Evaluation of the Effects of Glimepiride (Amaryl) on atherosclerosis progression in high cholesterol-fed male Rabbits

Najah R. Hadi, Ph.D, FRCP, FACP, Post Doc.(USA); Mohammad A. A. Hussein, MBChB,DM,CABM ; Fadhil A. Rezeg .MSc.
Department of pharmacology and therapeutics , College of Medicine , University of Kufa.

الخلاصة:
مرض تصلب الشرايين يعد ألان عملية التهاب مزمنة حيث أن كلا من الالتهاب و زيادة الإجهاد ألتأكسدي وما يسببه من تأكسد الدهون تساهم وبشكل أساسي في كل مراحل عملية تصلب الشرايين٠ يعتبر الگلامبيرايد من الجيل الثالث للسلفونيل يوريا قد يظهر نشاط مضاد للالتهاب عن طريق زيادة إنتاج النايترك اوكساید أو عن طريق تثبيط مسار السايكلواوكسيجينز. وعليه أن هذه الدراسة أجريت لتقييم تأثير دواء الگلامبيرايد على تقدم تصلب الشرايين المحدث في ذكور الأرانب من خلال تثبيط الالتهاب والإجهاد ألتأكسدي.
الطريقة: ثمانية عشر أرنبا محليا ذكرا استخدموا في الدراسة وتم توزيعهم على ثلاث مجاميع(ستة أرانب لكل مجموعة):
المجموعة الأولى :هي مجموعة السيطرة الطبيعية وأعطيت غذاء أرانب قياسي طبيعي.
المجموعة الثانية: أعطيت غذاء عالي الدسم يحتوي على ١ % كولسترول ولمدة عشرة أسابيع.**المجموعة الثالثة:** أعطيت غذاء عالي الدسم ١ % كولسترول مع الگلامبيرايد بجرعة ٠.١ ملغ/ كغم بالفم مرة واحدة قبل الإفطار الصباحي. تم أخذ نماذج من الدم قبل أعطاء الكولسترول وبعد كل أسبوعين من بدء إعطاء الكوليسترول٠وتم قياس الكولسترول الكلي والكليسريدات الثلاثية و الكولسترول عالي الكثافة و(hs-CRP,IL-6,TNF-α) ٠وفي نهاية الدراسة بعد عشرة أسابيع تم أخذ الشريان الأبهر و تم قياس مستوى عامل التأكسد (MDA) وكذلك مستوى (GTH) و بالإضافة إلى قياس سُمك جدار الشريان الأبهر.
النتائج:
إن العلاج بالگلامبيرايد لم يظهر تأثير معنوي ($p> ٠.٠٥$) على مقاييس الدهون بالمقارنة مع المجموعة التي أعطيت غذاء عالي الدسم ولم تعالج. بينما أظهر تأثيرا معنويا ($p < ٠.٠٥$) على الالتهاب من خلال منع ارتفاع (hs-CRP,IL-6, TNF-α) وعلى الإجهاد ألتأكسدي من خلال منع ارتفاع (MDA) ($p< ٠.٠٥$) .و منع نقصان (GTH) بالإضافة إلى تقليل الزيادة في سُمك جدار الشريان الأبهر.
الاستنتاج:
نتائج دراستنا أظهرت إن الگلامبيرايد منع تقدم تصلب الشرايين المحدث في ذكور الأرانب عالية الكولسترول من خلال تثبيط الالتهاب والإجهاد ألتأكسدي بدون التأثير على مستويات الدهن.

Abstract
Atherosclerosis is an inflammatory disease of the blood vessel wall, characterized in early stages by endothelial dysfunction, staffing and activation of monocyte/ macrophages. Glimepiride is one of the third generation sulphonylurea drugs useful for manage of diabetes mellitus type tow and it may exert anti inflammatory activity by induction of nitric oxide production or through selective suppression of the cyclooxygenase pathway.
Objectives: The objective of the present study was to assess the effect of glimepiride on atherosclerosis via interfering with inflammatory and oxidative pathways.
Methods: Eighteen local domestic male rabbits were involved in this study. The animals were randomly divided into three groups , Group I rabbits fed normal chow (oxiod) diet for 10 weeks. Group II rabbits fed with 1% cholesterol enriched diet . Group III rabbits fed with 1% cholesterol enriched diet together with Glimepiride (0.1mg/kg once daily before morning feed). Blood samples were collected before (0

time) and every two weeks on experimental diets for measurement of serum triglycerides (TG), total cholesterol (TC), HDL-C, high sensitive C-Reactive Protein (hsCRP),IL-6 and TNF-α level. At the end of 10weeks the aorta was removed for measurement of aortic (MDA), (GSH) and aortic intimal thickness.

Results: Glimepiride treatment don't show significant effect on lipid parameters compared with induced untreated group ($P > 0.05$). Glimepiride significantly reduced the elevation in hsCRP, IL-6, TNF-α, aortic MDA and aortic intimal thickness compared with induced untreated group ($P<0.05$).Also it restore aortic GSH level ($P <0.05$).

Conclusions: Glimepiride may reduce atherosclerosis progression in hypercholesterolemic rabbit via interfering with inflammatory and oxidative pathways without affecting lipid parameters.

Key words: glimepiride, inflammatory markers, oxidative stress, atherosclerosis,

Introduction

A main consequence of type two diabetes is accelerated onset of atherosclerosis, which an important risk factor in the progress of ischemic cardiovascular disease. However, successful management of hyperglycemia alone is usually inadequate to prevent cardiovascular events because many other recognized risk factors such as hyperlipidimia, hypertension, and obesity are often present concurrently in the diabetic population [(1)]. Also the incidence of atherosclerosis is 3–4 times greater in diabetics than non-diabetics at comparable plasma total cholesterol concentrations[(2)].Accordingly, prevention of onset and progression of atherosclerosis as well as tight glycemic control are essential goals of treatment in type two diabetic patients.

It has been extensively demonstrated that inflammation plays a central role in atherogenesis and mediating all stages of this disease from initiation through progression and, ultimately, the thrombotic complications of atherosclerosis[(3)].

Inflammation contributes to the formation and progression of atherosclerosis and the therapeutic potential of some anti-inflammatory drugs have been evaluated for possible anti atherosclerotic activity. Recent findings suggest that some agents with anti-inflammatory properties appear to have beneficial effects on atherosclerosis or subsequent risk for cardiovascular events[(4)].Sulfonylureas have been widely used in recent years as first-line treatment in type two diabetic patients whose blood glucose levels cannot be effectively controlled by diet alone. Glimepiride (Amaryl®), a third-generation sulfonylurea administered once daily, has been established to offer therapeutic advantages over other sulfonylureas in terms of glucose level-dependent insulinotropic action, insulin-sparing effects, and hypoglycemic risk [(5,6,7)]. Furthermore, glimepiride exhibits inhibitory effects on human platelets aggregation through selective suppression of the cyclooxygenase pathway, suggesting that it may have therapeutic potential in diabetic patients with enhanced platelet function [(8)]. In addition, there are several reports showing beneficial effects of sulfonylureas against development of cardiovascular disease in rabbits fed atherogenic diet[(9)] as well as in hyperglycemic patients[(10)]. Recently, glimepiride has been demonstrated to inhibit the development of atheromatous plaques in thoracic aorta of high-cholesterol fed rabbits .The mechanism by which glimepiride induces atheroprotective effects remains to be elucidated[(11)].

Method

Eighteen local domestic male rabbits were involved in this study. The animals were randomly divided in to three groups , **Group I** rabbits fed normal chow (oxiod) diet for 10 weeks. **Group II** rabbits fed with 1% cholesterol enriched diet for 10 weeks. **Group III** rabbits fed with 1% cholesterol enriched diet together with Glimepiride (0.1mg/kg once daily before morning feed for 10 week)[12].The drug prepared immediately before use as suspension in 5% starch. The blood samples were collected before (0 time) and every two weeks of experimental diets for measurement of serum triglycerides (TG), total cholesterol (TC), HDL-C, high sensitive C-Reactive Protein (hsCRP),IL-6 and TNF-α level. At the end of 10weeks the aorta was removed for measurement of aortic Malondialdehyde (MDA), reduced glutathione (GSH) and aortic intimal thickness.

Results

There was a slight insignificant increase in body weight of glimepiride receiving groups suggesting that food consumption probably was similar in all the groups and cholesterol or glimepiride had no effect on body weight. Also no significant changes was occur in the glucose concentration in the glimepiride receiving groups during the period of experiment. while there was significant increase in the level of blood glucose concentration in the induced untreated group and this may be due to that atherogenic diet reduce plasma insulin level and enhance gluconugenesis. as shown in table (1).

Compared with the control, levels of TC, TG, HDL-C, LDL-C, VLDL-C, atherogenic index, hsCRP, IL-6, TNF-α , MDA and aortic intimal thickness were increased and GSH was decreased in the animals with atherogenic diet ($P < 0.05$). Glimepiride treatment don't showed significant effect on lipid parameters compared with induced untreated group ($P > 0.05$).While glimepiride significantly reduced the elevation in hsCRP, IL-6, TNF-α ,aortic MDA and aortic intimal thickness compared with induced untreated group ($P < 0.05$). Also it restore aortic GSH level ($P < 0.05$) .as shown in table (2) ,table (3), table (4) and table (5).

Table 1 : changes of rabbits body weight in (kg) and blood sugar (mg/dl) of the three experimental groups. The data expressed as Mean ±SEM (N=6 in each group) Using paired T-test.

		Body weight(Kg)	Blood sugar(mg/dl)
Normal control	**Zero time**	**1.5±0.1**	**110±4.2**
	10 weeks	**1.5±0.09**	**100±6.2**
Induced untreated	**Zero time**	**1.55±0.1**	**110±6**
	10 weeks	**1.3±0.05**	**134±4***
glimepiride 0.1mg/kg	**Zero time**	**1.52±0.1**	**112±5.2**
	10 weeks	**1.64±0.05**	**124±5.7**

*p<0.05

Table 2 : **changes of rabbits serum lipid profile(TC, TG and HDL) in of the three experimental groups. The data expressed as Mean ±SEM (N=6 in each group) Using paired T-test .**

		TC(mg/dl)	TG(mg/dl)	HDL(mg/dl)
Normal control	Zero time	85±2.92	43.3±1.71	15.6±1.28
	10 weeks	88±3.26	45±3.5	16.5±1.4
Induced untreated	Zero time	90±1.65	44±2.87	16.2±1.29
	10 weeks	994±28*	324±20.5*	37±1.74*
glimepiride 0.1mg/kg	Zero time	91±3.36	42±2.17	16.4±1.37
	10 weeks	977±9.9	319±9.2*	42.5±3.08*

***p<0.05**

Table 3 : **changes of rabbits serum Inflammatory markers (hs-CRP , IL-6 and TNF- α) of the three experimental groups. The data expressed as Mean ±SEM (N=6 in each group) Using paired T-test .**

		hs-CRP(mg/l)	IL-6(pg/ml)	TNF- α(pg/ml)
Normal control	Zero time	3.5±0.5	0.75±0.1	0.6±0.09
	10 weeks	4.3±0.7	1.1±0.09	1.05±0.06
Induced untreated	Zero time	4.2±0.9	1.06±0.05	0.77±0.1
	10 weeks	20.7±1.7*	5.5±0.5*	7.05±0.44*
glimepiride 0.1mg/kg	Zero time	3.8±0.3	1.1±0.09	0.9±0.1
	10 weeks	7.4±1.1*	2.4±0.2*	2.5±0.2*

*p<0.05

Table 4 : the means of rabbits aortic Oxidative stress parameters (MDA and GTH) of the three experimental groups at the end of experiment. The data expressed as Mean ±SEM (N=6 in each group) Using paired T-test.

group	Aortic MDA (µmole/gm aorta)	Aortic GTH (nmole/mg aorta)
Normal control	1.4 ± O.14	38.3± 2.46
Induced untreated	7.3 ± O.55*	21.7± 1.75*
glimepiride 0.1mg/kg	3.6 ± O.44	30.4±2.2

*p<0.05

Table 5 : The means of rabbits aortic intima thickness of the three experimental groups at the end of experiment . The data expressed as Mean ±SEM (N=6 in each group) Using paired T-test.

group	Aortic intima thickness ((µm)
Normal control	32.4± 2.7
Induced untreated	334.82±53.5*
glimepiride 0.1mg/kg	203.03±22.35*

*p<0.05

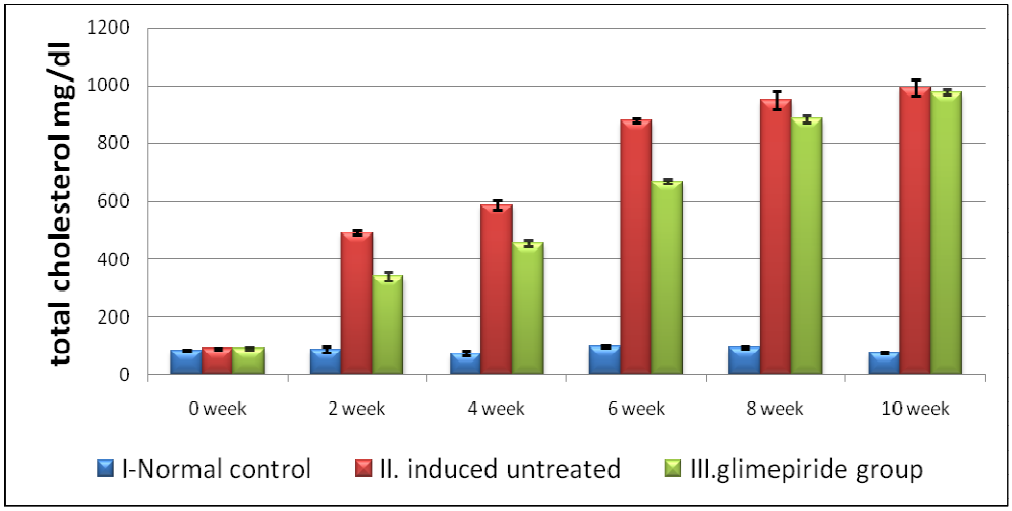


Fig (1): Effect of glimepiride 0.1mg/kg /day treatment on serum TC levels (mg/dl) during the experimental treatment.

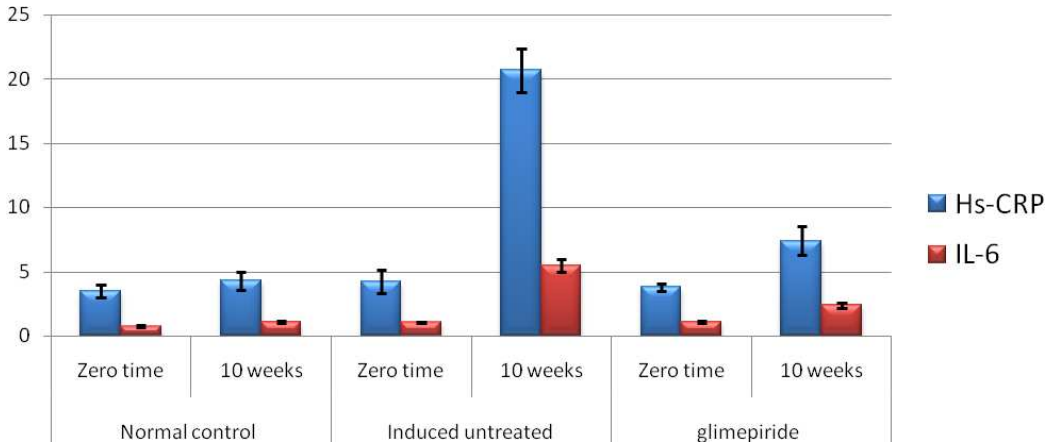


Figure (2): Effect of glimepiride 0.1mg/kg /day treatment on serum hs-CRP(mg/l) and serum IL-6 (pg/ml) in comparisons to the two control group (normal and control untreated).

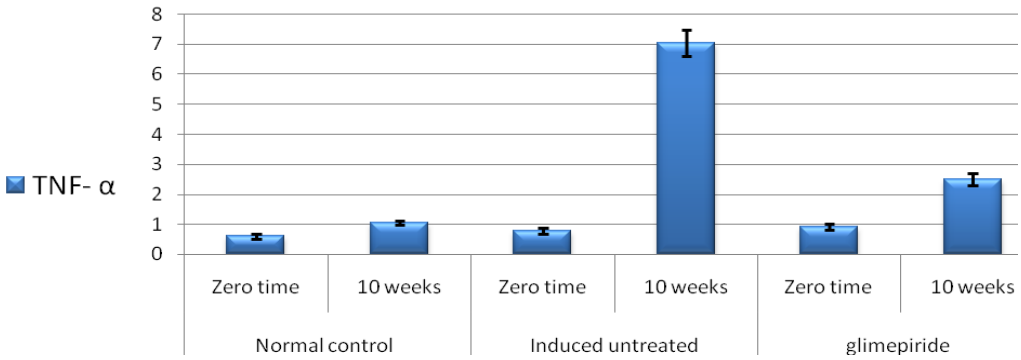


Figure (3): Effect of glimepiride 0.1mg/kg /day treatment on serum TNF-α (pg/ml) in comparisons to the two control group (normal and control untreated).

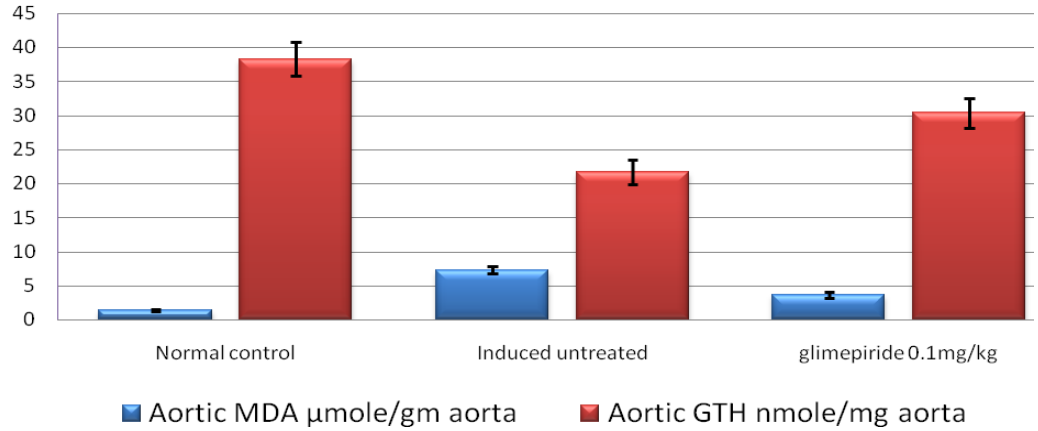


Figure (4): Effect of glimepiride 0.1mg/kg/ day treatment on aortic MDA µmole/gm aorta and aortic GTH nmole/mg aorta in comparisons to the two control group (normal and control untreated).

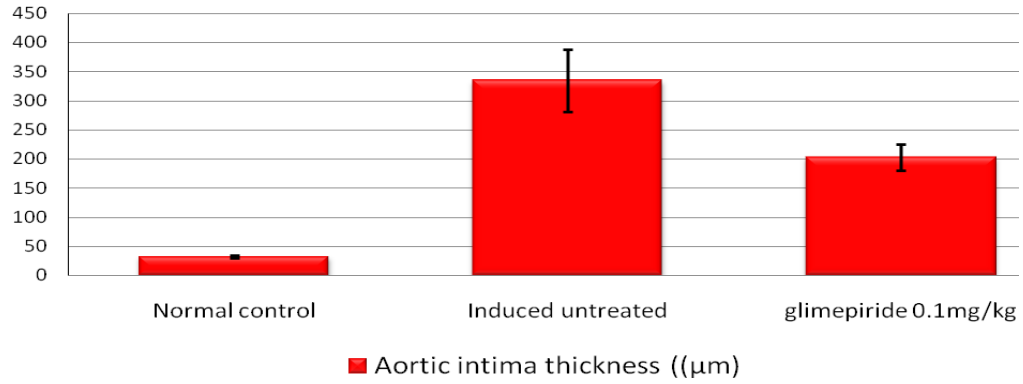
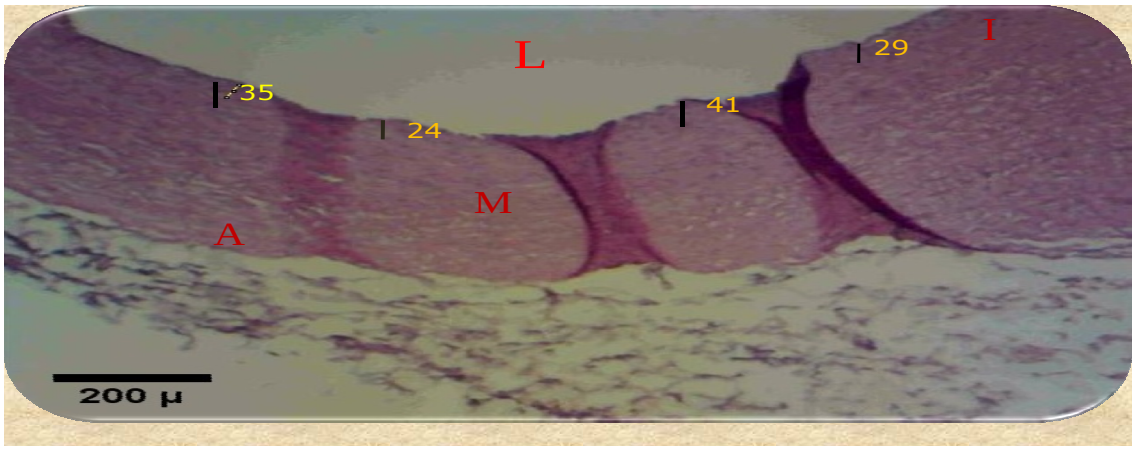
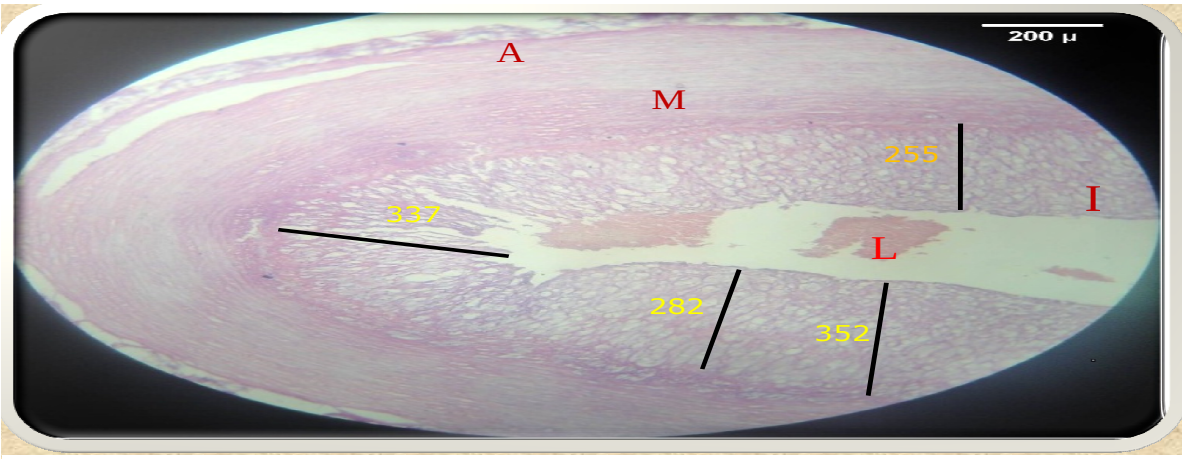


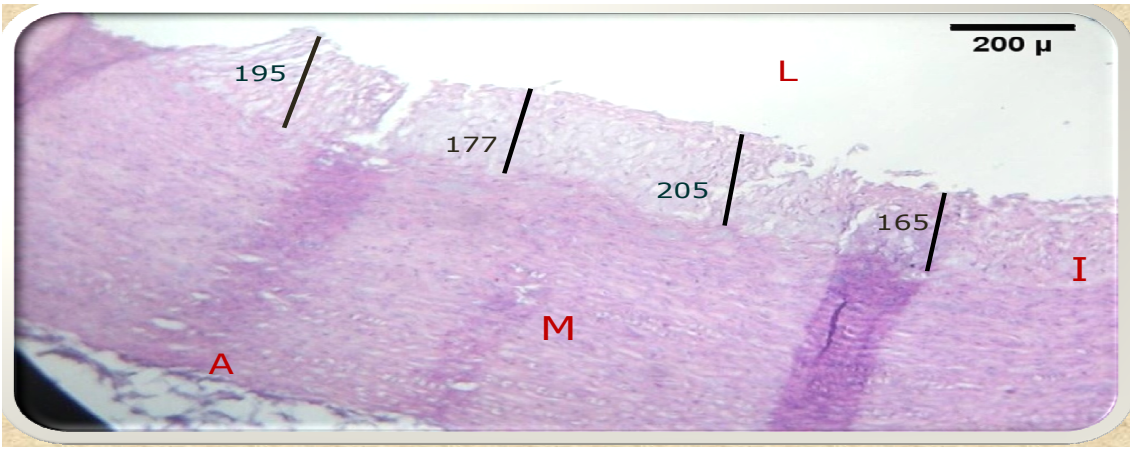
Figure (5): Effect of glimepiride 0.1mg/kg /day treatment on aortic intimal thickness level (µm) at the end of experiment.



Figure(6):photomicrograph of histomorphometric section in aortic arch of rabbits fed on normal diet for 10weeks (normal control) show appearance of arterial wall layers: Lumen(L) . Intact continuous endothelium ,Intima(I), regularly arranged smooth muscle fibers ,media(M) and adventitia(A). The section stained with haematoxylin and eosin (×10).



Figure(7) :photomicrograph of histomorphometric section in aortic arch of rabbits on atherogenic diet for 10 weeks(induced untreated) show diffuse intima thickening & confluence of lipid collections creates an extracellular dense accumulation of fat in a well determined area (I)intima,(M)media, (A)adventitia&(L) Lumen. The section stained with haematoxylin and eosin (×10).



Figure(8) :photomicrograph of histomorphometric section in aortic arche of Glimepiride hyperlipidimic rabbits. Also show significant decrease in The aortic intima thickness as compare to induced untreated (I)intima,(M)media,(A)adventitia&(L)lumen. The section stained with haematoxylin and eosin(×10).

Discussion

Accelerated atherosclerosis accompanied by high risk of premature mortality from cardiovascular disease (e.g. coronary heart disease) is a frequent and severe complication of type two diabetes[13]. Thus prevention of onset or progression of atherosclerosis as well as adequate glycemic control is mandatory for the management of patients with this entity. The present study was to assess the effect of glimepiride on atherosclerosis via interfering with inflammatory and oxidative pathways.

.In this study we demonstrated that treatment with glimepiride appeared to have no significant change on lipid parameters in comparison with the induced untreated group and this may be due to that the change in lipid parameter induced by high fat diet overrides any changes expected from glimepiride .

Also in the present study glimepiride treatment was significantly reduced the elevation of inflammatory markers (hs-CRP, IL-6 & TNF-α) in atherosclerosis model of hypercholesterolemic rabbit suggesting that glimepiride inhibit vascular inflammation induced by high cholesterol diet.

Glimepiride has been reported to show extra pancreatic actions including phosphorylation of insulin receptor substrate-1 which is a regulator of phosphaoltidylinosit 3-kinase (PI3-kinase) in adipocytes [(14)]. PI3-kinase has been shown to be involved in agonist-induced nitric oxide (NO) production in vascular endothelial cells[(15)]. Collectively, these findings suggest that glimepiride may stimulate NO production in endothelial cells through a PI3-kinase dependent pathway. Kenyon et al.(2002) had shown that the production of NO might be needed as a defensive factor in various models of acute or chronic inflammation [(16)].

Also in the present study glimepiride had significantly reduced aortic MDA level suggesting decrease in reactive oxygen species and subsequent lipid peroxidation. Also Glimepiride had significant effect on aortic GSH levels where prevents GSH depletion in hypercholesterolemic rabbit, and thus, maintain antioxidant balance which is important for vascular protection against lipid peroxide.

There are two possible explanations for antioxidant capacity of glimepiride ,firstly through inhibition of cellular cyclooxygenase pathways[(8)] ,and the second possible mechanism may be related to the fact that glimepiride has the property to up-regulate antioxidant enzyme gene like paraoxonase, superoxide dismutase and catalase gene through reducing the activation of the redox sensitive nuclear factor kappa-B (NF-κB),or through that glimepiride possessed agonistic activities for PPARγ [(17,18,19)].

More over glimepiride treatment significantly reduce aortic intima thickness compared with that in the induced untreated group. Atherosclerosis is now widely accepted as a chronic inflammatory process. Low-grade inflammation, enhanced oxidant stress and lipid peroxidation. The athero-protective effect of glimepiride is due to interfering with inflammatory and oxidative pathways in hypercholesterolemic rabbit. In addition to that glimepiride specifically inhibits some process in the early cellular events of atherosclerosis, which may include: adherence of circulating blood monocytes to vascular endothelial cells; migration of adherent monocytes into the sub endothelial space and their subsequent differentiation into macrophages; oxidative alteration of LDL mediated by vascular endothelial cells, smooth muscle cells, or macrophages; uptake of oxidative LDL by macrophages by scavenger receptors to generate foam cells [(20)]. Glimepiride have inhibitory effect on cell-mediated LDL oxidation, it may prevent all these processes & reduce aortic intimal thickness & inhibit progression of atherosclerotic lesion.

References

1.Lehto S, Ronnemaa T and Haffner SM. Dyslipidemia and hyperglycemia predict coronary heart disease events in middle aged patients with NIDDM. Diabetes 1997;46:1354–9.

2.Joseph L. Dixon, Siming Shen, James P. Vuchetich, Elzbieta Wysocka, Grace Y. Sun and Michael Sturek . Increased atherosclerosis in diabetic dyslipidemic swine ;protection by atorvastatin involves decreased VLDL triglycerides but minimal effects on the lipoprotein profile . Journal of Lipid Research 2002; 43: 1618-1629.

3.Libby P. and Ridker PM. Inflammation and atherosclerosis. Circulation. 2002;105:1135-114.

4.Dandona P, Dhindsa S. and Ghanim H. Angiotensin II and inflammation: the effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockade. J Hum Hypertens. 2007; 21:20–27

5. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998;352:837–53.

6. Geisen K. Special pharmacology of the new sulfonylurea glimepiride. Arzneim-Forsch/Drug Res 1988;38:1120–30.

7. Langtry H, Balfour J. Glimepiride—a review of its use in the management of type 2 diabetes mellitus. Drugs 1998;55:563–84.

8. Qi R, Ozaki Y and Satoh K. Sulphonylurea agents inhibit platelet aggregation and [Ca2+]i elevation induced by arachidonic acid. Biochem. Pharmacol 1995;49:1735–9.

9. Riveline J, Danchin N, Ledru F, Varroud-Vial M. and Charpentier G. Sulfonylureas and cardiovascular effects: from experimental data to clinical use. Available data in humans and clinical applications. Diab Metab. 2003;29(3):207–22

10. Kouichi. I., Masaki. W., Youhei. N., Takahiro. S., Nobuki. T., Masahiko.T. , et al. Efficacy of glimepiride in Japanese type 2 diabetic subjects.Diab. Res. Clin. Pract.2005; 68, 250–257.

11. Shakuto S, Sato Y, Ohshima K. and Yaguchi M. Atheroprotective effects of a new sulfonylurea of the third generation, glimepiride. Diabetic Complications 2001;15(Suppl. 1):68.

12.Shakuto S, Oshima K. and Tsuchiya E. Glimepiride exhibits prophylactic effect on atherosclerosis in cholesterol-fed rabbits. Atherosclerosis 2005;182 (2), 209–217,.

13.Beckman JA, Creager MA and Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology and management. JAMA 2002;287:2570–81.

14. Muller G, Jung C, Wied S, Welte S. and Frick W. Insulin-mimetic signaling by the sulfonylurea glimepiride and phosphoinositolglycans involves distinct mechanisms for redistribution of lipid raft components .Biochemistry 2001;40(48):14603–20.

15. Maejima Y, Ueba H. and Kuroki M. Src family kinases and nitric oxide production are required for hepatocyte growth factor-stimulated endothelial cell growth. Atherosclerosis 2003;167(1):89–95**.**

16. Kenyon. N.J., Vandervliet. A., Schock. B.C., Okamoto. T.,Mcgrew. G.M. and Last. J.A. .Susceptibility to ozoneinducedacute lung injury in iNOS-deficient mice. Am. J. Physiol. Lung Cell Mol. Physiol. 2002, 282, L540–L545.

17. Fan Y, Wang Y, Tang Z, Zhang H, Qin X, Zhu Y. et al . Suppression of pro-inflammatory adhesion molecules by PPAR-delta in human vascular endothelial cells. Arteriosclerosis, Thrombosis, and Vascular Biology 2008:28 (2), 315–321.

18. Schiekofer S, Rudofsky Jr G, Andrassy M, Schneider J, Chen J. and Isermann B. Glimepiride reduces mononuclear activation of the redox-sensitive transcription factor nuclear factor-kappa B. Diabetes, Obesity & Metabolism 2003; 5 (4), 251–261.

19. Fukuen S, Iwaki M, Yasui A, Makishima M, Matsuda M. and Shimomura I. . Sulfonylurea agents exhibit peroxisome proliferator-activated receptor gamma agonistic activity. The Journal of Biological Chemistry 2005:280 (25), 23653–23659.

20. Ross R. The pathogenesis of atherosclerosis: a perspective for the1990s. Nature 1993;362:801–9.