

Original Article

Protective effects of *Allium sativum* against defects of hypercholesterolemia on pregnant rats and their offspring

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Abstract: Sixty fertile female and male albino rats of Wistar strain (1 male/ 3 females) were used in the present study. The females were divided into four groups of ten rats each. Group 1 received water and standard feeds for thirty-four days. Group 2 was fed with a cholesterol-containing diet (1%) for two weeks prior to onset of gestation and maintained administration till parturition. produce atherosclerosis (34 days). Group 3 received intragastric administration of 100mg homogenate of garlic (*Allium sativum*)/kg body weight for three weeks prior to onset of gestation as well as throughout the gestation period. Group 4 intragastrically administered garlic for one week of group B and maintained with combined garlic-treatment for the mentioned period. At parturition, the pregnant were sacrificed and serum total cholesterol (TCL), triglycerides (TG), HDL, LDL and creatine kinase activity (CK) were determined. The total numbers of offspring were recorded and examined morphological for congenital abnormalities. Biopsies of heart and dorsal aorta of both pregnant and their offspring (1 day-age) were processed for investigation at light and transmission electron microscopy. The skeleton of the newborn of different experimental groups were stained with alizarin red s and morphometric assessment of mandibular and appendicular bone length. The study revealed that the myocardium of atherosclerotic mother exhibited leukocytic inflammatory cell infiltration associated with necrosis, eosinophilia of myocardial fibers, and edema of blood vessels. Ultrastructural studies revealed swelling of mitochondria, disruption of cristae in the myocardial muscle fibers. The dorsal aorta possessed accumulation of extra-cellular lipid in intima lining of endothelium. The collagenous fibrils in the tunica adventitia became fragile and loosely separated from each other. Numerous foamy lipid laden cells were detected within the tunica intima causing deterioration of the elastic fibers, resulting in fibrinoid necrosis. Oral supplementation with *Allium sativum* (100 mg / kg) ameliorated these effects in myocardium muscle of mothers and offspring; however the dorsal aorta of mothers showed partial amelioration. Hypercholesterolemic mothers exhibited marked alterations in serum TCL, TG, LDL and CK activity. Supplementation with *Allium sativum* ameliorated the drastic biochemical alterations. Concerning pregnancy, hypercholesterolemia increased the incidence of abortion and abnormalities of the newborn including decreased body weight, reduced ossification of axial (mandible) and appendicular bones. All these effects were markedly ameliorated by supplementation with *Allium sativum*. The author finally concluded that hypercholesterolemia exhibits pathological alterations of myocardial muscles reducing its optimal capacity for pumping blood to different body organs along with atherosclerosis of dorsal aorta which intern affect the progress of gestation and development of both morphological and skeletal abnormalities. *Allium sativum*-supplementation leads to amelioration of both mother and their offspring investigated parameters as a result of its antioxidant activity.

Keywords: Hypercholesterolemia, *Allium sativum*, cardiovascular, pregnant rats, offspring

Introduction

Familial hypercholesterolemia is a genetic disorder of lipoprotein metabolism characterized by very high plasma concentrations of low-density lipoprotein cholesterol, deposition of cholesterol in extravascular tissues, such as tendon xanthomas, and increased risk of premature coronary

heart disease [1]. Cardiovascular calcification and abnormal valve function are common consequences of hypercholesterolemia [2]. Hypercholesterolemia led to atherosclerosis which is described by an excessive inflammatory, fibro-fatty, proliferative response to damage of the artery wall involving several cell types, particularly smooth muscle cells, monocyte-derived

macrophages, T-lymphocytes and platelets [3].

Garlic (*Allium sativum*) belongs to the plant family *Liliaceae*, which is a genus of 500 species. Garlic (*Allium sativum*) has been grown around the world, from Mediterranean climates to Siberia. Ancient Egyptians used it as a form of currency; its medical and magical powers were described on the walls of ancient temples and on papyrus dating to 1500 BC. Garlic contains at least 33 sulfur compounds (Sulfur compounds: alliin, allicin, ajoene, allylpropyl disulfide, diallyl trisulfide, sallylcysteine, Vinylthiines, S- allylmercaptocystein, and others), several enzymes (allinase, peroxidases, myrosinase, and others), 17 amino acids (arginine and others), and trace minerals such as selenium, germanium, tellurium and other trace minerals [4,5].

Extracts of garlic was found to decrease serum cholesterol levels in humans, inhibit cholesterol biosynthesis, suppress LDL oxidation, lower plasma fibrinogen and increase fibrinolytic activity [6, 7, 8], and thus to possess antiatherosclerotic properties [9, 10].

Human studies on garlic's ability to prevent and possibly reverse atherosclerosis was tested in a randomized, double-blind, placebo-controlled, four-year study in which 152 men and women were given 900 mg garlic powder per day [11]. The authors selected subjects with significant plaque buildup and at least one additional cardiovascular risk factor (high LDL cholesterol levels). After four years-treatment, the atherosclerotic subjects receiving garlic-treatment (*A. sativum*) exhibited an average 2.6% reduction in plaque volume. Experimental studies on *A. sativum* were found to show a decrease in serum lipid levels in experimental atherosclerotic albino rats [12] and hyperlipidemic guinea pigs [13].

There have been discrepancies in the results of garlic and its extracts on experimentally induced hypercholesterolemia in animal models which may be due to differences in the concentrations of sulfur-containing compounds. These studies have shown that garlic is effective in reversing this condition and improving atherogenic effects on vascular endothelium. However, the effects of garlic have not been unequivocal in human trials, with some studies showing improvement in hyperlipidemia and others showing no effect. Additionally, it would be of the utmost value if other experimental factors such as the recruit-

ment of subjects, dietary and lifestyle of subjects, length of study, and methods of analysis among several studies be comparable [14, 15]

Thus, the present study investigates the effects of elevated cholesterol level during pregnancy in a rat model, on mothers and their offspring in order to observe the developmental origin of disease in childhood, and trials to ameliorate these effects by supplementation with *Allium sativum*.

Material and methods

Induction of hypercholesterolemia

Forty fertile male and virgin female albino rats of Wistar strain weighing approximately 150-180 g body weight were obtained from Hellwan Breeding Farm, Ministry of Health, and Egypt. They were kept under good ventilation with 12 hour light and dark cycle. Females were made pregnant by keeping them with healthy fertile male rats overnight (at a ratio of 1male /3females). On the next morning, vaginal smear were examined and the day of finding sperm was considered day 0 of pregnancy.

Rats in the control group were fed *ad libitum* on standard diet throughout the experimental period. However, experimental hypercholesterolemic animals were fed a cholesterol-enriched high-fat diet (15% cocoa butter, 2% cholesterol, 15 % sucrose, 15% corn starch and 4.7% cellulose. The time required to induce hypercholesterolemia was determined by monitoring their serum cholesterol level. The experimental animals were supplemented cholesterol enriched diet for 2 weeks before onset of gestation as well as till parturition (34 days).

Allium sativum treatment

Five gram freshly prepared *Allium sativum* was thoroughly homogenized with 50cc saline solution by a glass mortar and kept in refrigerator freshly prepared every week during experimentation. The crude homogenate was intragastrically administered to experimental hypercholesterolemic pregnant rats at doses of 100 mg/kg body wt starting from a week prior to onset of feeding on hypercholesterolemic diet (41 days).

Experimental work

The pregnant rats were arranged into four

groups, (n = 10 per group): normal control group, *Allium sativum* supplemented group, experimental hypercholesterolemic pregnant, and *Allium sativum* supplemented- hypercholesterolemic pregnant. Pregnant of both control and experimental groups were sacrificed at 15 days prenatal for resorption of fetuses as well as at parturition, in accordance with Egyptian Bioethics Committee regulations. The offspring of the experimental groups were weighed and examined morphologically for abnormalities. These were followed by immediately fixation in 10% phosphate buffered formalin Ph7.4 and processed for staining with Alizarin red S for ossified skeletal bones and examined in different groups. Besides, offspring and mothers were subjected to the followings investigation:

Biochemical assessments

At parturition, five pregnant mothers were sacrificed and blood was collected in non-heparinized tube and centrifuged at 2000 RPM and serum was collected. Serum levels of TCL [16], TG [17] and HDL [18] were determined. In case of LDL, it was calculated from the total cholesterol concentration (TC) and the HDL-cholesterol concentration and TG according to the equation described by Friedwald *et al* [19]. Serum LDLc (mg.dL = $Tc-HDLc-TG/5$, where 5 is a calculation factor.

Histological investigation of maternal tissues

Both control and experimental treated groups were sacrificed at parturition and dorsal aorta and heart were incised and fixed immediately in 10% formal saline for 24 hours, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and mounted in molten paraplast 58-62°C. Five µm histological sections were cut, stained with Harris hematoxylin & eosin and investigated under bright field Leitz microscope.

Transmission electron microscopic investigations (TEM)

At parturition, the maternal heart ventricles & dorsal aorta of both control and experimentally-treated groups were separated immediately fixed immediately in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4), post fixed in 1% osmium tetra oxide at 4°C for 1.5 hour. This was followed by dehydration in ascending grades of ethyl alcohol and

embedded in epoxy-resin. Ultrathin sections were cut with a diamond knife on a LKB microtome and mounted on grids, stained with uranyl acetate and Lead citrate and examined at Joel Transmission electron microscope.

Statistical analysis

All the grouped data were statically evaluated with statistical package for social sciences (SPSS) software. Statistical analysis was carried out by using MANOVA as well as between the same groups, followed by calculation of F values. P values of less than 0.05 were considered to indicate statistically significance. All the results were expressed as the mean ± standard error.

Results

Maternal biomarkers of hypercholesterolemia

Table 1 illustrates the effects of experimental hypercholesterolemia on serum TCL, TG, HDL, LDL and CK activity. Hypercholesterolemic mothers exhibited significant increase of serum TCL, TG, LDL and CK activity. Treatment with *Allium sativum* ameliorated the drastic effects but still not matched with the control value.

Histological observations of maternal tissues

Heart: At light microscopy, the control myocardium ventricle exhibits regularly attached muscle fibers with one or two nuclei, centrally located within the cell. Intercellular spaces are enclosed by delicate collagenous tissue containing blood capillaries. Between the ends of adjacent cardiac muscle cells, and the intercalated discs, specialized intercellular junctions are detected (**Figure 1A**). *Allium sativum* possessed normal histological architecture similar to control (**Figure 1A1**).

In contrast, the ventricle of experimental hypercholesterolemic rats showed histopathological changes manifested by irregular arrangement of muscle fibers with numerous necrotic zone infiltrated by inflammatory cells. The necrotic fibers become eosinophilic and their nuclei appeared to be dispersed in a zone of inflammatory cells. The blood vessel appeared swollen and edematous. Fat deposits were detected in between the muscle muscle fibers (**Figure 1A2**). Experimental hypercholesterolemic group supple-

Table 1. TCL, TG, HDL, LDL and CK of experimental hypercholesterolemic rat alone or in combination with *Allium-sativum*-treatment comparing with either control or *Allium sativum* group

	Groups	TCL	TG	HDL	LDL	CK
Mean± SE	G1	65.4±0.09	52.6±1.3	32.03±1.02	30.33±0.90	255.4±0.23
	G2	60.4±0.92	40.2±1.7	30.1±0.27	28.2±1.06	251.34±1.3
	G3	128.8±1.6	34.9±1.2	28.3±1.06	58±1.4	365.6±1.8
	G4	86.5±0.97	35.4±1.2	27.6±0.4	40.6±1.07	334.8±1.1
	S	S	S	S	S	S

Data were expressed as Mean ± SE (n=10). Abbreviations; G1, normal control; G2, *Allium sativum*-treatment; G3, Experimental hypercholesterolemia; G4, Experimental hypercholesterolemia ± *Allium sativum*; TCL, total cholesterol; TG, total glycerides ; HDL; high density lipoprotein; LDL, low density lipoprotein; CK, creatine phosphokinase. By applying one way ANOVA and determined F test. The data significant at $p < 0.05$.

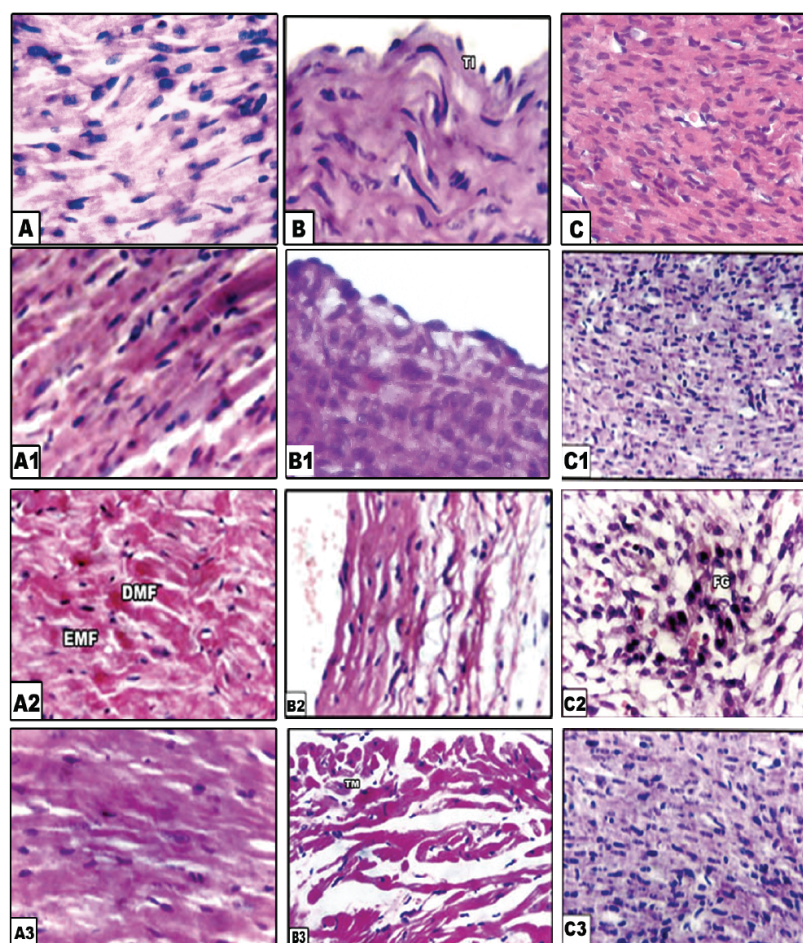


Figure 1. A-C3: Histological sections in both maternal heart (A-A2) and dorsal aorta(B-B2) as well as heart of offspring (1 day-old). (C-C2). A. Heart of control mother. A1. Heart of *Allium sativum* supplementation. A2. Hypercholesterolemic-heart. A3. Hypercholesterolemic and *Allium sativum* supplementation. B. Maternal control dorsal aorta. B1. Dorsal aorta of mother supplemented *Allium sativum*. B2. Maternal hypercholesterolemic dorsal aorta. B3. Aorta of hypercholesterolemic mother supplemented *Allium sativum*. C. Heart of control offspring. C1. Heart of offspring maternally supplemented *Allium sativum*. C2. Heart of offspring of hypercholesterolemic mother. C3. Heart of hypercholesterolemic mother supplemented *Allium sativum*. Hx-E X 250. Abbreviations; EMF, eosinophilic muscle fiber; DMF, degenerated muscle fiber.

mented *Allium sativum* showed amelioration of myocardial muscle fibers (**Figure 1A3**), and the histological appearance was almost similar to controls.

Ultrastructural observation

The control myocardial muscle possesses regularly arranged tightly backed bundles of muscle fibers with typical intercalated discs between them. The myofibrils are longitudinally divided into sarcomeres having regularly oriented me, Z, H and A bands which is composed of slender oriented filaments. Abundant distribution of mitochondria are detected in-between the myofibrils. Nuclei are frequently observed toward the middle of myocardial cells, but they are occasionally located near the sarcolemma (**Figure 2A**). *Allium sativum* supplemented group revealed similar normal observations.

In experimental hypercholesterolemic group, the myocardial muscle fibers exhibited disrupted muscle bands with almost missing in damaged muscle fibers. The myoblast nuclei possessed folded nuclear envelope and electron-dense chromatin material. The mitochondrial arrangement appeared distorted with considerable swelling and ill differentiated cisternae (**Figure 2A1&A2**). The mentioned hypercholesterolemic defects were ameliorated after supplementation of *Allium sativum* (**Figure 2A3**).

Dorsal aorta: The arterial wall of the control rats is consisted of three well-defined concentric layers that surround the arterial lumen, internal intima, middle media and the outer adventitia. The tunica intima consisted of a single layer of flattened endothelial cells supporting by a layer of collagenous tissue rich in elastin disposed in the form of both fibers and discontinuous sheets. The subendothelial supporting tissue contained scattered fibroblasts and other cells. The tunica media consisted principally of smooth muscle cells arranged in layers of circumferential orientation. The extra-cellular matrix consisted largely of elastic fibers and collagen. Tunica adventitia, the outermost layer of the artery, consisted of loose matrix of elastin; smooth muscle cells, fibroblasts, and collagen (**Figure 1 B**). Similar observation were detected post *Allium sativum*-supplementation (**Figure 1B1**).

In hypercholesterolemic mothers, endothelial

cells lining the tunica intima and the elastic fibers were sloughing and degenerated. Extracellular lipid (remnants of dead foam cells including small droplets) was detected in the deep intima layer. The tunica media smooth muscle cells become vacuolated and widely separated from each other as a result of increased accumulation of lipid loading cells. The collagenous fibrils in the tunica adventitia became fragile and loosely separated from each other (**Figure 1B2**).

On the other hand, experimental hypercholesterolemic group supplemented with *Allium sativum* showed partial amelioration of tunica intima which was still appeared fragile and enclosed by numerous lipid-loaden cells. However, the endothelial lining cells and elastic fibers of the tunica intima showed moderate regeneration. The tunica media appeared fragile and their content widely separated from each other. The myoblasts of the tunica adventitia exhibited moderate regenerated activity (**Figure 1B3**).

TEM

In the control, the endothelial cells lining the tunica appeared polygonal, with characteristic luminal protrusion. The cytoplasm is poor in Golgi complex and mitochondria. However, there is abundant distribution of cytoplasmic filaments, often arranged in bundles. A thin basal lamina separated the endothelial cells from the underlying tunica media which is composed of branched fibroblast – like cells and a network of fine collagenous and elastic microfibrils. The tunic media are markedly thickened. The tunica adventitia is considerably thick and consists of bundles of collagenous and elastic fibers. Longitudinally arranged smooth muscle cells are detected (**Figure 2B**).

In experimental hypercholesterolemic group, the endothelial cells appeared sloughing and degenerated. Numerous foamy lipid loaden cells were detected within the tunica intima causing deterioration of the elastic fibers. Fibrinoid necrosis developed in tunica intima with destruction of the internal elastica. The necrotic patches of tunica intima became surrounded by giant macrophages with signs of apoptic cell death. The tunica media possessed excessive accumulation of fatty vacuoles lying adjacent with each other forming a lipid core. The tunica media exhibited hyalinization and degenerated

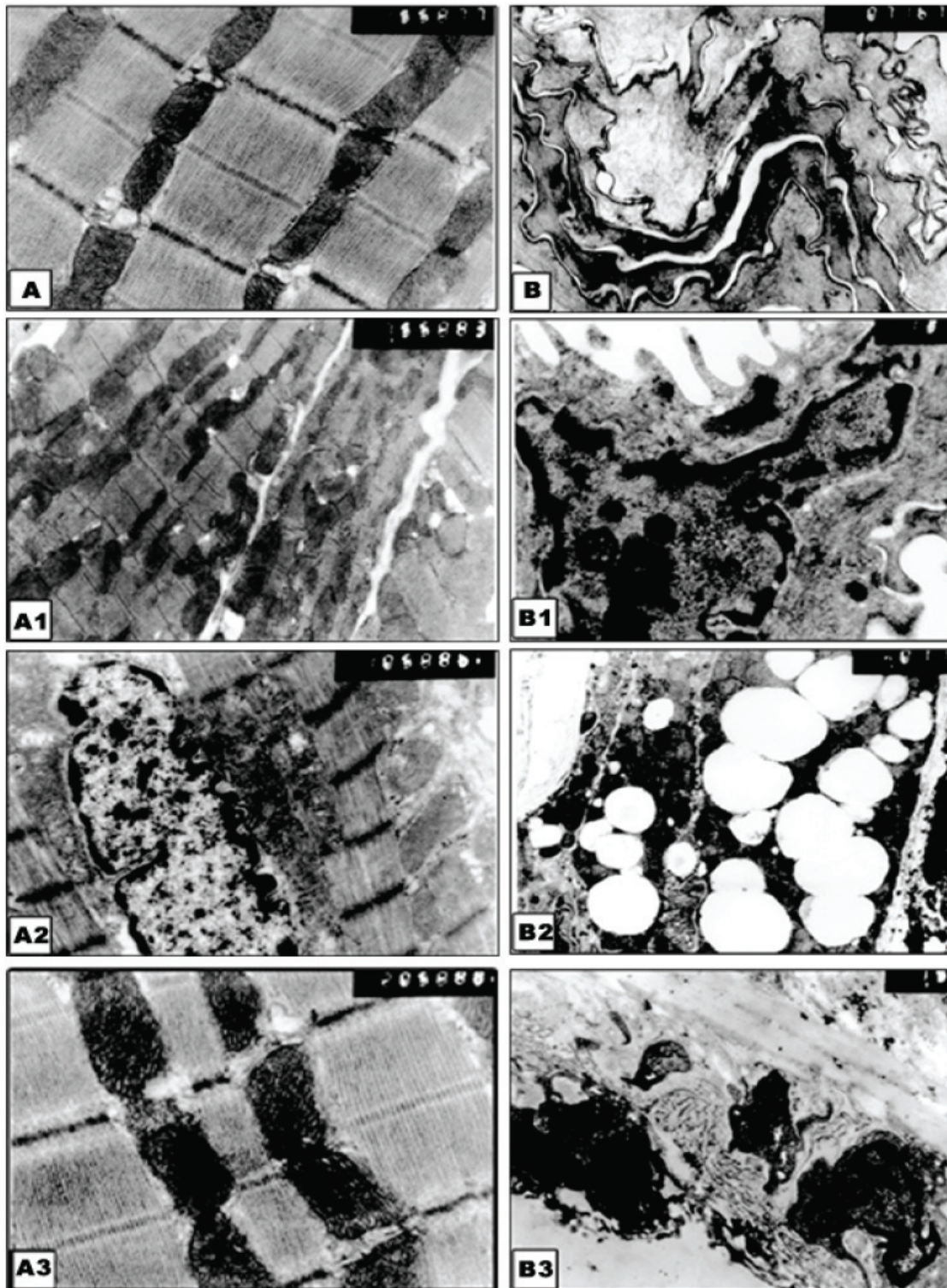


Figure 2. A-B3: Electron micrographs of maternal myocardial muscle (A-A2) and dorsal aorta(B-B2). A. Control myocardial muscle with close similarities to *Allium sativum* supplementation. 1&A2. Hypercholesterolaemic-myocardium. A3. Hypercholesterolaemic and *Allium sativum* supplemented myocardium. B. Maternal control dorsal aorta . B1&B2. Maternal hypercholesterolemic mother. B3. Maternal hypercholesterolemic mother supplemented *Allium sativum*. Lead citrate & uranyl acetate. X10000.

spots (**Figures 2B1 and B2**). In experimental hypercholesterolemic group supplemented *Allium sativum*, the dorsal aorta layer showed moderate amelioration compared to hypercholesterolemic group. The tunica intima partially restored its normal integrity except for the presence of small numbers of phagocytes in the affected area. The tunica media showed less activity of fibroblasts. Both of the tunica intima and media were still exhibited the presence of lipid-loaden cells (**Figure 2B3**).

Effects on pregnant mothers and their offspring

Experimental hypercholesterolemia raised the increased incidence of aborted mothers (4/ten mothers) and decreased the total number of offspring. The offspring of hypercholesterolemic exhibited marked depletion of body weight comparing to the controls. However, this effect was partially reversed after supplementation *Allium sativum*. Experimental hypercholesterolemia was found to increase rate of aborted mother as well as increased incidence of aborted fetuses. Morphologically, the pups revealed verities of congenital abnormalities including uni-and bilateral deformities of both fore-and hind limbs, kinky tail, malformed ear pinna, edematous skin and superficial haematomas. *Allium sativum* supplementation during pregnancy ameliorated these effects and the offspring lacked the apparent malformation (**Table 2 & Figure 3, A-C**).

Effects on ossification centers of skeleton of offspring

Offspring of hypercholesterolemic group showed retardation of ossification centers in axial and appendicular regions. The ossification centers of axial region included hyoid arch, incus, malleus, stapes and tympanic ring appeared missing. The appendicular bones clavicle, sternum and ischium appeared still lacking ossification. On the other hand, supplemented hypercholesterolemic mother with *Allium sativum* restored the progress of bone formation but was still not matched with the control. The ossified bones of mandibular, humerus, radio-ulna, femur, tibio-fibula, scapula and ilium were markedly decreased in offspring of hypercholesterolemic mothers. Supplementing hypercholesterolemic mothers with *Allium sativum* partially improved ossification (**Table 3, Figure 3, D-F**).

Histological observations on offspring

Heart: In offspring of control mothers, the ventri-

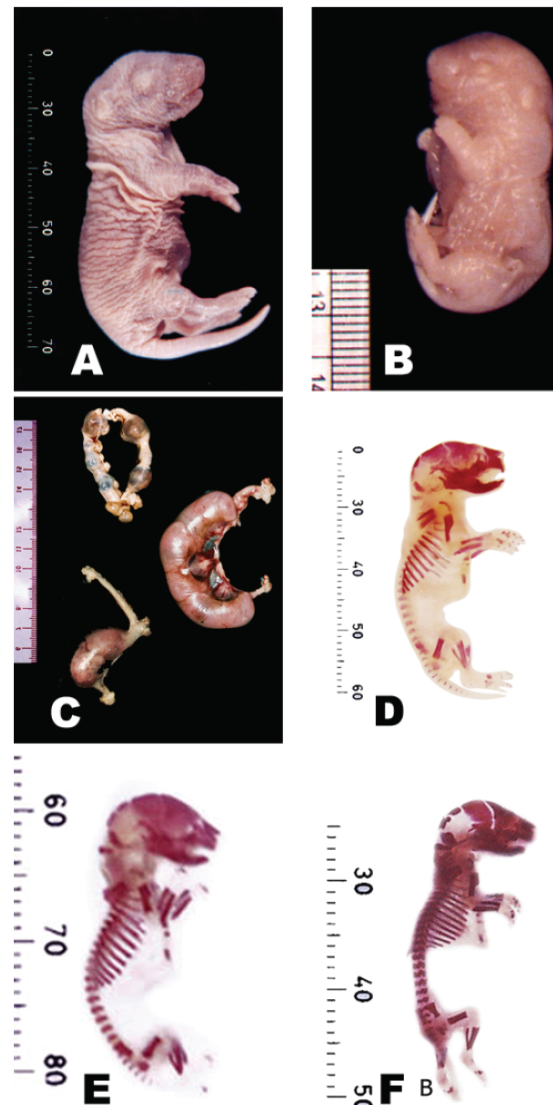


Figure 3. Gross morphology of control (A) and maternally hypercholesterolemic offspring. C. Patter of resorped fetuses of hypercholesterolemic mothers. D. Control alizarin-stained offspring showing normal ossified bone. E. Maternally hypercholesterolemic offspring showing reduced ossified bones. F. Skeleton of offspring of hypercholesterolemic mother supplemented *Allium sativum* Showing normal skeletal structure.

cle myocardial muscle is composed of regularly arranged myocardial muscle fibers with centrally located nuclei (**Figure 1C**). *Allium sativum* supplementation did not alter the normal pattern structure of offspring myocardium. In offspring of experimental hypercholesterolemia group, the myocardial muscle showed massive degeneration of fibers which assumed a reticular structural pattern with widely spread vacuo-

Table 2. Effect of experimental hypercholesterolemia alone or in combination with *Allium sativum* on mothers and their newborn and incidence of abnormalities

	G1	G2	G3	G4
Total number of mothers	10	10	16	10
Total number of aborted mothers	-	-	4 (25%)	-
Total number of completed pregnancy	-	10	10 (75%)	10
Total number of newborn	87	70	47	64
Mean number / dam	8.7	7	4.5	6
Mean body weight/dam	5.60±0.12	0.04±5.43	4.5±0.08	0.07±5.02
Malformed fore limb	-	-	-	-
Unilateral	-	-	3 (6.4%)	-
Bilateral	-	-	4(8.5%)	-
Unilateral malformed hind limb	-	-	3 (6.4%)	-
Kinky tail	-	-	8(17%)	-
Malformed ear pinna	-	-	2 (4.3%)	-
General edema	-	-	4 (8.5%)	-
Under wt. new born < 4 gm	-	-	-	-
Tympanic ring including incus, malleus and stapes	-	-	5 (10.6%)	2 (3.1%)
Clavicle	-	-	3 (6.4%)	-
Sternum	-	-	3 (6.4%)	-
Ischium	-	-	4 (8.5%)	-

G1, Control ; G2, *Allium sativum*-treated group ; G3, Hypercholesterolemic-treated group; G4, Hypercholesterolemia+*Allium sativum*.

lated spaces. Many of the cardiomyocytes showed apoptic cell death (**Figure 1C1**). Supplemented hypercholesterolemic mothers with *Allium sativum* ameliorated the histological picture of myocardial muscle fiber of their offspring to almost normal structural pattern (**Figure 1C2**).

Discussion

Hypercholesterolemia is an autosomal dominant disorder that causes severe elevations in total cholesterol which enhanced the developmental programming of atherosclerosis [1, 20]. Maternal hypercholesterolemia represent one of the major health problem influencing in complication of pregnancy as well as the presence of

severe developmental defects in offspring.

Experimental hypercholesterolemia was found to induce damage of cardiovascular organs started earlier in the myocardium and clarified by disrupting of its structural pattern including degeneration of muscle fibers, cell death of cardiomyocytes and leukocytic infiltration.

The present findings supported the work of Rózycka *et al* [21], Caligiuri *et al* [22] and Zhang *et al* [23] following feeding mice and rabbit on a hypercholesterolemic diet.

Ultrastructural findings revealed abnormal losing and degeneration of the structural pattern of sarcomeres associated with disorganized mito-

Table 3. Ossified length of mandible , girdles and for-& hind limb bones of offspring of hypercholesterolemic mother alone or in combination with *Allium sativum* supplementation

		G1	G2	G3	G4	P < 0.05
Mandibular		8.51±0.06	0.015±8.11	3.13±0.31	0.268±4.93	S
Girdles	Scapula	2.9 ± 1.02	2.73± 0.03	1.2 ± 0.07	1.78± 0.08	S
	Ilium	4.5±0.23	4.28±0.01	1.91±0.23	2.7±0.02	S
Fore limb	Humerus	5.02±0.63	4.9±0.06	2.3±0.11	0.09±3.68	S
	Radius	5.2±0.33	4.92±0.08	1.43±0.12	0.08±2.95	S
	Ulna	4.9±0.17	4.63±0.07	1.83±0.23	0.03±2.35	S
Hind limb	Femur	4.42±0.32	4.08±0.09	1.75±0.144	0.142±2.65	S
	Tibia	4.6±0.65	4.42±0.05	1.76±0.48	0.11±2.87	S
	Fibula	4.20±0.25	3.8±0.04	1.6±0.099	0.11±2.3	S

Data were expressed as Mean ± SE (n=10). Abbreviations; G1, normal control; G2, *Allium sativum*-treatment; G3, Experimental hypercholesterolemia; G4, Experimental hypercholesterolemia +*Allium sativum*. By applying one way ANOVA between the experimental groups as well as between each group and determined F test, significance was determined at p<0.05.

chondrial structure.

Oliveira *et al* [24] reported that mitochondria were the main intracellular sites of reactive oxygen species generation causing oxidative damage. Mitochondrial damage could affect cardiovascular cell function through the formation of reactive oxygen and nitrogen species, which are capable of oxidizing LDL (a key step in atherogenesis) and disrupting cellular energy production which led to cell death [24, 25], Wang *et al* [26] attribute Cardiomyocyte degeneration in hypercholesterolemic rabbits to the alterations of apoptosis markers including Bcl-2, Bax and caspase-3.

Besides, the dorsal aorta of hypercholesterolemic mother developed different grades of lesions manifested by massive deterioration of the endothelial lining cells associated with accumulation of lipid laden cells. The inflammatory cells were diffusely distributed in the inner lining layer.

Similar findings were reported following *in vitro* [27] and *in vivo* studies in rabbits [28, 29] and mice [23] fed on hypercholesterolemic diet. The association between hypercholesterolemia and impairment of cardiovascular functions may be attributed to the increased levels of superoxide in the aortas [30].

The observed maternal histopathological diseases of myocardium and dorsal aortic were supported by alterations of maternal hypercholesterolemic markers of serum TCL, LDL, TG and CK activity.

Excessive lipoprotein in the plasma tends to accumulate in the hyperplastic intima altering endothelial function and causing atherosclerosis [31].

The observed maternal defects were reflected in the myocardial histological picture of offspring with similar characteristic pathological alterations. Alkemade *et al* [32] demonstrated that maternal hypercholesterolemia during pregnancy increased atherogenic risk in the offspring which were highly susceptible for neointima formatting and thickening of cardiac arteries.

Supplementation of *Allium sativum* one week before and parallel with experimental hypercholesterolemia revealed amelioration of the histological picture of the myocardium, however, dorsal aorta possessed partial amelioration. Although, there was a considerable regeneration of the endothelial cells, the lipid laden-cells were still detected in both the tunica intima and tunica media. In addition, the observed findings of *Allium sativum* homogenate consumption showed an inverse correlation with the progres-

sion of the mentioned cardiovascular disease markers (TCL, TG, LDL, and HDL & CK). Similar findings were reported by Yeh and Liu [33].

These protective effects may be attributed to inhibition of enzymes involved in lipid synthesis, prevent lipid peroxidation and LDL, increase antioxidant [34]. Dillon [35] and Dillon *et al* [36] reported that garlic inhibited the *in vitro* oxidation of isolated human LDL by scavenging superoxide and inhibiting the formation of lipid peroxides.

From the present findings, there are a close relationship between maternal hypercholesterolemia and development of offspring myocardial degeneration as well as increased the incidence of different pattern of congenital abnormalities. As a result of the hypercholesterolemic stress, depletion of the body weights was detected in their offspring. The observed findings agree with the work of Mogren *et al* [37] who revealed a correlation between children atherosclerosis and low birth weight.

Overloading of cholesterol administration was influenced by a reduction of its synthesis and utilization by fetal tissues, impairing growth and consequently increased the incidence of developmental defects including morphological and delayed ossification of bones. The estimated axial and appendicular bones in offspring of hypercholesterolemic mother showed apparent reduction. The offspring growth defects may be associated to the damage of myocardium and development of atherosclerosis in dorsal aorta which reduces the carriage of nutrient and oxygen to tissues as well as the cytotoxicity of hypercholesterolemia. Developmental bone defects may be attributed to either disruption of vitamin D synthesis via inhibition of cholesterol synthesis as a result of decreased level of 7-dehydrocholesterol reductase [38] or defects in the formation of cell membrane domains [39] which regulate growth factor receptors. LDL receptor was found to exert its regulatory action on cellular cholesterol metabolism in fibroblasts through an interaction with a specific cell surface receptor and that this receptor is defective in homozygous familial hypercholesterolemia.

Following *in vitro* and *in vivo* studies on placenta, Bonet *et al* [40] and Stefulj *et al* [41] have shown that LDL oxidation is cytotoxic for placental cells, leads to cholesterol ester accu-

mulation in both placental macrophages and trophoblast and intern induce fetal growth defects.

Vicica *et al* [42] reported that cholesterol is synthesized by the liver and secreted as circulating lipoproteins and play a great role in osteoblast differentiation.

From the present findings, *Allium sativum* supplementation prior and throughout hypercholesterolemic treatment ameliorated light and ultrastructural component of myocardial muscle and dorsal aorta and confirmed by approximately normal matching of LDL, HDL, triglycerides and creatine phosphokinase level. These was intern reflected the normal morphological criteria, skeltogenesis and apparent almost normal histological pattern of their myocardium.

Amelioration of atherosclerosis by *Allium sativum* was also achieved in human [43, 44] and experimental animal [45]. The authors attributed amelioration to the scavenge of free radicals which protect membranes from damage and maintains cell integrity via lowering of cholesterol, anti-platelet activities, and thromboxane formation.

Finally, the authors concluded that *Allium sativum* supplementation to hypercholesterolemic pregnant rat ameliorated the drastic effects of hypercholesterolemia in myocardium, dorsal aorta and growth pattern of offspring.

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References

- [1] Goldstein JL, Hobbs HH, Brown MS. Familial Hypercholesterolemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The Metabolic and Molecular Bases of Inherited Disease. 8th ed. New York, NY: McGraw-Hill; 2001;2863-2913.
- [2] Vattikuti R. and Towler, DA. osteogenic regulation of vascular calcification: an early perspective. *Am J Physiol Endocrinol Metab* 2004;286 (5):E686-96.
- [3] Schwartz CJ, Valente AJ, Sprague EA, Kelley JL, Nerem RM. The pathogenesis of atherosclerosis: an overview. *Clin Cardiol* 1991;14(1):1-16.
- [4] Blania G. and Spangenberg B. Formation of allicin from dried garlic (*Allium sativum*): a simple HPTLC ethod for simultaneous determination of allicin and ajoene in dried garlic and garlic

- preparations. *Planta Med* 1991;57(4):371-375.
- [5] Newall CA, Anderson LA, Phillipson JD. Herbal medicines: a guide for health-care professionals. London:Pharmaceutical Press, 1996; ix, 296.
- [6] Bordia AK, Joshi HK, Sanadhya YK, Bhu N. Effect of essential oil of garlic on serum fibrinolytic activity in patients with coronary artery disease. *Atherosclerosis* 1977;28 (2): 155–159.
- [7] Bordia A, Sanadhya SK, Rathore AS and Bitu N. Essential oil of garlic on blood lipids and fibrinolytic activity in patients of coronary artery disease (Part 1). *J Assoc Phys Ind* 1978;26:327-331.
- [8] Harenberg J, Giese C and Zimmermann R. Effect of dried garlic on blood coagulation, fibrinolysis, platelet aggregation and serum cholesterol levels in patients with hyperlipoproteinemia. *Atherosclerosis* 1988;74(3): 247–249.
- [9] Lau B, Lam F, Wang CR. Effect of an odour-modified garlic preparation on blood lipids. *Nutr Res* 1987;7:139–149.
- [10] Phelps S and Harris W. Garlic supplementation and lipoprotein oxidation susceptibility. *Lipids* 1993;28(5): 475–477.
- [11] Koscielny J, Klubendorf D, Latza R, Schmitt R, Radtke H, Siegel G. and Kieseewetter H. The antiatherosclerotic effect of *Allium sativum*. *Atherosclerosis*. 1999;144(1):237-249.
- [12] Lata S, Saxena KK, Bhasin V, Saxena RS, Kumar A and Srivastava VK Beneficial effects of *Allium sativum*, *Allium cepa* and *Commiphora mukul* on experimental hyperlipidemia and atherosclerosis –a comparative evaluation. *J Postgrad Med* 1991;37 (3):132-135.
- [13] Choudhary, R. (2008). Beneficial effect off *Allium sativum* and *Allium tuberosum* on experimental hyperlipidemia and atherosclerosis. *Pak J Physiol* ;4(2):1-3.
- [14] Silagy C, Neil A. Garlic as a lipid lowering agent—a meta-analysis. *J R Coll Physicians Lond* 1994;28(1):39–45.
- [15] Warshafsky S, Kamer RS, Sivak SL. Effect of garlic on total serum cholesterol: a meta-analysis. *Ann Intern Med* 1993;119(7 Pt 1):599-605
- [16] Deeg R and Ziegenhorn J. Kinetic enzymic method for automated determination of total cholesterol in serum. *Clin Chem* 1983;29(10): 1798-1802.
- [17] Fossati P and Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem* 1982;28(10):2077-2080.
- [18] Grove TH. Effect of reagent PH on determination of the high-density lipoprotein cholesterol by precipitation with sodium phototungstate-magnesium. *Clin Chem* 1979;25 (4): 560-564.
- [19] Friedewald WT, Levy RI and Fredrickson DS. Estimation of low density lipoprotein cholesterol in plasma without use preparative ultracentrifuge. *Clin Chem* 1972;18 (6): 499-502.
- [20] Austin MA, Hutter CM, Zimmern RL, Humphries SE. Familial hypercholesterolemia and coronary heart disease: a HuGE association review. *Am J Epidemiol*. 2004;160 (5):421-429.
- [21] Rózycka Z, Lewicki Z and Wutzen J. Dynamics of lesions to the myocardium in experimental hypercholesterolemia. *Pol Tyg Lek*. 1989;44(10-11):247-52.
- [22] Caligiuri G, Levy B, Pernow J, Thorén P, Hansson GK. Myocardial infarction mediated by endothelin receptor signaling in hypercholesterolemic mice. *Proc Natl Acad Sci USA* 1999;96(12):6920-6924.
- [23] Zhang S, Picard MH, Vasile E, Zhu Y, Raffai RL, Weisgraber KH and Krieger M. Diet-induced occlusive coronary atherosclerosis, myocardial infarction, cardiac dysfunction, and premature death in scavenger receptor class B type I-deficient, hypomorphic apolipoprotein ER61 mice. *Circulation* 2005; 111(25):3457-3464.
- [24] Oliveira HC, Cosso RG, Alberici LC, Maciel EN, Salerno AG, Dorigheo GG, Velho JA, de Faria EC, Vercesi AE. Oxidative stress in atherosclerosis-prone mouse is due to low antioxidant capacity of mitochondria. *FASEB J* 2005;19(2):278-280.
- [25] Vercesi AE, Kowaltowski AJ, Oliveira HCF, Castilho RF. Mitochondrial Ca²⁺ transport, permeability transition and oxidative stress in cell death: implications in cardiotoxicity, neurodegeneration and dyslipidemias. *Front Biosci* 2006;11: 2554-2564.
- [26] Wang TD, Chen WJ, Su SS, Lo SC, Lin WW, Lee YT. Increased cardiomyocyte apoptosis following ischemia and reperfusion in diet-induced hypercholesterolemia: relation to Bcl-2 and Bax proteins and caspase-3 activity. *Lipids* 2002;37 (4):385-94.
- [27] Fontes CJ, Ameido L, Paiva I, Tavares P, Cabrita S, Wulfrath P, Matos BM and Teixeira F. Influence of 0.1 or 0.2 % cholesterol enriched diets on the induction of atherosclerosis and aorta reactivity in vitro. *J Cardiovasc. Pharmacol* 1998;31:690-699.
- [28] Ismail MF, Gad MZ, Hamdy MA. Study of the hypolipidemic properties of pectin, garlic and ginseng in hypercholesterolemic rabbits. *Pharmacol Res* 1999;39(2):157-66.
- [29] McConnell MV, Aikawa M, Maier SE, Ganz P, Libby P, Lee RT. MRI of rabbit atherosclerosis in response to dietary cholesterol lowering. *Arterioscler Thromb Vasc Biol* 1999;19:1956-1959.
- [30] Sun YP, Lu NC, Parmley WW and Hollenbeck CB. Effects of cholesterol diets on vascular function and atherogenesis in rabbits. *Proc Soc Exp Biol Med* 2000;224:166-171.
- [31] Stary HC. Composition and classification of human atherosclerotic lesions. *Virchows Arch. A Pathol Anat Histopathol* 1992;421:277–290.
- [32] Alkemade FE, Gittenberger-de Groot AC, Schiel AE, VanMunster JD, Hogers B, Van Vliet LS, Poelmann RE, Havekes LM, Willems van Dijk K, DeRuiter MC. Intrauterine exposure to maternal atherosclerotic risk factors increases the sus-

- ceptibility to atherosclerosis in adult life. *Arterioscler Thromb Vasc Biol* 2007;27(10):2228-2235.
- [33] Yeh YY and Liu L. Cholesterol-lowering effect of garlic extracts and organosulfur compounds: human and animal studies. *J Nutr* 2001;131:989S-993S.
- [34] Rahman K and Lowe GM. Garlic and Cardiovascular Disease: A Critical Review. *J Nutr* 2006;136(3): 736S-740S.
- [35] Dillon SA, Burmi RS, Lowe GM, Billington D, Rahman K. Antioxidant properties of aged garlic extract: an in vitro study incorporating human low density protein. *Life Sci* 2003;72:1583-94.
- [36] Dillon SA. Aged garlic extract as an antioxidant in cardiovascular disease [doctoral thesis]. Liverpool John Moores University, Liverpool, UK, 2002. pp 32-35.
- [37] Mogren I, Hogberg U, Stegmayr B, Lindahl B, Stenlund H. Fetal exposure, heredity and risk indicators for cardiovascular disease in a Swedish welfare cohort. *Int J Epidemiol* 2001;30(4):853-862.
- [38] Roux C, Wolf C, Mulliez N, Gaoua W, Cormier V, Chevy F and Citadelle D. Role of cholesterol in embryonic development. *Am J Clin Nutr* 2000;71(suppl):1270S-1279S.
- [39] Miyake Y, Tajima S, Funahashi T, Yamamoto A. Analysis of a recycling-impaired mutant of low density lipoprotein receptor in familial hypercholesterolemia. *J Biol Chem* 1989;264(28):16584-90.
- [40] Bonet B, Hauge-Gillenwater H, Zhu XD, Knopp RH. LDL oxidation and human placental trophoblast and macrophage cytotoxicity. *Proc Soc Exp Biol Med* 1998;217:203-211.
- [41] Stefulj J, Panzenboeck U, Becker T, Hirschmugl B, Schweinzer C, Lang I, Marsche G, Sadjak A, Lang U, Desoye G and Wadsack C. Human Endothelial Cells of the Placental Barrier Efficiently Deliver Cholesterol to the Fetal Circulation via ABCA1 and ABCG1. *Circ Res* 2009;104:600-608.
- [42] Viccica G, Vignali E, Marcocci C. Role of the cholesterol biosynthetic pathway in osteoblastic differentiation. *J Endocrinol Invest* 2007;30(6):8-12.
- [43] Kojuri J, Vosoughi AR, Akrami M. Effects of anethum graveolens and garlic on lipid profile in hyperlipidemic patients. *Lipids Health Dis*. 2007;6:1-5.
- [44] Butt MS, Sultan MT, Butt MS, Iqbal J. Garlic: nature's protection against physiological threats. *Crit Rev Food Sci Nutr* 2009;49(6):538-551.
- [45] Asdaq SM, Inamdar MN, Asad M. Effect of conventional antihypertensive drugs on hypolipidemic action of garlic in rats. *Indian J Exp Biol* 2009;47(3):176-181.