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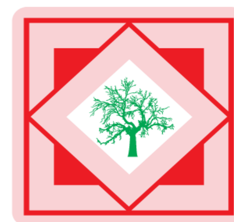

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Antioxidant and antiatherogenic impacts of *Catharanthus roseus* and *Hibiscus sabdariffa* on Cu^{++} mediated oxidation kinetics of low density lipoprotein

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ABSTRACT

The present study revealed that the ethanolic extracts of the leaves of Catharanthus roseus and the ethanolic extract of the flowers of Hibiscus sabdariffa exhibited satisfactory scavenging effect in all the radical scavenging assays in a concentration dependent manner. Oxidative damage to cholesterol component of the Low-Density Lipoprotein (LDL) leads to oxidized LDL by a series of consecutive events. Oxidised LDL acts as a trigger to initiate endothelial inflammation leading to Diabetes, atherosclerosis and vascular thrombosis (heart attack and stroke). Modified LDLs are produced during chemical modification that LDLs undergo after synthesis. Catharanthus roseus and Hibiscus sabdariffa inhibits the oxidative modification of LDL in normallipidemic subjects by 15.47% and 15.08% respectively. It has also been experimentally proved that these extracts are effective in lowering the formation of Malondialdehyde contents in LDL with plant extract, isolated from normallipidemic subjects. Though, these extracts do not show considerable effects in enhancing the catalytic activity of cellular enzymes such as Superoxide dismutase. In conclusion, based on our study of free radical scavenging and antioxidant properties of Catharanthus roseus and Hibiscus sabdariffa on copper mediated oxidative modification of LDL in normallipidemic subjects, administration of Catharanthus roseus and Hibiscus sabdariffa may be useful in the prevention and treatment of Diabetes, 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.

Key Words: *Catharanthus roseus, Hibiscus sabdariffa,* Antioxidant**,** oxidative modification of LDL

INTRODUCTION

One hundred years ago, the major threats to survival of our ancestors were caused by infectious agents. Today, the major threats to our survival are imposed upon us by synthetic chemicals, toxic heavy metals and the stress of modern living. Oxidative stress is fast becoming the nutritional and medical buzzword for the 21st century. Today, the prevailing throughout is “oxidative threat” or “oxidative stress” caused by free radicals. Cancer is the second most common cause of death after cardiovascular diseases (CVD) in most developed and in many developing countries, including India. In this country, every year around seven million new cases of cancer are being detected. Cancer is a major health problem worldwide, of all deaths, nine out of ten people die from cancer, heart attack, or stroke diseases caused by free radicals.

The body has the ability to produce endogenous antioxidants such as superoxide dismutase, catalase and glutathione peroxidase. Under normal circumstances, there is a balance between these endogenous antioxidants and the production of free radicals in the body. Compounds, which possess antioxidant properties, have the potential to decrease oxidative stress and thus may protect against smoking-induced pathology.

A free radical is defined as an atom or molecule that has one or more unpaired electron(s).These is formed during a variety of biochemical reactions and cellular functions. The steady state formation of pro-oxidants (free radicals) is normally balanced by a similar rate of consumption by antioxidants. Oxidative stress results from an imbalance between formation and neutralization of pro-oxidants. Excess production of free radicals and lower level of their scavengers in the body are one of the main causes of life threatening diseases such as Cancer, Cardiovascular diseases, Atherosclerosis etc. Various pathologic processes disrupt this balance by increasing the formation of free radicals in proportion to the available antioxidants (thus, oxidative stress) Example of increased free radical formation are immune cell activation, inflammation, ischemia, infection, cancer and so on. . Compounds that have been investigated are lipoic acid, taurine, ubiquinone, selenium, garlic, ginkgo biloba and polyphenols. Other compounds, which have been widely tried from their antioxidant properties, are vitamins such as alpha-tocopherol [1] and ascorbic acid. Free radical formation and the effect of this toxic molecule on cell function (which can result in cell death) are collectively called "oxidative stress". Oxidative stress is thought to contribute to the development of a wide range of disease including Alzheimer Parkinson, Rheumatic arthritis, heart attack, AIDS, stroke, Cancer, autoimmune disease etc [2]. Oxidative LDL exerts several biological effects that may contribute to the initiation and progression of the atherosclerotic process. The concentration, the size and the chemical modification of LDL are important for atherogenesis. Modified LDLs are produced during chemical modification that LDLs undergo after synthesis. Modifications take place in either plasma or in the inner layer of the artery and pertain to either the lipid or the protein fraction. Oxidative LDL exerts several biological effects that may contribute to the initiation and progression of the atherosclerotic process.

Oxidation takes place when naturally occurring antioxidant agents such as Vitamin E and β-carotenes that normally inhibit LDL oxidation do not occur. within the artery wall result in monocyte binding to endothelial cells, their entry into the vascular system, their differentiation into macrophages, and final conversion in to foamy cells. oxLDL also stimulate T-cells through the major histocompatability complex (MHC) and CD4+ helper T-cell receptor [3].

The term "antioxidant" has been defined by Gutteridge and Halliwell [2] as "any substance (synthetic and natural) that delays or inhibits oxidative damage to a largest molecule". Antioxidants are molecules which can safely interact with free radical and terminate the chain reaction before vital molecules are damaged. There are also molecules deserving the "antioxidant" team, because they act as chelating agents binding metal ions (redox activity). Antioxidant enzymes that catalyze free radical quenching reactions, and diet-derived antioxidants like ascorbic acid, Vitamin E, carotenoids, polyphenols and other low molecular weight compounds such as α-lipoic acid.

Catharanthus roseus is a medicinal plant in Ayurvedic medicine. Which belongs to family Apocynaceae which is commonly known as 'Madagascar periwinkle', vinca rosea or lachnera rosea in worldwide? The leaves are used traditionally in various regions of the world including India, West Indies as well as Nigeria to control diabetes [4]. It is an important source of indole alkaloids, which are present in all plant parts. Vinca is used for the treatment of diabetes, fever, malaria, throat infections, and chest complaints. It is also used for the regulation of menstrual cycles, and as a euphoriant. On the other hand, *Hibiscus sabdariffa* (Roselle) is one of the most common flower plants grown worldwide. There are more than 300 species of hibiscus around the world. Roselle is a member of family, Malvaceae. Roselle is an annual, erect, bushy, herbaceous shrub. Having smooth or nearly smooth, cylindrical, typically red stems. An imbalance between free radicals production and its scavengers in the body can lead to development of many numbers of diseases. Main cause of development of CVD and atherosclerosis is the oxidative modification of LDL. Oxidative modification of LDL may be a key early step in the pathogenesis of atherosclerosis. Evidence of oxidized LDL has been obtained from arterial walls of animal models of atherosclerosis and of CHD patients [5,6]. The levels of reactive oxygen species (ROS) are controlled by antioxidant enzymes, SOD, catalase, Gpx, Gred and non-enzymatic scavengers such as GSH.

In this study, we investigated the efficacy of antioxidant agent (plant extracts) *by* analyzing all the parameters in plasma LDL, HDL, MDA and *in-vitro* oxidizability of LDL in absence or presence of extracts of *Catharanthus roseus* and *Hibiscus sabdariffa.*

MATERIALS AND METHODS

Chemicals:-1-Chloro 2, 4-Dinitrobenzene was purchased from Central drug house, Pvt. Ltd. (India). All other chemicals used for this study were of analytical grade and obtained from HIMEDIA (India), Sisco (India), Ashirwad (India), Sigma-Aldrich (USA), Miles (USA), Acros (USA) and All other chemicals and reagents used in this study were of analytical grade.

METHODS

Aqueous Ethanolic Extraction of *Catharanthus roseus* leaves and Hibiscus sabdariffa flowers

The leaves of *Catharanthus roseus* and *Hibiscus sabdariffa* were collected from the campus of FRI Dehradun (Uttarakhand) During September-October of 2011,and further authenticated by Botanical survey of India, Dehradun. And the specimen was preserved in the herbarium voucher no- (*Catharanthus roseus-114135 Hibiscus sabdariffa-114134*) The leaves and flower samples were separated, weighed, washed, shredded and dried. The dried and powdered leaves and flower of *catharanthus roseus* and *hibiscus sabdariffa* (100g) each were separately Soxhlet extracted exhaustively with (95%) Ethanol. The extracts were concentrated to dryness under reduced pressure in a rotary evaporator to yield dried ethanolic extracts which were stored in desiccators and used for further studies.

Collection of blood and packed erythrocytes

Fresh Human blood sample was collected from the pathology laboratory of Sardar bhagwan Singh Instt.,Balawala,Dehradun. Packed erythrocytes hemolysate was prepared as described by [7] (1998). After the separation of plasma, 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 [8] was used for the isolation of plasma low density lipoprotein (LDL). The precipitation buffer consisted of 64 mM trisodium citrate adjusted to pH 5.05 with 5 N HCL, containing 50,000 IU/L heparin. 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 22^0C. The pellet was resuspended in 1.0 ml of 0.1 M sodium phosphate buffer, pH 7.4, containing 0.9 % NaCl.

The dual precipitation method of Patsch *et al*. [9] was used for the isolation of high density lipoprotein (HDL), HDL_2 and HDL_3 subtractions. According to this method 100 µl of reagent containing 1.0 g of dextran sulfate dissolved in 100 ml of 0.5 mM $MgCl_2$ solution was added to 1.0 ml of plasma, and vertexed-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 HDL_2 and HDL_3 subtractions. For HDL fraction, 100 µl of reagent containing 1.0 g of dextran sulfate dissolved in 100 ml of 1.5 mM $MgCl_2$ 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 HDL_3 fraction was removed, whereas the HDL_2 precipitate was dissolved in 0.1 M sodium phosphate buffer, pH 7.4, containing 0.9 % NaCl.

Determination of plasma cholesterol

Total cholesterol in plasma, LDL subtractions was determined as described by Annino and Giese [10], 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 *Boerhaavia diffusa* and *Paeonia emodie*

This was performed as described by Esterbauer *et al*. [11,12]. 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 *Paeonia emodie* and *Boerhaavia diffusa* (1mg/ml respectively). At time zero, the absorbance of lipoprotein samples was taken at 234 nm. Then, lipoprotein samples were mixed with $CuSO_4$ to a final concentration of 2.5 µM and incubated at 37^0C. 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, pH 7.4, stored at 4^0C and used for the assessment of conjugated dienes. The oxidation for LDL was carried out for 60 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 µmole MDA equivalent per mg LDL protein.

Measurement of plasma "total antioxidant power" (FRAP)
The method of Benzie and Strain [13] 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. 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, 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. 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 erythrocytes
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 [14].

The suspended erythrocytes in the washed blood sample were incubated with *Paeonia emodie* and *Boerhaavia diffusa* (50-100 µg/ml separately). 0.2 ml of packed erythrocytes were suspended in 3 ml of PBS.Then 1 ml of this lysate was added to 1 ml of 10% TCA (for precipitation of protein) and centrifuged for 5 minutes at 3,500 rpm. 1ml of supernatant was added to 1 ml of 0.6% TBA in 0.05m NaOH and boiled for 20 minutes at temperature greater than 90^0 C. After cooling the tubes to room temperature the absorbance of each sample was read against a reagent blank at 532 nm in spectrophotometer. Malondialdehyde was used as a standard (10^{-12} to 10^{-14}) for the calculation of MDA concentration [30].

Protein estimation
The protein was determined by the method of Bradford [15], using bovine serum albumin as standard. Aliquots of LDL and HDL, were first precipitated with 10 % TCA. The protein pellets were dissolved in 0.5 N NaOH and suitable aliquots were used for protein determination.

RESULTS

Average Value of Age, Weight, Height, Male, Female of Normal Subjects
As shown in Table 1, the average values of age, body weight, height, male and female of normal subjects (n=14) were 28 ± 1.82 year, 58.63 ± 3.03kg , 167.8 ± 1.97 cm, 9 and 5 respectively .

Average Value of Glucose and triglycerides (TGs) of normal subjects
As shown in Table 2, the average values of glucose and triglycerides of normal subjects were 115.47±1.89* mg/ml and 99.13±2.83*mg/ml respectively.

Average value of Total Cholesterol (TC), very low density lipoprotein (VLDL-C), low density lipoprotein (LDL-C) and non high density lipoprotein (HDL-C) of normal subjects
As shown in Table 3, the values of Total cholesterol, very low density lipoprotein, low density lipoprotein and non high density lipoprotein of normal subjects were 13.67 ± 1.67* mg/dl, 15.82 ± 0.43* mg/dl, 95.53± 1.71* mg/dl, and 92.65 ± 0.83*mg/dl respectively.

Average value of High density lipoprotein (HDL-C) and its sub fractions HDL_2-C and HDL_3-C of normal subjects
As shown in table 4, the values of High density lipoprotein and its sub fractions HDL_2-C and HDL_3-C of normal subjects were 47.63 ± 1.38* mg/dl, 15.64 ± 0.70* mg/dl, and 27.51 ± 0.73* mg/dl respectively.

TABLE1. Average Value of Age, Weight, Height, Male, Female of Normal Subjects (n=14)

PARAMETER	NORMAL (n=14)
Age	28 ± 1.82* yrs.
Body weight	58.63 ± 3.03* kg
Height	167.8±1.97*cm
Male	9
Female	5

**Values are mean± S.D. of normal subjects.*

Table 2. Average Values of Glucose and Triglycerides (TGs) of normal subjects

PARAMETER	NORMAL (mg/dl)
Glucose	115.47± 1.89*
TGs	99.13 ± 2.83*

**Values are mean± S.D. of normal subjects.*

Table 3. Average values of total cholesterol (TC), very low density lipoprotein (VLDL-C), low density lipoprotein (LDL-C) and non high density lipoprotein (HDL-C) of normal subjects

PARAMETER	NORMAL(mg/dl)
TC	136.57± 1.67*
VLDL-C	15.82 ± 0.43*
LDL-C	95.53± 1.71*
NON HDL-C	92.65± 0.83*

**Values are mean (mg/dl) ± S.D. of normal subjects.*

TABLE 4. Average value of high density lipoprotein (HDL-C) and its subfractions (HDL_2-C and HDL3-C) of normal subjects

PARAMETER	NORMAL(mg/dl)
HDL-C	47.63±1.38*
HDL_2–C	15.64±0.70*
HDL_3–C	27.51±0.73*

**values are mean (mg/dl) ± S.D. of normal subjects.*

Estimation of Protein of LDL, HDL, VLDL of normal subjects

As shown in table 5, the values of protein LDL, HDL, VLDL, of normal subjects were 1.732 ± 0.055* mg/dl, 1.319 ± 0.032* mg/dl, 0.817 ± 0.27* mg/dl respectively.

Average ratio value of LDL-C/HDL-C, HDL-C/LDL-C, TC/LDL-C, LDL-C/TC, TC/HDL-C, HDL-C/TC, HDL_2-C/HDL_3-C, and HDL_3-C/HDL_2-C in normal subjects

As shown in table 6, the values of LDL-C/HDL-C, HDL-C/LDL-C, TC/LDL-C, LDL-C/TC, TC/HDL-C, HDL-C/TC, HDL_2-C/HDL_3-C, and HDL_3-C/HDL_2-C in normal subjects were 3.101 ± 0.73*, 0.2532 ± 0.14*, 1.6295 ± 0.03*, 0.7402 ± 0.063*,

Measurement of total antioxidant power in *Catharanthus roseus* and *Hibiscus sabdariffa* at different concentrations

As shown in table 7, the values of antioxidant power in *Catharanthus roseus* + Ethanol at different concentration (10µl/ml to 100µl/ml) were 1.391n mole/mg, 1.561n mole/mg, 1.852n mole/mg, 1.8712n mole/mg, 1.911n mole/mg, 1.928n mole/mg, respectively. For *Hibiscus sabdariffa*+DMSO at different concentrations (10µl/ml to 100µl/ml) were 1.421n mole/mg, 1.732n mole/mg, 1.8023n mole/mg, 1.854n mole/mg, 1.89n mole/mg, 1.923n mole/mg, respectively. and *Hibiscus sabdariffa*+Ethanol at different concentrations (10µl/ml to 100µl/ml) were 1.469n mole/mg, 1.801n mole/mg, 1.827n mole/mg, 1.827n mole/mg, 1.863n mole/mg, 1.897n mole/mg, 1.902n mole/mg respectively. Then the result shows that, the *catharanthus roseus* extract has maximum antioxidant potential then other two plant extract.

Table 5. Estimation of protein of LDL, HDL, VLDL of normal subjects

PARAMETER	NORMAL(mg/dl)
LDL-P	1.732±0.055*
HDL-P	1 .319±0.032*
VLDL-P	0.817±0.27*

** Values are mean (mg/dl) ± S.D. of normal subjects.*

Table 6. Average ratio values of LDL-C/HDL-C, HDL-C/LDL, TC/LDL-C, LDL- C/TC, TC/HDL-C, HDL-C/TC, HDL_2-C/HDL_3-C, AND HDL_3- C/HDL_2-C of normal subjects

PARAMETER	NORMAL
LDL-C/HDL-C	3.101±0.73*
HDL-C/LDL-C	0.2532±0.14*
TC/LDL-C	1.6295±0.03*
LDL-C/TC	0.7402±0.063*
TC/HDL-C	3.1356±0.29*
HDL-C/TC	0.1326±0.211*
HDL_2–C/HDL_3–C	0.565±0.013*
HDL_3-C/HDL_2-C	3.1241±0.17*

**values are mean± S.D. of normal subjects.*

Table7. Measurement of total antioxidant power (FRAP method) in *Catharanthus roseus* and *Hibiscus sabdariffa* at different concentrations

conc. (µl/mg)	Total antioxidant power (n mole/mg)		
	C.R*+ETHANOL (mg/ml)	H.S**+DMSO#(mg/ml)	H.S**+ETHANOL (mg/ml)
10	1.391	1.421	1.469
20	1.5261 (+13.51%)	1.732 (+31.1%)	1.801 (+32.2%)
50	1.852 (+46.1%)	1.8023 (+38.13%)	1.827 (+35.8%)
60	1.8712 (+48.02%)	1.854 (+43.3%)	1.863 (+39.4%)
80	1.9116 (+52.06%)	1.899 (+47.8%)	1.897 (+42.8%)
100	1.928 (53.7%)	1.923 (+50.2%)	1.902 (+43.3%)

* *Catharanthus roseus*
** *Hibiscus sabdariffa*
# *Dimethyl sulphoxide*

Average value of Total Antioxidant power in low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), and its sub fractions (HDL_2-C and HDL_3-C), very low density lipoprotein (VLDL-C) of normal subjects

As shown in table 8, the values of antioxidant power in LDL-C, HDL-C, HDL_2-C and HDL_3-C, VLDL-C of normal subjects were 0.323 ± 0.015*n mole/mg, 1.751 ± 0.065*n mole/mg, 0.7168 ± 0.053*n mole/mg, 0.523 ± 0.033*n mole/mg, 1.1218 ± 0.093*n mole/mg respectively.

In vitro antioxidant effect of *Catharanthus roseus* on total antioxidant status in plasma isolated from normal subjects

As shown in table 9, the values of antioxidant effect of *Catharanthus roseus*+ ethanol on total antioxidant status in plasma isolated from normal subjects without drug are 0.7321± 0.032*n mole/mg, 0.5562±0.029*n mole/mg, and with drug were 1.1106 ± 0.029*n mole/mg, 0.6321± 0.0123*n mole/mg respectively. Thus, this demonstrated that, after addition of plant extract the total antioxidant power significantly increases. (14% to 52%).

Table 8. Average values of total antioxidant power in low density lipoprotein (LDL-C), High density lipoprotein (HDL-C), and its superfractions (HDL_2-C AND HDL_3-C), Very low density lipoprotein (VLDL-C) of normal subjects

LIPID LIPOPROTEIN	NORMAL (TAP) (n mole/mg)
LDL-C	0.323±0.015*
HDL-C	1.751±0.065*
HDL_2–C	0.718±0.053*
HDL_3–C	0.523±0.033*
VLDL-C	1.121±0.093*

* *Values are mean ± S.D. of normal subjects.*

Table 9. In *Vitro* Antioxidant effect of *Catharanthus roseus* on total antioxidant status in plasma isolated from normal subjects

Conc. (µl)	TOTAL ANTIOXIDANT POWER OF PLASMA	
	WITHOUT DRUG (n mole/mg)	WITH DRUG (n mole/mg) {C.R# + Ethanol (1mg/ml)}
10	0.7312±0.032*	1.1106±0.029* (+51.88%)
20	0.5562±0.029*	0.6321±0.0123* (+13.646%)

* *Values are mean ± S.D. of normal subjects.*
# *Catharanthus roseus*

In vitro antioxidant effect of *Hibiscus sabdariffa* on total antioxidant status in plasma isolated from normal subjects

As shown in table 10. the values of antioxidant effect of *Hibiscus sabdariffa*+DMSO on total antioxidant status in plasma isolated from normal subjects without drug were 0.8142± 0.064*n mole/mg, 0.5298± 0.046*n mole/mg, and with drug were 1.232± 0.016*n mole/mg, 0.5312± 0.025*n mole/mg respectively. Thus, demonstrated that, after addition of plant extract the total antioxidant power significantly increases. (0.26 % to 51%).

***In vitro* antioxidant effect of *Hibiscus sabdariffa* on total antioxidant status in plasma isolated from normal subjects**

As shown in table 11, the values of antioxidant effect of *Hibiscus sabdariffa*+Ethanol on total antioxidant status in plasma isolated from normal subjects without drug were 0.6142± 0.062*n mole/mg, 0.4321± 0.024*n mole/mg, and with drug were 1.3021± 0.012*n mole/mg, 0.987± 0.061*n mole/mg respectively. Thus, demonstrated that, after addition of plant extract the total antioxidant power significantly increases. (112 % to 128 %).

Table 10. *In Vitro* antioxidant effect of *Hibiscus sabdariffa* on total antioxidant status in plasma isolated from normal subjects

Conc. (µl)	TOTAL ANTIOXIDANT POWER OF PLASMA	
	WITHOUT DRUG (n mole/mg)	WITH DRUG (n mole/mg) {H.S# + Ethanol (1mg/ml)}
10	0.8142±0.064*	1.232±0.062* (+51.314%)
20	0.5298±0.046*	0.5312±0.025* (+0.26%)

** Values are mean ± S.D. of normal subjects.*
 # Hibiscus sabdariffa

Table 11. In *vitro* antioxidant effect of *Hibiscus sabdarifa* on total antioxidant status in plasma isolated from normal subjects

Conc. (µl)	TOTAL ANTIOXIDANT POWER OF PLASMA	
	WITHOUT DRUG (n mole/mg)	WITH DRUG (n mole/mg) {H.S# + Ethanol (1mg/ml)}
10	0.6142±0.062*	1.3021±0.012* (+111.99%)
20	0.4321±0.0024*	0.987± 0.061* (+128.41%)

** Values are mean ± S.D. of normal subjects.*
 # Hibiscus sabdariffa

***In vitro* antioxidant impact of *Catharanthus roseus* in basal and maximal value of conjugated diene formation in LDL oxidation isolated from normal subjects**

As shown in Table12., in absence of *catharanthus roseus*+Ethanol a significant increase in oxidative modification of LDL (conjugated diene formation) was observed from basal value (at 0 minute before oxidation) to maximal value (at 240 minute after adding 0.25mM $CuSO_4$) from 193.24 moles/mg protein-256.21 moles/mg protein i.e. increase by 33% (Fig. 8). Also, when the same sample was again subjected to oxidation with 0.25mM $CuSO_4$,but this time in presence of *catharanthus roseus*+Ethanol separately (10 µg/ml each), a significant decrease from basal to maximal values were observed (256.21 moles/mg protein -216.56 moles/mg protein) i.e., decrease in oxidative modification of LDL suspension isolated from normallipidemic subjects by *catharanthus roseus*+Ethanol by 15% (Fig. 8). This experiment suggests that at the concentration of 10 µg/ml, *catharanthus roseus* has a better antioxidant property in preventing the oxidative modification of LDL in normallipidemic subjects These results indicate a strong anti-oxidative protection of LDL by *cathranthus roseus*+Ethanol.

***In vitro* antioxidant impact of *Hibiscus sabdariffa* in basal and maximal value of conjugated diene formation in LDL oxidation isolated from normal subjects**

As shown in Table13., in absence of *hibiscus sabdariffa*+DMSO a significant increase in oxidative modification of LDL (conjugated diene formation) was observed from basal value (at 0 minute before oxidation) to maximal value (at 240 minute after adding 0.25mM $CuSO_4$) from 187.25 moles/mg protein-201.021 moles/mg protein i.e. increase by 8% (Fig.8). Also, when the same sample was again subjected to oxidation with 0.25mM $CuSO_4$,but this time in presence of *hibiscus sabdariffa*+DMSO separately (10 µg/ml each), a significant decrease from basal to maximal values were observed (201.021 moles/mg protein -197.11 moles/mg protein) i.e., decrease in oxidative modification of LDL suspension isolated from normallipidemic subjects by *hibiscus sabdariffa*+DMSO by 3% (Fig.8). This experiment suggests that at the concentration of 10 µg/ml, *hibiscus sabdariffa* has a better antioxidant property in preventing the oxidative modification of LDL in normallipidemic subjects These results indicate a strong anti-oxidative protection of LDL by *hibiscus sabdariffa*+DMSO.

***In vitro* antioxidant impact of *Hibiscus sabdariffa* in basal and maximal value of conjugated diene formation in LDL oxidation isolated from normal subjects**

As shown in Table14., in absence of *hibiscus sabdariffa*+Ethanol a significant increase in oxidative modification of LDL (conjugated diene formation) was observed from basal value (at 0 minute before oxidation) to maximal value (at 240 minute after adding 0.25mM $CuSO_4$) from 177.62 moles/mg protein-222.32 moles/mg protein i.e. increase by 26% (Fig.1). Also, when the same sample was again subjected to oxidation with 0.25mM $CuSO_4$,but this time in

presence of *hibiscus* sabdariffa+Ethanol separately (10 µg/ml each), a significant decrease from basal to maximal values were observed (222.32 moles/mg protein-189.63 moles/mg protein) i.e., decrease in oxidative modification of LDL suspension isolated from normallipidemic subjects by *hibiscus sabdariffa*+Ethanol by 15% (Fig.1). This experiment suggests that at the concentration of 10 µg/ml, *hibiscus sabdariffa* has a better antioxidant property in preventing the oxidative modification of LDL in normallipidemic subjects These results indicate a strong anti-oxidative protection of LDL by *hibiscus sabdariffa*+Ethanol.

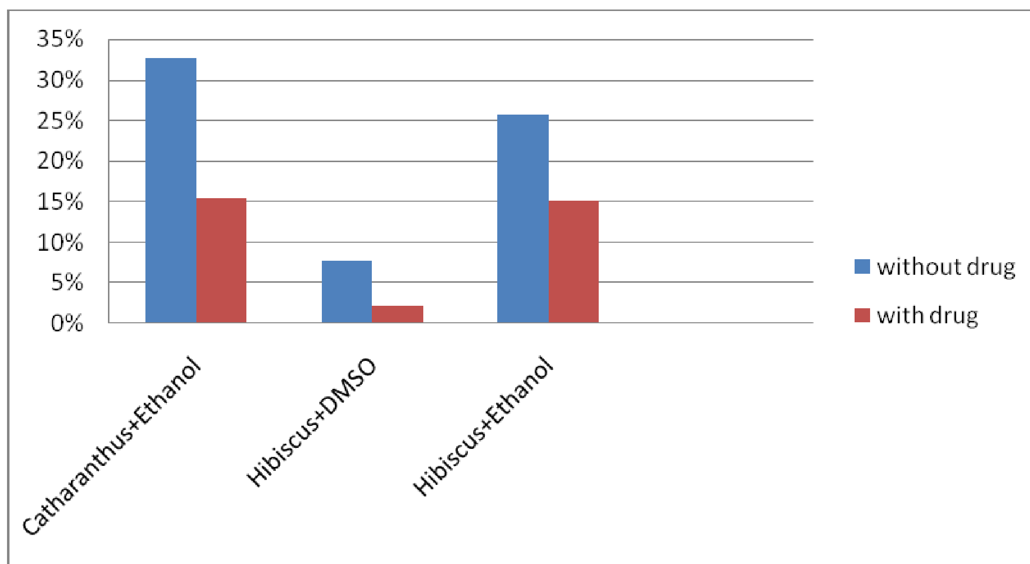

Fig.1. Antioxidant impact of catharanthus roseus+Ethanol, Hibiscus+DMSO, Hibiscus+Ethanol, in LDL oxidation of normal subjects

Table 12. In Vitro antioxidant impact of *Catharanthus roseus* on basal and maximal value of conjugated diene formation in LDL oxidation isolated from normal subjects

LDL oxidation at 37 ^{0}C (SAMPLE- 1)		
Conc. (µl)	**INCUBATION TIME at 37^0 C**	**CONJUGATE DIENE FORMATION (moles/mg protein)**
10	0' (Basal)	193.24 ± 0.031*
10	4hrs + $CuSo_4$ (Maximal)	256.21 ± 0.011*
10	$CuSo_4$ + Sample – 1 + 4hrs (Maximal)	216.56 ± 0.07*

** Values are mean ± S.D. of normal subjects.*
@ SAMPLE -1 ~ Catharanthus roseus + Ethanol(1mg/ml)

Table 13. In Vitro antioxidant impact of *Hibiscus sabdariffa* on basal and maximal value of conjugated diene formation in LDL oxidation isolated from normal subjects

LDL oxidation at 37 ^{0}C (SAMPLE- 2)		
Conc. (µl)	**INCUBATION TIME at 37^0 C**	**CONJUGATE DIENE FORMATION (n moles/mg protein)**
10	0' (Basal)	187.25± 0.11*
10	4shrs + $CuSo_4$ (Maximal)	201.021 ± 0.062*
10	$CuSo_4$+Sample–2 +4hrs	197.11 ± 0.031*

** Values are mean ± S.D. of normal subjects.*
@ SAMPLE -2 ~ Hibiscus sabdariffa + DMSO (1mg/ml).

Table 14. In Vitro antioxidant impact of *Hibiscus sabdariffa* on basal and maximal value of conjugated diene formation in LDL oxidation isolated from normal subjects

LDL oxidation at 37 ^{0}C (SAMPLE- 3)		
Conc. (µl)	**INCUBATION TIME at 37^0 C**	**CONJUGATE DIENE FORMATION (moles/mg protein)**
10	0' (Basal)	177.62± 0.32*
10	4shrs + $CuSo_4$ (Maximal)	222.32 ± 0.012*
10	$CuSo_4$+ Sample – 3 + 4hrs	189.63± 0.013*

@ SAMPLE -3 ~ Hibiscus sabdariffa + ETHANOL(1mg/ml).

In *vitro* antioxidant impact of *Catharanthus roseus* in basal value of Malondialdehyde (MDA) in LDL oxidation isolated from normal subjects

As shown in Table15., in absence of *catharanthus roseus*+Ethanol a significant increase in oxidative modification of LDL (conjugated diene formation) was observed from basal value (at 0 minute before oxidation) to maximal value (at 240 minute after adding 0.25mM $CuSO_4$) from 0.2976 moles/mg protein-0.7964 moles/mg protein i.e. increase by 168% (Fig.9). Also, when the same sample was again subjected to oxidation with 0.25mM $CuSO_4$,but this time in presence of *catharanthus roseus*+Ethanol separately (10 µg/ml each), a significant decrease from basal to maximal values were observed (0.7964 moles/mg protein -0.2132 moles/mg protein) i.e., decrease in oxidative modification of LDL suspension isolated from normallipidemic subjects by *catharanthus roseus*+Ethanol by 73% (Fig.9). This experiment suggests that at the concentration of 10 µg/ml, *catharanthus roseus* has a better antioxidant property in preventing the oxidative modification of LDL in normallipidemic subjects These results indicate a strong anti-oxidative protection of LDL by *cathranthus roseus*+Ethanol.

***In vitro* antioxidant impact of *Hibiscus sabdariffa* in basal value of Malondialdehyde (MDA) in LDL oxidation isolated from normal subjects**

As shown in Table16., in absence of *hibiscus sabdariffa*+DMSO a significant increase in oxidative modification of LDL (conjugated diene formation) was observed from basal value (at 0 minute before oxidation) to maximal value (at 240 minute after adding 0.25mM $CuSO_4$) from 0.1612 moles/mg protein-0.5964 moles/mg protein i.e. increase by 270% (Fig.9). Also, when the same sample was again subjected to oxidation with 0.25mM $CuSO_4$,but this time in presence of *hibiscus sabdariffa*+DMSO separately (10 µg/ml each), a significant decrease from basal to maximal values were observed (0.5964 moles/mg protein -0.2321 moles/mg protein) i.e., decrease in oxidative modification of LDL suspension isolated from normallipidemic subjects by *hibiscus sabdariffa*+DMSO by 61% (Fig.9). This experiment suggests that at the concentration of 10 µg/ml, *hibiscus sabdariffa* has a better antioxidant property in preventing the oxidative modification of LDL in normallipidemic subjects These results indicate a strong anti-oxidative protection of LDL by *hibiscus sabdariffa*+DMSO.

***In vitro* antioxidant impact of *Hibiscus sabdariffa* in basal value of Malondialdehyde (MDA) in LDL oxidation isolated from normal subjects**

As shown in Table17., in absence of *hibiscus sabdariffa*+Ethanol a significant increase in oxidative modification of LDL (conjugated diene formation) was observed from basal value (at 0 minute before oxidation) to maximal value (at 240 minute after adding 0.25mM $CuSO_4$) from 0.1692 moles/mg protein-0.4316 moles/mg protein i.e. increase by 155% (Fig.2). Also, when the same sample was again subjected to oxidation with 0.25mM $CuSO_4$,but this time in presence of *hibiscus* sabdariffa+Ethanol separately (10 µg/ml each), a significant decrease from basal to maximal values were observed (0.4316 moles/mg protein-0.3162 moles/mg protein) i.e., decrease in oxidative modification of LDL suspension isolated from normallipidemic subjects by *hibiscus sabdariffa*+Ethanol by 0.1154% (Fig.2). This experiment suggests that at the concentration of 10 µg/ml, *hibiscus sabdariffa* has a better antioxidant property in preventing the oxidative modification of LDL in normallipidemic subjects These results indicate a strong anti-oxidative protection of LDL by *hibiscus sabdariffa*+Ethanol.

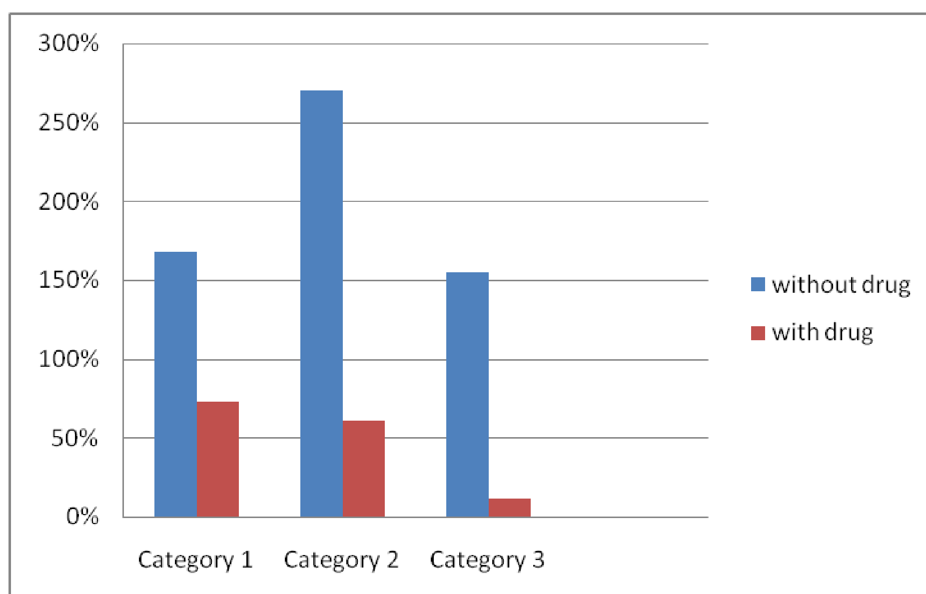

Fig.2. Antioxidant impact of MDA in LDL isolated from normal subjects

Table 15. In *Vitro* antioxidant impact of *Catharanthus roseus* on basal value of malondialdehyde (MDA) in LDL oxidation isolated from normal subjects

LDL oxidation at 37 ^{0}C (SAMPLE- 1)		
Conc. (µl)	**INCUBATION TIME at 37^0 C**	**MDA (moles/mg protein)**
10	0' (Basal)	0.2976± 0.001*
10	4shrs + $CuSo_4$ (Maximal)	0.7964± 0.032*
10	$CuSo_4$ + Sample – 1 + 4hrs (Maximal)	0.2132± 0.041*

** Values are mean ± S.D. of normal subjects.*
@ SAMPLE -1 ~ Catharanthus roseus + Ethanol (1mg/ml)

Table 16. In *Vitro* antioxidant impact of *Hibiscus subdariffa* on basal value of malondialdehyde (MDA) in LDL oxidation isolated from normal subjects

LDL oxidation at 37 ^{0}C (SAMPLE- 2)		
Conc. (µl)	**INCUBATION TIME at 37^0 C**	**MDA (moles/mg protein)**
10	0'	0.1612± 0.062*
10	4shrs + $CuSo_4$	0.5964 ± 0.002*
10	$CuSo_4$ + Sample – 2 + 4hrs	0.2321± 0.132*

** Values are mean ± S.D. of normal subjects.*
@ SAMPLE -2 ~ Hibiscus sabdariffa + DMSO(1mg/ml)

Table 17. In *Vitro* antioxidant impact of *Hibiscus sabdariffa* on basal value of malondialdehyde (MDA) in LDL oxidation isolated from normal subjects

LDL oxidation at 37 ^{0}C (SAMPLE- 3)		
Conc. (µl)	**INCUBATION TIME at 37^0 C**	**MDA (n moles/mg protein)**
10	0'	0.1692± 0.005*
10	4shrs + $CuSo_4$	0.4316±0.0023*
10	$CuSo_4$ + Sample – 3 + 4hrs	0.3162± 0.012*

** Values are mean ± S.D. of normal subjects.*
@ SAMPLE -3 ~ Hibiscus sabdariffa + Ethanol(1mg/ml).

DISCUSSION

At high concentrations, free radicals can cause structural damage to cells, proteins, nucleic acid, membranes and lipids, which exhibits a displacement towards the oxidative burst from phagocytes in which foreign components like proteins are denatured and destroyed. Many diseases such as rheumatid arthritis, pulmonary abnormalities, cardiovascular diseases, reproductive disorders, infertility, retinopathy, diabetes, neurodegenerative diseases, nephropathy and cancer. One of the major causes of these diseases is the oxidative stress within the cells which arises due to an imbalance between the level of antioxidants produced and its scavengers. My study on herbal and medicinal plants- *Catharanthus roseus* and *Hibiscus sabdariffa* shows that the extracts of these plants have a strong antioxidant property and thus they can be used in the prevention in *vivo* oxidative modification of LDL and thus lower the risk of developing lipid profile abnormality leading to various diseases.

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. 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=14) were 58.63 ± 3.03kg , 28 ± 1.82 year, 167.8 ± 1.97 cm, 9 and 5

The dyslipidemic profile of diabetics and affected individuals of other lipid abnormalities includes increased levels of plasma triglycerides (TG), total cholesterol (TC), very low density lipoprotein cholesterol (VLDL-C), low density lipoprotein cholesterol (LDL-C) and small dense (sd)-LDL-C, increased glycation of LDL and decreased plasma antiatherogenic high density lipoprotein (HDL) concentrations. Previous reports indicate that altered plasma lipoprotein profile in the excess atherosclerosis associated with DM may be most critical, because at any total cholesterol level, diabetics have 3- to 5-fold higher CAD mortality rates than do non-diabetic subjects. In addition,

80% of all type 2 diabetics will die of an atherosclerotic event. Our data shows that the average values of Glucose, $TG_{S,}$ TC, VLDL-C, LDL-C, Non HDL-C, HDL-C, HDL_2-C, HDL_3-C, LDL-P, HDL-P, and VLDL-P in normal lipidemic subjects were 115.47mg/dl, 99.13mg/dl, 136.57mg/dl, 15.82mg/dl, 95.53mg/dl, 92.65mg/dl, 47.63mg/dl, 15.64mg/dl, 27.51mg/dl, 1.732mg/dl, 1.319mg/dl, and 0.817mg/dl respectively. It has previously been established that LDL-C/HDL-C and HDL-C/TC ratios are good predictors for the presence and severity of CAD [16].

The average ratio values of TC/LDL-C, LDL-C/TC, HDL_2-C / HDL_3-C and HDL_3-C / HDL_2-C in normal lipidemic subjects were 1.6295, 0.7402, 0.565 and 3.1241 respectively.

Oxidative damage to cholesterol component of the Low-Density Lipoprotein (LDL) leads to oxidized LDL by a series of consecutive events. This induces endothelial dysfunction which promotes inflammation during atherosclerosis. Oxidised LDL acts as a trigger to initiate endothelial inflammation leading to atherosclerosis and vascular thrombosis (heart attack and stroke). Modified LDLs are produced during chemical modification that LDLs undergo after synthesis. Modifications take place either in the plasma or in the inner layer of the artery and pertain to either the lipid or the protein fraction, induced by hydrolytic or proteolytic enzymes, O, OH or O^2 radicals or other non-enzymatic mechanisms, modifications concern the production of lipoprotein-autoantibody complexes [17-18, 29-39].

A simple, automated test measuring the ferric reducing ability of plasma, the FRAP assay, is presented as novel method for assessing ''antioxidant power.'' 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 with antioxidant mixtures, including plasma, and with solutions containing one antioxidant in purified form [13]. My experiment shows that total antioxidant power of *Catharanthus roseus*+Ethanol, *Hibiscus sabdariffa*+DMSO, *Hibiscus sabdariffa*+Ethanol at different concentration (10µl/mg to 100µl/mg)in normallipidemic subjects were 1.928n mole/mg, 1.923n mole/mg, 1.902n mole/mg respectively. Demonstrating that *Catharanthus roseus* is a better antioxidant than *Hibiscus roseus* in experimental conditions. On the other hand the values of antioxidant power in LDL-C, HDL-C, HDL_2-C, HDL_3-C, and VLDL-C were 0.323n mole/mg, 1.751n mole/mg, 0.718n mole/mg, 0.523n mole/mg, 1.1218n mole/mg respectively.

We also examine the total antioxidant power of plasma with or without drug, we registered that after addition of plant extract *(catharanthus roseus*+ ethanol*, hibiscus sabdariffa* +DMSO*,* and *hibiscuss sabdariffa* +ethanol) the total antioxidant power significantly increased up to 0.26% to 128%.

Oxidative modification of lipoproteins is believed to play a central role in the pathogenesis of atherosclerosis [19-20, 29-39] (Berliner and Heinecke, 1996; Steinberg, 1997). Because plasma contains several antioxidants [21] (Frei, 1995) and lipoproteins with oxidative damage have been isolated from atherosclerotic lesions [19-20] (Berliner and Heinecke, 1996; Steinberg, 1997), lipoprotein oxidation generally is considered to occur in the vessel wall. Although lipid oxidation in the vessel wall is thought to occur as a result of a local deficiency of endogenous antioxidants or an excess of free metal ions, only limited data support these hypothesis. Research has shown that human atherosclerotic plaques contain massive amounts of lipid peroxidation products, despite the presence of large quantities of α-tocopherol (vitamin E) and ascorbate [22] (Suarna, 1995). Therefore, it is unclear whether oxidized lipoproteins originate in the arterial wall or are produced in the circulation and then enter the intimal space.

Conjugated diene (which measure the initial phase of lipid peroxidation), lipid hydroperoxide (intermediate product of lipid peroxidation) and MDA (which measure the degradation phase of lipid peroxidation) . The increase in plasma lipid peroxidation products is associated with a significant decline in plasma total antioxidants. The former suggests increased production of oxidants while later indicates diminished antioxidant defense. Both the changes indicate an existence of profound oxidative stress. Recently, Bloomer [23] has shown that young novice smokers (pack-year history of 3±2) have a lower plasma antioxidant capacity and exhibited a greater degree of lipid peroxidation compared to nonsmokers. My result indicates anti-oxidative protection of LDL by *Catharanthus roseus* and *hibiscus sabdariffa*+dmso, hibiscus sabdariffa+Ethanol were -16%, -2% and -15% inhibition, respectively. In absence of catharanthus roseus+ethanol, hibiscus sabdariffa+DMSO, and hibiscus sabdariffa+ethanol, a significant increase in oxidative modification of LDL was observed from basal value (at 0 minute before oxidation) to maximal value (at 240 minute after adding 0.25mM $CuSO_4$) from 193.24 moles/mg protein, 187.25moles/mg protein, 177.62moles/mg respectively to 256.21moles/mg, 201.021moles/mg, 222.32mols/mg protein respectively i.e. increase by 33%, 8%, 26%. But in the presence of *catharanthus roseus*+ethanol, hibiscus *sabdariffa sabdariffa*+DMSO and *Hibiscus sabdariffa*+Ethanol (10µg/ml) the increase in LDL oxidation decreased to 216.56moles/mg, 197.11moles/mg, 189.63 respectively. Thus this experiment implies that *Catharanthus roseus*+Ethanol and *Hibiscus sabdariffa* is more potent natural antioxidant in preventing the *in vitro* copper

mediated oxidative modification of LDL. Based on these results, it seems possible that oxygen radicals formed over and above the detoxifying capacity of plasma can be prevented by treatment with these extracts and hence block the peroxidative breakdown of phospholipid fatty acids and accumulation of MDA and therefore membrane damage.

The levels of reactive oxygen species (ROS) are controlled by antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx), glutathione reductase (Gred) and non-enzymatic scavengers such as glutathione (GSH. These enzymes are important in defending the body against free radicals as well as toxic substances by converting them to a form that can be readily excreted. Therefore, any changes in these enzymes could be potentially detrimental to the host by altering these defense mechanisms. low levels of ROS are vital for proper cell functioning, while excessive *in vivo* generation of these products can adversely affect cell functioning [24-25]. Malondialdehyde (MDA) is one of the final products of lipid peroxidation in human cells, and an increase in ROS causes over production of MDA, which is considered a surrogate marker of oxidative stress [26-27]. Increased MDA production in plasma is known to cause a decrease in the membrane fluidity of the membrane lipid bilayer and increased osmotic stability of cells [28].

My result indicates anti-oxidative protection of LDL by *Catharanthus roseus* and *hibiscus sabdariffa*+dmso, hibiscus sabdariffa+Ethanol were -73%, -61% and -0.115% inhibition, respectively. In absence of catharanthus roseus+ethanol, hibiscus sabdariffa+DMSO, and hibiscus sabdariffa+ethanol, a significant increase in oxidative modification of LDL was observed from basal value (at 0 minute before oxidation) to maximal value (at 240 minute after adding 0.25mM $CuSO_4$) from 0.2976moles/mg protein, 0.1612moles/mg protein, 0.1692moles/mg respectively to 0.7964moles/mg, 0.5964moles/mg, 0.4316mols/mg protein respectively i.e. increase by 167%, 270%, 155%. But in the presence of *catharanthus roseus*+ethanol, hibiscus *sabdariffa sabdariffa*+DMSO and *Hibiscus sabdariffa*+Ethanol (10µg/ml) the increase in LDL oxidation decreased to 0.2132, 0.2321, 0.3162(moles/mg) respectively. Thus this experiment implies that *Catharanthus roseus*+Ethanol and *Hibiscus sabdariffa*+DMSO is more potent natural antioxidant in preventing the *in vitro* copper mediated oxidative modification of LDL.

In conclusion, based on this study of free radical scavenging and antioxidant properties of *Catharanthus roseus* and *Hibiscus sabdariffa* on copper mediated oxidative modification of LDL in normallipidemic subjects, administration of *Catharanthus roseus* and *hibiscus sabdariffa* 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.

CONCLUSION

The present study revealed that the ethanolic extracts of the leaves of *Catharanthus roseus* and the ethanolic extract of the flowers of *Hibiscus sabdariffa* exhibited satisfactory scavenging effect in all the radical scavenging assays in a concentration dependent manner. However from this study it is concluded that the ethanolic extract of *Catharanthus roseus* had more antioxidant activity than *Hibiscus sabdariffa,* as evidenced from the various *in vitro* antioxidant assays. Free radicals can cause structural damage to cells, proteins, nucleic acid, membranes and lipids, which exhibits a displacement towards the oxidative burst from phagocytes in which foreign components like proteins are denatured and destroyed. Many diseases such as Pulmonary abnormalities, cardiovascular diseases, retinopathy, diabetes, neurodegenerative diseases, nephropathy, reproductive disorders, infertility, and cancer. One of the major causes of these diseases is the oxidative stress within the cells which arises due to an imbalance between the level of antioxidants produced and its scavengers. my study on herbal and medicinal plants- *Catharanthus roseus* and *Hibiscus sabdariffa* shows that the extracts of these plants have a strong antioxidant property and thus they can be used in the prevention in *in vivo* oxidative modification of LDL and thus lower the risk of developing lipid profile abnormality leading to various diseases.

Oxidative damage to cholesterol component of the Low-Density Lipoprotein (LDL) leads to oxidized LDL by a series of consecutive events. Oxidised LDL acts as a trigger to initiate endothelial inflammation leading to Diabetes, atherosclerosis and vascular thrombosis (heart attack and stroke). Modified LDLs are produced during chemical modification that LDLs undergo after synthesis. *Catharanthus roseus* and *Hibiscus sabdariffa* inhibits the oxidative modification of LDL in normallipidemic subjects by 15.47% and 15.08% respectively. It has also been experimentally proved that these extracts are effective in lowering the formation of Malondialdehyde contents in LDL with plant extract, isolated from normallipidemic subjects. Though, these extracts do not show considerable effects in enhancing the catalytic activity of cellular enzymes such as Superoxide dismutase.

In conclusion, based on our study of free radical scavenging and antioxidant properties of *Catharanthus roseus* and *Hibiscus sabdariffa* on copper mediated oxidative modification of LDL in normallipidemic subjects, administration of *Catharanthus roseus* and *Hibiscus sabdariffa* may be useful in the prevention and treatment of Diabetes,

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.

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