EFFECT OF GREEN TEA EXTRACT ON INJURED LIVER INDUCED BY ACETAAMINOPHEN IN RABBITS ADULT MALE

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ABSTRACT

Green tea is consider as a natural sources of antioxidants used to treat pathological changes occurring as a result of using of many drugs particularly in the liver tissue. Forty adult male rabbits were randomly divided into four groups (10 rabbits /group) and were treated daily for 30 days as follows: first group (c): is the control group , second group (T_1) : rabbits in this group were allowed to ad libitum supply of drinking water containing 2.5 gm /kg b.w of green tea extract , Third group (T2): rabbits in this group received 500 mg/kg b.w. of acetaminophen orally, fourth group (T_3) : rabbits in this group were allowed to ad libitum supply of drinking water containing 2.5 gm /kg b.w. of green tea extract and received 500mg/kg b.w. of Acetaminophen orally. blood samples were collected for measuring the liver enzymes, cholesterol and triglyceride in blood serum at 15 and 30 days of experience. The results of this study revealed that oral intubation of acetaminophen for 30 days caused hepatic dysfunction manifested by a significant elevation($P<0.05$) in the serum AST,ALT ALP cholesterol and triglyceride while the animals received GTE   with acetaminophen (T_3) for 30 days showed a considerable decrease($P<0.05$) in serum AST, ALT,ALP, cholesterol and triglyceride levels. The histological section showed pathological changes in the liver tissue in (T2) group while giving green tea with acetaminophen was effective in modified these changes into semi normal.

Keywords : Green tea , acetaminophen, ALT, AST, ALP, cholesterol and triglyceride.

تأثير مستخلص ألشاي الأخضر على ألتلف الكبدي ألمستحدث بالأست امينوفين في ذكور الأرانب البالغة

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المستخلص

يعد الشاي الأخضرمن المصادر الطبيعية لمضادات الاكسدة المستخدمة لعلاج التغيرات المرضية الحاصلة نتيجة استخدا م العديد من العقارات وبالخصوص في نسيج الكبد . نفذت التجربة على 40من ذكور الارانب البالغة وزعت عشوائيا" إلى اربعة مجاميع (10 أرانب / مجموعة) وعوملت يوميا" لمدة 30 يوم كالتالي : المجموعة الأولى (c) وعدت كمجموعة سيطرة ، المجموعة الثانية (T_1) : أعطيت (2.5 غرام/كلغم من وزن الجسم)من مستخلص الشاي الأخضر، المجموعة الثالثة (T 2) أعطيت (500 ملغم/كلغم من وزن الجسم) من الاسيت امينوفين ،المجموعة الرابعة (T3): أعطيت (2.5 غرام/كلغم من وزن الجسم)من مستخلص الشاي الأخضر مع (500 ملغم/كلغم من وزن الجسم) من الاسيت امينوفين .تم قياس انزيمات الكبد في مصل الدم خلال الفترات 15 و30 يوم , وفي نهاية التجربة أخذت مقاطع نسيجية من الكبد . أظهرت النتائج إن إعطاء الاسيت امينوفين لمدة 30 يوما" سبب خلل في وظيفة الكبد تمثلت بالزيادة المعنوية (0.05P >) في مستوى الأنزيمات الناقلة للامين ALT وAST وفعالية أنزيم الفوسفاتيز القاعدي ALP و ارتفاع في مستوى الكوليستيرول والدهون الثلاثية، كما أظهرت النتائج أن اعطاء مستخلص الشاي الأخضر مع الاسيت امينوفين في مجموعة (T_3) أدى إلى انخفاض معنوي (0.05P >) في مستوى الأنزيمات الناقلة للامين ALT وAST وفعالية أنزيم الفوسفاتيز القاعدي ALP و انخفاض مستوى الكوليستيرول والدهون الثلاثية . أظهرت نتائج الفحص النسيجي حصول تغيرات مرضية في نسيج الكبد للمجموعة التي أعطيت بالأست امينوفين (T_2) وان أعطاء الشاي الاخضر مع الاسيت امينوفين كان فعال في تعديل هذه التغيرات الى شبه الطبيعي.

الكلمات المفتاحية : مستخلص الشاي الاخضر، الاسيت امينوفين ،ALT,AST,ALP ، الكوليستيرول والدهون الثلاثية.

INTRODUCTION

Acetaminophen or Paracetamol is a commonly used as antipyretic (fever reducer) and analgesic (pain reliever), it is usually used for the relieve of pains, headaches and other minor aches and it is a main component in numerous cold and flu therapy. The beginning of analgesia is about 11-29 minutes after oral administration of paracetamol (18). It's half-life is 1- 4 hours, It is usually safe for use at recommended doses (1000 mg) per single dose and up to (4000 mg) per day for adult (20), acute over dose of paracetamol can cause acute fatal liver damage and rare individuals, a normal dose can do the same (2,12). Green tea is derived from unfermented leaves of *Camellia Sinensis* plant initially cultivated in east Asia, it is grows as large as a bush or tree. Nowadays *Camellia Sinensis* grows all over Asia and part of the middle East and Africa. People in Asia countries more ordinarily drink green tea. The healthy properties of green tea are mainly attributed to polyphenols which is chemicals with effective antioxidant properties. Actuality the antioxidant effects of polyphenols are greater than vitamin C (7). The polyphenol also caused in better flavor of green tea (16). Polyphenols in tea are identified as Catechins, Green tea have six main Catechins component (catechins, gallaogatechin, epicatechin, epicatechingallate, and epigallocatechingallate also known as EGCG) which is the most active, in addition green tea contain alkaloids like caffeine, theobromine and theophylline. These alkaloids give green tea effects of stimulant. Also there is an amino acid found in green tea known as L-theamine which has calming effects on the nervous system. Green tea boosts metabolic rates, accelerates fat oxidation and ameliorates insulin sensitivity and glucose tolerance (19,24). The green tea and its extract have been shown to fight obesity and lower LDL-C the two threat factor for cardiac disease and diabetes.(11,19). The theanin in the green tea might help the body′s immune system response when fighting infection by increasing the disease – fighting capacity of gamma delta T cells (13). The green tea polyphenols can help to prevent the growth of bacteria that cause in bad breath (8), also catechinpolyphenols may be elevate thermogenesis (the production of heat by the body) and so increases energy expenditure (1,4). The aim of present study was to examine the antioxidant capacity of green tea (*CamelliaSinensis*) extract (GTE) against acetaminophens induced liver injury in adult male rabbits.

MATRRIALS AND METHODS

A total number of 40 adult male rabbits (1000-1500 g) were used in this experiment. Animals in all stages of the research were housed in irons cages in a conditioned room (22-25⁰) in the animal house of the department of physiology and pharmacology at college of veterinary medicine-university of Baghdad. The animals were kept for ten days for adaptation with the experimental circumstances , Animals had free access to tap water and standard pellet diet all along the experimental time . Randomly the animals were divided into four identical groups (10 rabbits / group) and were treated every day for 30 days as follows : control group (C) : rabbits in this group were permitted to ad libitum provide of drinking water, treated group (T_1) : rabbits in this group were allowed to drink water containing 2.5 gm /kg b.w (8) of green tea water extract, treated group (T_2) : rabbits in this group received 500 mg/ kg b.w (1) of acetaminophen orally, treated group (T_3) : rabbits in this group were allowed to ad libitum supply of drinking water containing 2.5 gm /kg b.wof tea green extract and received 500 mg/ kg b.w. of Acetaminophen orally. Blood samples were collected at 15 and 30 days of the experisent, blood was collected directly from heart, blood specimens were preserved in tubes and centrifuged at 3500 rpm for 10 minutes, and then serum samples were a liquated and frosted at -20c⁰ till analysis, serum samples were used to measure the following parameters:- serum aspartate aminotransferase (AST) ,alanin aminotransferase (ALT) ,alkaline phosphatase (ALP) cholesterol and triglyceride.by using specialized kits from LINEAR company. For histological studies, rabbits were anesthetized, sacrificed by withdrawal of blood from heart, at once after scarification liver were excised, opened longitudinal and conserved in 10% neutral formalin buffer solution for preparing of histological sections (15) .

Preparing of green tea extract(GTE)

The dry green tea used in this experiment was obtained from local market. The green tea was made by soaking 25gm of the dry green tea in one litter of boiling tab water for 3-5 minutes, the solution was filtered to make 2.5% green tea extract and allowed to cool at room temperature (17).

Statistical Analysis Statistical analysis of data was performed using SAS(statistical analysis system-version 9.1). data were subjected to analysis using Two – way analysis of variance (ANOVA). Least significant differences (LSD) post hoc test was used (multiple comparison) to asses significant differences among means. P<0.05 was considered statistically significant (21).

RESULTS AND DISCUSSION

Table1 illustrated the mean values of alkaline phosphatase ALP (IU/L) in the control group and three treated groups all along the experimental time. After 15 days of treatment a significant ($p<0.05$) elevation in ALP was observed in animal received acetaminophen orally (500 mg/ kg b.w) with mean values of 285.40 ± 19.74 as compared to control group(72.00 ± 3.74), T_1(70.40 ± 19.70) and T_3(147.00 ± 17.72). At the same period of the experiment, oral administration of green tea in group T_3 caused a significant ($p<0.05$) depression in the ALP level (147.00 ± 17.72) in compared to T_2 group (285.40 ± 19.74). After 30 days of experiment there was also a considerable ($p<0.05$) increase in ALP levels in T_2 group (375.00 ± 62.57) as compared to control, T_1 and T_3 groups with mean values of 75.00 ± 3.53, 73.00 ± 62.57, and 127.80 ± 18.42 respectively.

Table 1. The effect of acetaminophen and green tea on serum alkaline phosphatase ALP (IU/L) in adult male rabbits

Days	Groups			
	Control	T_1	T_2	T_3
15	C72.00 ± 3.74a	C70.40±19.70a	A285.40±19.74b	B147.00±17.72a
30	C75.00±3.53a	C73.00±62.57a	A375.00 ± 62.57	B127.80±18.42b

Values are expressed as mean ± SE, n = 10 each group.

T_1: Animals received green tea extract 2.5 gm /kg b.w.

T_2 : Animals received acetaminophen 500 mg/ kg b.w.

T_3 : Animals received green tea extract 2.5 gm /kg b.w and acetaminophen 500 mg/ kg b.w

Capital different letters in the same row denoted that significant differences among groups ($p<0.05$).

Small different letters in the same column denoted that significant differences within groups ($p<0.05$).

Table2 clarified the mean values of serum aspartate aminotransferase (AST) (IU/L) of control and treated groups.A significant (P<0.05) increase in serum AST was found in group T_2 after 15 and 30 days of treatment (114.20 ± 28.18), (139.00 ± 28.27) as compared to the values in control ,T_1 and T_3 groups at the same period of time.Oral intake of green tea to T_3 group caused further significant (P<0.05) decline in serum AST along the experiment time (74.40 ± 5.18),(66.80 ± 6.66) respectively compared to the values in the other groups.

Table 2. The effect of acetaminophen and green tea on serum aspartate aminotransferase (AST) (IU/L) in adult male rabbits

Days	Groups			
	Control	T_1	T_2	T_3
15	C50.60 ± 5.59a	C48.10 ± 4.15a	A14.20 ± 28.18b	B74.40 ± 5.18a
30	C51.00 ± 3.91a	C47.23. ± 3.20a	A139.00 ± 28.27a	B66.80 ± 6.66b

Values are expressed as mean ± SE, n = 10 each group.

T_1: Animals received green tea extract 2.5 gm /kg b.w.

T_2 : Animals received acetaminophen 500 mg/ kg b.w.

T_3 : Animals received green tea extract 2.5 gm /kg b.w and acetaminophen 500 mg/ kg b.w.

Capital different letters in the same row denoted that significant differences among groups ($p<0.05$).

Small different letters in the same column denoted that significant differences within groups ($p<0.05$).

Table 3 pointed to the mean values of serum alanin aminotransferase (ALT) (IU/L) or different treated groups. Oral administration of acetaminophen caused significant ($p<0.05$) rise in serum alanin aminotransferase (ALT) in T_2 group along the experimentalperiod (120.00 ± 9.95),(140.80 ± 14.60) as compared

with other groups, while at the same period green tea intake caused a considerable decline ($p<0.05$) in serum alanin aminotransferase (ALT) (IU/L) in T_3 group (66.60 ± 8.88), (64.20 ± 11.34) as compared to other treated groups.

Table 3. The effect of acetaminophen and green tea on serum alanin aminotransferase (ALT) (IU/L) in adult male rabbits

Days	Groups			
	Control	T_1	T_2	T_3
15	C56.00 ± 4.11a	C54.00 ± 4.10a	A120.00± 9.95b	B66.60 ± 8.88a
30	C55.20 ± 2.13a	C50.15 ± 3.15a	A140.80 ± 14.60a	B64.20 ± 11.34a

Values are expressed as mean ± SE, n = 10 each group.

T_1: Animals received green tea extract 2.5 gm /kg b.w.

T_2 : Animals received acetaminophen 500 mg/ kg b.w.

T_3 : Animals received green tea extract 2.5 gm /kg b.w and acetaminophen 500 mg/ kg b.w.

Capital different letters in the same row denoted that significant differences among groups ($p<0.05$).

Small different letters in the same column denoted that significant differences within groups ($p<0.05$).

Table 4 showed the mean values of serum total cholesterol (mg/dl) in the control ,T_1, T_2 and T_3groups. After 15 and 30 days of treatment the results of animals who received acetaminophen orally (T_2)showed significant ($p<0.05$) increase in serum total cholesterol (59.80 ± 3.24), (80.20 ± 5.15) in compared to other groups .after 30 days of experiment, the results of animal group who take green tea and acetaminophen (T_3) showed a major ($p<0.05$) reduce in serum total cholesterol (41.40 ± 4.90) comparing to control, T_1 and T_2 groups (49.00 ± 4.48),(45.30 ± 2.65),(80.20 ± 5.15).

Table 4. The effect of acetaminophen and green tea on serum total cholesterol (mg/dl) in adult male rabbit

Days	Groups			
	Control	T_1	T_2	T_3
15	B51.40 ± 4.80a	B50.10 ± 3.90a	A59.80 ± 3.24b	B45.40 ± 5.44a
30	B49.00 ± 4.48a	BC45.30 ± 2.65a	A80.20 ± 5.15a	C41.40 ± 4.90a

Values are expressed as mean ± SE, n = 10 each group.

T_1: Animals received green tea extract 2.5 gm /kg b.w.

T_2 : Animals received acetaminophen 500 mg/ kg b.w.

T_3 : Animals received green tea extract 2.5 gm /kg b.w and acetaminophen 500 mg/ kg b.w.

Capital different letters in the same row denoted that significant differences among groups ($p<0.05$).

Small different letters in the same column denoted that significant differences within groups ($p<0.05$).

Table5 indicated to the mean values of serum triglyceride (mg/dl) of control and other experiment groups. During the experimental time 15 and 30 days there was a significant($p<0.05$) rise in serum triglyceride noticed in T_2 group (106.00 ± 12.97), (128.00 ± 27.15) in compared to control, T_1 and T_3 groups. Oral administration of green tea with acetaminophen (T_3 group) for more than fifteen days caused a significant ($p<0.05$) elevation in serum triglyceride with mean values of 61.60 ± 6.18 in compared to control, T_1 and T_2 (54.40 ± 5.60), (49.50 ± 4.12), (128.00 ± 27.15).

Table 5. The effect of acetaminophen and green tea on serum triglyceride (mg/dl) in adult male rabbit

Days	Groups			
	Control	T_1	T_2	T_3
15	B55.80 ± 3.02a	BC52.30 ± 3.40a	A106.00 ± 12.97b	B65.00 ± 7.67a
30	C54.40 ± 5.60a	C49.50 ± 4.12a	A128.00 ± 27.15a	B61.60 ± 6.18a

Values are expressed as mean ± SE, n = 10 each group.

T_1: Animals received green tea extract 2.5 gm / kg b.w.

T_2 : Animals received acetaminophen 500 mg/ kg b.w.

T_3 : Animals received green tea extract 2.5 gm / kg b.w and acetaminophen 500 mg/ kg b.w.

Capital different letters in the same row denoted that significant differences among groups (p<0.05).

Small different letters in the same column denoted that significant differences within groups (p<0.05).

The current experiment illustrate that acetaminophen caused considerable increase(p<0.05) in level of liver enzymes (ALP, ALT, and AST), serum cholesterol and triglyceride levels as compared to C,T_1 and T_3 groups. this rise was changed significantly (P<0.05) in the group which taken up the green tea and acetaminophen to a semi normal values. The role of paracetamol in causing hepatic injury is taking an important consideration, since Liver plays an essential function in organizing many of metabolic actions and making of different essential materials in the body. liver function enzymes (AST, ALT, and ALP) showed improve in activities after administration of acetaminophen for long period of time. The damage in the liver caused by acetaminophen associated with the fact that little amount of acetaminophen are turned into toxic metabolites. This toxic metabolites combine with liver proteins and result in cellular damage. The quantity of toxic metabolite formed and the capability of the liver to eliminate this metabolite before it joins to liver protein effect the degree of liver injury (14). There is a significant (P< 0.05) increase in the efficacy of plasma alkaline phosphatase (ALP) following an acute sever injury to the hepatic tissues which is existing in the lining membrane of the hepatocytes which has a cell membrane location related with the canalicular membrane injure (6). The raise in the alkaline phosphatase activity is maybe a reflexion of remained enzyme molecules on the cell membrane fragment emitted to the plasma as a result of liver damage. The elevated values of both AST and ALT enzymes might be attributed to the damage in the hepatocellular membrane possibly as a result of engaged of toxic metabolite of paracetamol with the protein and lipid components of the liver membrane (22). Treatment with green tea formed a significant (P<0.05) decline in the levels of serum AST , ALT and ALP as compared to acetaminophen treated group. These decrease point to the capability of green tea for stabilizing plasma membrane in addition to repair the hepatic tissue injuries occurred by oxidative stress. This consequence is acceptable with the ordinarily conventional opinion in which serum levels of transaminase go back to normal with regeneration of hepatocytes as well as curing of hepatic parenchyma (5). Alkaline phosphatase (ALP) is an enzyme localize within the bile ducts and utilize as index for estimation of hepatic excretory work, the serum activity of this enzyme is highly elevated in certain liver damage (7,23). The aptitude of green tea for lowering the serum activity of ALP proposes the steadiness of the biliary dysfunction in rabbits liver through injury prompted by acetaminophen(3,25). moreover green tea caused a significant (P<0.05) incline of serum triglyceride and cholesterol compared to paracetamol treated group. The diminution of serum cholesterol possibly because of the suppression of the absorption of cholesterol in the intestin by green tea leading to minimize the serum cholesterol concentration (9). The reduction in the triglyceride observed in recent study may be due to the fact that green tea restrains postprandial hypertriacylglycerolemia through the suppression of pancreatic lipase and subsequently delayed the absorption of fat (10). The histological sections of liver in the control group showed normal appearance (Fiqure-1), while there is a vacuolar degeneration and proliferation of kupffer cells and few RBCs in the sinusoids in

acetaminophen and green tea group(Fiqure-2), also there is sever necrosis in the liver parenchyma and infiltration of inflammatory cells around the portal area. There is also dilation of the central vein with focal aggregation of MNCS in liver parenchyma in acetaminophen group(Fiqure-3). There is a hydropic degeneration in the green tea group (Fiqure-4).
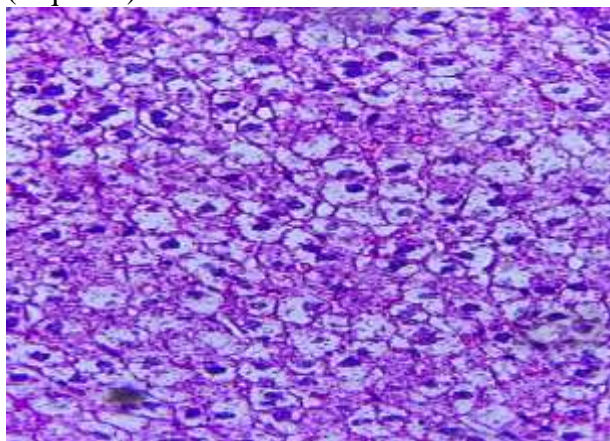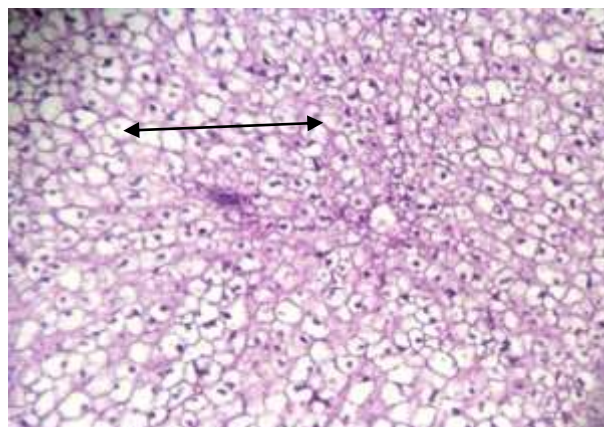

Figure1.Histological section in the liver of rabbit from control group (X400,H&E stain)
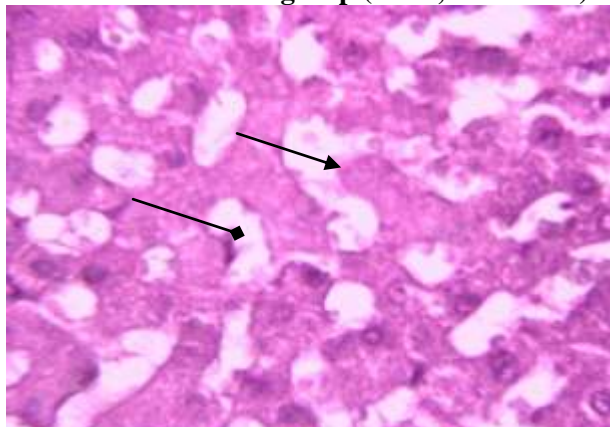
Figure2 . Histological section in the liver of rabbit From green tea and acetaminophen group, there is a proliferation of kupffer cells (⟶) and few RBCs in the sinusoids (➜). (X400,H&E stain)
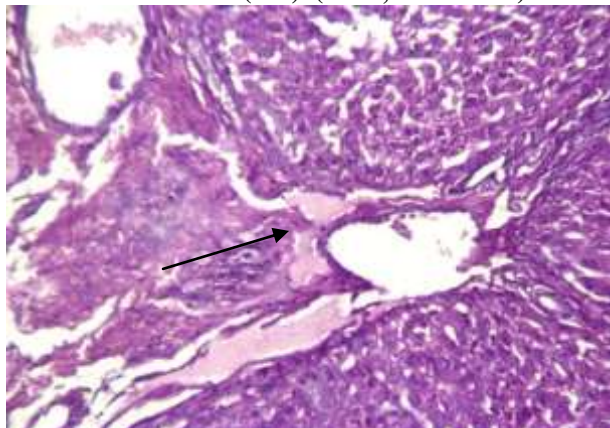
Figure 3. Histological section in the liver of rabbit from acetaminophen group, There is sever necrosis (⟶) in the liver parenchyma.. (X400,H&E stain)

Figure 4.Histological section in the liver of rabbit From green tea group, there is a hydropic degeneration (⟷). X400,H&E stain)

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