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The protective role of Epigallocatechin gallate on oxidative stress in Alzheimer rats chemically induced by Aluminum oxide nanoparticles

[1]Mohy eldin Abd el fattah,[2]Mohamed Ramadan Abdelgawad,[1]Basher Abd elghfar El boughdady
[1]Chemistry Department, Faculty of Science, Suez Canal University, Cairo, Egypt.[2]Biological Applications Department, Nuclear .Research Centre, Atomic Energy Authority, Cairo, Egypt.

Abstract
Alzheimer's disease (AD) is the most common cause of dementia. Epigallocatechin gallate (EGCG), as well as its most abundant polyphenol, is the most potent neuroprotective compound in green tea.
The aim of this study was to evaluate the possible protective effects of EGCG on oxidative stress in Alzheimer's disease induced by Aluminum oxide nanoparticles in rats. Eight groups of rats were used; Group1 (normal control n). Group 2 (AI_2O_3NPS-treated rats) . Group 3 (EGCG 5 mg/kg b.w -treated rats, n= 6). Group 4 (EGCG 10 mg/kg b.w-treated rats). Group 5 (Rivastigmine or Exelon-treated rats). Group 6 (AI_2O_3NPS + EGCG 5 mg/kg b.w). Group 7 (AI_2O_3NPS + EGCG 10 mg/kg b.w) . Group 8 (AI_2O_3NPS + Exelon, n=6). The study revealed that AI_2O_3NPS treated increase AChE, Aβ, BChE and MDA as well as the decrease in GSH, SOD and CAT. EGCG minimized the deteriorating effects of Aluminum oxide nanoparticles on biochemical parameters indicated by the decreased AChE, Aβ, BChE and MDA with the increased GSH, SOD and CAT.

Introduction
Alzheimer's disease (AD) is the most common cause of dementia and is a degenerative brain disease. Dementia is a syndrome that has a number of causes-a group of symptoms. Dementia's characteristic symptoms are memory, language, problem solving and other cognitive skills that affect the ability of a person to carry out everyday activities. These problems are caused by damage or destruction of nerve cells (neurons) in parts of the brain involved in cognitive function. Neurons in other parts of the brain are eventually damaged or destroyed in Alzheimer's disease, including those that allow a person to perform basic body functions such as walking and swallowing. People in the final stages of the disease are in bed and need 24-hour care [1]. Oxidative stress is a condition in which there is an imbalance of reactive oxygen species (ROS), reactive nitrogen species and antioxidant defenses [2, 3]. Increased oxidative stress is associated with normal aging, but it is further exacerbated in several neurodegenerative disorders including AD [4].
Nanoparticles (NPs) can be defined as materials that have at least one dimension less than 100 nm [5].Their small sizes allow cellular absorption and transcytosis into the blood and lymph circulation across epithelial and endothelial cells to reach potentially subtle target sites such as the brain, lymph nodes, bone marrow, heart and spleen [6, 7].
Epigallocatchin-3-gallate (EGCG) is the most abundant and active compound responsible for the majority of the role of green tea in promoting good health through various pathways ; as antioxidant, anti - inflammatory, antiatherogenic and also gene expression activity, it works through growth factor mediated pathways [8] .

Materials and Methods
Animals:
In the present study, adult male albino rats (120 ±20 g) from the animal house of Faculty of Veterinary Medicine Suez Canal University, Egypt, were used as experimental animals. The rats were grouped in special cages with six animals per cage and maintained under our laboratory conditions; temperature (23±2), with dark and light cycle (12/12h). Standard pellet diet and water were allowed free access *ad libitum*. The rats were adapted to laboratory conditions for 7 days before starting of experiment. All procedures of experiment were performed between 8-11 a.m.
Chemicals:
EGCG (M.W: 476.39, CAS Number: 989-51-5, Catalog No.: 4524, Batch No.: 2 B/189017) was purchased from Tocris Bioscience / clinilab company (4,160St. El-Etehad Square Riham Tower El-Maadi, Cairo, Egypt). Aluminum oxide nanoparticles (AI_2O_3NPS) from Egyptian Atomic Energy Authority, Inshas Science City. Chemicals used for analytical reagent grade were obtained from EGY-CHEM for lab technology, Badr city, Egypt and Biodiagnositc Company, Dokki, Giza, Egypt.
Experimental design Experimental design
The rats were randomly divided into 8 groups (6 rats for each group) according to the following design: Group 1; received 1ml saline 0.9% orally daily throughout the experiment and served as normal control group. Group (2); received AI_2O_3NPS alone in a dose 50 mg/ kg b.w intraperitoneally (i.p), for five weeks, served as positive control group [9] with little modefication. Group 3; received Epigallocatechin gallate alone in a dose (5 mg/kg b.w. i.v.) every day for five weeks .Group 4; received Epigallocatechin gallate alone in a dose (10 mg/kg b.w. i.v.) every day

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for five weeks [10]. Group 5; received Rivastigmine (Exelon) in a dose (0.3 mg/kg b.w. orally) for 36 days [11]. Group 6; received AI_2O_3NPS in a dose 50 mg/kg b.w intraperitoneally (i.p), for five weeks followed by simultaneous administration of Epigallocatechin gallate in a dose (5 mg/kg b.w. i.v.) every day for five weeks. Group 7; received AI_2O_3NPS in a dose (50 mg/kg b.w intraperitoneally (i.p), for five weeks followed by simultaneous administration of Epigallocatechin gallate in a dose (10 mg/kg b.w. i.v.) every day for five weeks. Group 8; received AI_2O_3NPS in a dose (50 mg/kg b.wintraperitoneally (i.p), for five weeks followed by simultaneous administration of Rivastigmine (Exelon) in a dose (0.3 mg/kg b.w. orally) for 36 days.

Biochemical Assays:

Lipid Peroxidation: Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content by a colorimetric method [12] .

Antioxidant Enzymes: Superoxide dismutase activity was determined according to the method of Nishikimi [13]. The method is based on the ability of SOD enzyme to inhibit the phenazine methosulphate-mediated reduction of nitro blue tetrazolium dye (NTB). Briefly, 0.05 mL sample was mixed with 1.0 mL buffer (pH 8.5), 0.1 mL nitro blue tetrazolium (NBT), and 0.1 mL NADH. The reaction was initiated by adding 0.01 mL phenazine methosulphate (PMs), and then increase in absorbance was read at 560 nm for five minutes.

Catalase activity was determined according to the method of Aebi [14]. The method is based on the decomposition of H2O2 by catalase. The sample containing catalase is incubated in the presence of a known concentration of H2O2. After incubation for exactly one minute, the reaction is quenched with sodium azide. The amount of H2O2 remaining in the reaction mixture is then determined by the oxidative coupling reaction of 4-aminophenazone (4-aminoantipyrene, AAP) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) in the presence of H2O2 and catalyzed by horseradish peroxidase (HRP).The resulting quinoneimine dye (N-(4-antipyrl)- 3-chloro-5-sulfonate-p-benzoquinonemonoimine) is measured at 510 nm.

Erythrocyte GSH was measured following as described by Beutler [15]. The method was based on the ability of the –SH group to reduce 5,5-dithiobis,2-nitrobenzoic acid (DTNB) and form a yellow coloured anionic product whose OD is measured at 412 nm. Concentration of GSH is expressed in milligram per millilitre packed RBCs and was determined from standard plot.

Alzheimer disease markers: Determination of ACHE content:ACHE was estimated by a colorimetric method using available commercial kit supplied by BioAssay Systems.

Determination of Butyryl cholinesterase: BChE was estimated by a colorimetric method using available commercial kit supplied by Biodiagnostic Company.

Determination of Aβ content: Determination of Aβ was assessed in brain tissue homogenate according to the manufacturer's instructions by using ELISA Kits supplied by LifeSpan BioSciences, Inc.

Statistical analysis:

The result values were expressed as means ± standard error (SE) for 6 rats in each group. Tabulation and graphics were designed using Microsoft Excel XP software. Data were statistically analyzed using Statistical Package for Social Science (SPSS) version 19, software. One-way analysis of variance (ANOVA) test was performed to statistical analysis for determining the statistical significant differences between means of different groups. Data were considered instatistically significant when the P values were > 0.05.

Results

Effect of Exelon and Epigallocatechin gallate on rats treated with Al2O3-NPs:

Effect on Alzheimer disease markers:

As shown in Table (1) and Figures (1), AChE activity showed a significant increase in rats treated with Al_2O_3-NPs in a dose 50 mg/ kg b.w intraperitoneally (i.p), five weeks to normal rats by 30.9% compared to normal control.

Al_2O_3-NPs treated with Exelon (0.3 mg/kg b.w) or Epigallocatechin gallate (5 mg/kg and 10 mg/kg) every day for five weeks were significantly decreased serum AChE activity as following (494.07 ± 2.22, 499.58 ± 3.7, 468.22 ± 4.54 vs. 594.75 ± 2.23) respectively compared to Al_2O_3-NPs group. The effect of Epigallocatechin gallate (5 mg/kg) with Al_2O_3-NPs is similar to the effect of Exelon on rats treated with Al_2O_3-NPs while, The effect of Epigallocatechin gallate (10 mg/kg) with Al_2O_3-NPs was more effect than Epigallocatechin gallate (5 mg/kg) or Exelon with Al_2O_3-NPs and returned nearly to the normal values.

As shown in Table (1) and Figure (2), BChE activity showed a significant increase in rats treated with Al_2O_3-NPs in a dose 50 mg/ kg b.w intraperitoneally (i.p) compared to normal control.

Al_2O_3-NPs treated with Exelon (0.3 mg/kg b.w) or Epigallocatechin gallate (5 mg/kg and 10 mg/kg) every day for five weeks were significantly decreased serum BChE activity as following (2134 ± 54.45, 2173 ± 67.6, 2100 ± 66.33 vs. 2493 ± 63.19) respectively compared to Al2O3-NPs group. The effect of Epigallocatechin gallate (5 mg/kg and 10 mg/kg) with

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Al2O3-NPs is similar to the effect of Exelon on rats treated with Al2O3-NPs.

As shown in Table (1) and Figure (3), Amyloid beta (Aβ) concentration showed a significant increase in rats treated with Al_2O_3-NPs in a dose 50 mg/ kg b.wintraperitoneally (i.p) compared to normal control. Al_2O_3-NPs treated with Exelon (0.3 mg/kg b.w) or Epigallocatechin gallate (5 mg/kg and 10 mg/kg) every day for five weeks were significantly decreased serum Amyloid beta (Aβ) concentration as following (18.35 ± 0.35, 18.73 ± 0.32, 16.85 ± 0.39 vs. 46.01 ± 1.71) respectively compared to Al_2O_3-NPs group. The effect of Epigallocatechin gallate (5 mg/kg) with Al_2O_3-NPs is similar to the effect of Exelon on rats treated with Al_2O_3-NPs. while, the effect of Epigallocatechin gallate (10 mg/kg) with Al2O3-NPs was more effective than Epigallocatechin gallate (5 mg/kg) or Exelon with Al_2O_3-NPs and returned nearly to the normal values.

Table (1): Tissue Acetylcholinesterase (AChE), serum Butyrylcholinesterase (BChE) and tissue Amyloid beta (Aβ) in control, AL_2O_3-NPS-treated rats, and AL_2O_3-NPS-treated rats and supplemented with Exelon and Epigallocatechingallate (n=6).

Parameters / Group	AChE (umol/g tissue)	BChE (U/L)	Aβ1-42 (pg/g tissue)
Control	454.28 ± 7.32 [c]	1683 ± 12.61 [c]	14.36 ± 0.38 [c]
Range (n=6)	(430-472)	(1641-1720)	(12.89-15.1)
AL_2O_3-NPS	594.75 ± 2.23 [a]	2493 ± 63.19 [a]	46.01 ± 1.71 [a]
Range (n=6)	(587.6-600)	(2358-2790)	(12.89-15.1)
%Change compared to control	30.9	48.1	220
Exelon	494.07 ± 2.22 [b]	2134 ± 54.45 [b]	18.35 ± 0.35 [b]
Range (n=6)	(488-500)	(2008-2358)	(17.5-19.8)
%Change compared to control	8.75	26.8	27.8
%Change compared to Al2O3-NPs	-16.9	-14.4	-16.1
EGCG (5 mg)	499.58 ± 3.7 [b]	2173 ± 67.6 [b]	18.73 ± 0.32 [b]
Range (n=6)	(488-510)	(2050-2480)	(17.9-20)
%Change compared to control	9.97	29.1	30.4
%Change compared to Al2O3-NPs	-16	-12.83	-59.3
EGCG (10 mg)	468.22 ± 4.54 [c]	2100 ± 66.33 [b]	16.85 ± 0.39 [b,c]
Range (n=6)	(455-483.4)	(1980-2360)	(15.9-18.5)
%Change compared to control	3.07	24.8	17.3
%Change compared to Al2O3-NPs	-21.27	-15.7	-63.4

Data presented as Mean ± SEM
Means have the same letters considered insignificant (P>0.05).

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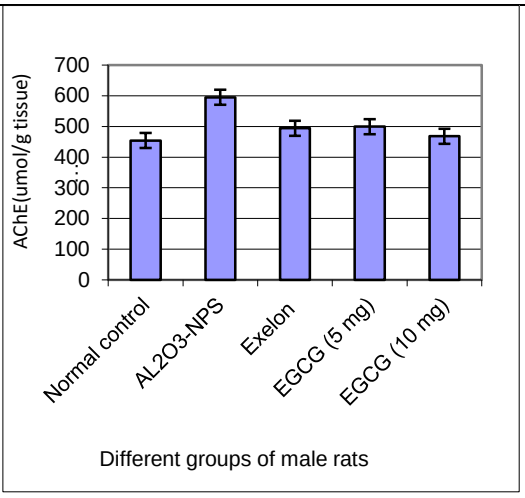

Figure 1: Mean tissue Acetylcholinesterase (AChE) activity (umol/g tissue) in normal control and different groups of AL_2O_3-NPS -treated rats.

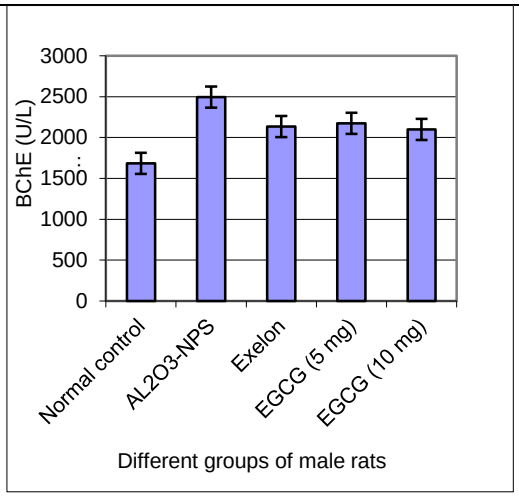

Figure 2: Mean serum Butyrylcholinesterase (BChE) activity (U/L) in normal control and different groups of AL2O3-NPS -treated rats.

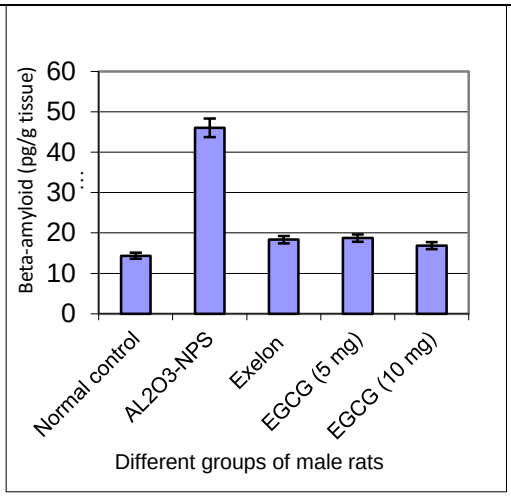

Figure 3: Mean tissue Amyloid beta (Aβ) concentration (pg/g tissue) in normal control and different groups of AL2O3-NPS -treated rats.

Effect on Tissue antioxidant enzymes:

Effect on glutathione (GSH) contents:

Results given in Table (2) and graphically illustrated in Figure (4) showed that the intraperitoneal injection of AL2O3-NPS in the previously mentioned dose and period to normal rats induced significantly decreased in GSH content from (19.84 ± 0.2 to 11.8 ± 0.21) when compared with the normal control group.

Exelon treated rats with AL2O3-NPS administration in the previously mentioned dose and period had significant increase in GSH content compared to AL2O3-NPS -treated rats.

GSH contents in rats treated with Epigallocatechin (5 mg) and (10 mg) with AL2O3-NPS administration were increased by 66.1% and 82.7% respectively compared to AL2O3-NPS -treated rats. Epigallocatechin (10 mg) more effective than Epigallocatechin (5 mg) and Exelon were increased by 82.7% vs 66.1% and 56.5% respectively.

Effect on superoxide dismutase (SOD) activity:

Results in Table (2) and Figure (5) showed that the intraperitoneal injection of AL2O3-NPS in the previously mentioned dose and period to normal rats induced decreased in SOD activity compared to normal control rats.

AL2O3-NPS -treated rates with Exelon in the previously mentioned dose and period had a significant increase in SOD activity compared to AL2O3-NPS-treated rats compared to AL2O3-NPS -treated rats. AL2O3-NPS -treated rates with Epigallocatechin gallate (5 mg and 10 mg) in the previously mentioned dose and period had a significant increase in SOD activity compared to AL2O3-NPS -treated rats. SOD activities of these rats restored to the values of normal group (346 ± 5.73 vs. 357 ± 8.83) respectively in case of rats treated with Epigallocatechin gallate (10 mg) treated with AL2O3-NPS administration and similar effect as Epigallocatechin gallate (5 mg) treated with AL2O3-NPS administration.

Effect on catalase (CAT) activity:

Results in Table (2) and Figure (6) showed that the intraperitoneal injection of AL2O3-NPS in the previously mentioned dose and period to normal rats induced significantly decreased in CAT activity by 50.8% compared to normal control rats. AL2O3-NPS -treated rates with Exelon and Epigallocatechin gallate (5 mg and 10 mg) in the previously mentioned dose with induced significantly increased in CAT activity by 69.7%, 76.1% and100% respectively compared to AL2O3-NPS - treated rats. AL2O3-NPS -treated rates with Epigallocatechin gallate (5 mg and 10 mg) in the previously mentioned dose were the same effective as AL2O3-NPS -treated rates with Exelon as following: (0.273 ± 0.029, 0.310 ± 0.008 vs. 0.263 ± 0.018) respectively.

Effect on lipid peroxidation (MDA) level:

Results in Table (2) and Figure (7) showed that intraperitoneal injection of AL2O3-NPS in the previously mentioned dose and period to normal rats induced significantly increased in malondialdehyde (MDA) level compared to normal control group. AL2O3-NPS -treated rates with Exelon and Epigallocatechin gallate (5 mg and 10 mg) in the previously mentioned dose with induced significantly decreased in malondialdehyde (MDA) level by 64.9%,59.8% and 65.9% respectively compared to AL2O3-NPS - treated rats. AL2O3-NPS -treated rates with Epigallocatechin gallate (5 mg and 10 mg) in the previously mentioned dose were the same effective as AL2O3-NPS -treated rates with Exelon as following: (22.89 ± 0.43, 19.43 ± 0.58 vs. 19.98 ± 0.31) respectively.

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Table (2): Tissue Glutathione, tissue antioxidant enzymes and tissue malodialdehyde in control, AL_2O_3-NPS-treated rats, and AL_2O_3-NPS-treated rats and supplemented with Exelon and Epigallocatechingallate (n=6).

Parameters / Group	GSH (mg/g tissue)	SOD (U/g tissue)	CAT (U/g tissue)	MDA (nmol/ g tissue)
Control	19.84 ± 0.2 [b]	357 ± 8.83 [a]	0.315 ± 0.027 [a]	10.43 ± 0.18 [c]
Range (n=6)	(19.2-20.5)	(320-380)	(0.21-0.42)	(9.9-11)
AL2O3-NPS	11.8 ± 0.21 [c]	233 ± 10.61 [b]	0.155 ± 0.012 [b]	57 ± 2.46 [a]
Range (n=6)	(11.1-12.5)	(210-280)	(0.11-0.19)	(45-63)
%Change compared to control	-40.5	-34.73	-50.8	446.5
Exelon	18.46 ± 0.16 [b]	314 ± 4.73 [c]	0.263 ± 0.018 [a]	19.98 ± 0.31 [b]
Range (n=6)	(17.9-18.9)	(300-330)	(0.20-0.31)	(18.7-20.9)
%Change compared to control	-6.9	-12	-16.5	91.5
%Change compared to Al2O3-NPs	56.5	34.76	69.7	-64.9
EGCG (5 mg)	19.6 ± 0.39 [b]	325 ± 5.37 [cd]	0.273 ± 0.029 [a]	22.89 ± 0.43 [b]
Range (n=6)	(18.1-20.9)	(314-346)	(0.18-0.40)	(21-24)
%Change compared to control	-1.2	-8.96	-13.3	119.4
%Change compared to Al2O3-NPs	66.1	39.48	76.1	-59.8
EGCG (10 mg)	21.56 ± 0.67 [a]	346 ± 5.73 [ad]	0.310 ± 0.008 [a]	19.43 ± 0.58 [b]
Range (n=6)	(18.5-23.2)	(330-360)	(0.29-0.35)	(18-22)
%Change compared to control	8.7	-3.1	-1.6	86.3
%Change compared to Al2O3-NPs	82.7	48.49	100	-65.9

Data presented as Mean ± SEM
Means have the same letters considered insignificant (P>0.05).

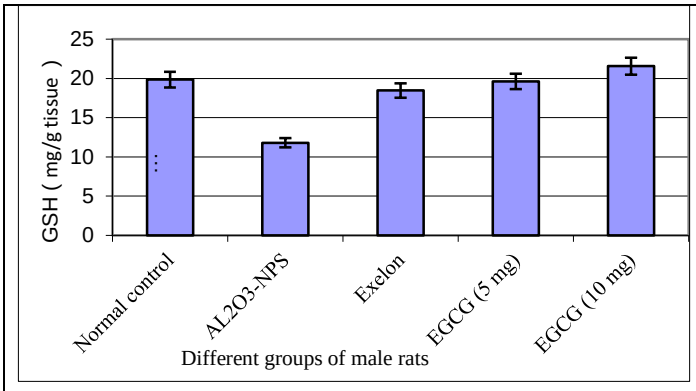

Figure 4: Mean Tissue Glutathione (GSH) concentration (mg/ g tissue) in normal control and different groups of AL2O3-NPS -treated rats.

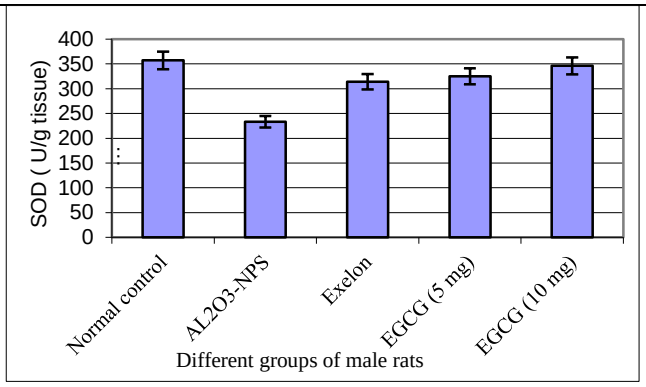

Figure 5: Mean tissue SOD concentration (U/g tissue) in normal control and different groups of AL2O3-NPS -treated rats.

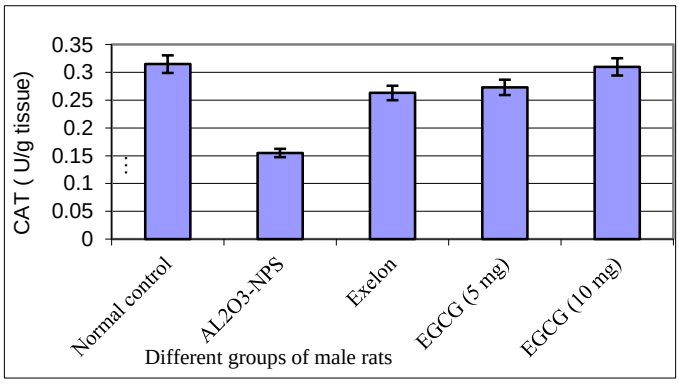

Figure 6: Mean tissue CAT activity (U/g tissue) in normal control and different groups of AL2O3-NPS -treated rats.

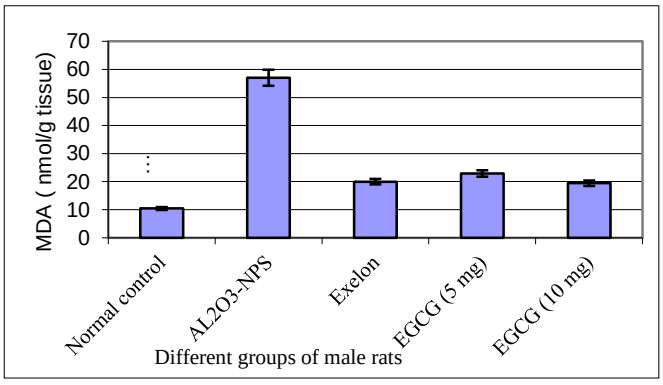

Figure 7: Mean tissue MDA concentration (nmol/gtissue) in normal control and different groups of AL2O3-NPS-treated rats.

Discussion

The present study was designed to investigate the protective role of Epigallocatechin gallate on oxidative stress in Alzheimer's disease by

aluminum oxide nanoparticles-induced neurotoxicity and brain damage in adult male albino rats. It is important that the protective agent is present in brain tissues after administration of aluminum oxide nanoparticles and damage occurs.
Green tea is one of human consumption's most popular drinks. Epidemiological studies have shown that green tea consumption is associated with a reduced risk of many chronic diseases, including cardiovascular diseases, diabetes and various cancers[16, 17]. Green tea's health benefits can be attributed primarily to catechins, its main bioactive components. Five major catechins have been identified in green tea, including catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG)[18, 19].
Epigallocatechin gallate is the most potent neuroprotective compound in green tea, along with its most abundant polyphenol [20]. Due to its structure of phenol rings, epigallocatechin gallate has a powerful antioxidant activity, acts as scavengers and free radical electron traps [21, 22], Preventing the formation of reactive oxygen species and reducing oxidative stress damage (Tipoe et al.,2007). EGCG can affect several potential diseases of Alzheimer's - related goals [23].
Oxidative stress is the most common toxicity mechanism associated with exposure to nanoparticles [24]. Nanoparticles induce oxidative stress, resulting in free radical production and antioxidant alteration. The formation of reactive oxygen species (ROS) in several cell lines involves various nanoparticles in interrupting mitochondrial function (Long et al., 2007; Kang et al., 2008; Park et al., 2008). ROS causes a number of lesions including protein, lipid and DNA oxidation (Shigenaga and Ames, 1991; Srikanth et al., 2014).
A set of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione -S - transeferase (GST) etc. accomplish the body's effective mechanism for preventing and neutralizing free radical damage. These intrinsic antioxidants protect the body against oxidative stress. Oxidative stress is caused by a relative oxidative free radical or reactive oxygen species (ROS) overproduction. Reactive oxygen species (ROS) formed in tissues result in lipid peroxidation and subsequent increase in MDA and other TBARS levels leading to cellular macromolecules degradation [25]. When the balance between antioxidant defense and ROS production is lost, oxidative stress is formed that deregulates cellular functions through a series of events leading to different pathological conditions. [26].
Superoxide dismutase (SOD) and catalase (CAT) play an important role in the metabolism of reactive oxygen species, thus protecting cells from oxidative stress [27]. Superoxide dismutase is a ubiquitous cellular enzyme, which disrupts superoxide radicals and dismutates superoxide radical to hydrogen peroxide and oxygen and are present in all cells with high amounts in erythrocytes [15]. It is the chief cellular defense mechanisms, against superoxide and hydrogen peroxide mediated lipid peroxidation. Superoxide dismutase (SOD) and catalase are involved in the clearance of superoxide and hydrogen peroxide radicals [28].
Glutathione (GSH) is an important non - protein thiol that plays an important role in the non - enzymatic antioxidant system in conjunction with GPx and GST [29]. Several authors have established that GSH is reduced in the brain, liver, kidney of rats exposed to micro-sized lead, Al, cisplatin and cadmium (Cd) [30-32]. GSH is known to protect cells against oxidative stress and any changes in GSH levels (either decrease or increase) indicate a disturbed oxidant status, and GSH synthesis increases when cells are challenged oxidatively [33]. As oxidative stress continues and the protein content of the tissue decreases significantly, as a result of the total protein oxidation by the Al in these tissues, GSH synthesis cannot efficiently supply the demand; therefore, GSH depletion occurs [34].
Lipid peroxidation can be defined as the oxidative deterioration of lipids. Lipid hydroperoxides are non - radical intermediates of unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol alone. These are formed in enzymatic or non-enzymatic reactions involving free radical [35].
In the present study, intraperitoneal injection of Al_2O_3-NPs to normal rats induced significantly decreased in GSH content, SOD and CAT activities and increased in MDA level in brain tissue and blood when compared with the control

group. Al2O3-NPs could induce free radical generation which would further initiate the lipid peroxidation process and damage cellular components. Previous studies have therefore shown that Al can induce lipid peroxidation in the brain that leads to neurodegeneration [36, 37], as confirmed by the increased levels of MDA and inhibition of the SOD, CAT and GSH activities in the brain [38]. As mentioned above, Al is a powerful pro - oxidant known in the cortex and hippocampus to enhance lipid peroxides [39]. Al has also been reported to induce lipid peroxidation and alter the physiological and biochemical properties of biological systems [39]. Furthermore, since oxidative damage is mediated by free radicals, it was necessary to examine the status of endogenous antioxidant enzymes, the first line of defense against free radical damage under oxidative stress conditions, as in malnutrition [40, 41].

AChE and BuChE are two different enzymes in the brain that are responsible for the hydrolysis of acetylcholine [42, 43]. Of these, AChE is the primary cholinesterase found mostly in nerve synaptic junctions and areas expressing intense activity in the adult cerebral cortex of the human brain , [44] whereas BuChE is located mainly in brain glial cells [45]and plays a major role in cholinergic mediation [46]. Butyrylcholinesterase's biological role is unknown [47, 48], but it has been suggested to be a toxin scavenger and to hydrolyze acetylcholine that escapes the action of acetylcholinesterase [49]. Butyrylcholinesterase is abundant in plasma and interstitial fluids of peripheral tissues, with moderate activity in the adult brain [47, 50]. Acetyl cholinesterase is widely distributed in neurons and axon terminals in brain and peripheral tissues, where it is responsible for terminating the action of acetylcholine [47, 51].

Results of the current study also showed that intraperitoneal injection of Al_2O_3-NPs significantly increased acetylcholinesterase , Amyloid beta (Aβ) content in the brain tissue and serum butyrylcholinesterase of control rats; AD rats showed significant increase in Aβ content than control rats. Al is known as a cholinotoxin agent and its neurotoxic effect could be applied by additional mechanisms such as: induction of oxidative stress, increased production of AChE due to a direct action of Aβ binding to nicotinic receptors or due to over expression of APP. Aβ induced by Al leads to increased AChE content in and around Aβ plaques [52] . Al is also bound by the Aβ and has been found to be co - located in the AD brain [53]. Aβ's neurotoxicity in any form may involve reactive oxygen species formation. Al is a pro - oxidant and, in the presence of iron, is known to promote the oxidation content of Aβ. It was also linked to the production of Aβ by the immune response. It is also associated with activating complement, which in turn has been associated with the enhanced aggregation of Aβ [53]. In addition, self - aggregation of Aβ due to Al administration can lead to the production of hydrogen peroxide and hydroxyl radical through certain chemical reactions and can lead to induction of membrane lipid peroxidation [54].

Inhibition of the activities of AChE and BChE enzymes has been widely established as a first - line treatment / management for the symptoms of neurodegenerative conditions such as Alzheimer's disease [55].

Results of the present study also showed that administration of EGCG significantly decreased Tissue Acetylcholinesterase (AChE), serum Butyrylcholinesterase (BChE), tissue Amyloid beta (Aβ) content and serum malondialdehyde (MDA) level, while significantly increased antioxidant enzymes GSH, SOD and CAT activity in AD rat model. The results presented are consistent with the results in which EGCG increased SOD content and protected against glycation end products by decreasing ROS and MDA [56]. Results also agree with the results showed that treatment with EGCG resulted in increased SOD content and decreased MDA in the hippocampus [57]. This effect caused by EGCG could be due to its powerful antioxidant property, since antioxidants were previously reported for their ability to alleviate oxidative damage [58, 59]. In addition to the donation of hydrogen atoms, the presence of four ring structures with 8 hydroxyl groups ; antioxidants can also inhibit oxidation through single electron transfer [60]. Also, due to chemical structure of EGCG, It is a radical scavenger and metal chelator that allows antioxidant effects to be executed directly [61, 62] Some studies have shown that EGCG may induce endogenous antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase. The gene expression was upregulated by EGCG or

the bioactivities of these antioxidants increased. EGCG could regulate antioxidant levels or activity directly or indirectly to reduce oxidative stress [63, 64].

Rivastigmine (Exelon) has been used as a standardized drug in which it is the only demonstrated pharmacological therapy for symptomatic AD treatment [65]. Treatment of AD rat groups with rivastigmine showed an improvement in oxidative stress status as a result of a significant reduction in brain AChE, BChE activity and Aβ content compared to AD - induced rats. In addition, rivastigmine produced a significant decrease in serum MDA, while significant increases in oxidative stress were reported in the glutathione, antioxidant enzymes (SOD, CAT activity) compared to the AD group. Rivastigmine protects behavioral changes, restores antioxidant enzymes in the brain and improves neurotocixity induced by mitochondrial enzymes [66].

Conclusion

The present study elucidated the beneficial effects of Epigallocatechin gallate of green tea evident by improvement of oxidative stress in rats induced by Aluminum oxide nanoparticles. So, our present work recommends the usage of green tea to overcome the abnormal changes in body functions. Since, green tea has been consumed over long periods without any known side effects, its possible role as an adjunct therapeutic agent against the renal defect due to its antioxidant activity.

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