International Journal of Phytomedicine 2 (2010) 267-276

http://www.arjournals.org/ijop.html

Research Article

ISSN: 0975-0185

Evaluation of Antioxidant and Antihyperlipidemic Activity of extracts Rich in Polyphenols

Shetgiri P.P[1], Darji. K.K[1], D'Mello P.M[1]*

***Corresponding author:**

P. M. D'Mello

Department of Pharmacognosy and Phytochemistry, Prin. K. M. Kundnani College of Pharmacy, Rambhau Salgaokar Marg. Cuffe parade, Colaba, Mumbai-400005, India.
Email – dmellopm(at)rediffmail.com
Tel – 022 2216436

Abstract

Polyphenols are phytochemicals present in plants that contribute to their antioxidant and different pharmacological activities. Flavonoids comprise of one class of polyphenols which play a beneficial role in preventing free radical damage and owe its antioxidant property. Lipid peroxidation and lipid-derived oxidized products have been implicated in the pathogenesis of a variety of human diseases. To clarify the role of oxidative stress in prevention of atherosclerosis and hypercholesterolemia, the antioxidant status of plant extracts of *Oroxylum indicum, Vitis vinifera* and Policosanol isolated from, *Saccharum officinarum* were selected for the study.

The extracts were evaluated by *in vitro* methods by monitoring the production of malondialdehyde as Thiobarbituric acid Reacting Substances (TBARs). The antihypperlipidemic activity was examined in cholesterol induced hyperlipidemic model using Albino Wistar rats.

The result of the study exhibited significant reduction in total cholesterol, triglycerides, LDL-C, VLDL-C levels and remarkable increase in the level of HDL-C when compared to standard lovastatin drug. The artherogenic index and LDL-C: HDL-C risk ratio was also reduced to significant extent in the group treated with extracts. The levels of SGOT and SGPT were estimated and found to be significantly less than that of hyperlipidemic control group.

The highest antihyperlipidemic activity was exhibited by total extract of the bark of *Oroxylum indicum* followed by extracts of fruits of *Vitis vinifera* and Policosanol isolated from sugarcane wax.

Keywords:- Polyphenols, Antihyperlipidemic, Antioxidant, *Oroxylum indicum, Vitis vinifera*, Policosanol

Introduction

Polyphenols are phytochemicals present in plants that contribute to their antioxidant and different pharmacological activities. Flavonoids belong to one class of the polyphenol family. Beneficial effects of polyphenols on human health are partly explained by their antioxidant activity. Because

doi:10.5138/ijpm.2010.0975.0185.02038

of the antioxidative property, it is suggested that polyphenols may delay or prevent the onset of diseases such as cancer, diabetes induced by free radicals [1]. They also inhibit low density lipoprotein (LDL) oxidation and platelet aggregation and are reported to have negative correlation with incidences of coronary heart disease [2]. Several medicinal plants contribute their activities due to the presence of flavonoids and polyphenols.

The present work is focused on antioxidant and antihyperlipidemic activity of plants of dried fruit of *Vitis vinifera* commonly known as black grapes, dried bark of *Oroxylum indicum* and policosanol isolated from sugarcane wax

Methods

Collection of plant material and Extraction:

The medicinal plants selected for the study included *Vitis vinifera* [3], *Oroxylum indicum* and Policosanol isolated from sugarcane waste. The bark of *Oroxylum indicum was* procured from local market at Pyaydhuni, Mumbai and was authenticated by at Nicholas Piramal India Ltd, Goregaon, Mumbai, while the other two plants *Vitis vinifera, Saccharum officinarum* which are commonly available were authenticated at the laboratory of Pharmacognosy & Phytochemistry, Prin. K. M Kundnani College of Pharmacy, Mumbai and used for the study. The voucher specimens have been maintained at the department. The dried materials of the bark of *Oroxylum indicum* fruits of *Vitis vinifera,* and waste of *Saccharum officinarum* were powdered and passed through 40 mesh to facilitate proper extraction and then subjected to soxhlet extraction using methanol as a solvent. The methanolic extracts were concentrated under vacuum at temperature of 50 degrees. The viscous crude extracts obtained were further reconstituted in water as a suspension in tween 80 to obtain aqueous suspensions that were used for the final study.

Quercetin, [6] a plant based polyphenol was isolated from the methanolic extract of *Vitis vinifera* by preparative HPLC. Policosanol [5, 6] was isolated from sugarcane wax by standard procedure. Total standardized extract of *Oroxylum indicum* in terms of ellagic acid equivalent [4] was used for the study using methanol as a solvent. All compounds isolated were identified and quantified by means of HPLC using standards and confirmed by IR. The quantified standardized extract were used for the study to determine their antioxidant activity and to study their role in prevention of hyperlipidemia and its effect on cardiovascular diseases

Reagents

The reagents used for antioxidants activity were thiobarbituric acid (TBA) purchased from Merck, Mumbai. Standard Quercetin purchased from Sigma Aldrich USA. The reagents required for anti hyperlipidemic studies were cholesterol obtained from Qualigens Fine Chemicals, crude edible coconut oil purchased from local market; standard drug Lovastatin was supplied as a gift sample by production department of Cipla pharmaceuticals, Mumbai. 1, 1 Diphenyl, 2picryl hydrzyl (DPPH) purchased from Sigma Aldrich USA. The kits for estimation of biological parameter like SGOT, SGPT, Triglycerides and cholesterol were obtained from span Bio Diagnostics, Mumbai. All the other chemicals used during the study were of laboratory grade.

HPLC Chemoprofiling

The extract of *Vitis vinifera* was standardized by HPLC using gradient phase system using C18 column and PDA detector and acetonitrile: water and acetic acid 0.2% as a mobile phase and policosanol was standardized by GC using dichloromethane and nitrogen as a gas. The extract of *Vitis vinifera* was quantified in terms of its quercetin content, Policosanol was quantified in terms of octacosanol content while *Oroxylum indicum* extract was standardized in terms of ellagic acid equivalent.

Animals

For antioxidant activity, Swiss Albino mice (25-30 G) of either sex were obtained from Haffkine Biopharmaceutical Corporation Ltd., Mumbai. For antihyperlipidemic study Albino Wistar rats

(120-150 G) of either sex male or female were obtained from Glenmark Pharmaceuticals Ltd, Mumbai. All the animals were housed under good hygienic conditions in the departmental animal house of Prin. K. M. Kundnani College of Pharmacy. Animals were maintained under standard environmental conditions (22-28 degrees centigrade, 60-70% relative humidity, 12 hr L: D cycle) and fed with standard diet and water *ad libitum*. Animals were allowed to acclimatize to experimental conditions by housing them for 8-10 days period prior to experimental study.

Evaluation of protocol

The experimental protocol was approved by the Institutional Animal Ethics Committee (Protocol No-091012) and the experimental work was carried out as per Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines. The antioxidant activity of plant was evaluated by different methods like hydroxyl, superoxide, DPPH scavenging activities.

Evaluation of antioxidant activity by lipid peroxidation method [7, 8]

The antioxidant activity of plant extracts was evaluated by calculating inhibition of lipid peroxidation which was performed using liver homogenate of mice. Mice liver homogenate (10%) was prepared by homogenizing the fresh liver in 0.15 M KCl solution. This fresh liver homogenate was mixed with 0.15 M KCL & tris hydrochloride buffer. The various extracts of *Vitis vinifera, Oroxylum indicum* and Policosanol at different concentrations were then added. Quercetin at various concentrations was used as standard. In vitro lipid peroxidation was initiated by addition of 100 µM ferrous sulphate and 100 µM Ascorbic acid. After incubation for 1 hour at 37 degrees, the reaction was terminated by addition of thiobarbituric acid and then boiled at 95 degrees for 15 minutes for development of colored complex. On cooling test tubes were centrifuged at 4000 rpm for 10 minutes. Absorbance of supernatant was determined colorimetrically at 532 nm and percent inhibition of TBARs formation was calculated with respect to control in which no test sample was added. The IC_{50} values were calculated for all test material by subjecting the results to linear regression.

Evaluation of antioxidant activity by DPPH method [9]

The antioxidant activity of plant extracts was evaluated by using 1, 1 Diphenyl, 2picryl hydrzyl (DPPH) as a free radical. The absorbance was monitored at 516nm. Quercetin was used as a standard.

Evaluation of antioxidant activity by deoxyribose method and NBT method

The hydroxyl radical scavenging activity of plant extracts was evaluated by using deoxyribose method. The absorbance was monitored at 532nm. Superoxide anions were generated with riboflavin-light-NBT system and the scavenging potential was assessed by measuring the reduction of farmazone formation as measured in terms of reduction in absorbance at 560 nm. Quercetin was used as a standard.

Evaluation of antihyperlipidemic activity:

The experimental protocol was approved by the Institutional Animal Ethics Committee and the experimental work was carried out as per CPCSEA guidelines. The doses were fixed after random trials conducted on the animals. The procedure was followed as per the method of Dhuley *et al* [10]. Albino Wistar rats (120-150 G) were randomly divided into five different groups each containing 6 to 8 animals as follows:

Group 1- Control group: Vehicle control

Group 2-Hyperlipidemic group: Cholesterol in coconut oil (25 mg/kg/ day) was administered to each rats of this group.

Group 3-Standard group: Lovastatin (10 mg/kg/day) was given along with

cholesterol in coconut oil.

Group 4-Test group: Rats of this group received *Policosanol aqueous* extract (200 mg/kg/day) along with cholesterol in coconut oil.

Group 5-Test group: Rats of this group received *Vitis vinifera aqueous* extract (200mg /kg/day) along with cholesterol in coconut oil.

Gr Group 6-Test group: Rats of this group received *Oroxylum indicum aqueous* extract (200mg/kg/day) along with cholesterol in coconut oil.

The cholesterol in coconut oil was administered to each animal except the vehicle control group daily at 10.00 am. The solution of extract and standard drug were prepared freshly every day and administered to the test animals in their respective groups at 3.00 pm. This process was followed for 28 days and the amount of food intake was monitored daily. At the end of the experimental studies animals were fasted for 12 hrs and the blood was collected by cardiac puncture under light ether anesthesia. Animals were then sacrificed and the livers were isolated and preserved in 10% formalin solution [11]. The biochemical parameters that were evaluated using Span Diagnostic Kits were Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C), High Density Lipoprotein Cholesterol (HDL) and Triglycerides (TAG) from the serum. The artherogenic Index and LDL-C: HDL-C ratios were calculated to determine the cardiac risk factors [12]. The activities of marker enzymes like Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) were also estimated.

Statistical Analysis

All the results were subjected to statistical analysis using one way Annova followed by Dunnet's multiple comparison tests against the hyperlipidemic group. The p values < 0.01 were considered statistically significant. All the values were expressed as mean$\pm$ S.E.M and compared with control group for estimation of antihyperlipidemic activity of test extracts.

Results & Discussion

Antioxidant Activity

Polyphenols and flavonoids comprise a ubiquitous class of alternative antioxidants and protect against the invasion of these free radicals. Literature survey sites the prevention role of these antioxidants in prevention of atherosclerosis and congestive heart diseases (1, 2). The findings in the present investigation have confirmed the contributory role of antioxidants in the lowering of cholesterol levels in experimental animals.

The antioxidant activity evaluation using *in vitro* methods revealed highest antioxidant activity for *Vitis vinifera* extract. The observed activity may be mainly due to the presence of polyphenols and flavonoids abundant in the extract like resveratrol and phytostilbenes, anthocyanins like delphinidin, cyanidin, flavonols like Quercetin, rutin and several biflavonids, carrotenoids and several Vitamin B derivatives as cited in the literature (13). The extract of *Oroxylum indicum* contains ellagic acid, beta sitosterol, coumaric acid and flavones like oroxylin which could be one of the mechanism by which it owes significant antioxidant activity by virtue of its polyphenol content (14). Policosanol is a mixture of long-chain aliphatic alcohols isolated from sugar cane (*Saccharum officinarum*) wax, whose

main component is octacosanol which contribute to less antioxidant activity as compared to polyphenolic components present in other extracts. (Table A)

Table -A. Antioxidant activity of extracts by *in vitro* lipid peroxidation & DPPH.

Extracts (Aqueous)	DPPH IC_{50} (µg/ml)	Superoxide radical scavenging IC_{50} (µg/ml)	Hydroxyl radical scavenging IC_{50} (µg/ml)	Lipid peroxidation IC_{50} (µg/ml)
Vitis vinifera extract	68.25	59.57	46.98	41.83
Policosanol Extract	91.76	112.74	97.06	101.57
Oroxylum indicum extract	76.30	65.48	52.23	53.29
Quercetin	22.63	42.56	33.32	29.61

HPLC Chemoprofiling.

The HPLC chromatogram (Figure- 1) shows Quercetin isolated from *Vitis vinifera* extract and comparison of the same with standard Quercetin purchased from Sigma Aldrich. The HPLC chromatogram (Figure 2) depicts policosanol isolated from sugarcane wax. Figure 3 depicts a spectrophotometric method for determination of polyphenols in terms of ellagic acid with the standard curve of ellagic acid a constituent present in *Oroxylum indicum.* These studies depict the use of standardization of the extract in terms of active contents.

Antihyperlipidemic activity.

Since hyperlipidemia, inflammation and obesity are closely related to atherosclerosis, therefore management of these factors together would be beneficial for overall treatment approach for atherosclerosis. Although, Indian system of medicine, especially Ayurveda has several

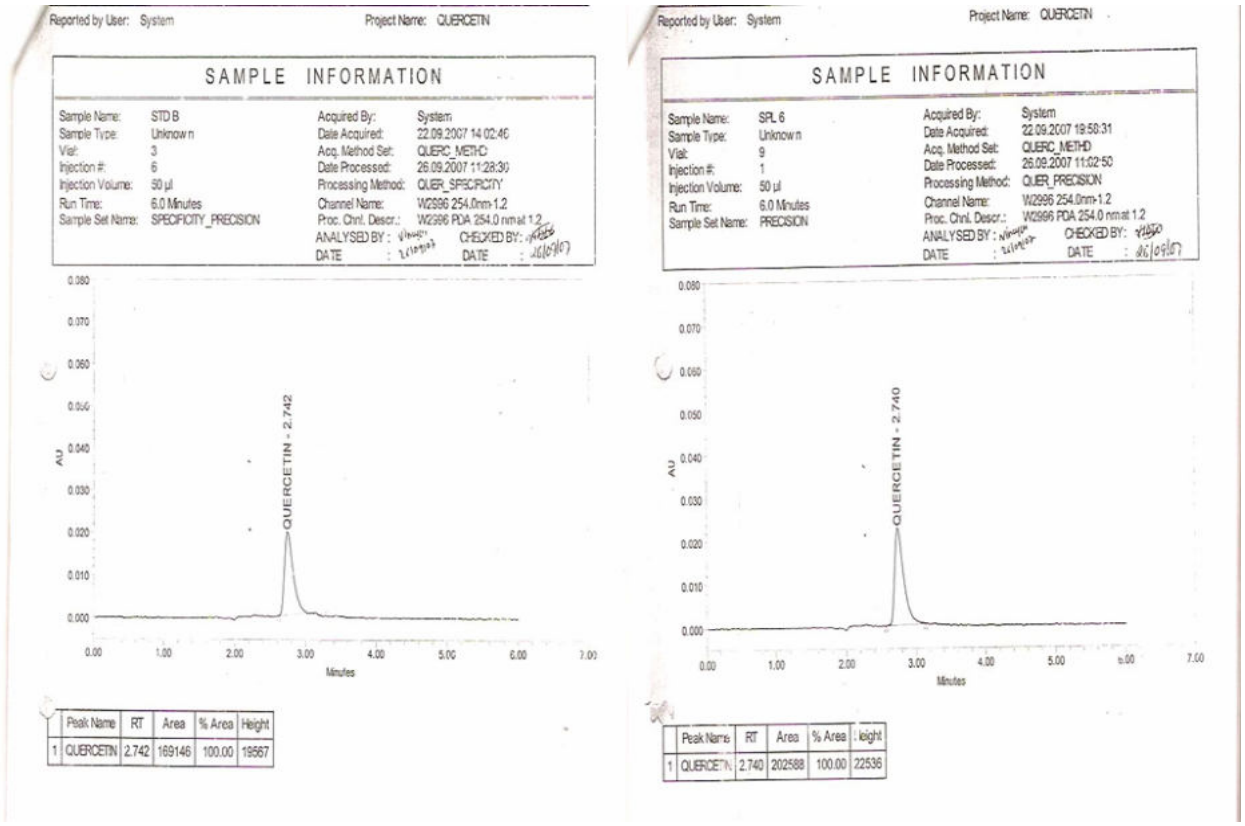
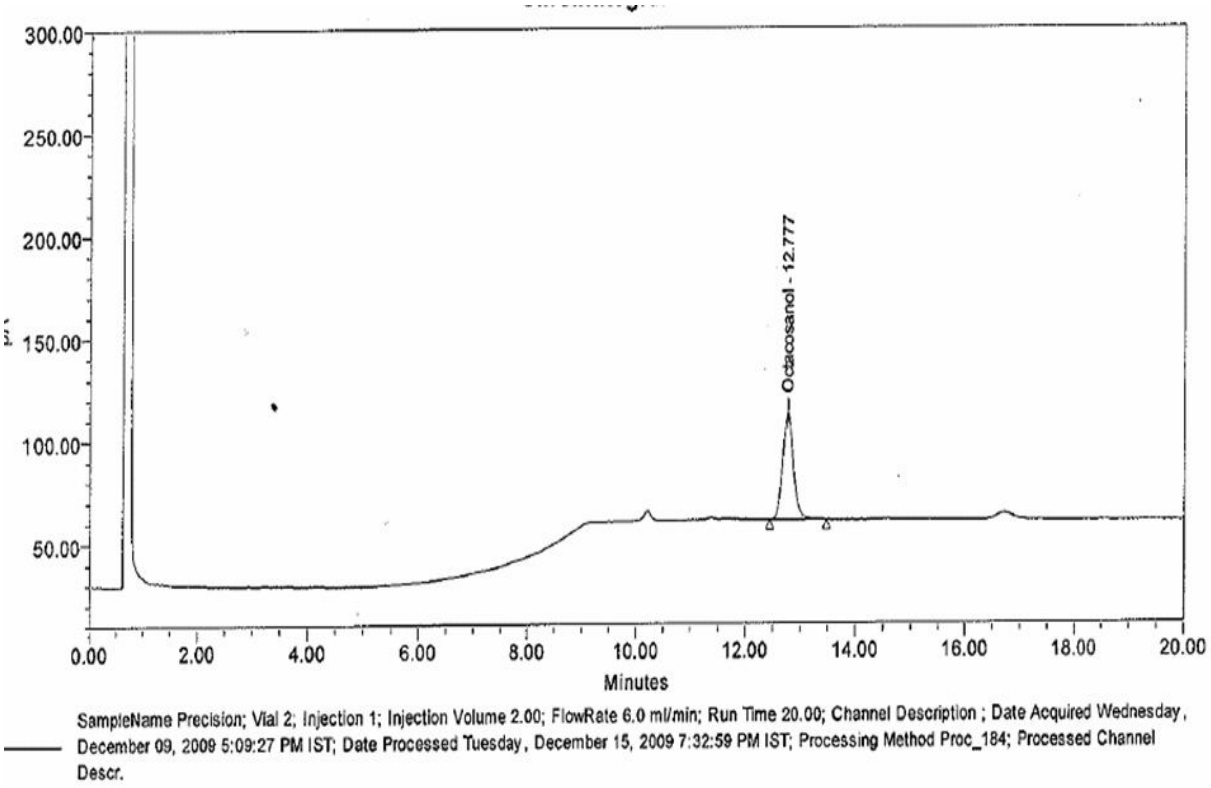


Figure 1 Depicting chromatogram of Quercetin and querctin standard isolated from *Vitis vinifera.*

medicinal plants with proven beneficial claims



Peak Results
Vial: 2

Peak Name	RT	Area	Height (µV)	% Area	USP Tailing	USP Plate Count	Injection	Vial	Int Type
Octacosanol	12.777	697	52	100.00	1.11	19841	1	2	BB

Figure 2 Depicting chromatogram of policosanol isolated from sugarcane wax

towards these pathological conditions, but most of them lack enough experimental data. In this paper, antioxidant, antihyperlipidemic, properties of plant extracts have been studied on several experimental models based on chemical tests, *in vitro* models, and *in vivo* experiments with normal animals.

Deranged serum lipid profile with high levels of triglycerides, total cholesterol, LDL cholesterol, and lower high density lipoproteins (HDL) are some of the basic causes, which should be targeted to prevent atherosclerotic disease. If not managed in the early stage, it leads to the formation of foam cells, resulting to the release of inflammatory cytokines and endothelial cells dysfunction. From pathological point, atherosclerosis may be characterized by the progressive accumulation of lipid and fibrous depositions in the vessel wall, loaded with monocyte-derived macrophages; smooth muscle derived foam cells and activated T cells.

A major culprit in the development of atherosclerosis is oxidized LDL (13) which contributes to increase in the level of cholesterol and triglycerides in the blood. The further step leads to development of atherosclerotic plaques leading to myocardial infarction. Several mechanisms have been a contributing factor in these cardiac ailments one of them being attack of free radicals in the body leading to atherosclerosis and congestive heart diseases (CHD). Free radicals may contribute to

atherogenesis by oxidizing low density lipoproteins (LDL) which then damage arterial walls [13]. The oxidation of LDL cholesterol is suspected to occur at the initial stages of atherosclerosis, and antioxidants have been shown to inhibit this oxidative reaction and prevent the body from these invading free radicals [14]. Common primary causes of hypercholesterolemia (specifically, high LDL cholesterol) include diet, obesity hypothyroidism (that is, low thyroid hormone levels), pregnancy, and kidney failure and attack of free radicals in the body. Common secondary causes of hypertriglyceridemia include diabetes, excess alcohol intake, obesity, and certain prescription medications (such as glucocorticoids and estrogen). Hyperlipidemia, along with diabetes, hypertension (high blood pressure), positive family history, and smoking are all major risk factors for coronary heart disease (15, 16).

Polyphenols and flavonoids act as alternative antioxidants and protect from these free radical damage thereby preventing atherosclerosis and cardiovascular diseases.

As free radicals generation & induction of inflammation (15, 17, 18) are the primary causes of atherogenesis, therefore antioxidant & anti hyperlipidemic potential of plant extracts were investigated on various experimental models. The principal radicals responsible for hyperlipidemia and progression of atherosclerosis were hydroxyl and superoxide radicals. Reduction in levels of these radicals in the body could cause decrease in the incidences of atherosclerosis and congestive heart diseases. An *in vitro* study showed that plant extracts significantly scavenged the superoxides and hydroxyl (OH) radicals in a standardized chemical system as described (Table A). The present study reveals that extracts of *Vitis vinifera* and *Oroxylum indicum* shows inhibition of hydroxyl, superoxide radicals and thiobarbituric acid reacting substances (TBARs) followed by policoosanol.

Table B: Effect of plant extracts on the serum lipid profile

Values are Mean± S.E. of 6 animals.
P-values: * < 0.01

Groups	TC (mg/dl)	TAG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Control group	61.00±1.14*	48.80±4.12*	39.60±0.58*	12.60±1.23*	8.640±1.42*
Hyperlipidemic group	120.00±1.41	190.6±3.61	16.36±0.53	65.52±1.48	38.12±0.72
Standard group lovastatin (10 mg/kg/day)	73.60±2.58*	90.00±6.23*	24.46±0.71*	31.13±2.34*	18.00±1.24*
Policosanol	67.60±2.48*	159.8±8.87*	29.91±4.43*	5.72±0.99*	31.96±1.77*
Vitis vinifera	64±2.82*	123.2±9.74*	32±1.83*	7.36±2.04*	24.64±1.94*
Oroxylum indicum	47±1*	51.60±2.05*	34.49±0.55*	2.91±1.12*	10.32±0.44*

In the present study the extract of *Oroxylum indicum* exhibited highest activity followed by *Vitis vinifera* followed by policosonal in cholesterol induced hyperlipidemic model in Albino Wistar rats, better than that observed with standard drug Lovastatin. The levels of TC, LDL-C, VLDL-C and TAG were found to be considerably less than standard Lovastatin drug (Table B). The atherogenic index and LDL-C: HDL-C ratio was found to be less than Lovastatin group (Table C) which could be one contributing factor in the development of atherosclerosis & congestive heart diseases (CHD).

In hyperlipidemic model, the purpose of inclusion of cholesterol and coconut oil is attributed to the very well established findings that addition of

dietary cholesterol along with saturated fats results in accumulation of intracellular cholesterol and its ester in the body tissues as coconut oil contains approximately 92% of saturated fatty acids (FA): of short chain 15%, medium chain 64.2% and long chain 12.2% [19]. Antihyperlipidemic agents which are active in cholesterol induced hyperlipidemic model function by one or more mechanisms.

The current therapy for treatment of hyperlipidemia mainly involves statins which are basically enzyme inhibitors [21, 20]. It is likely that they may inhibit many other essential enzymes and thereby causing serious side effects. The long term use of statins increases the risk of chronic toxic effects. While conventional statin drugs directly inhibit the enzyme HMG-CoA reductase, which is the rate-limiting enzyme in the hepatic metabolic pathway to cholesterol synthesis, policosonal limits this same enzyme indirectly. Policosanol has proven equivalent to or better than several statin drugs like simvastatin, pravastatin, lovastatin, probucol etc in reducing cholesterol levels and has fewer side effects. This dietary supplement also decreases several other risk factors for CHD by decreasing the oxidation of LDL cholesterol, platelet aggregation and endothelial damage.

Interestingly, these histological changes were in accordance of the lipid profile of these animals. There was significant decrease in triglyceride & cholesterol level accompanied with increase in HDL cholesterol (Table B, C).

All the plant extracts induced an increase in serum HDL-C levels when compared to hyperlipidemic group as well as to Lovastatin group (Table-B). The maximum activity was demonstrated by the extract of *Oroxylum indicum* which increased the level of HDL to 34mg/dl similar to that of the control group. The extract of *Vitis vinifera* and policosanol showed better activity and increased the level of HDL to 32 and 29mg/dl as similar to standard lovastatin group. HDL-C is beneficial for a number of reasons. The most important is its ability to drive a process called "reverse cholesterol transport". HDL helps to extract excess cholesterol deposited in blood vessel walls and deliver it back to the liver for elimination through the gastrointestinal tract. In general, the higher is the HDL-C, the greater is the capacity to remove cholesterol and prevent dangerous blockages from developing in blood vessels. HDL-C helps to keep blood vessels widened thereby promoting better blood flow [22, 23].

The SGOT and SGPT levels of the groups treated with plant extract were found to be significantly reduced as compared to hyperlipidemic group (Table C). The histopathological studies of the liver sections illustrate no granular degeneration and fatty infiltration due to cholesterol administration in the groups treated with the plant extracts as depicted in figure 3. The present

Table C: Effect of plant extracts on parameters like AI & LDL-C: HDL-C ratio and on enzyme activity like SGOT and SGPT. Values are Mean± S.E. of 6 animals.
P-values: * < 0.01

Groups	AI	$\frac{\text{LDL-C}}{\text{HDL-C}}$	SGPT	SGOT
Control group	1.540±0.01*	0.317±0.03*	35.55±6.48*	174.7±9.968*
Hyperlipidemic group	7.369±0.28	4.026±0.19	156.1±7.94	250.8±8.728
Standard group lovastatin (10 mg/kg/day)	3.017±0.12*	1.253±0.10*	43.64±4.75*	204.5±9.848*
Policosinol	2.258±0.10*	0.19±0.036*	68.02±1.72*	183.9±6.76*
Vitis vinifera	2.03±0.17*	0.244±0.081*	36.18±3.61*	110.2±4.05*
Oroxylum indicum	1.36±0.04*	0.084±0.035*	33.42±4.01*	108.4±5.93*

findings demonstrate leading antioxidant and antihyperlipidemic activity which can be explored further with respect to its mechanism of action to develop good antihyperlipidemic agents. Antioxidant activity is one of the mechanisms for lowering the lipid profile but a strong antihyperlipidemic activity exhibited by *Oroxylum indicum* indicates that there could be other constituents as well as flavonoids and polyphenols responsible for its anti hyperlipidemic activity.

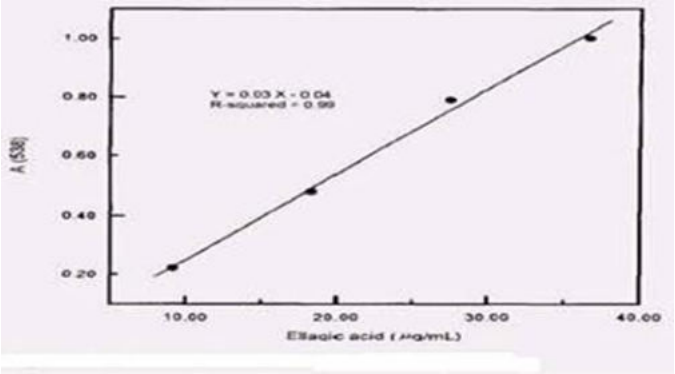


Figure 3 Depicting standard curve of ellagic acid isolated from *Oroxylum indicum* extract

In conclusion, the present study reveals that these plant extracts have very potential antioxidant activity and antihyperlipidemic activity due to the presence of flavonoids and polyphenols which could be one of the possible reasons in prevention of CHD and myocardial infarction.

Thus, based on the above scientific observations, it could be finally suggested that extracts could be targeted as a novel poly-herbal formulation with multi-targeted action as it collectively inhibits the process of atheroma formation, by regulating various steps in atherogenesis. It could acts either as (1) antioxidant (2) as hypolipidemic, through enhancing the HDL, (3) atheroma stabilizer through (a) lesser deposition of Ca in the plaque, (b) through maintenance of intactness of collagen cap and (c) through less proliferation of smooth muscle cells in the vascular wall.

Although, these individual plants are already in clinical use as single or in different formulation, but multi-centric clinical trial is required to use it as food supplement or as add on therapy to prevent atherosclerosis and hyper-lipidemia.

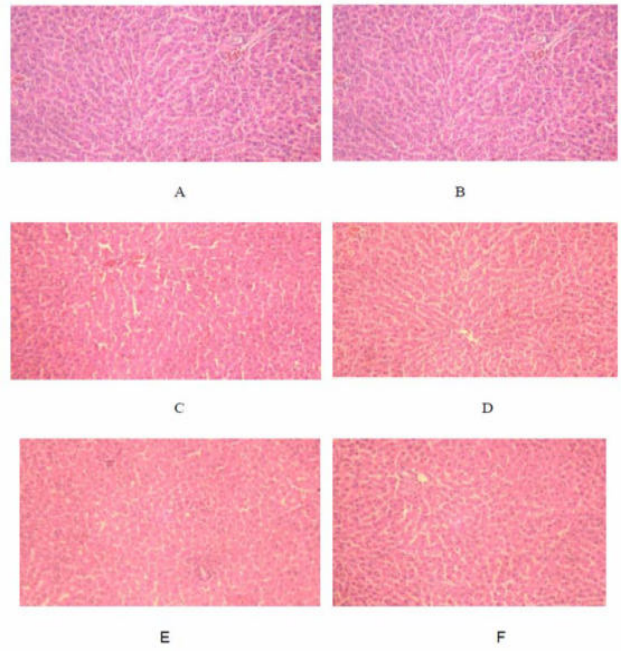


Figure 4 Hepatocytes of rats stained with hematoxylin and eosin (100 x magnification) – (A) control group showing normal architecture; (B) Hyperlipidemic group showing granular degeneration; (C) Lovastatin group showing mild diffuse granular degeneration; (D) *Policosanol*; (E) *Vitis vinifera extract*; (F) *Oroxylum indicum extract*

Acknowledgement

The authors would like to acknowledge HSNC Board and all those who have contributed directly or indirectly for the study.

Conflict of Interest Statement

The authors declare there is no conflict of interest.

Author Agreement

All authors have made substantial contributions and final approval of the conceptions, drafting, and final version.

References

1. Renaud S, De Lorgeril. M *Lancet* (1993) 339: 1523-1526.
2. Hoffman R, Garewal H *Arch. Inter. Med* (1995) 155: 241-246.
3. Hmamouchi M etal *Fitoterapia* (1996) 68: 113-114.
4. Gu, Q, Luo, H., Zheng, W., Liu, Z., and Huang, Y *Int. J. Syst. Evol. Microbiol.* (2006) 56:2193-2197.
5. Marinangeli CP, Kassis AN, Jain D, Ebine N, Cunnane SC, Jones PJ N *Br. J. Nutr.* (2007) 97 381–8.
6. Granja A, Hernandez J etal, 1997, U. S Patent No - 5663156 Mixtures of higher primary aliphatic alcohols, its obstention from sugarcane wax and its pharmaceutical uses.
7. Edwards RL, Lyon T, Litwin SE, Rabovsky A, Symons JD, Jalili T *J. Nutr.* (2007) 137: 2405–11.
8. Ohikawa H, Oshini N, Yagi K *Anal. Bio. chem.* (1979) 95: 351-358.
9. Devasagayam TPA, Boloor KK, Ramasarma T *Indian J Biochem Biophy,* (2003) 40: 300 -303.
10. Lamaison J, Ptitjean- Freylet *Pharm. Acta. Helvetia,* (1991) 66: 185-188.
11. Dhuley J, Naik SR, Rele S, Benarji A *Pharm. Pharmacol. commun*. (1999) 5 :689-670.
12. Wiliamson E, Okpako M, Evans FJ, Pharmacological methods in phytotherapy research, (1st edn) Wiley and sons publication USA. 1996.
13. P. Iacopini, M. Baldi,, Storchi, L. Sebastiani Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: Content, *in vitro* antioxidant activity and interactions. *Journal of Food Composition and Analysis* (2008) 21 589– 598
14. Roy MK, Nakahara K, Na TV, Trakoontivakorn G, Takenaka M, Isobe S, Tsushida T. Baicalein, a flavonoid extracted from a methanolic extract of Oroxylum indicum inhibits proliferation of a cancer cell line in vitro via induction of apoptosis. *Pharmazie* (2007) 62, 149-53.
15. Dahlen, G.H. Lp (a) lipoprotein in cardiovascular disease. (Review article and viewpoint).*Atherosclerosis* (1994) 108 111-126.
16. Edwards, C., Bouchier, I., Haslett, C. and Chilvers, E. Ischamic (Coronary) Heart Disease. *In*: Davidsons Principles and Practice of Medicine (1999). 18th ed. pp.245-266.
17. Farmer, I.A. and Gotto, A.M. Dyslipidemia and other risk factors for coronary artery disease. *In*: Heart Disease. A textbook of cardiovascular medicine. Braunwald (1997) 5th edition, pp.1126-1160.
18. Gensini, G.F., Comeglio, M. and Colella, A. Classical risk factors and emerging elements in the risk profile for coronary artery disease. *Euro. Heart J.* (1998) 19(Supp. A): A53-A61.
19. Ochani PC, D'Mello P *Indian J of experimental biology* (2009). 47: 276-282.
20. Ardlie N, Selley M, Simons L *Arterosclerosis* (1989) 76: 117-124.
21. Bagchi K, Puri S *Eastern Mediterranean Health Journal* (1998) 4: 350-360.
22. Augusti KT,Narayan A, Pillai LS *Indian J of experimental biology*, (2001) 39: 660-662.
23. Peter PT. The "Good Cholesterol" -High-Density Lipoprotein, Cardiology Patient Page, American Heart Association, Inc. 2005.