The protective role of Epigallocatechin gallate (EGCG) for renal damage by suppressing oxidative stress in rats induced by aluminum oxide nanoparticles

Mohy Eldin Abd El Fattah, Mohamed Ramadan Abdelgawad and Basher Abd Elghfar El Boughdady

Abstract
The aim of this study was to evaluate the possible Protective role of EGCG on Kidney Functions and Oxidative Stress in rats induced by Aluminum oxide nanoparticles. Eight groups of rats were used; Group 1, control. Group 2, received Al2O3-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 Al2O3-NPs followed by simultaneous administration of Epigallocatechin gallate in a dose (5 mg/kg b.w. i.v.). Group 6, received Al2O3-NPs followed by simultaneous administration of Epigallocatechin gallate in a dose (10 mg/kg b.w. i.v.). There was a highly significant elevation in serum of AST, ALT, ALP and GGT and while, a significant decrease in albumin and total protein level but no a significant change in globulin and A/G ratio in rats treated with Al2O3-NPs as compared to normal control rats. There was a highly significant elevation in serum of creatinine, urea and uric acid in rats treated with Al2O3-NPs compared to normal control rats. The concentrations of creatinine, urea and uric acid were significantly decreased in rats treated with Al2O3-NPs when compared to Al2O3-NPs-treated rats. Also, 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 Al2O3-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 Al2O3-NPs when compared to Al2O3-NPs–treated rats. Also, a significant change in globulin and A/G ratio in rats treated with Al2O3-NPs compared to normal control rats.

Keywords: Epigallocatechin gallate, (EGCG) suppressing oxidative

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 (Butterfield et al., 2002). 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 (Van Acker et al., 1996). 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 disorde, cancer, ischemia-reperfusion injury and stroke (M. E. Götz et al., 2001). Green tea contains polyphenols, including 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), Epigallocatechin (EGC) and Epigallocatechin-3-gallate (EGCG). Due to differences in origin and growing conditions, the amount of catechins differs in green tea leaves (Khokhar & Magnusdottir, 2002). Nanoparticles (NPs) may be defined as materials that have at least one dimension less than 100 nm (Balasubramaniam et al., 2009). They are desirable for industrial and healthcare applications because of their unique chemical, mechanical and biological properties (Oberdörster. 2005). Because of their unique chemical, mechanical, and biological properties they are desirable for industrial and healthcare 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, 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 Co., Dokki, Giza, Egypt.

Experimental design
The rats were randomly divided into 6 groups: Group 1; received 1 ml 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 (1974) using available commercial kit which was purchased from a local chemical company.

Procedure
1. 1.0 ml of working solution (R1: 1 volume + R2: 1 volume) was added to all tubes.
2. 100 µl of sample, 100 µl of standard were added to sample tube and standard tube.
3. 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 = Aspecimen or Astandard]

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 et al. (1962) using available commercial kit which was purchased from a local chemical company.

Procedure
1. 50 µl of reagent 2 and 1.0 ml of reagent 3 were added to blank tube.
2. 50 µl of reagent 2, 1.0 ml of reagent 3 and 10 µl of sample were added to sample tube.
3. 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
4. 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:

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 (1969) using available commercial kit which was purchased from a local chemical company.

Procedure
1. 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
2. 1.0 ml of reagent 2 was added to all tubes.
3. All tubes were mixed well.
4. 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

\[
\text{Uric acid concentration (mg/dl)} = \frac{A_{\text{specimen}}}{A_{\text{standard}}} \times 6
\]

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 according to Satoh (1978).

Antioxidant Enzymes: Superoxide dismutase activity was determined according to the method of Nishikimi et al., (1972). 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 (1985). 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 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 was 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-aminoantipyrine, 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 the method of Beutler, (1984). 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 instistically significant when the P values were > 0.05.

Results
- Effect of Epigallocatechin gallate on control rats

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.

![Fig 1: Mean Blood Glutathione (GSH) concentration (mg/dL) in normal control and Epigallocatechin gallate groups in normal rats.](Image)

![Fig 2: Mean Blood Superoxide dismutase (SOD) concentration (U/ml) in normal control and Epigallocatechin gallate groups in normal rats.](Image)

Table 1: Blood Glutathione, antioxidant enzymes and serum malodialdehyde in control, and normal rats treated with Epigallocatechin gallate (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 (301-358.1) | 346± 20.3 a (316-445) | 4.33± 0.22 a (3.7-5.1) |
| Exelon Range (n=6) | 23.28± 1.4 a (16.65-25.62) | 355.68± 10.33 a (320-386.8) | 350± 12.35 a (320-399) | 4.8± 0.23 a (4.17-5.5) |
| %Change compared to control | 5.87 | 11.64 | 1.16 | 10.8 |
| EGCG (10 mg) Range (n=6) | 24.50± 1.17 a (21.21-28.7) | 353.82± 12.05 ab (310-385) | 350± 13.33 a (289-380) | 4.38± 0.24 a (4.0-5.4) |
| % Change compared to control | 11.41 | 11.07 | 1.16 | 1.15 |

Data presented as Mean ± SEM

Means have the same letters considered insignificant (P>0.05).
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 decrease 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 decreased in CAT activity by 36.13% compared to normal control rats. AL2O3-NPs -treated rats 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 the intraperitoneal injection of Al2O3-NPs in the previously mentioned dose and period to normal rats induced significantly decreased in MDA level by 177.3% compared to normal control group. AL2O3-NPS -treated rates with (5 mg and 10 mg) in the previously mentioned dose had significant increase 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 Epigallocatechin gallate.

| Groups / Parameters | GSH(mg/dl)       | SOD(U/ml)         | CAT(U/L)       | MDA(mmol/ml) |
|---------------------|------------------|-------------------|----------------|--------------|
| Control             | 21.99 ± 0.62 a   | 318.57 ± 8.91 ab  | 346±20.3 a     | 4.33 ± 0.22 a|
| Range (n=6)         | (20.31-23.85)    | (301-358.1)       | (316-445)      | (3.7-5.1)    |
| AL2O3-NPS           | 14.75± 1.23 b    | 242.92±3.79 b     | 221±6.31 b     | 12.01±0.48 b |
| 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 c     | 309±23.18 c       | 399±8.96 c     | 9.75±4.16 c  |
| 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 AL2O3-NPs | 71.18             | 27.28             | 80.54          | -18.8        |
| MDA (n=6)           | 4.33 ± 0.22 a    | 9.75 ± 4.16 c     | 9.4 ± 0.39 b   | 117.1        |
| EGCG (10 mg)        | 22.99 ± 0.82 b   | 345±8.71 c        | 405.7±18.38 c  | 8.0-10.6     |
| 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 AL2O3-NPs | 55.86             | 42               | 83.57          | -21.73       |

Data presented as Mean ± SEM
Means have the same letters considered insignificant (P>0.05).
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 Range (n=6) | 0.65±0.006<sup>a</sup> (0.63–0.67) | 39 ± 2.6<sup>a</sup> (28–45) | 1.26 ± 0.02<sup>b</sup> (1.2–1.35) |
| EGCG (5 mg) Range (n=6) | 0.64 ± 0.25<sup>a</sup> (0.61–0.77) | 35 ± 1.54<sup>a</sup> (31–39) | 1.54 ± 0.09<sup>b</sup> (1.25–1.83) |
| %Change compared to control | 0.64±0.25<sup>a</sup> (0.61–0.77) | 35 ± 1.54<sup>a</sup> (31–39) | 1.54 ± 0.09<sup>b</sup> (1.25–1.83) |
| EGCG (10 mg) Range (n=6) | 0.62 ± 0.31<sup>a</sup> (0.55–0.75) | 32 ± 1.07<sup>a</sup> (29–36) | 1.35 ± 0.1<sup>b</sup> (1.0–1.68) |
| %Change compared to control | 0.62±0.31<sup>a</sup> (0.55–0.75) | 32 ± 1.07<sup>a</sup> (29–36) | 1.35 ± 0.1<sup>b</sup> (1.0–1.68) |

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

**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 | 0.65 ± 0.006 \( ^a \) (0.63 – 0.67) | 39 ± 2.6 \( ^d \) (28 – 45) | 1.26 ± 0.02 \( ^c \) (1.2 – 1.35) |
| AL2O3-NPS | 0.9 ± 0.012 \( ^a \) (0.88 – 0.95) | 60 ± 0.73 \( ^c \) (58 – 63) | 3.3 ± 0.11 \( ^c \) (2.9 – 3.62) |
| Change compared to control | 38.4 | 53.9 | 161.9 |
| AL2O3-NPS + EGCG (5 mg) | 0.7 ± 0.028 \( ^a \) (0.61 – 0.79) | 59 ± 3.3 \( ^c \) (49 – 72) | 1.61 ± 0.15 \( ^c \) (1.19 – 2.19) |
| Change compared to Al2O3-NPs | 7.7 | 51.2 | 29.3 |
| Change compared to AL2O3-NPS | -22.2 | -1.7 | -50.6 |
| AL2O3-NPS + EGCG (10 mg) | 0.6 ± 0.016 \( ^a \) (0.56 – 0.66) | 43 ± 3.7 \( ^d \) (30 – 55) | 1.4 ± 0.16 \( ^c \) (1.09 – 1.99) |
| Change compared to Al2O3-NPS | -7.7 | 10.2 | 11.1 |
| Change compared to AL2O3-NPS | -33.3 | -28.3 | -57.5 |

Data presented as Mean ± SEM

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

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Discussion

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 [15-18]. 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) [19, 20].

Catechins appear to be capable of producing and scavenging free radicals and showing their beneficial effects by combining the two mechanisms [21, 22]. Catechins’ antioxidant efficacy is exercised by (1) direct mechanisms - scavenging chelating ROS, metal ions ; and (2) indirect mechanisms - inducing antioxidant enzymes, inhibiting pro-oxidant enzymes, and producing detoxification enzymes and antioxidant enzymes in Phase II [23]. All catechins and their diastereoisomers have common chemical structures - phenolic hydroxyl groups that can stabilize free radicals [24]. Phenolic hydroxyl groups of catechins can react in a termination reaction with reactive oxygen and reactive nitrogen species that breaks the cycle of new radical’s generation. Catechins donate one phenolic OH group electron, thereby reducing free radicals and maintaining stability through the resonance of the resulting aroxyl radicals [25, 26]. The number of molecule hydroxyl groups is positively correlated with the antioxidant activity of phenolic compounds [27]. Catechins’ relative efficacy hierarchy as radical scavengers is EGCG > ECG > EGC > EC > C [27-29].

Epigallocatechin gallate is the most potent antioxidant compound in green tea, along with its most abundant polyphenol [30]. Due to its structure of phenol rings, Epigallocatechin gallate has a powerful antioxidant activity, acts as scavengers and free radical electron traps [31, 32]. Preventing the formation of reactive oxygen species and reducing oxidative stress damage [33].

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 (Barbier et al., 2005). 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 (Uehara et al., 2007).

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 (Yang et al., 2012).

In the present study, the concentration of creatinine, urea and uric acid were significantly decreased in rats treated with EGCG. 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 (Babu et al., 2008; Widlansky et al., 2007).

Conclusion

The present study elucidated the beneficial effects of green tea Epigallocatechin gallate evident by improvement of hepatic, renal and hematological parameters. 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 hepatotoxicity.

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