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Original Article

Effect of Combination of Polyherbal Extracts for treatment of Hyperlipidemia using Animal Model

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ABSTRACT

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to prevalence and severity of coronary heart diseases. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death. There are mainly two types of hyperlipidemia one is the primary hyperlipidemia caused due single genetic defect, and secondary hypolipidemia is diabetes, myxoedema, nephritic syndrome, chronic alcoholism, with use of drugs like corticosteroids, oral contraceptives, Beta- blocker*Justica adhatoda* leaves, *mimosa pudica* leaves, *Trigonella foenum-gracecum* seeds are traditionally used as antihyperlipidemic drugs as per Ayurvedic literature. Hence the present study is undertaken to investigate the anti hyperlipidemic effect of polyherbal preparation using the above three medicinal plants against high fat diet induced and poloxamer-407 induced hyperlipidemia in rats.

Keywords: Hyperlipidemia, *Justica adhatoda, mimosa pudica*

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INTRODUCTION

Hyperlipidemia has turned up as one of the major health concerns in the 21st century and is one of the leading causes of preventable death. It has reached epidemic proportions globally, with more than 1 billion adults overweight - at least 300 million of them clinically obese - and is a major contributor to the global burden of chronic disease and disability. Often coexisting in developing countries with under-nutrition, it is a complex condition, with serious social and psychological dimensions, affecting virtually all ages and socioeconomic groups (WHO, 2009).

Presently, the existing drugs used in CVD are associated with side effects such as abnormal liver disease, diarrheal, gastric irritation and nausea. Several herbs have been reported to reduce high blood cholesterol without negative side effects and they are relatively affordable [6]. This shows a need for planned activity guided phyto-pharmacological evaluation of herbal drugs.

The high expenses and side effects of hyperlipidemia medications have led many populaces to search for studies have been conducted to evaluate the effects of herb mentioned in ayurvedic text on hyperlipidemia. *Vasaka adhatoda* leaves, *mimosa pudica* leaves, *Trigonella foenum-gracecum* seeds are traditionally used as antihyperlipidemic drugs as per Ayurvedic literature. Hence the present study is undertaken to investigate the anti hyperlipidemic effect of polyherbal preparation using the above three medicinal plants against high fat diet induced and poloxamer-407 induced hyperlipidemia in rats.

The present study, "Effect of combination of polyherbal extracts for treatment of hyperlipidemia using animal models." was undertaken at the Department of Pharmacology, Appasaheb Birnale College of pharmacy, Sangli with the following objectives:

To evaluate antihyperlipidemic activity in combination of on hyperlipidemia. *Justica adhatoda* leaves, *mimosa pudica* leaves, *Trigonella foenum-gracecum* seeds extracts, in,

1. Poloxamer 407 induced hyperlipidemia.
2. High cholesterol diet induced hyperlipidemia.

MATERIALS AND METHODS

Procurement, authentification, drying and storage of plant material: The leaves of *Justica adhatoda* and *Mimosa pudica* leaves were collected in month September 2015 from local area of Sangli and *Trigonella foenum-graecum* seeds were purchased from local market of Sangli. The leaves and seeds were authenticated by Mr. M.D. WADMARE botanist of Smt. Kasturbai Walchand College (Arts-Science), Sangli. And voucher specimen deposited in same college. The leaves of *Justica adhatoda*, *Mimosa pudica* and seeds of *Trigonella foenum gracecum* were shade dried for a week then immediately the leaves and seeds are grinded and coarse powder was prepared by passing through the sieve no.85.

Preparation of extracts: Different extracts were prepared from *Justica Adhatoda* leaves, *Mimosa pudica* leaves and *Trigonella foenum-gracecum* by following methods.

Preparation of *Justica adhatoda* leaves extract: The powdered leaves of *A. vasica* were exhaustively extracted with 90% ethanol in a Soxhlet's appratus. The ethanolic extracts of *A. vasica* (AVE) thus obtained, were collected and concentrated using rotary evaporator under reduced pressure at less than 40°C. Then the extracts were stored until used for further study [54].

Preparation of *Mimosa Pudica* leaves extract: The coarsely powdered leaves (300 *g*) of *Mimosa pudica* was extracted to exhaustion in a soxhlet apparatus (9) at 50 ^{0} C with 500 mlof chloroform. The extract was filtered through a cotton plug, followed by whatman filter paper (No.1) and then concentrated by using a rotary evaporator at a low temperature (40 - 60^0*c)* and reduced pressure to provide chloroform extractive. Then the extract was kept in vaccume desicator to remove excess moisture present in extract [55].

Preparation of *Trigonella foenum-graecum* seeds extract: 500 g of dried and coarsely powdered seeds of Trigonella foenum-graecum was extracted with petroleum ether by maceration for two days. Filter and mark is extracted with 95% ethanol in soxhlet apparatus for 6 hr. Filter the filtrate. The filtrate was concentrated on water bath using petridish. The temperature was maintained at 55^0 C. Then the extract was kept in vaccume desicator to remove excess moisture present in extract [47].

PHYTOCHEMICAL ANALYSIS OF EXTRACTS:

Following chemical test were carried out for different extracts to identify the presence of various phytochemical constituents. (Kokate, 1991; Khandelwal, 2001 ;)

Test for alkaloids: Mayers test, Dragendroff's reagent, Wagners reagent, Hager's reagent.

Test for saponins: Foam test.

Test for cardiac glycosids: Baljet test, Legal's test.

Test for steroids: Salkowaski reaction, Libermann Burchard test.
Test for tannins and phenolic compounds: Diluted ferric chloride, Lead acetate, Potassium Dichromate, Aqueous bromine solution.
Test for flavonoids: Shinoda's test (Mg/HCL test-Magnesium turnings test), Resdue+lead acetate.
Test for Proteins- Biuret test, Millons test
Test for carbohydrates- Molish test, fhelings test.

Apparatus and equipments:

1. Labline variable volume micropipettes.
2. Water bath.
3. Tuberculin syringe,oral feeding needle.
4. Microcapillary tubes.
5. The common laboratory glassware of borosil glass, cotton, syringes, dissection instruments(sterile whenever necessary)

Table 1: List of Instruments

Sr. No.	Instrument	Manufacturer
1	UV-visible spectrophotometer	Jasco, model V-530
2	Incubator	Kumar model india
3	Laboratory centrifuge	Remi C-24
4	Animal weighing balance	Shimadzu
5	Analytical weighing balance	Shimadzu, Model-AX200
6	Biopac MP-35	Santa Barbara, CA, USA
7	Semi-autoanalyzer	MISPA PLUS

Drug solution:
The atorvastatin was prepared in distilled water.
The poloxamer was prepared in cold distilled water.
The ethanolic extract of Adhatoda vasika was prepared in distilled water.
The ethanolic extract of trigonella foenum-graecum was prepared in distilled water.
The chloroform extract of Mimosa pudica was prepared in suspension of gum acacia.

Dose selection: In order to deside the dose of plant extract it is essential to go through the toxicity study of the extract according to OECD guidelines. As per OECD guideline the LD50 of *Justica Adhatoda* is given as 2000mg/kg, LD50 dose of *Mimosa pudica* 2000mg/kg and LD50 of *Trigonella foenum-graecum* is given as 1000mg/kg. Therefore a dose below 1/10th of above dose was taken as safe dose i.e. 200mg/kg, 200mg/kg and 100mg/kg respectively.

Table 2: Drug used with route of administration:-

SR NO.	Drug Used	Dose	Route of administration
1	Atorvastatin	HCD-10mg/kg P-407-1.34mg/kg	Oral Oral
2	Poloxamer 407	500mg/kg	Intraperitoneal
3	High cholesterol diet	1ml/100gm	Oral
4	Plant extracts Justica adhatoda+ Mimosa pudica+ Trigonella foenum-graecum	200mg/kg+200mg/kg+100mg/kg	Oral

Table 3: Chemicals and kits used

SR NO.	Chemicals	Company
1	Atorvastatin	Cipla Ltd
2	Poloxamer	Sigma Aldrich.USA
3	Triglyceride kit	Biolab diagnostics Pvt, Ltd. Tarapur
4	Total cholesterol kit	Span Diagnostic Ltd.Surat
5	HDL cholesterol kit	Coral Clinical system, Goa
6	Cholesterol	Research-Lab Fine chem. Industries(Mumbai)
7	Cholic acid	Research-Lab Fine chem. Industries(Mumbai)
8	Pyrogallol	Sigma Aldrich,pvt.Ltd. Banglore
9	Anesthetic ether	Research-Lab Fine chem. Industries(Mumbai)
10	Tris buffer	Sigma Aldrich., Pvt. Ltd. Banglore
11	Ethanol	Research-Lab Fine chem. Industries(Mumbai)
12	Chloroform	Research-Lab Fine chem. Industries(Mumbai)

Animals used:

Species :Wistar rats
Age/weight :4-6 weeks (180-200gm)
Gender :Male/Female
Number to be used : 42
Number of days each animal will be housed :As per requirement

Experimental Animals: Wistar rats were obtained from the animal house of Pharmacology Dept. of Appasaheb Birnale College of Pharmacy, Sangli, were used with approval of the Institutional Animal Ethics Committee (Reg. No. IAEC/ABC/02/2015-16) of male rats weighing 180-200 gms were housed in groups of 3-4. All rats were fed with pelleted diet and tap water ad libitum. The animals were allowed to acclimatize under laboratory conditions prior to experimentation. Each group of animals was housed separately distinct throughout the study.

SCREENING METHODS (HYPOLIPIDEMIC STUDY)

HIGH CHOLESTEROL DIET (HCD) INDUCED HYPERLIPIDEMIA IN RAT:-

Preparation of High Cholesterol Diet (HCD): Diet cocktail contained 100g cholesterol, and 50g cholic acid in 1 liter of coconut oil. All ingredients were of the highest analytical grade and supplemented with egg yolk.

Experimental procedure: Twenty four albino rats weighing 180-200g were obtained from the animal house of Appasaheb Birnale College of pharmacy, Sangli. They were maintained at standard housing conditions and fed with commercial standard diet (Amrut agro Ltd, Sangli) and provided with water ad libitum during the experiment. Hypercholesterolemia was induced in rats by daily intragastric administration of cocktail diet (1ml/100gm) and also fed egg yolk. Serum cholesterol level was monitored monitored after a 14 hrs fasting by collecting blood samples by retro-orbital method under light anaesthesia with diethylether. Body weight was recorded on every 5th day. The amount of food intake was measured every day. After induction of hypercholesterolemia confirmed at a serum cholesterol level greater than 140mg/dl, the rats were further randomly subdivided into six groups. Each group contained six animals.

Inducing control group receives cocktail diet (1ml/100gm) for 20 consecutive days. Standard and test groups receive cocktail diet (1ml/100gm) for 10 consecutive days and from 11th to 20th day they receive treatment along with cocktail diet. Rats were fasted 8 hours after final sample treatment; various parameters were carried out as described below. Blood was collected by retro orbital sinus puncture under light ether anaesthesia. The blood was centrifuged at 3000 rpm for 10 minutes. Serum was separated and estimated for lipid parameters such as cholesterol, triglycerides and HDL using commercially available kits and analysed by Semi-autoanalyser[57].

Experimental Design:

Figure 4: Experimental design for HCD induced hyperlipidemia in rat.

Group	Treatment
I	Normal group(Vehicle or untreated)
II	Hyperlipidemic control(High fat diet 1ml/100gm)
III	High fat diet + Atorvastatin (10mg/kg p.o.)
IV	High fat diet + Plant extracts (500mg/kg p.o)

ESTIMATION:

Hemodynamic study: Blood pressure recording (non-invasive/ tail cuff method)[56]

Lipid profile in serum: The following lipids were estimated in plasma by using specific Kits from Biolab diagnostic kits.

a) Total Cholesterol (TC)
b) Triglycerides (TG)
c) HDL Cholesterol (HDL-C)
d) LDL Cholesterol (LDL-C)
e) Atherogenic index

Estimation of *In Vivo* Antioxidants activity using tissue Homogenate

Tissue Antioxidant Biomarkers: Malondialdehyde by method of Ohkawa et al (SOP No 9) [13]. Superoxide dismutase (SOD) enzyme activity by the method of Marklund et al (SOP No 10) [14]. Catalase (CAT) enzyme activity by the method of Aebi et al (SOP No11) [15]. Total protein by Biuret method (SOP No 12).

POLOXAMER 407 INDUCED HYPERLIPIDEMIA IN RATS:-

Preparartion of Poloxamer: The poloxamer was made at a final concentration of 500mg/kg

30%w/w by dissolving the powder in normal saline solution ; the solution was then kept refrigerated overnight to facilitate its dissolution. Needles and syringes used to administer poloxamer were cooled prior to administration to prevent poloxamer gelation within the syringe. Poloxamer was administered i.p to the rat [57].

Induction of Hyperlipidemia: Hyperlipidemia was induced by poloxamer (500mg/kg b. wt.) administered i.p

Experimental procedure: Experimental animals were divided into the normal control group, the control group from which hyperlipidemia was induced by use of poloxamer-407 (500mg/kg), and test group received poloxamer-407 with drug. To the normal group, feed and water were unlimitedly supplied. To the other groups, poloxamer-407 was administered at 3 days intervals (i.e. 1st, 4th, 7th, 10th, 13th, 16th, 19th day), from the first day of the experiment until the 21st day. Blood was collected by retro orbital sinus puncture under light ether anaesthesia. The blood was centrifuged at 3000 rpm for 10 minutes. Serum was separated and estimated for lipid parameters such as cholesterol, triglycerides, LDL, HDL and VLDL using commercially available kits and analysed by Semi-autoanalyser[57].

Experiment Design

The male wistar rats weighing 180-200 gm was used for the experimental study. The animals were divided into four groups of 6 animals each and fed orally.

Table 5: Experimental design for poloxamer-407 induced hyperlipidemia in rats.

Group	Treatment
I	Normal group(Vehicle or Untreated control)
II	Hyperlipidemic control (Poloxamer 500mg/kg, i.p.)
III	Poloxamer +Standard (Atorvastatin 1.34mg/kg, p.o.)
IV	Poloxamer + Plant extracts(500mg/kg p.o)

ESTIMATION

Hemodynamic study: Blood pressure recording (non-invasive/ tail cuff method)[56]

Lipid profile in serum: The following lipids were estimated in plasma by using specific Kits from Biolab diagnostic kits.

a) Total Cholesterol (TC)
b) Triglycerides (TG)
c) HDL Cholesterol (HDL-C)
d) LDL Cholesterol (LDL-C)
e) Atherogenic index

Estimation of *In Vivo* Antioxidants activity using tissue Homogenate: The following *In Vivo* antioxidants were estimated in tissue homogenates of aorta **Tissue Antioxidant Biomarkers:-**

Malondialdehyde by method of Ohkawa et al (SOP No 9) [13].

Superoxide dismutase (SOD) enzyme activity by the method of Marklund et al (SOP No 10) [14].

Catalase (CAT) enzyme activity by the method of Aebi et al (SOP No11) [15].

Total protein by Biuret method (SOP No 12).

Statistical Analysis: All the results were expressed as mean ± SEM. The Statistical significance between means was analysed using one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison post-test using Graph Pad software. P-values < 0.01 were considered significant.

DISCUSSIONS

Ayurveda is a system of Indian traditional form of alternative medicine. In 20th and 21 century due to side effects of synthetic drugs, there is an increasing interest in Ayurvedic proprietary medicines. The most essential criteria of herbal plants possess the phytopharmacological activity. The combination or substitution of herbal plants achieved the therapeutic effectiveness as compared to single herbal plant. It provide the greater scope of their utilization, easy availability, cost effectiveness, no side effects and most appropriate for clinical conditions, as per journals of medicinal plants 2013[58].

Preliminary phytochemical screening of *Justica adhatoda*, *trigonella foenum-graenum*, and *mimosa pudica* extracts showed that they contains alkaloids, glycosides, saponins, proteins and amino acids, flavonoids, phenolic compounds, tannins.

Hyperlipidemia, is condition of elevated serum lipoproteins, total cholesterol, and triglycerides, is a major risk factor of atherosclerosis, myocardial infarction and stroke. It is one of the major causes of premature death globally. It is associated with cardiovascular diseases which are promoting or increase the focus on development of novel and effective antihyperlipidemic drugs along with no any side effects [1].

There are various animal models are used to study the pathophysiological effects of hyperlipidemia. Genetic variants of HL can be induced by means of dietary and also by means of poloxamer-407. Earlier studies reported by Hetal R have also proved effectiveness of these models for induction of hyperlipidemia.

High Cholesterol Diet Induced Hyperlipidemia:
Rats fed with a diet supplemented with 100gcholesterol and 50gm Cholic acid in coconut oil withegg for 20 days served as the experimental model. Thisis in accord with previous finding reported by NaYoung Yoon et al; 2008 who showed that feeding ratswith high cholesterol diet for 7 days inducedhyperlipidemia. Similar results have been reported byHossam M.M. Arafa 2005, feeding rats with an HCDfor 7 consecutive days resulted in markedhypercholesterolemia. Also Palaninathan Varalakshmiet al; 2006 have demonstrated that feeding. wistar ratsfor 30 days with high cholesterol diet increased the serumlipids. The mechanism of action of cholic acid is twofold that it cause increase in cholesterol absorption and aconcomitant suppression of cholesterol 7a-hydroxlyaseactivity that results in decreased cholesterol excretion cholic acid improves cholesterol absorption by itsemulsifying property[59].

Diet containing Saturated fatty acids (Lard) increases the activity of HMG CoA-reductase, the rate determining enzyme in cholesterol biosynthesis; this may be due to higher availability of acetyl CoA, which stimulates the cholesterogenesis rate. Moreover, this could be associated with a down regulation in LDL receptors by the cholesterol in the diet, which could also explain the elevation of serum LDL cholesterol levels or non-HDL levels either by changing hepatic LDL receptor activity, the LDL production rate or both the activity of CETP, a key enzyme in reverse cholesterol transport and HDL metabolism increases in HFD and mediates the transfer of cholesteryl esters from HDL cholesterol to TG rich particles in exchange for TG. This leads to increased plasma concentrations of TGs & decreased concentrations of HDL cholesterol [5].

Body weight- The body weight was significantly increased in high cholesterol diet control group as compared to normal group. In present study, administration of combination of polyherbal extract 500mg/kg dose showed there was weight gain similar to normal group.

Haemodynamic study- The polyherbal extract shown significant reduction in systolic blood pressure, diastolic blood pressure, mean blood pressure. the present study have shown good preventive effect of polyherbal extract on hypertension as increased blood pressure is reduced in groups treated with extracts.

Lipid profile-
Total cholesterol and triglyceride-Therewas significant elevation in plasma TC and TG in high cholesterol diet treated group as compared to normal group. The high cholesterol diet induced in TC and TG concentration were significantly ($P<0.05$) suppressed by animal treated with atorvastatin and combination of polyherbal extract. Atorvastatin used as standard drug. Atorvastatin and polyherbal extract which shown marked reduction in TC and TG concentration as compared with control group.

LDL and VLDL- significant increase of serum LDL and VLDL level were detected in high cholesterol diet group compared to that of normal group. While decrease in serum LDL and VLDL observed in combination polyherbal extract and atorvastatin treated group compared to control group.

HDL and atherogenic index- Serum HDL-C and atherogenic index shown that there is an inverse proportion between the incidence of coronary artery disease (CAD) and the levels of HDL. Elevated level of HDL-C and decreased atherogenic index are considered for cardio protective effect.
The control group was shown decrease in HDL level as compared to normal group. The treatment of atorvastatin and polyherbal extracts showed that there is increase in level of HDL as compared to control group. The combination of polyherbal extract and atorvastatins showed the significant reduction in atherogenic index as compared to control.

The results showed that combination of polyherbal extractpossesses the significant antihyperlipidemic potential, which may be due to saponins, flavonoids, and phenolic compounds present in the extract. Reported investigation by A. Stark and Z. Madar has proved that saponins are responsible for inhibiting intestinal CH level. The selected plant also showed antihyperlipedemic activity probably due to presence of saponins as their chemical constituent.

Flavonoids present in the extract may enhance Lecithin Acyl Transferase (LCAT) activity. LCAT is responsible for the regulation of blood lipids. It is principle enzyme which is responsible for incorporation of cholesterol in to HDL. Saponnins also shows that the significance antihyperlipidemic activity with different mechanism like decrease the intestinal absorption of cholesterol by binding with

it and thus increasing its faecal elimination. Plant proteins also showed that control of the progression of hyperlipidemia as reported by Muhammad aslam and Rahila Najam. Most of these extracts contain that the vitamins which may show the antioxidant activity.

Poloxamer-407 Induced Hyperlipidemia:-
Hyperlipidemia can induce by means of poloxamer-407. It is , a non-ionic synthetic copolymer surfactant, provides an attractive means of inducing hyperlipidemia because of its rapid onset and seeming lack of overt toxicity; within 24 h of its intraperitoneal (i.p.) injection a profound hyperlipidemia state is achieved. It has been used to induce experimental hyperlipidemia in several rodent species including rat, mouse and rabbit. With chronic administration it has been shown to induce atherosclerosis in the mouse, a species which is quite resistance to its onset. P407 increases serum lipoproteins via its actions at various levels in lipid metabolism, largely by inhibiting lipoprotein lipase, which facilitates the hydrolysis of triglycerides (TG). Johnston et al investigated the effect of P407 on lipoprotein lipase activity and found that after 3 h of P407 i.p. injection in rats the enzyme activity decreased by 95% compared to normal saline treated controls [18].

PX-407 also causes indirect stimulation of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase which is involved in cholesterol biosynthesis. Several groups have used the P407 model of HL for the study of HL on pharmacokinetics and pharmacodynamics of a variety of drugs.

In present study polyherbal extract was administered to rats with hyperlipidemia induced by the administration of poloxamer-407. In order to ascertain whether blood lipid can be lowered.

Body weight- The body weight was significantly increased in PX-407 control group as compared to normal group. In present study, administration of combination of polyherbal extract 500mg/kg dose showed there was significant weight gain similar to normal group.

Hemodynamic study- The polyherbal extract shown significant reduction in systolic blood pressure, diastolic blood pressure, mean blood pressure.

Lipid profile-
Total cholesterol and triglyceride-Therewas significant elevation in plasma TC and TG in PX-407 treated group as compared to normal group. The PX-407 induced in TC and TG concentration were significantly ($P<0.05$) suppressed by animal treated with atorvastatin and combination of polyherbal extract. Atorvastatin used as standard drug. Atorvastatin and polyherbal extract which shown marked reduction in TC and TG concentration as compared with control group.

LDL and VLDL- significant increase of serum LDL and VLDL level were detected in PX-407 group compared to that of normal group. While decrease in serum LDL and VLDL observed in combination polyherbal extract and atorvastatin treated group compared to PX-407 control group.

HDL and atherogenic index- The control group was shown decrease in HDL level as compared to normal group. The treatment of atorvastatin and polyherbal extracts showed that there is increase in level of HDL as compared to control group. Ultimately the atherogenic index was also improved in polyherbal extract treated group.

The polyherbal extract also has significantly ability to lower these lipids markers and enhances HDL in poloxomer treated animals when compared to control group.

Present study showed that polyherbal extract treatment significantly lowered both plasma TC and TG level. The reduction of TC was associated with decrease of its LDL fraction which is the target of several drugs. The polyherbal extract supplementation also in significant elevation in HDL in serum which strengthens hypolipidemic effect of this extract.

ANTIOXIDANT STUDY
There is a drastic change in the life pattern with increasing number of subjects is at risk of vascular diseases and there is increase in evidence of oxidative stress. The loss of control of free radical formation from the mitochondria can contribute to the pathology of CVDs through a no of mechanisms including damage to mDNA enzyme degradation and apoptosis and thus contribute to human disease.

Dietary fat intake has been shown to be important in the development of human obesity and there are also experiment studies showing that high fat diet can be associated with increased oxidative stress in mammals. The production of free oxidative radicals is believed to induce endothelial dysfunction, an initial step of atherogenesis. Oxidative stress leads to oxidation of LDL (ox-LDL), whose uptake by macrophages is easier compared to non-oxidized lipoproteins. It has been proven that the main sources of oxidative substances and ROS in atherosclerotic vessels are macrophages and smooth muscle cells [65]. Indeed, hypercholesterolemia stimulates the production of

superoxide anion radicals from the smooth muscle cells of vessels, an event that leads to increased oxidation of LDL. In lipid peroxidation polyunsaturated fatty acids present in the membranes, phospholipids are particularly sensitive to attack by hydroxyl radicals and other oxidants. A single hydroxyl radical can result in the peroxidation of many polyunsaturated fatty acid molecules. . Lipid peroxidation is detected through the estimation of MDA [13].

Elevated level of malondialdehyde (MDA) an end product of lipid peroxidation observed in HCD fed and Poloxamer-407 treated rats indicates activation of lipid peroxidation system in aorta due to induction of hyperlipidemia [66]. From obtained results group treated with polyherbal extracts (500mg/kg) showed reduction in MDA level in HCD induced hyperlipidemia by 43.42% and PX-407 induced hyperlipidemia by25.19% as compared to control group.

SOD is the first line defense against free radical attack. Hypercholesterolemia has been reported to increase superoxide anion production in endothelium cells. SODs catalyze the rapid removal of superoxide radicals [62].

Catalase is an iron-containing enzyme found primarily in the cell small membrane enclosed cell compound called peroxisomes and convert hydrogen peroxide (H_2O_2) into molecular oxygen and water. Catalase plays important role in cellular protection by oxidative stress induced cell damage. In addition, catalase regulates the cell growth via activation of the extracellular signal regulated kinase (ERK) pathway, leading to the acceleration of the cell growth inhibited by stress [63].

SOD and CAT these are the supportive antioxidant enzymes which are defense against ROS. The present study reveals that marked decrease in level of SOD and CAT in high cholesterol diet fed and PX-407 induced hyperlipidemia. While administration of polyherbal extracts (500mg/kg) with PX-407 induced in animal treated with HCD and poloxamer were shown marked increase in SOD and CAT as compared to control group.

CONCLUSION

The present study revealed that effect of combination of polyherbal extracts of (*Justicaadhatoda* leaves +*Trigonella foenum-graenum* seeds + *Mimosa pudica* leaves) at a dose of (500mg/kg) in lipid profile rats decrease the associated cardiovascular risk by reducing blood pressure and weight gain. It also concluded that there is significant decrease in TC, TG, LDL, VLDL and significant increase in HDL level in serum lipid profile. Which shown its significant antihyperlipidemic activity. Moreover, it also decreases the MDA level and improves the enzymatic activity of SOD and CAT in aorta which probably produces anti atherogenic activity of extract. Preliminary phytochemical investigation of plant extracts revealed presence of tannins, flavonids, saponins, so antihyperlipidemic and antioxidant activity may be due to these constituents.

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