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The protective role of Epigallocatechin gallate (EGCG) on oxidative stress in normal and treated rats with aluminum oxide nanoparticles

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Abstract

The aim of this study was to evaluate the possible Protective role of EGCG on oxidative stress and kidney Functions in normal and treated rats with Aluminum oxide nanoparticles. Eight groups of rats were used; Group1, control. Group2, received Al_2O_3 NPS alone in a dose 50 mg/kg b.w i.p. Group 3, received Epigallocatechin gallate alone in a dose (5 mg/kg b.w. i.v.). Group 4, received Epigallocatechin gallate alone in a dose (10 mg/kg b.w. i.v.). Group 5, received Al_2O_3 NPS followed by simultaneous administration of Epigallocatechin gallate in a dose (5 mg/kg b.w. i.v.). Group 6, received Al_2O_3 NPS followed by simultaneous administration of Epigallocatechin gallate in a dose (10 mg/kg b.w. i.v.). The present study showed a highly significant decreased in GSH concentration, SOD activity and CAT activity but significantly increased in MDA level in blood for rats treated with Al_2O_3 -NPs compared to normal control rats. There was a highly significant increased GSH concentration, SOD and CAT activities while the level of MDA was decreased in rats treated with Exelon or Epigallocatechin gallate; 5 mg/kg and 10 mg/kg treated with Al_2O_3 -NPs when compared to Al_2O_3 -NPs - treated rats. Also, There was a highly significant elevation in serum of creatinine, urea and uric acid in rats treated with Al_2O_3 -NPs compared to normal control rats. The concentrations of creatinine, urea and uric acid were significantly decreased in rats treated with Exelon or Epigallocatechin gallate; 5 mg/kg and 10 mg/kg treated with Al_2O_3 -NPs when compared to Al_2O_3 -NPs-treated rats.

Keywords: Epigallocatechin gallate, oxidative stress

Introduction

Polyphenols are natural substances that are present in drinks obtained from plants, vegetables, and fruits, such as tea, and olive oil. The largest group of polyphenols are flavonoids which mainly divided into glycosylated derivative of anthocyanidin, anthocyanins, present in colorful flowers, fruits and anthoxantins. Anthoxantins are colorless compounds further divided into several categories including flavonols flavones, falvanols, flavans and is flavones [1]. Aromatic ring present in flavonoids is reduced to a heterocyclic ring then attached to a second aromatic ring. Antioxidant activity due to abundant phenolic hydroxyl groups on the aromatic ring, and the 3-OH is essential for the iron chelating activity of these compounds [2].

The importance of polyphenolic flavonoids in improving cell resistance to oxidative stress goes beyond simple scavenging and is most important for pathologies in which oxidative stress plays an important role. Numerous studies in the last 10 years have shown that polyphenols prevent or reduce the harmful effects of free radicals derived from oxygen associated with several chronic and stress - related human and animal diseases *in vitro* and *in vivo*. Oxidative stress is due to reactive oxygen species (ROS) generation and inflammation play a vital role in scientific disorders as arteriosclerosis, neurodegenerative disorders, cancer, ischemia-reperfusion injury and stroke [3, 4].

Green tea contains polyphenols, as flavonoids, flavanols, flavandiols and phenolic acids, which can account for approximately 30% of the dry weight. The majority of green tea polyphenols are flavonols commonly referred to as catechins. In green tea, four types of catechins are mainly detected: Epicatechin (EC), Epicatechin-3-gallate (ECG),

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Epigallocatechin (EGC) and Epigallocatechin-3-gallate (EGCG). Due to differences in origin and growing conditions, the amount of catechins differs in green tea leaves [5].

The product of biological processes is expected to include free radicals, reactive oxygen species and reactive nitrogen species (RNS). Free radicals are atoms that have unpaid valence electrons formed in the body during many different reactions. Although ROS and RNS play an important role in the signaling of biological cells, excess ROS or RNS eventually damage cells by producing lipid peroxidation, oxidation of DNA / RNA and oxidation of proteins [6]. Living organisms have a natural defensive system built against free radicals damage. Antioxidants are molecules that neutralize and scavenge free radicals to inhibit the oxidation of other molecules. This prevents cell damage from free radicals [6].

Nanoparticles (NPs) may be defined as materials that have at least one dimension less than 100 nm [7]. They are desirable for industrial and healthcare applications because of their unique chemical, mechanical and biological properties [8]. Because of their unique chemical, mechanical, and biological properties they are desirable for industrial and health care applications [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 (Al₂O₃NPS) 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 Biodiagnostic Company, Dokki, Giza, Egypt.

Experimental design

The rats were randomly divided into 6 groups: Group 1; received 1ml saline 0.9% orally daily throughout the experiment and served as normal control group. Group (2); received Al₂O₃NPS alone in a dose 50 mg/ kg b.w intraperitoneally (i.p), three times a week for three weeks, served as positive control group [9]. 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 for five weeks [10]. Group 5; received Al₂O₃NPS in a dose 50 mg/kg b.w intraperitoneally (i.p), three times a week for three weeks followed by simultaneous administration of Epigallocatechin gallate in a dose (5

mg/kg b.w. i.v.) every day for five weeks. Group 6; received Al₂O₃NPS in a dose (50 mg/kg b.w intraperitoneally (i.p), three times a week for three weeks followed by simultaneous administration of Epigallocatechin gallate in a dose (10 mg/kg b.w. i.v.) every day for five weeks.

Biochemical Assays

A. Kidney Functions

1. Determination of serum creatinine

The concentration of creatinine was determined by fixed rate colorimetric method as described by Henry [11] using available commercial kit which was purchased from a local chemical company.

Procedure

- 1.0 ml of working solution (R1: 1 volume + R2: 1 volume) was added to all tubes.
- 100 µl of sample, 100 µl of standard were added to sample tube and standard tube.
- All tubes were mixed well, the initial absorbance (A1) of the standard and specimen were read at 492 nm, and then after exactly 2 minutes, the absorbance (A2) of both standard and specimen were read again.

Calculation

[A2 - A1 = A specimen or A standard]

$$\text{Creatinine concentration (mg/dl)} = \frac{(A) \text{ Sample}}{(A) \text{ Standard}} \times 2.0$$

Where, 2 is the standard creatinine concentration.

2. Determination of serum urea

The level of urea was determined by a colorimetric method as described by Chaney and Marbach [12] using available commercial kit which was purchased from a local chemical company.

Procedure

- 50 µl of reagent 2 and 1.0 ml of reagent 3 were added to Blank tube.
- 50 µl of reagent 2, 1.0 ml of reagent 3 and 10 µl of sample were added to sample tube.
- 50 µl of reagent 2, 1.0 ml of reagent 3 and 10 µl of reagent 1 standard were added to standard tube, all tubes were mixed well
- After incubation for 3 min at 37°C. and then 200 µl of reagent 4 was added to all tubes. The absorbance of sample and standard tubes was read at λ 578 nm against blank.

Calculation

The level of urea in sample was calculated using the following equation:

$$\text{Urea (mg/dl)} = \frac{(A) \text{ Sample}}{(A) \text{ Standard}} \times 50,$$

Where, 50 is the standard urea concentration.

3. Determination of serum uric acid

The concentration of uric acid was determined by a colorimetric method as described by Trinder [13] using available commercial kit which was purchased from a local chemical company.

Procedure

- 20 µl of distilled water was added to blank tube, 20 µl of sample was added to sample tube and 20 µl of standard was added standard tube
- 1.0 ml of reagent 2 was added to all tubes.
- All tubes were mixed well.
- After incubation for 5 min. at 37°C. The absorbance of standard and sample tubes was read at 500 nm against blank.

Calculation

The level of uric acid was calculated using the following equation

$$\text{Uric acid concentration (mg/dl)} = \frac{\text{A specimen}}{\text{A standard}} \times 6$$

Where, 6.0 is the standard uric acid concentration.

B. Assessment of oxidative stress biomarkers

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 [14].

Antioxidant Enzymes: Superoxide dismutase activity was determined according to the method of Nishikimi [15]. 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 [16]. 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-antipyril)-3-chloro-5-sulfonate-p-benzoquinonemonoimine) is measured at 510 nm.

Erythrocyte GSH was measured following as described by Beutler [17]. 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.

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 statistically significant when the P values were > 0.05.

Results

• Effect of Epigallocatechin gallate on control rats Effect on blood antioxidant parameters

As shown in Table (1) and Figures (1,2,3 and 4), there was no significant variation in GSH, CAT, MDA and SOD activities compared to normal control group.

Table 1: Blood Glutathione, antioxidant enzymes and serum malondialdehyde in control, and normal rats treated with Epigallocatechingallate (n=6)

Groups / Parameters	GSH(mg/dl)	SOD(U/ml)	CAT(U/L)	MDA(nmol/ml)
Control Range (n=6)	21.99± 0.62 ^a (20.31-23.85)	318.57 ± 8.91 ^{bc} (301-358.1)	346± 20.3 ^a (316-445)	4.33± 0.22 ^a (3.7-5.1)
Exelon Range (n=6) %Change compared to control	23.28± 1.4 ^a (16.65-25.62) 5.87	355.68± 10.33 ^a (320.3-386.8) 11.64	350± 12.35 ^a (320-399) 1.16	4.8± 0.23 ^a (4.17-5.5) 10.8
EGCG (10 mg) Range (n=6) %Change compared to control	24.50± 1.17 ^a (21.21-28.7) 11.41	353.82 ± 12.05 ^{ab} (310-385) 11.07	350± 13.33 ^a (289-380) 1.16	4.38± 0.24 ^a (4.0-5.4) 1.15

Data presented as Mean ± SEM

Means have the same letters considered insignificant (P>0.05).

Fig 1: Mean Blood Glutathione (GSH) concentration (mg/dL) in normal control and Epigallocatechingallate groups in normal rats.

Fig 2: Mean Blood Superoxide dismutase (SOD) concentration (U/ml) in normal control and Epigallocatechingallate groups in normal rats.

Fig 3: Mean serum Catalase (CAT) Activity (U/L) in normal control and Epigallocatechingallate groups in normal

Fig 4: Mean serum MDA concentration (nmol/ml) normal control and Epigallocatechingallate groups in normal rats.

• Effect of Epigallocatechin gallate on rats treated with Al₂O₃-NPs

Effect on blood antioxidant enzymes

Effect on glutathione (GSH) contents

Results given in Table (2) and graphically illustrated in Figure (5) showed that the intraperitoneal injection of Al₂O₃-NPs in the previously mentioned dose and period to normal rats induced significantly decreased in GSH content when compared with the normal control group.

Rats treated with Exelon with Al₂O₃-NPs administration in the previously mentioned dose and period had significant increase in GSH content compared to Al₂O₃-NPs -treated rats.

The percentage of increase was 44.00% compared with the Al₂O₃-NPs -treated rats.

GSH contents in rats treated with Epigallocatechin (5 mg) and (10 mg) with Al₂O₃-NPs administration were increased by 71.18% and 55.86% respectively compared to Al₂O₃-NPs -treated rats.

Effect on superoxide dismutase (SOD) 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 decreased in SOD activity by 23.75% compared to normal control rats. Rats treated with Epigallocatechin gallate (5 mg and 10 mg) with Al₂O₃-NPs administration in the previously mentioned dose and period had increased in SOD activity compared to Al₂O₃-NPs -treated rats. SOD activities of these rats restored to the values of normal group (309 ± 23.18 , 345 ± 8.71 vs. 318.57 ± 8.91) respectively.

Effect on catalase (CAT) activity

Results in Table (2) and Figure (7) 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 36.13% compared to normal control rats.

Al₂O₃-NPS -treated rates with (5 mg and 10 mg) in the previously mentioned dose had significant increase in CAT activity compared to Al₂O₃-NPs -treated rats. CAT activities of these rats succeeded to restore the activities of CAT to normal values (399 ± 8.96 , 405.7 ± 18.38 vs. 346 ± 20.3) respectively.

Effect on lipid peroxidation (MDA) level

Results in Table (2) and Figure (8) showed that intraperitoneal injection of Al₂O₃-NPS in the previously mentioned dose and period to normal rats induced significantly increased in malondialdehyde (MDA) level by 177.3% compared to normal control group.

Al₂O₃-NPS -treated rates with Epigallocatechin gallate (5 mg and 10 mg) in the previously mentioned dose and period had a significant decrease in MDA level compared to Al₂O₃-NPS-treated rats. MDA levels of these rats returned nearly to the normal values.

Table 2: Blood Glutathione, antioxidant enzymes and serum malodialdehyde in control, AL₂O₃-NPS-treated rats, and AL₂O₃-NPS-treated rats and supplemented with Epigallocatechingallate.

Groups / Parameters	GSH(mg/dl)	SOD(U/ml)	CAT(U/L)	MDA (nmol/ml)
Control	21.99 ± 0.62 ^b	318.57 ± 8.91 ^{ab}	346 ± 20.3 ^a	4.33 ± 0.22 ^c
Range (n=6)	(20.31-23.85)	(301-358.1)	(316-445)	(3.7-5.1)
AL ₂ O ₃ -NPS	14.75 ± 1.23 ^c	242.92 ± 3.79 ^b	221 ± 6.31 ^b	12.01 ± 0.48 ^a
Range (n=6)	(10.24-18.6)	(231-255)	(205-245)	(10.5-13.8)
%Change compared to control	-32.92	-23.75	-36.13	177.3
EGCG (5 mg)	25.25 ± 0.56 ^a	309 ± 23.18 ^{ab}	399 ± 8.96 ^a	9.75 ± 4.16 ^b
Range (n=6)	(22.26-26.43)	(196.8-346.5)	(368-422.1)	(8.5-10.9)
%Change compared to control	14.82	-2.95	15.32	125.1
%Change compared to Al ₂ O ₃ -NPs	71.18	27.28	80.54	-18.8
EGCG (10 mg)	22.99 ± 0.82 ^{ab}	345 ± 8.71 ^a	405.7 ± 18.38 ^a	9.4 ± 0.39 ^b
Range (n=6)	(19.72-25.3)	(315.8-366.2)	(317.9-439)	(8.0-10.6)
%Change compared to control	-4.35	8.3	17.25	117.1
%Change compared to Al ₂ O ₃ -NPs	55.86	42	83.57	-21.73

Data presented as Mean ± SEM

Means have the same letters considered insignificant (P>0.05).

Fig 5: Mean Blood Glutathione (GSH) concentration (mg/dL) in normal control and different groups of AL₂O₃-NPS -treated rats. **Fig 7:** Mean serum CAT activity (U/L) in normal control and different groups of AL₂O₃-NPS -treated rats. **Fig 6:** Mean Blood SOD concentration (U/ml) in normal control and different groups of AL₂O₃-NPS -treated rats. **Fig 8:** Mean serum MDA concentration (nmol/ml) in normal control and different groups of AL₂O₃-NPS -treated rats.

• Effect of Exelon and Epigallocatechin gallate on kidney functions in control rats

In Table (3) and Figure (9, 10 and 11), There was no variation in creatinine and urea of normal rats treated with Epigallocatechin gallate (5 mg/kg and 10 mg/kg) compared to normal control rats.

Table 3: Renal functions in control, and normal rats treated with Epigallocatechin gallate

Groups / parameters	Creatinine(mg/dl)	Urea(mg/dl)	Uric acid(mg/dl)
Control	0.65 ± 0.006 ^a	39 ± 2.6 ^a	1.26 ± 0.02 ^b
Range (n=6)	(0.63-0.67)	(28-45)	(1.2-1.35)
EGCG (5 mg)	0.64 ± 0.25 ^a	35 ± 1.54 ^a	1.54 ± 0.09 ^{ab}
Range (n=6)	(0.61-0.77)	(31-39)	(1.25-1.83)
%Change compared to control	22.4	-10.25	22.2
EGCG (10 mg)	0.62 ± 0.31 ^a	32 ± 1.07 ^a	1.35 ± 0.1 ^b
Range (n=6)	(0.55-0.75)	(29-36)	(1.0-1.68)
%Change compared to control	-4.6	-17.9	7.1

Data presented as Mean ± SEM

Means have the same letters considered insignificant (P>0.05).

Fig 9: Mean serum creatinine concentration (mg/dL) in normal control and Epigallocatechin gallate groups in normal rats

Fig 10: Mean serum urea concentration (mg/dL) in normal control and Epigallocatechin gallate groups in normal rats.

Fig 11: Mean serum Uric acid concentration (mg/dL) in normal control and Epigallocatechin gallate groups in normal rats.

• **Effect of Exelon and Epigallocatechin gallate on kidney functions in rats treated with Al₂O₃-NPs**

Results given in Table (4) and graphically illustrated in Figures (12,13 and 14) showed that the intraperitoneal injection of Al₂O₃-NPs in the previously mentioned dose and period to normal rats induced significantly increased compared to normal control rats.

The concentration of creatinine, urea and uric acid were significantly decreased in Epigallocatechin (5 mg/kg or 10 mg/kg) treated with Al₂O₃-NPs administration for each when compared with the Al₂O₃-NPs group. The levels of creatinine, urea and uric acid of these rats returned nearly to the levels of control group in case of Epigallocatechin (10 mg/kg) than Epigallocatechin (5 mg/kg) treated with Al₂O₃-NPs.

Fig 13: Mean serum urea concentration (mg/dL) in normal control and different groups of AL₂O₃-NPS -treated rats.

Fig 12: Mean serum creatinine concentration (mg/dL) in normal control and different groups of AL₂O₃-NPS -treated rats.

Fig 14: Mean serum Uric acid concentration (mg/dL) in normal control and different groups of AL₂O₃-NPS -treated rats.

Table 6: Renal functions in control, AL₂O₃-NPS -treated rats, and AL₂O₃-NPS -treated rats and supplemented with Epigallocatechin gallate

Group / Parameters	Creatinine(mg/dl)	Urea(mg/dl)	Uric acid(mg/dl)
Control Range (n=6)	0.65 ± 0.006 ^a (0.63–0.67)	39 ± 2.6 ^{c,d} (28–45)	1.26 ± 0.02 ^c (1.2–1.35)
AL ₂ O ₃ -NPS Range (n=6)	0.9 ± 0.012 ^b	60 ± 0.73 ^a	3.3 ± 0.11 ^a
%Change compared to control	(0.88–0.95) 38.4	(58–63) 53.9	(2.9–3.62) 161.9
AL ₂ O ₃ -NPS + EGCG (5 mg)	0.7 ± 0.028 ^a	59 ± 3.53 ^a	1.63 ± 0.15 ^{b,c}
Range (n=6) %Change compared to control	(0.61–0.79) 7.7	(49–72) 51.2	(1.19–2.19) 29.3
%Change compared to AL ₂ O ₃ -NPs	-22.2	-1.7	-50.6
AL ₂ O ₃ -NPS + EGCG (10 mg)	0.6 ± 0.016 ^a	43 ± 3.7 ^{b,d}	1.4 ± 0.16 ^{b,c}
Range (n=6)	(0.56–0.66)	(30–55)	(1.09–1.99)
%Change compared to control	-7.7	10.2	11.1
%Change compared to AL ₂ O ₃ -NPs	-33.3	-28.3	-57.5

Data presented as Mean ± SEM

Means have the same letters considered insignificant (P>0.05).

Discussion

Green tea is one of the most popular drinks for human consumption. Epidemiological studies have shown that eating green tea is associated with a reduced risk of many chronic diseases, including cardiovascular diseases, diabetes and various cancers [18–21]. The health benefits of green tea can primarily be attributed to catechins, its main bioactive ingredients. In green tea, five major catechins including catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) were identified [22, 23].

By combining the two mechanisms, catechins appear to be able to produce and scavenge free radicals and show their beneficial effects [24, 25]. The antioxidant efficacy of catechins is exercised by (1) direct mechanisms-scavenging chelating ROS, metal ions; and (2) indirect mechanisms - inducing antioxidant enzymes, inhibiting pro - oxidant enzymes, and producing phase II enzymes and antioxidant enzymes [26]. The common chemical structures of all catechins and their diastereoisomers are phenolic hydroxyl groups that can stabilize free radicals [27]. Phenolic hydroxyl catechin groups may react with reactive oxygen and reactive nitrogen species in a termination reaction that breaks the cycle of the new generation of radicals. Catechins donate one phenolic OH group electron to reduce free radicals and maintain stability by resonating with the resulting aroxyl radicals [28, 29]. The number of hydroxyl groups of molecules correlates positively with the antioxidant activity of phenolic compounds [30]. The relative effectiveness hierarchy of catechins as radical scavengers is EGCG > ECG > EC > C [30–32].

Epigallocatechin gallate is the most potent antioxidant compound in green tea, along with its most abundant polyphenol [33]. Due to its structure of phenol rings, Epigallocatechin gallate has a powerful antioxidant activity, acts as scavengers and free radical electron traps [34, 35]. Preventing the formation of reactive oxygen species and reducing oxidative stress damage [36].

Oxidative stress is the most common toxicity mechanism associated with exposure to nanoparticles [37]. 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 [38, 39].

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 [40]. 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 [41].

Superoxide dismutase (SOD) and catalase (CAT) play an important role in the metabolism of reactive oxygen species, thus protecting cells from oxidative stress [42]. 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 [17]. 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 [43].

Glutathione (GSH) is an important non-protein thiol which in conjugation with GPx and GST, play an important role in the endogenous non - enzymatic antioxidant system [44]. Several authors have demonstrated that GSH is decreased in the brain, liver, kidney of rats exposed to micro-sized lead, Al, cisplatin and cadmium (Cd) [45–47]. GSH is known to protect cells against oxidative stress and any alteration in GSH levels (either a decrease or an increase) indicates a disturbed oxidant status, and when cells are oxidatively challenged, GSH synthesis increases [48]. As oxidative stress continues and the tissue protein contents get significantly depleted, 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 [49].

Lipid peroxidation can be defined as the oxidative deterioration of lipids. Lipid hydroperoxides are non-radical intermediates derived from unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol itself. These are formed in enzymatic or non-enzymatic reactions involving free radical [50].

In the present study, intraperitoneal injection of Al₂O₃-NPs to normal rats induced significantly decreased in GSH content, SOD and CAT activities and increased in MDA level in 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. Al has also been reported to induce lipid peroxidation and alter the physiological and biochemical properties of biological systems [51]. 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 [52, 53]. Oxidative stress is the most common mechanism by which toxicity occurs after exposure to NPs [37]. Our results showed that MDA levels increased significantly while SOD activity decreased in Al₂O₃-NP-treated rats [54]. Reported similar findings after administering oral Al to rats. In part, lipid peroxidation increases by inhibiting SOD activity [55].

Results of the present study also showed that administration of EGCG significantly decreased serum malondialdehyde (MDA) level, while significantly increased antioxidant enzymes blood GSH, SOD and CAT activity in AD rat model. The present results are in accordance with the results in which EGCG increased SOD content and protected against glycation end products induced neurotoxicity by decreasing ROS and MDA [56]. This effect elicited by EGCG might be due to its potent antioxidant property, as antioxidants have been reported previously for their ability to alleviate oxidative damage [57, 58]. The ability of presence of four ring structure with 8 hydroxyl groups in addition to hydrogen atom donation; antioxidants may also inhibit oxidation through single electron transfer [59]. Also, due to chemical structure of EGCG, it is a radical scavenger and metal chelator, which enables it to execute antioxidant effects directly [60, 61]. Some studies demonstrated that EGCG can induce endogenous antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase. EGCG upregulated the gene expression or elevated the bioactivities of these antioxidants. Thus, EGCG could directly or indirectly regulate the antioxidant levels or activity to reduce oxidative stress [62, 63].

The kidney is a complex organ made up of well-defined components that work in a highly coordinated way. It has been shown that a number of drugs, chemicals and heavy metals alter its structure and function, but acute and chronic intoxication has been shown to cause nephropathy with different levels of severity ranging from tubular dysfunction to acute renal failure [64]. The Nephrotoxicity is of critical concern when selecting new drug candidates during the early stage of drug development because of its unique metabolism; the kidney is an important target of the toxicity of drugs, xenobiotics and oxidative stress [65]. In the present study, intraperitoneal injection of Al₂O₃-NPs to normal rats induced nephrotoxicity and renal dysfunction as evidenced by significantly increased creatinine, urea and uric acid compared to normal control rats. Which suggested possible renal toxicity of alumina NPs, these results agree with [66]. In the present study, the concentration of creatinine, urea and uric acid were significantly decreased in rats treated with EGCG. This effect elicited by Epigallocatechin gallate (5 mg and 10 mg) might be due to its potent antioxidant property, as antioxidants have been reported previously for their ability to alleviate oxidative damage [57, 58].

Conclusion

The present study elucidated the beneficial effects of green tea Epigallocatechin gallate evident by improvement of

oxidative stress and renal damage 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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