Protictive effects of green tea and garlic on the morphological and oxidative stress changes of hippocampus in aged male and female albino rats

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Abstract

Objective: Green tea is an extremely popular drink and particularly rich in strong antioxidants, flavonoids. Also, garlic is important dietary and has medicinal role for centuries and possess both antioxidant and anti-inflammatory action in several oxidative stress conditions.

Materials and methods: Four groups of both male and female rats [young (3 months-old), aged (26 months-old), aged+green tea (200 mg/kg) and aged+garlic (100 mg/kg)] were used. Histological changes in the pyramidal cells of the hippocampus were examined. Levels of oxidized lipids (LPO), superoxide dismutase (SOD) activity and glutathione concentration (GSH) were measured in the serum.

Results: Irregular, shrunk and deeply stained large and small pyramidal cells with fragmented nuclei and basophilic cytoplasm observed in the hippocampus of aged male and female rats. Green tea and garlic administration preserve these changes in the pyramidal cells in both genders. Aging increased the level of LPO, decreased the activity of SOD and the concentration of GSH of both male and female rats versus those of young ones. Again, green tea and garlic ameliorated these changes.

Conclusion: our results showed that the protective role of green tea and garlic against oxidative stress parameters was more effective in males than females. Morphologically, garlic exerted preservation to the pyramidal cells of hippocampus more than green tea in both genders. This study suggests that the use of both green tea and garlic may inhibit the bodily changes related to aging.

Keywords: Aging, Garlic, Green tea, Lipid peroxidation, Antioxidants

Introduction

The aging process considered a unique feature of life cycle of virtually all multicellular organisms including many physiological actions to maintain the homeostasis and resulting in death. Although numerous hypotheses have been done to explain the aging process, many of them did not have sufficient empirical support to merit serious consideration [1].

It was postulated that, aging results from the accumulation of harmful effects caused by free radicals. The ability of an organism to overcome cellular damage induced by reactive oxygen species (ROS) plays a vital role in determining organismal life span [2]. ROS production by mitochondria and increased 8-oxo-dG content in the mtDNA are frequently detected in aged tissues. This suggesting that progressive accumulation

of oxidative DNA damage is a contributory factor to the aging process [3]. Consistently, [4] reported that increased oxidative damage in cells is associated with aging.

The hippocampus is the site of selection of important information and consolidation of spatial memory. Environmental sensory inputs received by the cortical sensory and association areas are transmitted in sequential order via the entorhinal cortex and hippocampal formation [dentate gyrus (DG), a series of *Cornu Ammonis* regions (CA3, CA2, CA1), subiculum] before finally transferred back to the cortical association areas [5].

CA1 cells are essential for associating information received gradually when there are considerable temporal intervals between components of stimuli. Of note, it is possible that CA1 and CA3 cells have an overlapping role in memory which

involves temporal ordering associated with spatial location [6]. Given the roles of the CA3 and CA1 regions in spatial memory formation, it follows that any disruption to the pyramidal cells of the CA2–CA3 and CA1 regions may cause spatial learning and memory deficits.

Garlic (*Allium sativum*) is important dietary and has medicinal role for centuries. The suggested pharmacological effects of garlic are hypolipidemic, hypoglycemic, anticoagulant, antihypertensive, antimicrobial, anticancer and antitumor, as an antidote for heavy metal poisoning, hepatoprotective and as an immunomodulator [7]. Organosulfur compounds, present in garlic, have significant antioxidant effects as well [8].

Green tea (*Camellia sinensis*) is an extremely popular drink in eastern countries, and polyphenols are natural plant flavonoids found in the tea plant leaves. Polyphenol antioxidants are known as catechins. The major tea catechins include epicatechin (EC), epigallocatechin (EGC), EC gallate (ECG) and EGC gallate (EGCG). Epigallocatechingallate is the most active antioxidant of the tea catechins and is responsible for the “green tea effect.” [9].

It was reported that, the polyphenols in green tea can neutralize free radicals and may reduce or even help to prevent some of the damage caused by ROS [10]. On the other hand, tea polyphenols have the ability to participate in vitamin E recycling [11]. Because of the presence of other antioxidant constituents in the green tea extract, it is more stable than pure epigallocatechingallate; one of the major constituents of green tea [12].

The aim of this study was to investigate the role of green tea extract and garlic as antioxidants against the effect of aging on the morphological changes of the hippocampus and the oxidative stress parameters (LPO, SOD and GSH) in the serum of aged male and female albino rats.

Materials and methods

Animals

A total number of 160 rats (80 males and 80 females) used in this study. 20 males and 20 females with initial ages of 3 months (young), 60 males and 60 females with the initial age of 22 months (aged). They were purchased from Assiut University Joint Animal Breeding Unit. Care and treatment of animals was approved and practices were performed according to approval of ethics regulation at the Assiut University. The animals were kept at temperature of $23\pm 2^{\circ}\text{C}$ and light cycle of 12:12 hours light: dark. All animals were given free access to standard chow and tap water.

Chemicals

2-thiobarbituric acid, 1,1,3,3-tetramethoxypropane, superoxide dismutase, epinephrine, glutathione, dimethyl sulfoxide and sodium dodecyl sulfate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Green tea was purchased from (Twinings of London, England). Garlic (Tomex 200 mg tab) was purchased from Sekem Pharmacy, Egypt. All other chemicals were of highest quality available.

Green tea and garlic preparations

The leaves of green tea (*Camellia Sinensis*) were boiled in distilled water (1:10 w/v) 5 min twice. The solution was called unfiltered, and then filtrates were combined, concentrated, and lyophilized. Ten grams of lyophilized aqueous extract were soaked in 1 liter of boiling distilled water to make 1% solution. The solution designated as green tea extract (GTE) was orally administered to rats by gastric tube at a dose of 200 mg/Kg [13].

Garlic (Tomex 200 mg tab) was dissolved in distilled water and was given orally to rats by gastric tube at a dose of 100 mg/kg [14].

Experimental design and procedures

The animals were divided into 4 groups for each gender, 20 rats for each; **group 1**; (3 mon- old) served as young control group (y. cont), **group2**; (26 mon-old) served as old group (Ag), **group 3**; (26 mon-old) orally received 200 mg/kg green tea extract per day (Ag+GT), and **group 4**; (26 mon-old) orally received 100 mg/kg garlic per day (Ag+Gr). The administration of green tea and garlic was repeated daily for 4 months.

Hippocampus preparation

The hippocampus specimens were fixed in formal alcohol. Blocks were made and paraffin sections were cut 5-7 μm thick and stained with haematoxylin and eosin. Sections were examined using light microscope and photographed. All methods were applied according to [15].

Biochemical examination

Blood samples were collected from rat orbital sinus for biochemical examinations. The samples were centrifuged at 2000g for 15 min for serum separation. Lipid peroxidation product (MDA) was measured by the method according to [16]. The activity of superoxide dismutase (SOD) was measured based on [17]. The concentration of glutathione (GSH) was measured based on [18].

Statistical analysis

Results were expressed as means + SEM. Differences between means were tested by one way ANOVA followed by the student- Newman- Keul t-test.

Results

Hippocampus histology

In male rats, the hippocampus composed of polymorphic cell layer, granular layer and molecular layer (**Figure 1a**). The dominant neurons in the hippocampus proper are the pyramidal cells. They are highly packed and variable in sizes (small and large). These cells have large vesicular nuclei and basophilic granular cytoplasm rich in Nissl's granules and prominent processes (**Figures 1a** and **1b**). Glial cells are distributed between the neurons and in the molecular layer (**Figure 1b**).

In Aged group, there is a decrease in the thickness and

Figure 1. Photomicrographs of hippocampus proper of male rats, (a and b) for y.cont. group. a: polymorphic cell layer (PL), granular cell layer (G), small pyramidal cell ($\uparrow$), neural process ($\blacktriangle$) and glial cells (Δ). b: large pyramidal cell ($\uparrow$), neural process ($\blacktriangle$) and glial cell (Δ). (c and d) for Ag group. c: shrunk cells with increased spaces around and between them ($\uparrow$), pyknotic and fragmented nuclei ($\blacktriangle$). d: shrunk and deeply stained pyramidal cell with pyknotic nuclei ($\uparrow$), some cells still have normal cytoplasm, processes and nuclei ($\blacktriangle$). (e and f) for Ag+GT group. e: normal small pyramidal cells ($\uparrow$), deeply stained cells with fragmented nuclei ($\blacktriangle$) and glial cells (Δ). f: degenerated large pyramidal cells ($\uparrow$), glial cells (Δ) and some pyramidal cells with vesicular nuclei ($\blacktriangle$). (g and h) for Ag+Gr group. g: increase in the thickness of pyramidal layer, large number of normally preserved small pyramidal cells ($\uparrow$), some shrunk and deeply stained cells ($\blacktriangle$) and glial cells (Δ). h: deeply stained shrunken large pyramidal cells ($\uparrow$), normal pyramidal cells with rounded vesicular nuclei ($\blacktriangle$) and glial cells (Δ). (H&E, Bar=50 μ m).

density of the granular layer. The pyramidal cells are irregular, shrunk, deeply stained and loosely packed with spaces around and in between them. Many cells have irregular nuclei and others with pyknotic and fragmented ones with basophilic cytoplasm. The nuclei of the glial cells are prominent (Figures 1c and 1d).

In (Ag+GT) group, some small pyramidal cells show nor-

mal appearance while others appear with deeply stained and fragmented nuclei (Figure 1e). Some degenerated large pyramidal cells between the preserved small cells and glial cells are observed. The preserved pyramidal cells appear with thick processes, vesicular nuclei and basophilic cytoplasm rich in Nissl's granules (Figure 1f).

In (Ag+Gr) group, there is an increase in the thickness of pyramidal layer. Large number of normally preserved small pyramidal cells with vesicular nuclei and basophilic granular cytoplasm are detected. Some cells are still shrunk with deeply stained cytoplasm. Normal glial cells with vesicular nuclei are clearly observed (Figure 1g). A lot of deeply stained shrunken large pyramidal cells are observed in Figure 1h. These cells have thick processes while others show normal vesicular rounded nuclei, basophilic granular cytoplasm and processes. Glial cells are normally present.

In female rats, the pyramidal cells are variable in sizes (small and large) in (y. cont.) group. These cells have vesicular nuclei; basophilic granular cytoplasm rich with Nissl's granules and prominent processes. Glial cells in molecular layer and in between the pyramidal cells are observed (Figures 2a and 2b).

In Ag group, there is apparent decrease in the thickness of the small pyramidal cells. The majority of the cells are irregular and deeply stained. Few cells have rounded vesicular nuclei and basophilic cytoplasm. Glial cells are observed (Figure 2c). Large pyramidal cells are shrunk, while some of them appear with deeply stained nuclei and others have fragmented nuclei (Figure 2d).

In Ag+GT group, many small pyramidal cells are shrunk and deeply stained. Other cells appear with rounded vesicular nuclei and basophilic granules. Normal glial cells are present (Figure 2e). Few large pyramidal cells are preserved and appear loosely packed. Other pyramidal cells are shrunk with increase spaces around them (Figure 2f).

In general, the architecture of the hippocampus of Ag+Gr group appears nearly like that in (y.cont.) group. Small pyramidal cells are normally preserved. These cells have rounded vesicular nuclei and basophilic granular cytoplasm while few cells are shrunk (Figure 2g). Many large pyramidal cells are preserved but still others have deeply stained cytoplasm and nuclei. Normal glial cells are present (Figure 2h).

Measurement of lipid peroxidation (LPO) in the serum

In male rats, the quantitative results are present in Table 1. In aged rats the level of lipid peroxidation (LPO) was increased (122.5%) significantly ($P < 0.001$) versus those of (y.cont.) group (as indicated by the increase in Malondialdehyde [MDA] levels). In (Ag+GT) group, decrease in the level of LPO (63.88%) occurred when compared with those of Ag group. This inhibition was statistically significant ($P < 0.001$). When compared with y. cont. group levels, there was non-significant difference between them (Table 1).

Garlic administration to aged rats (Ag+Gr) also decreased (53.64%) the level of LPO significantly ($P < 0.001$) than those of

Figure 2. Photomicrographs of hippocampus proper of female rats, (a and b) for y.cont. group: small (S) and large (L) pyramidal cells with round vesicular nuclei and basophilic granular cytoplasm with Nissl's granules (↑), cell processes (▲) and glial cells (Δ). (c and d) for Ag group. c: irregular cells, deeply stained with irregular nuclei (↑), few pyramidal cells with rounded vesicular nuclei (▲) and glial cells (Δ). d: shrunken large pyramidal cells (↑), some cells are with deeply stained nuclei, other with fragmented nuclei (▲). (e and f) for Ag+GT group. e: many shrunken and deeply stained small pyramidal cells (▲), other cells with rounded vesicular nuclei and basophilic granular cytoplasm (↑) and many glial cells are present (Δ). f: preserved large pyramidal cells (↑), others are shrunken with increase in space around them (▲). (g and h) for Ag+Gr group. g: preserved small pyramidal cells (↑), few cells are shrunken (▲) and many glial cells (Δ). h: preserved large pyramidal cells (↑), few cells with deeply stained cytoplasm and nuclei (▲) and many glial cells (Δ) (H&E, Bar=50 μm).

Ag group. There was a non-significant difference in the LPO level when compared with those of (y.cont.) group (**Table 1**).

There was non-significant differences occurred in the level of LPO between (Ag+GT) and (Ag+Gr) groups (**Table 1**).

In female rats, as shown in **Table 1**, there was an increase in oxidized lipids in aged rats (48.5%) versus those of y. cont. ones. Statistically, this increase was non-significant.

In (Ag+GT and Ag+Gr) groups, green tea and garlic admin-

istration did not exert any significant differences in the level of LPO versus those in Ag group. When comparing with those of (y.cont.) group, there was a significant increase ($P<0.001$) present in the level of LPO in both treated groups (**Table 1**).

The comparison between the two treated groups (Ag+GT and Ag+Gr) revealed that no significant differences present in the level of LPO between them (**Table 1**).

In comparison between the level of LPO of male and female rats, in y.cont.group there was non-significant difference between males and females. In Ag group, the LPO level increased (47.87%) significantly ($P<0.05$) in males than females. In Ag+GT groups, the level decreased (59.94%) significantly ($P<0.001$) in males than those of females. Also, significant ($P<0.001$) decrease (48.99%) was detected in the level of LPO of the male rats of Ag+Gr group than females of the same group (**Table 1**).

Measurement of Superoxide dismutase (SOD) activity in the serum

In male rats, the quantitative results are present in **Table 2**. In aged rats the activity of Superoxide dismutase (SOD) decreased (52.77%) significantly ($P<0.05$) versus those of young control group.

Green tea administration to aged rats (Ag+GT) increased the activity of SOD (67.53%) significantly ($P<0.001$) when compared with Ag group. When compared with y.cont. group, there was a significant ($P<0.001$) decrease in the SOD activity (**Table 2**).

In (Ag+Gr) group, garlic stimulated the activity of SOD (81.84 %) significantly ($P<0.001$) versus those of Ag group. There was a significant decrease ($P<0.001$) in the SOD activity when compared with y.cont. group ones (**Table 2**).

Non-significant increase (8.53%) occurred in the activity of SOD in Ag+Gr group versus those of Ag+GT groups (**Table 2**).

In female rats, as shown in **Table 2**, the activity of SOD decreased (39.50%) significantly ($P<0.001$) in Ag group versus those of (y.cont.) group.

Green tea administration to aged rats increased the activity of SOD significantly ($P<0.001$) by 55.67% when compared with Ag group. Non-significant differences occurred in the activity of SOD versus those of (y.cont.) group (**Table 2**).

In Ag+Gr group, the activity of SOD increased (1.77%) versus those in Ag group. Statistically, this stimulation was non-significant. When comparing with y.cont., there was significant ($P<0.001$) decrease in the activity of SOD occurred between them (**Table 2**).

Between (Ag+GT and Ag+Gr) groups, there was a non-significant decrease in the level of SOD (34.58%) in Ag+Gr versus those of Ag+GT (**Table 2**).

In comparison between the activity of SOD in male and female rats, in (y.cont.) group there was significant decrease ($P<0.001$) by 52.65% between males and females. In Ag group, there was non-significant difference between the two genders. In Ag+GT group, the activity of SOD increased

Table 1. Mean values $\pm$ SE of lipid peroxidation (MDA), stimulation and/or inhibition % in young, aged and aged-treated male and female rats.

Measurement Groups	Male		Female		♂ vs. ♀
	MDA (n mol/mg protein)	Inhibition % (I) Or Stimulation % (S)	MDA (n mol/mg protein)	Inhibition% (I) or Stimulation%(S)	
y. cont	0.3025 $\pm$ 0.051 ^a	-	0.3064 $\pm$ 0.041 ^a	-	P>0.05
Ag	0.673 $\pm$ 0.044 ^b	S=122.47vs.y.cont.	0.4551 $\pm$ 0.032 ^{ab}	S=48.53 vs. y.cont.	P<0.05
Ag+GT	0.2431 $\pm$ 0.019 ^{ac}	I=63.88. vs. Ag	0.6067 $\pm$ 0.041 ^b	S=33.31 vs. Ag	P<0.001
Ag+Gr	0.312 $\pm$ 0.021 ^{ac}	I=53.64 vs. Ag S=28.34 vs. Ag+GT	0.6117 $\pm$ 0.106 ^b	S=34.41 vs. Ag S=0.824vs.Ag+GT	P<0.001

Values in the same column with unlike superscript letters are significantly different at P<0.05.

Table 2. Mean values $\pm$ SE of Superoxide dismutase (SOD), stimulation and/or inhibition % in young, aged and aged-treated male and female rats.

Measurement Groups	Male		Female		♂ vs. ♀
	SOD U/min./ mg protein	Inhibition % (I) Or Stimulation % (S)	SOD U/min./ mg protein	Inhibition% (I) or Stimulation%(S)	
y. cont	285.6 $\pm$ 8.246 ^a	-	187.1 $\pm$ 5.774 ^a	-	P< 0.05
Ag	134.9 $\pm$ 6.654 ^b	I=52.77 vs.y.cont.	113.2 $\pm$ 7.508 ^b	I=39.50 vs. y.cont.	P>0.05
Ag+GT	226 $\pm$ 5.774 ^c	S=67.53 vs. Ag	176.1 $\pm$ 5.774 ^a	S=55.57 vs. Ag	P<0.05
Ag+Gr	245.3 $\pm$ 5.78 ^c	S=81.84 vs. Ag S=8.53 vs. Ag +GT	115.2 $\pm$ 8.663 ^b	S=1.77 vs. Ag I=34.58 vs. Ag+GT	P<0.05

Values in the same column with unlike superscript letters are significantly different at P<0.05.

(28.34%) significantly (P<0.001) in male rats than female ones. The Garlic administration to aged rats increased the activity of SOD (112.93%) significantly (P<0.001) in male rats versus females (**Table 2**).

Measurement of Glutathione (GSH) concentration in the serum

In male rats, the quantitative results are present in **Table 3**. In aged rats the concentration of glutathione (GSH) decreased (70.72%) significantly (P<0.05) versus those of (y.cont.) group. In (Ag+GT) group, green tea administration increased the concentration of GSH (17.34%) significantly (P<0.01) when compared with those of Ag group. When compared with y.cont. group, there was a significant decrease (P<0.001) in

GSH concentration (**Table 3**).

Garlic administration to aged rats (Ag+Gr) also increased significantly (P<0.001) the GSH concentration by 125.84% versus those of Ag group. There was a significant decrease (P<0.001) in the concentration of GSH when compared with y.cont.group ones (**Table 3**).

The comparison between the two treated groups (Ag+GT and Ag+Gr) revealed that the concentration of GSH was decreased (92.46%) significantly (P<0.001) in the animals given green tea (Ag+GT) versus those given garlic (Ag+Gr) (**Table 3**).

In female rats, as shown in **Table 3**, the concentration of GSH decreased (46.99%) significantly (P<0.001) in Ag group versus those of (y.cont.) group.

Green tea administration to aged rats (Ag+GT) decreased

Table 3. Mean values $\pm$ SE of Glutathione (GSH), stimulation and/or inhibition % in young, aged and aged-treated male and female rats.

Measurement Groups	Male		Female		♂ vs. ♀
	GSH μ g/ mg protein	Inhibition % (I) Or Stimulation % (S)	GSH μ g/ mg protein	Inhibition% (I) or Stimulation%(S)	
y. cont	166.8 $\pm$ 2.144 ^a	-	119.1 $\pm$ 2.613 ^a	-	P<0.001
Ag	48.8 $\pm$ 2.891 ^b	I=70.72 vs.y.cont.	63.1 $\pm$ 2.598 ^b	I=46.99 vs. y.cont.	P<0.01
Ag+GT	57.3 $\pm$ 1.357 ^c	S=17.34 vs. Ag	24.9 $\pm$ 2.895 ^c	I=60.58 vs. Ag	P<0.001
Ag+Gr	110.3 $\pm$ 2.903 ^d	S=125.84 vs. Ag S=92.46 vs. Ag+GT	29.1 $\pm$ 2.888 ^c	I=53.96 vs. Ag S=16.73vs.Ag+GT	P<0.001

Values in the same column with unlike superscript letters are significantly different at P<0.05.

significantly (P<0.001) the GSH concentration (60.58%) when compared with Ag group. Significant decrease (P<0.01) also occurred in the concentration of GSH when compared with y.cont. group (Table 3).

The administration of aged rats with garlic (Ag+Gr) decreased (53.96%)significantly (P<0.001) the concentration of GSH when compared with Ag group. When comparing with (y.cont.) group, there was significant decrease (P<0.001) in the GSH concentration (Table 3).

The comparison between the two treated groups (Ag+GT and Ag+Gr) revealed that there was non-significant differences occurred in the GSH concentration between them (Table 3).

In comparison between the concentration of GSH in male and female rats, there was a significant (P<0.001) increase by 40.05% between them in young control group. In Ag group, GSH concentration increased significantly (P<0.01) in females by 22.65% versus those in males. The administration of aged rats with green tea decreased significantly (P<0.001) the GSH concentration in males by (130.25%) versus those of females. In Ag+Gr group, there was significant (P<0.001) increase in the concentration of GSH by 279.43% in male rats versus female ones (Table 3).

Discussion

The simplest and the most popular region for studying the cortical network is the hippocampal CA1 area, because it has the least heterogeneous neuronal population, the somata and dendrites of principal neurons (pyramidal cells) are arranged into well-defined layers and the extrinsic and intrinsic inputs are segregated. The hippocampus is critically involved in learning and in memory formation for facts and events (explicit or declarative memory) [19].

In this study we focused on the hippocampus proper which is part of hippocampal formation. The hippocampal formation composed of dentate gyrus, subiculum in addition to the hippocampus proper. The hippocampus proper is formed of *Corno Ammonis*, CA1 and CA2 formed of zone of small pyramidal cells which is the dominant neurons in the hippocampus proper, CA3 and CA4 formed of zones of large pyramidal cells [20].

The degenerative changes were observed in this study appeared in the form of small, dense and deeply stained pyramidal cells in the pyramidal layer. This finding was in line with [21] who explained that the age related degenerative changes occur as a result of oxidative modification of DNA, protein, lipids and cellular molecules by reactive oxygen species (ROS). The appearance of these degenerative changes in the hippocampus proper leads to the affection in the spatial memory-related learning ability as they the main function of hippocampus. This in agreement with the finding of [22] who suggested that, changes in learning ability may be associated with the degeneration of cholinergic neurons in the hippocampus. Ma et al. [23] reported that the brain is more affected by oxidative damage while antioxidants defense ability decrease due to aging.

The present study cleared that the administration of green tea improves the histological appearance of the pyramidal cells. The preserved pyramidal cells appear with thick processes, vesicular nuclei and basophilic cytoplasm rich in Nissl's granules. Our finding come in line with the conclusion of [24 and 25] they suggested that green tea catechins and epigallocatechingallate (EGCG) have a protective effect against age-related neurological disease associated with ROS, small intestine, the liver, and it can cross the blood brain barrier to

lower the hippocampus reactive oxygen species concentration.

In the current study, garlic treated aged rats showed increase in the thickness of pyramidal layer and large number of normally preserved small pyramidal cells with vesicular nuclei and basophilic granular cytoplasm. These results confirmed previously by Borek [26].

The appearance of large number of glial cells with vesicular nuclei in both aged and treated groups in this study may explained as a compensatory role to preserve the neurons or help the affected neurons to perform their functions. This supported by the finding of [27] they cleared the role of glia and astrocytes in the activity of the neuronal networks. They showed that the microglia are activated in many neurodegenerative diseases, adding that whether they are helpful or harmful. The involvement of glial cells in brain aging was recently studied by [28 and 29], They observed that the glial genes shift their regional expression patterns, while the microglia-specific genes increase their expression upon aging.

In current study, there was a significant increase in the level of oxidized lipids in the serum of aged rats than that of those in young control group. In fact lipid peroxidation products have been used as indirect biomarkers of oxidative stress with aging [30]. According to [31], there is a great deal of interest in lipid peroxidation in terms of evaluating its importance as a primary mechanism of tissue injury and deterioration of a wide variety of cellular enzymes resulting in neurodegenerative process. Also, our results showed that in aged males the level of LPO in the serum was more than that in females. This may result from the protective role of estrogen against age-related diseases due to estrogen replacement [32].

In the present study, the administration of the aged male rats with green tea decrease the level of LPO significantly when compared with those of Aged group. When compared with y. cont. group levels, there was non-significant difference between them. The previous studies of [33,34] reported that GT reduces oxidative stress by virtue of its antioxidant properties thus improving the structural integrity of cell membrane and eventually alleviates the histopathological changes as well as the biochemical perturbations. These results suggest that catechins, epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and EGCG could have a direct (antioxidant) or indirect (increase of activity or expression) effect [35,36].

In our study, Garlic administration to aged male rats also decreased the level of LPO significantly than those of Ag group and there were non-significant differences when compared with those of y. cont. group. This result conform the result of [37] who observed that phytochemical from plant-rich diets, including garlic, provide important additional protection against oxidant damage. Garlic extract supplementation improves blood lipid profile, strengthens blood antioxidant potential, and also leads to a decrease in the level of oxidation product (MDA) in the blood samples, which demonstrates reduced oxidation reactions in the body [38,39].

[40] found that SOD activities were decreased with aging. Oxidative stress -which is results from aging in our study- is defined as a disturbance in the balance between the production of ROS and antioxidant defense systems as SOD. This result comes in line with our data which showed that the activity of Superoxide dismutase (SOD) in aged male and female rats decreased significantly versus those of young control group.

[41] suggested that different models of oxidative stress involve a biphasic response of the antioxidant enzyme activities. First, the enzymatic activities are markedly decreased but at longer times, in response to oxidative stress. Secondary, the activity levels are increased probably as a consequence of de novo synthesis or enzymatic activation. Our results are in agreement with this author in his first suggested phase.

In the current study, Green tea administration to aged male and female rats increased the activity of SOD when compared with Aged groups. These data confirmed by [42] they said that SOD plays an important role in attenuating oxidative damage in vivo, thereby delaying not only the onset of age-related degenerative disease, but also aging itself. In vivo study made by [43] intake of green tea extracts also increase the activity of superoxide dismutase in serum and the expression of catalase, these enzymes play a role in cellular protection against reactive oxygen species.

The administration of Garlic to aged male rats stimulated the activity of SOD significantly versus those of Ag group. These results are in agreement with many studies [44,45] who found that dietary supplement of garlic extract activated the antioxidant system and decreased peroxidation in aortic tissue in their experimental model. The water-soluble organo-sulfur compounds of garlic including S-allyl cysteine, S-allylmercaptocysteine, allicin, and selenium exhibited potent antioxidant and free radical scavenging activities in vitro and in vivo [46,47].

Our results revealed decrease in the concentration of glutathione (GSH) in aged male and female rats significantly versus those of young control group. These results come in agreements with [48] who suggested that a GSH-deficiency state is a general phenomenon in aging cells, organisms and tissues in rats due to a lack of the precursor amino acid, cysteine. Our results also showed that in Ag group, GSH concentration increased significantly in females versus those in males.

These results confirmed by [49] who found that the GSH levels in the females at 24 months were significantly higher than in the males. The reason for these gender-dependent differences in the maintenance of GSH levels during maturation and aging may be related to estrogen levels. Assuming that, this data is relevant to humans, an increase in g-glutamyltranspeptidase activity could account for a decreased GSH due to increased its degradation, decreased release from tissues, either as a consequence of decreased intracellular concentrations or due to decreased GSH transporter functions with aging [50].

In our study, the administration of the aged male rats with green tea and garlic increased the concentration of GSH

significantly when compared with those of Ag group. According to the finding of the present investigation, dietary supplementation of polyphenol-rich berries in transgenic mice was shown to modulate the expression of the catalytic subunit of γ -glutamylcysteine synthetase, leading to a significant increase in total glutathione concentrations in various organs [51].

The ability of garlic to enhance glutathione peroxidase, ROS scavenging may and provide protection from chronic diseases associated with disease and aging processes in liver and mammary tissue [52]. GSH is oxidized to its disulphide form, GSSG, which is then reduced back to GSH by GSSG-reductase. Garlic increased the intracellular level of GSH, decreased the intracellular level of GSSG, and increased the activity of GSSG-reductase and SOD [53]. The increased GSSG-reductase activity induced by garlic would accelerate regeneration of GSH from GSSG. This may explain the increase of intracellular GSH in the study.

Our results found that the protective role of green tea and garlic against the deterioration in the oxidative stress parameters were obvious in males than females, that may be resulted from the deficiency in the estrogen in aged females after menopause which play a protective role against oxidative stress and in turn triggering a lot of health problem in females [54].

Conclusion

Our results showed that the protective role of green tea and garlic against oxidative stress parameters was more effective in males than females. Morphologically, garlic exerted preservation to the pyramidal cells of hippocampus more than green tea in both genders. This study suggests that the use of both green tea and garlic may inhibit the bodily changes associated with aging.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	GHE	AAA	MMA
Research concept and design	✓	✓	--
Collection and/or assembly of data	✓	✓	--
Data analysis and interpretation	✓	✓	--
Writing the article	✓	✓	✓
Critical revision of the article	✓	✓	✓
Final approval of article	✓	✓	--
Statistical analysis	✓	✓	--

Publication history

EIC: Gaetano Giuseppe Magro, University of Catania, Italy.

Received: 03-April-2018 Final Revised: 03-May-2018

Accepted: 10-May-2018 Published: 17-May-2018

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Citation:

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http://dx.doi.org/10.7243/2055-091X-5-8