Hepatoprotective effect of green tea (Camellia sinensis) on female rats drenched with paracetamol

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Abstract

Hepatotoxicity is an acute adverse effect of paracetamol overdose which could be fatal, so in this research we studied the effect of paracetamol on liver enzymes (GPT, GOT, total protein , glucose ,uric acid) of female rats that drenched green tea. twenty laboratory female rats housed in plastic cages in animal house at Karbala university/ college of pharmacy , the animals divided randomly into five groups ,(G1 control, G2 drenched with 300 mg/kg green tea and 500 mg/kg paracetamol, G3 drenched with 500 mg/kg green tea and 500 mg/kg paracetamol , G4 drenched with 500 mg/kg paracetamol ,G5 drenched with 500 mg/kg green tea) , the present study found the ability of green tea to protect liver enzyme against the poisonous effect of paracetamol by reducing the higher value of GPT,GOT, Glucose, and evaluated the lower value of Uric acid and total protein causing by paracetamol.

تأثير الشاي الأخضر في حماية الكبد لإناث الجرذان المجرعة بالباراسيتامول

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مفتاح البحث : الشاي الأخضر ، البراسيتامول ، إنزيمات الكبد .

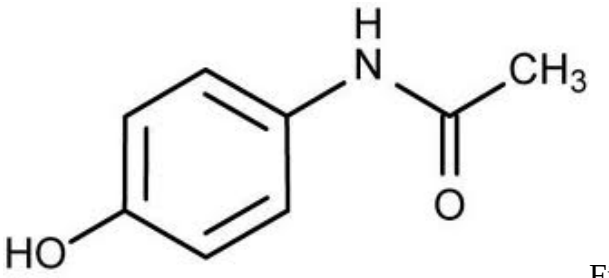
الخلاصة

يعد التسمم الكبدي نتيجة خطيرة للجرع المفرطة من البراسيتامول مؤديا الى الوفاة في معظم الحالات ،في هذا البحث درس تاثير هذا الدواء فيانزيمات الكبد لإناث الجرذان المختبرية المجرعة بالشاي الاخضر. حيث استخدم 20 جرذا مختبريا وضعت في اقفاص بلاستيكية في البيت الحيواني التابع لكلية الصيدلة /جامعة كربلاء2013 ،وقسمت الحيوانات عشوائيا الى خمسة مجاميع (مجموعة 1 مجموعة السيطرة ،مجموعة2 جرعت 300 ملغم/كغم شاي اخضر و500 ملغم/كغم براسيتامول ،المجموعة 3 جرعت500 ملغم/كغم شاي اخضر و500 ملغم/كغم براسيتامول ،مجموعة 4 جرعت500 ملغم/كغم براسيتامول ،المجموعة 5 جرعت 500 ملغم/كغم شاي اخضر),توصلت الدراسة الى قابلية اوراق الشاي الاخضر في حماية انزيمات الكبد من تسمم الباراسيتامول .

Introduction

Paracetamol (PCM) was discovered in Germany at the end of the 19th

century, but was not widely used until mid way through the 20thcentury,PCM is probably the most versatile and widely used asanalgesic and antipyretic drug worldwide(1), Its structure is shown in Figure 1 .Acetaminophen used in the symptomatic management of moderate pain and fever(2).When taken at recommended doses it has an excellent safety profile, notably lacking the gastrointestinal (GI) side effects of aspirin and ibuprofen (3).However, acute over dosage with acetaminophen, whether accidental or deliberate, is relatively common and can be extremely serious,Ingestion of 10–15 g of acetaminophen by adults may cause severe hepatocellular necrosis and doses of 20–25 g are potentially fatal (4).The WHO recommended dose is 100–500 mg (5).



Figer(1) paracetamol

Acetaminophen (APAP) is mainly metabolized in the liver to excretabl glucuronide and sulphate conjugates. APAP-induced hepatotoxicity has been attributed to the cytochrome p-450 generated reactive metabolite, N-acetyl-p-benzoquinoneimine (NAPQI), at therapeutic doses, NAPQI is removed by conjugation with glutathione (GSH) to form mercapturic acid.(6). However, once intracellular GSH reserves are depleted, multiple mechanisms ensure ultimate cell death (7),The therapeutic doses of acetaminophen have no serious side effects and the drug is well tolerated by the patients. Drug- drug interaction are not usually observed. Hepatotoxic effect of paracetamol is due to suicidal or Para suicidal intent coming from large overdoses of the drug taken (8). Centrilobular hepatic necrosis in animals was reported as a side effect of acetaminophen overdose (9).The most important organ in human body and considered as a chief site for regulating internal chemical environment is the liver, so the injury for this organ induced by hepatotoxicity has been recognized as a major toxicological problem for many years (10).also liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health ,But there are not much drug available for the treatment of liver disorders (11).

Herbs are being increasingly utilized to treat a wide variety of clinical diseases and this achieved with the herbal medicines derived from plant extracts (12).Natural antioxidants have protective effect against drug-induced toxicities and this concept should take in consideration especially whenever free radicals generation is involved (13).Green tea (Camellia sinensis), native of india, it is an annual herb, naturalized or wild throughout the world. The plant has a great value in medicine because the leaves are of use for making cigarettes and relief of asthma as fumigating powder (14)Green tea was supplemented in industrial products such as shampoo, soaps, creams, vitamins, drinks, lollipops and ice creams (15). Flavonol monomers are available in rich quantities in the

fresh tea leaves and these monomers are known as catechins such as epicatechins(16),The amount these compounds are 13.6 g/100g in green tea as compared with 4.2g/100 g dry weight in black tea (17).Catechins have a wide range of beneficial effects in cardiovascular diseases, they have the ability to prevent LDL oxidative susceptibility, serum lipid and lipoprotein concentration (18).Green tea can be effective for treatment of obesity (19) Chewing gum supplementation with green tea extract will be effective to oral cavity and periodontal disease in dentist medicine (20).Oxidative stress can be protected by catechins and the protective effect of green tea is belongs to the ability of its catechins to prevent the formation of oxygen radicals and scavenge free radicals such as peroxyl, hydroxyl, lipid radicals and superoxide anions (21,22,23).there is anevidence for the bioactivity of the phenolic compounds of green tea in vivo supports the notion that their antioxidant properties contribute to important role in the health protection and disease prevention.(24)

Materials and methods.

twentyadult laboratory female rats weighted between (180-250g), aged about 2-3 months were used as experimental animals in this study.they were housed in plastic cages in the animal house ofKarbala university/pharmacycollege. The animals placed on a 12-hour light/dark cycle,with food and water freely available . Rats were given an acclimation period up to one week before the beginning of experiment.

Plant extract preparation

The dried green teawere powdered by electrical miller. to prepare the extract, 150g of green tea powder was mixed with 1000 ml of 95% ethanol(1:10 w/v) placed on shaking water bath for 48h. The suspension was filtered through whatman No.1 filter paper and the residue vacuumed and evaporated in rotary evaporator. The dried extracts were stored at 4 C° until being used .(25)

Biochemical assay methods:

- Measurement of serum GPT activity:

Serum GPT activity was evaluated according to the method ofReitman ,S. and Frankel, S. (1975) (26) by using a ready made kit for this purpose .(spectrum company)

Principle: The method is based on the formation of hydrazine derivative of pyruvic acid as a result of reaction with 2,2-dinitrophenyl hydrazine , and the light absorbance of the product was measured spectrophotometrically at 540nm .

$$\alpha - \text{ketoglutarate} + \text{Alanine} \rightarrow \text{Glutamate} + \text{pyruvate}$$

-Measurement of serum GOT activity:

Serum GOT activity was evaluated according to the method ofReitman ,S. and Frankel, S.(1975) (26) by using a ready made kit for this purpose from (spectrum company).

Principle: The method is based on the formation of hydrazine derivative of pyruvic acid as a result of reaction with 2,2-dinitrophenyl hydrazine , and the light absorbance of the product was measured spectrophotometrically at 540nm .

α – ketoglutarate + L-aspartate → Glutamate + pyruvate

- Measurement of serum total protein activity:

Serum total protein activity was evaluated according to method ofWeichselbam , T. and Amer ,E. (1946)(27) by using a ready made kit for this purpose from (human company).

Principle: cupric ions react with protein in alkaline solution to form a purple complex . The absorbance of this complex is proportional to the protein concentration in sample at absorbance 520 nm.

-Measurement of serum uric acid activity:

Serum uric acid activity was evaluated according to method of Barham, D. and Trinder, P. (1972)(28) by using a ready made kit for this purpose from (spectrum company).

Principle : The assay is based upon the methods of modified trinder peroxidase assay using 3,5-dichloro-2-hydroxybenzenesulfonic acid (DCHB),the series of reactions involved in the assay system system is as follows:

1- uric acid is oxidized to allantion by uricase with production of hydrogen peroxide.

Uric acid $+O_2 +H_2O \rightarrow$ Allantion $+CO_2 + H_2O_2$

2- the peroxide react with 4-amino-antipyrine and (DCHB) in the presence of peroxidase to yield a quinoneimine dye. The sub change in absorbance at 546nm is proportional to uric acid concentration in the sample.

H_2O_2 + 4-AAP + DCHB → Quinoneimine $+H_2O$

- Measurement of serum Glucose:

Serum Glucose activity was evaluated according to method of Caraway, W.T. and Watts, N. B. (1987) (29) by using a ready made kit for this purpose from (spectrum company).

Principle: Glucose is determined after enzymatic oxidation in presence of glucose oxidase. The formed hydrogen peroxide reacts under catalysis of peroxidase (PAP) with phenol and 4-aminoantipyrine to form a red violet quinoneimine dye as indicator.

Glucose $+2H_2O + O_2 \rightarrow$ Gluconic acid + H_2O_2

$2H_2O_2$ + Phenol + 4-amino- antipyrine → $4H_2O$ + Quinoneimine

Animals design:

Rats were randomly distributed into five groups/four animals for each one, however these groups named G1(control) ,G2(treated with 3oo mg/kg green tea and 500 mg/kg paracetamol),G3(treated with 500 mg/kg green tea and 500 mg/kg paracetamol) ,G4 (treated with 500mg/kg paracetamol) and G5 (treated with 500 mg/kg green tea), so G1 received an oral dose of glucose water for 28 days, G2 received orally 300mg/kg green tea for 21 days and received in day 22 500mg/kg paracetamol to the day 28, G3 received orally 500mg/kg green tea for 21 days and received in day 22 500mg/kg paracetamol to the day 28 , G4 received glucose water for 21 days and received in day 22 500mg/kg paracetamol to the day 28, G5 received 500mg/kg green tea for 28 days. At the end of experiment all the animals were sacrificedand measure all the biochemicalindices.

Statistical analysis

Data were expressed as mean. Differences between control and other groups were tested for statistical significance using one-way analysis of variances (ANOVA). P-values of 0.05 or less were considered significant, statistical analysis was performed using SPSS for windows version (SPSS, Inc., Chicago, Illinois).

Result

table (1) illustrate a significant increase in the level of serum GPT, GOT, Glucose $p \leq 0.05$ in group treated with paracetamol(G4) only as compared with G1, G3,G5 and G2 , as compared with the other groups.

Groups	GPT(U/L)	GOT(U/L)	Glucose (mg/dl)
G1(control)	29.450 a	34.625 a	122.875 a
G2(300mg green tea +paracetamol)	89.175 b	90.375 b	164.325 b
G3(500mg green tea+ paracetamol)	43.950 b	39.850 b	147.075 b
G4(paracetamol)	112.850 b	163.150 b	187.800 b
G5(500mg green tea)	35.500 b	32.600 b	127.050 b
LSD	3.49	2.83	10.18

- Same small letter means no significant changing.
- Different small letter means significant changing.
- $p \leq 0.05$.

table (2) illustrate a significant decrease in the level of serum uric acid and total protein $p \leq 0.05$ in group treated with paracetamol (G4) only as compared with G1, G3,G5 and G2 , as compared with the other groups.

Groups	Uric acid(mg/dl)	Total protein(mg/dl)
G1(control)	2.350 a	7.125 a
G2(300mg green tea +paracetamol)	1.600 b	6.650 b
G3(500mg green tea+ paracetamol)	1.950 b	6.950 b
G4(paracetamol)	1.325 b	5.970 b
G5(500mg green tea)	2.075 b	7.050 b
LSD	0.32	0.34

- Same small letter means no significant changing.

- Different small letter means significant changing.
- $p \leq 0.05$.

Discussion

The results of the study demonstrated that the **Camellia sinensis** protects rats from acetaminophen-induced liver injury evident from the significant decrease in serum ALT, AST and glucose levels, these findings are in agreement with(16) Green tea has an excellent antioxidant effect due to the availability of flavonol monomers in rich quantities in the leaves of plant, and these chemical compounds known as catechins .Green tea extract may protect liver and brain cells against squeal of ethanol extract and this will lead to reduce the toxic effect of paracetamol in liver (30 ,10) .The plant obtained its activity from the present of flavonoids which have shown to possess various biological properties related to antioxidant mechanisms, by preventing of intracellular enzymes leakage resulting from cell membrane stability or cellular regeneration (31,32,33). The The lowering effect of glucose by the effect of green tea is agreement withFatemehHaidari et al (2012)(25),that the bioactive compounds found in green tea have anti-diabetic and antioxidant activities.

From the present study we found that the toxic effect of paracetamol on total protein and uric acid has a good indicator on the damage of liver and this result is agree with Thabrew, M and Joice, P. (1987)(33).The reduced levels of uric acid in PCM induced hepatotoxicity is probably due to increased utilization of uric acid against the increase in the production of free radicals (35).It is well known that toxicants like PCMproduce sufficient injury to hepatic parenchyma cells to decrease the level oftotal plasma protein content(36).JU-Hua et al (2004) (37) showed the activity of Green tea polyphenols in reduceing the severity of liver injury in association mechanism with lower concentrations of lipid peroxidation and proinflammatory nitric oxide–generated mediators. Green tea polyphenols can be a useful supplement in the treatment of liver disease and should be considered for liver conditions in which proinflammatory and oxidant stress responses are dominant.

Conclusions :

The present study concluded that green tea (**Camellia sinensis**) has a protective effect on liver against toxic agent such as paracetamol due to its antioxidant activity.

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