International Journal of Advanced Biochemistry Research

Beneficial effects of green tea Epigallocatechin gallate on liver functions and hematological parameters in normal and induced rats 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 beneficial effects of EGCG on Liver Functions and Hematological Parameters in rats induced by Aluminum oxide nanoparticles. Eight groups of rats were used; Group1, control. Group2, 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 ratio in rats treated with Al2O3-NPs as compared to normal control rats. Rats treated with Epigalocatechin gallate; 5 mg/kg and 10 mg/kg treated with Al2O3-NPs ameliorated liver functions (the above parameters) as compared to Al2O3-NPs-treated rats. Also, treatment with Epigallocatechin gallate; 5 mg/kg and 10 mg/kg with Al2O3-NPs -treated rats induced a significant increase in Hb concentration, RBCs count and PCV% compared to normal control rats but decreased in WBCs count and platelets compared to Al2O3-NPs -treated rats.

Keywords: Beneficial effects, Epigallocatechin gallate

Introduction

Green tea is one of the plant products that have significant effects on human health. Epigallocatechin gallate (EGCG), epicatechin (EC), epigallocatechin (EGC) and epicatechin gallate (ECG) are the major polyphenolic components in green tea [1]. In the biological system, EGGC, the main and most active component of green tea catechins, acts as an antioxidant [2] it is rapidly absorbed and distributed mainly in the mucous membranes of the small intestine and the liver; it may cross the blood brain barrier more interestingly [3]. In green tea, polyphenols can neutralize free radicals and can reduce or even help prevent some of the damage caused by reactive oxygen species (ROS) [4]. Long-term intake of green tea catechins may be important due to constant exposure of cells to oxidative stress. It has been reported that tea polyphenols have the ability to participate in vitamin E recycling besides directly quenching reactive oxygen species [5].

Epigallocatechin-3-gallate (EGCG) is the most abundant and active compound responsible for most of green tea’s role in promoting good health by acting through different pathways; as antioxidant, anti-inflammatory, antiatherogenic and also showing gene expression activity, functioning through growth factor mediated pathways [6]. Nanoparticles (NPs) may be defined as materials that have at least one dimension less than 100 nm [7]. 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 / clinlab company (4,160St. El-Etehad Square Riham Tower El-Maadi, Cairo, Egypt). Aluminum oxide nanoparticles (Al₂O₃NPS) from Egyptian Atomic Energy Authority, Ins has 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
Liver Functions
1. Determination of serum alanine aminotransferase (ALT)
Alanine aminotransferase (ALT) activity was determined by the kinetic method as described by [11] using available commercial kit which was purchased from a local chemical company.

Procedure
1. 100 µl of sample and 1.0 ml of working solution (R1: 9 volumes + R2: 1 volume) were added to dry test tubes.
2. The tubes were mixed and read at λ 365 nm after 1, 2, 3 minutes.
3. The change in mean absorbance per minute (ΔA/min) was determined.

Calculation
The activity of ALT was calculated using the following formula
ALT (U/L) = 1746 x ΔA

2. Determination of serum aspartate aminotransferase (AST)
Aspartate aminotransferase (AST) activity was determined by a kinetic method as described by [11] using available commercial kit which was purchased from a local chemical company.

Procedure
1. 100 µl of sample and 1.0 ml of working solution (R1: 9 volumes + R2: 1 volume) were added to dry test tubes.
2. The tubes were mixed and read at λ 405 nm after 1, 2, 3 minutes.
3. The change in mean absorbance per minute (ΔA/min) was determined.

Calculation
The activity of AST was calculated using the following formula
AST (U/L) = 1746 x ΔA

3. Determination of serum gamma glutamyl transferase (GGT)
Gamma glutamyl transferase (GGT) activity was determined by kinetic method as described by [11] using available commercial kit which was purchased from a local chemical company.

Procedure
1. 100 µl of sample and 1.0 ml of working solution (R1: 9 volumes + R2: 1 volume) were added to dry test tubes.
2. The tubes were mixed and read at λ 405 nm after 1, 2, 3 minutes.
3. The change in mean absorbance per minute (ΔA/min) was determined.

Calculation
The activity of γ-GT (GGT U/L) = 1158 x ΔA

4. Determination of serum alkaline phosphatase (ALP)
Alkaline phosphatase (ALP) activity was determined by kinetic method as described by [11] using available commercial kit which was purchased from a local chemical company.

Procedure
1. 10 µl of sample and 1.0 ml of working solution (R1: 5 volumes + R2: 1 volume) were added to dry test tubes.
2. Distilled water was used to adjust the instrument to zero.
3. The tubes were mixed, transferred in cuvette and incubated for 30 sec at 37oc.
4. ALP in sample was read at λ 405 nm after 1, 2, 3 minutes.
5. The change in mean absorbance per minute (ΔA/min) was determined.

Calculation
Alkaline phosphatase (ALP) was calculated using the following equation ALP (U/L) = 5454 x ΔA

5. Determination of serum Total protein
Total protein activity was determined by a colorimetric method as described by [12] using available commercial kit which was purchased from a local chemical company.

Procedure
1. Blank, sample and reagent 1 were added to dry test tube as follows:
2. All tubes were mixed well.
3. After incubation for 10 min. at 37oc, the absorbance of standard and sample tubes was read at λ 550 nm against blank.

| Tubes| Reagents | Blank (ml) | Standard (ml) | Sample (ml) |
|------|----------|------------|---------------|-------------|
| Reagent 2 | 1.0 | 1.0 | 1.0 |
| Sample | - | - | 0.025 |
| Standard | - | - | - |

**Calculation**
The level of Total protein was calculated using the following equation

\[ \text{Protein concentration (g/dl)} = \frac{(A) \text{Sample} - (A) \text{Standard}}{5.0} \]

Where, 5.0 is the standard concentration of total protein.

6. **Determination of serum albumin**
The level of albumin was determined by a colorimetric method as described by [13] using available commercial kit which was purchased from a local chemical company.

Procedure:
1. 10 µl of distilled water was added to blank tube.
2. 10 µl of sample was added to sample tube and 10 µl of reagent 1 was added to standard tube.
3. 1.0 ml of reagent 2 was added to all tubes.
4. All tubes were mixed well.
5. After incubation for 5 min at 25oc. The absorbance of sample and standard tubes was read at λ 623nm against blank.

**Calculation**
The level of albumin in sample was calculated using the following equation

\[ \text{Albumin concentration (g/dl)} = \frac{(A) \text{Sample} - (A) \text{Standard}}{4.0} \]

Where, 4.0 is the standard concentration of albumin.

7. **Determination of serum globulin**
Serum globulin concentration was obtained by subtracting the obtained albumin value from the corresponding total protein value for each sample as described by [13].

**A. Determination of hematological parameters**
Determination of hematological parameters was completed using cell counter equipment (Humacount, Germany) according to [14] provided by the following reagents:
- HC-DILUENT 17400/10 (20 litre).
- HC-LYSE 17400/20 (1 litre).
- HC-CLEANER 17400/30 (1 litre).
Hematological assays include hemoglobin (Hb), erythrocyte (RBCs) counts, leucocytic (WBCs) count and platelets count.

**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 in statistically significant when the P values were > 0.05.

**Results**
**Effect of Epigallocatechin gallate on liver functions in control rats**
As shown in Table (1) and Table (2) & Figures (1, 2, 4, 6, 7, 3, 5 and 8) there was no variation in the activity of ALT, GGT and alkaline phosphatase levels in normal rats treated Epigallocatechin gallate (5 mg/kg b.w. i.v. daily for five weeks & 10 mg/kg b.w. i.v. daily for five weeks ).

Treatment of normal rats with Epigallocatechin gallate 10 mg/kg induced significantly increased in the level of total protein by 24.4%, and while, no significant change in the level of total protein of normal rats treated with Epigallocatechin gallate 5 mg/kg compared to normal control rats and also significantly decreased in the level of A/G ratio in Epigallocatechin gallate (5 mg/kg and 10 mg/kg). There was no variation in the level of albumin and globulin of normal rats treated with Epigallocatechin gallate (5 mg/kg and 10 mg/kg) compared to normal control rats.

**Table 1:** Liver functions in control and normal rats treated Epigallocatechin gallate

| Groups / parameter | ALT(U/L) | AST(U/L) | ALP(U/L) | GGT(U/L) |
|--------------------|----------|----------|----------|----------|
| Control            | 60.1±3.4* | 117.1±2.5* | 384±35.4* | 3.1±0.26* |
| Range (n=6)        | (51–74)  | (109–124) | (280–495) | (2.3–3.9) |
| EGCG (5 mg)        | 57.5±4.0* | 132.5±4.6* | 314.7±9.3* | 3.4±0.2* |
| Range (n=6)        | (41.9–67.6) | (2.5–135) | (280–344) | (2.8–3.9) |
| %Change compared to control | -3.42 | 13.1 | -18 | 9.7 |
| EGCG (10 mg) Range (n=6) | 50.9±2.15* | 126.4±4.6* | 306.7±8.6* | 3.14±0.13* |
| %Change compared to control | (43.3–57) | (115–147) | (275–330) | (2.77–3.5) |

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

**Table 2:** Serum total protein, albumin, globulin level and A/G ratio in control, and normal rats treated with Epigallocatechin gallate

| Groups / parameter | T. protein(g/dl) | Albumin (mg/dl) | Globulin(g/dl) | A/G Ratio |
|--------------------|------------------|-----------------|----------------|-----------|
| Control            | 6.7±0.17 b       | 3.72 ± 0.05 *   | 3.0±0.18 b     | 1.27±0.09 b |
| Range (n=6)        | (6.2–7.2)        | (3.5–3.9)       | (2.3–3.5)      | (1.06–1.69) |
| EGCG (5 mg)        | 7.15 ± 0.09 a    | 3.88 ± 0.04 *   | 3.27 ± 0.11 b  | 1.2 ± 0.07 b |
| Range (n=6)        | (6.8–7.5)        | (3.6–4.3)       | (2.9–3.6)      | (1.03–1.48) |
| %Change compared to control | 6.5 | 4.3 | 9.0 | -5.5 |
| EGCG (10 mg) Range (n=6) | 8.35 ± 0.35 *  | 3.95 ± 0.04 *   | 4.77 ± 0.32 b  | 0.76±0.05 b |
| Range (n=6)        | (6.9–9.3)        | (3.8–4.1)       | (3.5–4.7)      | (0.6–0.97) |
| %Change compared to control | 24.4 | 6.2 | 59 | -40.1 |

Data presented as Mean ± SEM Means have the same letters considered insignificant (P>0.05).
Fig 1: Mean serum ALT activity (U/L) in normal control and Epigallocatechin gallate groups in normal rats.

Fig 2: Mean serum AST activity (U/L) in normal control and Epigallocatechin gallate groups in normal rats.

Fig 3: Mean serum alkaline phosphatase activity (U/L) in normal control and Epigallocatechin gallate groups in normal rats.

Fig 4: Mean serum GGT activity (U/L) in normal control and Epigallocatechin gallate groups in normal rats.

Fig 5: Mean serum Albumin concentration (g/dL) in normal control and Epigallocatechin gallate groups in normal rats.

Fig 6: Mean serum total protein concentration (g/dL) in normal control and Epigallocatechin gallate groups in normal rats.

Fig 7: Mean serum Globulin concentration (g/dL) in normal control and Epigallocatechin gallate groups in normal rats.

Fig 8: Mean A/G Ratio in normal control and Epigallocatechin gallate groups in normal rats.
Effect of Epigallocatechin gallate on liver functions in rats treated with Al2O3-NPs

Effect on serum ALT activity

As shown in Table (3) and Figure (9), ALT activity showed a significant increase in rats treated with Al2O3-NPs in a dose 50 mg/kg b.w intraperitoneally (i.p), three times a week for three weeks to normal rats by 152.2% compared to normal control. Al2O3-NPs treated rats with Epigallocatechin gallate (5 mg/kg and 10 mg/kg) every day for five weeks were significantly decreased serum ALT activity compared to Al2O3-NPs group.

Fig 9: Mean serum ALT activity (U/L) in normal control and different groups of Al2O3-NPs-treated rats.

Fig 10: Mean serum AST activity (U/L) in normal control and different groups of Al2O3-NPs-treated rats.

Fig 11: Mean serum GGT activity (U/L) in normal control and different groups of Al2O3-NPs-treated rats.

Fig 12: Mean serum ALP activity (U/L) in normal control and different groups of Al2O3-NPS-treated rats.

Fig 13: Mean serum total protein concentration (g/dL) in normal control and different groups of Al2O3-NPS-treated rats.

Fig 14: Mean serum Albumin concentration (g/dL) in normal control and different groups of Al2O3-NPS-treated rats.

Fig 15: Mean serum Globulin concentration (g/dL) in normal control and different groups of Al2O3-NPS-treated rats.
Effect on serum AST activity
In Table (3) and Figure (10), the administration of Al2O3-NPs in the selected dose and period to normal rats resulted in a significant elevation in AST activity from 117.1 ± 2.5 to 181.4 ± 6.5 recording 54.9% compared to normal control group. AL2O3-NPs-treated rats with Epigallocatechin (5 mg/kg and 10 mg/kg) induced a significant reduction of AST activity by 14.9% and 16% respectively compared to AL2O3-NPs group. No significant change between Epigallocatechin 5 mg/kg and 10 mg/kg treated rats with AL2O3-NPs.

Effect on GGT activity
In Table (3) and Figure (11), administration of Al2O3-NPs in the selected dose and period to normal control rats resulted in a significant elevation in GGT activity from 3.1 ± 0.26 to 11.4 ± 0.95 compared to normal control group, the percentage of increase in GGT activity was 266% compared to normal rats. AL2O3-NPs-treated rats with Epigallocatechin gallate (5 mg/kg and 10 mg/kg) in the previously mentioned dose and period induce a significant reduction of GGT activity compared to Al2O3-NPs group. No significant change between Epigallocatechin 5 mg/kg and 10 mg/kg treated with AL2O3-NPs.

Effect on Alkaline phosphatase (ALP) level
In Table (3) and Figure (12), injection of Al2O3-NPs to normal rats in the selected dose and period resulted in a significant elevation in the concentration of ALP from 384 ± 35.4 to 626.7 ± 22 recording 63% compared to control group. The concentration of ALP in rats of Al2O3-NPs-treated with Epigallocatechin gallate (5 mg/kg or 10 mg/kg) in the previously doses and period to Al2O3-NPs-treated rats resulted in a significant decrease of ALP concentration compared to Al2O3-NPs group. The levels of ALP in all rats treated with Epigallocatechin gallate treated with Al2O3-NPs were restored nearly to the level of normal control group.

Table 3: Liver functions in control, Al2O3-NPs-treated rats, and Al2O3-NPs-treated rats and supplemented with Epigallocatechin gallate

| Groups / parameter | ALT(U/L) | AST(U/L) | ALP (U/L) | GGT(U/L) |
|--------------------|----------|----------|-----------|----------|
| Control            | 60.1± 3.37b | 117.1± 2.5c | 384± 35.4b | 3.1± 0.26c |
| Range (n=6)        | (51–74) | (109–124) | (280–495) | (2.3–3.9) |
| AL2O3-NPS          | 151.6± 5.5b | 181.4± 6.5a | 626.7± 22a | 11.4± 0.95a |
| Range (n=6)        | (129–162) | (166.8–210) | (550–690) | (8.9–15) |
| %Change compared to control | 152.2 | 54.9 | 63 | 266 |
| AL2O3-NPS + EGCG (5 mg) | 61.6± 3.36b | 154.6± 3.3b | 421± 27.4b | 6.15± 0.22b |
| Range (n=6)        | (47.6–68) | (147.4–168) | (338–393) | (5.4–6.9) |
| %Change compared to control | 2.5 | 32 | 9.6 | 98.4 |
| AL2O3-NPS + EGCG (10 mg) | 53± 2.82b | 152.4± 5.2b | 360± 18.7b | 5.2± 0.2b |
| Range (n=6)        | (45-60.7) | (134–166.5) | (300–412) | (4.6–5.9) |
| %Change compared to Al2O3-NPs | -11.8 | 30.1 | -6.2 | 67.7 |
| %Change compared to Al2O3-NPs | -58.4 | -16 | -20.1 | -54.2 |

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

Table 4: Serum total protein, albumin and globulin level; and A/G ratio in control, Al2O3-NPs-treated rats, and Al2O3-NPs-treated rats and supplemented with Epigallocatechin gallate

| Groups / parameters | T. protein(g/dl) | Albumin(mg/dl) | Globulin(g/dl) | A/G Ratio |
|--------------------|-----------------|----------------|----------------|-----------|
| Control            | 67.7± 0.17     | 3.72± 0.05     | 3.0± 0.18     | 1.27± 0.09  |
| Range (n=6)        | (6.2–7.2)      | (3.5–3.9)      | (2.3–3.5)     | (1.06–1.69) |
| AL2O3-NPS          | 4.98± 0.27     | 2.5± 0.15      | 2.32± 0.24    | 1.2± 0.25   |
| Range (n=6)        | (3.9–4.9)      | (2.0–2.9)      | (1.2–2.8)     | (0.75–2.42) |
| %Change compared to control | -28.5 | -32.8 | -22.7 | -5.51 |
| AL2O3-NPS + EGCG (5 mg) | 7.58± 0.16b | 3.58± 0.08b | 4.0± 0.21b | 0.91± 0.07b |
| Range (n=6)        | (7.0–8.0)      | (3.2–3.8)      | (3.3–4.6)     | (0.69–1.12) |
| %Change compared to control | -12.9 | -33.3 | -28.3 |
| %Change compared to Al2O3-NPs | 52.2 | 43.2 | 72.4 |
| AL2O3-NPS + EGCG (10 mg) | 8.48± 0.19b | 3.84± 0.06b | 4.63± 0.16a | 0.83± 0.03ab |
| Range (n=6)        | (8.0–9.0)      | (3.6–4.0)      | (4.2–5.2)     | (0.75–0.93) |
| %Change compared to control | 26.3 | 54.3 | -34.6 |
| %Change compared to Al2O3-NPs | 70.2 | 53.6 | 99.5 |

Data presented as Mean ± SEM Means have the same letters considered insignificant (P>0.05).
**Effect on serum total protein**
As listed in Table (4) and graphically illustrated in Figure (13), intraperitoneal injection of Al2O3-NPs in the previously dose and period to normal rats induced a significant reduction in total protein level (6.71 ± 0.17 vs. 4.98 ± 0.27) compared to normal control group. The percentage of change was 25.8%. The levels of total protein in rats of Al2O3-NPs -treated with Epigallocatechin gallate (5mg/kg or 10 mg/kg) in the previously doses and period to Al2O3-NPs -treated rats resulted in a significant increase of total protein concentration compared to Al2O3-NPs group.

**Effect on serum albumin**
As listed in Table (4) and graphically illustrated in Figure (14), intraperitoneal injection of Al2O3-NPs in the previously dose and period to normal rats induced a significant fall in albumin level (2.5±0.15 vs. 3.72±0.05) compared to normal control group. The percentage of change was 32.8 % compared to normal control group. Treatment of Al2O3-NPs -treated rats with Epigallocatechin (5 mg/kg and 10 mg/kg) for each group in the previously mentioned dose and period significantly increased serum albumin level compared with the Al2O3-NPs group.

**Effect on serum globulin**
As listed in Table (4) and graphically illustrated in Figure (15), intraperitoneal injection of Al2O3-NPs in the previously dose and period to normal rats induced reduction in globulin level (2.32 ± 0.24 vs. 3.0 ± 0.18) compared to normal control group but no significant. The percentage of change was 22.7% compared to normal control group. Treatment of Al2O3-NPs -treated rats with Epigallocatechin (5 mg/kg and 10 mg/kg) for each group in the previously mentioned dose and period significantly increased serum globulin level compared with the Al2O3-NPs group.

**Effect on serum A/G ratio**
There was no variation in the level of A/G ratio of normal rats treated with Epigallocatechin gallate (5 mg/kg) and Al2O3-NPs compared to Al2O3-NPs -treated rats alone, Figure (16).

**3.15. Effect of Epigallocatechin gallate on hematological parameters in control rats**
In Table (5) and Figures (17, 18,19,20 and 21), the hematological parameters; WBCs and PLT were not changed in normal rats treated with Epigallocatechin gallate (5 mg) and Epigallocatechin gallate (10 mg) compared to normal control rats. Also Hb, RBCs and PCV were not changed in normal rats treated with Epigallocatechin gallate (10 mg) compared to normal control rats. Meanwhile, was present significant variation in case of Hb concentration in Epigallocatechin gallate (5 mg) compared to normal control rats as well as in case of PCV but no variation in case of RBCs count compared to normal rats.

**Table 5: Blood parameters in control and normal rats treated with Epigallocatechin gallate**

| Group / Parameters | Hb (g/dl) | RBCs(m/cmm) | PCV (%) | WBCs(10³/cmm) | PLT(10³/cmm) |
|--------------------|-----------|-------------|---------|----------------|--------------|
| Control | 16.7± 0.04ab | 9.66± 0.04ab | 48.5± 0.19a | 13.3± 0.07a | 711.1± 40.81a |
| Range (n=6) | (16.58–16.8) | (9.5–9.75) | (47.9–49) | (13.1–13.6) | (565–838) |
| EGCG (5 mg) | 16.3± 0.09b | 9.44± 0.07b | 45.9± 0.76b | 13.5± 0.23b | 735.6± 56.47b |
| Range (n=6) | (16–16.6) | (9.16–9.6) | (43.3–48) | (12.8–14.5) | (611–961) |
| %Change compared to control | -2.1 | 2.58 | -5.51 | 1.72 | 3.44 |
| EGCG (10 mg) | 16.85± 0.02a | 9.75± 0.09a | 49.02± 0.2a | 13.4± 0.2a | 746.1± 56.97a |
| Range (n=6) | (16.78–16.9) | (9.22–9.9) | (48.7–50) | (12.9–14.5) | (73–993) |
| %Change compared to control | 0.9 | 0.93 | 0.9 | 0.57 | 4.9 |

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

![Fig 17](image1.png) **Fig 17:** Mean blood Hb concentration (g/dl) of normal control and Epigallocatechin gallate groups in normal rats.

![Fig 18](image2.png) **Fig 18:** Mean blood RBCs count (mill./Cmm) of normal control and Epigallocatechin gallate groups in normal rats.
Effect of Epigallocatechin gallate on hematological parameters in rats treated with Al2O3-NPs

As shown in Tables (6) and Figures (22, 23, 24, 25 and 26), showed that intraperitoneal injection of Al2O3-NPs in the previously mentioned dose and period to normal rats induced significantly decreased in Hb concentration, RBCs count and PCV% by 22.15%, 21.9 and 26.66 respectively compared to normal control group, induced significantly increased in WBCs count by 7.12% compared to normal control group and no changes were detected in counts of platelets compared to normal control group. Treatment Epigallocatechin gallate (5 mg and 10 mg) with Al2O3-NPs -treated rats induced a significant increase in Hb concentration, RBCs count and PCV% comparison to Al2O3-NPs -treated rats and also, induced a significant decrease in WBCs count in comparison to Al2O3-NPs -treated rats. No significant change in Epigallocatechin gallate (5 mg and 10 mg) Al2O3-NPs -treated rats compared to normal control group. No changes were detected in counts of platelets compared to normal control group or Al2O3-NPs -treated rats in case of Epigallocatechin gallate (5 mg and 10 mg) Al2O3-NPs -treated rats.
**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], EGCG can affect several potential diseases of Alzheimer’s - related goals [31].

Liver is an organ that plays an important role in metabolism and has many functions in the body, including storage of glycogen, decomposition of red blood cells, and synthesis of plasma proteins, hormone production and detoxification. It is in the abdominal-pelvic region of the abdomen under the diaphragm. It produces bile, an alkaline compound that helps digest through lipid emulsification. The highly specialized tissues of the liver regulate a wide range of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are required for normal vital functions [34]. Liver is the main site of accumulation of aluminum in experimental animals [35]. The effect of aluminum on hepatic functions had been reported by [36, 37].

In our present study, Injection of Al2O3-NPs for 3 weeks, three times weekly to normal rats resulted in a significant elevation in the activities of AST, ALT, GGT and concentrations of ALP compared to normal control group. These results are in agreement with the results of [38]. ALT and AST are two important and sensitive indicators for hepatic damage. The ALT level increased in the alumina NPs treated rats and supplemented with Epigallocatechin gallate [39]. Due to its structure of phenol rings, Epigallocatechin gallate has a powerful antioxidant activity, acts as scavengers and free radical electron traps [31, 32].

**Table 6:** Blood parameters in control, AL2O3-NPS-treated rats, and AL2O3-NPS-treated rats and supplemented with Epigallocatechin gallate

| Group / Parameters | Hb (g/dl) | RBCs(m/cmm) | PCV (%) | WBCs(10³/cmm) | PLT(10³/cmm) |
|--------------------|----------|-------------|---------|---------------|--------------|
| Control            | 16 ± 0.04 | 9.6 ± 0.04  | 48 ± 0.19 | 13 ± 0.07     | 711 ± 40.81  |
| AL2O-NPS           | 13 ± 0.21 | 7.5 ± 0.05  | 35 ± 0.15 | 14 ± 0.12     | 593 ± 67.75  |
| NPS                | -        | -           | -       | -             | -            |
| Range (n=6)        | 12.4 ± 1.7 | 7.3 ± 0.66  | 35 ± 36  | 13.9 ± 14.8   | 373 ± 398    |
| %Change compared to control | -         | -           | -       | -             | -            |
| AL2O-NPS + ECGG (5 mg) | -        | -           | -       | -             | -            |
| Range (n=6)        | 14.0 ± 1.9 | 8.3 ± 0.6   | 39.8 ± 44.8 | 13.7 ± 13.9  | 515 ± 623.3  |
| %Change compared to control | -        | -           | -       | -             | -            |
| AL2O-NPS + ECGG (10 mg) | -        | -           | -       | -             | -            |
| Range (n=6)        | 14.9 ± 1.9 | 8.7 ± 0.6   | 45.8 ± 0.6  | 13.2 ± 0.05   | 709 ± 41.86  |
| %Change compared to control | -        | -           | -       | -             | -            |
| %Change compared to Al2O-NPs | -        | -           | -       | -             | -            |

*Fig 26: Mean blood PLT count (10³/Cmm) of normal control and different groups of AL2O3-NPS -treated rats.*

Due to its structure of phenol rings, Epigallocatechin gallate has a powerful antioxidant activity, acts as scavengers and free radical electron traps. Preventing the formation of reactive oxygen species and reducing oxidative stress damage. EGCG can affect several potential diseases of Alzheimer’s - related goals. Liver is an organ that plays an important role in metabolism and has many functions in the body, including storage of glycogen, decomposition of red blood cells, and synthesis of plasma proteins, hormone production and detoxification. It is in the abdominal-pelvic region of the abdomen under the diaphragm. It produces bile, an alkaline compound that helps digest through lipid emulsification. The highly specialized tissues of the liver regulate a wide range of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are required for normal vital functions. Liver is the main site of accumulation of aluminum in experimental animals. The effect of aluminum on hepatic functions had been reported. In our present study, Injection of Al2O3-NPs for 3 weeks, three times weekly to normal rats resulted in a significant elevation in the activities of AST, ALT, GGT and concentrations of ALP compared to normal control group. These results are in agreement with the results of ALT and AST are two important and sensitive indicators for hepatic damage. The ALT level increased in the alumina NPs treated rats and supplemented with Epigallocatechin gallate.
diseases. When the hepatocellular plasma membrane is damaged, the enzymes normally present in the cytosol are released into the blood stream [40]. This can be quantified to assess the type and extent of liver injury. The increased levels of serum liver enzymes indicated the increased permeability and damage or necrosis of hepatocytes [41]. In the present study, the treatment of Al2O3-NPs intoxicated rats with EGCG decreased serum activities of ALT; AST; GGT; and ALP. 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 [42, 43]. This ameliorative effect was due to the inhibition of ROS formation, which inhibits the peroxidation of membrane lipids and prevents the leakage of the enzyme of hepatic markers, and the administration of EGCG significantly improves the levels of enzymatic and non - enzymatic antioxidants in the liver, which further contributes to the hepatoprotective effect of the liver [44]. The present study results revealed that intraperitoneal injection of Al2O3-NPs to normal rats show significant decrease in the level of total protein and albumin compared to normal control group, a reduction in the level of correlation with the study carried out by Morsy, Abou El-Ala, et al., (2016). In the present study, treatment with EGCG to intoxicated rats showed increase of total protein and albumin compared to normal control group. Concerning the hematological parameters; showed that intraperitoneal injection of Al2O3-NPs in the previously mentioned dose and period to normal rats induced significantly decreased in Hb concentration and RBCs count compared to normal control group, induced significantly increased in WBCs count by compared to normal control group, that disagreed with those of Morsy, Abou El-Ala, et al., (2016), that showed marked increase in PCV% and increase in PCV%, decrease in WBCs count by compared to normal control group, these results agree with [38]. While no changes were detected in counts of platelets and increase in PCV% compared to normal control group, that disagreed with those of Morsy, Abou El-Ala, et al., (2016), that showed marked increase in PLT count and increase in PCV%. Aluminum may interfere with red cell synthesis and mature red blood cells at different stages. Nevertheless, persistent intoxication would cause compensatory mechanisms to be triggered, resulting in the restoration of hematocrit and hemoglobin concentrations [45]. Treatment with EGCG induced a significant increase in Hb, RBCs count and PCV%, decrease in WBCs count, and no changes were detected in counts of platelets compared to normal control group. This could be due to 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 [46].

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.

References

1. Weinreb O et al, Neurological mechanisms of green tea polyphenols in Alzheimer’s and Parkinson’s diseases.

2. Choi YT et al., The green tea polyphenol (-) Epigallocatechin gallate attenuates β-amyloid-induced neurotoxicity in cultured hippocampal neurons. Life sciences. 2001; 70(5):603-614.

3. Nakagawa K, T Miyazawa. Absorption and distribution of tea catechin, (-)-epigallocatechin-3-gallate, in the rat. Journal of nutritional science and vitaminology. 1997; 43(6):679-684.

4. Dullo AG et al, Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. The American journal of clinical nutrition. 1999; 70(6):1040-1045.

5. Zhu QY, et al., Regeneration of α-tocopherol in human low-density lipoprotein by green tea catechin. Journal of agricultural and food chemistry. 1999; 47(5):2020-2025.

6. Shixian Q, et al, Green tea extract thermogenesis-induced weight loss by Epigallocatechin gallate inhibition of catechol-O-methyltransferase. Journal of medicinal food, 2006; 9(4):451-458.

7. Balasubramanyam A, et al, In vivo genotoxicity assessment of aluminium oxide nanomaterials in rat peripheral blood cells using the comet assay and micronucleus test. Mutagenesis, 2009; 24(3):245-251.

8. Oberdörster G, E Oberdörster, J Oberdörster, Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environmental health perspectives. 2005; 113(7):823.

9. Shah SA, et al, Nanoscale-alumina induces oxidative stress and accelerates amyloid beta (Aβ) production in ICR female mice. Nanoscale. 2015; 7(37):15225-15237.

10. Rasoolijazi H et al, The beneficial effect of (-) epigallocatechin-3-gallate in an experimental model of Alzheimer’s disease in rat: A behavioral analysis. Iranian Biomedical Journal, 2007; 11(4):237-243.

11. Tietze W, Fundamentals of Clinical Chemistry 2nd Ed WB Saunders Co. Philadelphia, 1982.

12. Gornall AG, CJ Bardwell, MM David, Determination of serum proteins by means of the biuret reaction. Journal of biological chemistry. 1949; 177(2):751-766.

13. Doumas B, H Biggs, Standard methods of clinical chemistry. Academic Press, Chicago. 1972; 7:175-189.

14. Buttarello M, M Plebani, Automated blood cell counts: state of the art. American journal of clinical pathology. 2008; 130(1):104-116.

15. Gao YT, et al, Reduced risk of esophageal cancer associated with green tea consumption. JNCI: Journal of the National Cancer Institute. 1994; 86(11):855-858.

16. Iso H, et al, The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. Annals of Internal Medicine. 2006. 144(8):554-562.

17. Kuriyama S, et al, Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. Jama. 2006; 296(10):1255-1265.

18. Kapoor S Re: Green tea consumption and prostate cancer risk in Japanese men: a prospective study”. American journal of epidemiology. 2008; 168(1):119.
19. Seeram NP et al. Catechin and caffeine content of green tea dietary supplements and correlation with antioxidant capacity. Journal of Agricultural and Food Chemistry. 2006; 54(5):1599-1603.

20. Chen Q, Z Guo, J Zhao, Identification of green tea’s (Camellia sinensis (L.)) quality level according to measurement of main catechins and caffeine contents by HPLC and support vector classification pattern recognition. Journal of Pharmaceutical and Biomedical Analysis. 2008; 48(5):1321-1325.

21. Oliveira-Marques V et al., Modulation of NF-KB-Dependent Gene Expression by H2O2: A Major Role for a Simple Chemical Process in a Complex Biological Response. Antioxidants & redox signaling. 2009; 11(9):2043-2053.

22. Valko M et al., Free radicals and antioxidants in normal physiological functions and human disease. The international journal of biochemistry & cell biology, 2007; 39(1):44-84.

23. Youn HS et al., Suppression of MyD88-and TRIF-dependent signaling pathways of Toll-like receptor by (−)-epigallocatechin-3-gallate, a polyphenol component of green tea. Biochemical pharmacology, 2006; 72(7):850-859.

24. Fraga CG, et al., Basic biochemical mechanisms behind the health benefits of polyphenols. Molecular aspects of medicine. 2010; 31(6):435-445.

25. Fan FY, LX Sang, M Jiang, Catechins and their therapeutic benefits to inflammatory bowel disease. Molecules, 2017; 22(3):484.

26. Bors W et al., Flavonoids as antioxidants: Determination of radical-scavenging efficiencies, in Methods in enzymology. Elsevier. 1990, 343-355.

27. Rice-evans CA, et al., The relative antioxidant activities of plant-derived polyphenol flavonoids. Free radical research, 1995; 22(4):375-383.

28. Intra J, SM Kuo, Physiological levels of tea catechins increase cellular lipid antioxidant activity of vitamin C and vitamin E in human intestinal caco-2 cells. Chemico-biological interactions. 2007; 169(2):91-99.

29. Fujisawa S, Y Kadoma, Comparative study of the alkyl and peroxy radical scavenging activities of polyphenols. Chemosphere. 2006; 62(1):71-79.

30. Sutherland BA, RM Rahman, I Appleton, Mechanisms of action of green tea catechins, with a focus on ischemia-induced neurodegeneration. The Journal of nutritional biochemistry. 2006; 17(5):291-306.

31. Rice-Evans CA, NJ Miller, G Paganga, Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free radical biology and medicine, 1996; 20(7):933-956.

32. Chung JE, et al., Amplification of antioxidant activity of catechin by polycondensation with acetaldehyde. Biomacromolecules. 2004; 5(1):113-118.

33. Tipeo GL et al., Green tea polyphenols as an antioxidant and anti-inflammatory agent for cardiovascular protection. Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders), 2007; 7(2):135-144.

34. Hall C, E Hall, Human relations in education. 2003:

35. Spencer A et al., Aluminium deposition in liver and kidney following acute intravenous administration of aluminium chloride or citrate in conscious rats. Human & experimental toxicology. 1995; 14(10):787-794.

36. Klein GL et al., Altered glycine and taurine conjugation of bile acids following aluminium administration to rats. Journal of pediatric gastroenterology and nutrition. 1989; 9(3):361-364.

37. HM O et al., Aluminium toxicity in rats: The role of tannic acid as antioxidant. Ass. Univ. Bull. Environ. Res, 2003. 6(2).

38. Morsy GM, KS Abou El-Ala, AA Ali, Studies on fate and toxicity of nanoalumina in male albino rats: oxidative stress in the brain, liver and kidney. Toxicology and industrial health. 2016; 32(2):200-214.

39. Yang ST et al., Bioavailability and preliminary toxicity evaluations of alumina nanoparticles in vivo after oral exposure. Toxicology Research. 2012; 1(1):69-74.

40. Kuriake GC, MG Kurup, Hepatoprotective effect of Spirulina ionon paracetamol induced liver damage in rats. Asian J Exp Biol Sci. 2010; 1(3):614-623.

41. Pari L, A Suresh, Effect of grape (Vitis vinifera L.) leaf extract on alcohol induced oxidative stress in rats. Food and chemical toxicology, 2008; 46(5):1627-1634.

42. Babu A, V Pon, D Liu, Green tea catechins and cardiovascular health: an update. Current medicinal chemistry. 2008; 15(18):1840-1850.

43. Widlansky ME, et al, Acute EGCG supplementation reverses endothelial dysfunction in patients with coronary artery disease. Journal of the American College of Nutrition. 2007; 26(2):95-102.

44. Thangapandiyam S, S Miltonprabu, Epigallocatechin gallate effectively ameliorates fluoride-induced oxidative stress and DNA damage in the liver of rats. Canadian journal of physiology and pharmacology. 2013; 91(7):528-537.

45. Mahieu S et al, Aluminum toxicity. Hematological effects. Toxicology letters. 2000; 111(3):235-242.

46. Wright JS, ER Johnson, GA DiLabio, Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants. Journal of the American Chemical Society. 2001; 123(6):1173-1183.