

## **Effect of vildagliptin on atherosclerosis progression in high cholesterol –fed male rabbits**

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### **Abstract**

**Back ground:** Atherosclerosis is a disease of large and medium-sized muscular arteries and is a progressive disease characterized by the accumulation of lipids and fibrous elements in the large arteries.

**Objective:** This study was undertaken to assess the effect of vildagliptin on atherosclerosis via interfering with inflammatory and oxidative pathways

**Materials and Methods:** 18 local domestic male rabbits were included in this study. The animals were randomly divided into three groups (6rabbits in each group): Group I rabbits fed normal chow (oxiod) diet for 12 weeks. Group II rabbits fed 1% cholesterol enriched diet for 12 weeks. Group III rabbits fed with cholesterol enriched diet for 6 weeks, and then continued on cholesterol enriched diet and treated with vildagliptin 50mg/kg/day orally for the next 6 weeks. Blood samples were collected at the start of the study, at 6weeks of the study and then at the end of treatment course to measure Serum lipids profile [(TC), (TG), (HDL)] , hsCRP and TNF $\alpha$ . At the end of the study the aorta were removed for measurement of aortic MDA ,glutathione, sectioning for histopathology and measuring aortic intima- media thickness

**Results:** Treatment of rabbits with vildagliptin for 6 weeks results in a significant reduction ( $P<0.05$ ) in serum level of TC, TG, hsCRP and TNF $\alpha$  and a significant increase ( $P<0.05$ ) in serum HDL level. There was a significant reduction ( $P<0.05$ ) in aortic MDA and intima-media thickness, in comparison to the rabbits in the induced untreated control group. vildagliptin treatment cause significant increment ( $P<0.05$ ) in aortic GSH in comparison to induced untreated group .Regarding histopathological results, vildagliptin treatment for 6 weeks results in a significant reduction ( $P<0.05$ ) in

atherosclerotic lesions in comparison to the induced untreated group and significant reduction in aortic intima-media thickness ( $P < 0.05$ )

**Conclusions :** vildagliptin reduced atherosclerosis progression in hyperlipidemic rabbit via its effect on lipid parameters and interfering with inflammatory and oxidative stress pathway.

**Keywords:** vildagliptin, atherosclerosis, oxidative stress, inflammation

### الخلاصة:

يعتبر تصلب الشرايين المرض القاتل الرئيس على مستوى العالم. إن من أهم عوامل الخطورة لهذا المرض هي ارتفاع مستوى الدهون بالدم ومرض السكري وعوامل أخرى مثل الالتهابات المزمنة  
**هدف الدراسة:** لتقييم تأثير الفيلداكليبين على تقليل مستوى الدهون بالدم والالتهابات وعوامل التأكسد وتأثيرها على سُمك الطبقة الوسطية والداخلية للشريان الأبهري.

**الطريقة:** أدخلت في هذه الدراسة 18 أرنباً محلياً تم توزيع هذه الأرانب بصورة عشوائية إلى ثلاثة مجاميع المجموعة الأولى هي مجموعة السيطرة الطبيعية والمجموعة الثانية أعطيت غذاء عالي الدسم يحتوي 1% كولسترول طول فترة الدراسة (12) الأسبوع. المجموعة الثالثة أعطيت غذاء عالي الدسم لمدة ستة أسابيع، بعد ذلك أعطيت عقار الفيلداكليبين 50 ملغ/كغم لكل يوم عن طريق الفم. كل المجاميع أعطيت غذاء طبيعي طوال مدة التجربة (12 أسبوع).

**النتائج:** سبب تغذية الأرانب بالغذاء عالي الدسم الذي يحتوي 1% كولسترول لمدة اثني عشر أسبوعاً في زيادة معنوية ( $P < 0.05$ ) في مستوى الكولسترول الكلي والكليسيريدات الثلاثية البروتين المتفاعل نوع C وعامل الالتهاب  $TNF-\alpha$  في الدم. بالنسبة لنتائج الفحص النسيجي فإن جميع الأرانب التي تغذت على الغذاء عالي الدسم ظهر فيها مراحل مختلفة من تصلب الشرايين وكان هناك زيادة معنوية في نسبة التصلب ( $P < 0.05$ ) بالمقارنة مع مجموعة السيطرة الطبيعية. سبب معالجة الأرانب بعقار الفيلداكليبين لمدة ستة أسابيع انخفاضاً معنوياً ( $P < 0.05$ ) في الكولسترول الكلي والكليسيريدات الثلاثية ومستوى البروتين المتفاعل نوع C وعامل الالتهاب  $TNF-\alpha$  في الدم وعامل التأكسد المألون ثنائي الليدهايد وكذلك مستوى الكلوتوثاينون المختزل في الشريان الأبهري.

. بينما سبباً زيادة معنوية ( $P < 0.05$ ) في مستوى الكولسترول عالي الكثافة في الدم بالنسبة لنتائج الفحص النسيجي فإن عقار عقار الفيلداكليبين سبب انخفاضاً معنوياً ( $P < 0.05$ ) في مراحل تصلب الشرايين بالمقارنة مع مجموعة السيطرة غير المعالجة.

### Introduction :

Atherosclerosis is a multifactorial, multistep disease that involves chronic inflammation at every stage, from initiation to progression and,

eventually, plaque rupture.<sup>(1)</sup> Cardiovascular disease (CVD) is the leading cause of death and disability in developed nations and is increasing rapidly in the developing world. <sup>(1, 2)</sup>

Endothelial dysfunction is the initial step that allows diffusion of lipids and inflammatory cells (ie, monocytes, T lymphocytes) into the endothelial and subendothelial spaces. Secretion of cytokines and growth factors promotes intimal migration; SMC proliferation; and accumulation of collagen matrix, monocytes, and other white blood cells, forming an atheroma.<sup>(3)</sup>

**Incretin hormones:** The role of the gastrointestinal tract in regulating the secretion of insulin is demonstrated by the observation that insulin secretion is substantially increased in response to oral glucose, compared to intravenous glucose administration. This difference is known as the incretin effect these peptides are secreted from endocrine cells (L-cells) in the gastrointestinal tract, and are released in response to ingestion of food. The two main incretin hormones are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP),

DDP-IV is proteolytic enzyme widely expressed in many tissues, including the capillary bed of the gut mucosa responsible for rapid inactivation of (GLP-1).<sup>(4)</sup>

Incretin mimetics and DPP-4 inhibitors address the need to act on the underlying disease rather than on its symptoms. They enhance glucose-dependent insulin secretion by pancreatic beta cells, and consequently there is little risk of hypoglycemia. They also suppress elevated glucagon secretion and increase satiety. It has been hypothesized that they may restore beta-cell sensitivity to glucose, which could mean that they may be able to delay the onset of type 2 diabetes, slow its progression, and reduce its cardiovascular and metabolic complications<sup>(5)</sup>

vildagliptin is a potent, reversible, competitive inhibitor of DPP-4, with

high selectivity for DPP-4 over other peptidase enzymes<sup>(6)</sup> vildagliptin inhibits the activation of GLP-1 and GIP by Dpp-4 allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas

### **Materials and methods**

**Animals.** 18 local domestic male rabbits were included in this study from animal house of the faculty of medicine /kufa university . The animals were housed in cages and kept at room temperature and then randomly divided into three groups (6 rabbits in each group): Group I rabbits fed normal chow (oxiod) diet for 12 weeks. Group II rabbits fed 1% cholesterol enriched diet for 12 weeks. Group III rabbits fed with cholesterol enriched diet for 6 weeks, and then continued on cholesterol enriched diet and treated with vildagliptin 50mg/kg/day orally for the next 6 weeks. Blood samples were collected at the start of the study, at 6 weeks of the study and then at the end of treatment course for measurement of Serum lipids profile [(TC), (TG), (HDL)] , hsCRP, TNF $\alpha$  at the end of the study the aorta were removed for measurement of aortic MDA ,glutathione and Aortic intima- media thickness and sectioning for histopathology.

**Vildagliptin:** it was used in dose of 50mg/kg ,50 mg galvus tablet supplied by (Novartis) was suspended in distilled water and the dose was given according to body weight ,once daily through stomach tube.

### **Preparation of samples**

From each rabbit about 3 ml of blood was collected from the central ear artery without use of heparin after an overnight fasting. The blood sampling was done firstly at the start of the study i.e. at zero time and after 6 weeks of

the induction period, and then at end of treatment course (12week). The blood samples were allowed to clot at 37 C° and centrifuged at 3000 rpm for 15 min. Sera were taken, and analyzed for determination of serum total cholesterol, triglycerides, HDL-C , hs.C-reactive protein and TNF- $\alpha$ .

#### **Tissue preparation for oxidative stress measurement**

20% homogenates of tissues were prepared in phosphate buffer at pH 7.5 containing 1 mmol/l Na<sub>2</sub>EDTA. The homogenates were centrifuged at 20,000  $\times$ g at 4 C for 30 min and the supernatants were used for biochemical measurements of GSH &MDA level

#### **Histopathological procedure**

Autopsy of aortic (abdominal and thoracic aorta) sectioning were done at the end of the study (after 12 weeks), the histopathology is used to confirm the anti-atherogenic effect of sitagliptin in comparison with the control groups (normal control and induced untreated control).

The sections were examined by microscope under magnification power of ( $\times 4$ ,  $\times 10$  and

$\times 40$ ) then the histological changes were determined according to the American Heart Association classification of atherosclerosis<sup>(7)</sup> which divides atherosclerotic lesions into six types as follows:

Type I (initial) lesion: Isolated macrophage foam cells

Type II (fatty streak) lesion: Mainly intracellular lipid accumulation

Type III (intermediate) lesion: Type II changes and small extracellular lipid pools

Type IV (atheroma) lesion: Type II changes and core of extracellular lipid

Type V (fibro-atheroma) lesion: lipid core and fibrotic layer or multiple lipid cores and fibrotic layers

Type VI (complicated) lesion: complicated fibro-atheroma with hemorrhage or thrombus

#### **Statistical analysis.**

Data were expressed as mean  $\pm$  SEM; by using SPSS version 17, unpaired t-test was used to compare the mean values between different groups.

#### **Results**

##### **Effect of vildagliptin on serum lipid profile**

There was a statistically significant increase in serum TC, TG level ( $P < 0.05$ ) in all groups fed with cholesterol enriched diet for 6 weeks. At the end of 12 weeks, there was a statistically significant increase in serum TC level ( $P < 0.05$ ) in the induced untreated group continued to have cholesterol enriched diet for another 6 weeks, whereas groups treated with vildagliptin for 6 weeks showed a significant decrease in serum TC, TG level and significant increment in HDL level ( $P < 0.05$ ). As shown in table (1)

##### **Effect on aortic tissue reduced glutathione level (GSH) and MDA.**

At the end of study 12 weeks of high cholesterol diet the aortic GSH level was significantly decreased in induced untreated group (II) and significant increment in MDA level ( $P < 0.05$ ) in compared with normal control group.

For vildagliptin treated group (III) After 12 wks of high cholesterol diet, there was significant increment in the GSH level ( $P < 0.05$ ) associated with significant decrement of MDA level ( $P < 0.05$ ) As shown in table (2)

##### **Effect of vildagliptin on TNF- $\alpha$ and hsCRP**

Before the study, the baseline levels of serum hsCRP and TNF- $\alpha$  were statistically not significant among all groups. After 6 wks of high cholesterol diet, the TNF- $\alpha$  and hsCRP level

significantly increased ( $P<0.05$ ) in all group except normal group.

After 12 weeks, the hsCRP andTNF- $\alpha$  level significantly decreased in vildagliptin treated groups ( $P<0.05$ ) as compared with induced untreated group. As shown in table (3)

#### **Effect of vildagliptin on atherosclerosis and aortic intima-media thickness**

At the end of 12 weeks of high cholesterol diet rabbits treated with vildagliptin had a significant reduction

in the severity of atherosclerotic lesions in comparison with rabbits in the induced untreated group.

The level of aortic intima-media thickness(measured by histomorphometry) was significantly increased in induced untreated group (II), in compared with normal control ( $P<0.05$ ).

The aortic intima-media thickness level of vildagliptin treated (III) was significantly lower than that of induced untreated group (II). As shown in table (4) .

Table 1: Effect of cholesterol enriched diet, vildagliptin 50mg/kg/day on serum lipid profile level in mg/dl. The data expressed as mean  $\pm$ SEM

Group			TC mg/dl	TG (mg/dl)	HDL ( mg/dl)
I	Normal group	Zero time	54.3 $\pm$ 2.00	44.5 $\pm$ 0.6	16.2 $\pm$ 0.25
		6 weeks	56 $\pm$ 1.9	44.7 $\pm$ 0.9	15.8 $\pm$ 0.50
		12 weeks	57.2 $\pm$ 1.5	46.50 $\pm$ 1.0	16.4 $\pm$ 0.30
II	Induced untreated group	Zero time	52.5 $\pm$ 2.26	45.5 $\pm$ 1.8	15.9 $\pm$ 0.25
		6 weeks	596 $\pm$ 4.5*	159.3 $\pm$ 5.0*	20.0 $\pm$ 0.30*
		12 weeks	720 $\pm$ 9.5†	187.8 $\pm$ 8.0†	18.9 $\pm$ 0.75†
III	vildagliptin treated group	Zero time	54.7 $\pm$ 2.20	45.7 $\pm$ 1.5	16.9 $\pm$ 0.55
		6 weeks	598 $\pm$ 4.0*	159.5 $\pm$ 4.5*	18.2 $\pm$ 0.89*
		12 weeks	375 $\pm$ 2.7†	80.0 $\pm$ 2.50†	21.7 $\pm$ 1.10†

\*  $P<0.05$ (means at 6 weeks versus means at zero time)

†  $P<0.05$ (means at 12 weeks versus means at 6 weeks)

Table2: Changes in aortic oxidative stress (GSH in nmole/mg and MDAin  $\mu$ mole/gm)at the end of study. The data expressed as mean $\pm$ SEM

Groups		Aortic MDA level ( $\mu$ mole/gm)	Aortic GSH level (nmole/mg)
I	Normal group	1.9 $\pm$ 0.22	40.3 $\pm$ 2.4
II	Induced untreated group	9.0 $\pm$ 0.56*	20.9 $\pm$ 1.9*
III	vildagliptin treated group	3.3 $\pm$ 0.39	33.7 $\pm$ 2.2*

\* P<0.05(means at 6 weeks versus means at zero time)

† P<0.05(means at 12 weeks versus means at 6 weeks)

Table 3: Effect of cholesterol enriched diet, vildagliptin 50mg/kg/day on serum inflammatory marker (TNF- $\alpha$  level in pg/ml and hsCRP in mg/l) the data expressed as mean $\pm$ SEM

Group			TNF- $\alpha$ (pg/ml)	hsCRP(mg/l)
I	Normal group	Zero time	0.60 $\pm$ 0.09	3.0 $\pm$ 0.0
		6 weeks	1.08 $\pm$ 0.11	3.0 $\pm$ 0.0
		12 weeks	1.05 $\pm$ 0.06	3.0 $\pm$ 0.0
II	Induced untreated group	Zero time	0.77 $\pm$ 0.10	3.0 $\pm$ 0.0
		6 weeks	4.70 $\pm$ 0.54*	43 $\pm$ 1.8*
		12 weeks	7.05 $\pm$ 0.44†	57 $\pm$ 3.0†
III	vildagliptin treated group	Zero time	0.85 $\pm$ 0.05	3.0 $\pm$ 0.0
		6 weeks	4.60 $\pm$ 0.15*	41 $\pm$ 1.8*
		12 weeks	2.70 $\pm$ 0.17†	19 $\pm$ 1.0†

\* P<0.05(means at 6 weeks versus means at zero time)

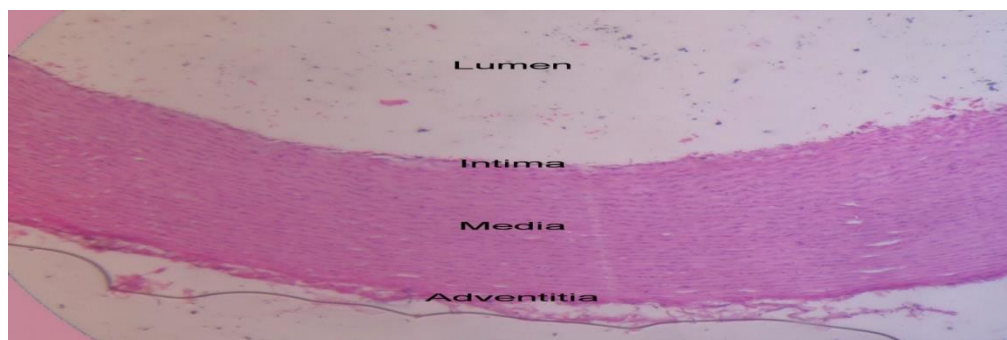
† P<0.05(means at 12 weeks versus means at 6 weeks)

Table 4:Changes in aortic intima-media thickness in ( $\mu$ m) at the end of the study the data expressed as mean $\pm$ SEM

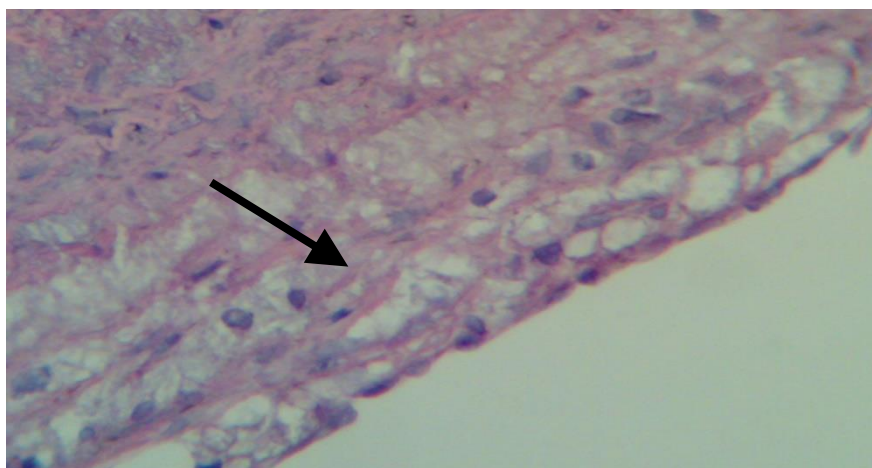
Groups		Aortic intima-media thickness ( $\mu\text{m}$ )
I	Normal group	$47.0 \pm 2.7$
II	Induced untreated group	$289 \pm 53.7^*$
III	vildagliptin treated group	$204 \pm 0.33^\dagger$

\*  $P < 0.05$  (means at 12 weeks versus means at zero time)

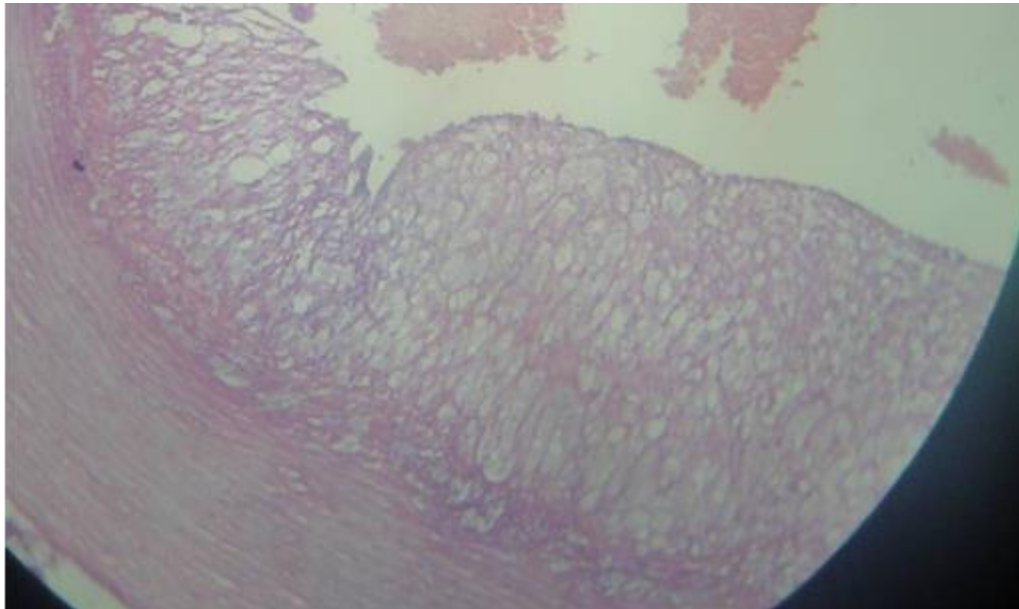
$^\dagger P < 0.05$  (means at 12 weeks versus means at 6 weeks)



**Figure (1):** photomicrograph of histomorphometric section in aortic arch shows the normal appearance of arterial wall layers. The section stained with haematoxylin and eosin ( $\times 10$ )



**Figure (2):** A cross section from aorta shows a narrowing of the arterial lumene by bulging of atherosclerotic plaque. (Type-5 atherosclerosis). The section stained with haematoxylin and eosin  $\times 40$



Figure(3) photomicrograph of histomorphometric section in aortic vildagliptin hyperlipidemic rabbits Show significant decrease in the aortic intima thickness as compare to induced untreated. section stained with haematoxylin and Eosin( $\times 40$  ).

## Discussion

In this study we demonstrate that high atherogenic diet cause significant increment in lipid parameter <sup>(8,9)</sup> (TC,TG,atherogenic index) in comparison with control group. Treatment with vildagliptin cause significant reduction in (TC,TG,atherogenic index) in comparison with induced untreated group this result constant with those reported by **Matikanianin et.al** <sup>(10)</sup> and monami et.al <sup>(11)</sup>

A significant increase inflammatory markers (hs.CRP,  $\text{TNF}\alpha$ ) level was found in rabbits fed with cholesterol enriched diet as compared with that in the normal control group. This result

is in agreement with that reported by **Howard and Culley** <sup>(12)</sup>, **Rajamannan et al.** <sup>(13)</sup> and **Sun et al.** <sup>(14)</sup>. The increase in serum CRP level is due to the fact that CRP is acute phase reactant that increases many folds during the inflammatory response to tissue injury, so it is increased by cholesterol enriched diet because cholesterol enriched diet causes the development of atherosclerosis which is a chronic inflammatory disease <sup>(15)</sup>.

vildagliptin treatment significantly reduce the elevation of inflammatory markers (hs.CRP,  $\text{TNF}\alpha$ ) in atherosclerosis model of hypercholesterolemic rabbit suggesting that vildagliptin inhibit vascular



inflammation induced by high atherogenic diet these results constant with those reported by and Bolli et .al<sup>(16)</sup> and Rizzo et.al<sup>(17)</sup>

In our study atherosclerosis was associated with increases in the levels of the lipid peroxidation product MDA, and decrease in the level of GSH in aortic tissue suggesting an increase in the levels or activity of oxygen radicals. MDA and GSH have been considered as specific indicators of oxidative status<sup>(18)</sup>. MDA level is widely utilized as a marker of lipid peroxidation and its measurement gives adirect evidence for LDL oxidation and is important in predicting free radical-induced injury, Therefore, the observed elevation in tissue MDA may be attributed to hyperlipidemia that enhances the processes of lipid peroxidation. . Hypercholesterolemia could increase the levels of ROS through stimulation of polymorph- nuclear leukocytes (PMNLs) and dysfunction of endothelial cells<sup>(19,20)</sup>.

Furthermore hypercholesterolemia, especially if prolonged, results in vascular oxidant burden<sup>(21,22)</sup>, which could favor GSH depletion because of enhanced oxidation of the tripeptide or its consumption by electrophilic

compounds like lipoperoxidation aldehydes<sup>(23,24)</sup>.

vildagliptin treatment had significantly reduced aortic MDA level suggesting decrease in ROS and subsequent lipid peroxidation. Also vildagliptin had significant effect on aortic GSH levels where prevents GSH depletion in hypercholesterolemic rabbit, and thus, maintain antioxidant reserve which is important for vascular protection against lipid peroxide<sup>(18)</sup>

In rabbits treated with vildagliptin there was a significant reduction in the severity of atherosclerotic lesions<sup>(17,25)</sup> in comparison with rabbits in the induced untreated group also there is significant decrement in aortic intima media thickness ( $P<0.05$ ) of vildagliptin treated group compared with that of the induced untreated group. In our study we found that vildagliptin exert anti inflammatory effect by reducing (hsCRP, &TNF- $\alpha$ ) and had antioxidant effect by reducing lipid peroxide (MDA) and enhancing GsH . So these findings may provide answers how vildagliptin reduce aortic intima-media thickness via suppression of systemic inflammatory response and oxidative stress.

## **Conclusion**

The results of present study reveal that sitagliptin possess antihypertensive effects in experimentally induced atherosclerosis via interfering with inflammatory and oxidative pathways

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