World Journal of Pharmaceutical and Life Sciences

www.wjpls.org

Impact Factor: 8.21 Coden USA: WJPLA7

FREE RADICAL SCAVENGING AND ANTIOXIDANT IMPACT OF *AZADIRACHTA INDICA* ON COPPER MEDIATED OXIDATIVE MODIFICATION OF LOW DENSITY LIPOPROTEIN NORMAL/ DIABETIC LIPIDEMIC SUBJECTS

Arvind Yadav[1], Umar Farooq[2] and Amir Khan*[1,2]

[1]Department of Biotechnology & Biochemistry, Division of Life Science, Sardar Bhagwan Singh University Dehradun, 248161, Uttarakhand, India.
[2]Oral Medicine and Allied Dental Sciences Department, Faculty of Dentistry, Taif University, Taif, KSA.

***Corresponding Author: Amir Khan**

Department of Biotechnology and Biochemistry, Division of Life Science, Sardar Bhagwan Singh University Dehradun, 248161, Uttarakhand, India.
DOI: https://doi.org/10.5281/zenodo.19906001

How to cite this Article: Arvind Yadav[1], Umar Farooq[2] and Amir Khan*[1,2]. (2026). Free Radical Scavenging and Antioxidant Impact of Azadirachta Indica on Copper Mediated Oxidative Modification of Low Density Lipoprotein Normal/ Diabetic Lipidemic Subjects. World Journal of Pharmaceutical and Life Sciences, 12(5), 233–250.

Article Received on 05/04/2026 **Article Revised on 25/04/2026** **Article Published on 01/05/2026**

ABSTRACT

Diabetes can lead to serious complications and premature death, but people with diabetes, working together with their support network and their health care providers, can take steps to control the disease and lower the risk of complications. A diabetes epidemic is underway. An estimated 30 million people world-wide had diabetes in 1985. By 1995, this number had shot up to 135 million. The latest WHO estimate (for the number of people with diabetes, world-wide, in 2000) is 177 million. This will increase to at least 300 million by 2025. The number of deaths attributed to diabetes was previously estimated at just over 800,000. Atherosclerosis and coronary heart disease have been considered as major health problem worldwide. The clinical presentations of atherosclerosis mainly involve the coronary and carotid arteries, which remain the leading causes of morbidity and mortality in both men and women of all racial groups with Coronary Heart Disease (CHD) the leading cause of death worldwide. Excessive dietary lipids and cholesterol are the major factor of relevance for the development of hypertriglyceridemia and hypercholesterolemia, two important cardiovascular risk factors. Abnormalities in lipid profiles, folate metabolism and other traditional risk factors (e.g., diabetes mellitus and hypertension) play a rather peripheral role and serve to amplify the atherosclerotic process initiated by persistence of infection and inflammation. Infection and inflammation are accompanied by cytokine induced alterations in lipid and lipoprotein metabolism. Of note, inflammatory cytokines are increased and play a pathogenic role in a variety of very common disorders, such as diabetes, obesity, metabolic syndrome, hypertension, chronic heart failure, chronic renal failure, and atherosclerosis. Based on our study of free radical scavenging and antioxidant properties of ethanolic Neem (leaves), Aq. Neem (leaves), Aq. Niboli, Aq. Bark and ethanolic Bark on copper mediated oxidative modification of LDL in normallipidemic subjects, administration of ethanolic Neem (leaves), Aq. Neem(leaves), Aq. Niboli, Aq. Bark and ethanolic Bark may be useful in the prevention and treatment of dyslipidemia/hyperlipidemia, atherosclerosis and other oxidative stress mediated diseases. In addition, use of these herbal plant extracts will be efficacious, cost effective and involves no risk of side-effects.

KEYWORDS: Coronary Heart Disease, Diabetes, Hypertension, Atherosclerosis.

INTRODUCTION

Diabetes is a group of diseases marked by high levels of blood glucose resulting from defects in insulin production, insulin action, or both. Diabetes can lead to serious complications and premature death, but people with diabetes, working together with their support network and their health care providers, can take steps to control the disease and lower the risk of complications.

A diabetes epidemic is underway. An estimated 30

million people world-wide had diabetes in 1985. By 1995, this number had shot up to 135 million. The latest WHO estimate (for the number of people with diabetes, world-wide, in 2000) is 177 million. This will increase to at least 300 million by 2025. The number of deaths attributed to diabetes was previously estimated at just over 800,000. However, it has long been known that the number of deaths related to diabetes is considerably underestimated. A more plausible figure is likely to be around 4 million deaths per year related to the presence of the disorder. This is about 9% of the global total. Many of these diabetes related deaths are from cardiovascular complications. Most of them are premature deaths when the people concerned are economically contributing to society. This situation is increasingly outstretching the health-care resources devoted to diabetes.

According to recent estimates, approximately 285 million people worldwide (6.6%) in the 20–79 year age group will have diabetes in 2010 and by 2030, 438 million people (7.8%) of the adult population, is expected to have diabetes.(1) The largest increases will take place in the regions dominated by developing economies.

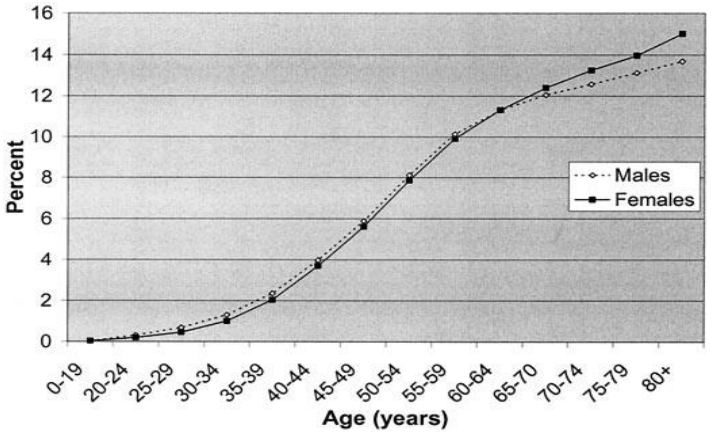

Figure 1: Global diabetes prevalence by age and sex for 2000.

METHOD

Aqueous, Ethanolic extraction of Leaves, Bark and seed of *Azadirachta indica*

The fresh dried stems, Bark and seed of *Azadirachta indica* were collected from market of Dehradun (Uttrakhand). 100 gm roots crushed to make powder and were then subjected to extraction using 70% hydro-alcoholic solvent (95% ethanol: 5% distilled water). The final extract was filtered and the remaining alcohol was allowed to evaporate.

Collection of blood and plasma

Fresh Human blood sample was collected from the pathology laboratory of Sardar Bhagwan Singh Instt., Balawala, Dehradun. Plasma was separated by centrifugation at 2500 rpm and the packed erythrocytes obtained were washed thrice with physiological saline and a portion of washed erythrocytes was lysed in hypotonic (10mM) sodium phosphate buffer, pH 7.4. A portion of the washed packed erythrocytes was stored at 4^0C for future use.

Fractionation of plasma lipoproteins

The precipitation method described by Wieland and Seidel (1989) was used for the isolation of plasma low density lipoprotein (LDL).

Precipitation buffer for LDL isolation

The precipitation buffer consisted of 64 mM trisodium citrate adjusted to pH 5.05 with 5 N HCL, then50,000 IU/L heparin is added. It is freshly prepared.

LDL precipitation

Before precipitation of LDL, plasma samples and precipitation reagent were allowed to equilibrate to room temperature. One ml of plasma sample was added to 7.0 ml of heparin-citrate buffer. After mixing with a vortex mixer, the suspension was allowed to stand for 10 min at 22^0C. The insoluble LDLs were then sedimented by centrifugation at 1,500 rpm for 10 min at 220C. The pellet was resuspended in 1.0 ml of 0.1 M sodium phosphate buffer, pH 7.4, containing 0.9 % NaCl.

Precipitation buffer for HDL isolation

Precipitation buffer consist of Reagent A and Reagent B which is freshly prepared. Reagent A was prepared by dissolving 1.0 g of dextran sulfate in 100 ml of 0.5 mM MgCl2 solution.

Reagent B was prepared by dissolving 10 g of dextran sulfate in 100 ml of 1.5 mM MgCl2 solution.

The dual precipitation method of Patsch *et al*. (1989) was used for the isolation of high density lipoprotein (HDL), HDL2 and HDL3 subfractions was added to 1.0 ml of plasma, and vortexed-mixed for 3 sec. After an incubation period of 10 min at 22^0C, the mixture was centrifuged at 1500 rpm for 15 min at 22^0C and the supernatant containing HDL was removed. The clear

supernatant was used for the analysis of HDL-C as well as for the isolation of HDL2 and HDL3 subtractions. For HDL fraction, 100 µl of reagent containing 1.0 g of dextran sulfate dissolved in 100 ml of 1.5 mM MgCl2 solution was added to 1.0 ml of HDL supernatant, and vertexes-mixed for 3 sec. Following an incubation of 10 min at 22^0C, the mixture was centrifuged at 1500 rpm for 20 min at 22^0C and the supernatant containing HDL3 fraction was removed, whereas the HDL2 precipitate was dissolved in 0.1 M sodium phosphate buffer saline (pH 7.4).

Determination of plasma cholesterol

Total cholesterol in plasma, LDL subtractions was determined with a minor modification. For the determination of cholesterol in plasma and lipoproteins, 0.1 volume of plasma was mixed with 1 volume of isopropanol, allowed to stand for 5 min and centrifuged at 3,000 rpm for 10 min. A suitable aliquot of isopropanol extract was used for cholesterol determination in a total volume of 0.75 ml. To each tube 0.25 ml of 7.03 mM ferric chloride dissolved in glacial acetic acid, was added, mixed instantly, followed by the addition of 0.8 ml of sulphuric acid with thorough mixing. After 5 min, the absorbance was read at 550 nm in a Beckman DU 640 spectrophotometer. The cholesterol content in the samples was determined by using a cholesterol standard.

Measurement of *in vitro* Cu++mediated oxidation of LDL in the absence or presence of Azadirachta indica in normallipidemic and dyslipidemic subjects

Experiment was performed as described by **Esterbauer *et al*. (1989; 1992).** The *in vitro* Cu++-mediated susceptibility of isolated LDL to oxidation was assessed by determining the formation of conjugated diene. Prior to oxidation studies LDL samples were dialyzed against 5 mM phosphate buffer saline (PBS), pH 7.4, for 24 h. The incubation mixture contained LDL (3 mg TC/dl) in the absence or presence of *Azadirachta indica* (1mg/ml). At time zero, the absorbance of lipoprotein samples was taken at 234 nm. Then, lipoprotein samples were mixed with CuSO4 to a final concentration of 2.5 mM and incubated at 370C. In one series, at different time intervals of oxidation, 1.0 ml aliquots from LDL incubation mixture were taken out, mixed with 0.5 mM EDTA in eppendorff tubes, pH 7.4, stored at 40C and used for the assessment of conjugated dienes. The oxidation for LDL was carried out for 200 minutes. The formation of conjugated dienes in each aliquot was measured by monitoring absorbance at 234 nm in a Beckman DU 640 spectrophotometer. Conjugated dienes was calculated by using an extinction coefficient of 2.52 x 10^4 M^{-1} cm-1 and expressed as µM MDA equivalent per mg LDL protein.

Measurement of plasma "total antioxidant power" (FRAP)

The method of Benzie and Strain (1996) was used for measuring the ferric reducing ability of plasma, the FRAP assay, which estimate the "total antioxidant power", with minor modification. Ferric to ferrous ion reduction at low pH results in the formation of a colored ferrous-tripyridyl-triazine complex.

Preparation of FRAP reagent

FRAP reagent was freshly prepared by mixing 10.0 ml of 22.78 mM sodium acetate buffer (pH 3.6), 1.0 ml of 20 mM ferric chloride and 1.0 ml of 10 mM 2, 4, 6-tripyridyl-s-triazine solution prepared in 40 mM HCl.

The assay was carried out in a total volume of 1.0 ml containing a suitable aliquot of plasma in 0.1 ml and 900 µl of freshly prepared FRAP reagent. Before starting the reaction, both FRAP reagent and plasma samples were pre-incubated for 5 min at 30^0C. Incubation was done for 5 min at 30^0C and absorbance was recorded at 593 nm against a reagent blank in spectrophotometer. Ferrous sulphate (1mM) was used as a standard for calculating the "total antioxidant power"

Determination of Malondialdehyde in Plasma

MDA is the most abundant individual aldehyde resulting from lipid peroxidation and its determination by TBA is the most common method of estimating lipid peroxidation (Esterbauer H. and Cheeseman K.H., 1990).

100 µl of normal and diabetic plasma sample were taken separately with Azadirachta indica in triplicate .1ml of TBA(0.375%) and TCA (15%) was added and incubated in boiling water bath for 15 minutes (for precipitation of proteins). After cooling the tubes to room temperature the absorbance of each sample was read against a reagent blank at 532 nm in spectrophotometer. Molar extinction coefficient (2.5 × 10^4 $M^{-1}cm^{-}1$) used for the calculation of MDA concentration (Liu *et al.*, 1982).

Protein estimation in lipoprotein ApoB **100 and HDL**

Preparation of Bradford dye

50 mg Bradford dye (CBR-250) was dissolved in 25 ml of 95% ethanol and stirred for 2 hours.50 ml of o–phosphoric acid was added to the solution and was diluted to 500ml with distilled water. Again stirred for 30minutes.

Precipitation of protein from lipoprotein ApoB 100 and HDL of Normal and Diabetic subjects

100 µl of LDL suspension was taken and 1ml of TCA was added and incubated at room temperature. Solution was centrifuged at 12,000 rpm for 5 minutes. Pellet obtained was dissolved in 0.5 N NaOH.

Same procedure was repeated for precipitation of proteins in HDL.

The protein was determined by the method of Bradford (1976), using bovine serum albumin as standard. Aliquots of LDL and HDL (HDL-3, HDL2-C, HDL_3-C) in triplicate for normal and diabetic samples, were first precipitated with 10 % TCA separately. The protein pellets were dissolved in 0.5 N NaOH and absorbance

was measured in a spectrophotometer at 595 nm.

RESULTS

Average Values of Age, Weight, Height, Male, Female of Normal and diabetic subjects

The average values of body weight, age, male and female of normal subjects (n=45) were 59.42 ± 8.39kg, 32 ± 1.86years, 26 and 19 respectively and of diabetic subjects (n=60) were 64 ± 9.41kg, 58± 3.56, 29 and 31 respectively as shown in table1.

Table 1: Average Values of Age, Weight, Height, Male, Female of Normal and diabetic subjects.

S. No.	PARAMETER	NORMAL (n= 45)	DIABETIC (n= 60)
1.	Age	32 ± 1.86	58 ± 3.56
2.	Body weight	59.42 ± 8.39	64 ± 9.41
3.	Male	26	29
4.	Female	19	31
5.	Drugs used by the patients (Pre treated)	-	Human insulin, dayonil, Metformin, pioglitazone, Glynase, glyciphase etc.

Total phenolic content of Aq. and ethanolic extracts of *Azadirachta indica*

Total phenolic content of aqueous and ethanolic extract of Azadirachta indica leaves, bark and niboli in grams in 100ml of extract were as shown in table 2.

Table 2: Total Phenolic Content in Aqueous and Ethanolic Extract of Azadirachta Indica.

Plant extract	Phenolic content (g/100ml)
Ethanolic Neem (Leaves)	0.990
Aqueous Neem (Leaves)	0.70
Aqueous (Niboli)	0.860
Aqueous Neem (Bark)	0.650
Ethanolic Neem (bark)	1.380

Average value of TC, Total protein, LDL-C, HDL-3, HDL_2-C, HDL_3-C, HDL protein and non – HDL cholesterol in normal and diabetic subject

As shown in table2, the average values of TC, LDL-C, total protein in plasma and LDL, HDL-C, HDL_2-C ,HDL_3-C ,HDL_3 protein, HDL_2C protein, HDL_3 C protein, Non HDL-C in normal lipidemic subjects were 42.80 ± 2.39 µg/ml, 14.9 ± 2.09 µg/ml, 1.212 ± 0.066 µg/ml, 1.179 ± 0.348µg/ml, 5.36 ± 0.46 µg/ml, 1.186 ± 0.34 µg/ml, 3.172 ± 0.53 µg/ml, 0.301 ± 0.111µg/ml,0.164 ± 0.00014µg/ml,0.601 ± 0.018µg/ml and 37.44 ± 1.93µg/ml respectively. On the other hand, the average values of TC, LDL-C, total protein in plasma and LDL, HDL-C, HDL2-C, HDL3-C, HDL 3 protien, HDL_2C protein, HDL_3 C protein, Non HDL-C in hyperlipidemic subjects were 114.66 ± 2.24µg/ml, 10.89 ± 0.53µg/ml, 1.190 ± 0.015µg/ml, 1.046 ± 0.28µg/ml, 7.025 ± 0.63µg/ml, 1.514 ± 0.51µg/ml, 4.33 ± 0.247 µg/ml, 0.332 ± 0.0016 µg/ml, 0.183 ± 0.036 µg/ml, 0.304 ± 0.0007 µg/ml, 107.63 ± 1.61 µg/ml respectively.

Table 3: Average values of TC, Total protein, LDL-C, HDL-3, HDL_2-C, HDL_3-C, HDL and non – HDL cholesterol protein in normal and diabetic subject.

S.NO	PARAMETERS	NORMAL VALUE (µg/ml)	DAIBETIC VALUE (µg/ml)
1.	Total cholesterol in plasma	42.80 ± 2.39	114.66 ± 2.24
2.	Low Density Lipoprotein – Cholesterol (LDL- C)	14.9 ± 2.09	10.89 ± 0.53
3.	Total protein in plasma	1.212 ±0 .066	1.190 ± 0.015
4.	Total protein in LDL	1.179 ± 0.348	1.046 ± 0.28
5.	High Density Lipoprotein cholesterol (HDL)	5.36 ± 0.46	7.025 ± 0.63
6.	High Density Lipoprotein2 cholesterol(HDL_2-C)	1.186 ± 0.34	1.514 ± 0.51
7.	High Density Lipoprotein3 Cholesterol(HDL_3-C)	3.172 ± 0.53	4.33 ± 0.247
8.	High Density Lipoprotein-protein(HDL)	0.301 ± 0.111	0.332 ± 0.0016
9.	High density Lipoprotein – protein(HDL_2-C)	0.164 ± 0.00014	0.183 ± 0.036
10.	High Density Lipoprotein – protein(HDL_3 –C)	0.601 ± 0.018	0.304 ± 0.0007
11.	Non- HDL - cholesterol	37.44 ± 1.93	107.63 ± 1.61 *

*****Indirectly calculated values

All values are mean ± S.D from pooled serum of normal subjects (n=45)

Average Ratio Value of TC/LDL –C, LDL –C /TC, HDL_2 – C /HDL_3 –C and HDL_3 –C /HDL_2 – C

As shown in table 4, the average ratio Value of TC/LDL –C, LDL –C /TC, HDL_2 – C/HDL_3 –C and HDL_3 –C /HDL_2 – C in normallipidemic subjects were 1.15, 0.866, 0.233, 3.66 and no significantly registered in dyslipidemic subjects were 1.055, 0.950, 0.602, 1.66 respectively.

Table 4. Average Ratio Value of TC/LDL –C, LDL –C /TC, HDL_2 – C /HDL_3 –C and HDL_3 –C /HDL_2 – C.

S.NO.	PARAMETER	NORMAL SUBJECTS	DIABETIC VALUE
1.	TC/LDL-C	1.15	1.055
2.	LDL-C/TC	0.866	0.950
3.	HDL2-C/HDL3-C	0.233	0.602
4.	HDL3-C/HDL2-C	3.66	1.66

In *vitro* copper mediated oxidative modification of LDL (at 37°C) isolated from normallipidemic and hyperlipidemic subjects

Copper mediated LDL oxidation begins first with a lag phase during which protective endogenous anti-oxidants are consumed by initiating free radical species. After the consumption of all endogenous anti-oxidants, a lipid radical propagated peroxidation chain reaction begins in which them polyunsaturated fatty acids contained in the LDL are rapidly oxidized to lipid hydroperoxides. Copper is redox active metal that can participate in electron transfer reactions with the consequent production of oxidant species capable of oxidizing cell components. Copper can catalyze the formation of the highly reactive hydroxyl radicals from hydrogen peroxide via the Haber-Weiss reaction and decompose lipid peroxides to peroxyl and alkoxyl radicals, which favor the propagation of lipid peroxidation. Addition of extract/drug in LDL suspension, are capable of inhibiting LDL oxidation.

Table 5: Cu^{2+} mediated oxidation of LDL in the presence or absence of glucose.

LDL oxidation at 37° C Time (min.)	Conjugated diene formation without glucose (µM/ml)	Conjugated diene formation with glucose (µM/ml)
0'	284.14	284.14
10'	315.08	452.96
20'	315.96	453.56
40'	333.92	454.76
80'	369.46	460.70
120'	375.19	476.59
Fold/%	+1.32 fold or +25.06 %	+1.67 fold or +40.17%

As shown in table5. In absence of glucose, 1.32 fold (+25.06%) increase in oxidative modification of LDL was observed from basal value (284.14µM/ml) to maximal value (375.19µM/ml) at 120 min. In presence of glucose LDL oxidation was increase up to +21.27%. We observed that glucose increased the LDL oxidation (CD formation- µM/ml).

Table 6(a): Cu^{2+} mediated oxidation of LDL in the presence or absence of ethanolic Neem (leaves) in normal subjects.

Time (Min.)	Conjugated diene formation without Eth. Neem (leaves) (µM/ml)	Conjugated diene formation with Eth. Neem (leaves) (µM/ml)
0'	285.11	285.11
10'	376.19	314.48
20'	371.43	316.66
40'	377.77	334.12
60'	383.13	367.46
80'	385.31	369.04
100'	392.65	376.19
120'	403.22	398.00
140'	427.97	420.83
160'	442.85	433.75
180'	444.64	449.44
200'	457.73	453.01
Fold/ %	+1.60 fold or +37.71%	+1.58 fold or +37.06%

As shown in table 6(a)., In absence of Eth. Neem (leaves) 1.60 fold or (+37.71%) increase in oxidative modification of LDL was observed from basal value (285.11µM/ml) to maximal value (432.73 µM/ml) at 200

min, after adding 2.5 mM $CuSO_4$. But in presence of Eth. Neem (leaves), this increase was only 37.06%(285.11 µM/ml – 453.01µM/ml), in normallipidemic subjects. On the other hand, LDL oxidation decreases up to 1.03% with Eth. Neem (leaves), when compare to without Eth. Neem (leaves).

Table 6(b): Cu^{2+} mediated oxidation of LDL in the presence or absence of Eth. Neem (leaves) in diabetic subjects.

Time (minutes)	Conjugated diene formation without Eth. Neem (leaves) (µM/ml)	Conjugated diene formation with Eth. Neem (leaves) (µM/ml)
0'	510.12	510.12
10'	483.33	483.33
20'	506.34	470.23
40'	518.45	475.19
60'	527.41	477.77
80'	543.35	486.30
100'	558.12	499.00
120'	569.31	513.64
140'	587.30	526.63
160'	589.46	539.31
180'	594.30	543.12
200'	599.22	552.57
Fold/ %	+1.17 fold or +14.86%	1.03 fold or 8.32%

As shown in table6 (b). In absence of Eth. Neem (leaves), 1.17fold(+14.86%) increase in oxidative modification of LDL was observed from basal value (510.12µM/ml) to maximal value (597.22µM/ml)at 200 min. after adding 2.5 mM $CuSO_4$. But in presence of E. Neem (leaves) this increase was significantly reduced to 7.78% in hyperlipidemic subjects.

Table 7(a): Cu^{2+} mediated oxidation of LDL in the presence or absence of Aqueous Neem (leaves) in normal subjects.

Time (minutes)	Conjugated diene formation without Aq. Neem (leaves) (µM/ml)	Conjugated diene formation with Aq. Neem (leaves) (µM/ml)
0'	285.11	285.11
10'	307.19	306.35
20'	319.43	334.32
40'	338.77	369.52
60'	354.13	379.54
80'	376.31	396.89
100'	383.65	408.76
120'	404.21	421.83
140'	428.97	434.96
160'	441.85	444.05
180'	445.64	454.86
200'	457.73	467.92
Fold /%	+1.60 fold or +37.71%	+1.64 fold or +39.06%

As shown in table 7(a), In absence of Aq. Neem (leaves) +1.60fold (+37.71%) increase in oxidative modification of LDL was observed from basal value (285.11µM/ml) to maximal value (457.73 µM/ml) at 200 min. after adding 2.5 mM $CuSO_4$. But in presence of Aq. Neem (leaves) this increase was only 39.06%(285.11 µM/ml – 467.92µM/ml), in normallipidemic subjects. We registered no significant reduction after adding the aq. Neem (leaves).

Table 7(b): Cu^{2+} mediated oxidation of LDL in the presence or absence of Aqueous Neem (leaves) in diabetic subjects.

Time (min)	Conjugated diene formation without Aq. Neem (leaves) (µM/ml)	Conjugated diene formation with Aq. Neem (leaves) (µM/ml)
0	443.65	443.65
10	465.32	451.49
20	588.36	489.78

40	598.95	518.85
60	603.09	534.38
80	613.35	552.19
100	623.19	598.87
120	627.80	604.80
140	633.31	615.00
160	640.69	623.46
180	641.90	629.57
200	657.29	639.77
Fold / %	+1.48 fold or +32.50%	+1.44 fold or +30.65%

As shown in table 7(b). In absence of Aq. Neem (leaves),+1.48 fold(+32.50%) increase in oxidative modification of LDL was observed from basal value (443.65µM/ml) to maximal value (657.29µM/ml)at 200 min, after adding 2.5 mM $CuSO_4$.But in presence of Aq. niboli this increase was significantly reduced to 2.66% in hyperlipidemic subjects.

Table 8(a).Cu^{2+} mediated oxidation of LDL in the presence or absence of Ethanolic Bark in normal subjects.

Time (minutes)	Conjugated diene formation without eth. Bark (µM/ml)	Conjugated diene formation with eth. Bark (µM/ml)
0	284.15	284.15
10	295.99	298.77
20	302.43	304.93
40	338.77	327.30
60	363.18	363.10
80	385.39	388.50
100	391.65	402.87
120	401.91	428.01
140	428.97	434.63
160	443.85	453.46
180	445.65	483.47
200	457.74	511.13
Fold/ %	+1.61 fold or 37.92%	+ 1.79 fold or 44.40%

As shown in table8(a).,in absence of Eth. Bark, +1.61 fold(+37.92%) increase in oxidative modification of LDL was observed from basal value (284.15µM/ml) to maximal value (457.74 µM/ml)at 200 min, after adding 2.5 mM $CuSO_4$.But in presence of Eth. Bark, this increase was 44.40%(284.15 µM/ml 511.13 µM/ml), in normallipidemic subjects.

Table 8(b).Cu^{2+} mediated oxidation of LDL in the presence or absence of Ethanolic Bark in diabetic subjects.

Time (minutes)	Conjugated diene formation without Eth. Bark (µM/ml)	Conjugated diene formation with Eth. Bark (µM/ml)
0	444.64	444.64
10	483.33	462.34
20	506.34	473.05
40	518.45	483.01
60	529.00	499.01
80	535.35	509.81
100	540.11	529.35
120	550.31	539.57
140	557.30	547.38
160	572.60	468.25
180	587.30	569.84
200	597.22	578.30
Fold/%	+25.54% or + 1.34 fold	+23.11% or 1.30 fold

As shown in table 8(b), In absence of Eth. Bark 1.34 fold (+25.54%) increase in oxidative modification of LDL was observed from basal value (444.64µM/ml) to maximal value (597.22µM/ml) at 200 min. after adding 2.5 mM $CuSO_4$.But in presence of Eth. Bark, this increase was significantly reduced to 3.16% in

hyperlipidemic subjects.

Table 9(a).Cu^{2+} mediated oxidation of LDL in the presence or absence of aqueous niboli in normal subjects.

Time (minutes)	Conjugated diene formation without aq. Niboli (µM/ml)	Conjugated diene formation with aq. Niboli (µM/ml)
0	285.11	285.11
10	298.19	309.28
20	309.43	326.39
40	337.07	345.68
60	346.13	356.90
80	359.31	370.09
100	367.65	387.57
120	389.02	398.57
140	409.97	402.98
160	421.85	408.58
180	445.64	421.95
200	466.73	427.50
Fold/ %	+1.63 fold or 38.91%	+ 1.49 fold or 33.30%

As shown in table12,in absence of Aq. Niboli ,+1.63 fold(+38.91%) increase in oxidative modification of LDL was observed from basal value (285.11µM/ml) to maximal value (466.73 µM/ml)at 200 min. after adding 2.5 mM $CuSO_4$.But in presence of Aq. Niboli, this increase was only 8.40% in normallipidemic subjects.

Table 9b.Cu^{2+} mediated oxidation of LDL in the presence or absence of aqueous Niboli in diabetic subjects.

Time (minutes)	Conjugated diene formation without drug(µM/ml)	Conjugated diene formation with drug(µM/ml)
0	444.64	444.64
10	464.32	481.95
20	478.34	485.32
40	487.45	507.54
60	499.90	513.57
80	505.35	537.28
100	511.11	539.41
120	524.31	541.36
140	541.30	546.69
160	561.60	549.12
180	588.30	555.24
200	598.22	565.36
Fold / %	+25.67% or 1.34 fold	+21.35% or 1.27 fold

As shown in table 9(b), In absence of Aq. Niboli, 1.34 fold (+25.67%) increase in oxidative modification of LDL was observed from basal value (444.64µM/ml) to maximal value (598.22µM/ml) at 200 min. after adding 2.5 mM $CuSO_4$.But in presence of Aq. Niboli, this increase was significantly reduced to 5.49% in normallipidemic subjects.

Table 10(a). Cu^{2+} mediated oxidation of LDL in the presence or absence of aqueous bark in normal subjects.

Time (minutes)	Conjugated diene formation without aq. Bark (µM/ml)	Conjugated diene formation with aq. Bark (µM/ml)
0'	285.11	285.11
10'	298.39	305.56
20'	307.93	327.97
40'	327.07	339.88
60'	343.33	342.46
80'	365.91	373.68
100'	373.65	399.93
120'	385.22	413.74
140'	399.97	431.95
160'	408.85	453.77
180'	427.64	455.36

200'	436.73	461.25
Fold/ %	+34.17% or 1.53 fold	+38.19% or 1.61 fold

As shown in table 10(a), In absence of Aq. Bark, 1.53 fold(+34.17%) increase in oxidative modification of LDL was observed from basal value (285.11µM/ml) to maximal value (436.73 µM/ml)at 200 min, after adding 2.5 mM $CuSO_4$. But in presence of Aq. bark, this increase was only 38.19% (285.11 µM/ml – 461.25µM/ml), in normallipidemic subjects.

Table 10(b): Cu^{2+} mediated oxidation of LDL in the presence or absence of Aqueous Bark in diabetic subjects.

Time (minutes)	Conjugated diene formation without aq. Bark (µM/ml)	Conjugated diene formation with aq. Bark (µM/ml)
0	444.64	444.64
10	463.73	479.69
20	479.34	481.51
40	497.45	489.69
60	511.90	492.66
80	523.35	503.07
100	537.11	519.65
120	546.31	533.65
140	557.30	538.50
160	561.60	541.55
180	569.30	550.80
200	581.22	578.57
Fold/%	+23.49% or +1.30 fold	+ 23.15% +1.30 fold

As shown in table 10(b), In absence of Aq. bark, 1.30 fold (+23.49%) increase in oxidative modification of LDL was observed from basal value (444.64µM/ml) to maximal value (581.22µM/ml) at 200 min, after adding 2.5 mM $CuSO_4$. But in presence of Aq. bark, this increase was slightly reduced to 0.45% in hyperlipidemic subjects.

Total free radical scavenging (FRAP) property at different time intervals of plasma isolated from normal and diabetic subjects

Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. Absorbance changes are linear over a wide concentration range and time with antioxidant mixtures, including plasma, and with solutions containing one antioxidant in purified form. The FRAP assay offers a putative index of antioxidant, or reducing, potential of biological fluids.

Table 13: Total free radical scavenging property at different time intervals of plasma isolated from normal and diabetic subjects.

Time in min.	Total antioxidant power (µM/ml) of plasma (Normal subjects)	Increase in fold	Total antioxidant power(µM/ml) of plasma (Diabetic subjects)	Increase in fold
0'	276.88	1.48 fold increase	186.88	0.76 fold decrease
1'	437.25		174.9 0	
2'	444.24		168.86	
3'	449.90		164.13	
4'	556.88		154.15	
5'	410.98		143.55	

As shown in table13. With increase in time interval (from 0' – 6'), total antioxidant in normal subjects, increased from 276.88 µM/ml to 431.003 µM/ml (0.35 fold increase). While in case of diabetic subjects, it decreased from186.88 µM/ml to 143.55µM/ml (0.76fold decrease). At 0 min. plasma of normal subject showed 32.62% more total antioxidant power than the plasma of diabetic subjects and at 200 minutes total antioxidant power increased by 66.90% than diabetic subjects.

Table 14: Total free radical scavenging property at different time intervals of plasma isolated from normal and diabetic subjects in presence and absence of ethanolic Neem (leaves).

Time in min.	Total antioxidant power (µM/ml) of plasma (Normal subjects)	Increase in fold	Total antioxidant power(µM/ml) of plasma (Diabetic subjects)	Increase in fold
0'	513.08	2.10 fold increase	358.46	1.56 fold increase
1'	747.29		447.30	
2'	867.28		488.45	
3'	956.76		516.77	
4'	1031.02		538.98	
5'	1078.38		562.03	

As shown in table14. With increase in time interval (from 0' – 6') in the presence of Eth. Neem (leaves), total antioxidant in normal subjects, increased from 513.08µM/ml to 1078.38µM/ml (2.10 fold increase). While in case of diabetic subjects, it increased from358.46 µM/ml to 562.03 µM/ml (1.56fold increase). At 0 min. plasma of normal subject showed 29.78% more total antioxidant power than the plasma of diabetic subjects and at 200 minutes total antioxidant power increased by 47.48% than diabetic subjects.

Table 15: Total free radical scavenging property at different time intervals of plasma isolated from normal and diabetic subjects in presence of Aqueous Neem (leaves).

Time in min.	Total antioxidant power (µM/ml) of plasma (Normal subjects)	Increase in fold	Total antioxidant power(µM/ml) of plasma (Diabetic subjects)	Increase in fold
0'	231.47	2.30fold increase	274.25	2.33 fold increase
1'	333.72		417.77	
2'	408.04		497.03	
3'	447.14		558.51	
4'	489.30		599.82	
5'	534.03		639.77	

As shown in table15. With increase in time interval (from 0' – 6') in the presence of Aq. Neem (leaves), total antioxidant in normal subjects, increased from 231.47µM/ml to 534.03 µM/ml(2.30 fold increase). While in case of diabetic subjects, it increased from 274.25 µM/ml to 639.77 µM/ml(2.33fold increase). At 0 min., plasma of normal subject showed 56.65% more total antioxidant power than the plasma of diabetic subjects and at 200 minutes total antioxidant power increased by 57.13% than diabetic subjects.

Table 16: Total free radical scavenging property at different time intervals of plasma isolated from normal and diabetic subjects in presence of Aqueous Niboli.

Time in min.	Total antioxidant power (µM/ml) of plasma (Normal subjects)	Increase in fold	Total antioxidant power (µM/ml) of plasma (Diabetic subjects)	Increase in fold
0'	455.67	1.83 fold increase	366.42	1.31 fold increase
1'	613.56		389.98	
2'	696.50		421.90	
3'	748.08		449.40	
4'	789.08		467.82	
5'	838.08		483.61	

As shown in table16. With increase in time interval (from 0' – 6') in the presence of Aq. Niboli, total antioxidant in normal subjects, increased from 455.67µM/ml to 838.08 µM/ml(1.83 fold increase). While in case of diabetic subjects, it increased from 366.42µM/ml to 483.61µM/ml (1.31fold increase). At 0 min., plasma of normal subject showed 45.63% more total antioxidant power than the plasma of diabetic subjects and at 200 minutes total antioxidant power increased by 24.23% than diabetic subjects.

Table 17: Total free radical scavenging property at different time intervals of plasma isolated from normal and diabetic subjects in presence of ethanolic Bark.

Time in min.	Total antioxidant power (µM/ml) of plasma (Normal subjects)	Increase in fold	Total antioxidant power(µM/ml) of plasma (Diabetic subjects)	Increase in fold
0’	288.30	2.06 fold increase	275.60	2.39 fold increase
1’	408.14		438.40	
2’	468.40		511.56	
3’	517.70		571.56	
4’	557.30		621.93	
5’	595.14		659.98	

As shown in table17. With increase in time interval (from 0’ – 6’) in the presence of Eth. Bark total antioxidant in normal subjects, increased from 288.30µM/ml to 595.14µM/ml(2.06 fold increase). While in case of diabetic subjects, it increased from 275.60 µM/ml to 659.98µM/ml (2.39fold increase). At 0 min. plasma of normal subject showed 58.24% more total antioxidant power than the plasma of diabetic subjects but at 200 minutes total antioxidant power of diabetic subjects increased by 10.12% than normal subjects.

Table 18: Total free radical scavenging property at different time intervals of plasma isolated from normal and diabetic subjects in presence of aqueous bark.

Time in min.	Total antioxidant power (µM/ml) of plasma (Normal subjects)	Increase in fold	Total antioxidant power(µM/ml) of plasma (Diabetic subjects)	Increase in fold
0’	156.78	3.00 fold increase	216.78	2.44 fold increase
1’	167.84		377.72	
2’	337.98		428.46	
3’	394.67		459.93	
4’	436.77		497.68	
5’	471.51		529.35	

As shown in table18. With increase in time interval (from 0’ – 6’) in the presence of aq. Bark, total antioxidant in normal subjects, increased from 156.78µM/ml to 471.51 µM/ml(3.00 fold increase). While in case of diabetic subjects, it increased from 216.78µM/ml to 529.35µM/ml (2.44fold increase). At 0 min. plasma of normal subject showed 66.75% total antioxidant power than the plasma of diabetic subjects showed 59.04 and at 200 minutes total antioxidant power reduced by 10.77% than diabetic subjects in the presence of aq. Bark.

Table 19: Total free radical scavenging property at different time intervals of LDL isolated from normal and diabetic subjects.

Time in min.	Total antioxidant power (µM/ml) of LDL (Normal subjects)	Increase in fold	Total antioxidant power(µM/ml) of LDL(Diabetic subjects)	Increase in fold
0’	400.56	1.51 fold increase	295.25	1.23 fold increase
1’	488.98		331.51	
2’	524.14		342.04	
3’	551.61		346.70	
4’	573.56		359.64	
5’	607.82		364.40	

As shown in table19. with increase in time interval (from 0’ – 6’) in the absence of any extract, total antioxidant in LDL of normal subjects, increased from 400.56µM/ml to 607.82 µM/ml(1.51fold increase). While in case of diabetic subjects, it increased from 295.25µM/ml to 364.40 µM/ml (1.23fold increase).

At 0 min., plasma of normal subject showed 34.10% more total antioxidant power than the plasma of diabetic subjects and at 200 minutes, total antioxidant power in

normal subjects increased by 40.77% than diabetic subjects.

Table 20: Total free radical scavenging property at different time intervals of LDL isolated from normal and diabetic subjects in the presence of Ethanolic Neem (leaves).

Time in min.	Total antioxidant power (µM/ml) of LDL (Normal subjects)	Increase in fold	Total antioxidant power(µM/ml) of LDL (Diabetic subjects)	Increase in fold
0'	478.90	1.14 fold increase	497.30	1.17 fold increase
1'	506.30		534.72	
2'	518.93		553.14	
3'	531.56		566.20	
4'	541.03		576.80	
5'	547.35		590.50	

As shown in table20. With increase in time interval (from 0' – 6') in the presence of Eth. Neem (leaves) total antioxidant in LDL of normal subjects, increased from 478.90µM/ml to 547.35µM/ml (1.14fold increase). While in case of diabetic subjects, it increased from 497.30 µM/ml to 590.50µM/ml (1.17fold increase).

At 0 min, plasma of diabetic subject showed 3.69% more total antioxidant power than the plasma of normal subjects and at 200 minutes, total antioxidant power in normal subjects decreased by 40.77% than diabetic subjects.

Table 21: Total free radical scavenging property at different time intervals of LDL isolated from normal and diabetic subjects in presence of Aqueous Neem (leaves).

Time in min.	Total antioxidant power (µM/ml) of LDL (Normal subjects) (µm/ml/min)	Increase in fold	Total antioxidant power(µM/ml) of LDL (Diabetic subjects)	Increase in fold
0'	548.40	1.19 fold increase	621.03	1.21 fold increase
1'	596.77		678.50	
2'	621.83		719.92	
3'	637.70		732.66	
4'	646.34		745.18	
5'	655.87		753.66	

As shown in table21. With increase in time interval (from 0' – 6') in the presence of aq. Neem (leaves) total antioxidant in LDL of normal subjects, increased from 548.40µM/ml to 655.87 µM/ml(1.19fold increase). While in case of diabetic subjects, it increased from 621.03 µM/ml to 753.66 µM/ml (1.21fold increase).

At 0 min. plasma of diabetic subject showed 11.69% more total antioxidant power than the plasma of normal subjects and at 200 minutes, total antioxidant power in normal subjects reduced by 12.71% than diabetic subjects.

Table 22: Total free radical scavenging property at different time intervals of LDL isolated from normal and diabetic subjects in presence of Aqueous Niboli.

Time in min.	Total antioxidant power (µM/ml) of LDL (Normal subjects)	Increase in fold	Total antioxidant power(µM/ml) of LDL (Diabetic subjects)	Increase in fold
0'	466.70	1.19 fold increase	474.67	1.16 fold increase
1'	507.30		485.19	
2'	514.14		495.72	
3'	536.77		503.56	
4'	546.24		537.70	
5'	555.72		551.50	

As shown in table22, with increase in time interval (from 0' – 6'), in the presence of aq. niboli, total antioxidant power in LDL of normal subjects, increased from 466.70µM/ml to 555.72 µM/ml(1.19fold increase). While in case of diabetic subjects, it increased from 474.67µM/ml to 551.50 µM/ml(1.16 fold increase).

At 0 min. plasma of normal subject showed1.68 % less total antioxidant power than the plasma of diabetic subjects, but at 200 minutes, total antioxidant power in

normal subjects slightly increased by 0.76% than diabetic subjects.

It has been graphically represented in fig25.

Table 23: Total free radical scavenging property at different time intervals of LDL isolated from normal and diabetic subjects in presence of Aqueous Bark.

Time in min.	Total antioxidant power (µM/ml) of LDL (Normal subjects)	Increase in fold	Total antioxidant power(µM/ml) of LDL (Diabetic subjects)	Increase in fold
0'	415.30		483.72	
1'	417.35		503.56	
2'	428.46	1.10 fold increase	515.66	1.11 fold increase
3'	445.19		497.82	
4'	453.09		529.45	
5'	459.35		537.77	

As shown in table23. With increase in time interval (from 0' – 6') in the presence of Aq. bark, total antioxidant in LDL of normal subjects, increased from 415.30µM/ml to 459.35 µM/ml(1.10 fold increase). While in case of diabetic subjects, it increased from 483.72µM/ml to 537.77 µM/ml (1.11fold increase).

Total antioxidant power of plasma of normal subject at 0', reduced by 14.11% than the plasma of diabetic subjects also at 200 minutes, total antioxidant power in normal subjects reduced by 14.63% than diabetic subjects.

Table 24: Total free radical scavenging property at different time intervals of LDL isolated from normal and diabetic subjects in presence of ethanolic Bark.

Time in min.	Total antioxidant power (µM/ml) of LDL (Normal subjects)	Increase in fold	Total antioxidant power(µM/ml) of LDL (Diabetic subjects)	Increase in fold
0'	476.80		337.93	
1'	548.35		270.90	
2'	592.03	1.37 fold increase	263.09	0.76 fold decrease
3'	619.40		267.04	
4'	638.34		259.46	
5'	657.24		259.46	

As shown in table24. With increase in time interval (from 0' – 6') in the presence of Eth. Bark, total antioxidant in LDL of normal subjects, increased from 476.80µM/ml to 657.24 µM/ml (1.37fold increase). While in case of diabetic subjects, it increased from 337.93 µM/ml to 259.46 µM/ml (0.76fold increase).

At 0 min. plasma total antioxidant power of diabetic subject was much less than the plasma of normal subjects (+29.06%) also, total antioxidant power at 200 minutes, in normal subjects increased drastically by 60.40% than diabetic subjects.

Malondialdehyde formation in plasma of normal and diabetic patient's presence or absence of drugs

When peroxidized material is heated with thiobarbituric acid (TBA), a colored complex formed between malondialdehyde (MDA) and TBA. Even when little MDA is present, large amounts of MDA-TBA adduct can be formed. This is because lipid peroxides break down to release MDA during the test conditions. Iron is not essential for the breakdown of the peroxides but is essential for the formation of TBA reactivity. This can be related to the ability of iron to decompose lipid peroxides with the release of peroxy radicals, which are precursors of MDA which are measured spectrophotometrically.

Table 25: Formation of malondialdehyde in plasma of normal and diabetic patient's presence or absence of drugs.

In vitro treatment	Baseline MDA Formation in normal patients at 535 nm (nM/mg/ml)	Baseline MDA Formation in diabetic patients at 535 nm (nM/mg/ml)
Without drug	266.87 ± 0.552	342.26 ± 0.611
Ethanolic Neem(leaves)	258.93 ± 0.474	297.62 ± 0.572
Aqueous Neem(leaves)	260.92 ± 0.352	312.51 ± 0.604
Ethanolic Bark	270.84 ± 0.550	328.96 ± 0.715

Aqueous Bark	262.09 ± 0.430	279.96 ± 0.403
Aqueous Niboli	232.15 ± 0.291	334.43 ± 0.179

*All the values are mean of triplicate readings ± S.D pooled from plasma of normal and diabetic subjects.

Baseline MDA formation at 535 nm, in normal and diabetic subjects without extracts were 265.87 ± 0.552 nM/mg/ml and 341.26 ± 0.611 nM/mg/ml respectively. After addition of extracts, baseline MDA formation in normal subjects was significantly reduced. Reduction in baseline value MDA after addition of E. neem (leaves), Aq. neem (leaves), E. bark, Aq. bark, in normal LDL were 43.64%, 29.75, 11.9%, 61.5%, 7.93%, 2.06%, 53.57% respectively, and reduction in baseline value after addition of E. neem (leaves), Aq. neem (leaves), E. bark, Aq. bark, in normal and diabetic respectively. This is graphically represented in fig.28.

Table 26: Formation of malondialdehyde in LDL of normal and diabetic patient's presence or absence of drugs in the presence or absence of drugs.

In vitro treatment in LDL	Baseline MDA Formation in normal patients at 535 nm (nm/mg/ml LDL suspension)	Baseline MDA Formation in diabetic patients at 535 nm (nm/mg/ml LDL suspension)
LDL alone	188.49 ± 0.066	271.02 ± 0.059
LDL with glucose	295.63 ± 0.520	553.57 ± 0.326
Ethanolic Neem (leaves)	216.26 ± 0.472	234.12 ± 0.433
Aqueous Neem (leaves)	261.90 ± 0.326	248.01 ± 0.514
Ethanolic Bark	248.01 ± 0.441	257.93 ± 0.613
Aqueous Bark	206.35 ± 0.207	242.06 ± 0.341
Aqueous Niboli	250.00 ± 0.691	257.93 ± 0.702

*All the values are mean of triplicate readings ± S.D pooled from plasma of normal and diabetic subjects.

In normal and diabetic subjects, baseline MDA formation at 535 nm without extracts were188.49 ± 0.066nM/mg/ml and 271.02 ± 0.059nM/mg/ml respectively. After addition of extracts, baseline MDA formation in normal subjects was significantly reduced. Reduction in baseline value MDA after addition of Eth. Neem (leaves), Aq. Neem (leaves), Aq. Niboli, Aq. Bark and Eth. Bark in normal LDL were 26.84%, 11.41%, 16.07%, 30.2%, 15.4%, 40.27%, 35.57% respectively, and reduction in baseline value after addition Eth. Neem (leaves), Aq. Neem (leaves), Aq. Niboli, Aq. Bark and Eth. Bark in normal and diabetic 57.7%, 55.19%, 53.40%, 56.27%, 53.40%, 64.87% and 60.57% respectively.

This is graphically represented in fig.29.

DISCUSSIONS

Atherosclerosis and coronary heart disease have been considered as major health problem worldwide. The clinical presentations of atherosclerosis mainly involve the coronary and carotid arteries, which remain the leading causes of morbidity and mortality in both men and women of all racial groups with Coronary Heart Disease (CHD) the leading cause of death worldwide. Excessive dietary lipids and cholesterol are the major factor of relevance for the development of hypertriglyceridemia and hypercholesterolemia, two important cardiovascular risk factors. Abnormalities in lipid profiles, folate metabolism and other traditional risk factors (e.g., diabetes mellitus and hypertension) play a rather peripheral role and serve to amplify the atherosclerotic process initiated by persistence of infection and inflammation. Infection and inflammation are accompanied by cytokine induced alterations in lipid and lipoprotein metabolism. Of note, inflammatory cytokines are increased and play a pathogenic role in a variety of very common disorders, such as diabetes, obesity, metabolic syndrome, hypertension, chronic heart failure, chronic renal failure, and atherosclerosis.

In my base line study of various physiochemical parameters, the average values of physical parameters such as body weight, age, height, number of male and female in normal subjects (n=45) were 59.83±8.40kg, 32± 1.86years, 26 and 19 respectively, and of diabetic subjects (n=60) were 64± 9.41kg, 58± 3.56, 29 and 31 respectively.

Statistical evaluation of the lipid profile among the normal and diabetic patients was compared. The normal range for total cholesterol should be 150-200 mg dl^{-1}. In the present study, the results showed that the lipid and the lipoprotein profiles of the diabetics were higher than that of the controls and they were comparable with the findings of Idogun et al., (2007) and Albrki et al., (2007).

The average values of TC, LDL-C, total protein in plasma and LDL, HDL-C, HDL_2-C ,HDL_3-C ,HDL-C protien, HDL_2C protein,HDL_3 C protein, Non HDL-C in normal lipidemic subjects were 42.80 ± 2.39 µg/ml, 14.9 ± 2.09 µg/ml, 1.212 ± 0.066 µg/ml, 1.179 ± 0.348µg/ml, 5.36 ± 0.46 µg/ml, 1.186 ± 0.34 µg/ml , 3.172 ± 0.53 µg/ml ,0.301 ± 0.111µg/ml,0.164 ± 0.00014µg/ml,0.601 ± 0.018µg/ml and 37.44 ± 1.93µg/ml respectively.

The average values of TC, LDL-C, total protein in plasma and LDL, HDL-C, HDL_2-C ,HDL_3-C ,HDL

protien, HDL_2C protein, HDL_3C protein, Non HDL-C in hyperipidemic subjects were 114.66 ± 2.24µg/ml, 10.89 ± 0.53µg/ml,1.190 ± 0.015µg/ml, 1.046 ± 0.28µg/ml, 7.025 ± 0.63µg/ml, 1.514 ± 0.51µg/ml, 4.33 ± 0.247 µg/ml, 0.332 ± 0.0016 µg/ml, 0.183 ± 0.036 µg/ml , 0.304 ± 0.0007 µg/ml, 107.63 ± 1.61 µg/ml respectively. The average ratio Value of TC/LDL –C ,LDL –C /TC , HDL_2 – C /HDL_3 –C and HDL_3 –C /HDL_2 – C in normallipidemic subjects were 1.15, 0.866, 0.233 ,3.66 and of dyslipidemic subjects were 1.055,0.950,0.602,1.66 respectively.

Dyslipidemia was observed in the diabetic population, but that HDL-C was not significantly decreased. Lipid disorders are very common in both insulin dependent and non-insulin dependent diabetic mellitus (Prabodh et al., 2012).

The chronic hyperglycemia, insulin resistance and abnormal lipoprotein profiles found in diabetes may contribute to a decrease of bioavability of vascular nitric oxide (NO), impairing endothelium- dependent vasodilatation documented in and in humans with diabetes (Listy, 2000). NO possesses a variety of antiatherogenic properties, and loss of these protective mechanisms may lead to an increase in susceptibility to vascular disease. In vitro cell mediated oxidative processes usually require the presence of transition metal ions to oxidize the LDL (Daugherty and Roselar., 1995). Oxidized Oxidized LDL may contribute to the progression of atherosclerosis by enhancing endothelial injury by inducing foam cell generation and smooth muscle proliferation, it also initiate endothelial inflammation leading to atherosclerosis and CVD. Modifications take place either in plasma or in the inner layer of the artery (Khan et al., 2012).

Vascular plants are able to synthesize organic molecules/phytochemical, referred to as "secondary metabolites". These molecules are involved in a variety of roles in the life span of plants, ranging from structural ones to protection. Phenolic compounds are regarded as one such group that is synthesized by plants during development and in response to conditions such as infection, wounding, UV radiation, etc. Phenolic acids are aromatic secondary plant metabolites widely distributed throughout the plant kingdom (Stalikas, 2007). Percentage yield of phenol in ethanolic Neem (leaves), Aq. Neem (leaves), Aq. Niboli, Aq. Bark and ethanolic Bark were 0.99%, 0.70%, 0.86%, 0.65%, 1.38% respectively.

In my present investigation it was found that in absence of glucose, 1.32 fold (+25.06%) increase in oxidative modification of LDL was observed from basal value (284.14µM/ml) to maximal value (376.19µM/ml) at 120min after adding 2.5mM $CuSO_4$. But in case of LDL+ Glucose, this was increased was of 40.17 %(284.14 µM/ml- 476.59 µM/ml) which suggests that evaluated glucose level promote LDL oxidation in normal plasma.

In vitro treatment was given to both normallipidemic and dyslipidemic subjects with natural herbal Aq. and Ethanolic extracts of Neem (leaves), Niboli, and Bark and significant decrease in LDL oxidation was observed. In case of ethanolic Neem (leaves) LDL oxidation was reduced by 22.85% in normallipidemic subjects and dyslipidemic subjects by 28.74. When in vitro treatment was given with Aq. Neem (leaves). LDL oxidation was decreased by 4% in normallipidemic subjects and in dyslipidemic subjects LDL oxidation was reduced greatly by 32.4%.

Aq. niboli reduced oxidation of LDL by 18.92% and 4.64% in normal and diabetic subjects respectively. Aq. Bark reduced LDL oxidation by 18.84% in normallipidemic subjects by and 9.54% in dyslipidemic subjects whereas ethanolic bark reduced it only by 2.28% in normallipidemic and in dyslipidemic it was reduced by 2.53% only.

FRAP is a novel method for assessing "antioxidant power" in which Ferric ion is reduced to ferrous ion at low pH and lead to formation of colored ferrous tripyridyltriazine complex (Benzien and Strain .,1996).

Total antioxidant power of normal plasma, without any extract increased from 277.88 µM/ml to 431.003 µM/ml(0.35 fold increase) with time (from 0' to 5') whereas in diabetic patients it decreased from 185.78 µM/ml to 142.62 µM/ml (0.23fold decrease) with time (from 0' to 5').

CONCLUSION

Diabetes mellitus is known to be a state of excess generation of free radicals contributed by several mechanisms including hyperglycemia and impaired natural antioxidant status that causes oxidative stress which may constitute the key and common event in the pathogenesis of secondary diabetic complications oxidative stress as a "disturbance in the pro-oxidant/antioxidant balance. Oxidative stress exceeding the antioxidant capacity level may induce oxidative damage; it is responsible for atherosclerosis, cardiovascular diseases, pulmonary abnormality, retinopathy, kidney failure and neurodegenerative disorders.

In conclusion, based on our study of free radical scavenging and antioxidant properties of ethanolic Neem (leaves), Aq. Neem (leaves), Aq. Niboli, Aq. Bark and ethanolic Bark on copper mediated oxidative modification of LDL in normallipidemic subjects, administration of ethanolic Neem (leaves), Aq. Neem(leaves), Aq. Niboli, Aq. Bark and ethanolic Bark may be useful in the prevention and treatment of dyslipidemia/hyperlipidemia, atherosclerosis and other oxidative stress mediated diseases. In addition, use of these herbal plant extracts will be efficacious, cost effective and involves no risk of side-effects.

REFRENCES

1. ADA (American Diabetes Association). Diagnosis and classification of *diabetes mellitus*. *Diabetes Care.*, 2005; 28(1): S37–S43.
2. Agarwal, A. Gupta, S. Sharma, R.K. Role of oxidative stress in female reproduction. *Reprod Biol Endocrinol*, 2005; 14: 3-28.
3. Ahmad, M. Sher, H. 2004. Medicinally important wild plants of Chitral, medicinally important wild plants in view of ethnobotanical study of district Chitral.
4. Aikens, J. & Dix, T.A. Perhydroxyl radical (HOO*) Initiated lipid-peroxidation – The role of fatty-acid hydroperoxides. *J. Biol. Chem*., 1991; 266: 15091-15098.
5. Alam *et al*., Potential hypoglycemic effect of *swertia chirata* and indian subcontinental herb with important medicinal value. *Pharmacologyonline*. 2011; 2: 642-647.
6. Alam, K.D., M.S. Ali, S. Mahjabeen, S. Parvin, M.A. Akbar and R. Ahamed: Analgesic activities of ethanol extract of leaf, stem and their different fractions of *Swertia chirata*. *Pak. J. Pharm.* Sci., 2010; 23: 455-457.
7. Allred, E. N., Bleecker, E. R., Chaitman, B. R. Short-term effects of carbon monoxide exposure on the exercise performance of subjects with coronary artery disease. *N Engl J Med*., 1989; 321: 1426-1432.
8. Al-Mamari, A. *Oman Medical Journal*, 2009; 24: 173-178.
9. Altan, V.M. The pharmacology of diabetic complications. *Current Medicinal Chemistry*, 2003; *1*, 10: 1317–1327.
10. Andersen, H. R., Nielsen, J. B., Nielsen, F. and Grandjean, P. Antioxidative enzyme activities in human erythrocytes. *Clin Chem*., 1997; 43: 562-8.
11. Ankara, Ecz. Fak. Derg ., An overview of ascorbic acid biochemistry, 2009; 38(3): 233–255.
12. Ashok BT, Ali R. The aging paradox: Free radical theory of aging. Exp Gerontol, 1999; 34: 293–303.
13. Atta-ur-Rahman, Choudhary, M.I. and Thomsen, J.W. Manual of bioassay techniques for natural product research. Harward Academic Press, Amsterdam, 1999; 82-84.
14. Bailey CJ, Day C: Traditional plant medicines as treatments for diabetes. *Diabetes Care*, 1989; 12: 553–564.
15. Bautista, A.P. Free radicals, chemokines, and cell injury in HIV-1 and SIV infections and alcoholic hepatitis. *Free Radic Biol Med.*, Dec. 15, 2001; 31(12): 1527-32.
16. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes, 1991; 40: 405–412.
17. Beg, Z. H., Stonik, J. A. and Brewer, H. B., Jr. Characterization and regulation of reductase kinase, a protein kinase that modulates the enzymic activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase Proc. Natl. Acad. Sci., USA., 1979; 76: 4375-4379.
18. Behl, C., Davis, J.B., Lesley, R., Schubert, D. Hydrogen peroxide mediates amyloid ß protein activity. Cell., 1994; **77**: 817-27.
19. Benoit J. Arsenault, *et.al.*, Lipitor. *J Am Coll Cardio*l. 2011; 57(1): 63-69.
20. Benzie, I., F. F. and Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochem*, 1996; **239**: 70-76.
21. Bhattarai, K.R. & Shrestha. *Identification, trade & economic significance of Chiraito of Nepal.* A paper presented at workshop of the Utilization of NTFPs for Environment Conservation & Economic Development in Nepal.Chopra, R. N., Chopra, I. C, Handa, K. L. and Kapur, L. D. (eds), *Indigenous Drugs of India*, U.N. Dhur and Sons, Kolkata, 1997; 1958: 51–595.
22. Esterbauer, H., Gebicki, J., Puhl, H. and Jugens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radic. Bio. Med*., 1992; **13**: 341-390.
23. Esterbauer, H., Striegel, G., Puhl, H., Oberreither, S., Rotheneder, M., El Saadani, M. and Jurgens, G. The role of vitamin E and carotenoids in preventing oxidation of low-density lipoproteins. *Ann. N. Y. Acad. Sci*., 1989; **570**: 254-267.
24. Fasano, M., Bergamasco, B., Lopiano, L., Modifications of the ironneuromelanin in Parkinson"s disease. *J Neurochem*, 2006; **96**: 909-16.
25. Friedwald, W. T., Levy, R. I., Fredrickson, D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of preparative ultracentrifugation. *Clin. Chem*., 1972; **18**: 499-502.
26. Gofman, J. W., deLalla, O. and Glazier, F., Freeman, N. K., Lindgren, F. T., Nichols, A. V., Strisower, E. H., Tamplin, A. R. The serum lipoprotein transport system in healthy persons metabolic disorders, atherosclerosis, and coronary artery disease. Plasma., 1954; **2**: 413-416.
27. Gutteridge JM, Quinlan GJ. *J Appl Biochem.* Aug-Oct., 1983; **5**(4-5): 293-9.
28. Halliwell, B., Free Radicals, Antioxidants, and Human Disease: Curiosity, Cause, or Consequence. *Lancet*, 1994; 344: 721-724.
29. Hara, A., Chater, O. & Williams, L.H.J. An Enumeration of flowering plants of Nepal., 1982; **3**: 96: 97.
30. Hatch, G.E, Asthma, Inhaled Oxidants, and Dietary Antioxidants. *Am J Clin Nutr*., 1995; **61**(3): 625S-630S.
31. Hodgkinson, A. D., Bartlett, T., Oates, P. J., Millward, B. A., and Demaine, A. G. The response of antioxidant genes to hyperglycemia is abnormal in patients with type 1diabetes and diabetic nephropathy. *Diabetes*, 2003; **52:** 846–851.

32. Hogg, J. S., Lohmann, D. H., & Russell, K. E. Can.*J. Chem*., 1961; **39:** 1588-1594.
33. Huuskonen J, Olkkonen VM, Jauhiainen M, Ehnholm, C., The impact of phospholipid transfer protein (PLTP) on HDL metabolism. Atherosclerosis, 155: 269-81. *J. Chem.*, 2001; **39:** 1588-1594.
34. Jacob, R.A the Integrated Antioxidant System. *Nutr Res.*, 1995; 15(5): 755-766.
35. Joshi, P. and Dhawan. V., *Swertia chirayita* – an overview. *Current Science*, August 2005; **89**(4): 25.
36. Kagen, V.E., et al., Dihydrolipoic Acid–a Universal Antioxidant Both in the Membrane and in the Aqueous Phase. *Biochem Pharmacol*, 1992; 44: 1637-1649.
37. Kalaivalan, K.N, et.al., Lipid peroxidation in type 2 diabetes mellitus. *Int J diab Dev*., 2006; **26**(1).
38. Kehrer, J.P. and Smith, C.V. Free Radicals in Biology: Sources, Reactivities, and Roles in the Etiology of Human Diseases. Ch., 1994; 2: 25-62, In: Natural Antioxidants in Human Health and Disease. ed. Frei, B. Academic Press: San Diego.
39. Khan, A., Ishaq, F., Chandel, S.A. and Chhettri, S. Effect of dietry tocotrienolon Antioxidant status and LDL oxidation in rats exposed to cigarette smoke. *Asian Journal of clinical Nutrition*., 2012; 1-12.
40. Leitersdorf E. Selective cholesterol absorption inhibition: A novel strategy in lipid-lowering management. Int J Clin Pract., 2002; **56**(2): 116 – 119.
41. Lipman TH, Hayman LL, Fabian CV, DiFazio DA, Hale PM, Goldsmith BM. Risk factors for cardiovascular disease in children with type I diabetes. *Nurs Res*., 2000; **49**(3): 160-166.
42. Listy Bratisl Lek. The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular disease, 2000; **101**(10): 541.551.
43. Liu T, Stern A, Roberts LJ. The isoprostanes: Novel prostanglandin-like products of the free radical catalyzed peroxidation of arachidonic acid. J Biomed Sci., 1999; 6: 226–35.
44. M. Jung, M. Park, H-Ch. Lee, Y. Kang, E.S.Kang, S.K. Kim. Antidiabetic agents from medicinal plants, *Curr. Med. Chem.*, 2006; **13:** 1203–1218.
45. M.A Ghaffari and T. Ghiasvand. Kinetic study of low density lipoprotein oxidation by copper. *Indian Journal of Clinical Biochemistry*, 2010; **25**(1): 29-36.
46. Marshall, WJ. Lipids and Lipoproteins. *Clinical Chemistry. London*: *Mosby*, 1995; 213-228.
47. Martin A, Frei B. Both intracellular and extracellular vitamin C inhibit atherogenic modification of LDL by human vascular endothelial cells. *Arterioscler Thromb Vasc*, 1997.
48. National institute of diabetes and digestive and kidney diseases. 2 Diabetic Neuropathies: The Nerve Damage of Diabetes. *NIH Publication*, February 2009; 09–3185.
49. Neuzil J, Thomas SR, Stocker R. Requirement for promotion, or inhibition of α- tocopherol of radical induced initiation of plasma lipoprotein lipid peroxidation. Free Radic Biol Med., 1997; 22: 57–71.
50. Osganian SK, Stampfer MJ, Rimm E, et al. *J Am Coll Cardiol.*, 2003; **42**(2): 246-252. "Vitamin C and risk of coronary heart disease in women".
51. *Other Purposes*, VCH, Weinheim, Germany, 1995; 1–696.
52. Packer, L. and Witt, E.H. Antioxidant Properties and Clinical Implications of Alpha-Lipoic Acid. *Biothionls in Health and Disease. New York*: Marcel Dekker, Inc., 1995; 479-516.
53. Pant N, Jain DC, Bhakuni RS. Some chemical constituents of *Swertia chirata*. *Indian J Chem.*, 2002; **41B**: 1980-1986.
54. Patsch, W., Brown, S. A., Morrisett, J. D., Gotto, Jr., A. M. and Patsch, J. R. A dual-precipitation method evaluated for measurement of cholesterol in high-density lipoprotein subfractions HDL2 and HDL3 in human plasma. *Clin. Chem.*, 1989; **35**: 265-270.
55. Prior, R.L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G., & Mainland, C.M. *J. Agric. Food Chem*., 1998; **46**: 2686-2693.
56. Purohit, K; Vijay, Bahuguna; Tiwari, Deepti; Tiwari, Alka; Andola C. Haris, Negi S.Jagmohan; Chauhan S. Rajendra, 2013.
57. Roxb. ex Fleming Karsten on the verge of extinction in the Himalayan region. *Current Science*, **104**: 2.
58. Sargeant LA, Wareham NJ, Bengham. S. Vitamin C and hyperglycemia in the European prospective investigation into cancer. *Diabetes care*., 2000; **23**: 726-732.
59. Sastre J, Pellardo FV, Vina J. Glutathione, oxidative stress and aging. Age., 1996; 19: 129–39.
60. Schmutterer, H. (ed.), *The Neem Tree: Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes*, VCH, Weinheim, Germany, 1995; 1–696.
61. Shapiro K., Gong WC. Natural products used for diabetes. *J Am Pharm Assoc.;* **42**: 217–226.
62. Sies, H. and Stahl, W. Vitamins E and C, beta carotene, and other carotenoids as antioxidants. *Am J Clin Nutr.*, 1995; **62**: 1315S-21S.
63. Sies, H. *et al*., Antioxidant Function of Vitamins. *Ann NY Acad Sci*., 1992; **669**: 7-20.
64. Srikantaiah *et al*., 1977; Edwards *et al*., 1979.
65. Stalikas, D. Constantine. Extraction, separation, and detection methods, 2007.
66. Stanaway SERS, Gill DV. Protein glycosylation in diabetes mellitus: biochemical and clinical consideration. *Diabet Int.*, 2001; 11: 73-76.
67. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol.
68. Vinson, J.A., Hao, Y., Su, X., & Zubik, *J. Agri. Food Chem.*, 1998; **46**: 3630-3634.
69. Wadkar *et.al*., Anti-diabetic potential and indian medicinal plants. *Journal of Herbal Medicine and Toxicology*, 2008; **2**(1): 45-50.

70. Wang, H., Cao, G., & Prior, R.L. *J. Agric. Food Chem.*, 1996; **44**: 701-703.
71. Weeks BS *et al*. Absorption rates and free radical scavenging values of vitamin C-lipid metabolites in human lymphoblastic cells. *Med Sci Monit*, 2007; **13**(10): BR205-210.
72. Wieland, H. and Seidel, D. A simple method for precipitation of low-density lipoproteins. *Journal of Lipid Research*, 1989; **24**: 904-909.
73. Witztum JL, Steinberg D. The oxidative modification hypothesis of atherosclerosis: does it hold for humans? *Trends Cardiovasc Med.*, 2001; **11**: 93-102.
74. World Health Organization. *Traditional medicine*. WHO, Geneva, 2008; 134.
75. Wright JV, Suen RM, Kirk FR. Comparative studies of "Ester C" versus L-ascorbic acid. *International Clinical Nutrition Review*, 1990; **10**: 267–70.
76. Ya BQ. *et al.* "Protective effect of swerchirin on hematopioesis in 60 Co-irrdiated mice" *Phytomedicine*, 1999; 6: 85-8.
77. Yokoyama, M., Seo T., Park, T; Yagyu H., Y. Hu; Son N.H; Augustus, A.S ; R. Vikramadithyan, RK., R. Ramakrishnan., Pulawa LK; Eckel RH., and Goldberg IJ; Effects of lipoprotein lipase and statins on cholesterol uptake into heart and skeletal muscle. *J. Lipid Res*., 2007; **48**: 646–655.
78. Zhang, Z., Apse, K., Pang, J., and Stanton, R. C. High glucose inhibits glucose-6-phosphate dehydrogenase via cAMP in aortic endothelial cells. *J. Biol. Chem.*, 2000; **275:** 40042–40047.