Study The Effect of Thiadiazole and Thiazine Derivatives as Antiatherogenic and Antioxidant Agents in Induced Hyperlipidemic Mice

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Abstract

This study conducted to examine the effect of two newly synthesized compounds[3-(5-(ethylthio)-1,3,4-thiadiazol-2-yl)-2,3-dihydro-2-(3-nitrophenyl)benzo[1-3-e] thiazin-4-one (compound I) and 5(4-dimethyl amino) benzylidene amino)-1,3,4-thiadiazole-2-thiol(compound II)] as antiatherogenic and antioxidant agents, *In vivo* study.

The study was carried out on sixty male Wister mice aged seven to eight weeks their weight ranged(180-200 g). The mice were grouped as: group(1)(G1): control group (20 mice).Group(2)(G2):consisted of 40 mice in which the mice were daily administered cholesterol (25mg/kg/day) in coconut oil 6% and creamy cheese for 28 days. Lipid profile (total cholesterol (Tch), triglyceride (TG), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol VLDL-c), was measured for 12 of mice chosen randomely from G2 to assure hyperlipidemia. Then group2 (G2) subdivided into two groups: group (2.A): (14 mice) which the mice were daily treated with (10^{-4})M of compound (I)via drinking water for 20 days. Group(2.B):(14 mice) which the mice were daily treated with (10^{-5})M of compound II via drinking water for 20 days. Lipid profile, catalase, total oxidative status (TOS), total antioxdant status (TAS) , and atherogenic index of plasma (AIP) were determined in all studied groups. The results showed significant

elevation in levels of Tch, TG, LDL and VLDL, while there are significant reduction in HDL-c levels in G2 comparing to G1. After orally administration of compounds I and II from the mice, the results revealed that the levels of Tch, TG, LDL and VLDL were reduced while the levels of HDL-c was elevated. Catalase activity showed significant decrease in G2 when compared with G1. Also, a significant elevation was found in AIP, TOS and TAS in G2 when comparing to G1. Also, significant elevation was noticed in catalase activity after administration of compounds I and II, in addition to reduction in levels of AIP, TOS, and TAS. The conclusion could be drawn from this study that compounds I and II could be considered as antiatherogenic and antioxidant agents.

Key words: Lipid lowering compounds, Thiazine & Thiadiazole derivatives, *In vivo* study.

Introduction

Hyperlipidaemia is an increase in the lipids, and lipoproteins[(1)]. The combination of certain risk factors such as dyslipidemia can act multiplicatively or synergistically to increase the risk of atherosclerosis and heart disease [(2)].

Oxidant generation is part of the normal metabolism of many types of cells and it is critical for cell homeostastis. To protect itself against exposure to noxious oxidants. The airway mucosa has developed an antioxidant system[(3)] . Cells infiltrating the nasal mucosa in patients with AR produce a variety of mediators, including reactive oxygen species (ROS). This leads to an imbalance between the oxidative forces and the antioxidant defense systems which is believed to favor an oxidative injury that has been causes tissue damage [(4-6)].

Thiazines and Thiadiazole compounds have gained importance in medicinal and pharmaceutical fields due to a broad spectrum of biological activities like antioxidants, even at small concentration and thus have diverse physiology role in the body. Antioxidant act as radical scavengers and help in converting the radicals to less species and promote the repair of relative oxygen species induced damage to cell structures [(7-10)].

The aim of the present study is to examine the effect of some derivatives of thiadiazole and thiazine which may be considered as antiatherogenic and antioxidant agents.

These compounds were prepared in previous study[11].

Subjects and Methods

The studied groups comprised with sixty male Wister mice obtained from animal house , from the college of Medicine, Baghdad University, the mice divided into group(1): which consist of 20 mice as control,(G 2): consist of 40 mice in which the mice were daily administered cholesterol (25mg/kg/day)[12] in coconut oil 6% and creamy cheese for 28 days. Lipid profile were measured for 12 mice chosen randomly from G2 to assure hyperlipidemia. After that G2 were subdivided into two groups as follows:

G2A: (14 mice) in which the mice were daily treated with (10^{-4})M of compound (I)via drinking water for 20 days.G2B: (14 mice) in which the mice were daily treated with (10^{-5})M of compound II via drinking water for 20 days.

The chosen concentration was based on *in vitro* and *in vivo* previous studies which given the best inhibition from the other concentrations[13,14].

The blood samples were collected and blood serum was prepared by centrifuging blood at 2600 rpm for 15 min. Serum was frozen until the assay. Total cholesterol[15] , high density lipoprotein- cholesterol (HDL-c)[16] and triglyceride (TG) [17] were determined by using commercial kits (Randox, France).

Atherogenic index of plasma (AIP) calculated from the formula[18] :

log(TG/HDL-c).

Catalase activity was measured by using the method that depends on its ability of H_2O_2 decomposition to give H_2O and O_2[19]. Serum TOS was also determined using a automated measurement method, developed by Erel[20]. in this method, oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by abundant glycerol molecules in the reaction medium. The ferric ion forms a colored complex with xylenol organ in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules in the sample. The assay is calibrated with hydrogen peroxide and results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 Eq/L). Serum TAS was determined using automated method [21] involving the

production of hydroxyl radical, the most potent of biological radicals. The antioxidant effect of the sample against the potent free-radical reactions initiated by the hydroxyl radicals produced is measured. Student t-test was used to examine the differences of mean. The results were expressed as mean ±SD and P≤ 0.05 was considered significant.

Result and Discussion

Table (1) show the levels of lipid profile in G1, G2, G2A and G 2B.The results showed significant elevation in levels of Tch, TG, LDL, VLDL, and AIP while there were significant reduction in HDL levels in G2 comparing to control group(G1). The results revealed that the levels of Tch, TG, LDL and VLDL were reduced while the levels of HDL-c was elevated after administration of compounds I and II in G2A and G2B respectively. Antihyperlipidemic agents which are active in cholesterol induced hyperlipidemic model function by one or more mechanisms. Therefore, higher intake of dietary cholesterol increases serum lipid profile by down regulation of LDL-c receptor synthesis thus decreasing uptake of LDL-c via these receptors[22].

Some researches demonstrated that the actions may be due to increased inhibition of intestinal absorption of cholesterol, interference with lipoprotein production, increased expression of hepatic LDL receptors and their protection etc. leading to an increased removal of LDL-c from the blood and its increased degradation and catabolism of cholesterol from the body[23].

The logarithamatically transformed ratio of plasma TG to HDL correlated closely with the LDL particle size and could sever as an indicator of the atherogenic lipoprotein phenotype [24]. AIP indicates a balance between the actual concentration of plasma TG and HDL. Clinical studies have shown that AIP predicts cardiovascular risk and it is an easily available cardiovascular risk marker and a useful measure of the response to treatment[25].

Table (2) show the catalase activity, levels of TOS and TAS were significant decrease in G2 in levels of TOS and TAS when compared with G1. Also, a significant increase was found G2 compared with G1 in patient group. Catalase is the enzyme, which protects the cells from accumulating of hydrogen peroxide by dismutating it to form water

and oxygen or by using it as an oxidant in which it works as peroxidase[26].

After orally administration of compounds I and II to the mice, the results revealed that the levels of Tch, TG, LDL and VLDL were reduced while the levels of HDL-c was elevated. Catalase activity showed significant decrease in G2 when compared with G1. Also, a significant elevation was found in AIP, TOS and TAS when comparing to group 2. A, significant elevation was noticed in catalase activity after administration of compounds I and II, in addition to reduction in levels of AIP, TOS, TAS and AIP.

The main difficulties associated with the use of oxidative stress markers are the complexity and cost of the methods and equipment required. Since the measurement of individual antioxidant molecules is not feasible and since the antioxidant effects of these molecules are additive, we measured total oxidative status (TOS) and total antioxidant status(TAS)[27,28]. Although the complete mechanism of this elevation is unknown, it is proposed that TAS was increased as to compensate for the increase in total oxidative stess[29].

The conclusion could be drawn from this study that compounds I and II could be considered as antiatherogenic and antioxidant agents.

References

1. Camphell, M.K. and Farrel SO., Biochemistry Thomson Brooks learing. 7th ed, (2014).
2. Zamorano, J. and Edwards, J. Combining antihypertensive and antihyperlipidemic agents-optimizing cardiovascular risk factor management. Int. Blood Pressure Cont. ; 4:55-71(2011).
3. Chole, R.H. Patil, R.N. Basak, A. Palandurkar, K. and Bhowate, R. Estimation of serum malondialdyde in oral cancer and precancer and its association with healthy individuals gender, alcohol, and tobacco abuse. J. cancer Res. Ther; 6:487-91(2010).
4. Emin, O. Hasan, A. Aysegul ,D. and Rusen, D. Total antioxidant status and their relationship to total IgE levels and Eosinophil counts in children with allergic rhinitis. J. Allergol Clin. Immunol; 22:188-192(2012).
5. Marple, B.F. Allergic rhinitis and inflammatory airway disease: interactions within the unified airspace. Am J. Rhinol Allergy; 24:249-54(2010).
6. Deepa, D.S. Subhas, B.G. Shishir, R.M. and Preethi ,B. Estimation of serum malondialdehyde in potentially malignant disorders and post-antioxidant treated patients: A biochemical study.164:100-31(2013).

7. Gupta, S. Reddy, M.V. and Harinath, B.C. Role of oxidation stress and antioxidants in aetiopathogenesis and management of oral submucous fibrosis. Indian J Clin Biochem. dian J 19: 138-41(2004).
8. Bala. S. Uppal, G. Kamboj, S. Saini, V. and Prasad, D.N. Design, characterization, computational studies, and pharmacological evaluation of substituted-N`-[(IE) substitutedphenylmethyli-dene]benzohydraide analogs. Med. Chem. Res. 21:1-3(2012).
9. Shivrj, S.B. and Sambhaji. V.P. Simple and Efficient Synthesis of Novel Fused Bicgclic Heterocycles Pyrimido-Thiazine and Their Derivatives. Organic Chem. Cur. Res.;1:5(2012).
10. Miri, R. Razzaghi-asl, N. and Mohammadi, M.K. QM study and conformational analysis of an isatin Schiff base as a potential cytotoxic agent. J of Mol. Mod. ; 19:727-735(2013).
11. Ruwaidah, S. Ali, H.S. and Khalid, F.A. (2013) Synthesis and Characterization of new Heterocyclic Compounds Derived from 1,3,4-Thiadiazole and their antibacterial study Athesis Submitted to the Council of college of Education for a pure Science, Ibn- Al-Haitham, University of Baghdad, in partial fulfillment of requirements for the Degree of Master of Science in Chemistry.
12. Deng, R. Food and food supplements with hypocholesterolemic effects, Recent patents ,FoodNutr. Agric.;1:15-24(2009).
13. Zeinab, M. Geihd, H. Tamara, A. Kinetic Study of the Effect Some Novel Lipid Lowering Compounds on Creatine Kinase and 3-Hydroxy-3-Methyl-Glutaryl-Co enzyme A Reductase Activities, J of Nat. Sci. Res.; 4(10),99-105(2014).
14. Zeinab, M. Geihd, H. Tamara, A. Kinetic Study of the Effect of Some Novel Lipid Lowering Compounds on Creatine Kinase and 3-Hydroxy-3-Methyl-Glutaryl-CoA Reductase Activities In Mice Induced Hyperlipidemia, Ibn Al-haitham Jour. Pure & Apple. Sci.; 1(28): 1-12(2015).
15. Allain, c. Poon, L. and Chan, S. Enzymatic determination of total serum cholesterol. J. Clin. Chem.; 20:(4)270-275(1974).
16. Burstien, M. Scholinck, H. and Morfin, R. Rapid method for the isolation of lipoproteins for human serum by precipitation with polyanions. J. Lipids Res.;11:583-595(1970).
17. Bucolo, G. and Divid, H. Quantitive determination of serum triglycerides by the use of enzymes. Clin. Chem.; 19(5):467-482(1973).
18. Ikewnch, C. Alternation of plasma lipid profile and atherogenic index of cholesterol loaded rats: implications for the management of obesity and cardiovascular disease. Biokemistri; 21(2):96-102(2009).
19. Aebi, A. E. "eatalase" In Bergnmeryer H. v. Ed, Methods of Enjymatic Analysis, 3rd Edition vol 3, academia press, New York, 273-386(1933).
20. Erel, O.A. new automated colorimetric method for measuring total oxidant status. Clin Biochem.; 38:1103-11(2005).

21. Erel, O.A. a novel automated method to measure total antioxidant response against potent free radical reactions. Clin. Biochem.; 37: 112-9(2004).
22. Dhandea, R.S. Kaikinia, A.A. Patila, K.a. and Kadama, V. Antihyperlipidemic Activity of Bambusa Bambos (Druce.) and swertia chirata (Buch-ham) in cholesterol suspension induced hypercholesterolemia in rats. Maharashtra;6(1): 607-610(2014).
23. Ochani, PC. and Mello, PD. Antioxidant and antihyperlipidemic activity of Hibiscus sabdariffa Linn. Leaves and calyces extracts in rats. Indian J. of Exp. Bio. ;47:276-282(2009).
24. Vera, B. Johnson, B. Issam, Z. William, J. and Diane, V. The TG/HDL Cholesterol Ratio Predicts All Cause Mortality in Women With Suspected Myocardial Ischemia A Report from the Women's Ischemia Syndrome Evaluation (WISE). Am Heart J. ; 157(3): 548-555(2009).
25. Jeffrey, S. Screening for Cardiovascular Risk in Asymptomatic Patients. J. of the American college of cardiology; 55(12): 1169-1177(2010).
26. Hasa, F. and Esraa , H. Oxidative stress and the antioxidant mechanisms of Iraqi patients with polycystic ovary syndrome (POS). Iraqi J comm. med. ; 9(2): 196-200(2010).
27. Emrullah ,B. Ali, B. Esra, E. Mustafa, A. Yasemin, O. Sevilay, S. Mehmet, H. Pelin, Y. and Frauk, O. Changes of total antioxidant capacity and toal oxidant status of aqueous humor in diabetes patients and correlations with diabetic retinopathy. Int J ophthalmol; 6(4): 531-536(2013).
28. Fatemeh, B. Sedighe, S. Mahtab, B. Lili, K. Maryam, M. Mohammad, T. and Nima, R. Total Antioxidant status in patients with major B-Thalassemia. Iran J Pediatr; 21(2):159-165(2011).
29. Kaya s, H L., Keskin, B., Ustuner, I . and Avsar ,A. Reduced total antioxidant status in postterm pregnancies. Quarterly medical journal; 17(1): 55-59(2013).

Table(1): The Levels of Lipid profile in all studied Groups.

Parameters / Groups	G1 n=12	G2 n=12	G2A n=12	G2B n=12
Tch (mg/dl)	155±2.4	600±20.5	340±0.97	172±0.91
TG (mg/dl)	100.3±5.1	325.01±15.6	299±9.3	205±10.3
HDL (mg/dl)	43±6.9	25.6±4.2	40.1±6.1	50.3±4.01
LDL (mg/dl)	62.1±3.4	510±9.3	240±2.7	81±2.4
VLDL (mg/dl)	30.1±0.19	65.3±0.25	59.8±0.43	41.6±0.74
AIP	0.36±0.13	1.10±0.56	0.87±0.17	0.60±0.40

P-values <0.05 was considered statistically significant

Table(2): The level of oxidant-antioxidant status in all studied Groups.

Parameters / Groups	G1 n=12	G2 n=12	G2A n=12	G2B n=12
Catalase (U/ mg)	0.82±0.04	0.09±0.001	0.63±0.03	0.54±0.01
TAS (mmol/L)	1.61±0.34	5.54±0.63	1.71±0.23	1.83±0.4
TOS (µmol/L)	7.29±1.5	12.9±3.3	0.49±0.03	0.94±0.03

P-values <0.05 was considered statistically significant

دراسة تأثير مشتقات جديدة للثياديازول والثيازين كعامل مضاد لتصلب الشرايين ومضاد الاكسده في الفئران المحثة بارتفاع الدهون

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الخلاصة:

الهدف من هده الدراسة هو تقدير تأثير بعض مشتقات الثياديازول والثيازين الجديدة التحضير كمضاد لتصلب الشرايين ومضادات الأكسدة بالاضافة الى قياس صورة الدهون في الفئران المختبرية التي تم حث ارتفاع الدهون لديها بواسطة تغديتهم بالاغدية الغنية بالكولستيرول . تم اختبار تأثير المركب (I) بتركيز(10^{-4}) مولاري والمركب (II) *بتركيز* (10^{-5}) مولاري داخل الخليةالحية .

حيث أجريت الدراسة على ستين فأرآ من الذكور الذين تتراوح اعمارهم بين سبعة الى ثمانية اسابيع (180−200 g) والتي تم الحصول عليها من البيت الحيواني في كليه الطب ,جامعة بغداد .تم تقسيم الفئران المختبرية كما يلي:

المجموعة الاولى: مجموعة السيطرة(20 فأرا") والمجموعة الثانية: تتكون من (40 فأرة) التي كانت تعطى يوميا الكولسترول (25mg/kg/day),زيت جوز الهند% 6 وجبن كامل الدسم لمدة (28)يوم . تم قياس صورة الدهون في 12 فأر من المجموعة الثانية تم اختيارم عشوائيا لتشخيص ارتفاع الدهون فيها. ثم قسمت المجموعة الثانية الى مجموعتين على النحو التالي:

المجموعة 2A: (14 فأرة) حيث عولجت هذه الفئران يوميا بالمركب الاول بتركيز(10^{-4}) مولاري عن طريق الشرب لمدة (20 يوم).المجموعة 2B: (14 فأرة) حيث عولجت هذه الفئران يوميا بالمركب الثاني بتركيز(10^{-5}) مولاري عن طريق الشرب لمدة (20 يوم). وقد أجريت هذه الدراسة على ستين ذكر من الفئران الذين تتراوح أعمارهم بين سبعة إلى ثمانية أسابيع وزنهم تراوحت (180−200 غرام). مستوى الدهون في الدم، الكتاليز، وتم تحديد سعة الاكسدة الكلية (TOS) ، سعه مضادات الاكسده الكليه (TAS)، ومؤشر تصلب الشرايين من البلازما (AIP) في كل المجموعات المدروسة .أظهرت النتائج ارتفاع كبير في مستويات الكولستيرول، الدهون الثلاثيه، الدهون البروتينيه الواطئه الكثافه و الدهون البروتينيه الواطئه الكثافه جدا ، في حين أن هناك انخفاض كبير في مستويات الدهون البروتينيه العاليه الكثافه في المجموعه الثانيه مقارنة مع المجموعه الاولى بعد اعطاء إدارة المركب الأول والثاني للفئران وقد لوحظ وجود ارتفاع كبير من سعه الاكسده الكلية (TOS)، سعه مضادات الاكسده الكلي (TAS)عند المقارنة بالمجموعة الثانيه و أيضا لوحظ ارتفاع كبير في نشاط الكتاليز بعد ادارته من المركب الأول والثاني، بالإضافة إلى انخفاض في مستويات وتم تحديد سعه الاكسدة الكليه (TOS) ، مضادات الاكسده الكلية (TAS)، ومؤشر تصلب الشرايين من البلازما (AIP) ويمكن الاستنتاج من هذه الدراسة بأن المركب الاول والثاني يمكن ان يقدر كعامل مضاد لتصلب الشرايين ومضاد الاكسدة .

الكلمات المفاتحيه: مركبات خفض الدهون، مشتقات الثياديازول والثيازين في الخليه الحيه.