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Studying the effective dose of polyphenols extracted from green tea in ameliorating the deleterious effect of iron overload in female rats

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Summary

experiment aimed at studying the effective dose of total polyphenol extracted from green tea in ameliorating the deleterious effect of iron overload in rats, where forty eight adult female rats were randomly divided into eight equal groups (each of six) and injected intraperitoneally (i.p.) with iron dextran (100mg/kg B.W each 72 hour and treated orally for one month successive increasing dose of GTPs daily (75, 100, 125, 150, 175, 200, and 225 mg/kg B.W.). Measurement of serum iron, albumin, reduced glutathione, peroxynitrate concentration and catalase activity was used as a guide for the detection of the effective dose of total polyphenol that ameliorating the deleterious effect of iron overload. According to this study, the maximum effective dose of total poly phenol extracted from green tea that ameliorating the deleterious effect of iron overload in female rats were found to be equal to 200mg/kg B.W.due to results showed that maximal reduction in serum Iron concentration in groups (T5, T6 and T7) which received total polyphenol orally (175, 200 and 225) mg/kg respectively with mean value equal to (345.87±12.48, 331.73±4.09and 327.27±4.99) respectively as compared with control group (308.68±12.03) after 30 days of treatment, also the best and successive increments in antioxidant enzyme and reduction in free radical status shown in doses of (200 and 225) mg/kg polyphenol, and no significant statistic differences between this groups in all above parameter.

تحديد الجرعة المؤثرة للفينولات المتعددة المستخلصة من الشاي الاخضر التي تخفف التاثير الضار لفرط الحديد في أناث الجرذان

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الخلاصة

هدفت هذه التجربة تحديد الجرعة المؤثرة للبوليفينولات المتعددة المستخلصة من الشاي الأخضر في تخفيف الأثر الضار لفرط الحديد في اناث الجرذان ، حيث قسمت ثمانية واربعون من اناث الجرذان عشوائياً الى ثمانية مجاميع متساوية (ستة لكل مجموعة) وحقنت بالتجويف الخلبي (100ملغ / كغ من وزن الجسم) من ديكسترين الحديد كل 72 ساعة واستمرت المعالجه يومياً لمدة شهر بجرع متزايده من الفينولات المتعدده وكالاتي : 75، 100، 125، 150، 175، 200 ، و 225 ملغ / كغ من وزن الجسم. تم قياس تركيز الحديد ، الالبومين، الكلوتاثايون المختزل ، البيروكسي نايتريت في مصل الدم ونشاط انزيم الكتليز كدليل لتحديد الجرعة المؤثره من مادة البوليفينولات المتعدده والتي تخفيف الأثر الضار لفرط الحديد. وبلغت الجرعة المؤثرة من للبوليفينولات المتعددة مساوية ل(200 ملغ / كغم من وزن الجسم) .الان النتائج اظهرت اعلا تخفيف لتركيز حديد مصل الدم حدث في المجاميع(175, 200 و 225) ملغم/كغم بعد مرور 30 يوم من العلاج , كذلك افضل زيادة في تركيز انزمات موانع الاكسدة لوحظ في التراكيز (200 و 225) ملغم/كغم فينولات متعددة وعدم وجود فرق معنوي ($P>0.05$) بين مجموعة 200 و225 ملغم /كغم فينولات متعددة لهذة المعايير.

Introduction

Iron overload is common among transfusion-dependent patients who do not receive effective iron chelation therapy. Each unit of blood contains 200mg of heme iron, more than 100 times the iron, normally absorbed from the diet on a given day. Signs of iron overload

can usually be seen after 10-12 transfusions. (1) The common clinical complaints in iron overload include lethargy, weight loss, change in skin color, loss of libido, abdominal pain and joint pain (2).

States of iron overload can result from several disorders, some inherited like, hemochromatosis and some acquired like, iron-loading anemia (thalassemia major), dietary iron overload, parenteral iron overload and chronic liver disease (hepatitis C, alcoholic liver disease) (3). Accordingly, the potential hazards of enhanced oxidative stress in animal receiving iron therapy as well as the effect of excess iron on various risk factors for cardiovascular disease and the expected DNA damage deserved further appercial.

Tea is the most popular drink after water, consumed everyday by millions of people around the world. Tea is generally consumed in the forms of green, oolong, and black tea. It is the most popular non-alcoholic beverage in the world (4).

The green tea contains 30 to 42% polyphenols on the dry weight basis and a cup of green tea contains about 300 to 400 mg of polyphenols The natural polyphenols in green tea include (-)-epigallocatechin-3- gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3- gallate (ECG), and epicatechin (EC) (Park et al., 2010). The public awareness of the health giving properties of tea has increased recently.The majority of beneficial effects of tea have been attributed to primary polyphenolic constituents of green tea. Strong antioxidant potential of these polyphenols is thought to mediate most of the beneficial effects of tea. Green tea possesses significant antioxidant, anti-inflammatory (5), antimicrobial (6), antihypertensive (7) and thermogenic (8) properties. Recent human studies suggest that green tea may contribute to a reduction in the risk of cardiovascular disease and some forms of cancer, as well as to the promotion of oral health. The health benefits in cancer (9), arthritis (10), diabetes (11) and obesity (12). The results of these investigations have indicated that green tea catechins have a great potential to be developed as therapeutic agents. Chelating agent may protect against excessive iron catalyzed oxidative damage (13). Currently, there are two chelating agents available for the management the iron overload: desferioxamine, which need to be administered parenterally, and oral deferipron, which possessed less advantage (14). Both agents have been shown to have antioxidant and cytoprotective effects (15). However, many side effect had been detected following such treatment including bloody urine , loss of hearing , cataract ,liver and kidney damage (16).Accordingly, an attempt to substitute such agents with natural antioxidant like green tea in ameliorating the deleterious effect of iron overload in rats was the aim of this study.

Materials and Methods

Extraction of polyphenolic compounts from green tea leaves was performed according to Markham (17) in two steps as following:

1. 200mg of of green tea leaves were crushed with 400mL of mixture methanol 95% and distill water (9:1), mixed for 18h in magnetic stirrer at room temprature, then filtered under vacuum using whatman No. (1).
2. The filtrate residues from step one was mixed again with 200mL of mixture methanol 95% and distill water (1:1) for 18h in magnetic stirrer at room temprature and the filtered was collected as described in step one. Then the filtrate collected in step 1 and 2 was evaporated in incubator (42oC) to reach one- third of original volumes .

The concentrated extract was separated from low organic materials by addition of chloroform 20:100 (extract: chloroform) in separatory funnel, then the maxture was left for one hour to seperate in to two layers: lower layer contain Chloroform and upper layer contain(total polyphenol). The upper layer was separated with chloroform 10:100 (extract: chloroform), from the upper layer, total polyphenol was collected and dried at 40 °C incubator.

Forty eight adult female rats were randomly divided (each of six) and were handled as follows for one month:

Group C: Rats of this group were injected Intraperitoneally (I.P.) with saline solution twice/week plus normal saline orally each day served as control.
Groups T1, T2, T3, T4,T5,T6,T7 and T8 were injected I.P. with (100mg/kg B.W.) of iron dextran every 72 hour and given orally 75, 100, 125, 150, 175, 200 and 225 mg/kg B.W. of crude polyphenols extracted from green tea daily respectively. Fasting Blood sample were collected on day 15 and 30 of the of experiment for measuring the followings parameters: A)Serum iron concentration. b) Serum Albumin enzymatically measured using enzymatic assay kit (Linear Chemicals, Barcelona; Spain). C) Reduced glutathione concentration according to Burtis and Ashood 18. D) Catalase activity (19).E) Serum peroxynitrate concentration (20).

Statistical analysis of data was performed on the basis of Two-Way Analysis of Variance (ANOVA) using a significant level of ($P<0.05$). Specific group differences were determined using least significant differences (LSD) as described.

Results

Serum iron concentration (mg/dL) : Upon oral administration of 75,100,125,150,175 and 200 mg/kg B.W. of polyphenols to IO rats, a significant increase ($P<0.05$) in the SI concentration (table 4-5 and figure 4-5) was observed in groups that received the above doses as compared to the control group on days 15. Significant differences ($P<0.05$) between some of these groups were also exist. At the same time, oral gavages of polyphenol causing significant decrease ($P<0.05$) in SI concentration in iron overloaded rats at the doses of 150, 175 and 200 in groups T4, T5 and T6 respectively compared to the values in groups T1, T2 and T3 after 15 day. While polyphenol at the doses of 225mg/kg B.W. in group T7 caused normalization of the value (333.50±2.18) near that of the control group (305.14±23.79).

While oral gavages of polyphenol for 30 days caused a significant decrease ($P<0.05$) in SI concentration in groups T5, T6 and T7 comparing to groups T1, T2, T3 and T4 respectively. Besides, no significant difference ($P>0.05$) in the value of this parameter was observed in groups T5 (345.87±12.48), T6 (331.73±4.09) and T7 (327.27±4.99) after polyphenol gavages when compared to the control group (308.68±12.03).

Table 1: Effect of different doses of total polyphenol extracted from green tea on serum iron concentration (mg/dL) of iron overload treated rats.

Days / Groups	15 days	30 days
C	305.14±23.79Aa	308.68±12.03Aa
T1	500.85±42.22Db	438.65±1.20BDa
T2	476.85±21.01BDa	436.58±8.40BDa
T3	472.49±16.82Bb	413.79±16.07BCa
T4	428.73±9.24Cb	383.05±29.90CDa
T5	366.07±19.18Da	345.87±12.48ADa
T6	359.24±8.97Da	331.73±4.09Aa
T7	333.50±2.18ADa	327.27±4.99Aa

-LSD=42.515. - C= control. -T1= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 75 mg/kg. -T2= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 100 mg/kg. -T3=Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 125 mg/kg. -T4= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 150 mg/kg. -T5= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 175 mg/kg. -T6= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 200 mg/kg. -T7= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 225mg/kg. - Capital letters denote differences between groups, P<0.05 vs. control. - Small letters denote differences within group.

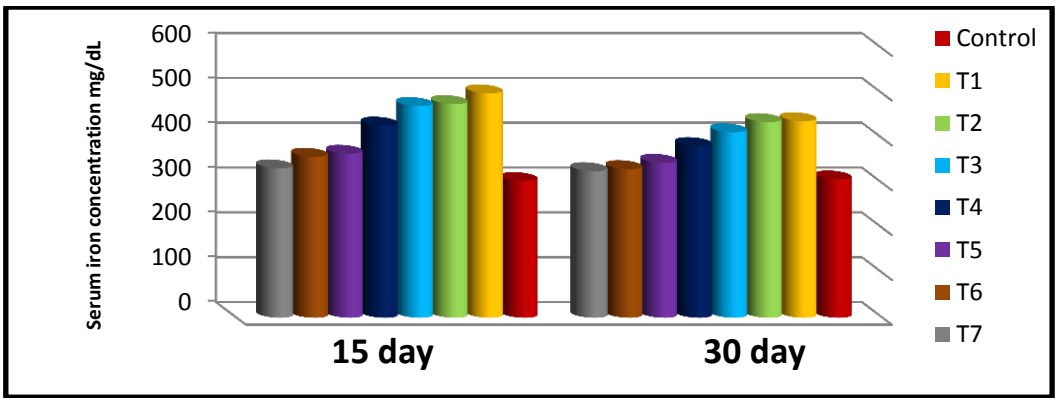

Figure 1: Effect of different doses of total polyphenol extracted from green tea for 15 and 30 days on serum iron concentration (mg/dL) of iron overload treated rats.

Serum Albumin concentration (g/L):

Table (2) and Figure (2) illustrate the mean value of serum albumin concentration in IO treated rats received different doses of polyphenol for 30 days.

The result showed that different doses of polyphenol caused dose depended significant ($P<0.05$) increase in serum albumin concentration in groups T4, T5, T6 and T7 comparing to T1 group at day 15 of the experiment. While non significant elevation ($P>0.05$) in this parameters was observed on day 30 in all treated groups comparing to the control. Highest significant elevation ($P<0.05$) in serum albumin was observed in group T7 (**24.41±1.35)** which received 225 mg/kg B.W. on day 15. With exception to groups T7 at day 15, within the time no significant differences ($P>0.05$) was observed in the remaining treated groups along the experimental period.

Table 2: Effect of different doses of total polyphenol extracted from Green tea on Serum Albumin concentration (g/L) of iron overload treated rats.

Days / Groups	15 days	30 days
C	22.46±1.53CDEb	19.81±0.55 Aa
T1	18.53±1.23Aa	19.31±1.47 Aa
T2	20.01±0.38ABa	19.46±0.47 Aa
T3	20.41±1.18ABCa	19.95±0.97 Aa
T4	20.89±0.49BCa	21.17±0.33 Aa
T5	21.79±0.50BCDa	21.02±0.28 Aa
T6	23.17±0.09DEa	21.22±0.15 Aa
T7	24.41±1.35Db	21.31±0.73Aa

LSD=2.062. - C= control. -T1= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 75 mg/kg. -T2= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 100 mg/kg. -T3=Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 125 mg/kg. -T4= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 150 mg/kg. -T5= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 175 mg/kg. -T6= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 200 mg/kg. -T7= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 225mg/kg. - Capital letters denote differences between groups, P<0.05 vs. control. - Small letters denote differences within group.

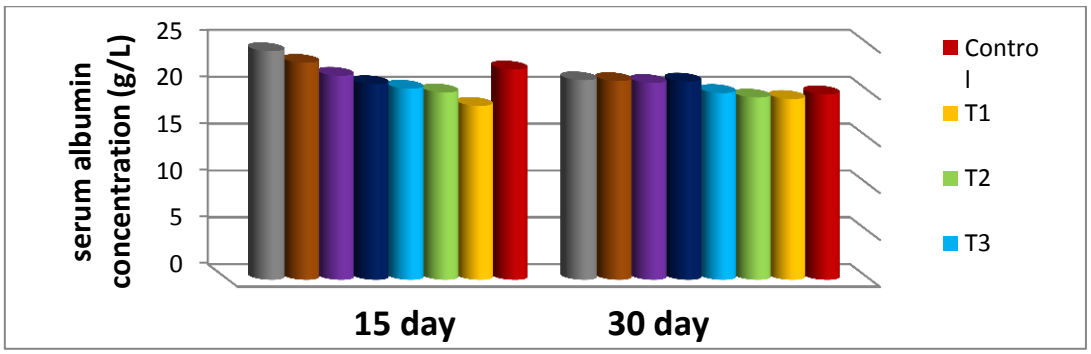

Figure 2: Effect of different doses of total polyphenol extracted from green tea for 15 and 30 days on serum albumin concentration (g/L) of iron overload treated rats.

Serum reduced glutathione (GSH) concentration (µmol/l):

Depending on the results clarified in table (3) and figure (3), with exception to groups T6 and T7, oral gavages of polyphenol extracted from green tea at doses of 175, 100, 125 and 150 mg/kg B.W. failed to elevated the depressed reduced GSH concentration in iron overloaded (IO) animals in groups T1, T2, T3 and T4 when compared to the control group on day 15. While oral gavages of poly phenol at doses of 200 and 225 mg/kg B.W. in groups T6 and T7 respectively caused significant increase ($P<0.05$) in reduced GSH concentration compared to groups T1, T2, T3 and T4. Besides, continuous gavages of poly phenol to IO rats for 30 days caused significant elevation ($P<0.05$) in this parameter in groups T4, T5, T6 and T7 comparing to groups T1 and T2. Significant increase ($P<0.05$) was observed in group T7 (**50.00±3.53**) comparing to control group (**40.00±2.85).**

Table 3: Effect of different doses of total polyphenol extracted from Green tea on Serum reduced glutathione (GSH) concentration(µmol/l) of iron overload treated rats.

Days / Groups	15 days	30 days
C	373.87±23.71Aa	382.58±14.41Aa
T1	255.67±18.42Ba	262.84±11.13Ba
T2	259.20±14.69Ba	291.82±13.19BCa
T3	263.96±19.22BCa	316.67±7.39CDb
T4	272.22±18.99BCDa	321.68±14.55CDb
T5	287.60±9.56BCDa	333.77±10.58Db
T6	296.82±15.29CDa	349.76±5.80ACDb
T7	300.17±10.91Da	358.80±12.07ADb

-LSD=7.111. - C= control. -T1= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 75 mg/kg. - T2= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 100 mg/kg. -T3=Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 125 mg/kg. -T4= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 150 mg/kg. -T5= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 175 mg/kg. -T6= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 200 mg/kg. -T7= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 225mg/kg. - Capital letters denote differences between groups, P<0.05 vs. control. - Small letters denote differences within group.

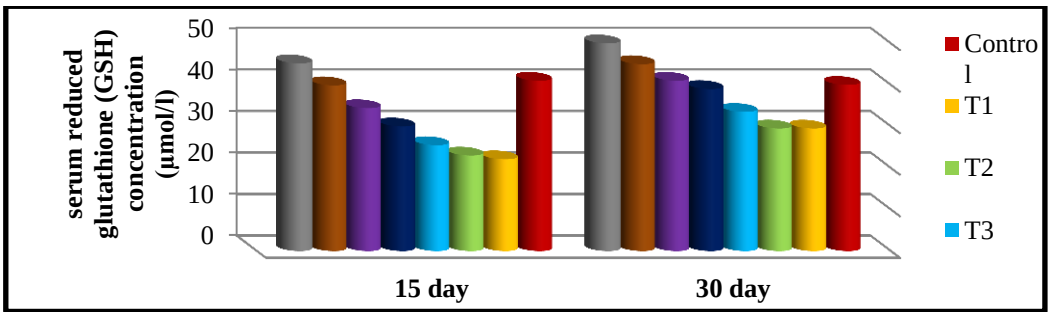

Figure 3: Effect of different doses of total polyphenol extracted from green tea for 15 and 30 days on serum reduced glutathione (GSH) concentration (µmol/l) of iron overload treated rats.

Serum Catalase Activity (kU/l):

The mean value of serum catalase activity in different treated and control groups is clarified in table (4) and figure (4). Meanwhile, successive increasing in doses of polyphenol administered to IO rats especially at groups T6 and T7 which received 200 and 225 mg/kg B.W. of poly phenol respectively, caused a significant increase ($P<0.05$) in serum catalase activity on day 15 comparing to groups T1 and T2 which received 75 and 100 mg/kg B.W. of poly phenol respectively. Continues gavages of polyphenol at doses of 200 and 225 mg/kg B.W. for 30 days caused correction of serum catalase activity near that of control group with mean value of (349.76±5.80) and (358.80±12.07) for groups T6 and T7 respectively.

Within the time, significant differences ($P<0.05$) were observed in the activity of serum catalase in groups (T3, T4, T5, T6 and T7) when compared on days 15 and 30 of the experiment.

Table 4: Effect of different doses of total polyphenol extracted from Green tea on Serum Catalase Activity (kU/l) of iron overload treated rats.

Days / Groups	15 days	30 days
C	41.00±2.03Aa	40.00±2.85Aa
T1	22.10±0.95Ba	29.50±3.00Bb
T2	23.00±1.19Ba	29.50±5.30Ba
T3	25.50±1.45Ba	33.50±3.02ABb
T4	30.00±3.25BCa	39.00±4.22ACb
T5	34.40±1.79CDa	41.00±3.58ACa
T6	39.9±1.80ADEa	45.00±3.53ACDa
T7	45.2±2.87AEa	50.00±3.53Da

LSD=34.22. - C= control. -T1= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 75 mg/kg. -T2= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 100 mg/kg. -T3=Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 125 mg/kg. -T4= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 150 mg/kg. -T5= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 175 mg/kg. -T6= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 200 mg/kg. -T7= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 225mg/kg. - Capital letters denote differences between groups, P<0.05 vs. control. - Small letters denote differences within group.

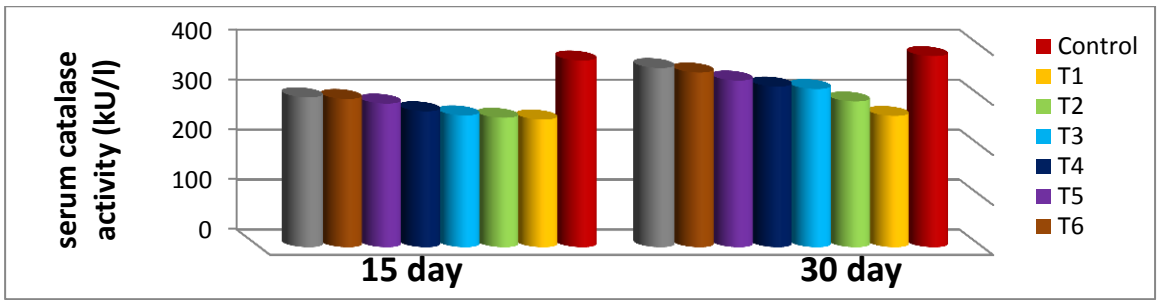

Figure 4: Effect of different doses of total polyphenol extracted from green tea for 15 and 30 days on serum catalase activity (kU/l) of iron overload treated rats.

4.2.5. Serum Peroxy nitrate radical concentration (M/L).

The concentration of serum peroxynitrite radical concentration in the iron overloaded rats after daily oral administration of successive increasing polyphenol doses for month is shown in table (5) and Figure (5). Significant decrease ($P<0.05$) in serum peroxynitrite radical concentration was observed in groups T3 to T7 which received 125 to 225 mg/kg B.W. of polyphenol in addition to 100mg/kg B.W. of iron dextran comparing to groups T1 and T2 which received 75 and 100 mg/kg B.W. of polyphenol on day 15. The values in groups T6 (32.65±4.19) and T7 (30.45±3.73) became close to that of the control group (24.81±1.51).

Continuous gavages of polyphenol for 30 days caused a significant depression($P<0.05$) in this parameter in most treated groups (T1, T2 and T3) compared to day 15. No significant differences ($P<0.05$) were observed in these values in groups T6 with the mean value of (29.90±0.55) and T7 (29.01±1.61) compared to the control group (23.17±2.85).

Table 5: Effect of different doses of total polyphenol Extracted from Green tea on Serum Peroxy nitrate radical Concentration (M/L) of iron overload treated rats.

Days / Groups	15	30
C	24.81±1.51Aa	23.17±2.85Aa
T1	105.45±2.33Bb	51.81±8.42Ba
T2	102.30±9.81Bb	47.51±4.23BCa
T3	89.60±8.76Cb	43.40±4.62BCa
T4	47.04±8.74Da	42.61±4.83BCa
T5	38.81±2.15DEa	36.21±3.99CDa
T6	32.65±4.19AEa	29.90±0.55ADa
T7	30.45±3.73AEa	29.01±1.61ADa

-LSD=12.59. - C= control. -T1= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 75 mg/kg. -T2= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 100 mg/kg. -T3=Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 125 mg/kg. -T4= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 150 mg/kg. -T5= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 175 mg/kg. -T6= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 200 mg/kg. -T7= Animal Injected iron dextran 100 mg/kg B.W + Polyphenol 225mg/kg. - Capital letters denote differences between groups, P<0.05 vs. control. - Small letters denote differences within group.

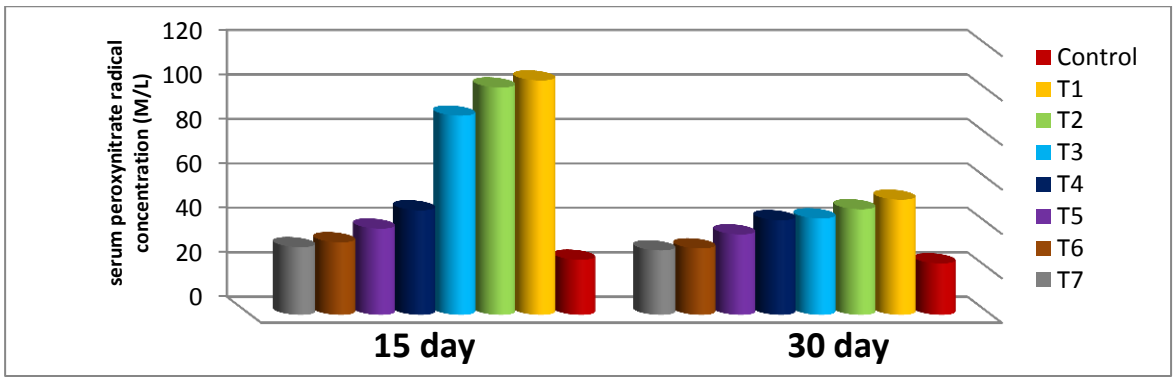

Figure 5: Effect of different doses of total polyphenol extracted from green tea for 15 and 30 days on serum peroxynitrite radical concentration (M/L) of iron overload treated rats.

Discussion

The data obtained from the results demonstrated that oral administration of total polyphenol extracted from green tea led to graded reduction response in the serum iron concentration. Changes were observed from the first blood drawn after 15 days of starting of experiment. This may be attributed largely to the antioxidant activity of green tea polyphenols (GTPS). One of the postulated mechanisms was chelation of prooxidant metals such as iron by GTPS. This mechanism may impair the utilization of dietary iron (22) coincide with its depression. Polyphenolic compound of green tea contain a catechol- like structure, which has both a potent free radical scavenger and iron chelator activities (23).

Albumin represents the major and predominant antioxidant in plasma (24). It represents 70% of the free radical-trapping activity of serum (25).Glutathione (GSH) is the major and the most abundant intracellular antioxidant that could be reduced dramatically in response to oxidative stress (26). While catalase (CAT) is a heme protein which protects the tissues from highly reactive hydroxyl radicals (27).The result of the present study pointed to the fact that GTPs at doses of 75, 100 and 125 mg/kg B.W. failed to counteract the prooxidative effect of iron overload induced by 100mg/kg B.W. of iron dextran, accompanied by significant decrease in serum catalase activity and serum concentration of GSH and albumin with elevation in serum peroxynitrite concentration comparing to animals received high doses of GTPs (200 and 225 mg/kg B.W.). These results have been documented by many authors, where iron injection to the rats resulted in a significant decrease in the level of endogenous antioxidant determinate like reduced glutathione, catalase (28) and albumin (29) due to IO status. Moreover, glutathione reductase enzyme which converts oxidized glutathione (GSSG) to reduced GSH is also found to be significantly reduced in beta-thalassemia characterized by iron overload**(**30).

Excess free iron is suggested to increase production of superoxide and hydroxyl radical via the fenton reaction and oxidative stress development (28), correlated with a drastic depletion of cysteine from the glutathione pool, and a progressive decline of serum selenium that is correlated with decrease serum glutathione (31)**.** The observed decrease in the serum albumin concentration after iron administration indicated oxidation of serum albumin suggested that albumin has protective mechanism in response to iron induce ROS generation (32). Besides such observation could be ascribed to changes in protein and free amino acid metabolism and their synthesis in the injured liver cells and /or increased protein degradation (27) due to iron overload.

The loss of CAT activity impairs cellular protection against the endogenously generated H2O2, and increases iron mediated injury to the thalassemic erythrocyte membranes (33).The mechanism(s) underlying the decreased CAT activity was proposed by different investigators who suggested that nicotinamide adenine dinucleotide phosphatase (NADPH) is important in maintaining CAT activity, and that the loss of NADPH due to IO would adversely affect erythrocyte enzyme activity (34).

Peroxynitrite radical concentration was significantly increased in groups T1,T2 and T3 where IO toxicity was predominant. Free iron catalyzes the formation of highly reactive compounds such as nitric oxide (NO), hydroxyl radical, hydrogen peroxide, and peroxynitrite (35). Peroxynitrite, when generated in excess, may damage cells by oxidizing or nitrating cellular components and oxidation of cofactors of antioxidant enzyme either by direct or free-radical-dependent mechanisms lead to antioxidant depletion of superoxide dismutase, glutathione reductase, and glutathione (36). This fact is observed in our result, where a significant elevation in peroxynitrite is coincided with subsequent depletion in glutathione after IO. On the other hand, gavages of highest doses of GTPs in the present study to IO rats restore the antioxidant stats of the animal with significant elevation in serum GSH, albumin concentration and catalase activity and significant depression in peroxynitrite concentration.

It has been documented that green tea could act as potent exogenous antioxidant as well as iron chelating agents, leading to graded increase in serum GSH concentration (37) and catalase activity (38). The antioxidant properties of green tea are attributed to the presence of polyphenols, which contain a catechol- like structure, which is both a potent free radical scavenger and iron chelator activities (39). In addition, after the oxidation of GTPs like catechins, due to their reaction with free radicals, a dimerized product is formed, which has been shown to have increased superoxide scavenging and iron-chelating potential (40)**.**Accordingly lowering iron level by GTPs will lead to increase in antioxidant/oxidant status and elevation of albumin and other measured antioxidant.

Regarding the concentration of peroxynitrite radical which was decreased gradually with increasing the doses of GTPs, indicating their antioxidant effect. Flavonoids, such as (-)-epicatechin, which occurs in green tea as monomer or in the form of oligomers, can contribute to cellular defense against peroxynitrite (41). As well, there is substantial evidence that the catechins' anti-inflammatory effects may be due, in part, to their scavenging of NO and reduction of NO synthase (NOS) activity (42). Depending on the results shown in tables 1, 2, 3, 4 and 5, a maximal decrease in serum iron concentration was observed in groups (T5, T6 and T7) which received total polyphenol orally at doses of 175, 200, 225 mg/kg respectively, also the best and successive increases in antioxidant enzyme and reduction in free radical status was shown in doses of (200 and 225) mg/kg polyphenol, and there was no significant statistic differences between these groups in all studied parameters.

Accordingly, the maximum effective dose of crude polyphenols extracted from green tea that ameliorates deleterious effects of iron overloaded was equal to 200mg/kg B.W.

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