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Study the Effects of Different Concentrations of Aqueous Green Tea Extract on Rats' Livers: *In vivo* Study

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Abstract:

Green tea (*Camellia sinensis*) has received a considerable attention in the health benefit owing to its anti-oxidant properties both *in vivo* and *in vitro* against drug-induced toxicities. However, there are many scientific evidences suggest that green tea or its catechins at higher concentration produced pro-oxidative effect *in vivo* and *in vitro*.

This study was designed to assess whether or not the aqueous green tea extract AGTE) used in various concentrations may have pro-oxidant effect in rats liver.

Aqueous extract of green tea was freshly prepared daily by soaking the required amount of green tea leaves for 10 min in 100 ml distilled water at 90°C to obtain 2.5 %, 5 % and 10 % concentrations, respectively of aqueous solutions. Twenty four white albino rats of both sexes, weighing 200-250 g were used in this study and allocated into two groups; group I- Six rats were fed tap water by feeding bottle for 7 days; this group was served as control group; group II- Eighteen rats were fed different concentrations of AGTE by feeding bottle as only source of drinking fluid, this group was served to demonstrate the possible pro-oxidant effect of AGTE on the liver of rats as follows: A-Six rats were fed 2.5% AGTE, B-Six rats were fed 5 % AGTE, C-Six rats were fed 10 % AGTE.

The parameters of oxidative stress, malondialdehyde (MDA) and reduced glutathione (GSH) were evaluated in the liver tissue homogenate. Serum activities of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphates (ALP) were assessed .

Analysis of data revealed that, rats orally administered AGTE in concentrations (5 % and 10 %) produced significant reduction in the content of MDA in liver tissue homogenate (62.42 % and 12.4 % respectively) compared to control animals, while a non significant difference was observed in animals administered 2.5 % AGTE compared to control group. Meanwhile, 10 % AGTE produced significant decrease in levels of GSH in liver tissue homogenate, while non significant differences concerning GSH levels in liver tissue homogenate in rats administered 2.5% and 5% AGTE compared to control animals (59.25%).

Regarding the serum activities of AST, ALT and ALP, rats administrated an oral concentration of 2.5% AGTE for 7 days showed a non-significant difference in the serum activity of ALT compared to control group, while there was a significant decrease in the serum AST activity (64.48%) and ALP (65.4%) compared to control group. Rats administrated an oral concentration of 5% AGTE for 7 days showed a non-significant difference in serum activity of AST, ALT and ALP compared to control group. Administration of an oral concentration of 10 % AGTE for 7 days to rats showed non-significant difference in the serum activity of AST, ALT compared to control group, while there was a significant decrease in the serum ALP activity (58.57 %) compared to the control group.

According to the results obtained from this study, it could be concluded that AGTE possesses an antioxidant- rather than pro-oxidant-effect *in vivo*, manifested by a decrease in the content of MDA in liver tissue homogenate, and in serum activities of AST and ALP.

الخلاصة:

الشاي الاخضر (*Camellia sinensis*) لاقى اهتماما واسعا نظرا لفائدته الصحية المتمثلة بخاصيته كمضاد للأكسدة في كل من داخل الجسم الحي *(in vivo)* وخارج الجسم الحي *(in vitro)* ضد الادوية التي تستحدث تسمما الا ان هناك ادلة علمية كثيره تشير الى ان الشاي الاخضر او احد مكوناته الرئيسية من الفينول المتعدد وهو (EGCG) يسبب تاثيرات تاكسدية في داخل و خارج الجسم الحي بتراكيز مختلفة.

صممت هذه الدراسة لتقييم فيما اذا المستخلص المائي للشاي الأخضر الذي استخدم بتراكيز مختلفة قد يكون له تاثيرات تاكسدية على الكبد في الجرذان.

تم تحضير المستخلص المائي للشاي الاخضر يوميا وذلك بتنقيع الكمية المطلوبة من اوراق الشاي ولمدة عشر دقائق في 100 ملليتر من الماء المقطر عند 90 درجة مئوية للحصول على تراكيز(2.5%، 5%، 10%) على التوالي من المحلول المائي. تم استخدام اربعة وعشرون جرذا من (white albino) من كلا الجنسين و تتراوح اوزانهم من 200-250 جم و تم تقسيمها الى مجموعتين :

المجموعة الاولى: ستة جرذان تم اعطائها ماء من الحنفية كمصدر وحيد للشرب لمدة سبعة ايام، واعتبرت هذه مجموعة سيطرة.

المجموعة الثانية: ثمانية عشر جرذا تم اعطائها تراكيز مختلفة من المستخلص المائي للشاي الاخضر كمصدر وحيد للشرب، وتم استخدام هذه المجموعة لتبيين التاثيرات التاكسدية المتوقعة للتراكيز المختلفة من المستخلص المائي للشاي الاخضر على الكبد في الجرذان بعد ان وزعت كالتالي:

المجموعة (أ): ستة جرذان تم اعطائها تركيز% 2.5 من المستخلص المائي للشاي الاخضر، المجموعة (ب) ستة جرذان تم اعطائها تركيز % 5 من المستخلص المائي للشاي الاخضر، المجموعة (ج) ستة جرذان تم اعطائها تركيز % 10 من المستخلص المائي للشاي الاخضر، كمصدر وحيد للشرب.

تم قياس الادلة الخاصة بظاهرة فرط الاكسده مثل تراكيز ال MDA و ال GSH في انسجة الكبد كما تم قياس مستوي ال AST,ALT and ALP في مصل الجسم.

اظهر تحليل النتائج ان الجرذان التي تم اعطائها تراكيز (% 5 ,% 10) من المستخلص المائي للشاي الاخضر قد شهدت هبوط ملموس في مستوى ال MDA في انسجة الكبد للجرذان، وكانت نسبة الهبوط (% 62.42 ,% 12.4) وعلى التوالي مقارنة بمجموعة السيطرة ولكن لا يوجد تغير في مستوى ال MDA في الجرذان التي تم اعطائها % 2.5 مستخلص المائي للشاي الأخضر مقارنة بمجموعة السيطرة، وفي نفس الوقت فان المستخلص المائي للشاي الاخضر بتركيز (% 10) سبب هبوط ملموس في مستوى ال GSH (% 59.25) مقارنة بمجموعة السيطره بينما ليس هناك تغيير ملموس في مستوى ال GSH في الحيوانات التي اعطييت (% 2.5,% 5) من المستخلص المائي للشاي الأخضر مقارنة بمجموعه السيطرة.

بخصوص فعالية ال AST, ALT and ALP في مصل الجسم، المجموعة الثانية– أ: الجرذان التي اعطيت % 2.5 من المستخلص المائي للشاي الأخضر اظهرت تغيير غير ملموس في فعالية ال ALT مقارنة بمجموعة السيطرة، بينما كان هناك هبوط ملموس في فعالية ال AST (% 64.48) و كذلك مستوى فعالية ال ALP بواقع (% 65.4) مقارنة بمجموعة السيطرة.

المجموعة الثانية–ب: الجرذان التي اعطيت % 5 من المستخلص المائي للشاي الأخضر اظهرت تغيير غير ملموس في مستوى فعالية ال AST, ALT and ALP في مصل الدم مقارنة بمجموعة السيطرة.

المجموعة الثانية–ج: الجرذان التي اعطيت % 10 من المستخلص المائي للشاي الأخضر اظهرت تغيير غير ملموس في مستوى فعالية ال AST,ALT في مصل الدم مقارنة بمجموعة السيطرة، بينما كان هناك هبوط ملموس في مستوى فعالية ال ALP في مصل الدم (% 58.57) مقارنة بمجموعة السيطرة.

Introduction:

Tea plant (*Camellia sinensis*) belongs to the family *theacaceae* which is distributed through tropical and subtropical areas [1]. Tea leaves are immediately heated with rolling after harvest to inactivate the enzyme, polyphenol oxidase, which is capable of oxidizing the tea catechins to oligomeric and polymeric derivatives, e. g., theaflavins and thearubigins [2], Green tea represents approx. mately 20% of world tea consumption. Its extracts now a day are widely used as dietary supplements[3]. Green tea contains poly phenolic compounds particularly the flav onoids, which includes mainly catechins (flavon-3-ol), like (-)- epigallocatechin gallate (EGCG) {an active constituent and make up the highest proportion (85%) and also the component with the highest antioxidant properties [4]}, (-)- epigallocatechin (EGC), (-)- epicatechin gallate (ECG), and (-) – epica techin (EC) [5]. Other compounds obtainable in green tea are the flavonols

(quercetin, kaemp ferol and rutin), phenolic acids like (Gallic acid), theanine, and flavour compounds [6] .Morever, green tea contains volatile oils, vitamins like (B,C,E and folic acid); xanthine bases (caffein and theophylline), minerals and trace elements (Ca,Mg,Cr,Fe,Cu,Zn and Se)[7]. Herbal polyphenolic compounds in the cell can function as an antioxidant and pro-oxidant by scavenging reactive oxygen species via enzymatic and non-enzymatic reactions [8]. The antioxidant potential of green tea polyphenols' is directly related to the combination of aromatic rings and hydroxyl groups that make up their structure, and is a result of binding and neutralization of free radicals by the hydroxyl groups. In addition, green tea polyphenols stimulate the activity of hepatic detoxification enzymes, thereby promoting detoxification of xenobiotic compounds [9]. The catechins ability for chelating redox-active transition metal ions like iron and copper [10] and prevents their participation in Fenton and Haber-Weiss reactions[11,12]. Also contributed to the antiox idant activity of green tea. Additionally, green tea polyphenols may indirectly function as antioxidants through inhibition of the redox-sensitive transcription factors [13] and induction of antioxidant enzymes, such as glutathion S-trans ferases, superoxide dismutases and catalase[14]. However, there is increasing evid ences to suggest the pro-oxidative effect of polyphenols *in vitro*, in which the tea catechins are unstable in cell culture under alkaline conditions where it undergoes oxidative poly merization and auto-oxidation with co-generation of H_2O_2[15,16]. Thus, this study was designed to assess whether or not the aqueous green tea extract AGTE used in various concentrations may have pro-oxidant effect in rats liver.

Materials and Methods:

Aqueous green tea extract (AGTE) was made according to method of Maity *et al.*[17], by soaking for 10 min 2.5, 5 and 10 mg , respectively of green tea leaves in 100 ml of distilled water whose temperature was 90^0C to obtain soluble polyphenols dissolved in aqueous extract. Solution was freshly prepared on daily basis and then filtered to obtain the final 2.5,5 and10%, respectively of AGTE. This solution substituted water as sole source of drinking fluid in the tested animal groups.

Twenty four white albino rats of both sexes, weighing 200-250 g were used in this study. They were obtained from and maintained in the Animal House of the College of Pharmacy, University of Baghdad under controlled temperature. The animals were fed commercial pallets. Additionally, those that are selected as control group were fed tap water and the other groups were fed AGTE as only source of drinking fluid and kept in separated cages (one animal/cage) and allocated as follows:

Group-1: Six rats were fed tap water by feeding bottle for 7 days. The animals were euthanized by anesthetic ether on day 8; this group was served as control group.

Group-2: Eighteen rats were fed different concentrations of AGTE by feeding bottle as only source of drinking fluid and then they were euthanized by anesthetic ether on day 8 to demonstrate the possible pro-oxidant effect of green tea extract (AGTE) on the liver of rats as follows:

A-Six rats were fed 2.5% (AGTE) by feeding bottle.

B-Six rats were fed 5% (AGTE) by feeding bottle.

C-Six rats were fed 10% (AGTE) by feeding bottle.

After the animals have been euthanized by anesthetic ether, livers were quickly excised, homogenated and utilized for the assessment of MDA content [18] and GSH levels [19]. In addition, blood was collected by intra-cardiac puncture, centrifuged at 3000 rpm for 15 min to obtain serum, which was utilized for the estimation of both AST and ALT [20] in addition to ALP activities [21]. Data were expressed as mean ± SEM. Statistical

significance and differences from control were evaluated by t-test, where stastical probability of $p<0.05$ was considered to be significant.

Results:

Table 1and figures 1 and 2 showed that rats administered oral concentration of 2.5 % AGTE for 7days showed a non-significant differences ($p>0.05$) in MDA contents and in GSH levels in liver tissue homogenate compared to control group. **2**Rats administered an oral concentration of 5 % AGTE for 7days showed a significant decrease ($p<0.05$) in MDA content as compared to control group (62.42 %) and a non significant difference in the level of GSH in liver tissue homogenate compared to the control group ($p >0.05$) as shown in table 1 and figures 1 and 2. Rats administered an oral concentration of 10 % AGTE for 7 days showed a significant decrease in MDA content (12.4%) and in the GSH level (59.25%) in liver tissue homo genate, respectively; compared to the control group ($p<0.05$) as shown in table 1 and figures1and2.

Table- 1: The effect of oral administration of various Concentrations of AGTE on MDA contents and GSH levels in rats' liver homogenate.

Groups	MDA (µmol/g tissue)	GSH (µmol/gtissue)
Control (n=6)	0.31375±2.23074	6.24±34.24
Green tea 2.5 % (n=6)	0.25819±2.09999	2.16±27.23
Green tea 5 % (n=6)	* 0.26592±83824 .0	24.11±0.62
Green tea 10% (n=6)	* 0.21726±1.95394	* 3.31±13.95

- data were presented as MEAN ± SEM
- n= number of animals
-*: $p < 0.05$ with respect to control group.

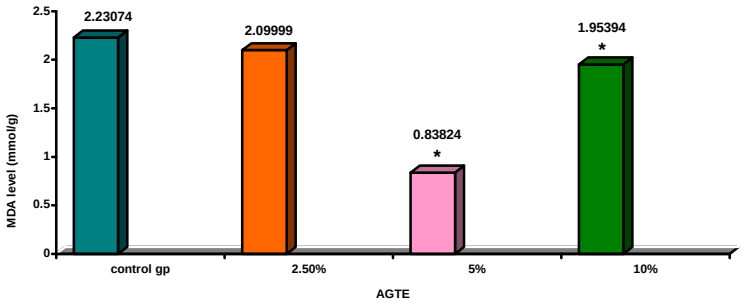

Figure-1: Bar chart comparing the effects of different concentrations of Aqueous green tea extracts administration for 7 days on liver MDA Contents.
* P< 0.05 significant difference with respect to control group

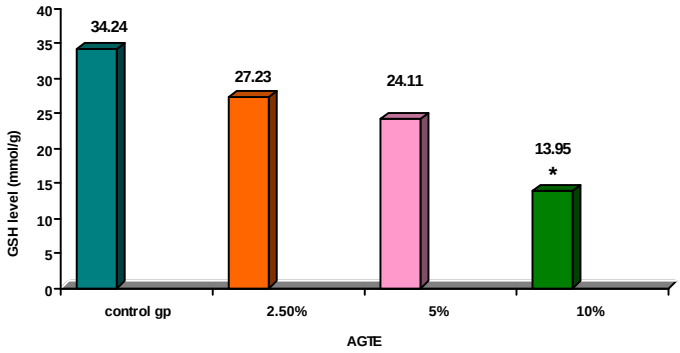

Figure-2: Bar chart comparing the effects of different concentrations of Aqueous green tea extracts administration for 7 days on liver GSH Contents.
* P< 0.05 significant difference with respect to control group.

Rats administered an oral concen tration of 2.5 % AGTE for 7 days showed a non-significant difference in the serum activity of ALT(p>0.05)compared to control group, while there was asignificant decrease in the serum AST activity (64.48%) and ALP (65.4 %)compared to control group (p<0.05) as shown in table 2 and figures 3, 4 and 5. Rats administered an oral concentration of 5% AGTE for 7days showed non-significant differences in the serum activity of AST, ALT and ALP(p>0.05) compared to control group as showed in table 2 and figures 3, 4 and5.Rats administered an oral concentration of 10 % AGTE for 7days showed non- significant differences in the serum activity of AST, ALT(p>0.05) compared to control group, while there was a significant decrease in the serum ALP activity (58.57 %) compared to the control group(p<0.05) as showed in table-2 and figures3, 4 and 5.

Table -2: The effect of oral administration of various concentration of AGT on serum activities of AST, ALT and ALP in rats.

Group		AST (U/L)	ALT (U/L)	ALP (U/L)
Control	**(n=6)**	204.6±35.05	25.5±2.61	46.71±8.67
Green tea	**2.5 %**	72.66±22.76*	27.16±1.57	16.16±3.74*
Green tea	**5 %**	182.66±16.77	23.5±2.28	38.28±6.14
Green tea	**10 %**	147.83±19.95	21±1.12	19.35±2.77*

-Data were represented as mean ± SEM.
-n=number of animals
-*: p< 0.05 significant difference compared to control group

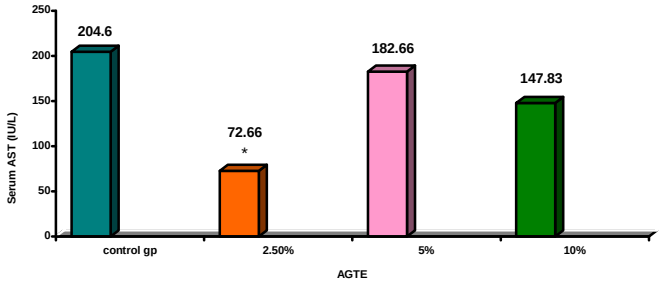

Figure-3: Bar chart comparing the effects of different concentrations of aqueous green tea extract administrated for 7 days on serum AST Level.
* P< 0.05 significant difference with respect to control group

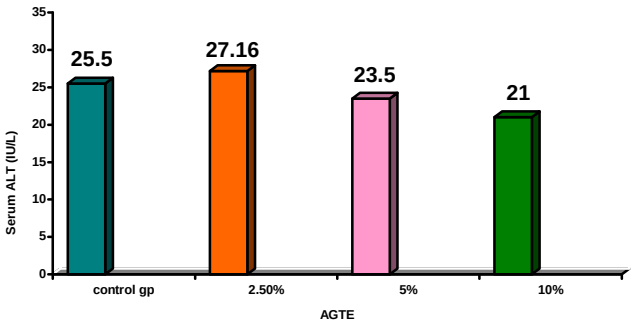

Figure-4: Bar chart comparing the effects of different concentrations of aqueous Green tea extract administrated for 7 days on serum ALT level.

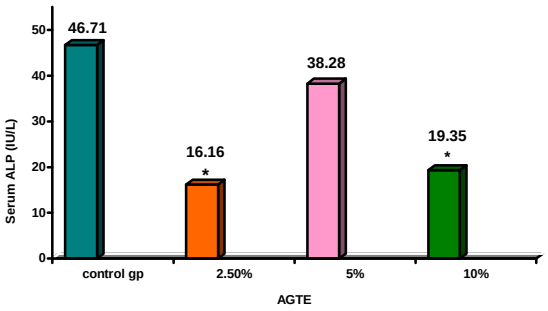

Figure-5: Bar chart comparing the effects of different concentrations of aqueous Green tea extract administrated for 7 days on serum ALP level.
*: P< 0.05 significant difference with respect to control group

Discussion:

There were conflicting data concerning the action of tea catechins as they have dual action as antioxidant (hydrogen donor) and pro-oxidant (Auto-oxidation) [15,19]. Green tea flavor noids might produce anti-oxidant effect by protecting the liver from toxicity through inhibition of oxidative damage as they act as one-electron donors and serve as derivatives of conjugated ring structure with hydroxyl groups that have the scavenge many free radicals involved in oxidative processes[22]. Moreover, it was demonstrated that green tea inhibits lipid peroxidation and induces the activity of anti-oxidant enzymes such as SOD, catalase and GPX[23]. The results of this study demonstrated that the antioxidant effect of various concen trations of AGTE as assessed by lower contents of MDA in liver tissue homogenate compared to control group, where the highest level of protection where produced by 5% AGTE in which there was a significant decline in content of MDA in liver tissue homogenate (62.42%) as compared to control group (Table 1and figure1). An *in vitro* study was performed by Ko *et al* demonstrated that, green tea extracts significantly reduce the levels of GSH, and increase the level of oxidized glutathione (GSSG) in G6PD - deficient erythrocytes in a dose-dependent manner[24]. Moreover, at higher doses, EGCG, a major catechin of green tea can induce oxidative stress *in vivo* where the catechin is believed to be oxidized to form EGCG quinone, which can react with glutathione to form the thiol conjugates[25]. Green tea has a potential to reduce the severity of liver cirrhosis in association with decr eased lipid peroxidation, restored anti-oxidant system and the liver enzymes[26]. Additionally, other study showed that green tea polyph enols reduce the severity of liver injury in association with lower concentration of lipid peroxidation and pro-inflammatory nitric-oxide generated mediators, and it is

useful in the treatment of liver diseases and conditions in which proinflammatory and oxidative stress response [27]. The decrease in the serum activity of AST shown in this study after the administration of AGTE, may suggest that the release of such enzyme is inhibited, probably by a chemical component in the tea extract that stabilizing the cellular membrane [28]. While there was no significant difference in the serum activity of ALT compared to control group (Table-2) and figures 3and4. The result of this study demonstrated that, AGTE at various concen trations produce a significant decline in serum ALP as compared to control group ($p<0.05$), and a clear decline was observed with AGTE at 2.5%on serum ALP (65.4%)as shown in table-2 and figure-5. It was demonstrated that, green tea extract is effective scavengers of reactive oxygen species and may also function indirectly as antioxidant through the effects on transcription factors and enzyme activities [29]. To our knowledge this is the first *in vivo* study that examines the effect of administration of various concentrations of AGTE in normal liver tissues. Thus, we did not have the chance to compare the results of this work with other reports.

Conclusion:

According to the results obtained from this study, it could be concluded that aqueous green tea extract possesses an antioxidant effect, manifested by a decrease in the content of MDA in liver tissue homogenate, and in serum AST and ALP activities.

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