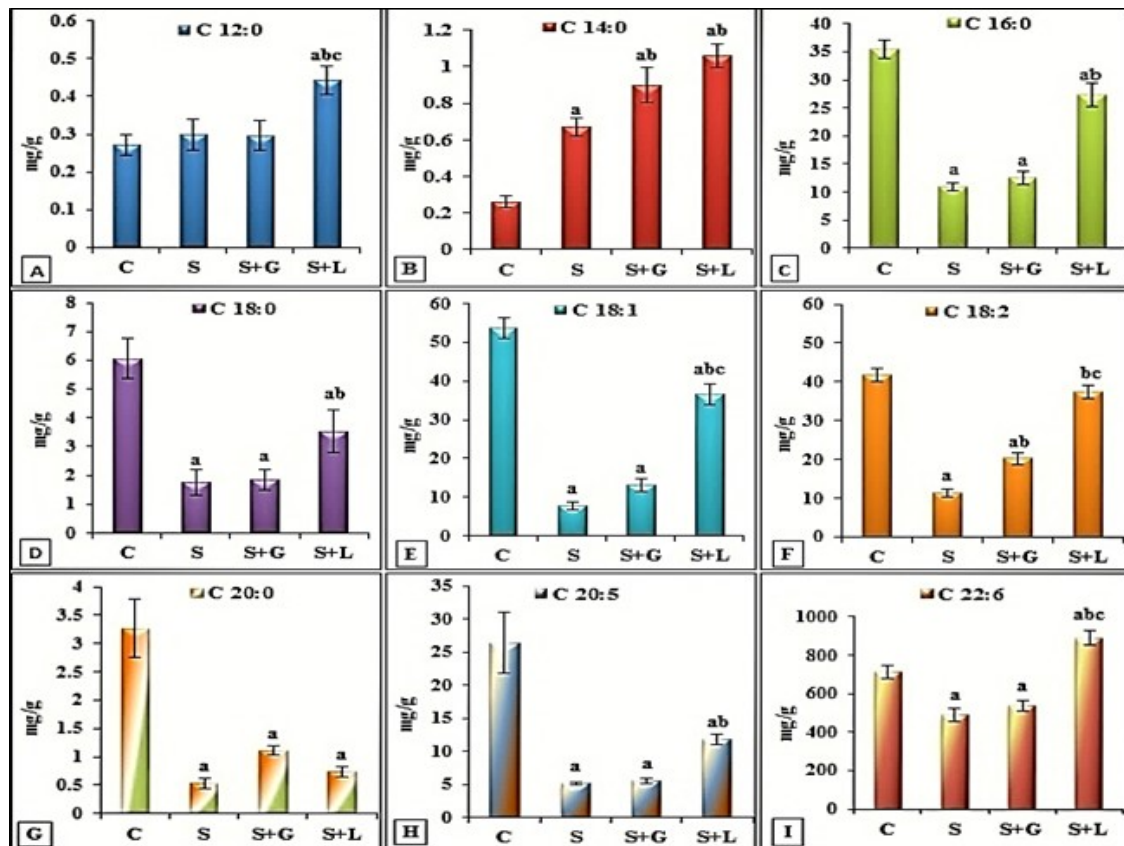


Figure 1. (A-I): Cortex free fatty acids content. Data expressed as mean \pm SE. Superscript represent significance at $p \leq 0.05$ as (a) from control (c), (b) from senile group (S), from senile+ginger group (S+G)

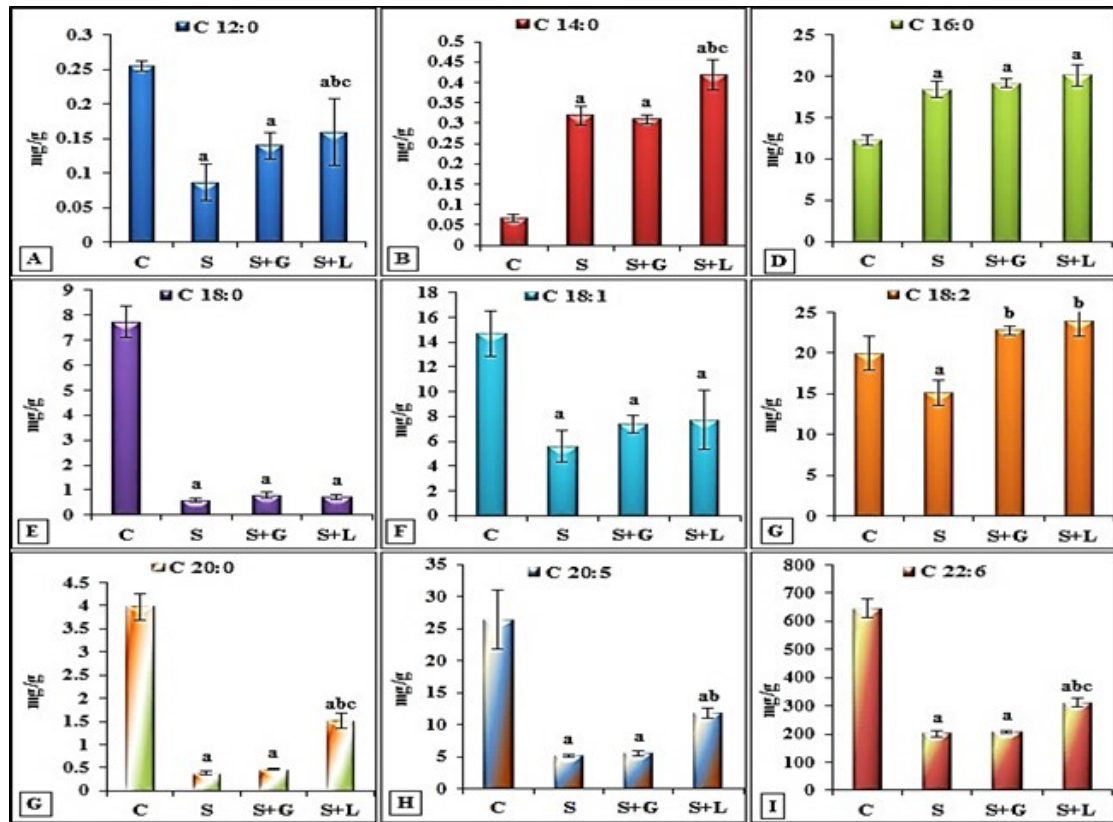


Figure 2. (A-I): Hippocampus free fatty acids content. Data expressed as mean \pm SE. Superscript represent significance at $p \leq 0.05$ as (a) from control (C), (b) from senile group (S), from senile+ginger group (S+G)

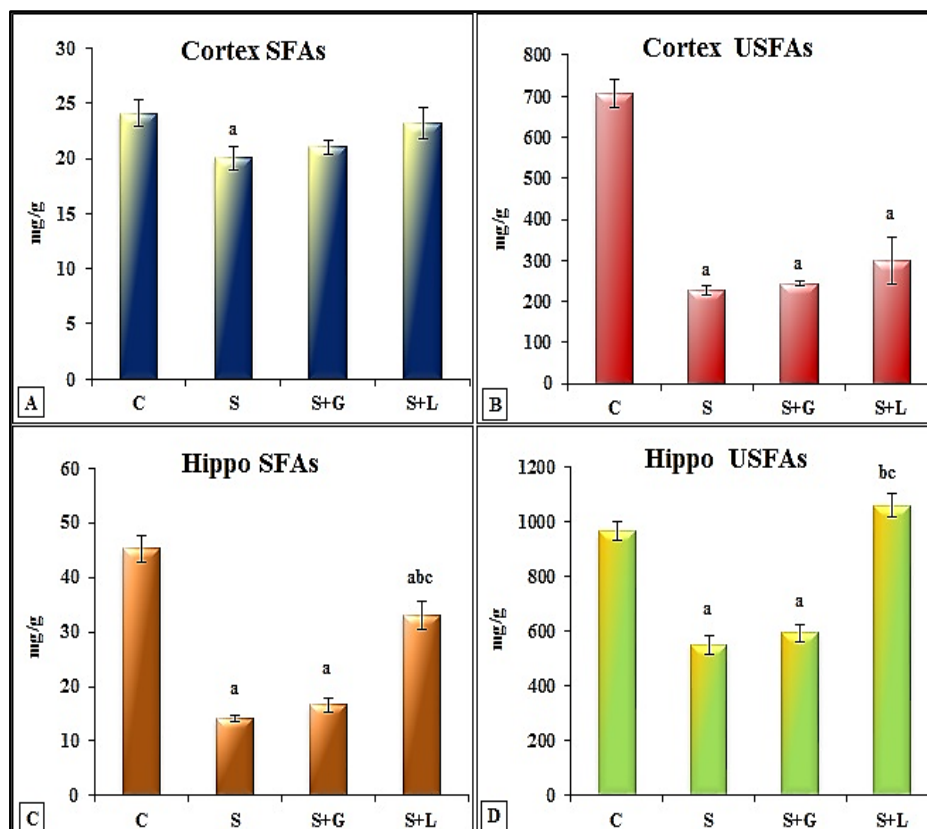


Figure 3. (A-D): Cortex and hippocampus saturated fatty acids (SFAs) and unsaturated fatty acids (USFAs) content. Data expressed as mean \pm SE. Superscript represent significance at $p \leq 0.05$ as (a) from control (C), (b) from senile group (S), from senile+ginger group (S+G)

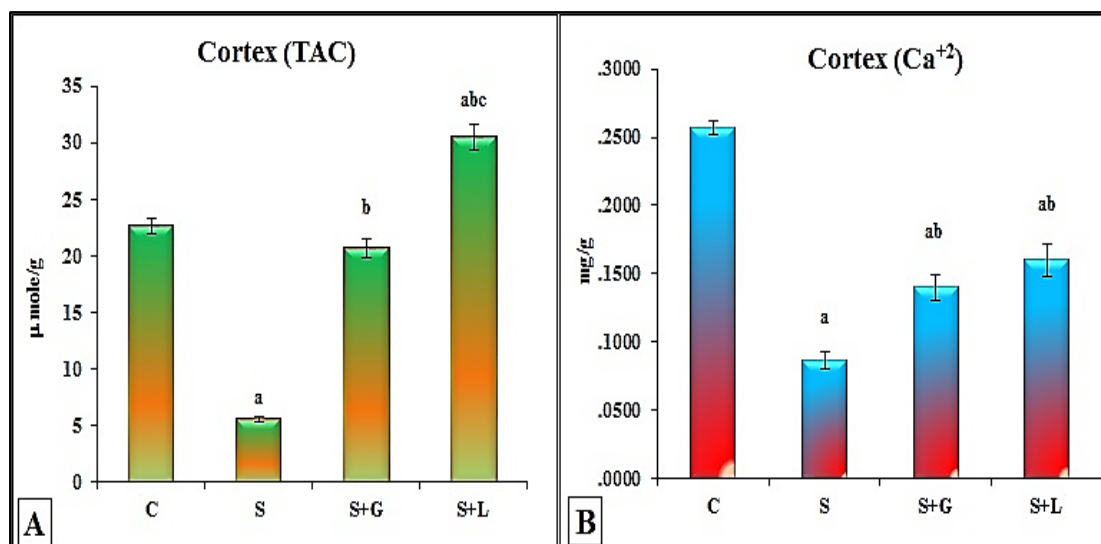


Figure 4. (A & B): Cortex total antioxidant capacity (TAC) and calcium ions contents (Ca^{+2}) content. Data expressed as mean \pm SE. Superscript represent significance at $p \leq 0.05$ as (a) from control(c), (b) from senile group (S), from senile+ginger group (S+G)

Biochemical Assay: Following the completion of the experiments, the rats sacrificed after 12 hours from the last dose by rapid decapitation. Brain cortex and hippocampus excised for the determination of free fatty acids as methyl esters by GC according to the method previously described in Firlag et al., (2013). Using column HP-5 (Methyl siloxane). Cortex also used for the determination of Ca^{+} contents according to the method in Arafa (2010) and the total antioxidant capacity according to the method of Koracevic *et al.* (2001).

Statistical Analysis: Reported values represent means \pm SE. Statistical analysis evaluated by one-way ANOVA. Once a significant F test obtained, LSD comparisons performed to assess the significance of differences among various treatment groups. Statistical Processor System Support "SPSS" for Windows software, Release 21.0 (SPSS, Chicago, IL) was used.

3. Results and Discussion

The data depicted for senile female group showed in cortex (Figure 1 (A-I)) significant decrease in SFAs (palmitic, stearic and arachidic) and UFA (oleic, linoleic, EPA and DHA) and a significant increase in myristic acid. While showed, significant decrease in SFAs (lauric, stearic and arachidic) and UFA (oleic, linoleic, EPA and DHA), but significant increase in SFAs, myristic and palmitic in hippocampus (Figure 2 (A-I)) as compared to the adult control values. The data in Figure 3 (A-D) demonstrated the significant decrease in cortex and hippocampus total saturated (SFAs) and unsaturated (UFAs) fatty acids in senile female rats as compared to the control values. In cortex, TAC and Ca^{+2} contents decreased significantly in senile group as compared to the control values (Figure 4(A and B)).

The results confirmed the age-related metabolic changes in lipids, resulted in variations in lipid content that also

reflected changes in the proportions of gray and white matter as well as the increase in the oxidative stress results in a lower concentration of n-3 PUFA in the nervous system (Ledesma et al., 2012). The ratio of saturated to unsaturated fatty acids is one of the key factors influencing cell membrane fluidity. Then perturbations in the fatty acid ratio have implications for neurological diseases (Hazel and Williams, 1990; Yehuda et al., 2002). The essential fatty acids linoleic acid and alpha-linolenic acid (SC-PUFAs) (- 3 fatty acid) are required for normal nerve impulse transmission and brain function and serve as precursors of other PUFAs. The levels of two of these metabolites, DHA and arachidonic acid decreased in aged rats. A significant age-dependent decrease in the levels and turnover of other PUFAs observed in the hippocampus and cortex (Yehuda et al., 2005; Bazan et al., 2011; Ledesma et al., 2012; Fabelo et al., 2014). Oleic acid and palmitoleic acid are the major monounsaturated fatty acids in fat depots and membrane phospholipids. These fatty acids synthesized by the stearoyl-CoA desaturase. The ratio of stearic acid to oleic acid is one of the factors influencing membrane fluidity and cell-cell interaction. Abnormal alteration of this ratio play a role in several physiological and disease states including diabetes, cardiovascular disease, obesity, hypertension, neurological diseases, immune disorders, cancer, and aging (Ntambi, 1999). Rioux et al., (2008), suggested the contribution of high dietary myristic acid and the increased tissue storage of α -linolenic acid and the overall bioavailability of (n-3) polyunsaturated fatty acids in the brain. Lin et al., (2012), indicated through pre-dementia studies that decrease in EPA level is a risk factor for cognitive impairment.

Different studies discussed the age-related variations in the total antioxidant defenses in cortex and hippocampus. Also the oxidative stress-related neurodegenerative disorders (Siqueira et al., 2005; Muthuswamy et al., 2006; Murali and Panneerselvam, 2007; Badr El-Din et al., 2010).

Previously, Rogasevskaia and Coorsen, (2006) tried to explore potential dysfunctional changes in regulatory mechanisms that may contribute to a breakdown in calcium homeostasis in aging. The study of Tanaka *et al.*, (1996) provided evidence for that age-related decrease in presynaptic functions. Reduction in calcium influx via voltage-dependent calcium channels followed by decreased acetylcholine (ACh) release from synapses in spite of the abundance of ACh within the synapses. Wainwright (2002) reported that deficiencies of EFA influenced specific neurotransmitter systems in animals, particularly the dopamine systems of the frontal cortex. As well as associated behaviors and also the age-related reduced contents of cortex and hippocampus neurotransmitters which influenced by the FFA composition (Hegazy & Ali, 2011).

Ginger treatment exhibited the significant increase compared to the senile group values. In the cortex, myristic and linoleic acids (Figure 1 (A-I)), and in hippocampus linoleic acid (USFA), (Figure 2 (A-I)). The data in Figure 3 (A-D) demonstrated not statistically different increase in cortex and hippocampus SFAs and UFAs in ginger treated group as compared to the senile group values. Cortex TAC and Ca^{+2} contents significantly increased in ginger group as compared to the senile group values (Figure 4(A and B)). Results confirmed the antioxidant potential of the ginger due to its active ingredients especially essential oils (Yeh *et al.*, 2014). Brain tissue has 60% lipid, and it has a remarkable high-energy consumption (Crowford, 1993). In addition, these tissues are more susceptible to oxidative damage than other tissues (Foloyd *et al.*, 2001). It is in line with the neuroprotective effect of ginger that may affect neurotransmitters which playing crucial role in learning and memory. In addition to, interaction with inhibitory and excitatory system and calcium channel inhibition. Beyond the antioxidant effect (Stoilova *et al.*, 2007; Waggas, 2009; Jittiwat and Wattanathorn, 2012; Hosseini and Mirazi, 2014).

Lipoic acid treatment represented the significant increase in cortex SFAs (lauric, myristic, palmitic, stearic and USFAs, oleic, linoleic, EPA and DHA) (Figure 1 (A-I)), Also in hippocampus SFAs (lauric, myristic and arachidic and USFAs, linoleic acid, EPA and DHA) (Figure 2 (A-I)) as compared to the senile group values. The data in Figure 3 (A-D) demonstrated a significant increase in hippocampus SFAs and UFAs in lipoic treated group as compared to values of senile and ginger treated groups. Cortical TAC significantly increased in lipoic group as compared to the values of senile and ginger treated groups and Ca^{+2} contents increased significantly compared to senile group value (Figure 4(A and B)). Alpha-lipoic acid treatment previously proved impact in reducing oxidant production in rat brain regions in arsenic and stressed rats (Shila *et al.*, 2005; Akpinar *et al.*, 2008). Its beneficial effect in both preventing and reversing abnormalities in an aging brain associated with normalization of lipid peroxidation and partial restoration in the activities of various enzymatic antioxidants. Suggesting it could improve brain antioxidant functions in the elderly

(Arivazhagan *et al.*, 2002). Celik and Ozkaya (2002) suggested that the lipoic acid had a therapeutic effect via treatment guinea pig oxidative stress- model by H_2O_2 . Suggesting action by limiting damage from the oxidation reaction in unsaturated fatty acids.

4. Conclusions

The results suggested protective effect of ginger and alpha lipoic acid in senile female rats. That through modulation of fatty acids in cortex and hippocampus, as well as the cortical free calcium ions content. Such could protect the senile rats from aging diseases and their supplementation as complimentary could improve brain antioxidant functions in the senile rats.

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