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Phytochemical investigation and experimental evaluation of Panchavalkala for Lekhana Karma w.s.r. to Antihyperlipedemic Activity

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ABSTRACT

Panchavalkala the barks of five trees i.e., Nyagrodha (*Ficus benghalensis L.*), Udumbara (*Ficus racemosa L.*), Ashwatha (*Ficus religiosa L.*), Plaksha (*Ficus virens Aiton*) and Parisha (*Thespesia populnea (L.) Sol.ex Correa*) are also known as Pancha Ksheeri Vrikshas in use since Vedic period. The plant samples were collected in Sharad Ritu (late autumn) from their natural habitat, extracted using ethanol and water and subjected for the phytochemical analysis. The study was aimed to evaluate Antihyperlipidemic effect (*Lekhana Karma*) of Panchavalkala Bark extracts individually and in combination (1:1:1:1:1) both in ethanolic and aqueous extracts on serum lipid profile in Cafeteria diet (HFD) induced hyperlipidemia in SD rats. The animals were divided into fifteen groups of six animals each. The normal control group continued to be fed laboratory pellet chow ad libitum. The cafeteria diet-control group received the cafeteria diet in addition to the normal pellet diet (NPD). The remaining 13 groups were fed with the cafeteria diet and NPD along with standard control Atorvastatin 10mg/kg and the test drug p.o for 30 days. Treatment with the test substance PVK AE and PVK EE at doses of 200mg/kg body weight p.o. controlled and regulated the HFD induced weight gain, glucose and lipids in the blood when compared against high fat diet control ($P < 0.05$). PVK bark extracts are capable of exhibiting significant antihyperlipidemic activity in HFD induced SD rats. Taken together, this study strongly suggests that PVK bark extracts might be an efficient way for treatment of hyperlipidemia.

Key words: High fat diet, Panchavalkala, Atorvastatin, Hyperlipidemia, Aqueous extract, Ethanolic extract, Anti hyperlipidemic

INTRODUCTION

Hyperlipidemia is a secondary metabolic dysregulation associated with diabetes. Besides the cause-effect relationship with diabetes, elevated serum levels of triglycerides, cholesterol and LDL are major risk factors

for the premature development of cardiovascular diseases like atherosclerosis, hypertension, coronary heart disease etc.^[1]

Hyperlipidemia is one of the greatest risk factors contributing to the prevalence and severity of atherosclerosis and subsequent coronary heart disease.^[2] Treatment of hyperlipidemia involves diet control, exercise, and the use of lipid-lowering diets and drugs.^[3] The most commonly employed drugs for treatment of hyperlipidemia include hydroxyl methyl glutarate coenzyme A (HMG-CoA) reductase inhibitors, also called as statins. Other drugs employed for treatment of hyperlipidemia include bile acid sequestrants (anion-exchange resins) such as cholestyramine and colestipol; fibrates such as clofibrate, gemfibrozil, fenofibrate, ciprofibrate, and bezafibrate; niacin; cholesterol absorption inhibitors such as ezetimibe; and omega-3-fatty acids.^[4]

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Liver synthesizes two-third of the total cholesterol made in the body. Endogenous cholesterol biosynthesis in the liver is mainly controlled by rate limiting enzyme, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid.^[5] Drugs that lower cholesterol level mainly work by inhibiting the HMG-CoA reductase activity.^[6,7] Despite the significant clinical benefits provided by statins^[8], many patients do not achieve the recommended low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol target goals.^[9]

Moreover, the use of statins is not preferred in more than 40% of patients, mostly due to the occurrence of several side effects including myalgia, myopathy, liver disease, and rhabdomyolysis in more severe cases.^[10,11] This limits the use of statins and incites a search for new natural drugs to combat hypercholesterolemia as well as cholesterol induced oxidative stress and atherosclerosis.

Elevated lipid levels result from increased absorption through the gut or enhanced endogenous synthesis. Therefore, two ways are feasible to reduce hyperlipidemia either to block endogenous synthesis or to decrease absorption. Both factors can be evaluated in normal animals without artificial diets. Thus, there is still need for development of better antihyperlipidemic agents.

Medicinal plants are potential sources of therapeutic compounds and plant products are frequently considered to be less toxic and free from side effects than synthetic ones. Ficus species are versatile sources of bioactive metabolites such as flavonoids, phenolic acids, tannins, alkaloids, glycosides, coumarins, triterpenoids, sterols and vitamin E.^[12]

Panchavalkalas are group of trees belong to Moraceae family found all over India. Their barks have ethno pharmacological uses for the treatment of Diarrhea, hemorrhage, coughs, wound healing properties and also used as an antiseptic, antidiabetic, antioxidant and anti-hyperlipidemic agent.^[13] And also several works have demonstrated the anti-hyperlipidemic, antioxidant and antidiabetic activity.^[14] The current list

of compounds found in PVK barks include the presence of Tannins, Flavonoids, Beta sitosterol and Stegmasterol, Polyphenols such as Gallic acid, Ellagic acid, Caffeic acid, Burgeptol and etc.^[15-17]

Thus the present study was designed to evaluate Anti hyperlipidemic effect of *Panchavalkala Dravyas* individually and in combination (1:1:1:1:1) both in ethanolic and aqueous extracts on serum lipid profile in Cafeteria diet (HFD) induced hyperlipidemia in SD rats.

OBJECTIVE

The objective of the study is to evaluate the efficacy of Aqueous and Ethanolic extract of *Panchavalkalas*, *Vata*, *Udumbara*, *Aswatta*, *Plaksha* and *Pareesha* together and individually for anti-hyperlipidemic activity in cafeteria induced SD rats.

MATERIALS AND METHODS

Plant material

The barks of *Panchavalkala* trees i.e., *Nyagrodha* (*Ficus benghalensis* L.), *Udumbara* (*Ficus racemosa* L.), *Ashwatha* (*Ficus religiosa* L.), *Plaksha* (*Ficus virens* Aiton.), *Parisha* (*Thespesia populnea* (L.) Sol.ex Correa were collected in *Sharad Rutu* i.e., late autumn season (November to January) from their natural habitat, Dhanvantari Vana Bangalore University Campus and Reserve Forest, Jaraka Bande Kaval, Bangalore North. The specimens were authenticated by Dr. Ravikumar K. Taxonomist and Senior Botanist's from FRLHT TDU, Yelahanka Bangalore – 560064. Quantity sufficient matured barks of *Panchavalkalas* were cut in to small pieces separately and were dried under mild sunlight covering thin cloth for 2 days and later shade dried for 10 days. Then the dried barks were powdered using pulverizer at Sanjeevini Pharma Kengeri, Bangalore. The phytochemical analysis, quantification of marker compounds and in vitro study is carried out at Skanda Life Sciences Pvt. Ltd., Bangalore-560091.

Formulation details - Preparation of aqueous and alcoholic extract

20 g of dried sample powder-PVK weighted and dissolved in 100 ml water or 100 ml of ethanol for aqueous and alcoholic extract in 500ml beaker with

Aluminium foil covered on it. Then the beaker was kept on hot water bath at 50°C for 4 hours. After incubation period the extract was filtered with Whatman filter paper, and the filtrate was collected in 50ml beaker. Residue present over the filtrate was taken for further use. Then the filtrate was kept at 70°C for few hours until the extract got completely dried and turned into semisolid form. This semi solid sample was weighted and used for dosing.

Phytochemical Analysis

Various Chemical tests were carried out for detection of organic chemical constituents Phyto chemical examinations were carried out for all the extracts as per the standard methods.^[18,19]

Study of marker compounds Caffeic acid and Gallic acid through HPLC analysis

- A.** Caffeic acid standard (Sigma Aldrich, 99% w/v) stock solution (1mg/ml) was prepared in MEOH. Caffeic acid was quantified using the peak area after HPLC analysis. Chromatography analysis for the quantification of the chemical marker Caffeic acid in *Ficus virens* Ait., *Ficus religiosa* L., *Ficus benghalensis* L. and *Thespesia populnea* L. was conducted using an instrument (Shimadzu LC-MS Prominence 20AT) at ambient temperature (40°C) on an C18 column (250 × 4.6 mm, 5.0 µm particle size). The mobile phase linear comprised a mixture of Acetonitrile and HPLC grade water (60:40 v/v). The flow rate was 0.1 ml/min. The detector wavelength was set to 325 nm, and the injection volume was 10 µl [20].
- B.** Gallic acid standard (Sigma Aldrich, 99% W/v) stock solution (1mg/ml) was prepared in MEOH. Gallic acid was quantified using the peak area after HPLC analysis. Chromatography analysis for the quantification of the chemical marker Gallic acid in *Thespesia populnea* L. and *Ficus racemose* L. was conducted using an instrument (Shimadzu LC-MS Prominence 20AT) at ambient temperature (40°C) on an C18 column (250 × 4.6 mm, 5.0 µm particle size). The mobile phase linear comprised a mixture of Acetonitrile and HPLC grade water (60:40 v/v). The flow rate was 1 ml/min. The detector

wavelength was set to 271 nm, and the injection volume was 10 µl. The peaks were detected at 271 nm and were identified by comparing the retention time with standard Gallic acid.^[21]

Study Guidelines

All the animal experiments were conducted at Invivo Biosciences Bangalore-560091 Study No.IE-105 according to the ethical norms approved by CPCSEA guidelines for research in small animals 1165/PO/RcBiBt-S/NRCL/08/CPCSEA, dated 9th November 2018, Ministry of social justice and empowerment, Government of India and ethical clearance was granted by Institutional animal ethical committee in resolution no. IE-105/69/2019 held on 23rd November 2019.

Test Substance

Name of Test Substance: Aqueous extract of *Panchavalkalas* in combination, *Vata*, *Udumbara*, *Aswatta*, *Plaksha* and *Pareesh*, Ethanolic extract of *Panchavalkalas* in combination, *Vata*, *Udumbara*, *Aswatta*, *Plaksha* and *Pareesha*.

Chemical Name (IUPAC): NA

Purity: NA

Manufactured Date and batch no: NA

Recommended Storage condition: Room temperature

Test System:

Species: Rat

Strain: Sprague Dawley

Justification for the selection of species: Literature survey suggests that rabbit will be used to evaluate the anti-atherosclerosis activity.

No. of groups: 15

No. of groups: 6

Body weight: 170-200gm

Identification: By cage card, crystal picric acid color body marking.

Acclimatization: 5 days

Treatment protocol and group allocation:

The animals were divided into fifteen groups of six animals each and individually housed in cages. The normal control group continued to be fed a laboratory pellet chow ad libitum. The cafeteria diet-control group received the cafeteria diet in addition to the normal pellet diet (NPD). The Standard control group received Atorvastatin along with HFD and NPD. The remaining 12 groups were fed with the cafeteria diet and NPD along with the test drug 200mg/kg body weight p.o. Treatment was continued for 30 days. The animals were weighed at the start of the experiment and on 30th day thereafter.

Husbandry Conditions:

Animals were housed under standard air-conditioned laboratory conditions.

- Temperature: Maximum: 24°C and minimum 23°C
- Relative humidity: Maximum: 63% and minimum 48%
- 12 h light and 12 h dark cycle.

The maximum and minimum temperature and relative humidity in the experimental room was recorded once daily.

Equipment Details and Chemicals:

- Weighing balance: Make: Weigh well, model: WWTT
- Biochemical analyzer: Make: Robonik, Model: Prietest- COMPACT
- Glucose Kit: Make: Robonik, Lot no: 191431
- TG kit: Make: Robonik, Lot no: 192902
- Total Cholesterol: Make: Asritha (CH-10/1019)
- HDL: Make: Robonik: Lot no: 191705
- Atorvastatin: Ranbaxy Laboratories Ltd. Gurgaon

Housing

Rat: Polypropylene cages with provision for water bottle holder and feed hopper with corn cobs as bedding material.

Water ad libitum: Aqua guard water in polypropylene bottles

Diets:

The SD Rats were fed with Cafeteria diet consisted of three diets (a) 48 g of condensed milk, 48 g of bread, (b) 18 g of chocolate, 36 g of dried coconut, 36 g of biscuit and (c) 48 g of cheese, 60 g of potatoes. The three diets were presented to the rats on day 1, 2 and 3 respectively and then repeated for 30 days in the same succession in addition to normal pellet chow diet.^[22]

Preparation of standard drugs and administration:

Atorvastatin 10 mg/kg was used as the reference standard drug for evaluating the antihyperlipidemic activity which was made into suspension in distilled water using Tween-80 as a suspending agent. The test formulations were administered orally.

Blood biochemical analysis:

After the dosing period of 30 days the blood was collected from all the animals by retro-orbital puncture from the ether-anesthetized rats and subjected to centrifugation to obtain the serum for bio chemical analysis. The serum levels of cholesterol and lipoproteins were estimated using ERBA estimation kits. The biochemical parameters like serum glucose level, Triglyceride, Total cholesterol, HDL, VLDL and LDL cholesterol were measured using standard protocol method.^[23,24] Finally, all the animals were sacrificed, and gross necropsy was performed. The organ weights of Liver, Kidney, Heart and Lungs were measured. Liver was removed and stored in formalin and was processed for histopathology.

Estimation of Body weight and organ weights:

Animals were weighed before and after 30 days and later sacrificed with an overdose of diethyl ether. The liver, heart, kidney and lungs were quickly removed and weighed. The effect of PVK bark extracts on cholesterol ratios, Atherogenic Index of Plasma and change in body weight were calculated and analysis done as per the protocol.

Atherogenic index of Plasma is calculated using formula

$$AIP = \text{Log} (\text{serum triglyceride} / \text{serum HDLc})^{[25]}$$

Estimation of Lipid ratios:

Lipid ratios can provide information on risk factors difficult to quantify by routine analyses and could be a better mirror of the metabolic and clinical interactions between lipid fractions. These ratios could be potential indicators for risk stratification of ICAS (Intracranial atherosclerotic stenosis) and provide guidance in lipid-lowering therapy.

Histopathology: The liver tissue was stored at -80°C for further histopathology analysis.

Slide preparation procedure: Tissues were washed for formalin clearing over-night in running water. Tissues were prepared for staining after serial dilution of alcohol by rehydration and dehydration method. The tissues were embedded in paraffin blocks for sectioning in microtome. The sections were made at $4\ \mu$ thickness suitable for staining.

Staining procedure: The tissue fixed slides were deparaffinized using xylene and further rehydrated using serially diluted alcohol. Further sections were stained using Eosin-hematoxylin. The slides were dehydrated by serially diluted alcohol. Microscopical examinations of H&E (hematoxylin and eosin) stained slides were conducted by research microscope at 40x resolution for better visibility and detailed examination. The changes are recorded based on circulatory changes, infiltrative changes and proliferative changes.

Data Compilation and Statistical Analysis

Average of all the data were compiled and SEM were calculated. All the data was analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. Values <0.05 were considered as statistically significant.^[26]

RESULTS

Phytochemical screening:

Preliminary phytochemical screening of the aqueous and Ethanolic extracts of PVK together and separately

revealed the presence of different Phyto constituents which are represented in Table 1 and Table 2.

Table 1: Results of Phytochemical analysis of AE of Panchavalkala

Types of tests	Aqueous extract					
	<i>F.religiosa</i>	<i>T. populnea</i>	<i>F.virens</i>	<i>F.benghalensis</i>	<i>F.racemosa</i>	PV K
Alkaloid	+	+	+	+	+	+
Carbohydrate	+	+	-	+	+	+
Tannin	+	+	+	+	+	+
Terpenoid	+	+	+	-	+	+
Glycoside	-	-	+	-	+	+
Steroid	+	+	+	+	+	+
Saponin	-	+	-	+	+	+
Flavonoid	+	+	-	+	+	+
Myllon's test	+	+	+	+	+	+
Starch test	-	-	-	-	-	-
Resin test	+	+	+	+	+	+
Carboxylic acid test	-	+	-	+	-	-
Phenol test curcuminid	+	+	+	+	+	+

Note: + : Indicates presence of chemical constituents, - : Indicate the absence of chemical constituents

Table 2: Results of Phytochemical analysis of EE of Panchavalkala

Types of tests	Ethanollic extract					
	<i>F.religiosa</i>	<i>T. populenea</i>	<i>F.virens</i>	<i>F.benghalensis</i>	<i>F.racemosa</i>	PVK
Alkaloid	+	+	+	+	+	+
Carbohydrate	+	+	-	-	+	+
Tannin	-	+	+	+	+	+
Terpenoid	+	-	+	+	+	+
Glycoside	-	+	+	-	+	+
Steroid	+	+	+	+	+	+
Saponin	-	+	+	+	+	+
Flavanoid	+	-	+	+	+	+
Myllon's test	+	+	+	-	+	+
Starch test	-	-	-	-	-	-
Resin test	+	+	+	+	+	+
Carboxylic acid test	-	+	-	-	-	-
Phenol test-curcumoid	+	+	+	+	+	+

Note: + : Indicates presence of chemical constituents, - : Indicate the absence of chemical constituents

HPLC analysis of the marker compounds:

Caffeic acid and Gallic acid was quantified using the Retention time (RT) and the peak area after HPLC analysis of given samples in comparison to the standard sample. The results are summarized in Table-3 and 4.

Table 3: Caffeic acid content of test sample

Sample	Stock	R.T	Area (mv* S)	Caffeic acid content (µg /ml of sample)	Dilution factor	Caffeic acid content (µg /g of sample)
Caffeic acid	100 µg /ml	1.8	629.00	-	-	-
FicusvirensAit.	10g/50 ml	1.9	11.46	1.82	1	0.182
Ficusreligiosa L.	10g/50 ml	1.7	291.00	46.26	1	4.626
Ficusbenghalensis L.	10g/50 ml	1.8	207.00	32.91	1	3.291
Thespasiopulnea L.	10g/50 ml	1.953	248.00	39.43	1	3.943

Table 4: Gallic acid content of test sample

Sample	Stock	R.T	Area (mv* S)	Gallic acid content (µg /ml of sample)	Dilution factor	Gallic acid content (µg /g of sample)
Gallic acid	100 µg /ml	2.4	482.00	-	-	-
Ficus racemose L.	10g/50 ml	2.1	10.49	1.67	1	0.167
Thespasiopulnea L.	10g/50 ml	2.653	1.13	0.18	1	0.018

Table 5. Effect of PVK bark extracts on Lipid profile of rats fed with trial drug for 4 weeks

Treatment	Serum Glucose (mg/dL)	TG (mg/dL)	TC (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control	88.88 ± 1.26	88.02 ± 0.45	105.03 ± 1.50	61.33 ± 2.12	26.10 ± 3.14	17.60 ± 0.09
HFD control	179.78 ± 11.51	168.90 ± 3.05	181.93 ± 12.50	28.25 ± 3.15	119.90 ± 14.52	33.78 ± 0.61

Standard control	93.57 ± 2.04**	97.58 ± 2.75**	116.5 ± 2.35**	57.90 ± 4.28**	39.12 ± 4.36**	19.52 ± 0.55**
AE PVK	122.73 ± 2.97**	124.13 ± 3.25**	152.37 ± 5.97*	48.13 ± 1.70**	79.41 ± 6.96**	24.83 ± 0.65**
EE PVK	118.55 ± 3.72**	117.38 ± 2.08**	134.82 ± 2.98**	58.13 ± 2.60**	53.21 ± 4.82**	23.48 ± 0.42**
AE Vata	150.00 ± 14.86*	150.15 ± 2.47**	163.18 ± 3.50 ^{ns}	40.88 ± 4.78*	92.27 ± 5.40*	30.03 ± 0.49**
EE Vata	130.32 ± 2.79**	135.50 ± 4.38**	157.52 ± 6.62 ^{ns}	51.10 ± 3.78**	79.32 ± 9.85**	27.10 ± 0.88**
AE Udumbara	146.68 ± 7.29*	149.37 ± 2.35**	153.35 ± 3.44*	44.12 ± 1.81*	79.36 ± 3.24**	29.87 ± 0.47**
EE Udumbara	131.58 ± 2.31**	133.72 ± 1.33**	143.58 ± 0.95*	53.48 ± 0.81**	63.36 ± 1.33**	26.74 ± 0.27**
AE Aswatta	165.00 ± 4.08 ^{ns}	153.98 ± 2.60*	144.37 ± 9.81**	48.88 ± 1.82**	64.69 ± 11.10**	30.80 ± 0.52*
EE Aswatta	135.55 ± 2.63**	137.02 ± 2.58**	144.20 ± 0.94**	54.57 ± 0.77**	62.23 ± 0.45**	27.40 ± 0.52**
AE Plaksha	168.80 ± 6.33 ^{ns}	156.20 ± 2.85*	148.75 ± 1.09**	50.75 ± 0.87**	66.76 ± 1.61**	31.24 ± 0.57*
EE Plaksha	129.95 ± 3.81**	132.97 ± 3.60**	147.85 ± 2.04**	54.68 ± 1.15**	66.57 ± 3.26**	26.59 ± 0.72**
AE Pareesh a	139.73 ± 8.94**	155.02 ± 4.22*	147.48 ± 3.07**	42.07 ± 2.69*	74.41 ± 1.25**	31.00 ± 0.84*

EE Pareesh a	133.38 ± 5.68**	152.45 ± 3.64**	136.48 ± 2.19**	49.33 ± 3.78**	56.66 ± 4.59**	30.49 ± 0.73*
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Data were expressed in Mean ± SEM, n=6; Data was analyzed using one-way ANOVA followed by Dunnett’s multiple comparison test. Values <0.05 were considered statistically significant. *p< 0.05, **p<0.01, ***p<0.001, Compared with HFD control.

Table 6. Effect of PVK bark extracts on cholesterol ratios, total Atherogenic Index of Plasma and change in liver and body weight.

Treatment	TC/HD L	LDL/HD L	AIP	Liver Weight in gm	Change in Body Weight (in gm)
Control	1.71** *	0.43***	-0.2***	13.27 ± 0.71	47.33±2.75
HFD control	6.44#	4.24#	0.42#	18.62 ± 0.53	59.83±2.86
Standard control	2.01** *	0.68***	-0.13** *	14.72 ± 0.35** *	40.83±3.12**
AE PVK	3.17**	1.65***	0.05**	16.82 ± 0.26**	48.83±2.6
EE PVK	2.32** *	0.92***	-0.06** *	15.82 ± 0.23** *	45.83±2.8*
AE Vata	3.99**	2.26***	0.2**	16.83 ± 0.28**	50.34±3.45
EE Vata	3.08**	1.55***	0.07** *	17.57 ± 0.20**	44.83±2.65*
AE Udumbara	3.48**	1.80***	0.17#	18.02 ± 0.19 ^{ns}	47.16±4.83
EE Udumbara	2.68** *	1.18***	0.04**	16.78 ± 0.21**	49.33±2.40
AE Aswatta	2.95** *	1.32***	0.14#	16.67 ± 0.22** *	51.66±3.23

EE Aswatta	2.64** *	1.14***	0.04** *	17.20 ± 0.25*	45.17±2.29*
AE Plaksha	2.93** *	1.32***	0.13#	17.03 ± 0.20**	47.67±2.37*
EE Plaksha	2.70** *	1.22***	0.02** *	16.80 ± 0.22**	45.34±2.82*
AE Pareesha	3.51**	1.77***	0.21#	16.55 ± 0.26** *	50.83±2.81
EE Pareesha	2.77** *	1.15***	0.13#	16.90 ± 0.26**	44.83±2.96*

A. Low Risk: TC/HDL-c ratio < 3, B. Intermediate Risk: TC/HDL-c ratio < 4, C. High Risk: TC/HDL-c ratio > 5

A. Low Risk: LDL/HDL-c ratio < 2.5, B. Intermediate Risk: LDL/HDL-c ratio < 3, C. High Risk: LDL/HDL-c ratio > 4

A. Low Risk: AIP < 0.11, B. Intermediate Risk: AIP = 0.11-0.21, C. High Risk: AIP > 0.21

Liver weight: Data were expressed in Mean ± SEM, Values P<0.05 were considered statistically significant

Body weight: Data were expressed in Mean ± SEM, A (Significant) ***, B (Moderate) **, C (Poor) #

Figure 1: Histopathology of Liver Tissue - Control, HFD Control, Standard Control, AE PVK, EE PVK and AE Vata

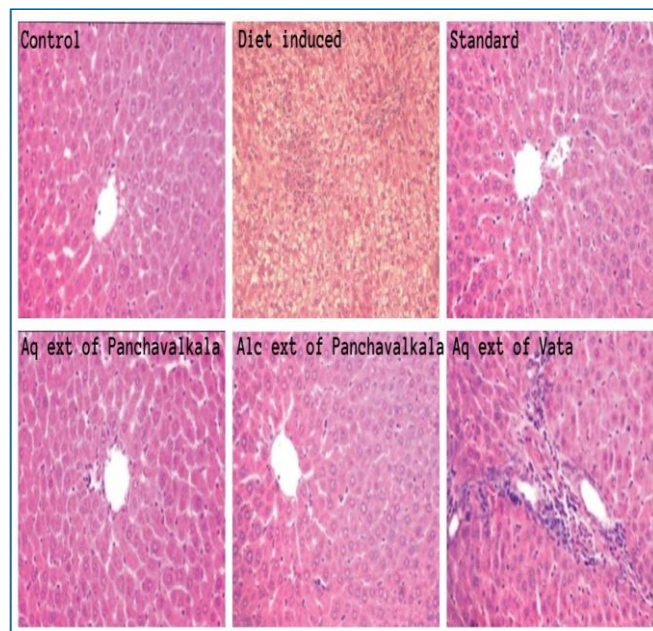


Figure 2: Histopathology of Liver tissue - EE Vata, AE Udumbara, EE Udumbara, AE Aswatta, EE Aswatta and AE Plaksha

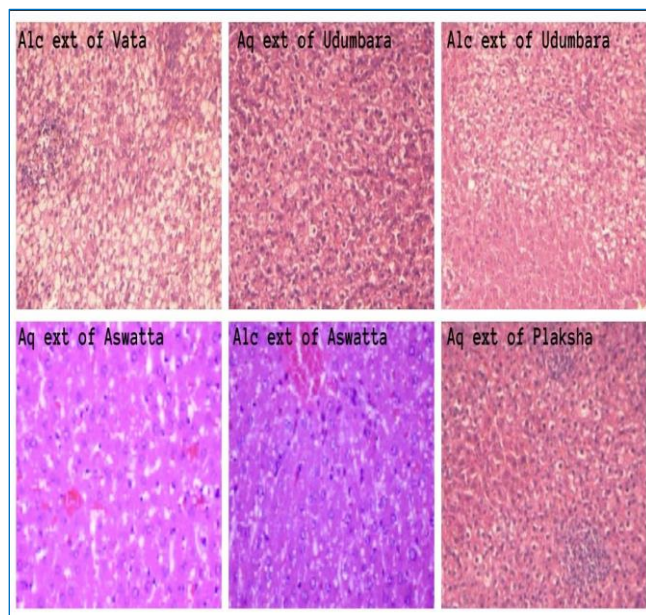
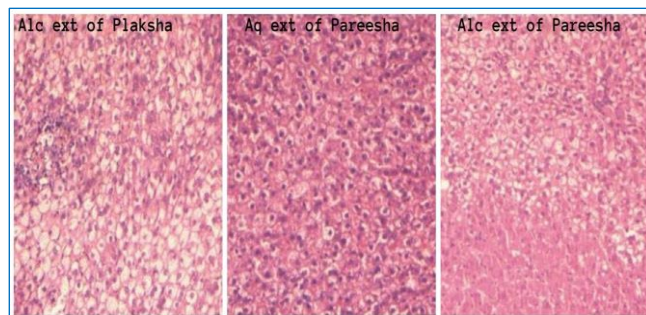


Figure 3: Histopathology of Liver Tissue- EE Plaksha, AE Pareesha and EE of Pareesha



DISCUSSION

Plant has long been a very important source of drug and many plants have been screened if they contain compounds with therapeutic activity.^[27] In the preliminary phyto-chemical analysis, we detected the presence of Tannins, Flavonoids and polyphenols classes in large quantities (Table no. 1 & 2). As per the quality standards of Indian medicinal plants and available literature Caffeic acid and Gallic acid was chosen as a marker and we proceeded to quantify and validate *Panchavalkalas* compound individually screening for the presence of these markers for the purity and quantitative analysis of the source of raw materials used for the study (Table no. 3 & 4).^[33]

A high-fat diet causes cholesterol levels to increase in susceptible people, which leads to obesity.^[28] Hyperlipidemia is a well-known risk factor for cardiovascular diseases, especially atherosclerotic coronary artery disease (CAD), which is one of the major causes of premature death globally.^[29] Several studies revealed that an increase in HDL cholesterol and a decrease in TC, LDL cholesterol and TG are associated with a decreased risk of ischemic heart diseases.^[30]

Feeding rats with cafeteria diet inevitably causes weight gain compared to normal pellet chow diet.^[31] A cafeteria diet induced obesity model is the simplest obesity-induced model and possibly the one that most closely resembles the reality of hyperlipidemia and obesity in humans.^[32] As expected, a high fat Cafeteria diet significantly increased the levels of total lipids TC, TG and LDL-C in the serum and liver of rats, compared to animals on a normal diet. It also substantially increases the blood glucose levels when compared to the control group. When the PVK extract was co-administered with the high cholesterol diet, the levels of these lipids and Glucose were significantly reduced whereas plasma HDL-C was increased thereby confirming the anti-hyperlipidemic efficacy of the extracts (Table-5).

Among the extracts PVK Ethanolic Extract is showing as promising candidate for anti-hyperlipidemic activity. Among all the extracted fractions, PVK Ethanolic Extract showed most potent antidiabetic, and antihyperlipidemic activity (Table - 5). In vitro experimentation has also proven a significant antioxidant and pancreatic lipase inhibition activity of PVK Ethanolic Extract when compared to PVK Aqueous Extract and Ethanolic, aqueous extracts of individual drugs separately.^[33,34] Polyphenols represent the major class for the pancreatic lipase inhibitor. They bind to the enzyme by polyvalent sites present in them. When compared to PVK Aqueous Extract, PVK Ethanolic Extract has better extraction and solubility of Phenolic compounds the key phyto-constituents responsible for anti-hyperlipidemic action.

It is well accepted that enzymes are the major regulators of the lipid metabolism; wherein HMG-CoA

reductase is one of the most clinically important enzymes involved in the cholesterol biosynthetic pathway. Changes in the reductase activity are closely related to changes in the overall rate of cholesterol synthesis. This suggests that the inhibition of HMG-CoA reductase would be an effective mean to lower plasma cholesterol.^[35] Thus, this enzyme is the target of the widely available cholesterol lowering drugs known, collectively, as statins.^[36] Although, most of the HMG-CoA reductase inhibitors have some adverse side effects, PVK Ethanolic Extract having rich bioactive substances such as Flavonoid's, Polyphenols, Tannins, etc. inhibits HMG-CoA reductase activity, and produces antihyperlipidemic action. Our results are in concordance with earlier reports demonstrating that the reduction in HMG-CoA reductase activity is responsible for the hypolipidemic property of natural agents.^[37] This lipid lowering activity might be due to the pleiotropic effect that the compound exhibits through reduced HMG-CoA reductase activity, with significant antioxidant property and increased lipoprotein lipase activity of PVK responsible for hydrolysis of plasma lipids. PVK Ethanolic Extract showed marked anti-hyperlipidemic activity properties and might be a drug candidate.

The reduction of total cholesterol by the PVK extract was associated with a decrease of its LDL fraction (LDL-C) (Table-5), which is the target of several hypolipidemic drugs. This result suggests that the cholesterol-lowering effect of the extract may result from the rapid catabolism of LDL-C through its hepatic receptors for final elimination in the form of bile acids as demonstrated by Khanna et al.^[38]

Elevated serum TG is considered as an independent risk factor for cardiovascular diseases.^[39] In the triglyceride metabolism di-acyl-glycerol transferase (DAGT) and lipoprotein lipase plays an important role. Triglyceride synthesis is mainly regulated by di-acyl-glycerol transferase (DAGT) in many tissues. Lipoprotein lipase (LPL) is the rate-limiting enzyme in the hydrolysis of triglyceride-rich lipoproteins.^[40] In the present study, triglyceride levels are significantly reduced by Atorvastatin and test drug group administered rats (Table-5). Thus, the test drug might

have either inhibitory action on synthesis of TG by inhibiting DAGT or stimulating catalytic action of lipoprotein lipase.

A significant decline in the serum TG was observed in PVK treated rats. The mechanism by which PVK lower the serum TG concentration could be either by decreasing VLDL synthesis, by channeling VLDL through pathways other than to LDL or an increase in lipoprotein lipase activity. PVK supplementation suppressed fat absorption, consequently resulting in increased excretion of TG in feces and this could partly contribute to its lipid lowering effect.

There was marked increase in the level of serum total cholesterol, triglycerides, LDL, VLDL and decrease in the level of good cholesterol carrier HDL in the animals treated with HFD- atherogenic diet. Elevated level of blood cholesterol especially LDL was the major risk factor for the coronary heart disease and HDL as cardio protective protein. Treatment with PVK Aqueous Extract and Ethanolic Extract 200 mg/kg Body weight significantly decreased the level of cholesterol, triglycerides, VLDL-C (Table-5) and LDL-C as compared to hyperlipidemic (HFD) control. There was significant increase in HDL as compared to control. This effect may be due to the increased anti hyperlipidemic and antioxidant effect of PVK.

PVK demonstrated a significant antioxidant effect and also pancreatic lipase inhibition activity which is proven in in-vitro study^[46,47] clearly demonstrate the potential interference of the test drug in absorption of dietary cholesterol. From the earlier studies it has been reported that the Cholesterol acetyl transferase-2 (ACAT-2) is an isoenzyme found in the intestine and liver, where cellular free cholesterol is esterifies before triglyceride rich lipoproteins are assembled. In intestine ACAT-2 regulates the absorption of dietary cholesterol.^[41]

The LDL particles are arising mainly from larger triglyceride rich apo-B lipoproteins and catabolism of VLDL, which accounts for the high plasma concentration of LDL. In the hyper triglyceridemia two third of plasma cholesterol is found in the LDL. And the plasma clearance of LDL particle is mediated primarily

by LDL receptors located in the liver, which removes more than 75% of LDL from the plasma. In the present study the test drug PVK together and individually both EE and AE have shown significant reduction in the LDL-C level (Table-5). It might due to its capacity to induce a greater number of LDL receptors in the liver and thereby decreasing the plasma LDL- C level. Hence the hypolipidemic effect could be due to an increased catabolism of cholesterol in to bile acids in liver. Another possible mechanism is inhibition of HMG CoA reductase a rate limiting enzyme in the biosynthesis of cholesterol.^[42] Thus, the test drug might have either inhibitory action on absorption of dietary cholesterol or has inhibitory action on HMG CoA reductase enzyme. Furthermore, the fraction of *Ficus virens* which was identified as Octadecanyl-O- α D-glucopyranosyl (6' \rightarrow 1'')-O- α -D-glucopyranoside a new and completely different structure than the other inhibitors have been studied for in silico molecular interaction and inhibitory action on HMG CoA reductase enzyme and confirms the uncompetitive mode of inhibition observed in vitro study.^[43]

HDL is considered as protective lipoproteins that decrease the risk of coronary heart disease. This protective effect may result from participation of HDL in reverse cholesterol transport, the process by which the excess cholesterol is acquired from cells and transferred to the liver for excretion.^[44] In the present study the Atorvastatin has shown significantly increase in the HDL- C level and test drug-PVK has showed a moderate elevation (Table-5). Thus, test drug has shown moderate protective activity.

In the same way, oxidative stress also plays a pivotal role in atherosclerosis and subsequent CVD. Therefore, finding an HMG-CoA reductase inhibitor with antioxidant property is of great potential in the treatment and management of hypercholesterolemia and cholesterol induced oxidative stress.

Dyslipidemia is very common in type 2 diabetes (T2DM) mellitus affecting around 72%-85% patients.^[45] Lipid abnormalities in patients with diabetes, often termed "diabetic dyslipidemia", are typically characterized by high total cholesterol (T-Chol), high

triglycerides (Tg), low high density lipoprotein cholesterol (HDL-C) and increased levels of small dense LDL particles.

Many lipoprotein abnormalities are seen in untreated, hyperglycemic diabetic patient. The NIDDM patient with mild fasting hyperglycemia commonly has mild hypertriglyceridemia due to over production of TG rich lipoproteins in the liver, associated with decreased HDL-C. Improvement in glucose control, in the absence of weight gain, leads to lower triglyceride and higher HDL-c levels.^[46]

Among the PVK *F. benghalensis*, *F. racemosa*, *F. religiosa*, *Ficusvirens* and *Thespsia populenea* exhibited remarkable antidiabetic properties with various mechanisms of action. Moreover, Ficus species are versatile sources of bioactive metabolites such as flavonoids, phenolic acids, tannins, alkaloids, glycosides, coumarins, triterpenoids, sterols and vitamin E. These extracts having various phytochemical compounds significantly have enhanced insulin secretion and subsequently reduced blood glucose level (Table-5). Enhancement of glucose utilization in muscle, up-regulation of glucose transporters, enhancement of fat accumulation, activation of nuclear reporters, and reduction of hepatic glycogen are regarded as important mechanisms for controlling diabetes (El-Abhar and Schaaln, 2014, Tabatabaei-Malazy et al., 2012). PVK having kashayarasa and rookshaguna does inhibition of glucose absorption in the intestinal tract, enhance glucose uptake from the blood.^[47]

Epidemiologic studies have shown a correlation between an increased consumption of phenolic antioxidants and a reduced risk of CVD.^[48] In the present study high fat diet (CD) group has shown significant increase in the total cholesterol and Glucose as compared to the normal control. The Atorvastatin and test drug (PVK) administration at 200mg/kg body weight has considerably reduced the elevated total cholesterol level/ Triglycerides, LDL-C and Glucose.

Animals fed with HCD showed increase in body weight (Table-6), glucose and lipids in the blood (Table-5) compared to those fed with normal chow diet. PVK

Ethanollic Extract Treated group, *Ashwata* Ethanollic Extract and group treated with standard drug atorvastatin has shown moderate weight loss when compared with HFD Control ($P<0.05$). There is loss of liver weight in groups treated with Standard drug Atorvastatin, Test drug PVK Ethanollic Extract, *Ashwata* Aqueous Extract and *Pareesha* Aqueous Extract when compared with HFD Control ($P<0.01$). Decrease in liver weight and body weight is due to loss of cholesterol, due to anti-hyperlipidemic and antioxidant activity of the PVK test drug. However, the test drug did not show any significant changes in the relative body weight and organ weight such as kidneys, heart and lungs (Table-6).

HFD produced significant increase in Liver weight when compared to the control group where in the weight gain of kidneys, heart and lungs were not significant.

Histopathological study of Liver tissue is carried out in all groups to demonstrate effects of PVK extracts on liver tissue. In the control group which represents Hematoxylin eosin staining of normal liver with glomerulus. It shows normal histological tissue architecture of regular classical hepatic lobules with central vein and the peripheral portal area. Each hepatic lobule consisted of normal hepatocytes cords from the center to the periphery (Fig.1) HFD fed animals revealed numerous histopathological changes indicating severe hepatic steatosis (accumulation of lipid droplets in hepatic tissue), disrupted liver architecture marked central vein dilation, congestion of portal veins and blood sinusoids (Fig.1). The hepatic parenchyma of the standard treatment received group showed features of moderate hepatic steatosis and hydropic degeneration (Fig.1). The hepatic architecture was normal in the group treated with Aqueous extract of PVK and the cell borders were visible and the size of sinusoids was normal (Fig.1). Similarly, animals treated with Alcoholic extract of PVK group showed improvement of the hepatic architecture. For instance, most hepatocytes had regular nuclei, the amount and size of vacuoles decreased considerably, the cell borders were visible, and the size of sinusoids was normal (Fig.1). Similarly in other individual groups treated with single drugs of PVK both in aqueous and

Ethanol extracts are showing significant changes when compared to HFD control group (Fig.2&3). Earlier reports also demonstrate that lipid drops are usually accumulated in hepatic tissue, called hepatic steatosis under the progress of atherosclerosis especially hyperlipidemia stage. However, enzymes such as Superoxide dismutase and catalase which contribute to the antioxidant defense mechanism and reducing excess lipid drops (Lee et.al.2002; Devi & Sharma, 2004). In the present study, these data directly confirm that treated groups can keep hepatocytes normal by preventing or reducing excess lipid formation in HFD rats.

It is very important to study the lipid ratios along with the lipid profile in order to predict the cardiovascular risk and choose the appropriate line of treatment and drugs. Risk categories and target levels for Total Cholesterol/HDL-C, LDL-C/HDL-C in primary and secondary prevention stratified by gender is taken in to consideration. The target of TC/HDL-C ratio in males is <4.5 and <3.5 in primary and secondary prevention where as in females it is <4.0 and <3.0. The target of LDL-C/HDL-C ratio in males is <3.0 and <2.5 in primary and secondary prevention where as in females it is <2.5 and <2.0.^[49,50]

Thus, the implication of the effects of PVK Bark extracts on Lipid ratio is calculated and is shown (Table-6). There is a significant change in the ratio of trial groups when compared to HFD control group. In TC/HDL-C ratio of all groups have shown significant reduction in the value except AE of PVK, *Vata*, *Udumbura*, *Pareesha* and EE of *Vata*. Whereas all the trial groups have shown a significant reduction of LDL-C/HDL-C ratio when compared to HFD control.

Although atherogenesis is a multifactorial process, the atherogenic index of plasma (AIP) is a critical index that can be used as a stand-alone index for cardiac risk estimation. Changes in the levels of any lipid profile make the individuals more susceptible for atherosclerotic complications.^[51]

The implication of the effects of PVK Bark extracts on Lipid ratio is calculated as per standard methods^[52] and are shown (Table - 6). There is a significant change in

the ratio of trial groups when compared to HFD control group. AIP is significantly reduced in both EE, PVK and AE. PVK treated groups when compared to HFD control group (Table No. 6). Similarly, there is decrease in AIP index in the groups treated with EE *Vata*, *Udumbura*, *Aswatha*, *Plaksha* and AE of *Vata* where as it is not significant in AE of *Udumbura*, *Aswatha*, *Plaksha*, *Pareesha* and EE of *Pareesha*.

CONCLUSION

The present study showed PVK Ethanol Extract has significant antihyperlipidemic activity due to presence of Flavonoids, Tannins, and polyphenols. PVK have all such properties like Antidiabetic, Antioxidant and Pancreatic lipase enzyme inhibition activity. When it is compared with Atorvastatin, in vivo study has proven it has significantly reduced the lipids and brought changes in liver architecture. The present study has proven *Lekhana* and *Medhogni Karma* / Antihyperlipidemic activity of PVK which demonstrate the interference in the whole set of the pathophysiology involved in metabolic disease like diabetes, dyslipidemia and hyperlipidemia in particular. Hence it can be exploited as an antihyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of *Medorogas* which includes Hyperlipidemias.

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