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# STUDY OF ANTIATHEROSCLEROTIC ACTIVITY OF POLYHERBAL PREPARATION USING RAT AS AN EXPERIMENTAL ANIMAL MODEL

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## ABSTRACT

Atherosclerosis is one of the risk factors for coronary artery disease. The present study highlights the antiatherosclerotic activity of Methanolic extract of polyherbal preparation in experimentally induced atherosclerotic rats. Atherosclerosis was developed in male Albino Wistar rats, which were randomly divided into five groups of six animals each; by feeding with high cholesterol diet for 21 days. Group 1 received normal diet. Group 2 received high cholesterol diet (HCD) which served as control. Group 5 served as standard, administered with Atorvastatin (10 mg/kg) along with HCD and Group 3 and Group 4 were administered with methanolic extract of polyherbal preparation (300 mg/kg and 600 mg/kg) along with HCD. Methanolic extract of polyherbal preparation reduced the raised serum level of total cholesterol, triglyceride , LDL ,VLDL and increased the serum HDL level as compared to the control group(High cholesterol group).There was increased HMG CoA/Mevalonate ratio in liver. There was also depletion of GSH content and increased level of SOD and MDA in liver. Methanolic extract of polyherbal preparation treated groups (300 mg/kg and 600 mg/kg) exhibited less damage to endothelial lining of aorta as compared to control group (High Cholesterol Diet),which may be attributed to hypo-cholesterolemic activity of polyherbal preparation, which may be attributed to hypocholesterolemic activity of polyherbal preparation.

**INTRODUCTION:** Atherosclerosis is a disease of blood vessels and known colloquially as “hardening of the arteries”. It is characterized by the accumulation of fatty substance, cholesterol, cellular waste products, calcium and other substances in the inner lining of an artery. Major complications of atherosclerosis include angina pectoris, myocardial infarction and stroke, which are recognized as leading causes of morbidity and mortality in Western countries. The World Health Organization (WHO) predicted that heart diseases and stroke are becoming more deadly, with a projected combined death of 24 million by 2030 [1].

Due to accumulation of fat, cholesterol and other substances, plaque builds up inside the arteries. Arteries are blood vessels that carry oxygen-rich blood to the heart and other parts of the body.

Over time, plaque hardens and narrows the arteries. This limits the flow of oxygen-rich blood to the organs and other parts of the body. Atherosclerosis can lead to serious problems, including heart attack, stroke, or even death [2]. The development of atherosclerosis is a complex and multistep process.

There are a number of genetic, metabolic, and environmental factors involved in the formation and evolution of the atherosclerotic plaque. Lipoprotein oxidation and oxidative processes in general play an important role in the pathogenesis of atherosclerosis [3].

Disorders of lipid metabolism are menifested by elevation of the plasma concentration of the various lipid and lipoprotein fractions (total and LDL cholesterol, VLDL cholesterol, triglycerides and chylomicrons) they result, predominantly in cardiovascular diseases. So hyperlipidemia is one of the major cause of atherosclerosis and associated conditions [4].

Synthetic antilipidemic drugs clearly reduce cardiovascular mortality but are expensive and sometimes highly prone to side effects. However they are not covered by most health care insurers when used primarily for prophylactic purpose [5]. The plant kingdom represents a rich storehouse of organic compounds,many of which have been used for medicinal and other purpose. There exists a plethora of knowledge, information and benefits of herbal drugs in our ancient literature of Ayurvedic and Unani medicines. Ethanomedicines are replete with description of plant medicines and the grandma's pouch that has been called from years of medical wisdom is excellent proof of efficacy of the experimental medicines [6].

*Acorus calamus* has been indicated in the Indian System of Medicine (ISM) to be useful in the aggravation of 'medas', its hypolipidemic activity has been explored [7]. Its antiatherosclerotic effect may be due to presence of saponin which forms complex with cholesterol by binding plasma lipid and thereby alters cholesterol metabolism and absorption [8]. *Curcuma longa* shows hypocholesterolemic effect due to curcumin which enhance excretion of cholesterol in bile. It also protect LDL from oxidation [9].

*Cyperus rotundus* having antioxidant and anti-atherosclerotic effect due to presence of flavanoids, polyphenols and terpenes which reduce absorption of total cholesterol and triglycerides [10]. *Picrirhiza kurroa having ayurvedic properties of* tikta rasa, laguruksha guna, and katuvipaka. Based on these properties it is having antioxidant and antiatherosclerotic effect [11]. *Plumbago zeylanica,* due to presence of plumbagin, prevents accumulation of cholesterol and triglyceride in liver and aorta and regress atheromatous plaque in thoracic and abdominal aortas [12].

Herbal medicinal preparations play an especially important role in prevention of atherosclerosis. Their therapeutic action is directed against important mechanisms involved in the development of atherosclerosis. The herbal alternative have a very low incident of side effects and can be recommended for medically supervised self-treatment [5].

The aim of the current investigation is to evaluate the antioxidant and antiatherosclerotic activity of the polyherbal formulation containing following six herbal drugs using rat as experimental model.

1) *Acorus calamus*
2) *Curcuma longa*
3) *Cyperus rotundus*
4) *Picrorrhiza kurroa*
5) *Plumbago zeylanica*

**MATERIAL AND METHODS:**

**Plant Material:** Methanolic extracts of all six plants were procured from AMSAR PVT. LTD., Indore.

**Composition of Polyherbal Preparation:**

| Sr. no. | Sanskrit Name | Plant Name | Family | Part Used | Strength |
|---|---|---|---|---|---|
| 1. | Sadgrantha | *Acorus calamus* | Araceae | Rhizome | 25% |
| 2. | Nisa | *Curcuma long* | Zingiberaceae | Rhizome | 12.5% |
| 3. | Mustaka | *Cyperus rotundus* | Cyperaceae | Rhizome | 25% |
| 4. | Katurohin | *Picrorrhiza Kurroa* | Scoruphulariaceae | Rhizome | 25% |
| 5. | Jvalanakhya | *Plumbago Zeylanica* | Plumbaginaceae | Root | 12.5% |

Dried powdered methanolic extracts of all five plants were mixed according to the above praportation. Freshly prepared aqueous solution of this mixed extract (PHE) was used for experimental study.

**Drugs and Chemicals:** Greetish sample of Atorvastatin was obtained from Astron Pharmaceutical Contract Research Organization,Ahmedabad. All other reagents and chemicals were of analytical grade.

**Animals:** Healthy male albino rats (250-350g) of Wistar strain were used for the study with the approval of Institute's animal ethics committee. The animals were purchased from Jay Research Foundation,Surat. The animals were housed in a large spacious cage, bedded with husk and were given food and water. The animal house was ventilated with a 12hr light/dark cycle, throughout the experimental period. Animal experimentation was conducted according to the current institutional regulations. The animals were maintained on a commercial rat feed manufactured by M/s. Pranav Agro Industries Ltd., India, under the trade name 'Amrut rat feed'. The feed contains 5% fat, 21% protein, 55% nitrogen free extract, 4% fiber (wt/wt) with adequate vitamin and mineral content.

After the completion of duration of study (21 days), the animals were fasted overnight and sacrificed by cervical decapitation. Their livers were dissected out on a petri dish kept in an ice-bath to keep the sample fresh. Sufficient care was tsken to complete the entire procedure within 5-7 min to prevent loss of the enzyme activity in liver samples. The aorta was excised immediately, washed with cold saline. The aorta was fixed by 10% formalin for histopathological studies

**Acute Toxicity Studies:** The methanolic extract of polyherbal preparation showed no mortality in Albino Wistar rats up to the dose 3000 mg/kg for duration of 24 hours.Hence,as per OECD guidelines $1/10^{th}$ and $1/5^{th}$ of 3000mg/kg i.e. 300mg/kg and 600mg/kg were selected as doses for the study.

**Methods:**

**Preparation of High Cholesterol Diet (HCD)** [13]: The HCD consist of 95% commercial rat feed, 4% cholesterol and 1% cholic acid.

**Experimental design:** Rats (n=30) were randomized into following groups:

| Group | Treatment |
|---|---|
| I | They were administered with vehicle (saline) for 21 days. |
| II | They were administered with saline for 21 days along with High cholesterol diet. |
| III | Atorvastatin (10mg/kg/day, p.o.) was administered for 21 days along with High cholesterol diet [14]. |
| IV | Methanolic extract of Polyherbal preparation (300 mg/kg/day,p.o.in distilled water) was administered for 21 days along with High cholesterol diet. |
| V | Methanolic extract of Polyherbal preparation (600 mg/kg/day, p.o.in distilled water) was administered for 21 days along with High cholesterol diet. |

After the duration of treatment blood was collected from the retro-orbital sinus and tests were done for listed biochemical parameters.

**Measurement of various Parameters:**

**Physical Parameters [15]:** The body weight was recorded on the first day and then last day of the study period in each group.

**Biochemical Estimations [16]:** Lipid parameters were determined in blood serum. At the end of 21 days,animals were fasted overnight and blood was collected from retro orbital plexus under light ether anaesthesia, centrifuged at 2500 rpm for 20 minutes. The serum obtained will be kept at $4^{o}C$ until used.

The quantitative estimation of lipid profile was carried out using Infinite triglycerides liquid for triglycerides, Infinite cholesterol liquid for total cholesterol and Autozyme for HDL-C, ACCUREX in Brambhatt laboratory, Anand.

Estimation of VLDL-C and LDL-C will be done by using the Friedward's formula.

VLDL-C = Triglycerides/5

LDL-C = Total cholesterol – (HDL-C + VLDL-C)

**Measurement of Coronary Disease Risk Factor** [17]: *Atherogenic Index (AI),* which is a measure of the

atherogenic potential of an agent, was calculated using the following formula and the results were tabulated.

$$\text{Atherogenic Index} = \frac{\text{Total serum triglyceride}}{\text{Total serum HDL-C}}$$

$$\% \text{ Protection} = \frac{\text{AI of control - AI of treated group}}{\text{AI of control}} \times 100$$

**Assay of HMG CoA Reductase Activity** [18]: The method described by Rao and Ramkrishan will be used for the estimation of HMG-CoA reductase activity

The tissue homogenate will be prepared by homogenizing 1 gm of tissue (liver) with 10 ml of saline arsenate solution. Mix equal volumes of the fresh 10% tissue homogenate and diluted perchloric acid. Allow to stand for 5 minute and centrifuge (2000 rpm, 10 minutes). Treat 1.0 ml of filtrate with 0.5ml of freshly prepared hydroxylamine reagent (alkaline hydroxylamine reagent in the case of HMG-CoA), mix, after 5 minute add 1.5 ml of ferric chloride reagent to the same tube and shake well. Take readings after 10 minutes at 540 nm vs. a similarly treated saline/arsenate blank.

**Detrrmination of Antioxidant Parameters**: For estimation of antioxidant parameters,the livers were homogenized (10%w/v) in ice cold phosphate buffer and centrifuged at 2000 rpm for 5 min at 4 ˚C in a cooling centrifuge (Remi Instruments,Mumbai).After centrifugation,the supernatant was separated and stored for further analysis.

**Assay for Thiobarbituric acid reactive substance (Maleic dialdehyde)** [19]: Lipid peroxidation was estimated colorimetrically in the liver by quantifying TBARS according to the method of Ohkawa and Ohishi. For the estimation,0.5 ml of supernatant was treated with 0.2 ml of of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid adjusted to pH 3.5 with NaOH and 1.5 ml of 0.8% solution of thiobarbituric acid.The volume of the mixture was adjusted to 4.0 ml with distelled water and heated in a water bath at 85˚C for 60 min. Light pink color was developed.After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of mixture of n-butenol and pyridine (15:1,V/V) was added.

The mixture was shaken vigorously and them centrifuged at 4000 rpm for 5 min. After centrifugation, the organic layer was separated and its absorbance was read at 532 nm using a UV-visible spectrophotometer (UV-1700, Schimadzu, Japan) against a reagent blank. The amount of TBARS was calculated by using $1.56\times10^{5}M^{-1}cm^{-1}$ as molar extinction co-efficient and the level of lipid peroxidation was expressed as nmol of maleic dialdehyde/mg of protein (MDA).

**Assay for Reduced Glutathion Content** [20]: Reduced glutathione (GSH) was determined colorimetrically by the method of Ellman. The Ellman's reagent was prepared by dissolving 39.6 mg DTNB in 10 ml phosphate buffer (ph=7). For estimation of GSH, 0.5 ml of supernatant was treated with 3.0 mt of ethanol and 0.5 ml of Ellman's reagent. The absorbance of the developed yellow color was read at 412 nm using a UV-visible spectrophotometer (UV-1700, Schimadzu, Japan) against a reagent blank. The reduced glutathione content was calculated by using $13,600M^{-1}$ $cm^{-1}$ as the molar extinction co-efficient as nmol GSH formed/mg protein.

**Determination of Superoxide Dismutase Activity** [21]: Superoxide dismutase activity was assayed in terms of its ability to inhibit the radical-mediated autoxidation of epinephrine; using the method described by Misra and Fridovich.

The reaction mixture consisted of 0.5 ml of carbonate buffer (pH=9.7), 0.5 ml of supernatant, 0.1 mi of EDTA solution ($1\times10^{-4}$ M) and 0.1 ml of Epinephrine solution ($3\times10^{-3}$ M).The changes in the absorbance of this solution was read at 480 nm for 3 min at 30 sec intervals using a UV-visible spectrophotometer (UV-1700, Schimadzu, Japan) against reagent blank. The activity of superoxide dismutase was calculated by using 4020 $M^{-1}$ $cm^{-1}$ as molar extinction co-efficient and expressed as Units/min/mg of protein.

**Histopathology of Aorta:** For histopathology, the rats were sacrificed by cervical decapitation and their aortas were dissected out. During the procedure,ice was used to keep the aorta samples fresh and avoid any degradation. The aortas were stored in 10% formaline solution and sent to a local pathological laboratory for hematoxyline and eosine staining.

**Statistical Analysis:** The results are expressed as mean ± standard error of mean (SEM).The data were analyzed using one-way analysis of variance (one-way ANOVA) followed by Tukey's test for comparison between groups. The criterion for statistical significance was $p<0.05$.

## RESULTS:

**TABLE 1: EFFECT OF METHANOLIC EXTRACT OF POLYHERBAL PREPARATION ON BODY WEIGHT (GM) IN ALBINO WISTAR RATS**

| | Body Weight (gm) | |
|---|---|---|
| **Groups** | **Before treatment** | **After treatment** |
| I | 311.7 ± 4.01 | 321.7 ± 4.01 |
| II | 313.3 ± 4.94 | 366.7 ± 4.94 |
| III | 313.3 ± 4.94 | 325.0 ± 4.28 |
| IV | 310.8 ± 3.74 | 343.3 ± 3.33 |
| V | 313.3 ± 4.94 | 343.3 ± 4.21 |

**TABLE 2: EFFECT OF METHANOLIC EXTRACT OF POLYHERBAL PREPARATION ON SERUM LIPID PROFILE IN ALBINO WISTAR RATS**

| Groups | Serum Total Cholesterol (mg/dl) | Serum Triglyceride (mg/dl) | Serum HDL (mg/dl) | Serum LDL (mg/dl) | Serum VLDL (mg/dl) |
|---|---|---|---|---|---|
| I | **91.98 ± 0.50** | 121.70 ± 0.78 | 36.57 ± 0.38 | 71.40 ± 0.44 | 20.07 ± 0.44 |
| II | **178.70 ± 0.73** * | 160.20 ± 0.60 * | 25.65 ± 0.52 * | 95.32 ± 0.40 * | 31.78 ± 0.38 * |
| III | 101.30 ± 0.98 # | 114.70 ± 1.00 # | 42.80 ± 0.29 # | 77.63 ± 0.46 # | 23.92 ± 0.45 # |
| IV | 147.70 ± 0.89 # | 147.90 ± 0.49 # | 38.22 ± 0.27 # | 87.82 ± 0.56 # | 28.23 ± 0.17 # |
| V | 135.20 ± 0.98 # | 139.80 ± 0.57 # | 40.20 ± 0.30 # | 81.10 ± 0.34 # | 26.38 ± 0.20 # |

Expressed as mean ± SEM, (n=6) for each group. Data were analyzed by One way ANOVA followed by Tukey test; * $p<0.001$, represent significant difference when compared with normal group; # $p<0.001$, represent significant difference when compared with control group(High cholesterol diet).

**TABLE 3: EFFECT OF METHANOLIC EXTRACT OF POLYHERBAL PREPARATION ON CORONARY RISK FACTORS**

| Groups | Ahterigenic Index | % Protection |
|---|---|---|
| I | 3.32 ± 0.04 | - |
| II | 6.26 ± 0.13 * | - |
| III | 2.67 ± 0.02 # | 57.08 ± 1.24 % |
| IV | 3.87 ± 0.02 # | 38.03 ± 1.65 % |
| V | 3.47 ± 0.02 # | 44.32 ± 1.31 % |

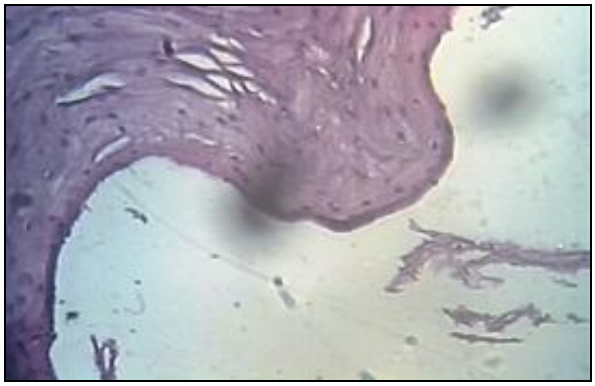
Expressed as mean ± SEM, (n=6) for each group. * $p<0.001$, represent significant difference when compared with normal group; # $p<0.001$,represent significant difference when compared with control group(High cholesterol diet).

**TABLE 4: EFFECT OF METHANOLIC EXTRACT OF POLYHERBAL PREPARATION ON HMGCoA/MEVALONATE RATIO AND ANTIOXIDANT ENZYME LEVELS IN LIVER**

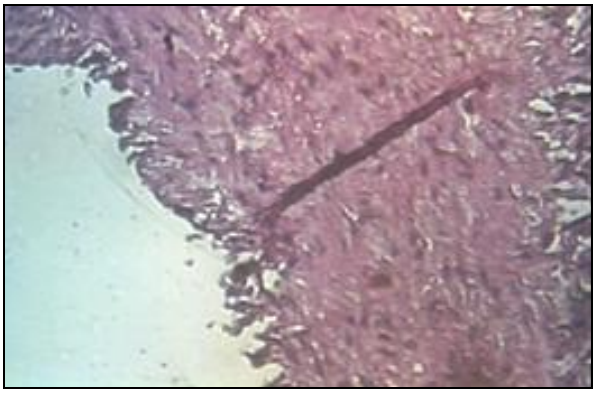
| Groups | HMG-CoA/Mevalonate ratio | SOD (µg/100 mg protein) | GSH (µg/mg protein) | MDA (nmol/mg protein) |
|---|---|---|---|---|
| I | 0.37 ± 0.01 | 13.07 ± 0.17 | 5.66 ± 0.13 | 3.30 ± 0.07 |
| II | 0.40 ± 0.04 | 8.05 ± 0.30 * | 2.59 ± 0.17 * | 9.75 ± 0.16 * |
| III | 2.07 ± 0.21 * | 11.92 ± 0.08 # | 4.85 ± 0.09 # | 4.37 ± 0.12 # |
| IV | 1.12 ± 0.04 * | 9.09 ± 0.10 ## | 3.50 ± 0.22 # | 6.83 ± 0.12 # |
| V | 1.51 ± 0.08 * | 10.45 ± 0.25 # | 4.13 ± 0.17 # | 5.50 ± 0.09 # |

Expressed as mean ± SEM, (n=6) for each group. * $p<0.001$, represent significant difference when compared with normal group; # $p<0.001$, ## $p<0.05$, represent significant difference when compared with control group (High cholesterol diet).

**Histopathology of Aorta:** In histopathology study, normal group showed no pathological changes in endothelial lining. Control group (High cholesterol diet) showed severe damage to endothelial lining. Methanolic extract of polyherbal preparation treated groups (300 mg/kg and 600 mg/kg) showed less damage to the endothelial lining as compared to control group. Atorvastatin treated group showed mild damage to the endothelial lining.



**GROUP- 1**



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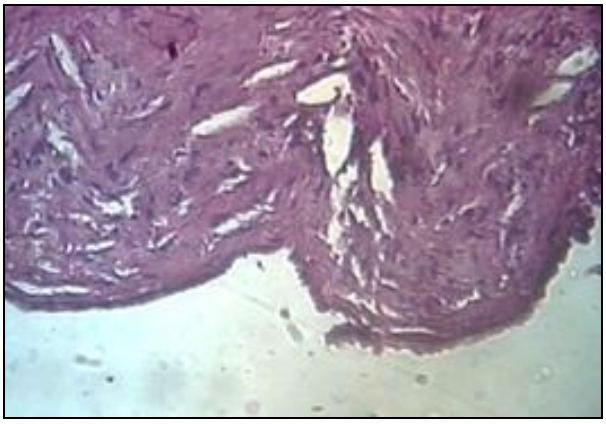

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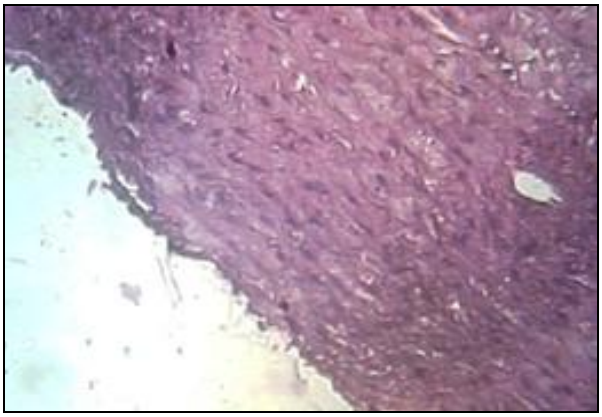
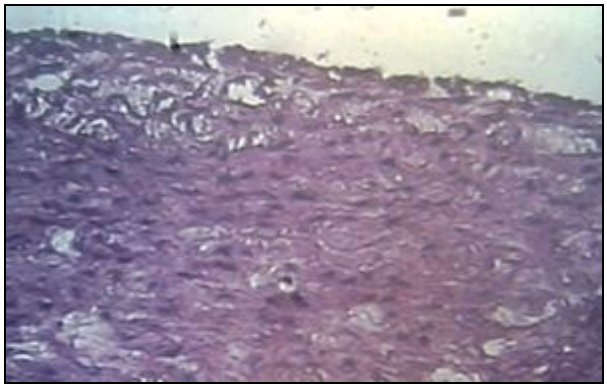


**Group- 4**



**Group- 5**

**DISCUSSION:** In present study,the antiatherosclerotic activity of methanolic extract of polyherbal preparation was evaluated in rats receiving high cholesterol diet (HCD). Determination of body weight in experimentally induced atherosclerosis is considered to be a positive factor to find out the prognosis of disease [22]. Results indicated increase in body weight of animals from the beginning to the end of the experiment in all five groups, but at the end, increase in body weight is low in methanolic extract of polyherbal preparation treated groups as compared to high cholesterol diet treated group.

Cholesterol is an essential structural element of the biological membranes.In addition, it is the precursor of many compounds such as synthesis of bile acids,steroid hormones and vitamin D. Despite this, high concentration of serum cholesterol increases the risk of coronary heart dieses (CHD) [23].

Our study demonstrated that rats fed with a high cholesterol diet (control group) exhibited a higher level of total cholesterol in serum as compared to rats fed with a standard laboratory diet (Normal group); while oral administration of methanolic extract of polyherbal preparation dose (300 mg/kg, 600 mg/kg, p.o) reduced the raised level of total cholesterol in serum.

In our study, the administration of methanolic extract of polyherbal preparation dose (300 mg/kg,600 mg/kg, p.o) significantly lowered triglyceride and VLDL level in serum. It is widely accepted that the elevation of plasma LDL-C level is major risk factor for CHD [24]. Direct correlation between LDL-C level and atherosclerosis as well as the reversibility of the related pathological events by lowering the serum level of LDL-C has already been reported [25].

The results indicated that, the high concentration of LDL-C in serum was significantly reduced by oral administration of methanolic extract of polyherbal preparation; which might constitute a good candidate for the treatment of atherosclerosis by lowering serum LDL-C level.

Another risk factor for developing atherosclerosis is the reduced serum level of HDL-C. This effect which is largely attributed to its central role to reserve cholesterol transport, a process whereby excess cell cholesterol is up taken and which is subsequently processed by HDL-C particles for further delivery to the liver for metabolism [26]. Therefore, it is logical that an increase in HDL-C level can contribute to lower the risk of atherosclerosis [27]. The results clearly indicated that methanolic extract of polyherbal preparation was capable of increasing level of HDL-C in serum.

High atherogenic index (A.I.) is believed to be an important for atherosclerosis. Our data clearly indicated that methanolic extract of polyherbal preparation is capable of potentially decreasing this risk factor. Similar results were reported by chloroform extract of *Mimosa pudica* Leaves [28].

HMG CoA reductase is the rate limiting enzyme in the cholesterol biosynthetic pathway. It converts HMG CoA to mevalonate. In the present study, HMG CoA reductase activity was indirectly measured in terms of the ratio between HMG CoA and mevalonate [18]. The ratio was found to be inversely prapotional to HMG CoA reductase activity, indicated that an increase in the ratio inferred a decrease in the enzyme activity.The methanolic extract of polyherbal preparation produced a significant and dose dependant (300 mg/kg and 600 mg/kg,p.o.) increase in HMG CoA/mevalonate ratio in liver as compared to the normal group.Similar results were also reported with ethanolic extract of *Ficus religiosa* Linn leaves [29].

Many reports have demonstrated that high Cholesterol diet-induced atherosclerosis has tight relations with vascular damage and oxidative stress, which is involved in the pathogenesis of a great number of diseases [30]. Atherosclerosis leads to tissue oxidant stress, due to reduction in antioxidant capacity and free radical load generated by high cholesterol diet.

Recently, some clinical studies suggest dyslipidaemia as one of the major risk factors for coronary disease, while preclinical observations demonstrate that atherosclerosis promotes accumulation of oxidised low-density lipoprotein (Ox-LDL) in the arterial wall, which plays a major role in the initiation and progression of the cardiovascular dysfunction associated with atherosclerosis [31]. Since oxidation of LDL plays a significant role in atherogenesis, amelioration of oxidative stress is equally important as controlling or decreasing dyslipidaemia. In this work, we investigated activities of three major defensive antioxidant agents.

SOD is the first line of defence against free radicals, while GSH-Px is responsible for most of the decomposition of lipid peroxides in cells and may thus protect the cell from the deleterious effects of peroxides. In agreement with previous reports [32], it was observed a significant decrease in the accumulation of SOD and GSH-Px in atherosclerotic rats, compared to those in the normal group. In addition, the content of MDA, the product of lipid peroxidation, was elevated in the atherosclerotic rats.

Taken together with the above results, the imbalance between oxidative stress generation and antioxidants formation could occur after feeding a high cholesterol diet. Nevertheless, Methanolic extract of polyherbal preparation (300 mg/kg,p.o. and 600 mg/kg,p.o.) could prevent this pathological process, which indicated its therapeutic and preventive effect on hepatosteatosis induced by high cholesterol diet.

It was derived from present study that curcuma longa extract has a hypocholesterolemic effect on rats fed on a high cholesterol diet to induce experimental atherosclerosis. This effect could be explained on the basis of an enhanced excretion of cholesterol in bile by curcumin with a concomitant reduction in bile cholesterol saturation and elevated faecal fat excretion [33]. It means that the excess cholesterol from diet is removed by excretion. However, in the control group cholesterol is accumulated in plasma and tissue. Furthermore, it has been suggested that *Curcuma longa* extract produces a decrease in triglyceride levels in human [34].

It is generally assumed that some antioxidants can prevent atherosclerosis by protecting LDL from oxidation [35] and are also associated with an antihypercholesterolemic effect. For the above reason, It was investigated whether curcuma longa extract could influence the development of atherosclerotic lesions after its antioxidant activity and a hypocholesterolemic effect was found. In present study the same positive effect conformed its antiatherosclerotic activity.

*Picrirhiza kurroa* contains iridoid glycosides (including picroside I, II, III, pikuroside, kutkoside and 6-feruloyl catalpol), cucurbitacin glycosides, androsin, apocynin, and other organic acids such as vanillic and cinnamic acids. It is having ayurvedic properties of tikta rasa, laguruksha guna, and katuvipaka. Based on these properties, one may anticipate its pharmacodynamic activity on lipids specifically related to lipid disorders [36]. Hypolipidemic, free radical scavenger, antioxidant and anti-inflammatory effect of Picrorhiza kurroa has been investigated [37]. The current study further confirmed its antioxidant and hypolipidemic activity.

It has been demonstrated that saponins which are present in *Acorus calamus*, are known to form complexes with cholesterol by binding plasma lipids, there by altering cholesterol metabolism and lipid absorption [38]. The presence of tannins and saponin in *Capparis deciduas* causes the inhibition of lipid absorption [39]. So here, antiatherosclerotic effect of Acorus calamus might be due to presence of saponins.

It also has been reported that treatment with Plumbagin, an active constituent of *Plumbago zeylanica*, prevented the accumulation of cholesterol and triglyceride in liver and aorta and regressed atheromatous plaque of the thoracic as well as abdominal aortas [40, 41].

Plumbagin also having antiplatelet activity which is useful in many coronary heart diseses [42]. In present study, it was found that treatment group showed reduced cholesterol and triglyceride level as compare to normal group.

*Cyperus rotundus* significantly reduces total cholesterol as compared to the normal control. The activity may be due to the presence of flavanoid compounds.

In addition, *Cyperus rotundus* containing tannins, polyphenols, flavanolols, terpenes, alkaloids, and saponins. Its antioxidant property may be attributed to these phytochemicals [43].

**CONCLUSION:** It can be concluded from the present study that methanolic extract of polyherbal preparation at the dose of 300 mg/kg and 600 mg/kg,p.o. shows antiatherosclerotic activity in high cholesterol diet model in rats. Methanolic extract of polyherbal preparation may have antiatherosclerotic activity due to inhibition of the HMG CoA enzyme pathway and its antioxidant effect.

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**REFERENCES:**

1. Pilaipark C, Panya K, Yupin S, Srichan P, Noppawan PM, Laddawal P, Piyanee R, Supath S, Klai-upsorn SP: The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. Leaves. Journal of Ethnopharmacology 2008; 2: 47-52.
2. National heart Lung and Blood Institute. People Science Health. U.S. Department Health and Human Services 2011.
3. Ramıirez MC, Mesa MD, Aguilera MC, Quiles JL, Baro L., Ramirez CL, Martinez E, Gil A**:** Oral administration of a turmeric extract inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental atherosclerosis. Atherosclerosis 1999; 147: 371-378.
4. Bennett PN, Brown MJ. Clinical Pharmacology. London: Churchill Livingston. Ninth Edition 2003: 581.
5. Karin K, Christopher H. Pocket Guide to Herbal Medicine. Health and Fitness. Thieme 2004: 72-73.
6. Pandey HP, Bhavaprakasa: Chaukambha sauskrut sansthan. Varasani, Edition 2,Vol. 25,2006: 407.
7. Reshma SP, Sushma A: Mengi Hypolipidemic activity of *Acorus calamus* L. in rats. Fitoterapia 2002; 73: 451-455.
8. Johns T, Chapman LI, Amason JT,Mata R,Romeo JT:Phytochemistry of medicinal plants. Kluwer Academic Publishers,Third Edition 1995.
9. Ramıirez MC, Mesa MD, Aguilera MC, Quiles JL, Baro L, Ramirez CL, Martinez E, Gil A**:** Oral administration of a turmeric extract inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental atherosclerosis. Atherosclerosis 1999; 147: 371-378.
10. Soumaya K, Ines B, Ribai B, Mohamed B, Ines S, Jihed B, Amor M, Marie-Geneviève D, Kamel G, Leila C: Chemicalinvestigation of different extracts and essential oil from the tubers of (Tunisian) *Cyperus rotundus*. Correlation with their antiradical and antimutagenic properties.Annals of Microbiology 2007; 57: 657-664.
11. Hyeung SL, Hyo CA, Sae KK**:** Hypolipemic effect of water extracts of *Picrorrhiza rhizoma* in PX-407 induced hyperlipemic ICR mouse model with hepatoprotective effects.A prevention study. Journal of Ethnopharmacology 2006; 105: 380-386.

12. Vijayakumar R,Senthilvelan M, Ravindran R, Sheela DR: *Plumbago zeylanica* action on blood coagulation profile with and without blood volume reduction. Vascular Pharmacology 2006; 45: 86-90.
13. Mohana T, Navin AV, Jayachandaran KS, Sivasitambaram ND: Protective effect of grape seed proanthocyanidins against cholesterol cholic acid diet-induced hypercholesterolemia in rats. Cardiovascular Pathology 2011; 20: 361–368.
14. Shirya R,Kristine DU,Gary FL:Effect of Atorvastatin on High-Density Lipoprotein Apolipoprotein A-I Production and Clearance in the New Zealand White Rabbit. Circulation 2002; 106: 2955-2960.
15. Novelli ELB, Diniz YS, Galhardi CM, Ebaid GMX, Rodrigues HG, Mani F, Fernandes AAH, Cicogna AC, Novelli Filho JLVB: Anthropometrical parameters and markers of obesity in rats. Lab. Animal 2007; 41: 111–119.
16. Ching-Yee K, Candy Ngai-Yan W, Mabel Yin-Chun Y, Peter Hoi-Fu Y, Alice Lai Shan A, Christina Chui-Wa P, Sai-Wang S, Tsz-Yan L: Consumption of dried fruit of *Crataegus pinnatifida* (hawthorn) suppresses high-cholesterol diet-induced hypercholesterolemia in rats. Journal of functional foods 2010; 2: 179 –186.
17. Ahire AE, Laddha KS: Hypolipidemic effects of Carthamus tinctorius in rats. Indian Drugs 2005; 42: 545-546.
18. Venugopal A, Ramarishna S: Indirect assessment of Hydroxymethylglutaryl-CoA reductase (NADPH) activity in liver. Clin.Chem 2005; 21: 1523-1525.
19. Ohkawa H,Ohishi W,Yahik K: Assay of lipid peroxidation in animal tissues by thiobarbituric acid reaction.Anal. Biochem 1999; 95: 351-358.
20. Ellman GC: Tissue sulfhydryl group. Arch. Biochem.Biophys 1995; 82: 70-77.
21. Misra H, Fridovich I: The role of superoxide anion in the autoxidation of epinephrine and a simple assay for uperoxide dismutase. J. Biol. Chem 1997; 247: 3170-3175.
22. Mohana T, Navin AV, Jayachandaran KS,Sivasitambaram ND: Protective effect of grape seed proanthocyanidins against cholesterol cholic acid diet-induced hypercholesterolemia in rats. Cardiovascular Pathology 2011; 20: 361–368.
23. Libby P, Aikawa M, Schonbeck U: Cholesterol and atherosclerosis. Biomed. Biochim Acta 2000; 15: 299-309.
24. Bbrliner JA, Hetnecke JW: The role of oxidised lipoproteins in atherosclerosis.Free Radic Biol Med 1996; 20: 707-727.
25. Ross R: Atherosclerosis: an inflammatory dieses. N. Engl J. Med 1999; 340: 115-126.
26. Martinez LO, Jacquet S, Terce F, Collex X, Perret B, Barbaras R: New insight on the molecular mechanisms of high-density lipoprotein cellular interactions. Cell Mol Life Sci 2004; 61: 2343-2360.
27. Nofer JR, Kehrel B, Fobker M, Levkau B, Assmann G, Eckardstein AV: HDL and atherosclerosis: Beyond reverse cholesterol transport.Atherosclerosis 2002; 6: 1-16.
28. Rekha R, Ekambaram K: Hypolipidemic Activity of Chloroform Extract of *Mimosa pudica* Leaves. Avicenna Journal of Medical Biotechnology 2010; 2: 215-221.
29. Manal AH**,** Tahrir ST, Dokki, C: Beneficial effect of *Ficus religiosa* Linn. on high-fat-diet-induced hypercholesterolemia in rats. 2011; 129: 162-170.
30. Yan MX, Li YQ,Meng M,Ren HB,Kou Y: Long-term high-fat diet induces pancreatic injuries via pancreatic microcirculatory disturbances and oxidative stress in rats with hyperlipidemia. Biochemical and Biophysical Research Communications 2006; 347: 192–199.
31. Czako L, Szabolcs A, Vajda A, Csati S,Venglovecz V, Rakonczay ZJ: Hyperlipidemia induced by a cholesterol-rich diet aggravates necrotizing pancreatitis in rats.European Journal of Pharmacology 2007; 572: 74–81.
32. Visavadiya NP, Narasimhacharya AVRL: Hypocholesteremic and antioxidant effects of *Withania somnifera* (Dunal) in hypercholesteremic rats. Phytomedicine 2007; 14: 136–142.
33. Rao SD, Chandrasekhar N, Styanarayana MN and Srinivasan M:Effect of curcumin on serum and liver cholesterol level in rat.J Nutr 1970; 100: 1307–1315.
34. Deshpande UR, Joseph LJ, Manjure SS, Samuel AM, Pillai D, Bhide SV: Effects of turmeric extract on lipid profile in human subjects.Med Sci Res 1997; 25: 695–698.
35. Viana M,Barbas C,Bonel B,Bonet MV, Castro M, Fraile MV: *In vitro* effects of a flavonoid rich extract on LDL oxidation. Atherosclerosis 1996; 123: 83–91.
36. Koolman AH, Bloks VW, Oosterveer MH, Jonas I, Kuipers F, Sauer PJ: Metabolic responses to long-term pharmacological inhibition of CB1-receptor activity in mice in relation to dietary fat composition.Int J Obes (Lond) 2010; 34: 374–384.
37. Musso G, Gambino R, Cassader M: Non-alcoholic fatty liver disease from pathogenesis to management: An update.Obes Rev 2010; 11: 430–445.
38. Oakenfull D: Saponin in food.Food Chem 1981; 6: 19-40.
39. Goyal R, Grewal RB: The influence of Teent (*C. decidua*) on human plasma triglyceride, total lipids and phospholipids.Nutr. Health 2003; 17: 71-76.
40. Sharma I, Gusain D, Dixit VP: Hypolipidaemic and antiatherosclerotic effects of plumbagin in rabbits. Ind. J. Physiol. Pharmacol 1991; 35: 10-14.
41. Sudha R, Pendurkar S, Mengi A*:* Antihyperlipidemic effect of aqueous extract of *Plumbago zeylanica* roots in diet-induced hyperlipidemic rat 2009; 47: 1004-1010.
42. Vijayakumar R, Senthilvelan M, Ravindran R, Sheela Devi R**:** *Plumbago zeylanica* action on blood coagulation profile with and without blood volume reduction. Vascular Pharmacology 2006; 45: 86-90.
43. Atiat ME, Farok MA, Usama EM, Yasser ME, Rasha HH: Studies of effect of using saffron, cyperus, manuka honey and their combination on rats suffering from hyperglycemia. 2011, 27: 2295-2298.


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