

## Research Article



## Evaluation the Effects of Sitagliptin on Atherosclerosis Progression in High Cholesterol-Fed Male Rabbits

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### ABSTRACT

The aim of the study was to assess the effect of sitagliptin on atherosclerosis via interfering with inflammatory and oxidative pathways. Twenty four 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 12 weeks. Group II rabbits fed with 1% cholesterol enriched diet. Group III rabbits fed with 1% cholesterol enriched diet together with sitagliptin (10 mg/kg once daily before morning feed). Blood samples were collected before (0 time) and every four weeks of experimental diets for measurement of serum triglycerides, total cholesterol, high density lipoprotein cholesterol, high sensitive C-Reactive Protein, endothelin-1 and intracellular adhesion molecule-1 level. At the end of 12 weeks the aorta was removed for measurement of aortic Malondialdehyde, reduced glutathione and aortic intimal thickness. Sitagliptin treatment did show a non significant effect on lipid parameters compared with induced untreated group ( $P > 0.05$ ). It was significantly reduced the elevation in high sensitive C-Reactive Protein, endothelin-1, intracellular adhesion molecule-1 level, aortic MDA and aortic intimal thickness compared with induced untreated group ( $P < 0.05$ ), and they restore aortic reduced glutathione level ( $P < 0.05$ ). Sitagliptin may reduce atherosclerosis progression in hyper cholesterolemic rabbit via interfering with inflammatory and oxidative pathways without affecting lipid parameters.

**Keywords:** Atherosclerosis, Inflammatory markers, Oxidative stress, Sitagliptin.

### INTRODUCTION

The main consequence of diabetes is accelerated onset of atherosclerosis, which an essential risk factor in the progress of ischemic cardiovascular disease. However, successful treatment of hyperglycemia alone is usually inadequate to prevent cardiovascular events because many other risk factors such as hypertension, hyperlipidemia and obesity are often present concurrently in the diabetic population.<sup>1</sup> Also the incidence of atherosclerosis is 3–4 times greater in diabetics than non-diabetics at comparable plasma cholesterol levels. Accordingly, prevention of onset and progression of atherosclerosis as well as good glycemic control are essential goals of management diabetic patients.<sup>2</sup> It has been widely demonstrated that inflammation plays an essential role in atherogenesis and mediating all stages of this disease from initiation through progression and, ultimately, the thrombotic complications of atherosclerosis.<sup>3</sup> Inflammation contributes to the development and progression of atherosclerosis and the therapeutic potential of some anti-inflammatory agents have been evaluated for possible anti atherosclerotic activity. Recent findings suggest that some drugs with anti-inflammatory action appear to have beneficial effects on atherosclerosis or subsequent risk for cardiovascular events.<sup>4</sup> Atherosclerosis is a complex process, and it is possibly mediated by high-fat diet and sedentary lifestyle.<sup>5</sup> Atherosclerosis is a progressive structural and functional vascular disease that initiates molecular and

cellular events mediated by endothelial dysfunction, resulting in reduction of nitric oxide production, increased endothelin-1 [ET-1], cyclooxygenase activity and inflammation.<sup>6</sup> Sitagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor represent a new class of antidiabetic drugs reflecting their ability to expand the biological actions of incretin hormones like Glucagon-like peptide-1 (GLP-1).<sup>7</sup>

Dipeptidyl peptidase-4 (DPP-4) inhibitors improve glucose metabolism through the increment of glucagon-like peptide-1 (GLP-1) receptor signaling, which enhances insulin secretion and decreases glucagon secretion in the pancreas.<sup>8</sup> Sitagliptin has been shown to decrease HbA1c level by about 0.7% points versus placebo. It is slightly less effective than metformin when used as a monotherapy. It does not cause weight gain and has less hypoglycemia compared to sulfonylureas. It is used as a second line drug (in combination with other drugs) after the combination of diet/exercise and metformin fails.<sup>9</sup>

Several researches have shown the beneficial effects of GLP-1 on the cardiovascular system.<sup>10</sup> Experimental studies have founded that DPP-4 inhibitors, as well as GLP-1 receptor agonists, decrease monocytes/macrophages accumulation in the arterial wall by reducing the inflammatory response in monocytes/macrophages, thereby contributing to the reduction of atherosclerotic lesions in apolipoprotein E-knockout mice, a murine atherosclerotic model.<sup>11</sup> The monocyte–macrophage system, which plays a central role in the pathogenesis of inflammation and atherosclerosis,



shows at least two distinct phenotypes of differentiation: pro-inflammatory (M1) and anti-inflammatory (M2).<sup>12</sup>

## MATERIAL AND METHODS

Twenty four local domestic male rabbits were involved in this study. The animals were randomly divided into three groups include: Group I rabbits fed normal chow (oxiod) diet for 12 weeks. Group II rabbits fed with 1% cholesterol enriched diet for 12 weeks. Group III rabbits fed with 1% cholesterol enriched diet together with sitagliptin (10 mg/kg once daily before morning feed for 12 week). The blood samples were collected before (0 time) and every four weeks on experimental diets for measurement of serum triglycerides (TG), total cholesterol (TC), HDL-C, intracellular adhesion molecule-1(ICAM-1), endothelin-1 (ED-1) and high sensitive C-Reactive Protein (hsCRP), level. At the end of 12 weeks the aorta was removed for determination of aortic intimal thickness aortic Malondialdehyde (MDA) and reduced glutathione (GSH).

## RESULTS

There was a slight insignificant increase in body weight of sitagliptin receiving group suggesting that food consumption probably was same in all the groups and cholesterol or sitagliptin had no effect on body weight. Compared with the control, levels of TC, TG, HDL-C, LDL-C, VLDL-C, atherogenic index, ICAM-1, hsCRP, ED-1, aortic intimal thickness and aortic MDA were increased and aortic GSH were decreased in the animals with atherogenic diet ( $P < 0.05$ ). Sitagliptin treatment don't show significant effect on lipid parameters compared with induced untreated group ( $P > 0.05$ ). Sitagliptin was decreased the elevation in hsCRP, ED-1, ICAM-1, aortic MDA and aortic intimal thickness compared with induced untreated group ( $P < 0.05$ ). Also they restore aortic GSH level ( $P < 0.05$ ) as shown in table (1), table (2), and table (3).

**Table 1:** Changes of rabbit's serum lipid profile (TC, TG and HDL) of the three experimental groups. The data was expressed as Mean  $\pm$  SEM (N=8 in each group) Using paired T-test.

		TC(mg/dl)	TG(mg/dl)	HDL(mg/dl)
Normal control	Zero time	80 $\pm$ 1.92	36.3 $\pm$ 4.56	13.7 $\pm$ 3.8
	12 weeks	85 $\pm$ 4.26	35.6 $\pm$ 4.2	14.5 $\pm$ 1.8
Induced untreated	Zero time	95 $\pm$ 1.75	35 $\pm$ 3.7	14.2 $\pm$ 1.9
	12 weeks	99 $\pm$ 31*	348 $\pm$ 16.3*	39 $\pm$ 5.4*
Sitagliptin 10mg/kg	Zero time	91 $\pm$ 1.92	34.9 $\pm$ 6.9	13.7 $\pm$ 4.6
	12 weeks	88 $\pm$ 34*	329 $\pm$ 5.7*	43.6 $\pm$ 2.9*

\* $p < 0.05$

## DISCUSSION

Accelerated atherosclerosis attended by high risk of premature mortality from cardiovascular disease (e.g. coronary heart disease) is a common and severe complication of type two diabetes.<sup>13</sup> Thus prevention of onset or development of atherosclerosis as well as a good

glycemic control is mandatory for the treatment of patients with this entity. The current study was to assess the effect of sitagliptin on atherosclerosis via interfering with inflammatory and oxidative pathways. Our results indicated that 12 weeks consumption of 1% high-cholesterol diet increased all serum cholesterol profile fractions and enhanced formation of atherosclerotic lesions including the thickening of the intima and/or build up of lipid droplets under endothelial cells in carotid artery. Moreover, the results of the current study shown that the level of ICAM-1 and other inflammatory markers were significantly higher in the atherosclerotic rabbits and sitagliptin treatment could significantly decrease it.

**Table 2:** Changes of rabbit's serum inflammatory markers (hs-CRP, ED-1 and ICAM-1) of the three experimental groups. The data was expressed as Mean  $\pm$  SEM (N=8 in each group) Using paired T-test.

		hs-CRP(mg/l)	ED-1(pg/ml)	ICAM-1(pg/ml)
Normal control	Zero time	2.8 $\pm$ 0.7	0.41 $\pm$ 0.3	7.6 $\pm$ 1.09
	12 weeks	2.9 $\pm$ 0.9	0.45 $\pm$ 0.2	7.5 $\pm$ 0.6
Induced untreated	Zero time	3.1 $\pm$ 0.5	0.45 $\pm$ 0.12	7.7 $\pm$ 0.9
	12weeks	22.6 $\pm$ 2.4*	1.6 $\pm$ 0.5*	25.7 $\pm$ 0.9*
Sitagliptin 10 mg/kg	Zero time	3.1 $\pm$ 0.8	0.46 $\pm$ 0.18	8.4 $\pm$ 1.05
	12weeks	13.4 $\pm$ 3.1*	0.89 $\pm$ 0.1*	17.5 $\pm$ 1.2*

\* $p < 0.05$

**Table 3:** The means of rabbits aortic Oxidative stress parameters (MDA and GTH) and aortic intima thickness of the three experimental groups at the end of experiment. The data was expressed as Mean  $\pm$ SEM (N=8 in each group) Using paired T-test.

Group	Aortic MDA $\mu$ mole/gm aorta	Aortic GTH nmole/mg aorta	Aortic intima thickness ( $\mu$ m)
Normal control	2.1 $\pm$ 0.24	37.3 $\pm$ 2.6	29.4 $\pm$ 3.7
Induced untreated	10.2 $\pm$ 0.51*	19.8 $\pm$ 1.5*	356.2 $\pm$ 32.5*
Sitagliptin 10 mg/kg	4.35 $\pm$ 0.72**	30.3 $\pm$ 2.8**	199.6 $\pm$ 24.3**

\* $p < 0.05$ ; \*\*  $p < 0.05$  as compare to induced untreated

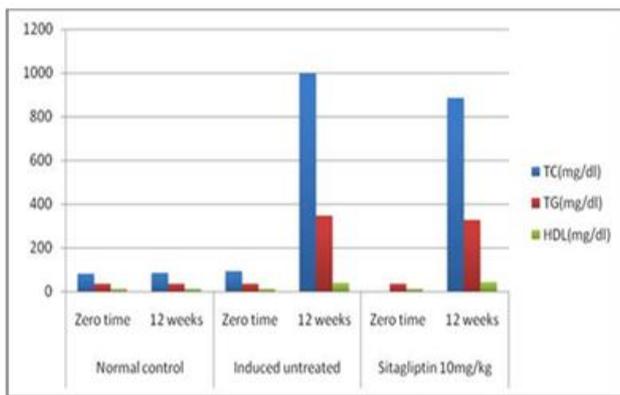
## Effects of sitagliptin on study parameters

### Effect of sitagliptin on lipid profile

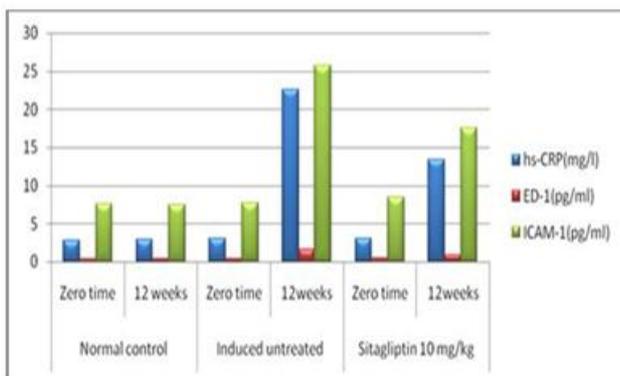
In the present study sitagliptin had no significant effects on lipid profile in comparing with induced untreated group. This is disagreement with Yoshiko Sakamoto et al., 2013 who had found that the blood pressure and serum levels of triglycerides and total cholesterol were significantly decreased in Patients with diabetes mellitus type 2 taking sitagliptine.<sup>14</sup> The finding of this study may be explained by the effect of high cholesterol diet can



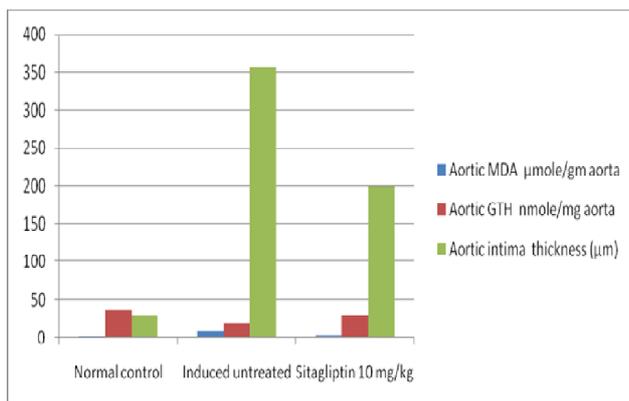
masks any changes expected from sitagliptin on lipid parameter (R).



**Figure 1:** Effect of sitagliptin 10 mg/kg /day treatment on serum lipid profile level (TC (mg/dl), TG levels (mg/dl) and HDL (mg/dl)) during the experimental treatment.



**Figure 2:** Effect of sitagliptin 10 mg/kg /day treatment on serum hs-CRP (mg/l), serum ED-1 (pg/ml) and serum ICAM-1 (pg/ml) in comparisons to the two control group (normal and induced untreated).

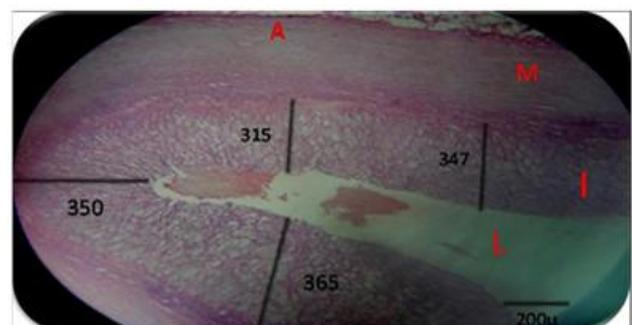


**Figure 3:** Effect of sitagliptin 10 mg/kg /day and treatment on aortic MDA μmole/gm aorta, aortic GTH nmole/mg aorta and aortic intimal thickness level (μm) in comparisons to the two control group (normal and induced untreated).

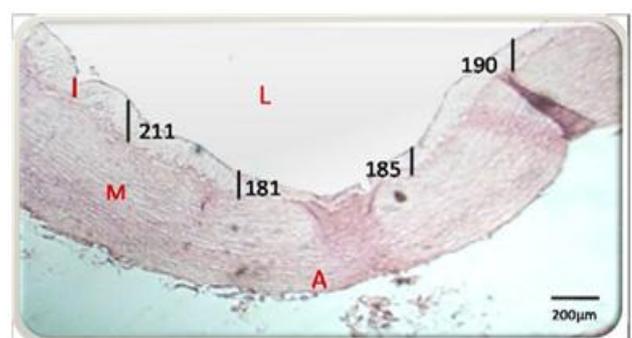
**Effect of Sitagliptin on inflammatory parameters (hsCRP, ED-1, ICAM-1)**

Sitagliptin, as one of the DPP-4 inhibitors, has been found to play a defensive role in the cardiovascular disease

included atherosclerosis.<sup>15</sup> But the mechanisms through which sitagliptin satisfy the progress of atherosclerosis are complex and still not completely understood. The result of our study demonstrated that there was a significant effect of Sitagliptin on inflammatory markers (hsCRP, ED-1 and ICAM-1) in rabbit on high fat diet. Suggesting a possible anti-inflammatory activity of Sitagliptin on the vascular inflammatory responses enhanced by high fat diet and this may explain its action on atherosclerosis progression. Also this is in agreement with Zeng et al., 2014 who had found that sitagliptin significantly decreased the expression of inflammatory cytokines (monocyte chemoattractant protein-1 and interleukin-6) in the aorta, as well as the serum levels of soluble vascular cell adhesion molecule-1 and P-selectin in apolipoprotein-E-knockout male mice.<sup>16</sup> Sitagliptin can activate the AMPK (AMP-activated protein kinase) pathway and inhibit MAPK (mitogen activated protein kinase) signaling by enhancing the phosphorylation of AMPK in the aorta. As a result, sitagliptin decreases serum soluble VCAM-1 and P-selectin levels, which play a central role in regulating the binding of leukocytes to endothelial cells as a key initial step in the development of atherosclerosis, and also decrease the expression of inflammation factors such as MCP-1 and IL-6. As is well known, inflammation in the vascular endothelium and following leukocyte recruitment are initiating events in the progression of atherosclerosis.<sup>16</sup>



**Figure(4) :** photomicrograph of histomorphometric section in aortic arch of rabbits on atherogenic diet for 12 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(5):** photomicrograph of histomorphometric section in aortic arche of sitagliptine treated groups. Show significant decrease in The aortic intima thickness as compare to induced untreated (L)lumen, (I)intima ,(M) media, (A) adventitia & section stained with haematoxylin and Eosin (× 10).

### Effect of Sitagliptin on oxidative stress

In the current study sitagliptin significantly reduced the elevation of serum MDA induced in high cholesterol-fed rabbits suggesting reduced in ROS and subsequent lipid peroxidation. Also sitagliptin significantly increased GSH level so it prevented GSH depletion in hypercholesterolemic rabbit, and thus, maintained antioxidant balance which is essential for vascular protection against lipid peroxide. This is in agreement with Chen et al., 2013 who found that treatment with sitagliptin can protect kidney from ischemia-reperfusion injury through suppressing oxidative stress and inflammatory reaction.<sup>17</sup> sitagliptin, an oral hyperglycaemic agent, has been found to be capable of increasing circulating GLP-1 levels via reducing DPP-IV activity GLP-1 analogues have been reported to have both antioxidative properties and anti-inflammatory properties, thereby contributing to its anti-inflammatory and anti-atherosclerotic cardiovascular protective effect.<sup>10</sup>

### Effect of Sitagliptin on aortic intima thickness

The present study demonstrated that sitagliptin treatment significantly suppress the increase in intimal thickness enhanced by atherogenic diet in rabbits as compared with induced untreated group.

This is in agreement with (Zeng et al., 2014) who indicates that sitagliptin can decrease the area of the atherosclerotic lesion, possibly by regulating the AMP-activated protein kinase (AMPK) and mitogen-activated protein kinase (MAPK) pathways and then reducing leukocyte –endothelial cell interaction and inflammatory reactions.<sup>16</sup> Sitagliptin can decrease the development of atherosclerotic lesion and changing the histological composition of atherosclerotic plaques by reducing the content of collagen fibre and tending to decrease smooth muscle cells and macrophages in the aorta. These results are consistent with previous studies on the anti atherosclerotic effects of DPP-4 inhibitors in animals.<sup>15</sup> It has been found that sitagliptin is cardio protective in the diabetic Akita mouse even at low doses.<sup>18</sup> A pooled analysis of 25 randomised clinical trials show that chronic treatment with sitagliptin may have cardio protective action in diabetes patients presenting with acute coronary syndrome.<sup>19,20</sup> This finding may provide mechanistic answers how sitagliptin modulates atherosclerotic lesions via number of pathways, including the inhibition of AMPK and MAPK pathways as well as the reduction of systemic inflammatory response and oxidative stress. Furthermore, This study demonstrated that sitagliptin prevent lipid peroxidation as shown by lowering serum MDA level which may be further contribute to its protective effect against hypercholesterolemic atherosclerosis.

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