RESEARCH ARTICLE

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The Effect of Intragastric Gavage of High Dose Green Tea Extract on Serum Status of Magnesium, Calcium, and Zinc

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Abstract

Objective: Green tea (GT) contains polyphenolic flavonoids, different minerals like magnesium, calcium, and zinc, vitamins, amino acids, carbohydrates, proteins, and others. It has a different health benefit. The aim of the present study was to investigate the effect of intragastric gavage of a high dose GT extract on serum biochemical analysis of magnesium, calcium, and zincin juvenile Wistar albino rats. **Methods:** Twelve rats were used in the study and divided in to two equal groups. All the animals in the control group were intragastically gavaged by distilled water and continues for ten days, from day 24 to day 34 of age, while the animals in the study group were intragastically gavaged by GT extract (300mg/kg/day) which continues also for ten days from day 24 to day 34 of age. On day 34 of age, and two hours after the last dose, the rats were anaesthetized and blood collection by cardiac puncture was taken. **Results:** The results showed that the intragastric gavage of a high dose of GT extract caused a non-significant increase in serum magnesium, and calcium levels ($p>0.05$), but a significant increase in zinc serum level was seen($p< 0.05$). **Conclusion:** GT can cause a significant increase in zinc serum level, and this may explain the significant role of GT in the response to different oxidative stress. It is recommended to measure the Zn serum level in rats after a period longer than two hrs from the time of the last dose of intragastric gavage of GT extract.

Keywords: Camellia sinensis- biochemical analysis- magnesium- calcium- zinc

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Introduction

Tea is considered the most consumed beverages in the world next to the water. Millions of people in all over the world consumed every day more than three billion cups of tea. Each year throughout the world, the tea leaves are produced in about 2.5 million tons and about 20% of the products is GT, which is consumed mainly in Asia, United States, Europe, and some parts of North Africa as explained by (Balentine et al., 1997). GT leaves, young leaves, and terminal buds are picked from the Camellia sinensis bushes two times per year during spring and early summer (De Costa et al., 2007). The first step in GT processing is to inactivate the enzyme by dry heating in which most of chlorophyll is preserved with a limited oxidation of all types of catechins (Nakovet al., 2020). While the black tea is a fully fermented tea in which the catechins are oxidized to the arubigins and theaflavins which are the causative factor for the color and the typical taste of this tea. The oxidation of catechins in oolong tea, which is the third type of tea, occurs to a much less extent than those for the black tea, as it was explained by (Khokhar et al., 2002; Yadav et al., 2020).

Chemical composition of GT and its leaves is the same, but different factors can affect the composition of the leaves like the season, climate, age of the plan, and the type horticultural practices (Sabhaponditet al., 2012).

The polyphenolic flavonoids are account for about 40% of the dry weight of GT (Kochmanet al., 2021). The other constituents are minerals (like Mg, Ca, and Zn), different vitamins, amino acids, proteins, sterol, carbohydrates, lipids, pigments, and volatile compounds etc., as it was explained by Lee et al., (2014) and Musial et al., (2020).

GT with its constituents shows a significant antioxidant property and this led to their evaluation in a large number of illnesses to reduce the risk of different diseases "as it was explained by Ikbal et al., (2020) and Yan et al., (2020). It has recently attracted significant attention for its health benefits as anti-cancer, as it was explained by Bimonteet al., (2020) and Khaleel et al., (2020), anti-diabetic (Maruyamaet al., 2009), anti-microbial (Songet al., 2005; Kimet al., 2008) and anti-hypertensive activities (Luoet al., 2020). In addition, it promotes bone formation by increasing osteoblast activity and inhibiting

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osteoblast formation (Shen et al., 2009), decreasing obesity (Ohishiet al., 2021), and act as a neuroprotective (Maoet al., 2019).In general, the use of GT extracts has been well tolerated in human clinical trials. Others found that a higher consumption of GT can cause liver or pancreas damage (Takabayashiet al., 2004), induced goiter in rats (Sakamoto et al., 2001), serum iron reduction by 26%, and significantly increased the serum zinc by 24%"as it was explained by Hamdaoui et al., (1997) and Nelson and Poulter (2004), and increases the manganese absorption (Record et al., 1996). GT has been considered as a medicine since ancient times. Its extracts are also sold as supplements with different diets.

The benefits of the GT and its different constituents are continuously under investigation in recent years. The aim of the present research was to study the effect of intragastric gavage of GT extract (300 mg/kg/day) on serum biochemical analysis of magnesium, calcium and zinc in Wistar albino rats.

Materials and Methods

Rats and housing

The number of the animals used in the research was 12 male Wistar rats, aged 24 days, and weighing 35-45 grams. The source of the animals was from College of Pharmacy, University of Karbala, and Laboratory Animal Research Center, Iraq. All rats were kept in a standard room condition. The project was approved by the Institutional Animal Care and Use Committee/USM and Hilla University College, Babylon/ Iraq.

Preparation of green tea extract

GT extract was prepared by the addition of 2.5 grams of GT, Alwazah, Sri Lanka to a boiling water (50 ml) and steeped for twenty minutes until cooled to room temperature then after that filtered.GT extract was daily prepared (Rahmanet al., 2019), and used in an oral dose of 300mg/kg/day as it was explained by (Arteel et al., 2002; Abdel-Raheem et al., 2010).

Design of the experiment

Rats were divided randomly into two equal groups. Table 1 shows the design of the study.

Rat's anesthesia, blood collection and euthanization

On day 34 of age, and two hours after the last dose, rats were anaesthetized with intra muscular injection of 5 mg xylazine and 100 mg ketamine (Parasuraman et al., 2010). For each animal, a new needle and syringe was used. Check for anesthesia was done by lack of spontaneous movement and response to different stimuli. The blood collection by cardiac puncture was taken from the ventricle of the heart, and very slowly to avoid the collapsing of the heart. The rat was placed on its back. The heart is present nearly one centimeter above the level of the lowest rib. The syringe was hold and the needle was then inserted between the two ribs and watched carefully for the drop of blood to ascertain that it was inside the heart (Figure 1). The rats were immediately euthanized post blood collection. Bilateral thoracotomy with scissor was done to insure death. The whole blood was collected in a test tube and allowed for clotting. Then all the samples were centrifuged in a refrigerated centrifuge at 4°C for 10 minutes at 3,000 RCF. The serum was placed on ice for detection, but when the serum was not analyzed on the same day of blood collection, the serum was stored at –80°C (Abdelhalim et al., 2020).

Biochemical analysis

A. Serum magnesium level

Serum magnesium was quantified by a photometric assay procedure and following the protocol of Magnesium Assay Kit (Germany), by using a fully automatic Abbott Arcchitect c4000 clinical chemistry analyzer.

Principles of procedure: With isocitrate dehydrogenase, the magnesium present in the sample serves as a cofactor in an enzymatic reaction. Rate of the increase in absorption at 340 nm due to the production of NADPH is proportional to the concentration of magnesium, Isocitrate dehydrogenase concentration=2.2 U/ml, D-isocitrate acid concentration=1.47 mg/ml, NADP concentration=8.37 mg/ml.

$$\text{D-isocitric acid} + \text{NADP} \xrightarrow[\text{Mg}^{2+}]{\text{Isocitrate dehydrogenase}} \text{2-oxoglutarate} + CO_2 + \text{NADPH}$$

Serum magnesium concentration was assayed by routine laboratory method using Architect system autoanalyzer which calculate the magnesium concentration as follows:

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} = \text{mg/dL magnesium}$$

B. Serum calcium level

Serum calcium was quantified according to Arsenazo III photometric method and following the protocol of Calcium Assay Kit (Germany), by using a fully automatic Abbott Arcchitect c4000 clinical chemistry analyzer.

Principles of procedure: In an acid solution, the arsenazo-III dye can react with calcium to generate a purple- blue complex. At 660 nm, the color that developed was measured, and which is proportional to the sample's calcium concentration. Arsenazolll dye concentration=0.94 mmol/l.

$$\text{Arsenazo III} + \text{Calcium} \xrightarrow{\text{pH 6.5}} \text{Arsenazo III-Calcium complex}$$

Serum calcium concentration was assayed by routine laboratory method using Architect system autoanalyzer which calculate the calcium concentration as follows:

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} = \text{mg/dL Calcium}$$

C. Serum zinc level

Serum zinc was quantified by colorimetric assay procedure using Dirui Auto-Chemistry Analyzer, and following the protocol of Zinc Colorimetric Assay Kit, DIRUI (China).

Principles of procedure:Zinc forms a purple complex with 2-(5-nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamine) phenol disodium salt (Synonym Nitro-PAPS) in an alkaline media. It reached absorption peak at 570nm.The amount of Zn present in the sample is proportional to the intensity of the complex produced. Nitro-PAPS concentration=0.4 mmol/l.

$$\text{Zinc} + \text{Nitro - PAPS} \xrightarrow[\text{Medium}]{\text{Alkaline}} \text{Purple Coloured complex}$$

After that we referred to the Dirui system for result calculation. A supplementary calibrator was used. 2-point calibration was conducted with purified water and calibrator according to the instruction of calibrator. The calibration curve was formed after tests.

$$\text{Sample concentration } (\mu g/dl) = \frac{A_{\text{Sample tube}}}{A_{\text{Calibration tube}}} \times \text{Calibrator Concentration } (\mu g/dl)$$

Data analysis

All results were given as mean ± standard deviation. Using Statistical Package for the Social Science; SPSS Inc., version 24, the statistical calculations was done. To analyze the significant differences on biochemical data, the non-parametric statistical Kruskal-Wallis test was used. P-Value less than or equal to 0.05 was considered statistically significant.

Results

The present study revealed that mean serum levels for rat's magnesium, calcium, and Zinc in the control group were 2.258±0.199 mg/dl, 9.416±0.369 mg/dl, and 95.51±1.81 µg/dl respectively. While the mean serum levels for rat's magnesium, calcium, and zinc in the study group were 2.45±0.156,9.692±0.593, and 101.71±2.55 respectively.

Statistical analysis showed that the intragastric gavage of 300 mg/kg b.w of GT extract can cause a non-significant increase in the serum magnesium and calcium levels ($p>0.05$), but the Zinc serum level showed a significant increase ($p< 0.05$) as seen in Table 2.

Discussion

The present study revealed that mean serum levels for rat's magnesium, calcium, and Zinc in the control group were 2.258±0.199 mg/dl, 9.416±0.369 mg/dl, and 95.51±1.81 µg/dl respectively. While the mean serum levels for rat's magnesium, calcium, and zinc in the study group were 2.45±0.156,9.692±0.593, and 101.71±2.55 respectively.Previous studies found that the normal serum concentration of magnesium and calcium in male rats was 2.26 ± 0.12 mg/dl (Parvizi et al., 2014) and 9.9±0.2mg/dl (Bover et al., 1994) respectively. Ahmed study (2015) found that the normal serum level of zinc in control rats was 115.08 ±2.04 ug/dl.

Hamdaoui et al., (1997) research found that 50 and 100 g/l of GT decoction for six weeks can increased

Table 1. Experimental Design of the Study

Group	Description
Control Group	On day 24 of age, all the animals were intragastically gavaged by distilled water from day 24 to day 34 of age.
Study Group	On day 24 of age, all the animals were intragastically gavaged by GT extract (Figure 2A) from day 24 to day 34 of age.

Table 2. Biochemical Analysis for Rat's Serum in Different Studied Groups

Groups	Mean± SD with level of significance					
	Magnesium mg/dl	P-value	Calcium mg/dl	P-value	Zinc ug/dl	P-value
Control Group	2.258±0.199	0.117	9.416±0.369	0.601	95.51±1.81	0.009*
Study Group	2.45±0.156		9.692±0.593		101.71±2.55	

*Significant p<0.05

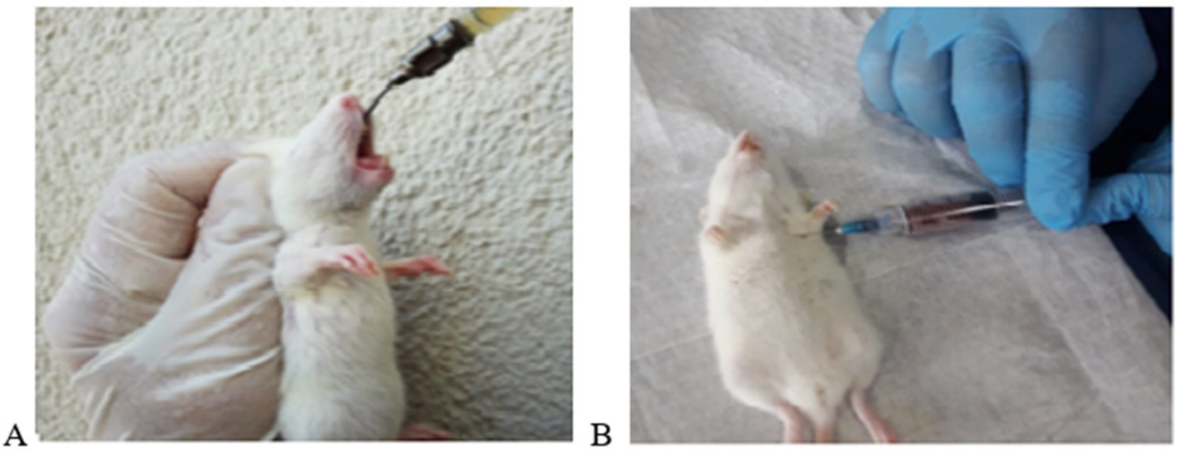

Figure 1. A, Administration of green tea extract by intragastric gavage to the rats; B, Blood collection by cardiac puncture.

serum zinc in rats from 118±4 in control group to 129±5 and 146.6 ug/dl in the 50 and 100 g/l of GT decoction groups respectively. Meki et al., (2009) also found that the serum zinc was increased in GT extract treated rats with rheumatoid arthritis compared with control group. The increased serum zinc level may be due to the improved antioxidant activity as a result of GT treatment. These results disagree with that of Record et al., (1996) study in which they showed no change in hematological level of zinc after the GT extract consumption by rats.

In Suliburska et al., (2012)study, patients were assigned to receive 379 mg of GT extract for three months. The study showed that the magnesium, calcium, and zinc serum levels showed a significant increase. But Al-Othman et al., (2012) study showed a nonsignificant increase in serum calcium level after 1,2, and 3 months intake of GT. Basu et al., (2013) results also noted a non-significant difference at baseline and after 8 weeks in case of serum zinc in GT extract groups versus adults with the metabolic syndrome. Other found that after GT consumption, the GT constituent reached peak level in about 1.5 to 2.5 hrs (Yang et al.,1998).

The different absorption rates of zinc present between populations groups based on the diet type. Zinc is absorbed efficiently if it is administered in aqueous solutions, whereas in case of solid diets, the absorption is less efficient (Krebs, 2000). From the low zinc diet, Istfan et al (1983) study revealed that the zinc absorption was 92%, but from the high zinc diet, it was 81%in young human.

In conclusion, the research was to investigated the effect of intragastric gavage of a high dose GT extract on serum biochemical analysis of magnesium, calcium, and zinc in juvenile Wistar albino rats.

- GT extract (300mg/kg/day) can caused a non-significant increase in serum magnesium and calcium serum levels, but a significant increase in serum zinc level was seen.
- The increased serum zinc level may cause improvement in the antioxidant activity in different diseases as a result of GT treatment.
- The findings of the current study are opposing the findings of the previous studies which reported that the consumption of GT can causes a significant increase or a non-significant change in serum levels of magnesium, calcium, or zinc due to different methodology and approaches that were used, thus the findings of the current research has novelty.
- The researchers recommend measuring the Zn serum level in rats after a period longer than two hrs from the time of the last dose of intragastric gavage of GT extract.

Author Contribution Statement

Dr Ramizu Bin Shaari, as a main supervisor for the PhD study, Dr Mohamad Arif Awang Nawi and Dr Ali Mihsen Hussein Al-Yassiri as a co-advisors, Ameera Kamal Khaleel as a PhD student in USM, contributed to conception, design, interpretation, and writing the manuscript. All authors have read and agreed to the published version of the manuscript.

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Ethical considerations

This study was approved by the Institutional Animal Care and Use Committee/USM and Hilla University College, Babylon/ Iraq.

Availability of Data

The data sets generated and/or analyzed during the study are not publicly available.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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