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Comparison of Simvastatin and Rosuvastatin in Oxidation and Antioxidant Levels in Male Rabbits Infected with Experimental Lipid Disorder

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ABSTRACT

The study was carried out in the animal house of Samarra University, This study included evaluation of two drugs as antioxidants outside the body. As well as comparing the effect each of them on oxidation and antioxidant of infected male rabbits with experimental lipid disorder by treated them with Triton x100. The 48 domestic rabbits were used, divided into 6 groups and each group containing 8 rabbits. The level of oxidation and antioxidant was measured malondialdihyde, peroxy nitrite and ceroplasmin. The results showed that simvastatin has a high antioxidant capacity compared with rosuvastatin which has a lower susceptibility to the standard substance (vitamin C). The results showed a significant increase of P≤0.05 in the concentration of malon dialdehyde and peroxy nitrite, and a significant decrease P≤0.05 in the concentration of ceroplasmin in the serum of the infected group, also the results showed a significant decrease P≤0.05 in malon dialdehyde in the serum of all groups compared to the infected group. The fifth group which was treated with rosuvastatin demonstrated a significant decrease P≤0.05 in malon dialdehyde compared with the other groups. The results exhibited a significant decrease P≤0.05 in the concentration of pyroxy nitrite root in the serum of all groups compared to the infected group without significant differences. The results showed a significant increase of P≤0.05 in ceroplasmin concentration in the serum of all groups compared with the infected group.

Introduction

Atherosclerosis is a chronic disease lead to hardening of the artery wall and increases its thickness and narrowing the diameter to reduce the passage of oxygen-laden blood and reduces the ability of the artery to expand and contract due to accumulation of fat, cholesterol, calcium and other blood-borne substances. They represent fat-filled cells known as foam cells as a primary lesion of atherosclerosis, where it represents fatty carrots of cholesterol in the muscle fibers smooth lining of the blood vessels and heart muscles which is the first factor for the formation of porridge or Thrombus [1].

The oxidation of low-density lipoproteins is the key step for the development of atherosclerosis. Oxidation of Low density lipoprotein – cholestrol LDL-c is indicated by the high level of malon dialdehyde (MDA), which indicates oxidative damage to the body. It has been found that there are many cells that have the ability to oxidize LDL-c. These cells are phagocytic cells, single cells, neutrophil cells and smooth muscle cells. The production of large amounts of free radicals increases the damage of the ventricular lining and the heart muscle [2].

Many drugs have been used that can restore the lining of the vessel which has been adversely affected by the risk factors and thus reduced the rate of response. These substances may be nutritious, such as plants containing estrogens such as pomegranate, garlic, oatmeal, flax and others[3], or anti-platelet aggregates such as Aspirin[4], As well as drug treatments such as Statins and derivatives[5].

Simvastatin is one of the Statins, which is not soluble in water and used as a drug against hyperlipidemia and hypercholesterolemia, lowering its level in the blood. And its direct effect in lowering cholesterol by

inhibiting its production in the liver by inhibiting the enzyme 3-hydroxy-3-methelgiutaryl coenzyme A reductase (HMG-CoA reductase), and regulate the construction of LDL-c receptors on liver cells [6] In addition, Simvastatin has a role in reducing oxidative stress by influencing NADPH oxidase and stop the effect of effective oxygen varieties by regulating the work of antioxidant enzymes thus the equation of free radicals[7].

Rosuvastatin is a statin dissolved in water. It contains a large number of binding sites with HMG-COA reductase, which shows a greater inhibitory activity of the enzyme and thus the synthesis of cholesterol, and restricts the formation of low-density lipoproteins - LDL-c[8],as well as its play an important role in antioxidant capacity and oxidative damage[9]

Materials and methods

Fourty eight adult male rabbits were used, 8-10 months age, and weight (1200-1500) g., the rabbits were housed under constant environmental condition (22-25 C°), 12 h/ dark/ light cycle. They were divided homogeneously to six groups. The first group (G1) is a normal control group that was given water and a standard diet. The second group (G2) is the pateints group, which was injected with triton x100. The third and fourth group (G3) and (G4) were injected with triton x100 and treated and given rosuvastatin (3 mg / kg / day) and simvastatin (3mg/kg/ day), respectively. The fifth and sixth group (G5) and (G6) were given rosuvastatin (3 mg / kg / day) and simvastatin (3 mg / kg / day), respectively. At the end of the experiment, blood was drawn via cardiac puncture technique using disposable medical syringe (5 ml). Blood samples were kept with sterilized gel tube without anticoagulant, and left for 15-30 mints at room temperature 25C° to allow to blood coagulation, and serum was collected by centrifugation at (3000 rpm) for 15 minutes and frozen at (-20 C°) for biochemical testes wich include: Reducing power assay: the two drugs was estimated depending on (Oyaizu) assay [10], Phosphomolybdenum method: where the molybdenum complex is formed in low PH and at high temperatures [11], Determination of serum Malon dialdehyde (MDA):by using the modified assay which adopted by Guid and Shah [12][13]. , Determination of serum peroxy nitrite:by using the modified assay which adopted by Vanuffelen *et al* [14][15], Determination of serum ceroplasmin:used the assay of Sunderman and Nomato [16][17].

Results

A-Study the susceptibility of drugs as antioxidants outside the body.

An evaluation study was conducted for the drugs Rosuvastatin and Simvastatin as an antioxidant outside the body. The results showed that the standard material (ascorbic acid) has a high reduction power compared to the drugs. The standard material demonstrated an increase in absorption with increased concentration, ascorbic acid exhibited a good linear relationship (R2=0.9975) compared to the simvastatin, where the linear relationship was (R2=0.933). Rosuvastatin was the linear relationship (R2= 0.9519) as shown in figure (1). The results show that simvastatin has a high antioxidant potential compared with rosuvastatin, which has a lower susceptibility to the standard material that has the highest potential for reduction.

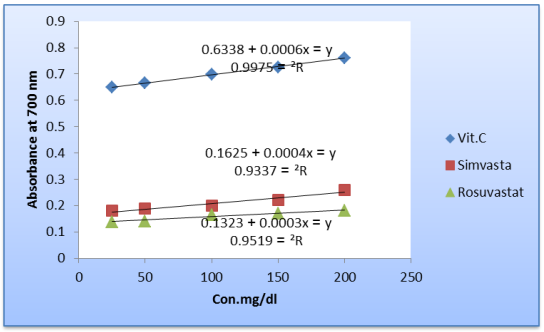

Figure (1) the reducing power of the simvastatin and rosuvastatin compared to the standard substance (ascorbic acid)

With regard to ability of the two drugs to reduce the molybdenum, the results showed that ascorbic acid had a high reduction power compared to the two drugs. The linear relationship of the ascorbic acid was (R2=0.9963), while the linear relationship of the simvastatin and rosuvastatin was (R2=0.9878) and (R2=0.9867) respectively, as shown in Figure (2). The results expose that Simvastatin has a high antioxidant potential compared to rosuvastatin, which has a lower tolerability compared with the standard material that has the highest molybdenum reduction.

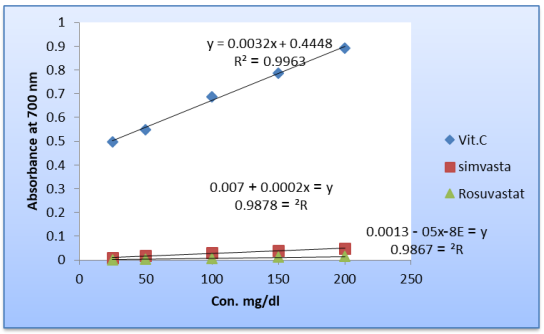

Figure (2) ability of ascorbic acid (standard material) , drug of simvastatin and rosuvastatin in molybdenum reduction

b- Effect of Simvastatin and Rosuvastatin on the level of oxidation and its antagonists in local rabbit serum (inside the body).

Table 1 shows that the mean ± standard deviation of the MDA level in G1 was (8.036 ± 0.489) micromole / L. While G2 was (11.060 ± 1.945) micromole / L. Either the G3, G4 were (7.415±1.682) ، (8.095 ± 2.172)) micromole / L, Respectively. While G5, G6 were (6.476 ± 0.901) ، (9.530 ± 1.108) micromole / L, Respectively.

The mean ± standard deviation of the level of pyroxy nitrite in G1 was (22.157±2.565) micromole / L, While the infected group which treated with triton

was (34.430±7.469) micromole / L. Either the G3 ، G4 were (28.908 ± 5.290) (26.535 ± 5.637) respectively. While G5, G6 were (23.737 ± 6.636) (27.666 ± 4.532) micromole / L, respectively.

The mean ± standard deviation of serum ceroplasmin level in G1 was (0.11729 ± 0.00890) g/L. While G2 was (0.05800 ± 0.01673) g/L. Either the G3, G4 were (0.07800 ± 0.00683), (0.08000 ± 0.01414) g/L respectively. While G5, G6 were (0.07000 ± 0.01155), (0.08214 ± 0.01438) g/L respectively.

Table (1) Mean ± standard deviation of concentrate (MDA, pyroxy nitrite, ceroplasmin) in serum of treated rabbits that treated with simvastatin and rosuvastatin

test / Group	MDA (µmol/L)	Peroxy (µmol/L	Ceroplasmin (g/L)
G1	8.036±0.489 bc	22.157±2.565c	0.117±0.008 a
G2	11.060±1.945 a	34.430±7.469 a	0.058±0.016 c
G3	7.415±1.682 cd	28.908±5.290 ab	0.078±0.006 b
G4	8.095±2.172 bc	26.535±5.637bc	0.080±0.014 b
G5	6.476±0.901d	23.737±6.636bc	0.070±0.011 b
G6	9.530±1.108 b	27.666±4.532bc	0.082±0.014b

*Similar characters indicate no significant differences.
* Different characters indicate Significant differences.
*mean± Standard deviation

The results showed a significant increase P≤0.05 in MDA level in the serum of G2 compared to G1, also the results showed a significant decrease P≤0.05 in the serum of all groups compared to the infected group and control, except for G3 and G4, which was no significant differences observed with the control group,as shown in figure (3).

The results showed a significant increase P≤0.05 in the level of peroxy nitrite in the serum of G2 compared to G1. Also the results showed a significant decrease P≤0.05 in the level of pyroxy nitrite in serum of G4, G5, G6 compared to the infected group. While those groups did not show any significant differences with G1 except for G3 which showed a significant increase compared with G1, as shown in figure (4)

The results indicated a significant decrease P≤0.05 in the level of serum ceroplasmin in the infected group compared to the control group. The results also showed a significant increase P≤0.05 in the level of serum ceroplasmin in all serum groups compared to G2. In addition there was a significant decrease P≤0.05 in all serum groups compared to G1 intact, as shown in figure (5)

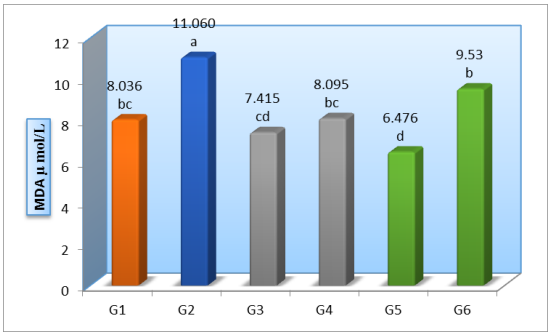

Figure (3) Mean concentration of serum MDA in control group and treatment groups.

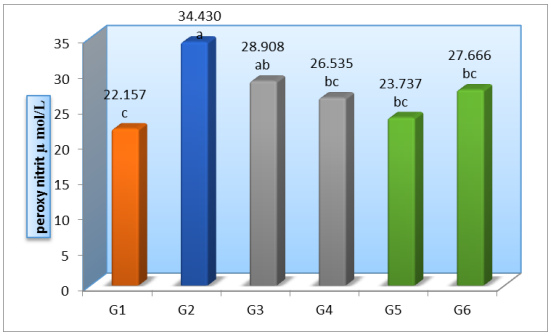

Figure (4)mean concentration serum peroxy nitrite in control group and treatment groups.

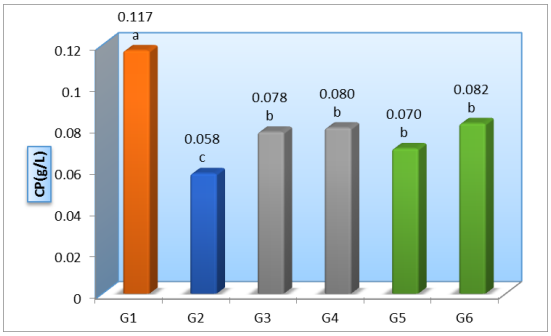

Figure (5) mean concentration serum ceroplasmin in control group and treatment groups.

Discussion

The high concentration of MDA in G2 is lead to the fact that hyperlipidemia develops or causes oxidative stress , due to an increase in the free radical roots such as superoxide root and hydrogen which inhibits the body's antioxidant enzymes. The oxidative modification of LDL-c and its accumulation in serum is a primary event in the proceeding of atherosclerosis [18].

Levels of lipid peroxidation and antioxidant status linked with high TC and LDL-c, or may the LDL's increase oxidation in the circulatory system, lipid peroxidation leads to increase the ability of LDL-c to cause atherosclerosis [19].

The statins reduce the concentration of MDA without affecting on concentrations nitric oxide synthase and endothelial nitric oxide synthase, The most possible explanations for the statins is to reduce oxidative stress by increasing the antioxidant activity due to the pharmacologic properties of the statins [20].

The decrease in the level of pyroxy nitrite probably return to the role of indirect removal to (ONOO·), by stimulating the antioxidant enzymes and raise their concentrations in the body, especially the catalase enzyme and superoxide dismutase (SOD). The enzyme SOD competition with nitric oxide (NO) to bind with the superoxide oxide ($O2^-$) and results hydrogen peroxide H_2O_2, which is removed by the catalase enzyme [21][22].

The reason for the low concentration of ceroplasin in the serum of G2 is that the body has effective defense mechanisms, to modify the free radicals damage. This is done by the treatment of internal enzymatic or non-enzymatic factors. The results revealed a decrease in the level of ceroplasmin in the group that injected with Triton X-100 due to increased consumption of antioxidants to encounter the production of free radicals during cases of hypercholesterolemia or hyperlipidemia. There is a positive relationship between concentrations of total cholesterol and triglycerides with the production of free radical roots [23][24]. Also the reason for the increase in concentration of ceroplasmin in the treated groups is the statin has the ability to catch many of the metal elements, including iron and copper, converting the iron ion Fe^{+2} to Fe^{+3}, which enter the process of oxidation within the body of the organism body, also the ceroplasmin linked with the copper and making it restricted and unable to participate in the process of oxidation [25].

Simvastatin plays an important role in reducing oxidative stress and its resistance from free radicals as it increases antioxidant enzymes [26]. The drug also plays a role by influencing the enzyme NADPH Oxidase and closing the way to the effect of reactive oxygen species (ROS) by regulating the antioxidant enzymes [27][28].

Conclusion

1- Simvastatin and Rosuvastatin play an important role as external antioxidants, simvastatin had a bigger role compared with rosuvastatin, which has a lower tolerance.

2- The two drugs had an important role in reducing the oxidative stress factors and increase the antioxidant factor. Simvastatin played an important role in the reduction of MDA compared to rosuvastatin, also Simvastatin played a role in the reduction of peroxy nitrite and increase the ceroplasmin level.

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مقارنة عقاري Simvastatin وRosuvastatin على مستوى الأكسدة ومضاداتها في ذكور الأرانب المصابة باضطراب الدهون التجريبي

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الملخص

أجريت التجربة في البيت الحيواني لجامعة سامراء من الفترة 1/10/2018– 4/11/2018 ، وتضمنت الدراسة الحالية تقييم كل من العقارين كمضادات اكسدة خارج الجسم. كذلك مقارنة تأثير كل منها على الأكسدة ومضاداتها في ذكور الأرانب المصابة بأضطراب الدهون التجريبي والمعاملة بمادة الترايتون 100X. تم استخدام ذكور الأرانب المحلية والبالغ عددها 48 أرنب، وقسمت الى 6 مجاميع (كل مجموعة تحتوي على 8 أرانب). تم تقييم كل من العقارين كمضادات اكسدة خارج الجسم بأستخدام طريقتي القوى الأختزالية وفوسفات الموليبديوم، واتضح من خلال النتائج ان الـSimvastatin يمتلك قابلية مضادة للأكسدة عالية مقارنة بعقار Rosuvastatin الذي يمتلك قابلية اقل مقارنة بالمادة القياسية (فيتامين C). كما وتم قياس مستوى الاكسدة ومضاداتها داخل الجسم (المالون ثنائي الالديهايد، البيروكسي نتريت، السيروبلازمين). واظهرت النتائج ارتفاعاً معنوياً $P\leq0.05$ في تركيز المالون ثنائي الألديهايد وجذر البيروكسي نتريت وانخفاضاً معنوياً $P\leq0.05$ في تركيز السيروبلازمين في مصل دم G2، كما واظهرت النتائج حصول انخفاض معنوي للمالون ثنائي الالديهايد في مصل دم كافة المجاميع مقارنة بـ G2، واظهرت G5 انخفاض معنوي $P\leq0.05$ في المالون ثنائي الالديهايد مقارنة ببقية المجاميع، كما واظهرت النتائج حصول انخفاض معنوي$P\leq0.05$ في تركيز جذر البيروكسي نتريت في مصل دم كافة المجاميع مقارنة بـG2 دون وجود فروق معنوية، كما وأظهرت النتائج حصول ارتفاع معنوي $P\leq0.05$ في تركيز السيروبلازمين في مصل دم كافة المجاميع مقارنة بـG2.