EFFECT OF GALLIC ACID ON LIPID PROFILE AND ANTIOXIDANT STATUS IN CADMIUM CHLORIDE TREATED RATS

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ABSTRACT

This study was aimed to explore the role of gallic acid (GA) in ameliorating in reducing adverse effects of cadmium chloride ($CdCl_2$) on antioxidant status and lipid profile in adult male rats. Twenty-eight (28) adult male rats were divided randomly into four equal groups; they were daily handled for 30 days, as follows: control group (C), received tap water only. (G1), received 100ppm of $CdCl_2$ in drinking tap water, animals in proceeding groups were given in addition to $CdCl_2$ in drinking water the following: intraperitoneal injection of GA 100 mg/kg. daily (G2 group) and the combination of GA and $CdCl_2$ were given to rats in group (G3) in the same pattern. At the end of the experiment, fasting blood samples were collected and serum was isolated for measuring of antioxidant status and lipid profile. The results showed that administration $CdCl_2$ (G1 group) caused a case of dyslipidemia illustrated by significant elevation in serum cholesterol concentration in lipoprotein low density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-C), total cholesterol (TC), triglyceride (TAG) and non-HDL-C accompanied with significant decrease in cholesterol of high density lipoprotein (HDL-C) concentrations. The results also revealed a significant elevation in lipid indices including, coronary risk index (CRI), and cardiovascular risk index (CVRI) in $CdCl_2$ exposed rats. While significant elevation in malondialdehyde (MDA) and reduction in (GSH) concentrations observed in the same group comparing to gallic acid and control group, indicating a case of oxidative stress. The current results also recorded that intraperitoneal injection of GA against $CdCl_2$ caused amelioration of all previously estimated parameters.

Key Words: GSH, MDA, Dyslipidemia, Lipoproteins, Oxidative stress.

مجلة العلوم الزراعية العراقية -2023 :54(3):735-740 رمضان وآخرون

تأثير حمض الجاليك على صورة الدهون وحالة مانعات الأكسدة في الجرذان المعاملة بكلوريد الكادميوم

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المستخلص

هدفت الدراسة للتحري عن دور حمض الجاليك (GA) في تخفيف التأثيرات الضارة لكلوريد الكادميوم ($CdCl_2$) على حالة مضادات الأكسدة و صورة الدهون في ذكور الجرذان البالغة. تم تقسيم ثمانية وعشرين من ذكور الجرذان البالغة بشكل عشوائي إلى أربع مجموعات متساوية وتم التعامل معها يوميًا لمدة 30 يومًا، على النحو التالي: مجموعة السيطرة (C) تلقت ماء الصنبور فقط. المجموعة (G1)، أعطيت 100 جزء في المليون من $CdCl_2$ مع مياه الشرب، وأعطيت الحيوانات في المجاميع التالية بالإضافة إلى $CdCl_2$ في مياه الشرب كما يلي: الحقن داخل الصفاق (i / p) 100 ملغم / كغم / يوم / منGA للمجموعة G2 و تم إعطاء مزيج من GA و $CdCl_2$ للجرذان في المجموعة (G3) بنفس الجرعات المذكورة سابقًا وطريقة الإعطاء. في نهاية التجربة جمعت عينات الدم بعد التصويم وتم عزل مصل الدم لتقدير صورة الدهون وحالة مضادات الأكسدة. أظهرت النتائج أن كلوريد الكادميوم (المجموعة G1) تسبب في حالة من اضطراب البروتينات الشحمية في مصل الدم تجلت بارتفاع معنوي في تراكيز المصل من الدهون الثلاثية (TAG) والكوليسترول الكلي (TC) والبروتين الدهني منخفض الكثافة (LDL−C) والبروتين الدهني منخفض الكثافة جدًا. ارتفاع الكوليسترول في البروتين منخفض الكثافة جدا (VLDL−C) في غير HDL−C مع انخفاض كبير في تراكيز البروتين الدهني عالي الكثافة (HDL−C) في الدم. أظهرت النتائج أيضًا ارتفاعًا معنويا في مؤشر تصلب الشرايين (AI)، ومؤشر مخاطر الشريان التاجي (CRI) ومؤشر مخاطر القلب والأوعية الدموية (CVRI) في الجرذان المعرضة لكوريد الكادميوم. في حين لوحظت حالة الإجهاد التأكسدي والتي تميزت بانخفاض في تركيز ال GSH وارتفاع تركيز MDA في مصل الدم في نفس المجموعة مقارنة بحمض الجاليك ومجموعة التحكم. سجلت النتائج الحالية أيضًا أن حقن حمض الجاليك في الصفاق أو مزيج من كل من حمض الجاليك وكلوريد الكادميوم تسبب في تحسين جميع المعايير المقدرة مسبقًا.

الكلمات المفتاحيه :كلوتاثايون، مالونداديهاد، أضطراب الدهون، الشحوم البروتينية، اجهاد تاكسدي.

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INTRODUCTION

Cadmium (Cd) is regarded as environmental heavy toxic metals and a common industrial pollutant (28). Excessive release of Cd to the environment have increase the risk of human exposure via inhalation and ingestion, attributed to its presence in air, food as well as tobacco leaves (38). Hepatic and renal dysfunction were observe after prolong exposure to Cd (10,13). Besides, vascular damage (18), testicular toxicity (27) anemia, osteoporosis, and bone fractures (11) were also reported. Toxicity of Cd has been associated with oxidative stress via it high affinity to thiol group specially of GSH which result in alteration in intracellular redox status, releasing of lipid peroxides (LPO) products such as MDA and hydroperoxides that stimulating deleterious effect on essential biochemical processes (3). Gallic acid (GA) is a natural phenolic acid percent in different plant including; grapes, pomegranate and green tea (22). GA and its derivatives possess numerous uses in the food, cosmetic, printing, pharmaceutical and dyeing industries (29). It has been documented that consumption of foods rich in GA, induced hypoglycemia and attenuate the health hazard effects in obese individuals (36). GA has been implicated as antioxidant (34), antibacterial (21) and antitumor (23) agents. In addition to its protection against myocardial infarction (19). Besides, protective role of GA on testicular damage (1) and DNA fragmentation against cyclophosphamide was recorded in mice (25).

MATERIALS AND METHODS

A total of 28 adult Wistar male rats were randomly divided into four equal groups and were treated daily for 30 days as follows: Group C: Rats of this group were allowed to *ad libitum* supply of drinking water (control group), Group G1: Rats of this group were allowed to *ad libitum* supply of drinking water containing 100 ppm of $CdCl_2$; animals in proceeding groups were given in addition to $CdCl_2$ in drinking water, intraperitoneal (i/p) injection of 100 mg/kg/day/ of GA (G2group), while the combination of both GA and $CdCl_2$ were given to animal in group (G3), in the same previous mentioned doses and method of administration. Fasting blood (for 8-12 hrs.) samples were collected (by cardiac puncture technique) at the end experiment, centrifuged at 2500rpm for 15 minutes, and then serum samples were liquated and frozen at -20 °C until analysis. Serum lipid profile including: TAG, TC, HDL-C were measured using enzymatic kits (Linear chemicals, Barcelona /Spain); LDL-C, VLDL-C and non-HDL-C concentrations according to Friedewald *et al.*, (12). Atherogenic index (AI), coronary risk index (CRI) and cardiovascular risk index (CVRI) were measured according to Abbott *et al.*, and Alladi *et al.*, (2,6). Serum GSH and MDA concentrations were measured using ELISA kits (My BioSource, USA) according to Burtis and Ashwood (8). Statistical analysis of data was performed on the basis of One-Way Analysis of Variance (ANOVA) using a significant level of ($P \leq 0.01$). Specific group differences were determined using least significant differences (LSD) as described by Snedecor and Cochran (37).

RESULTS AND DISCUSSION

The mean values of serum lipid profile concentrations in the control and treated groups at zero and after 30 days of treatment were clarified in Table 1. The result showed that i/p injection of gallic acid (G2) or combination of both (G3) for 30 days caused significant ($P \leq 0.01$) decrease in serum TC, TAG, VLDL-c and LDL-c concentration comparing to the values in G1 group that received $CdCl_2$ alone. Furthermore, serum HDL-c concentrations decreased significantly ($P \leq 0.05$) in the G1 treated group as compared to the control and other treated groups. Cardiovascular risk index (CVRI) and Atherogenic index (AI), (Table 2), non HDL-C and coronary risk index (CRI) (Table 3) showed a significant elevation ($P \leq 0.01$) in $CdCl_2$ group comparing to GA and control groups.

Table 1. Effect of gallic acid on serum lipid profile concentrations in normal and cadmium chloride treated rats

Groups	Mean ± SE (mg/dl)				
	LDL-C	HDL-C	VLDL-C	Total Cholesterol	Triglyceride
Control	59.93 ±0.67 b	42.06 ±0.65 b	19.04 ±0.17 b	121.03 ±0.35 b	95.21 ±0.87 b
G1: $CdCl_2$	86.78 ±0.78 a	29.87 ±0.26 d	25.74 ±0.32 a	142.40 ±0.73 a	128.72 ±1.60 a
G2: Gallic acid	31.67 ±0.82 c	54.41 ±0.53 a	14.47 ±0.19 d	100.56 ±0.51 d	72.39 ±0.98 d
G3: $CdCl_2$ + Gallic acid	59.89 ±1.60 b	36.07 ±0.44 c	18.06 ±0.16 c	114.02 ±1.42 c	90.30 ±0.84 c
LSD value	3.0349 **	1.455 **	0.655 **	2.503 **	3.275 **
Means having with the different letters in same column differed significantly ** (P≤0.01).					

Values are expresses as mean ± SE. n= 7 / each groups. C: Control group rats were given tap water, G1: received 100ppm of $CdCl_2$ in drinking tap water, G2: Rats were injection intraperitoneally (i/p) of 100 mg/kg/day/ of GA, G3: rats were given combination of GA and $CdCl_2$ for 30 day.

Table 2. Effect of gallic acid on coronary vascular risk index (CVR1) and Atherogenic index in normal and cadmium chloride treated rats

Groups	Mean ± SE (ratio)	
	Coronary vascular risk index (CVR1)	Atherogenic index
Control	2.26 ±0.03 c	1.420 ±0.04 c
G1: $CdCl_2$	4.31 ±0.08 a	2.90 ±0.05 a
G2: Gallic acid	1.33 ±0.02 d	0.578 ±0.02 d
G3: $CdCl_2$ + Gallic acid	2.50 ±0.04 b	1.66 ±0.06 b
LSD value	0.156 **	0.129 **
Means having with the different letters in same column differed significantly ** (P≤0.01).		

Values are expresses as mean ± SE. n= 7 / each groups. C: Control group rats were given tap water, G1: received 100ppm of $CdCl_2$ in drinking tap water, G2: Rats were injection intraperitoneally (i/p) of 100 mg/kg/day/ of GA, G3: rats were given combination of GA and $CdCl_2$ for 30 day.

Table 3. Effect of gallic acid on non HDL-C and CRI in normal and cadmium chloride treated rats

Groups	Mean ± SE	
	non HDL-C (mg/dl)	CRI (ratio)
Control	78.97 ±0.64 b	2.87 ±0.04 c
G1: $CdCl_2$	112.52 ±0.95 a	4.64 ±0.13 a
G2: Gallic acid	47.49 ±1.81 c	1.84 ±0.02 d
G3: $CdCl_2$ + Gallic acid	77.95 ±1.72 b	3.16 ±0.06 b
LSD value	4.017 **	0.234 **
Means having with the different letters in same column differed significantly ** (P≤0.01).		

Values are expresses as mean ± SE. n= 7 / each groups. C: Control group rats were given tap water, G1: received 100ppm of $CdCl_2$ in drinking tap water, G2: Rats were injection intraperitoneally (i/p) of 100 mg/kg/day/ of GA, G3: rats were given combination of GA and $CdCl_2$ for 30 day.

Dyslipidemia and oxidative stress were the major deleterious effects induced by Cd administration in the current study. It has been documented that several enzymes correlate with lipid metabolism has been inhibited by Cd leading to dyslipidemia and atherosclerosis. An elevation in serum hepatic activity hydroxyl methyl glutaryl-CoA reductase (HMG-CoA) was observed after Cd administration, which can alter cholesterol synthesis and lipid metabolisms (5). The results also showed dyslipidemia caused by Cd in the current study was accompanied with reduction in antioxidant status, documented that oxidative stress induced by Cd could be a mechanism for its dyslipidemic effect (35). In the present study restoration of lipid profile by GA indicating its hypolipidemic effect. The inhibitory effect of gallic acid-derivatives rich fruit, pomegranate (*Punica granatum*) extracts on α-amylase, lipase, α-glycosidase and trypsin enzyme activities (40) has been reported. Another mechanism for hypolipidemic effect of GA could be due to its inhibitory effect on the rate limiting enzymes (HMG-CoA reductase) in cholesterol synthesis (4), in addition to its hypoglycemic effect (33), that lead to decrease in TAG formation (16). Lipid indices evaluation (AI, CRI and CVRI) is used to document the role of lipid profile as risk factor for cardiac vascular diseases (15,39). Their elevation by $CdCl_2$ indicating its cardiotoxic effects. While, reduction in these indices by GA indicating its beneficial supplement in improving lipid profile and cardiovascular risk. The result in Table 4 showed significant ($P \leq 0.01$) elevation in serum GSH concentration and decrease in serum MDA in G2 and G3group comparing to G1 treated group at the end of the experiment.

Table 4. Effect of gallic acid on serum glutathione (GSH) and malondialdehyde (MDA) concentrations in normal and cadmium chloride treated rats

Groups	Mean ± SE (umol/liter)	
	GSH	MDA
Control	25.05 ±0.35 b	10.59 ±0.25 c
G1: $CdCl_2$	18.20 ±0.27 c	15.84 ±0.22 a
G2: Gallic acid	35.89 ±0.76 a	7.74 ±0.29 d
G3: $CdCl_2$ + Gallic acid	24.87 ±0.37 b	11.89 ±0.21 b
LSD value	1.406 **	0.721 **

Means having with the different letters in same column differed significantly ** ($P \leq 0.01$).

Values are expresses as mean ± SE. n= 7 / each groups. C: Control group rats were given tap water, G1: received 100ppm of $CdCl_2$ in drinking tap water, G2: Rats were injection intraperitoneally (i/p) of 100 mg/kg/day/ of GA, G3: rats were given combination of GA and $CdCl_2$ for 30 day. The results of current study also indicated elevation in serum MDA and depletion in GSH concentration in $CdCl_2$ treated group which documented that oxidative stress is possible mechanism for Cd toxicity (7,26). Cadmium being a Fenton metal (24), decrease the activity of antioxidant enzyme, bind to thiol groups of GSH, caused its depletion (9,30), this further weakened the body antioxidant defense system causing over production of reactive oxygen species ,with subsequent enhancing of LPO followed by elevation in MDA level (32) as documented in this study. Besides, inhibiting pancreatic lipase activity, improving glucose uptake, and adipogenesis blocking by GA (17) could be a mechanism. Our findings showed that GA administration caused a rise in GSH and decrease in MDA levels indicating its antioxidant activity (31). It has characteristics of the strong free radical scavenging and antioxidant activities, boosting the concentrations of non-enzymatic antioxidant system, such as GSH and it can protect different organs and tissues from injury and damages induced by oxidative stress (14,20). In conclusion, the present study the beneficial role of GA as antioxidant and hypolipidemic effect against Cd exposure in rats.

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