

Evaluation of Anti-Hyperlipidemic Activity of Extracted Alpha-linolenic Acid from Natural *Linum Usitatissimum* Compared with Atorvastatin in High Fat Diet Induced Rats

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Abstract

Linum Usitatissimum (LU) has a lipid-lowering action in both normal and diabetic animals. Because OS leaves are rich in oil, the present study was conducted to explain the anti-hyperlipidemic and organ-protective effect of LU fixed oil in rats fed with a high fat (HF) diet. LU fixed oil was extracted by hexane and the fatty acids composition identified by GC-MS. Four groups of male Wister rats included a normal control group, a high fat fed-diet (HF) group, a HF group treated with LU fixed oil, and a HF group treated with a reference drug Atorvastatin. The results show that LU fixed oil contains five kinds of fatty acids, of which alpha-linolenic acid was the major fatty acid. LU fixed oil depressed high serum levels of total cholesterol, triglyceride, LDL-C, and AI, whereas no significant effect on HDL-C was observed. LU fixed oil also suppressed high levels of liver cholesterol and triglyceride with no significant effect on both lipids in feces. In addition, LU fixed oil normalized the high serum levels of LDH and CK-MB but no significant effect on high serum levels of ALT, AST, and ALP was obtained. We conclude that treatment with LU fixed oil during the last three weeks of HF diet feeding decreased the high serum lipid profile and expressed anti arterogenic and cardio protective actions against hyperlipidemia. The anti-hyperlipidemic action of OS fixed oil was mainly resulted from the suppression of liver lipid synthesis. Linolenic acid and linoleic acid contained in LU fixed oil were possibly responsible for both lipid-lowering and cardiac protective action against hyperlipidemia.

Keywords: Anti-Hyperlipidemic Activity, *Linum Usitatissimum*, Linolenic Acid, Atorvastatin

Introduction

Hyperlipidemia is an excess of fatty substances called lipids, largely cholesterol and triglycerides, in the blood. It is also called hyperlipoproteinemia because these fatty substances travel in the blood attached to proteins. This is the only way that these fatty substances can remain dissolved while in circulation.

Hyperlipidemia disease has afflicted humankind since antiquity. The treasure house of plant kingdom has a number of plants to treat this ailment. The indigenous system of medicine provides an abundant data about plants available for treatment of hyperlipidemia. A lot of work has been carried out by researches on various plants to evident their effectiveness in hyperlipidemia. But still lots many are left which are used in the indigenous system but no systematic studies regarding their pharmacology have been carried out. One such natural medicine in indigenous system of medicine claimed to be useful in treatment of high fat deposition in various organs. Alpha-linolenic acid is natural medicine extracts from capsicum

and other pepper plants the systematic pharmacological study is carried out to support its hyperlipidemic activity. Current study is under taken to evaluate whether the Alpha-linolenic acid possess anti-oxidant and anti hyperlipidemic activity and able to increase liver protection. So the purpose of present study is to evaluate effect of Alpha-linolenic acid on hyperlipidemia.

Here the parameters included are

- Oxidative stress in liver
- Serum cholesterol and HDL cholesterol and Total protein levels
- Histopathology of liver and carotid artery in rats

Experimental Material and Methods Animal Husbandry

All experiments and protocols described in present study were approved by the Institutional Animal Ethical Committee (IAEC) of S. V. University, Tirupathi and with permission from Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), ministry of Social Justice and Empowerment, Government of India.

Animals

Female Wister rats (100 ± 25) were housed in group of 6 animals and maintained under standardized condition (12-hours light/dark cycle, 24°C) and fed with high fat diet food and purified drinking water ad libitum.

Chemicals

Alpha-linolenic acid (sigma), Epinephrine, DTNB (sigma), Thiobarbituric acid (TBA) and Tri chloro acetic acid, Hydrogen peroxide (SD fine chemicals Ltd), Sodium dihydrogen phosphate, potassium dihydrogen phosphate, Tris buffer, All other reagents used were of analytical grade

Instrumentation

UV spectra were recorded in Cyber Spec UV-Visible spectrophotometer

Experimental Design

The experiment conducted for 45 days.

Wister rats (n = 24) are divided in to 4groups as per following

Group 1: (Normal) Received normal diet

Group 2: high fat diet (egg white, vegetable fat, cakes, potatoes, cholesterol) and drinking tap water

Group 3: 0.20% Alpha-linolenic acid + high fat diet treated

Group 4: 0.05mg Atorvastatin + high fat diet

Bio-Chemical Studies: Parameters Measured

Serum Analytical Methods

- Estimation of total cholesterol
- Estimation of HDL cholesterol

Tissue (Liver) Bio Chemical Methods

- Antioxidant and pro-oxidant enzymes
- Super oxide dismutase (SOD) (by the method Misra, Fridovich L, et al. 1972)
- Catalase (Colowick, et al. 1984)
- Glutathione (GSH) (by the method Moran M.S et al., 1984)
- Malondialdehyde (MDA) (by the method Slater TF and Sawyer BC et al., 1971)

Physical Methods

- Body weight

Histopathology

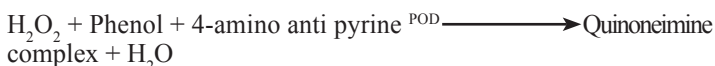
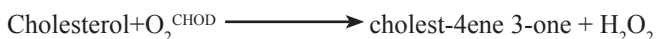
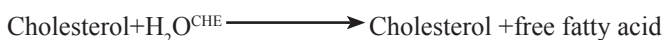
- Liver
- Carotid artery

Bio Chemical Studies

Serum Analytical Methods

Estimation of Total cholesterol-MR (Chod-Pod/ Phosphotungstate Method)

Principle



Procedure

Pipette into 3 test tubes labeled Blank (B), Standard(S), and Total cholesterol (T_c) as shown below (Table 1).

Mix well and incubate for 5min at 37°C or 10min at R.T. Read the absorbance of standard(S), Total cholesterol (T_c) against blank at 505nm or with green filter (500-540nm)

Calculations

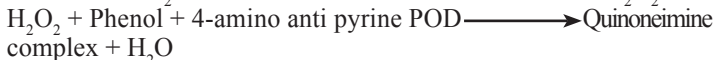
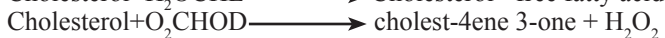
Total cholesterol (in mg/dl) = (abs. of T_c / abs. of S) X 200

Table 1: Estimation of Total cholesterol-MR

Reagent	1.0ml procedure			3.0 ml procedure		
	B	S	T_c	B	S	T_c
Cholesterol reagent (1)	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
Cholesterol standard(2) (conc. 200mg/dl)	--	10 μ l	--	-	20 μ l	--
Specimen	--	--	10 μ l	--	--	20 μ l
Distilled water	--	--	--	2.0ml	2.0ml	2.0ml

Estimation of HDL

Principle



On addition of precipitating reagent to the serum, followed by centrifugation, HDL fraction remains in the supernatant while the lipoproteins precipitate out.

Procedure

Step 1: Pipette into the centrifuge tube (Table 2)

Mix well and allow to stand at RT for 5min. Centrifuge at 3000rpm for 10min to get a clear supernatant. If supernatant is not clear (high TGL level) dilute the sample 1:1 normal saline and multiply the result with 2.

Table 2: Estimation of HDL

Serum / plasma	0.2ml
Precipitating reagent(3)	0.3ml

Step 2: Pipette into 3 test tubes labeled Blank (B), Standard (S), HDL cholesterol (TH) a shown below (Table 3)

Mix well and incubate for 5min at 37°C or 10min at R.T. Read the absorbance of standard(S), HDL cholesterol (T_H) against blank at 505nm or with green filter (500-540nm)

Table 3: Estimation of HDL

Reagent	1.0ml procedure			3.0 ml procedure		
	B	S	T_H	B	S	T_H
Cholesterol reagent (1)	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
HDL Cholesterol standarad(4) (conc. 50mg/dl)	--	100 μ l	--	-	200 μ l	--
Supernatant (from step1)	--	--	100 μ l	--	--	200 μ l
Distilled water	--	--	--	2.2ml	2.0ml	2.0ml

Calculations

HDL cholesterol (in mg/dl) = (abs. of TH/ abd. of S) X 50 (Clin, Chem 20,470. (Allain, C.C. et al., 1974)).

Tissue (Liver) Bio Chemical Methods

Preparation of Homogenate

The animals were sacrificed and liver isolated and weighed, the homogenate is prepared as follows

Reagent

1.0.25M sucrose solution: 85.87gm of sucrose was dissolved in 1000ml of distilled water. 2.10mM buffer solution: 1.2gm. of tris was dissolved in 900ml of distilled water, pH was adjusted to 7.4 with 1M Hcl and dilute up to 1000ml.

Procedure

Liver was separated and kept in cold condition were cross chopped with surgical scalpel in to fine slices and was chilled in the cold 0.25M sucrose, quickly blotted with filter paper. The tissue was minced and homogenized in ice cold 10mM tris HCL buffer (to pH 7.4) at a concentration of 10% (w/v) with 25 strokes of tight Teflon pestle of glass homogenizer at a speed of 2500 rpm. The prolonged homogenization under hypotonic condition was designed to disrupt as far as possible the ventricular structure of cells so as to release soluble protein and leave only membrane and non-vascular matter in a sedimentable form It was then centrifuged in cooling centrifuge at 5000 RPM for 20°C, temperature was maintained at -4°C during the centrifugation, clear supernatant was separated and used to estimate SOD, Catalase, Glutathione, MDA.

Evaluation of Antioxidant Status

- Super oxide dismutase (SOD)
- Catalase
- Reduced Glutathione (GSH)
- Evaluation of Pro-Oxidant

Super Oxide Dismutase (SOD)

SOD was estimated by the method of Misra and Fridovich (1972)

Principle

Rate of auto oxidation of epinephrine and the sensitivity of this auto oxidation to inhibition by SOD were augmented as pH was raised from 7.8-10.2, O₂ generated by xanthine oxidase reaction, caused by the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per O₂ introduced. the auto oxidation of epinephrine proceeds by at least two distinct pathways only one of which is free radical chain reaction involving O₂ and hence inhabitable by SOD.

Procedure

0.5ml of sample was diluted with diluted with 0.5ml of distilled water, to this 0.25ml ethanol, 0.5ml of chloroform (all reagents chilled) was added .the mixture was shaken for 1min and centrifuged at 2000rpm for 20min. The enzymatic activity in supernatant was determined. To 0.05ml of carbonate buffer (0.05M, pH 10.2) and 0.5ml of EDTA (0.49M) was added. The reaction was initiated by the addition of 0.4ml of epinephrine and the change in optical density/min was measured at 480nm. SOD activity was expressed as units / mg protein change in optical density/min. 50% inhibition of epinephrine to adrenochrome transition by enzyme is taken the

enzyme unit. Calibration curve was prepared by using 10 -125 units of SOD.

Formula

$$\text{SOD} = (0.025 - Y) / (Y \times 50) \times 100$$

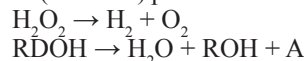
Where,

$$Y = \text{Final reading} - \text{Initial reading}$$

Catalase

Catalase was estimated by Hugo E. Aebi method: Hydrogen peroxide: hydrogen-peroxidoreduatase

In U.V range H₂O₂ can be followed directly by the decrease in absorbance (O.D 240) per unit time is a measure of catalase activity.



Decomposition of H₂O₂ = Decrease in absorbance at 240 nm

Procedure

Dilute homogenate 20 times with Phosphate buffer pH 7.0

Blank Test

1. 4ml homogenate diluted
1. 2ml homogenate diluted
2. 2ml Phosphate buffer pH 7.0
2. -----
3. -----
3. 1ml H₂O₂ (8.5 micro lit. in 2.5ml Phosphate buffer ;(50mM/l; pH 7.0).

Calculation

$$\text{Log A} / \text{B} \times 2297.3$$

Where,

A: Initial absorbance

B: final absorbance (after 30 seconds)

Units = μ moles of H₂O₂ consumed /min/mg

Reduced Glutathione (GSH)

Reduced Glutathione was determined by the method of (Moran et al., 2008)

Procedure

To 1ml of sample 1ml of 10%TCA was added .The precipitated fraction was centrifuged and to 0.5ml supernatant, 2ml of DTNB reagent was added. The final volume was made up to 3ml with phosphate buffer. The colour developed was read at 412nm. The amount of glutathione was expressed as μg of GSH/mg protein reduced glutathione was used as standard (100μg/ml).

Calculation

$$X = (Y-0.0046) / 0.0034$$

Where,

$$Y = \text{Absorbance of test sample}$$

Evaluation of Pro-Oxidant

Lipid peroxidation (Malondialdehyde formation)

Malandialdehyde formation was estimated by the method of (Slater and sawyer et al. 1971)

Procedure

2ml of sample was mixed with 2ml of 20% trichloroacetic acid and kept in ice for 15min. The precipitate was separated by centrifugation and 2ml of samples of clear supernatant solution were mixed with 2ml of aq. 0.67 thiobarbituric acid. This mixture was then heated on a boiling water bath for 10min. It was then cooled in ice for 5min and absorbance was read at 535 nm. The values expressed as nm of MDA formed/mg of protein. Values are normalized to protein content of tissue.

Calculation

$$X = (Y+0.002) / 0.0026086$$

Where,

Y = Absorbance difference of final (after 3min) and initial reading of test sample

Physical Methods

Body Weight

The animals weight were taken on day 1 and then animals weights were again taken on day 45 and compared the difference between the day 1 and day 45.

Histopathology

Liver and carotid artery were collected after the rats were sacrificed in 10% formal saline solution and utilized for the histopathological studies.

Liver and carotid artery were separated from Normal, high fat diet treated, and Alpha-linolenic acid + high fat diet treated and Atorvastatin +high fat diet treated groups after rats were sacrificed and blotted free of blood and tissue fluids. They are fixed in bovine's fluid (picric acid: Formalin: Acetic acid in the ratio of 75:52:5. After 24 hours the tissue were washed thoroughly in 70 % alcohol and then dehydrated in ascending grades of alcohol (70,100%). Dehydration in absolute alcohol was followed by treatment of tissue with toluene-xylol (50:50) followed by 10 %, 50%, 70%, 90% paraffin wax in toluene and finally to changes in 100 % paraffin wax,60-62 C followed by embedding of tissue in wax.

5-15 micro-meter thick section were serially cut in leitz microtome in horizontal plane and mounted in glass slide with the help of egg albumin in glycerin solution (50%v/v). The section were deparaffinated in xylol and downgraded through 100, 90, 50 and 30% alcohol and then finally in water. They were then stained with 105 hematoxylin for 3-5minutes and staining was intensified by running water. The hematoxylin stained section was stained with 10% eosin for two minutes and were then quickly passes through ascending grades of alcohol and finally treated with xylol followed by mounting in DPX. The sections were observed and desired area was photographed in an Olympus microscope. The sections were observed under 40X magnifications.

Statistical Analysis

All the data expressed as mean \pm SEM. Statistical significance between more than two groups was tested using one way ANOVA followed by the Turkey test using computer based fitting program (Prism, Graph pad). Statistical significant was determined at $P < 0.05$ [1].

Results

Results of Serum Analytical Parameters

Cholesterol

Administration of high fat diet and cholesterol in rats show significant ($P < 0.001$) increase in the cholesterol levels in blood serum compared to respective Normal group. Treatment with Alpha-linolenic acid and Atorvastatin show significant ($P < 0.001$) reduction in the amount of cholesterol in Alpha-linolenic acid + high fat diet treated group and atorvastatin + high fat diet group compared to respective high fat diet group.

Cholesterol levels in blood serum of all groups Normal, high fat diet treated group, Alpha-linolenic acid + high fat diet administered group and Atorvastatin + high fat treated group (Table 4).

All values shown are mean \pm SEM and $n = 6$.

*** $P < 0.001$ Compared to high fat diet treated group.

Table No.4: Cholesterol

Groups	Values (cholesterol mg/dl)
Normal	133.6 \pm 24.06 ***
High fat diet treated group	521.6 \pm 13.9
Alpha-linolenic acid + high fat diet treated group	101.8 \pm 5.951 ***
Atrovastatin + high fat treated group.	132.8 \pm 18.55 ***

Effect of Alpha-linolenic acid on Total Cholesterol levels of all groups Normal, high fat diet, high fat diet+ Alpha-linolenic acid, and high fat diet + atorvastatin groups.

All values shown are mean \pm SEM and $n = 6$.

*** $P < 0.001$ Compared to group.

HDL Cholesterol

There was a significant ($P < 0.001$) decrease in the HDL cholesterol levels in blood serum of high fat diet treated group compared to Normal group. There was a significant ($P < 0.001$) increase in the amount of HDL cholesterol levels in Alpha-linolenic acid + high fat diet treated group and atorvastatin + high fat diet treated groups compared to respective high fat diet treated group.

HDL cholesterol in blood serum of all groups Normal, high fat diet treated group, Alpha-linolenic acid + high fat diet administered group and Atorvastatin + high fat treated group (Table 5).

All values shown are mean \pm SEM and $n = 6$.

*** $P < 0.001$ Compared to high fat diet treated group.

Table 5: HDL cholesterol

Groups	Values(HDL Cholesterol mg/dl)
Normal	18.59 \pm 3.037 ***
High fat diet treated group	8.547 \pm 0.934
Alpha-linolenic acid + high fat diet treated group	24.47 \pm 1.879 ***
Atrovastatin+ high fat treated group.	27.76 \pm 2.86 ***

All values shown are mean \pm SEM and $n = 6$.

*** $P < 0.001$ Compared to high fat diet treated group.

Effect of Alpha-linolenic acid on HDL Cholesterol levels of all groups Normal, high fat diet, high fat diet+ Alpha-linolenic acid, and high fat diet + atorvastatin groups

All values shown are mean \pm SEM and n = 6.

** P < 0.001 Compared high fat diet group

Total Protein

There was a significant (P < 0.002) increase in the Total Protein levels in blood serum of high fat diet treated group compared to Normal group. There was a significant (P < 0.002) decrease in the amount of Total Protein levels in Alpha-linolenic acid + high fat diet treated group and atorvastatin + high fat diet treated groups compared to respective high fat diet treated group.

Total protein levels in blood serum of all groups Normal, high fat diet treated group, Alpha-linolenic acid + high fat diet administered group and Atorvastatin + high fat treated group (Table 6).

Table 6: Total protein

Groups	Values(total protein)
Normal	10.20 \pm 1.363 ***
High fat diet treated group	17.54 \pm 1.633
Alpha-linolenic acid + high fat diet treated group	9.070 \pm 0.7082 ***
Atorvastatin+ high fat treated group.	2.216 \pm 0.9048 ***

All values shown are mean \pm SEM and n = 6.

*** P < 0.002 Compared to high fat diet treated group.

The oxidative stress was measured in rat liver homogenate on 45th day in Normal, high fat diet treated group, Alpha-linolenic acid (0.2%) + high fat diet administered group and Atorvastatin(0.5mg) + high fat treated group.

Effect of Alpha-linolenic acid on Total protein levels of all groups Normal, high fat diet, high fat diet + Alpha-linolenic acid, and high fat diet + atorvastatin groups.

All values shown are mean \pm SEM and n = 6.

*** P < 0.002 Compared to High fat diet group.

Anti-Hyper Lipidemic Activity of Alpha-Linolenic Acid in High Fat Diet Induced Rats

Results of Tissue (liver) Parameters

There was a significant (P < 0.01) decrease in the in the SOD levels in high fat diet treated group compared to respective Normal group. There was a significant (P < 0.01), (P < 0.01) increase in SOD levels in Alpha-linolenic acid (0.2%) + high fat diet administered group and Atorvastatin(0.05mg) + high fat treated groups compared to respective high fat diet treated group.

Superoxide Dismutase

SOD levels in liver of all groups Normal, high fat diet treated group, Alpha-linolenic acid (0.2%) + high fat diet administered group and Atorvastatin(0.05mg) + high fat treated group (Table 7).

Effect of Alpha-linolenic acid on SOD levels of all groups Normal, high fat diet, high fat diet+ Alpha-linolenic acid, and High fat diet + atorvastatin groups.

All values shown are mean \pm SEM and n = 6.

** P < 0.01, *** P < 0.001 to high fat diet group.

Table 7: Superoxide Dismutase

Groups	Values (Units/gram tissue)
Normal	20.74 \pm 5.863 ***
High fat diet treated group	3.992 \pm 1.835
Alpha-linolenic acid + high fat diet treated group	16.29 \pm 2.697 **
Atorvastatin + high fat treated group	16.58 \pm 3.106 **

All values shown are mean \pm SEM and n = 6.

** P < 0.01, *** P < 0.001 Compared to high fat diet treated group .

Catalase

There was a significant (P < 0.001) decrease in the catalase levels in high fat diet treated compared to respective Normal group. There was significant increase (P < 0.01) in catalase levels in Alpha-linolenic acid (0.2%) + high fat diet administered group and Atorvastatin (0.05mg) + high fat treated groups compared to respective high fat diet treated group.

Catalase levels in liver of all groups Normal, high fat diet treated group, Alpha-linolenic acid (0.2%) + high fat diet administered group and Atorvastatin(0.05mg) + high fat treated group (Table 8).

Table 8: Catalase

Groups	Values(μ moles of H ₂ O ₂ consumed/ min/gram tissue)
Normal	292.51 \pm 3.6 ***
High fat diet treated group	188.21 \pm 2.4
Alpha-linolenic acid + high fat diet treated group	213.26 \pm 2.45 **
Atorvastatin+ high fat treated group.	229.56 \pm 3.65 ***

All values shown are mean \pm SEM and n = 6.

** P < 0.01, *** P < 0.001 Compared to high fate diet treated.

Effect of Alpha-linolenic acid on CATALASE levels of all groups Normal, high fat diet, high fat diet+ Alpha-linolenic acid, and high fat diet + atorvastatin groups.

All values shown are mean \pm SEM and n = 6.

** P < 0.01, *** P < 0.001 Compared to high fat diet group

Glutathione

There was a significant decrease (P < 0.01) in the GSH levels in high fat diet group compared to respective Normal group. There was a significant increase (P < 0.01) (P < 0.001) in GSH levels in Alpha-linolenic acid (0.2%) + high fat diet administered group and Atorvastatin(0.05mg) + high fat treated groups compared to respective high fat diet treated group.

Glutathione levels in liver of all groups Normal, high fat diet treated group, Alpha-linolenic acid (0.2%) + high fat diet administered group and Atorvastatin(0.05mg) + high fat treated group (Table 9).

Table 9: Glutathione

Groups	Values (μg of GSH / g tissue)
Normal	112.6 \pm 10.07 * * *
High fat diet treated group	75.37 \pm 5.092
Alpha-linolenic acid + high fat diet treated group	99.55 \pm 10.09 * *
Atrovastatin+ high fat treated group.	106.6 \pm 9.134 * * *

All values shown are mean \pm SEM and n = 6.
* * * P < 0.01, * * * P < 0.001 Compared to high fat diet treated.

Effect of Alpha-linolenic acid on GLUTATHIONE levels of all groups Normal, high fat diet, high fat diet + Alpha-linolenic acid, and high fat diet + atorvastatin groups.

All values shown are mean \pm SEM and n = 6.
* * P < 0.01, * * * P < 0.001 Compared to high fat diet group.

Malondialdehyde

There was a significant increase (P < 0.001) in the MDA levels in high fat diet group compared to respective Normal group. There was a significant (P < 0.05), (P < 0.01) decrease in MDA levels in Alpha-linolenic acid (0.2%) + high fat diet administered group and Atorvastatin(0.05mg) + high fat treated groups compared to respective high fat diet treated group.

Malondialdehyde levels in liver of all groups Normal, high fat diet treated group, Alpha-linolenic acid (0.2%) + high fat diet administered group and Atorvastatin(0.05mg) + high fat treated group (Table 10).

Table 10: Malondialdehyde

Groups	Values (nm of MDA / g tissue)
Normal	1.937 \pm 0.2214 * * *
High fat diet treated group	3.421 \pm 0.2786
Alpha-linolenic acid + high fat diet treated group	1.821 \pm 0.22 * * *
Atrovastatin+ high fat treated group.	1.327 \pm 0.2048 * * *

All values shown are mean \pm SEM and n = 6.
* * * P < 0.001 Compared to high fat diet treated

Effect of Alpha-linolenic acid on MDA levels of all groups Normal, high fat diet, high fat diet+ Alpha-linolenic acid, and high fat diet + atorvastatin groups.

All values shown are mean \pm SEM and n = 6.
* * * P < 0.001 Compared to high fat diet group.

Body Weight

Body weight of all groups Normal, high fat diet treated group, Alpha-linolenic acid + high fat diet administered group and Atorvastatin + high fat treated group on day 1. (table 8) Administration of high fat diet and cholesterol in rats show significant (P < 0.001) increase in bodyweight in high fat diet treated group compared to respective Normal group. There was a significant (P < 0.001) decrease in body weight in Alpha-linolenic acid + high fat diet treated group and atorvastatin + high fat diet treated groups compared to respective high fat diet treated group after 45 days (Table 11).

Table 11: Bodyweight

Groups	Body weight in grams	
	On day 1	On day 45
Normal	100.8 \pm 5.23	120.7 \pm 5.74 * * *
High fat diet treated group	98.3 \pm 8.207	171.7 \pm 6.009
Alpha-linolenic acid + high fat diet treated group	112.3 \pm 4.041	145.0 \pm 4.28* * *
Atrovastatin+ high fat treated group	113.3 \pm 10.54	143.0 \pm 6.66* * *

Effect of Alpha-linolenic acid on body weight levels of all groups Normal, high fat diet, high fat diet+ Alpha-linolenic acid, and high fat diet + atorvastatin groups.

All values shown are mean \pm SEM and n = 6.
* * * P < 0.0001 Compared to high fat diet group.

Histopathological Findings

Liver

On histological examination of liver section of high fat diet groups showed fat deposition in majority of cells accompanied by white globules formation, extensive intercellular hemorrhage. These histological observations support the presence of cholesterol deposition. On administration of Alpha-linolenic acid and atorvastatin treated groups significantly decrease the cholesterol formation are observed all most similar like normal liver section.

Carotid Artery

In examination of carotid artery of high fat diet groups showed fat deposition in the artery and it shows presence of fatty patches and may be thrombus formation, and congestion of blood vessels. These histological observations support that the deposition of cholesterol. On administration of Alpha-linolenic acid and atorvastatin treated groups significantly decrease the cholesterol deposition observed all most similar like normal carotid artery.

Discussion

Hyperlipidemia has plagued humans from antiquity and constitutes a major health problem. Despite dramatic progress in both medical and surgical areas, still management of Hyperlipidemia is not complete. The goal of the medical treatment is the prevention of Hyperlipidemia with synthetic drugs, each suffers from their own disadvantages, still offering a wide scope of research in this particular area. Many research laboratories are pursuing investigations in Anti Hyperlipidemia in both the preclinical and clinical areas. Various factors involved in fat deposition are still being recognized and evaluated. The aim of the present study is also an attempt in this direction.

Despite considerable effort on the part of a number of investigators, there has been only a limited success in developing an ideal animal model of lipidemic disease that faithfully mimics human Hyperlipidemia. Various procedures have been reported by numerous investigators from time to time such as selective diets, High fat diets, drug induced Hyperlipidemia in experimental animals.

In the animal model, Hyperlipidemia induces not only fat deposition damage to the cells of organs that leads generation of free radicals. The experimental model selected for the present study is administration High fat diet every day and cholesterol (0.5%) orally

at every alternative day.

The present study on Hyperlipidemia and oxidative stress was carried out on the female albino rats of Wister strain. The project was aimed to study the Anti-hyperlipidemia and oxidative stress and protective role of Alpha-linolenic acid in hyperlipidemia. Biochemical parameters of hyperlipidemia and oxidative stress were analyzed from liver homogenate and serum. Histopathological study was carried out to confirm the biological changes.

In the present study, administration of High fat diet to rats caused significant increase in oxidative stress i.e. decreased activity of SOD, Catalase and decreased levels of GSH while increased levels of LPO. Administration of High fat diet to rats also increased deposition of fat in liver and various blood vessels.

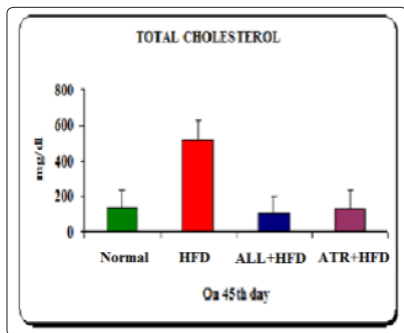


Figure 1: Cholesterol

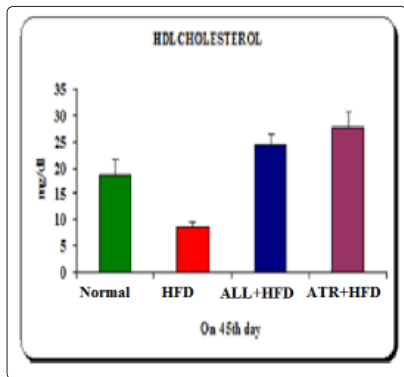


Figure 2: HDL Cholesterol

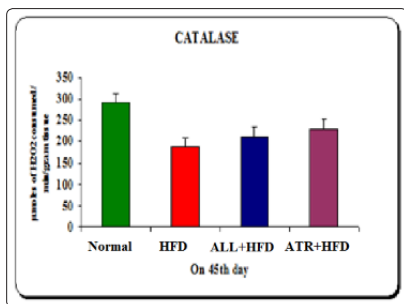


Figure 3: Superoxide Dismutase

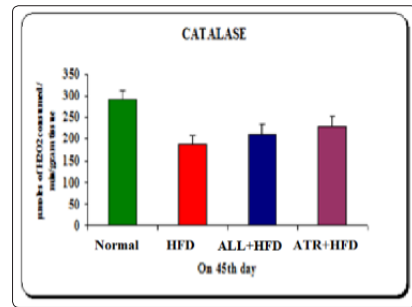


Figure 4: Catalase

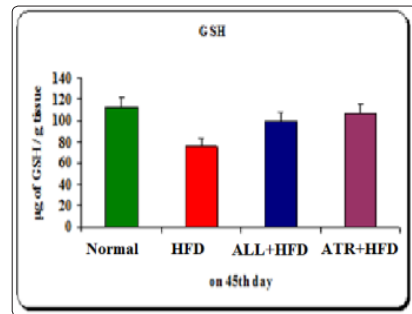


Figure 5: Glutathione

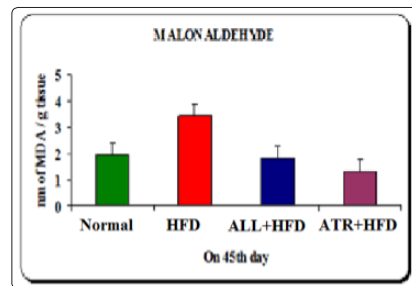


Figure 6: Malondialdehyde

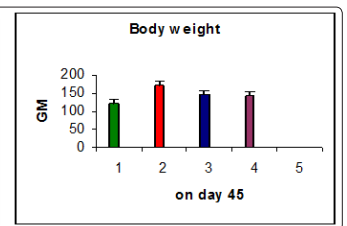
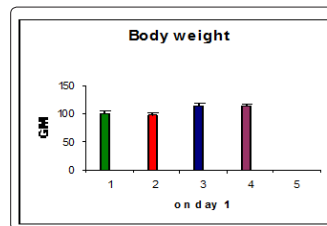


Figure 7: Body weight

Photo micro graphs of livers
Histopathology

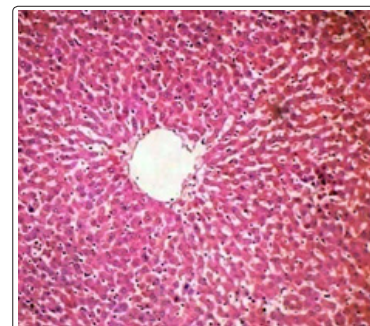


Figure 8: Normal liver

Photo Micro Graphs of Carotid Artery

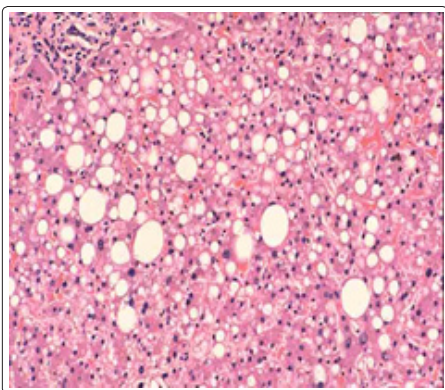


Figure 9: HFD Induced

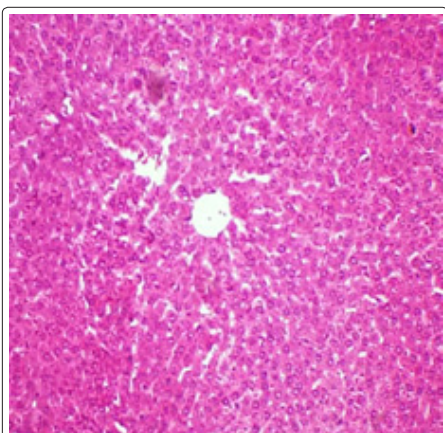


Figure 10: Alpha-linolenic acid +HFD

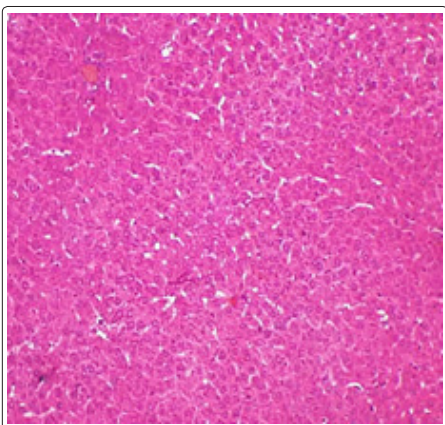


Figure 11: Atrovastatin +HFD

HFD = High Fat Diet

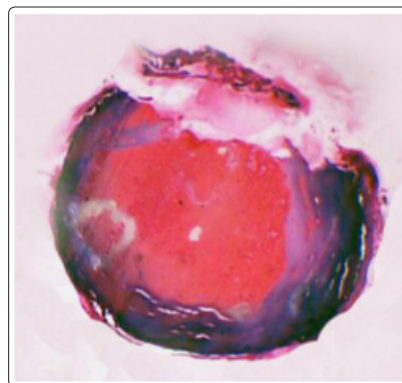


Figure 12: Normal Carotid artery

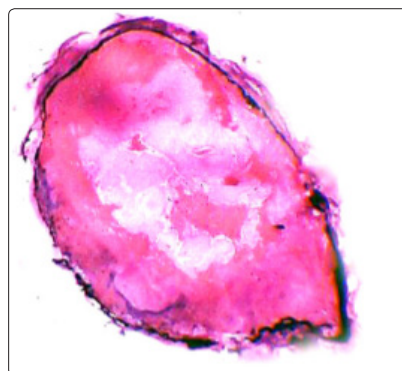


Figure 13: HFD induced

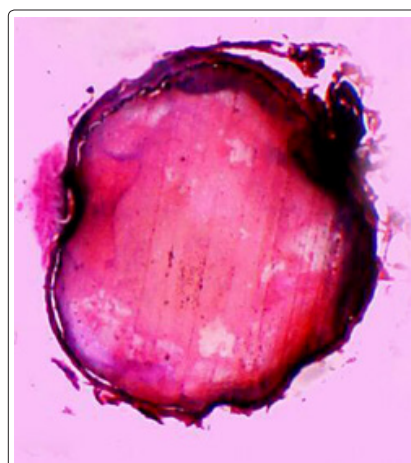


Figure 14: Alpha-linolenic acid + HFD

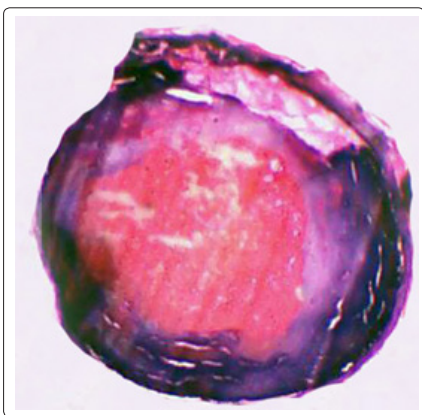


Figure 15: Atrovastatin +HFD

HFD = High Fat Diet

Conclusion

All synthetic drugs like fibrates and statins have their own adverse effects for example atorvastatin have constipation, sleep disturbances, abnormal liver function values and rhabdomyolysis with acute renal failure and it has following drug interactions. Drugs like erythromycin,azole antifungals, cyclosporine, or niacin that interfere with metabolism or its protein binding may increase serum concentration of atorvastatin and risk of myopathy. To over from these problems we use natural synthetic drugs like Alpha-linolenic acid.

The present study finds out the role of anti-hyperlipidemic and antioxidant activity of Alpha-linolenic acid in hyperlipidemia [2-10].

From the Present Study the Following Conclusions Can Be Made

Administration of high fat diet + cholesterol (0.5%) increase in fat formation and fat deposition in liver and carotid artery. It also increases the levels of biomarkers of oxidative stress in the liver. The increase in serum cholesterol and total protein in high fat diet + cholesterol (0.5%) group rats were also observed.

In conclusion, the study seemed to reveal that high fat and high anti-oxidant nutrition play the aggregative and alleviative roles respectively in the context of lipid and oxidative stress status as high fat diet feeding for 45 days resulted in raising both lipid and oxidative stress status in blood and hepatic tissues. However these effects were considerable lowered by high all antioxidants diets each when fed in combination with high fat diet.

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