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RESEARCH ARTICLE

HYPOLIPIDEMIC EFFECT OF PIPER NIGRUM AND ZINGIBER OFFICINALE (MIXED – EXTRACT) : AN EXPERIMENTAL STUDY

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ABSTRACT

The present study was planned to know the effect of mixed aqueous extract of *piper nigrum* & *zingiberofficinale* in high fat diet (HFD) induced hyperlipidemic albino rats. Phytochemical analysis was done separately on each aqueous extract. Alkaloids, saponins, tannins, phlobatanins, anthrax quinones, & cardenolides were found in both plants where as cardiac glycosides, steroids, &terpenoids were absent. Forty albino rats were randomly distributed into four groups (A-D) of ten albino rats each. All were fed with high fat diet (HFD) & water ad libitum. Group A was negative control group& Group D was positive control group and were not administered any extract. Whereas Group B & Group C were test groups, treated by mixed aqueous extract (*P.Nigrum + Z.Officinale*) at 400mg/kg.body weight & 800mg/kg.body weight respectively for forty-two days.The results showed that the treatment with mixed aqueous of ZO & PN at a dose of 800mg/kg body weight successfully reduced the elevated serum lipids more than the dose of 400mg/kg.body weight & that of simvastatin.These findings indicate that the mixed aqueous extract of ZO & PN can be used in hyperlipidaemia, a major cause of CVD. Thus, herbs PN & ZO may be useful in future as a leading compound for development of new drugs, after clinical trials.

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INTRODUCTION

Although clinical awareness is improved, public alertness is raised and health innovations are widely used, cardiac problems remains the leading cause of death worldwide. (Khatib *et al*., 2011) According to the 'Framingham Heart Study', dyslipidemia which can grade from hypercholesterolemia to hyperlipoproteinmia is one of the many modifiable risk factors for coronary heart disease (CHD), stroke and various vascular diseases.(Uma Bhandari *et al*., 2005)H yperlipidemia is widespread and todays health problem. Hyperlipidemia is a main cause of atherosclerosis and atherosclerosis associated conditions, such as coronary heart disease (CHD), ischemic cerebrovascular disease, and peripheral vascular disease. (Hitesh K. Dhamija and Ankit S. Chauhan, 2011) Recently World Health Organization (WHO) has declared that by 2020, 60% of cardiovascular cases will be of Indian origin.(Jose *et al*., 2012)It is projected that in 2020 cardiovascular disease will be the reason of over 40% deaths in India as compared to 24% in 1990. It causes 17.3 million deaths per year worldwide. Due to 3 million deaths owing to cardiovascular diseases every year, India will be the "Heart Disease Capital of the World" in recent years. (India set to be "heart disease capital of world", say doctors: Express news service, 2012)

Cardiovascular diseases (CVD) are epidemic in India. Within Asian countries, the occurrence of CVD has increased by a factor of 10 within last 4 decades. The incidence of coronary artery disease (CAD) in India was found to be 3-4% in rural areas and 8-10% in metropolitan areas with a total of 29.8 million affected according to population-based cross sectional surveys done in 2003. (Subramanian *et al*., 2012) In India, the main reason for this epidemic effect is lifestyle changes such

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as- sedentary, high stress jobs, enhancement in socioeconomic status results in unhealthy diets rich in fats and the addictions like smoking, alcohol and tobacco. So, the changed life style, absence of exercise, high calorie food leads to high incidence of hyperlipidemia, which unfortunately leads to atherosclerosis and cardiovascular diseases. (SrinivasNammi *et al*., 2009)

Many researchers reported the highest prevalence of hypercholesterolemia, a well known risk factor for CVD in all age groups. Unfortunately hypercholesterolemia, in young adults (i.e.30 to 50 years), to be one of the major contributors of cardiovascular disease. This has a significant effect on the national productivity.

Therapy with Statins is the first choice for lowering the lipid levels. Treatment of hyperlipidemia with statins has become an integral part of management of vascular diseases. Statins have become the leading prescription drugs of today. Indications of statins are considerably comprehensive over the last five years, succeeding the publication of the many multicenter prospective trials. Multiple statins exist in the Indian market like atorvastatin, simvastatin, pravastatin, pitavastatin, fluvastatin, cerivastatin and rosuvastatin. (Shirin Adel and Jamuna Prakash, 2010)

Statin like simvastatin, a widely used group of hypocholesterolemic drug, has shown a useful effect in reducing cardiovascular related complications and deaths in patients with or without coronary artery disease as well as with or without high cholesterol levels. The primary mechanism of action as cardio protective effect is likely through its effectively inhibiting lipid peroxidation, preservation of anti-oxidant enzymes as well as scavenging of free radicals. Along with therapeutic effects, statins also known to have dose dependant side effects like- liver damage, rhabdomyolysis and kidney failure. (Khatib *et al*., 2011)Thus, treatment with synthetic drugs in such patient's leads to various and undesirable side effects. The current available synthetic hypolipidemic drugs have their own limitations and undesirable side effects, and the costs of these drugs are very high. It will take almost a decade to develop a new synthetic drug with fewer side effects with low cost. So, new drugs with hypolipidemic effect are in great demand from ancient herbal medicines.

Plants are employed in variety of systems of medicines in our country still as in different countries. Republic of India is acknowledge because the "Emporium of medicinal plants". The utilization of plants to treat numerous diseases in Republic of India dates back to the days of Rig-Veda religious writing (3500 to 1800 B.C). Later, the monumental Ayurvedic words like Charaksamhita and Sushrutasamhita followed by different writing and Siddha treatises have incorporated nearly 700 medicinal plants used in several medicinal preparations employed in the management of health care. In reality these systems are in apply even in remote areas of our country for hundreds of years. (Ghosh2011)Until synthetic medicine was developed in nineteenth century, herbs were the premise for nearly all medical treatment. Today, herbs are still found in forty percentage of prescriptions, and also the interest to be used of plant remedies rather than chemical medicine is increasing.(Shirin Adel and JamunaPrakash, 2010)

India has been well-known from prehistoric times because the "Land of spices". Spices and herbs have tremendous necessary in lifestyle as ingredients in food, alcoholic beverages, medicine, perfumery, cosmetics, and coloring agents and additionally as farming plants. Spices impart flavour, aroma and color and so, they're a vital part of Indian food. Spices show a range of health advantages together with carminative action, hypolipidemic result, anti-diabetic, anti-lithogenic, anti-oxidant, anti-inflammatory drug, anti-mutagenic and anti-carcinogenic properties. The hypocholesterolemic and anti-oxidant properties have so much reaching nutraceutical and therapeutic price. Most of the medicinal values are attributed to the secondary metabolites gift in spices. Hence, spices are more and more being noticed for their medical properties and so their potential as a functional food has tremendous scope. (RaneRajashree *et al*., 2013) *pepper nigrum* and zingiberofficinale are both common as well widely used spices. Black-pepper (*piper nigrum*), additionally referred to as the "King of spices", is one in all the oldest and world's most significant spices utilized in ancient Indian Ayurvedic and Siddha medicines, additionally as in varied therapeutic measures within the ancient system of drugs. Piperine, principle organic constituent of black-pepper exhibits numerous pharmacologic properties and was evaluated for its potential to boost the bioavailability of drugs and nutrients in animals and humans. Rauscher determined that piperine exhibits anti-oxidant activity in streptozotocin elicited diabetic rats and alters lipid peroxidation and glutathione metabolism within the liver and intestine in varied experimental conditions.(Ramasamy Subramaniam Vijaykumar and Namasivayam Nalini, 2006)

Ginger, the root stock of *zingiberofficenale*, is one amongst the foremost widely used species of the ginger family (zingiberaceae) and could be a common condiment for varied foods and beverages. Ginger incorporates a long history of medicinal use since 2500 years back in China and India for conditions like headaches, nausea, rheumatism, colds and inflammatory diseases. The foremost pungent compounds in ginger from studies of the lipophilic rhizome extracts have yielded probably active gingerols, which may be changed to shogaols, zingerone and paradol that have anti-oxidant, anti-cancer, anti-inflammatory, angiogenesis, and anti-atherosclerotic properties.(Vani Gupta *et al*., 2013) These drugs have less or no undesirable side effects, easy availability and low cost. Therefore phyto constituents isolated from such herbs may acts as a lead compound for new pharmaceuticals.Thus considering the economic conditions and cheapness of these herbal products present study is designed to determine the hypolipidemic effect of mixed aqueous extract of *piper-nigrum* and *zingeberofficinale* in albino rats. Since, albino rats have similar metabolic patterns as that of human beings.

MATERIALS AND METHODS

Raw material procurement

The dried, unbleached, fine powders of *Z.Officinale* rhizome and *P.nigrum* fruits were purchased from The Ayurvedeeya Arkashala Ltd. Satara, India, a manufacturer of quality Ayurvedic medicines since 1926. The raw materials were authenticated at the Department of Pharmacology,

BharatiVidyapeeth Deemed University Medical College and Hospital, Sangli.

Preparation of mixed aqueous extract

Aqueous extract of *Z.Officinale* and *P.nigrum* was prepared separately as per the method of Olayemi and opaleye (1999). This was carried out by measuring 80 Gms. Of each of fine grounded powder of *Z.Officinale* and *P.nigrum* on an electronic weighing balance. This was dispensed into two beakers, each containing 1000 ml of distilled water. These were soaked for 72 hours, after which the solutions were carefully filtered with muslin cloth into a sterilized container of 1000 ml each and the filtrates obtained were stored in the refrigerator at a temperature of 4^0C until required.

Preparation of High fat diet

High fat diet (HFD) was prepared by mixing the usual standard diet (in the form of rodent-pallet) with hydrogenated sun-flower oil, which was purchased from local market.

Chemicals

Simvastatin 'tablet zosta' was purchased from Bharati Vidyapeeth Hospital Pharmacy, Sangli; manufactured by US Vitamins Ltd, India.

Qualitative Phytochemical Screening of *Zingiber Officinale* and *Piper Nigrum*

The phytochemical analysis of the aqueous extract of *zingiber-officianale and piper nigrum*for presence of alkaloids, saponins, tannins, cardiac glycosides, phenols and phlobatanins were assayed by using standard methods described by 'Trease and Evans' (1978, 1989) (Trease and Evans, 1978; Treaseand Evans, 1989) and 'Harborne and Williams, (2000) (Harborne and Williams, 2000) with slight modification.

Animal Experimentation (In vivo studies)

Forty healthy adult albino rats, weighing between 190 to 220 Gms. were obtained from 'Central Animal House.' Bharati Vidyapeeth Deemed University Medical College and Hospital, Sangli. They were housed in custom modified polypropylene cages having stainless steel vertical mesh that divides cage into 40 compartments. Each animal was housed in each compartment so that, the animals were be able to see each other, thus isolation – stress if any, was minimized. The animal facilities were well maintained and ventilated at an ambient temperature of 24± 2 ^{o}c having 50-60% relative humidity with 12 hrs light and dark cycle. They were weight matched and divided into 4 groups of 10 animals each according to their body-weight.The animals were fed with usual standard palleted diet was supplied by 'Pranav Agro Industries Limited, Kupwad MIDC, Sangli and water ad libitum for about seven days. After seven days of acclimatization, they were again weight matched & randomly selected for four experimental groups. The use and care of animals in the experimental protocol had been approved by the Local Institutional Animal Ethics Committee (Regd. No-972/po/ac/06/ CPCSEA) following the guidelines of the committee for the purpose of control and supervision of Experiments on Animals (CPCSEA), Govt. of India.

Dose selection

Human dose of tablet zosta, 40mg/kg body weight was changed into animal dose of 3.6mg/kg.body weight.

Experimental protocol

Forty albino rats were divided into four groups of ten animals each.

Group A (Negative control group): fed with HFD & water ad Libitum.

Group B (Test control group 1): fed with HFD & water ad Libitum. + mixed aqueous extract of PN & ZO at dose of 400mg/kg. body weight/day.

Group C (Test control group 2): fed with HFD & water ad Libitum. + mixed aqueous extract of PN & ZO at dose of 800mg/kg. body weight/day.

Group D (Positive control group): fed with HFD & water ad Libitum. + 3.6mg/kg body weight of simvastatin.

All administrations were given orally for an experimental period of 42 days.

Blood sample collection & experimental parameters

Initially, and at the end of experimental period of 42 days, following over-night abstaining for 12 hrs, 2 to 4 ml of blood samples were collected from retro-bulbar plexus of the medial canthus of the eye. It is done by puncturing the retro-bulbar plexus of the eye. The blood was collected into sterile, labeled plain containers. After clotting and retracting, the blood samples were centrifuged at 3000 rpm for 5 min. and the supernatant sera samples were separated and used for Lipid profile and serum glutamate pyruvate transaminase (SGPT) determinations.

Determination of serum cholesterol :(CHOD / PAP method)

Total cholesterol in serum was determined by using an enzymatic colorimetric assay based on cholesterol oxidase method as per the manufacturer's instruction by using a machine Auto Quant automated clinical chemistry analyzer.[16]

Determination of serum HDL-Cholesterol

Serum HDL- cholesterol in serum was determined by using an enzymatic colorimetric assay based on cholesterol oxidase method as per the manufacturer's instruction by using a machine Auto Quant automated clinical chemistry analyzer.[17]

Determination of serum LDL-Cholesterol

Serum LDL-Cholesterol was calculated indirectly by the "Friedewald's equation"

LDL = Total Cholesterol – (HDL + TGL/5)

Determination of serum VLDL-Cholesterol

Serum VLDL-Cholesterol was calculated indirectly by the "Friedewald's equation" VLDL = Triglycerides / 5

Determination of serum triacylglycerol: (GPO-PAP-method)

Serum triacylglycerol was measured using an enzymatic colorimetric assay based on GPO- DAOs method following the manufacturer's instruction by using a machine Auto Quant automated clinical chemistry analyzer. (Burtis *et al*., 1994; CLSI/NCCLS ep25- A, CLSI/NCCLSEP5-A_2)

Determination of serum glutamate pyruvate transaminase (SGPT/ALT)

Serum SGPT/ALT was measured quantitatively by enzymatic colorimetric assay supported "Reitman's and frankel" methodology following the manufacturer's instruction by using a machine Auto Quant automatic clinical chemistry analyzer.

Statistical Analysis

Statistical Analysis of the information was carried out by using appropriate statistically matched tests ike "ANOVA" and "Paired and unpaired- t tests."

RERULTS

Phytochemical Analysis

Phytochemical analysis of the aqueous extract of plant samples showed the presence of alkaloids, saponins, tannins, phlobatanins, Anthraquinones & cardenolides were found in each plants whereas cardiac glycosides, steroids & Terpenoids were absent.

DISCUSSION

Cardiovascular diseases continue the most reason behind morbidity and mortality in India.The role of lipids as vital factors in the pathological process of CVD has currently been firmly established.

Table 1. Phytochemical Analysis of *P.nigrum* and *Z. officenale*

S.No.	Phytochemical	*Z.Officinale*	*P.Nigrum*
1	Alkaloids	+	+
2	saponins	+	+
3	Tannins	+	+
4	Phlobatanins	+	+
5	Anthraquinones	+	+
6	Cardiac glycosides	-	-
7	Legal test	+	+
8	Lieberman's Test	-	-
9	Salkowski Test	-	-

Key: Present = + Absent = -

Table 2. Initial and final concentration of serum cholesterol levels (mg/dl.) ofAlbino rats in the experimental period of 42 days

GROUP	INITIAL	FINAL	t	p	Significance
	MEAN ± SD	MEAN ± SD			
A	78.60 ± 8.42	131.10 ± 14.07	-17.624	0.000	Highly Significant
B	79.30 ± 7.54	105.80 ± 11.71	-13.545	0.000	Highly Significant
C	78.80 ± 5.27	64.20 ± 10.00	6.095	0.000	Highly Significant
D	82.80 ± 12.7	75.20 ± 8.35	2.115	0.064	Not Significant

$p < 0.05$ = Significant; $p > 0.05$ = Not Significant

Table 3. Comparison of initial and final Cholesterol of group A with group B, group C, and group D

Cholesterol	INITIAL					FINAL				
	Mean ± SD	Std. Error	T value	p value	Significance	Mean ± SD	Std. Error	t value	p value	Significance
A	78.60 ± 8.42	2.66	-0.196	0.847	Not Significant	131.10 ± 14.07	4.45	4.37	0.000	Highly significant
B	79.30 ± 7.54	2.39				105.80 ± 11.71	3.70			
A	78.60 ± 8.42	2.66	-0.064	0.95	Not Significant	131.10 ± 14.07	4.45	12.254	0.000	Highly significant
C	78.80 ± 5.27	1.67				64.20 ± 10.00	3.16			
A	78.60 ± 8.42	2.66	0.907	0.377	Not Significant	131.10 ± 14.07	4.45	8.047	0.000	Highly significant
D	75.20 ± 8.35	2.64				82.80 ± 12.73	4.03			

Values are expressed as mean ± SD.

Table 4.Initial and final concentration of serum HDL levels (mg/dl.) of albino rats in the experimental period of 42 days

GROUP	INITIAL	FINAL	t	p	Significance
	MEAN ± SD	MEAN ± SD			
A	22.40 ± 1.90	17.00 ± 2.21	7.521	0.000	Highly Significant
B	22.90 ± 2.18	24.92 ± 2.56	-2.268	0.050	Significant
C	23.80 ± 2.70	29.50 ± 4.40	-5.697	0.000	Highly Significant
D	22.70 ± 1.57	25.80 ± 2.20	-4.122	0.003	Highly Significant

$p < 0.05$ = Significant ; $p > 0.05$ = Not Significant

Table 5. Comparison of initial and final HDL cholesterol of group a with group B, group C and group D

HDL	INITIAL					FINAL				
	Mean ± SD	Std. Error	t value	p value	Significance	Mean ± SD	Std. Error	t value	p value	Significance
A	22.40 ± 1.90	0.60	-0.547	0.591	Not Significant	17.00 ± 2.21	0.70	-7.388	0.000	Highly significant
B	22.90 ± 2.18	0.69				24.90 ± 2.56	0.81			
A	22.40 ± 1.90	0.60	-1.342	0.196	Not Significant	17.00 ± 2.21	0.70	-8.022	0.000	Highly significant
C	23.80 ± 2.70	0.85				29.50 ± 4.40	1.39			
A	22.40 ± 1.90	0.60	-0.386	0.704	Not Significant	17.00 ± 2.21	0.70	-8.92	0.000	Highly significant
D	22.70 ± 1.57	0.50				25.80 ±2.20	0.70			

Values are expressed as mean ± SD.

Table 6. Initial and final concentration of serum VLDL levels (mg/dl.) of albino rats in the experimental period of 42 days

GROUP	INITIAL	FINAL	t	p	Significance
	MEAN ± SD	MEAN ± SD			
A	23.80 ± 2.67	28.92 ± 3.30	-7.767	0.000	Highly Significant
B	24.68 ± 2.65	23.82 ± 3.66	1.2	0.261	Not Significant
C	26.80 ± 2.12	19.36 ± 2.89	8.106	0.000	Highly Significant
D	24.72 ± 2.44	23.34 ± 3.47	2.01	0.075	Not Significant

p< 0.05 = Significant ; p > 0.05 = Not Significant

Table 7. Comparison of initial and final VLDL of group A with group B, group C, and group D

VLDL	INITIAL					FINAL				
	Mean ± SD	Std. Error	t Value	P value	Significance	Mean ± SD	Std. Error	t value	p value	Significance
A	23.80 ± 2.67	0.84	-0.74	0.469	Not Significant	28.92 ± 3.30	1.04	3.271	0.004	Highly significant
B	24.68 ± 2.65	0.84				23.82 ± 3.66	1.16			
A	23.80 ± 2.67	0.84	-2.782	0.012	Not Significant	28.92 ± 3.30	1.04	6.895	0.000	Highly significant
C	26.80 ± 2.12	0.67				19.36 ±2.89	0.91			
A	23.80 ± 2.67	0.84	-0.804	0.432	Not Significant	23.92 ±3.30	1.04	3.682	0.002	Highly significant
D	24.72 ± 2.44	0.77				23.34 ± 3.47	1.10			

Values are expressed as mean ± SD.

Table 8. Initial and final concentration of serum LDL levels (mg/dl.) of albino rats in the experimental period of 42 days

GROUP	INITIAL	FINAL	t	p	Significance
	MEAN ± SD	MEAN ± SD			
A	32.40 ± 7.09	85.18 ± 13.34	-17.001	0.000	Highly Significant
B	31.72 ± 7.69	57.08 ± 13.99	-9.352	0.000	Highly Significant
C	28.20 ± 3.11	16.54 ± 9.99	4.118	0.003	Highly Significant
D	27.78 ± 8.60	33.66 ± 10.25	-2.097	0.065	Not Significant

p< 0.05 = Significant ; p > 0.05 = Not Significant

Table 9. Comparison of initial and final LDL of group A with group B, group C, and group D

LDL	INITIAL					FINAL				
	Mean ± SD	Std. Error	t value	p value	Significance	Mean ± SD	Std. Error	t value	p value	Significance
A	32.40 ± 7.09	2.24	0.206	0.839	Not Significant	85.18 ±13.34	4.22	4.597	0.000	Highly significant
B	31.72 ± 7.69	2.43				57.08 ± 13.99	4.42			
A	32.40 ± 7.09	2.24	1.715	0.104	Not Significant	85.18 ± 13.34	4.22	13.026	0.000	Highly significant
C	28.20 ± 3.11	0.98				16.54 ± 9.99	3.16			
A	32.40 ± 7.09	2.24	1.31	0.207	Not Significant	85.18 ± 13.34	4.22	9.684	0.000	Highly significant
D	27.78 ± 8.60	7.72				33.66 ±10.25	3.24			

Values are expressed as mean ± SD.

Table 10. Initial and final concentration of serum Triacylglycerol levels (mg/dl.) of albino rats in the experimental period of 42 days

GROUP	INITIAL	FINAL	t	p	Significance
	MEAN ± SD	MEAN ± SD			
A	119.00 ± 13.35	144.60 ± 16.50	-7.764	0.000	Highly Significant
B	123.40 ± 13.23	119.10 ± 18.32	1.2	0.261	Not Significant
C	134.00 ± 10.60	96.80 ± 14.44	8.106	0.261	Not Significant
D	123.60 ± 12.21	116.70 ± 17.37	2.01	0.075	Not Significant

p< 0.05 = Significant ; p > 0.05 = Not Significant

Table 11. Comparison of initial and final TG of group A with group B, group C and group D

TG	INITIAL					FINAL				
	Mean ± SD	Std. Error	t value	p value	Significance	Mean ± SD	Std. Error	t value	p value	Significance
A	119.00 ± 13.35	4.22	-0.74	0.469	Not Significant	144.60 ± 16.50	5.22	3.271	0.004	Highly significant
B	123.40 ± 13.23	4.19				119.10 ± 18.32	5.79			
A	119.00 ± 13.35	4.22	-2.782	0.012	Not Significant	144.60 ± 16.50	5.22	6.895	0.000	Highly significant
C	134.00 ± 10.60	3.35				96.80 ±14.44	4.57			
A	119.00 ± 13.35	4.22	-0.804	0.432	Not Significant	144.60 ± 16.50	5.22	3.682	0.002	Highly significant
D	123.60 ± 12.21	3.86				116.70 ± 17.37	5.49			

Values are expressed as mean ± SD.

Table 12. Initial and final concentration of serum SGPT levels (U/L) of albino rats in the experimental period of 42 days

GROUP	INITIAL	FINAL	t	p	Significance
	MEAN ± SD	MEAN ±SD			
A	28.10 ± 7.10	51.56 ± 14.14	-9.596	0.000	Highly Significant
B	31.11 ± 7.29	41.76 ± 7.91	-3.507	0.007	Highly Significant
C	28.99 ± 5.56	26.80 ± 7.46	0.874	0.405	Not Significant
D	26.22 ± 5.75	55.21 ±14.96	-8.881	0.000	Highly Significant

p< 0.05 = Significant ; p > 0.05 = Not Significant

Table 13. Comparison of initial and final SGPT of group A with group B, group C, and group D

SGPT	INITIAL					FINAL				
	Mean ± SD	Std. Error	t value	p value	Significance	Mean ± SD	Std. Error	Value	p value	Significance
A	28.10 ± 7.10	2.25	-0.935	0.362	Not Significant	51.56 ± 14.14	4.47	1.912	0.072	Not significant
B	31.11 ± 7.29	2.31				41.76 ± 7.91	2.50			
A	28.10 ± 7.10	2.25	-0.312	0.759	Not Significant	51.56 ± 14.14	4.47	4.896	0.000	Highly significant
C	28.99 ± 5.56	1.76				26.80 ± 7.46	2.36			
A	28.10 ± 7.10	2.25	0.65	0.524	Not Significant	51.56 ± 14.14	4.47	-0.561	0.582	Not significant
D	26.22 ± 5.75	1.82				55.21 ±14.96	4.73			

Values are expressed as mean ± SD.

Lipoprotein composition is continuously changed during intravascular Lipoprotein transit through the agency of epithelium lipases (LPL, liver lipase), LCAT, cholesterol ester transfer protein and phospholipids (PL). Modification within the regulation of those enzymes as a result of genetic and environmental factors like dietary cholesterols and saturated fat might cause clinical abnormality like Hyperlipidemia. Symptom of Hyperlipidemia mirrors the onset of abnormalities in lipid metabolism secondary to the manifestation and progression of the CVD within the patient. Hyperlipidemia is the most vital modifiable risk factor responsible for hardening and induration of the arteries, and this is associated with different cardiac conditions like CHD, IHD – etc.

Completely different approaches were used to treat the CVD. Popular among them are medicinal drug therapy, diet therapy and recently herbal medicine therapy with spice.*Piper-nigrum* and *zingiberofficenale* are world's most vital ancient spices. they're widely used for varied ailments. The beneficial properties of each plant come back from their chemical composition (i.e. phytochemicals).In the our experimental study, we found the hypolipidemic property of mixed aqueous extract of *piper nigrum* and *zingiberofficenale*, at completely different concentrations in HFD- fed albino rats, a metabolic model for hyperlipidemia. Since, experimental albino rats have similar metabolic patterns as that of human-beings. it's rational to use this hyperlipidemic model to look at the beneficial effects of treatment with *piper nigrum* and *zingiberofficenale*.
The study was conducted within the Department of Biochemistry, BharatiVidyapeeth, Medical College and Hospital Sangli. It had been included total 40- albino rats, out of them twenty were treated with mixed aqueous extract of *pipernigrum* and *zingiberofficenale* and twenty were controls. To check the effect of deferential doses of *piper nigrum* and *zingiberofficenale* as well as synthetic drug simvastatin. These results of B, C, and D groups were compared with group A, which was negative control group within the study.

Serum total cholesterol (mg/dl.)

Table 2: Shows the initial and final serum total cholesterol levels (mg/dl.) in the different experimental groups.Prior to treatment, serum total cholesterol concentrations were not statistically different among all the groups. As all animals were with same dietary and environmental conditions.

Table 3: Shows, comparison of initial and final total cholesterol concentration of group A with group B, group C and group D after treatment finally at the end of 42 days.

Group B, C and D showed significant reduced total cholesterol concentration than group A i.e. negative control group. This may be due to the effect of treatment. Our results of decreased cholesterol were in line with that of Reza Alizadh- Navaei, and coworkers (2008).(Reza Alizadeh Navaei *et al*., 2008) The extracts in B group with concentration of 400mg/kg body weight/day and C group with concentration of 800mg/kg body weight /day were sufficient to lower the cholesterol concentration in respective groups. *Piper nigrum* and *zingiberofficinale* both decreases the absorption of cholesterol and increases the excretion of cholesterol as bile acids. (Reza Alizadeh Navaei *et al*., 2008) The conversion of cholesterol into bile acids is an important pathway of eliminating cholesterol from body. Albino rats fed with zingiberofficinale showed significantly elevated activity of hepatic cholesterol-7-hydroxylase which is the rate limiting enzyme in bile acid synthesis. (Reza AlizadehNavaei *et al*., 2008) Simultaneous piperine supplementation along with the high fat diet significantly reduced the activity of HMG CoA reductase indicating the potential cholesterol-lowering effect of piperine. (Vijaykumar Subramaniam Ramasamy and Namasivayam Nalini, 2006)

Cholesterol synthesis is related to exaggerated activity of the lipoprotein LDL receptor, that successively results in increased removal of lipoprotein LDL from plasma, leading to reduced plasma cholesterol concentration. (Reza AlizadehNavaei *et al*., 2008)

Thus, may be because of inhibition of HMG CoA reductase enzyme and removal of cholesterol by increased lipoprotein HDL concentration, cholesterol concentration reduced considerably and exaggerated conversion and excretion of cholesterol as bile acid could results in reduced cholesterol concentration.

Serum HDL Cholesterol

The levels of HDL Cholesterol studied in different groups of rats are shown in Table 4 & 5**:** The serum HDL concentrations before the treatment were same among the all groups.

The concentration of HDL cholesterol in B group increased with high significance than group A, similar highly significant elevation was observed in group C and D also, when compared to these groups with group A at the end of experimental period of 42 days.The low HDL cholesterol values in group A than group B, C and D may because of high fat diet. In this A group no synthetic drug or herbal extract was used to reduce lipid levels. Hence the HDL cholesterol values may be lower than all other groups.

In group B and C, where piper nigrum and zingiberofficinale extract was used that leads to raised HDL cholesterol level. The mean concentration of HDL in C group was 29.5 ± 4.40 mg/dl and in B group it was 24.90 ± 2.56 mg/dl as compared to 17.00 ± 2.21 of group A. This also indicates that the dose of group C (i.e. 800 mg/kg body weight /day) was additionally effective to improve the HDL cholesterol levels.

The piperine, principle alkaloid of piper nigrum inhibits lipid accumulation by dramatically modifying the enzymes like LCAT. [(23)]This modulation of LCAT by extract may be responsible for the increase in HDL concentrations.We also observed that simvastatin was also increases the HDL concentration in group D, But not statically significant than that of extract with 800mg/kg body weight /day concentration.

Serum VLDL Cholesterol

Table 6: Shows initial and final concentration of serum VLDL levels of albino rats in the experimental period of 42 days with mean ± SD. Initially there was no significant difference in VLDL concentration among all groups.

Table 7: Shows comparison of initial and final VLDL of group A with group B, C and D.

The concentration of VLDL cholesterol in group B reported highly significant decrease ($p<0.000$) than group A, where as similar highly significant decrease was observed in group C and group D, when compared with group A finally at the end of study.The increased concentration of VLDL in group A as compared to group B, C and D may because of high fat diet and water ad libitum. Piperine modulates lipoprotein lipase activity (LPL). (Shah *et al*., 2011) Lipoprotein lipase hydrolyses a portion of Triacylglycerol (TAG) present in Chylomicrons and VLDL to liberate free fatty acids and glycerol. (Satyanarayana, 2005)Therefore, decreased VLDL in group B and C may be due to increased lipoprotein lipase activity which hydrolyze VLDL and reduces its concentration. This activity of lipoprotein lipase may be more in group C with extract 800 mg/kg body weight concentration. That's why the VLDL decreases as more than group B and D ($p<0.000$). The mean concentration of VLDL in group B was 23.82 ± 3.66 and that of group D was 23.34 ± 3.47 while in group C it was 19.36 ± 2.89, therefore extract with 800 mg/kg body weight concentration may be more effective to reduce VLDL than 400 mg/kg body weight /day dose.Simvastatin treated group D act as positive control which also has VLDL values more than group B.

Serum LDL Cholesterol

Table 8: Shows initial and final concentration of serum LDL levels of albino rats in the experimental period of 42 days with mean ± SD.

Initially at 0 day there was no difference in serum LDL levels of albino rats this happened because all animals were with same dietary and environmental conditions and they were with no treatment, either synthetic or herbal.

Table 9: Shows comparison of initial and final LDL of group A with group B, and group C and group D. The concentration of LDL cholesterol in group B decreased with high significance than group A, similar highly significant decrease was observed in group C and D also, when compared to these groups with group A at the end of experimental period of 42 days. As piperine modulates the activity of LCAT; the LDL cholesterol from peripheral tissues can be transported to liver. This may be the reason for decreased LDL levels in group B, C, D than that of group A. Again 800 mg/kg body weight /day concentration of mixed aqueous extract showed exaggerated effect. More decrease in LDL concentration in C than group B and D as compared with group A.

Serum Triacylglycerol

Table 10: Shows Initial and final concentration of serum Triacylglycerol levels of albino rats in the experimental period of 42 days.

Prior to treatment, serum total Triacylglycerol concentrations were not statistically different among all the groups. As all animals were with same dietary and environmental conditions and they were with no treatment, either synthetic or herbal.

Table 11: Shows Comparison of initial and final Triacylglycerol of group A with group B, group C and group D. Piperine modulates activity of lipoprotein lipase which hydrolyzes the TAG present in Chylomicrons and VLDL to liberate free fatty acid and glycerol.(Satyanarayana, 2005) Hence increased hydrolysis of TAG by lipoprotein lipase may be the reason for lowering TAG concentration in B, C, and D group as compared with A in our study.

Table 12: Shows Initial and final activity of serum SGPT of albino rats in the experimental period of 42 days. Initially at 0 day there was no statistical difference in serum SGPT activity of albino rats this was due to all animals were with same dietary and environmental conditions and they were with no treatment, either synthetic or herbal medicine.

Table 13: Shows comparison of initial and final SGPT of group A with group B, group C and group D. When final SGPT of group A was compared with final SGPT of group B there was no significant difference between SGPT activities of these

two groups. Final SGPT activity of group C was decreased with high significance than that of group A (negative control), and in D group there is no significant difference between activity of SGPT of these two groups.

Simvastatin synthetic drug has known hepatotoxic side effect hence SGPT activity increased finally than initially in group D (positive control group). SGPT activity is high than normal in group A and group D. Group A fed with high fat diet leads to fat load for in liver for its metabolism therefore the liver function was hampered and this leads to increased SGPT.B group also shows no significant difference while C group shows highly significant decrease in SGPT than positive and negative control. This may be due to decrease in cholesterol, LDL, VLDL and increased HDL concentration i.e. hypolipidemic effect of extract at higher concentration. So when fat load in liver decrease; the SGPT activity may normalize.

Summary and Conclusion

We assessed the hypolipidemic effects in the experimental animals in terms of measurement of biochemical parameters like serum cholesterol, serum HDL, serum LDL, serum VLDL, & serum SGPT etc. The results were compared with the study group which was treated with synthetic statin drug against herbal compounds. In our study, we found the hypolipidemic effect of *piper nigrum*and *zingiberofficinale* in high fat diet fed albino rats, a metabolic model. Since, albino rats have same metabolic patterns as that of human beings.On comparison of initial levels of serum cholesterol with final values, it was observed that group A showing highly significant increased levels this may be due to feeding of high fat diet. Groups C and D shows decreased levels, proves the hypolipidemic effects of drugs herbal and statin. High values in group B may be cause of failure of action of herbal extracts which may be due to low dose.

We found that, HDL-C is increased with significance in group B, C and D as compared to negative control group A. The mean concentration of HDL-C in group C is highly significant than that of group B. This means that the higher dose of group C (800mg/kg/day) is more practical. Group D (negative control) also increases the HDL-C levels but not as much as that of group C. Our results are in line with Shreya *et al*. (2011)We conjointly noted that, increased concentration of LDL-C in group A because it was negative control group. Test group C showed exaggerated effect as compared with group B and D, it may be due to modulated activity of LCAT by phytochemicals present in extract of both plants. And as a result LDL-C from peripheral tissues can be transported to liver.In case of TAG, we found concentration of TAG is reduced with high significance in group B, C and D as compared with A. This may be due to increased hydrolysis of TAG by lipoprotein lipase, caused by a piperine which may present in extract. We also assessed the liver function by measuring SGPT activity. We found that, final result of SGPT activity of group C is more marked than group B and negative control group A. This may be due to higher dose of extract and increased HDL-C in test group C. Thus, herbs *pipernigrum* and *zingiberofficenale* could also be helpful in future as a number one compounds for development of recent medicine, which may ready to treat hyperlipidemia, a serious reason behind CVD, after clinical trials.

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