THE EFFECT OF GALLIC ACID AS PROTECTIVE AGENT ON SOME BIOCHEMICAL PARAMETERS INDUCED HYPERCHOLESTEROLEMIA IN LABORATORY MALE RATS

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ABSTRACT

The role of gallic acid was studied to alleviating some biochemical parameters alterations in hyper cholesterolemia in rats. For this purpose 24 sexually adult male rats were divided randomly in to 4 groups (6 rats each). The experiment was conducted at the animal house of the Veterinary Medicine College–University of Basrah, the control group, rats were injected intraperitoneally (I.P.) with 0.9 % normal saline (N.S)and were supply with the stander ration.

The first treated group were injected intraperitoneally (I.P.) with 100 mg/ kg gallic acid dissolve in 0.5 ml distilled water daily and were supply with the stander ration.

The second treated group were supplied with the stander ration in addition to 1.5% cholesterol of the stander ration .

The third treated group were supplied with standard ration in addition to 1.5% cholesterol, and injected intraperitoneally (I.P.) after one hour with 100 mg/kg gallic acid dissolved in 0.5 ml distilled water

The obtained results indicated that the administration gallic acid as protective agent on high cholesterol diet tend to alleviated and improved lipid profile by a significant reduction in the serum total cholesterol (TC),LDL-C and VLDL compared with animals fed cholesterol 1,5% diet, hepatic enzymes activates of AST &ALT in the protective group almost were at the range of normal values and the protective urea blood value reach almost as the normal level as control group.

INTRODUCTION

Gallic acid (GA) is an endogenous product found in large amounts in tea leaves (1) . GA is a strong antioxidant that possesses antimutagenic and anticarcinogenic activities (2) and(3).

It has been known that obesity is implicated in various diseases, including type II diabetes, hypertension, cancer and CHD (4).

Obesity is a conditions decreases antioxidant capacity, by elevated levels of cholesterol (TC), triglycerides (TG), Low Density Lipid (LDL), Very Low Density Lipid (VLDL) and also significantly increased the level of High Density Lipid (HDL).(5),and lowering the levels of antioxidant enzymes (catalase, glutathione peroxidase (GPx) and glutathione reductase (6) and (7).

According to (5) results, that showed that treatment with GA significantly reduced the elevated levels CHL, TG, LDL,VLDL and also significantly increased the level of HDL.

According to l (5) who taken the anti-stress effect of gallic acid on immobilization induced-stress in male albino Wistar.

The result showed that treatment with GA significantly reduced the elevated levels of plasma and tissue cholesterol (CHL), triglycerides (TG), Low Density Lipid (LDL), Very Low Density Lipid (VLDL) and also significantly increased the level of High Density Lipid (HDL).

Study of (8) was showed that the treatment with doxorubicin increased the levels of serum cardiac and lipid biomarker which were brought down by gallic acid treatment.

According to (9) study revealed that treatment with gallic acid effectively reduced elevated LDL, VLDL and increased HDL levels .

Also in the study of (10), the anti-obesity effect of GAin an animal model of diet-induced obesity was investigated. GA was given as a supplement at the levels of 50 and 100 mg/kg rat for a period of 10 weeks, the results showed that the AST and ALP in the high food diet (HFD) groups were significantly decreased as compared with the HFD group. Also the intake of GA (50 and 100 mg/kg rat) for 10 weeks in Wister rats did not affect the serum AST and ALP.

According to (11) , pointed out that GA decreased Pb induced oxidative damages not by decreasing Pb bioaccumulation, but by improving antioxidant defenses, thus GA may be promising in the treatment of Pb intoxications.

Study by (12) showed that, lindane treated rats showed high level of urea than control animals. But given gallic acid after lindane treatment was decreased the level of urea was significantly, which could be due to the protective effect of gallic acid.

MATERIALS AND METHODS

The experiment was conducted at the animal house of the Veterinary Medicine College–University of Basrah. Twenty four males rats sexually mature, 6-8 weeks old, and of 140-200 grams weights were used. They were given free access to the different dietary formulations (table 1) and water ad labium for four weeks. The rats were allowed to use the experimental diet for one week before the start of experiment.

Table (1) Components of experimental diets (gm/kg diet)

Ingredients groups	Control group	Gallic group	Cholestrol group 1.5%	Protective group	Therapeutic group	Negative Control group
Casein	200	200	200	200	200	200
Corn starch	600	600	600	600	600	600
Vitamins and minerals mix.	50	50	50	50	50	50
Corn oil	50	50	50	50	50	50
Cellulose	50	50	50	50	50	50
Cholesterol	0	0	15	15	15	15

Rats were divided randomly into 4 equal groups (6 rats in each group) and treated for 4 weeks as following:

1-The control group In this group, 6 male rats were injected intraperitoneally (I.P.) with 0.9 % normal saline (N.S) and were supply with the stander ration .

2- The first treated group were injected intraperitoneally (I.P.) with 100 mg/ kg gallic acid dissolve in 0.5 ml distill water by insulin syringe daily and were supply with the stander ration .

3- The second treated group were supply with the stander ration in addition to 1,5% cholesterol of the stander ration .

4- The third treated group were supply with standard ration in addition to 1,5% cholesterol, and were injected intraperitoneally (I.P.) after one hour with 100 mg/kg gallic acid dissolve in 0.5 ml distill water by insulin syringe.

After anesthetization of animal by placing rat inside tightly closed container which contain cotton soaked with chloroform as alighted anesthesia.

Blood samples were collected from the heart by direct heart puncture. Blood samples were poured into plain tubes centrifuged at (3000 rpm for 15 minutes) to separate the serum .The serum then was poured into specific tubes Ependr of which stored at -4 ˚C until it can used for different biochemical parameters measurements, such as TC, HDL-C,LDL-C ,VLDL-C,Triglyceride, AST, ALT, and urea.

RESULTS

The effects of gallic I.P injection on serum lipid profile was presented in table (2). It seems that gallic injection with normal diet has no effect on the serum total cholesterol (TC), triglyceride, LDL-C and VLDL concentration and HDL-C compared with control animals. Fed animal cholesterol 1.5% diet led to elevated in (TC) and LDL-C and VLDL, compared with the control group , No significant changes were seen in concentration of HDL and triglyceride .When the gallic was injected after almost one hour of cholesterol administration in rats food ,a significantly reduction ($P \leq 0.05$) in the serum TC,LDL-C and VLDL compared with animals fed cholesterol 1.5% diet. No significant changes were observed in concentration of HDL and triglyceride.

Table (2) The effect of gallic acid on lipid profile in induced hypercholesterolemia rats : (Mean ± SD)

Groups / parameter	TC mg/dl	HDL –C mg/dl	TG mg/dl	LDL –C mg/dl	VLDL mg/dl
Control group	63.83 ± 6.04 bc	20.00 ± 5.69 ab	129.83 ± 10.40 a	18.00 ± 6.22 bc	25.83 ± 1.94 b
Gallic group	56.33 ± 4.88 c	17.83 ± 4.40 b	130.66 ± 24.49 a	12.33 ± 2.16 c	26.16 ± 4.79 b
Cholesterol group	92.16 ± 14.30 a	25.00 ± 7.12 a	113.16 ± 13.94 ab	44.66 ± 9.56 a	29.50 ± 2.66 a
Protective group	66.16 ± 3.48 bc	20.66 ± 5.68 ab	94.50 ± 19.92 b	26.50 ± 2.66 b	19.00 ± 3.79 c

LSD	12.83	7.16	19.83	9.33	3.66

Different small letter represent significant difference at (P≤0.05).

Activities of AST and ALT in the serum are generally tested as indicators for liver functions .The effect of gallic and a high cholesterol diet on AST and ALT of rats during the period of protective are shown in table (3).The enzyme activity of AST was not significantly affected by gallic compared with control group . The enzyme activity of AST is declined significantly in the serum of hypercholesterolemia rats compared with control and gallic group . When the gallic was injected after almost one hour of cholesterol administration in rats food, the AST elevated significantly (P≤0.05) compared with cholesterol group , and the protective values reach almost as the normal activity of the AST compared with control group. The enzyme activity of ALT was not affected significantly in the serum of gallic group compared with control group . But the enzyme activity of ALT was significantly elevated in the serum of hypercholesterolemia rats compared with control and gallic group. No significant affected was seen in the enzyme activity of ALT when the gallic was injected after almost one hour of cholesterol administration in rats food, compared with cholesterol group, but enzyme activity of ALT in the protective group almost was at the range of normal values compared with control and gallic groups.

Table (3) The effect of gallic acid on the Aspartate aminotansferas (AST),Alanin aminotransferase (ALT) activities (Mean ± SD) .

Groups / parameter	**AST (GOT)u/l**	**ALT (GPT)u/l**
Control group	99.50 ± 18.38 a	17.66 ± 6.68 b
Gallic group	87.00 ± 33.22 a	13.50 ± 3.83 b
Cholesterol group	42.66 ± 17.82 b	24.00 ± 4.51 a
Protective group	117.83 ± 35.82 a	21.00 ± 3.28 ab
LSD	30.83	6.00

Different small letter represent significant difference at (P≤0.05).

As shown in table (4), the level of blood urea in serum of male rats was obviously significantly increased (P≤0.05) by gallic compared with control group . The value of blood urea in hypercholesterolemia rats was reduced significantly (P≤0.05) compared with gallic and control group . When the gallic was injected after almost one hour of cholesterol administration in rats food, the blood urea elevated significantly (P≤0.05) compared with cholesterol group , and the protective urea blood value reach almost as the normal level as control group.

Table (4) The effect of gallic acid on the Blood Urea (Mean ± SD) .

Groups / parameter	Blood Urea mg/dl
Control group	40.16 ± 6.85 b
Gallic group	56.83 ± 8.70 a
Cholesterol group	23.33 ± 6.12 c
Protective group	47.00 ± 10.37 b
LSD	9.83

Different small letter represent significant difference at (P≤0.05).

DISCUSSION

The present study results showed that gallic injection with normal diet has no effect on the serum total cholesterol (TC), triglyceride, LDL-C and VLDL concentration and HDL-C compared with control animals when administrated for four weeks (Table 2) .Fed animal cholesterol 1.5% diet led to elevated in (TC) and LDL-C and VLDL, compared with the control group, No significant changes were seen in concentration of HDL and triglyceride . The elevation of TC may be due to increased rate of intestinal cholesterol absorption (13). and subsequently ,increased dietary cholesterol intake (14) .Lard fat is rich in saturated fatty acids, which known to increase serum and LDL-cholesterol, in addition to monounsaturated fatty acids, which can increase serum triacylglycerol's,(15)and (16). The high level of LDL-

cholesterol found in hypercholesterolemia rats may be attributed to a down regulation in LDL receptors by cholesterol and saturated fatty acids included in the diet (17). The present results run in parallel with those of other investigators Jang (18) and (19) and (10), except in TG and HDL . According to (6) that the obesity adversely affects plasma lipids, especially by increasing TG and decreasing the level of HDL-

cholesterol. The high cholesterol might lead to an increase in the synthesis of phospholipids and cholesterol esters in rats(20). The(21) indicated that the blood level of LDL-cholesterol and its oxidation are related to cardiovascular risk and the LDL-cholesterol level of blood is an index of health.

When the gallic was injected , significantly reduction in the serum total cholesterol TC ,LDL-C and VLDL was seen compared with animals fed cholesterol 1.5% diet . These results agree with results reported by (10), which also found that the GA groups had significantly decreased levels of TG, phospholipids, total cholesterol and LDL-cholesterol. This lipid profile was decreased by gallic acid is due to inhibition of hepatic cholesterol biosynthesis, increased fecal bile acid secretion and stimulation of receptor mediated catabolism of serum LDL cholesterol ,and due to lipogenesis inhibiting effect of gallic acid (8). According to (5), that reducing the elevated levels of cholesterol, triglyceride, LDL and VLDL and significantly increased HDL levels might be due to inhibition of stimulation of sympathetic nervous system.

The enzyme activity of AST is declined significantly but the enzyme activity of ALT was significantly elevated in animals administered high cholesterol diet for four weeks were observed in present study, this could be a single indicating occurrence of liver disorder (table 3).The disorder of liver may be caused by damage of the integrity of the heart and liver due to oxidative stress of high cholesterol diet which led to leakage into the animals serum (22).

Approximately 80% of AST in hepatocytes appear to be located in mitochondria(23), whereas ALT is thought to be predominantly non mitochondrial and has been postulated that in hepatocellular injury, when the hepatocytes plasmatic (but not the

mitochondrial membrane is damaged) cytoplasmic AST and ALT are released into serum with more sever hepatocellular injury (24), whereas mitochondrial membrane damage may resulted in the release of mitochondrial AST and elevating the AST/ALT ratio (25,26). According to (27), the elevated serum ALT levels in the absence of viral hepatitis has been reported to lead to a higher risk of cardiovascular disease with the risk greater in women . Studies have shown that increased serum enzyme activity is a reflection of cellular damage and alteration of functional membrane integrity,(28). The current results were almost similar to the results of her (29),and (19) studies who they reported that serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were significantly high in high-cholesterol fed diet than in normal rats.

When the gallic acid was injected after almost one hour of cholesterol administration in rats food , the AST elevated significantly but the ALT not affected .

The dosage used in the present study was consistent with those in many other studies

on the inhibitory effect of intake of phenolic acids in rats(30) and (31). The antioxidative effect of gallic acted as protective to preserve the damage in the hepatocyte that occurs due to high cholesterol diet. Gallic was able to ameliorate the effect after four weeks of treatment and may be its effect would be better if the period of treatment was extended. This results came in agreement with (10,19) and l(32) .

The present results shown in tabls (4) that the values of blood urea in serum of male rats was obviously significantly increased by gallic compared with control group . Elevated blood urea is known to be correlated with an increased protein catabolism in mammals and/or the conversion of ammonia to urea as a result of increased synthesis of arginase enzyme involved in urea production (33). The value of blood urea in hypercholesterolemia rats was reduced significantly compared with gallic acid and control group . Urea is an end product of protein and amino acid metabolism. Kidney filters excess urea into the urine and in sweat, but some goes into

the blood stream as serum urea. It is a well-known fact that, if blood urea levels are low, a problem centered in the liver might be suspected because urea is produced in the liver. Conversely, high blood levels of urea suggest that the kidney is not excreting urea normally (34). Kidney is vulnerable to damage because of larger perfusion and the increased concentration of excreted compounds that occur in renal tubular cells (35). When the gallic acid was injected after almost one hour of cholesterol administration in rats food, the blood urea elevated significantly compared with cholesterol group , and the protective urea blood value reach almost as the normal level as control group. The renoprotective effect of gallic acid from antioxidant and anti-inflammatory properties was demonstrated in many experimental studies in acute kidney injury against lindane (12), sodium fluoride (36) and chronic kidney disease (37).

It seems that nephrotoxicity is evidenced by the presence of renal lesions and of the decrease in urea concentration in serum (38).

التأثير الوقائي للحامض الاميني الكالكع لبعض المعايير البيوكيماوية التي يسببها ارتفاع الكولسترول المستحدث في الجرذان المختبرية

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الخلاصة

اشتملت الدراسة على تقييم دور الحامض الاميني الكالك من خلال التغيرات في بعض الاختبارات البايوكيميائيه في الجرذان المختبرية ذات الكوليسترول العالي لهذاالغرض صممت الدراسه لدراسة (24) من ذكور الجرذان

المختبريه البالغه عشوائيا والتي قسمت الى اربعة مجاميع متساوية لكل مجموعه (ستةحيوانات /مجموعه) ، جهزت مجموعة السيطرة بالعليقة المتوازنة بالإضافة الى حقن كل جرذ تحت ا لبريتون

(i.p) بماء مقطر ذو تركيز 0,9% ،

اما بالنسبة للمجموعة المعاملة الاولى فقد حقنت تحت البيرتون بحامض الكالك ذوالتركيز (100ملغم /كغم من وزن الجرذ) يوميا مذابة في (0,5مل) من الماء المقطر اضافة الى تجهيزها بالعليقه المتوازنه الاعتيادية ، واضيف الكولستيرول بنسبة 1,5% الى عليقة حيوانات المجموعة الثالثة ، بينما اضيف الكوليسترول الى عليقة المجموعة الثالثة بنسبة 1,5% ثم حقنت بعد ساعة واحده من وقت التغذيه بالحامض الاميني الكالك ذوالتركيز (100ملغم /كغم من وزن الجرذ) يوميا لمدة شهر.

أظهرت النتائج ان إعطاء حامض الكالك كعامل وقائي للجرذان المغذاة على عليقة عالية الدهون يحسن من فرط

الدهون عن طريق الانخفاض الواضح في معدلال دهون الكلي VLDL &LDL, (TC) بالمقارنة مع الحيوانات التي تتغذى على عليقة مضاف لها الكوليستيرول بتركيز 1,5%. فيما كانت فعالية انزيمات الكبد (AST&ALTفي المجموعة الوقائية ان اغلبيتها تميل الى المعدلات الطبيعية عند مقارنتها مع مجموعة السيطرة وكذلك بالنسبة لتركيز اليوريا التي تصبح قريبةجدا من المعدلات الطبيعية لمجموعة السيطرة.

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