**Summary and Conclusion**

Present work aimed mainly to:

1. Study some factors dealing with induced hyperlipemia and in experimental atherosclerosis in experimental rats. This is done through long term supplementation of diet enriched with saturated fats and cholesterol then followed by alloxan administration.
2. Reflection of this on certain changes recorded for selected biochemical parameters in blood and aortic tissues. This was followed by histopathological studies for aorta using different stains. The study included two parts.

**First part**: deal with the effect of certain drugs focused on:

1. Angiotensin converting enzyme inhibitor (ACEI)
2. Angiotensin type 1 receptor blocker
3. Role of natural antioxidant represented by green tea extract, catechin either alone or in combination with drugs (1&2).

**Second part**: effect of dietary components having various degrees of unsaturated fatty acids.

**Biochemical markers selected were**:

**Serum**: Glucose, lipid profile, NO, CRP, chemokines expression (MCP-1 and RANTES).

**Plasma**: susceptibility of non HDL-C to oxidation.

**Aorta tissues**: Glutathione (GSH), super oxide dismutase (SOD) enzymes.

**Histological study**: using different stains

* 1. Hematoxylin and eosin: to illustrate any changes like cellular infiltration, foam cells, and thickening changes in arterial walls.
	2. Alcian blue: to study glycosaminoglycans distribution in vessel wall.
	3. Masson trichrome: to study collagen distribution as related to smooth muscle cells.
	4. Modified Taenzer-unna Orcein method: to study elasticity and corrugation of fibers.

**Experimental design:** The study was performed in two parts

**The first part:** Rats were fed chow diet supplemented with 3% saturated fatty acid 1%cholestrol and 0.5% cholic acid, and 0.01% thiouracil for four months. Subsequently received alloxan; 150 mg/ kg, IP(1 dose), after. Acclimatization, rats were categorized as follow:

Group (1): Hypercholesterolemic hyperglycemic rats received enalapril

Group (2): Hypercholesterolemic hyperglycemic rats received losartan

Group (3): Hypercholesterolemi hyperglycemic rats received catechin

Group (4): Hypercholesterolemic hyperglycemic rats received enalapril and losartan

Group (5): Hypercholesterolemic hyperglycemic rats received enalapril, losartan and atechin

Treatment regimen was continued for 4 and 8 weeks

**Results**

1- Rats received hypercholesterolemic diet followed by alloxan injection demonstrated significant increase in glucose, lipid profile (TC, TAG, LDL), susceptibility of non-HDL-C to oxidation, atherogenic indexes (TC/HDL, LDL/HDL), inflammatory markers (CRP), chemokine expression. Moreover significant decrease was demonstrated in NO and aortic antioxidant content (GSH and SOD).

2- Histopathological examination revealed massive cellular infiltration, foam cell formation, and marked distribution of glycosaminoglycans allover the aortic layers. Increased collagen synthesis and degradation joined with loss of elastic fibers corrugation was also observed.

**1-ACEI (Enalapril)**

Enalapril administration induced significant decrease in serum glucose levels (after 8 weeks). Significant decrease in TC, TG, LDL,atherogenic indexes, and susceptibility of non-HDL-C to oxidation was observed, while HDL-C demonstrated non significant effect. Significant increase in NO, aortic GSH and SOD was also achieved. MCP-1 and RANTES revealed significant decrease in comparison to control group.

Histopathological examination illustrated moderate distribution of foam cells, cellular infiltrate, and glycosaminoglycans. Collagen fibers amount and elasticity demonstrated moderate improvement.

**2 –AT1RA (Losartan):**

Losartan administration induced significant decrease in serum glucose (after 8 weeks). Hyperlipemia and atherogenic indexes (TC/HDL, LDL/HDL) show non significant change. While 8 weeks of continual administration significantly decreased susceptibility of non HDL–C to oxidation, MCP-1, and RANTES. However it increased NO level. CRP, aortic GSH and SOD illustrated non significant change.

Histopathological examination showed massive infiltration of intima and media with foam cells, cellular infiltrate and glycosaminoglycans. Massive increase in collagen fibers and loss of elastic fibers corrugation was also observed.

**3- Antioxidant (catechin):**

catechin administration significantly decreased serum glucose, TC, TAG, LDL and the atherogenic ratios (LDL/HDL and TC/HDL) meanwhile HDL-C recorded non significant change. Susceptibility of non HDL oxidation, CRP, MCP-1 and RANTES showed significant decrease. However aortic GSH and SOD content recorded significant increase in comparison to control group.

Histopathological examination showed moderate cellular infiltration in intima, media and adventitia with marked improvement in foam cells amount, joined with minimal amount of glycosaminoglycans. Minimal amount of collagen fibers and marked improvement of fibers elasticity and corrugation were also observed.

**4–Combination of enlalapril and losartan:**

Combined administration of these drugs induced significant decrease in serum glucose (after 8 weeks), TC, TAG, LDL, atherogenic indexes (TC/HDL and LDL/HDL), CRP, MCP-1, and RANTES.

NO and aortic GSH and SOD content demonstrated significant increase in comparison to control group.

Histopathological examination illustrated moderate improvement in cellular infiltration and foam cell formation. glycosaminoglycans distribution was moderate in intima, media and adventitia. Relative improvement in collagen appearance with moderate loss of corrugation of elastic fibers was also recoded.

**5- Combination of enalapril, losartan and catechin:**

Tritherapy administration induced significant decrease in blood glucose, TC, TAG, LDL, susceptibility of non HDL-C to oxidation, atherogenic indexes (TC/HDL and LDL/HDL), CRP, MCP-1 and RANTES. NO level, aortic GSH and SOD demonstrated significant increase in comparison to control group.

Histological examination showed marked improvement in both of cellular infilteration and foam cell formation. Minimal amount of glycosaminoglycans was observed in intima, media and adventitia. Collagen amount was decreased greatly and elastic fibers appeared weavy with marked improvement in corrugation.

**The second Part:**

Animals received chow diet consisted of 21% saturated fat enriched with 1% cholesterol, 0.5% cholic acid, and 0.01% thiouracil for four monthes. Atherogenic rats were randomly divided into three experimental groups.

First group received chow diet supplemented with 10% v/w OO

Second group received chow diet enriched with 10 % v/w of SO

Last group received chow diet enriched with 10 % v/w FO

This dietary regimen was continued for 8 weeks.

**Results:**

 Atherogenic diet induced dyslpidemia state represented by elevated TC, LDLC, TAG and marked increase in oxidative stress markers. Increased LDL oxidation susceptibility and decreased aortic antioxidant enzymes (SOD and GSH) are examples. CRP, MCP-1, and RANTES showed significant increase and greatly exaggerated in comparison with baseline data.

Histopathological examination of the atherosclerotic aorta showed thick intima with thick and irregular endothelium subendothelial basal lamina (SBL), multiple cellular infiltrations and foam cells in addition to white longitudinal streaks as compared to normal aorta. Also there was marked increase in the positively stained area of glycosaminoglycan in intima, media and adventitia compared to control rats. Massive increase in amount of collagen combined with loss of corrugation of elastic fibers was also observed.

1. **Virgin olive oil (OO) :**

Rats received OOillustrated moderate decrease in lipogram pattern, atherogenic index, inflammatory marker (CRP) and chemokine expression (MCP-1 and RANTES). Decreased OX-LDL and increased antioxidant enzymes (SOD and GSH) in comparison to atherogenic control group was also observed.

Histopathological examination showedrelatively thin intima with thin endothelium. Reduced infiltration, vacuolations, white longitudinal streaks of intima and media with  minimal infiltration with glycosaminoglycan. Significant amelioration of collagen amount with obvious corrugation increase.

 **2- Sunflower oil (SO):**

Rats received SO enriched diet illustrated non significant change in the lipogram pattern and atherogenic index after 4 and 8 weeks. Antioxidant effect was mild. This was manifested by decreased lipoprotein susceptibility to oxidation and aortic GSH increase after 4 and 8 weeks. Aortic SOD recorded significant increase after 8 weeks only. MCP-1 Significantly decreased after 4 and 8 weeks additionally RANTES (after 8 weeks) in comparison to atherogenic diet group.

Histopathological examination showed marked cellular infiltration and foam cell formation in intima and media joined with marked increase in glycosaminoglycans amount. Obvious amount of collagen and loss of corrugation of elastic fibers was still evident.

**3- Omega 3 fatty acid (FO):**

Supplementation with FO significantly reduced lipogram pattern and atherogenic indexes. CRP, MCP-1, and RANTES recorded significant decrease. Moderate decrease in susceptibility of lipoprotein to oxidation joined with aortic GSH and SOD increase were also observed after 4 and 8 weeks.

Histopathological examination illustrated few cellular infiltrate and few white longitudinal streaks are observed in media. Additionally relative reduction in glycosaminoglycan distribution as compared to atherosclerotic group. Moderate collagen amount and corrugation of elastic fibers was also observed in the aortic stained rings.

 **Conclusion**

* Atherosclerosis disease represents the net results of certain biological reactions in the blood and effectively pronounced later in the arterial walls. This does not neglect the early changes that probably occur between the two (blood and tissue).
* Hyperlipemic state was induced in experimental rats though long intake of diet supplemented with cholesterol and saturated fatty acids.
* Rennin angiotensin system may play vital role in induction phase of atherosclerosis. This may be through significant increase in chemokines (MCP-1 and RANTES), oxidative stress, and antioxidant enzymes decrease.
* Inflammatory markers, chemokines from one side showed positive correlation with hyperlipemia, oxidative stress markers and from the other side with antioxidant enzymes.
* Enalapril and losartan effects were focused clearly on MCP-1, RANRES and CRP joined with minimal effects on lipogram pattern and oxidative stress markers, this may leave an impression that angiotensin pathway was not alone in sequences leading to induced atherosclerosis. Therefore it may require the collaboration of other factors.
* Catechin (green tea extract) appeared as hypolypemic antioxidants, decreased the susceptibly of non HDL-C to oxidatiation represents an example takes in consideration that it have immunogenic and chemoattractant properties (MCP-1 and RANTES)
* Combined administration of enalapril, losartan, catechin induced certain improvement in biochemical markers additionally aortic tissues.
* Catechin being effective and known hypolipemic agents in turn, its inclusion in such combination was additive as potential treatment. This was manifested and viewed through decreased foam cells (catechin effect), cellular infiltration degree (enalapril & losartan) and collagen amount joined with marked improvements in elastic fibers corrugation.
* Diet containing higher degree of unsaturated fatty acids was greatly effective. Marked improvement produced in biochemical parameters studied (lipogram pattern, oxidative stress, inflammatory makers and chemokines expression in turn pattern of cellular aorta are confirmative.
* Although omega 3 fatty acids have hypolipemic, antioxidant and anti-inflammatory properties, olive oil appeared more effective. The later have antioxidant (polyphenolic content) and moderate anti-inflammatory properties.

This was clearly evident, reflected through certain improvement produced in cellular pattern of the aorta (decreased atherosclerotic lesions) and comparably more than omega 3 fatty acids and lastly sunflower oil.