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The atheroprotective effects of genistein in hypercholestrolemic male rabbit

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Objective The occurrence of this disease is related to different risk factors such as cardiovascular issues and elevated levels of plasma cholesterol, hypertension, diabetes mellitus and many others.
Methods Three groups of domestic male rabbits, six in each group, were studied. Each group constituted a different diet condition where group I had a normal chow diet; group II a 1% cholesterol-diet, group III a 1% cholesterol-diet and Genistein. The level of serum total cholesterol (TC), triglycerides (TG) and High density lipoprotein HDL-C serum interleukin-6 (IL-6), serum high sensitive C-reactive protein (hs-CRP), serum monocyte chemo-attractant protein type 1 High mobility group box 1 (MCP-1) and HMG-box1 was monitored by collecting blood samples at the start of the study, 28 and 56 days. Then, the aorta was removed to be examined (histopathology) for atherosclerosis lesion and thickening in the aortic intima-media.
Results In comparison to the control group (I), levels of TC, TG, low-density lipoprotein (LDL) cholestrol, very LDL cholestrol, hs-CRP, IL-6, MCP-1 and HMG-box1 had increased while serum HDL-C had reduced in the animals that followed a high-fat diet. Histologically, the aortic intimal thickening and atherosclerosis lesions has increased in the induced-untreated animals. The Genistein treated group showed a substantial decrease of lipid parameters in comparison with the induced-untreated group. Genistein counteracted the changes in hs-CRP, IL-6, MCP-1 and HMG-box1 in compared with the induced-untreated group ($P < 0.05$). Histomorphometric measurements indicated that Genistein significantly minimizes the thickness of the aortic intima-media and atherosclerosis lesions in comparison to the animals on a high-fat diet.
Conclusion The outcomes of this investigation show that Genistein significantly decrease the progression of atherosclerosis in hypercholesterolemic animals via inhibition of inflammatory markers and reduced levels of lipid parameters.
Keywords genistein, atherosclerosis, inflammation

Introduction

Atherosclerosis is a serious, chronic inflammatory disease where arteries become clogged with lipids (plaques or atheroma).[1] It begins with the accumulation of fatty substances in the arteries, this leading to the hardening and narrowing of said arteries.[2] The initial step in the pathogenesis of atherosclerosis is the lipid retention followed by chronic inflammation at sensitive sites in the major walls of arteries, this resulting in fatty streaks, which then develop into fibroatheromas.[3,4] Endothelial dysfunction indicates the start of this process.

A variety of mediators induce endothelial dysfunction, some of which are currently unknown, others known and related to established cardiovascular risk factors such as dyslipidemia, smoking, diabetes mellitus, aging and hypertension.[5] Other risk factors also contribute, for example lipids in the blood, low-density lipoprotein (LDL) and very LDL (VLDL) bind to endothelial cells and oxidize in the subendothelial space. T cells and monocytes bind to the endothelial cells and migrate to the subendothelial space, where monocytes engulf the oxidized LDL and transformed into foam cells. This process represents the first stage after which macrophages further elaborate proinflammatory cytokines that recruit smooth muscle cells, this producing an increase in smooth muscle cells and replication in dense extracellular matrix. The resulting atherosclerosis lesion is a subendothelial fibrous plaque composed of a lipid core surrounded by connective tissue fibers and smooth muscle cells.[6] Normal adaptive vascular responses, such as the release of endothelium-derived NO, are subsequently impaired. Endothelial activation results in inflammatory cells and the adhesion of platelets that can release proatherogenic growth factors. These factors result in the migration and proliferation of vascular smooth muscle cells (VSMCs) and the increased generation of extracellular matrix. Lymphocytes and monocytes invade the vessel walls, contributing to the processes of inflammation that results in atherosclerosis lesion formation. Forms of (LDL) cholesterol that accumulate in the vessel walls, may be taken up by macrophages and other vascular cells, resulting in foam cell formation.[7,8]

Inflammation plays an important role in plaque initiation, disruption and progression. Levels of C-reactive protein (CRP), an acute phase protein released from the liver in response to infection or inflammation, is a predictor of cardiovascular events.[9,10] Various forms of vascular injury, including elevated levels of modified (LDL), stimulate proinflammatory effects resulting in the recruitment of T lymphocytes and monocytes, the earliest feature of atherosclerosis lesion formation.[11] This infiltration by inflammatory cells is mediated by various chemokines including interleukins (IL) and monocyte chemo-attractant protein type 1 (MCP-1).[12] These monocytes change into macrophages and up-regulate their scavenger receptor expression, allowing them to accumulate lipids which results in the formation of fatty streaks and foam cells. These activated macrophages release growth factors and numerous cytokines including tumor necrosis factor-α, interleukins (IL, especially IL-1β, IL-6 and IL-8), and MCP-1. These factors, which can stimulate adhesion molecule expression, macrophage

activation, leukocyte migration, VSMC proliferation and vascular permeability, are normally expressed in the vessel wall, but their concentrations are significantly higher in atherosclerosis plaque.[13]

A wide body of literature showed that Genistein, which is an isoflavone phytoestrogen, has a central role in the regulation of different biological activities, acting as an inhibitor for tyrosine kinase proteins.[14] With this in mind, many researchers are investigating its potential action in the treatment of diseases such as the cancer, and its effect on skeletal and cardiovascular health. These investigations have focused on the potential hypolipidemic, anti-inflammatory, antioxidative and estrogenic effects of the Genistein.[15]

Materials and Methods

Three groups of domestic male rabbits, six in each group, were studied, each under different conditions. Group I was the control group kept on a normal chow diet; group II was given a 1% of cholesterol-diet, group III a 1% cholesterol-diet and Genistein. Levels of serum total cholesterol (TC), triglycerides (TG) and HDL-C serum IL-6, serum high sensitive CRP (hs-CRP), serum MCP-1 and serum HMG-box1 was monitored by collecting blood samples at the start of the study, 28 and 56 days. At the end of the experiment, the aorta was removed to be examined (histopathology) for atherosclerotic changes or changes in the thickness of aortic intima-media.

Blood Sampling

The sampling process involved taking 5 ml of blood from the central ear artery of the rabbits following overnight fasting. Blood sampling was carried out at zero time, 28 and 56 days. The samples of blood were permitted to clot at 37°C, then cenə trifuged for 12 min at 6000 rpm. The isolated serum was analyzed to measure serums TC, TG, HDL-C, LDL-C, VLDL-C, IL-6 and CRP, HMG-box1, MCP-1.

Tissue Sampling for Histopathology

At the end of the experiment (56 days), the rabbits were euthanized using chloroform. Their chest walls were dissected for resection of the aorta. The removed connective tissue samples, after removal of adherent fat, were immediately fixed in a 10% xylene solution. Following fixation tissue sampling, the samples were processed in the usual method. Samples were observed with a microscope under a magnification power of 10× and 40×, the histopathological alterations determined. Atherosclee rosis lesions were categorized into different phases as outlined by the American Heart Association classifications.[16]

Results

Influence of Genistein on Serum Lipid Profiles

After 28 days, the results revealed a substantial increase in the lipid profile of the rabbits that followed a high cholesterol diet. Over the same period, the Genistein-treated rabbits showed a substantial decrease in serum lipids in comparison to the untreated rabbits (Table 1).

Influence of Genistein on hs-CRP, IL-6 and MCP1

According to the statistical analyses, at the beginning of the study, the groups of rabbits showed non-significant differences in levels of serum hs-CRP, IL-6 and MCP-1. However, substantial differences ($P < 0.05$) in levels of hs-CRP, IL-6 and MCP-1 were recorded after 28 days where a significant increase was noticed in the high cholesterol diet animals. After 56 days, the Genistein-treated rabbits showed a substantial decrease ($P < 0.05$) in the levels of hs-CRP, IL-6 and MCP-1 (Table 2).

Influence of Genistein on HMG-box1

Prior to the experiment, the levels of serum HMG-box1 in the rabbits, were statistically analyzed, the results showing non-significant differences. After 28 days, the high cholesterol diet group showed a substantial increase in HMG-box1 levels. After 56 days, the Genistein-treated rabbits showed a clear increase in the level of HMG-box1 (Table 3).

Influence of Genistein on Aortic Intima-media Thickness

After 56 days, the high cholesterol diet rabbits showed a clear increase in the thickness of the aortic intima-media, as shown in Figs. 1 and 2. The Genistein-treated rabbits had a lower thickening in the aortic intima-media (Figure 3 and Table 4).

Table 1. **Influence of cholesterol-enriched diet and Genistein 10 mg/kg/day on serum lipid profile. Values are represented as means ± SEM (Six rabbits in each group)**

		TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)
Normal control	Zero time	48.8 ± 3.0	49.5 ± 2.1	21.0 ± 1.7
	28 days	47.0 ± 3.8	50.8 ± 3.4	20.1 ± 1.5
	8 weeks	46.5 ± 2.4	44.3 ± 0.8	21.5 ± 0.8
Induced-untreated	Zero time	46.3 ± 3.7	57.3 ± 1.8	20.2 ± 1.4
	28 days	625.0 ± 12.1*	123.7 ± 17.8*	13.9 ± 0.5*
	8 weeks	859.0 ± 73.8†	172.0 ± 6.7†	12.0 ± 0.2†
Genistein 10 mg/kg	Zero time	51.0 ± 2.7	51.5 ± 1.8	21.8 ± 1.1
	28 days	663.8 ± 17.8*	138.5 ± 17.0*	12.3 ± 0.4*
	8 weeks	271.8 ± 50.6†	85.8 ± 12.3†	17.1 ± 0.9†

*Means at 28 days significant to means at 0 days; †Means at 56 days means to averages at 28 days.

Table 2. **Influence of cholesterol-enriched diet and Genistein 10 mg/kg/day on serum inflammatory marker (hs-CRP, IL-6, MCP-1). Values are represented as means ± SEM (Six rabbits in each group)**

		hs-CRP mg/l	IL-6 pg/l	MCP-1
Normal control	Zero time	3.0 ± 0.2	1.3 ± 0.1	0.7 ± 0.1
	28 days	3.4 ± 0.1	1.3 ± 0.1	1.0 ± 0.1
	8 weeks	3.3 ± 0.2	1.2 ± 0.2	0.7 ± 0.1
Induced-untreated	Zero time	3.0 ± 0.2	1.5 ± 0.3	1.0 ± 0.2
	28 days	5.5 ± 0.2*	4.7 ± 0.3*	4.2 ± 0.3*
	8 weeks	7.5 ± 0.2†	6.6 ± 0.3†	5.6 ± 0.2†
Genistein 10 mg/kg	Zero time	3.1 ± 0.1	1.3 ± 0.3	1.2 ± 0.2
	28 days	6.1 ± 0.3*	4.8 ± 0.3*	4.6 ± 0.2*
	8 weeks	5.3 ± 0.3†	2.5 ± 0.4†	1.6 ± 0.1†

*Means at 28 days significant to means at 0 days, †Means at 56 days significant to means at 28 days.

Table 3. **Influence of cholesterol-enriched diet and Genistein 10 mg/kg/day on serum inflammatory marker (HMG-box1). Values are represented as means ± SEM (Six rabbits in each group)**

		HMG-box1
Normal control	Zero time	0.7 ± 0.03
	28 days	0.8 ± 0.1
	8 weeks	0.7 ± 0.2
Induced-untreated	Zero time	0.8 ± 0.1
	28 days	2.9 ± 0.04*
	8 weeks	4.1 ± 0.09†
Genistein 10 mg/kg	Zero time	0.7 ± 0.6
	28 days	3.8 ± 0.08*
	8 weeks	1.9 ± 0.4†

*Means at 28 days significant to means at 0 days; †Means at 56 days significant to means at 28 days.

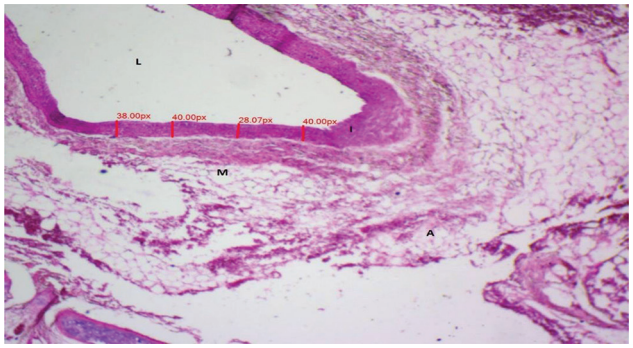

Fig. 1 **Photomicrograph of histophotometric section in aortic arch of rabbit fed on normal diet for 8 weeks (normal control) show the normal intimal thickness and intact continuous endothelium. Stained with haematoxylin and Eosin (10×), where, I: intima of the aorta, M: media of the aorta, A: adventitia of the aorta and L: the lumen of the aorta.**

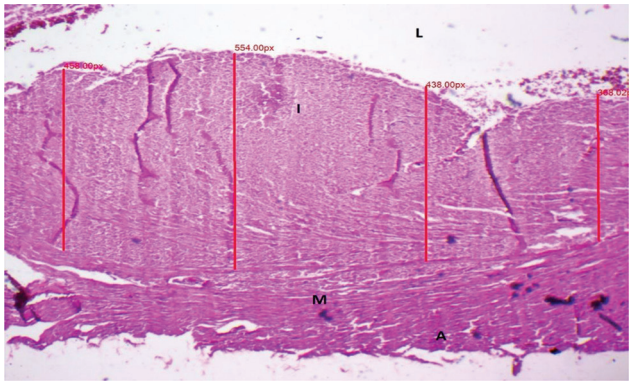

Fig. 2 **Photomicrograph of histomorphometric section in aortic arch of rabbits on hypercholeterolemic diet for 8 weeks (induced-untreated) show diffuse intimal thickening and in completely coalesced extracellular lipid underneath a layers of macrophages and smooth muscle cells. The section stained with hematoxylin and eosin (10×), where, I: intima of the aorta, M: media of the aorta, A: adventitia of the aorta and L: the lumen of the aorta.**

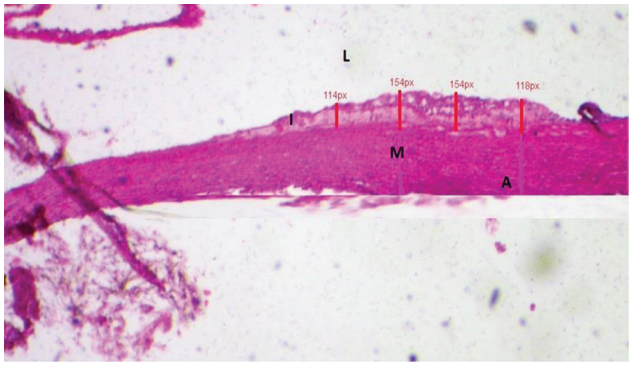

Fig. 3 **Photomicrograph of histomorphometric section in aortic arch of Genistein hyperlipidemic rabbits. The section stained with hematoxylin and eosin (10×), where, I: intima of the aorta, M: media of the aorta, A: adventitia of the aorta and L: the lumen of the aorta.**

Discussion

The outcome of the current study reveals a direct relationship between a cholesterol-enriched diet and serum TC, TG, LDL-C, VLDL levels, where a clear increase has been noted in these serums after 56 days. Levels of HDL-C decreased significantly. Similar results were found by Prasad and Lee[17] and Nigrisa and Paolo.[18] Additionally, the outcomes of this investigation indicate a direct relationship between Genistein and the levels of serums TC, TG, HDL-C, LDL-C, VLDL-C, which agrees with the results

Table 4. **Thickening values in aortic intima-media (px) after 56 days. Values are represented as means ± SEM (Six rabbits in each group)**

Group	Aortic intimal thickness (µm)
Normal control	36.5 ± 2.8
Dietary induced-untreated	454.6 ± 38.3*
Genistein group	135.0 ± 11.0†

*Means for induced-untreated animals significant to control group.
†Means for Genistein-treated animals significant to induced-untreated group.

of Eftekhari et al.[19] and Tang et al.[20] These results demonstrate that cholesterol levels lower when increasing LDL receptor activity, this increasing the absorption of LDL from the blood into the liver. Genistein reduces the activity of enzymes involved in fatty acid synthesis, such as fatty acid synthase, resulting in decreased serum triglyceride and VLDL. In terms of levels of hs-CRP, the results here agree with the results of Zhao and Wu,[21] in that a remarkable increase was noted in levels of hs-CRP in comparison to rabbits on a normal diet. However, Genistein additives substantially decrease the hs-CRP plasma level in comparison to normal animals, these findings in line with Kim and Lim.[22] Cholesterol-enriched diet caused significant increased levels of MCP-1 in comparison to control group, this in agreement with Chen et al.[23] who showed that MCP-1 expression increased after a 3-week, high cholesterol diet in comparison to a control group. It has been proved that the MCP-1 plays an essential role in atherosclerosis disease, MCP-1 is expressed mainly through endothelial and inflammatory cells. The expression level is upregulated after tissue injury and proinflammatory stimuli, both correlating with atherosclerotic disease.[24] The generation of MCP-1 substantially decreased in the Genistein treated animals, in comparison with the induced-untreated rabbits, this also in agreement with Kim and Lim[22] who showed that a comparatively low amount of Genistein might decrease MCP-1 through suppression of NF-κB activation. In the current investigation, a clear increase was noted in levels of IL-6 in the hypercholesterolemic rabbits. This could be attributed to the fact that hypercholesterolaemia causes endothelial microinflammation, which increases the proinflammatory cytokine IL-6. These results are in agreement with those of Uich et al.[25] and Elwakkad et al.[26] In the current study, Genistein treatment caused a significant reduction of IL-6 levels, this supported by Ibrahim et al.[27] and Palanisamy et al.[28] These collective results demonstrate that Genistein caused a substantial reduction in serum IL-6 in nonalcoholic steatohepatitis rats induced by a high fat diet (HFD). Genistein presents anti-inflammatory activity by reducing IKB-α phosphorylation in a nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and inhibitor of kappa light polypeptide gene enhancer in B-cells (IKKB). In these studies, it was found that aortic intimal thickening and atherosclerosis area in hypercholesterolemic animals was substantially decreased by treatment with Genistein. Comparable outcomes were found by Wang et al.[29]

Conclusion

The findings of this investigation demonstrated that Genistein significantly decreased atherosclerosis progression in hypercholesterolemic rabbits via inhibition of inflammatory markers, reduced levels of lipid parameters.

Conflict of Interest

None.

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