

To Evaluate the effect of *Marich* (*Piper nigrum.*, Linn.) on experimental model of high fat diet induced Hyperlipidemia.

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ABSTRACT:

Ayurveda conceives and describes the basic and applied aspects and principles of life process, health and diseases and its management in terms of its own principles and approaches. Because of luxurious life and sedentary habits body fats along with cholesterols are increasing in the body, which invites the disorders like hypertension, heart diseases, and hyperlipidemia. Hyperlipidemia is a condition in which the levels of lipoproteins, i.e cholesterols, triglycerides or both are raised in plasma to the extent that it may have adverse effect in health leading to life expectancy. *Marich* is very old drug known to Indians from long time and its iniquity goes beyond 2000-3000 years. *Marich* is useful remedy in many disorders, it helps in the *puran* of *dhatu*s , It is helpful in the shaman of diseases and in maintaining good health , The experimental study was carried out for

42 days. In which for first 21days the obesity was induced by creating high fat induced obesity experimental model of Wister rats male, which was further treated for next 21 days with three different test drug dosage of *Marich* (A), of 90mg/kg., *Marich* (B) of 180mg/kg and *Marich* (C) of 270mg/kg were used. For obesity induction Vanaspati Ghee (Dalda) and coconut oil (Parachute) was used in which daily pellets were soaked overnight. The Lipids were recorded , the blood samples were collected on 0, 21 and 42 days respectively. Standard Drug Atorvastatin was used. The blood samples were send for histopathological results and the statistical analysis was done with *Annova* method. Obesity was induced till day'21 and again was reduced satisfactorily by *Marich* group(A) and group (C) showed maximum satisfactory results with histopathological changes.

KEYWORDS:

Marich, HFD, , hyperlipidemia, Lipids.

INTRODUCTION:

Hyperlipidemia is an elevation of lipids (fats) in the bloodstream. These lipids include cholesterol, cholesterol esters (compounds), phospholipids and Triglycerides. Hyperlipidemia^(9,10) is a condition in which the levels of lipids in Plasma are increased. It is of utmost significance because it leads to *Atherosclerosis* of vessels (arterial walls) leading to vascular accidents. Moreover, lipid levels vary with age, sex and nutritional status. Adolescence causes more change in males than in females. Levels of plasma lipids tend to rise from the third to seventh decade, particularly in affluent societies. Diagnosis of hyperlipidemia⁽¹¹⁾ is done via blood measurement of Cholesterol, triglyceride, LDL, VLDL, and HDL. LDL and VLDL can be measured indirectly by Applying the formula of FRIEDEWALD for calculation as $LDL = \text{Total cholesterol} - HDL - VLDL$. $VLDL = \text{triglycerides} / 5$ Where all the values are measured in milligrams per deciliter. According to vedic literature (Upanishad Bhoj 2/2/77). *Brahmans* should not sell *Maricha*⁽¹⁾ and pippali. It means the utility of *Maricha* is very less compared to that of *pippali* in the *Rugveda*. However, in the *Samhitta* period the utility of black pepper is more realized and used extensively in the therapeutics. The drug is also mentioned

in the work of *Kalidas* (4th century A.D.), *Bramha Bhatta* (7th century A.D), & in *Aamrakosha*, (6th century A.D.) In Gupt kala some important spicy drugs were being transported from India to Arab country among them *Maricha*⁽¹⁾ is one. *Brihatraye* extensively described this plant as appetizer, carminative and *Charakacharya* has described in following *gana's* as *Deepaniya*, *Shoolaprashamana*, *Krimighna*, *Shirovirechanopaga*, *Shirovirechan*, *aharopayogi*. *Suhsrutacharya* in following *gana's* *Pippaliyadi*, *Trikatu*⁽²⁾, *Shirovirechan* and further *Vagbhatt* has mentioned *Shirovirechanagana*, *Vatsakadi*, *Pippaliyadi*, *Deepaniya*, It is responsible for destroying *Visha*, *Jantu*, *kapha*, and *vata* from body. It was an important item of export and was found on ports. It is black in colour. It is useful in scraping *kapha* from the body. It is *Katuramatmak* and taken in the weight of one Kola i.e. 10gms.

AIM:

To evaluate the effect of *Piper nigrum churna* on High Fat Diet induced obesity model in the male wistar rats with Lipid profile, Total cholesterol, TG (Triglyceride), HDL (High Density Lipo Protein), LDL (Low Density Lipo Protein), VLDL (Very low Density Lipo Protein), (0,21,42) comes under the aim and objectives of the study.

NIGHANTU-CLASSIFICATION MARICHA

<i>DHANVANTARI</i>	<i>Shatpushpadi varga</i>
<i>MADANPALA</i>	<i>Shuntyadi varga</i>
<i>RAJ</i>	<i>Pippalyadi varga</i>
<i>KAIVYADEV</i>	<i>Oushadi varga</i>

WBHAVAPRAKASH	Haritakyadi varga
MAHAAUSHADHA	Mahaaushada varga
ADARSH NIGHANTU	Aadrakadi varga
ABHIDANA	Katukskand

RASA PANCHAK OF MARICHA::

RASA	VEERYA	VIPAK	GUNA	DOSHA
Katu	Ushna	Madhur,Katu	Laghu	Kaphaghna, Vataghna

Hyperlipidemia⁽⁴⁾ is a condition in which the levels of lipoproteins, i.e cholesterol, triglycerides or both are raised in plasma to the extent that it may have adverse effect in health leading to life expectancy. Following activities have been studied on *Maricha*⁽⁴⁾. Oxidative stress, Antioxidant, Anti thyroid, *Hypercholesterimia*, Dyslipidemia, Lipid lowering Anti amoebic *Acardicidal* Pharmacodynamics. Hyperlipidemia model in the male Wistar rats using following parameters, Weight (weekly i.e. day 0,7, 14, 21, 28, 35, 42), Fasting blood sugar levels (day- 0, 21, 42) Histopathological changes VLDL, LDL HDL Triglyceride Total cholesterol, liver, adipocytes⁽³⁾.

Before starting the experimental study the permission of the Institutional Animal Ethics Committee for Animal Experimentation was obtained. The permission of the Institutional Animal Ethics Committee for Animal Experimentation was obtained at SGRS College of Pharmacy, Saswad. The Experimental study was done on High Fat Diet Model⁽⁵⁾ for 42 days (Ref: M.P. Shyamala, Antioxidant potential of the *Syzygium aromaticum (gaertn.) Linn.* (Cloves) in rats fed with high fat diet Indian Journal of Pharmacology 2003;35; 99-103.) The study was carried out in 36 Wistar Male rats; weighing up to 180-200 gm. They were divided into 6 groups as mentioned below.

MATERIALS AND METHODS:

TABLE.NO.1—Table showing groups of Wistar rats for experiment

Group	Name of group	No. of animals	Group description
1	Normal control	6	Normal diet
2	Normal control	6	HFD 10ml/kg
3	Standard control	6	Atorvastatin 1.2mg/kg/day
4	Formulation	6	Dose 1 (90 mg/Kg) — HFD + <i>Maricha churna</i> — Day22 - Day 42
5	Formulation	6	Dose 2 (180 mg/Kg) — HFD + <i>Maricha churna</i> — Day22 - Day 42
6	Formulation	6	Dose 3 (270 mg/Kg) — HFD + <i>Maricha churna</i> — Day 22- Day 42

EXPERIMENTAL EVALUATION

Sample selected and purchased as per the API guidelines. ,Analysis done as per the guidelines given in API. Drug identification and Authentication done at Department of Botany, Pune University. The plan of work is divided as follows:1) Collection of Samples, 2) Identification 3) Authentication, 4)Standardization 5) *Pharmacognostical* study 6)Experimental study. Market samples of the drugs collected from 3 different vendors. Marked as Sample A, B & C. Authentication of the samples done at Department of Botany, Pune University, Pune, Maharashtra., further *pharmacognostical* study was carried out.

DESCRIPTION ABOUT GROUPS:

1. Group 1 receive normal diet and served as normal control.
2. Group 2 receive 10 ml/kg/ body weight of HFD⁽⁵⁾ (Coconut oil + *Vanaspati* ghee 2:3) throughout the study i.e. for 42 days.
3. Group 3 receive Atorvastatin (1.2 mg/kg/day for 21 days)(i.e. from 21st day of the study till the end of the study). This group will act as positive control group.
4. Group 4, 5 and 6 receive aqueous extract of *Marich* churna 50,100 and 150 mg/Kg respectively for 21 days (i.e. from 21stday of the study till the end of the study).
5. Obesity get induced by the 21stday of the experiment ,to reveal *hyperlipidemic* changes Blood samples taken, after 21stday these groups receive the treatments as mentioned above along with HFD till the Day 42.

Period for animals are given to adjust in the animal house with regular water and feed before handling then for any kind of experiment. After this period the animals are selected on random basis for experiment.

The randomly selected animal are then marked with number tags on cages or on their body parts like head, tail, left or right paw are marked using picric acid solution so it becomes easy to identify animals.

Animals were maintained at room temperature at 25degree Celsius, with 12 hrs. day and dark cycles. Standard laboratory diet was given with an unlimited water supply of drinking water.

The Pallets were soaked overnight in *Vanaspati* Ghee (Dalda) and Coconut Oil(Parachute),this feed was given for 42 days to Disease control Group

To Test drug Group Animals this feed was given for 21 days for obesity induction involving hyperlipidemia as per *dalda* and oil diet. Normal control group was not given this feed.

HFD INDUCTION:

There are 4 types of experimental models to induce obesity, they are as Follows.

1. Food Induced Obesity - In this method the obesity is induced by feeding the animals with food with high starch and fat content so naturally the obesity is induced in todays world major reason of obesity induction is heavy intake of starchy and fatty food like oil corns ,chips, oily and fast food so using this method is easy and cheapest method of obesity induction so this method is selected for the study the animals were administered with *Vanaspati* Ghee (*Dalda*) and Coconut Oil

(Parachute), which gradually cause hyperlipidemia

2. Hypothalamic method-*Hyperphagia* in rats has been reported after hypothalamic lesions by surgical techniques, such hypothalamic lesions are prepared which leads to obesity induction and further leading with hyperlipidemia
3. *Gold-Thio Glucose* - In this method intraperitoneal or intra muscular injection of *gold-thio-glucose* induces obesity in mice.
4. Monosodium Gluconate - Monosodium Gluconate injections are given subcutaneously to animals to induce obesity by causing adiposity.

The animals were sacrificed after blood collection by retro-orbital sinus puncture on day42. The serum was separated at 3800rpm for 15 min at 25 degrees Celsius in Remiss cooling microfuge and samples are stored at -20degree Celsius until use. Liver and adipocytes were quickly transferred to ice, cold, phosphate buffered saline(ph. 7.4) and EDTA solution. The organs were blotted free from blood and tissue Fluids and weighted on S. *Chaimdzu* scale.

OBSERVATIONS:

Physical properties of *Marich* shows it is slightly soluble in *Jala*. Organoleptic observation shows that *Marich Churna*⁽²⁾ has Brown-blackish color, Pungent taste and odour. The changes in Thin layer Chromatography shows that Yellow and Violet color are seen which shows the drug are chemical components present in it. Lipids of animals recorded on day 0, 7, 14, 21, 28, and 42 increase of Triglyceride. Cholesterol, lipids of disease control group recorded; gradual changes has been seen in increase of levels. Readings for same , of animals recorded in disease control group shows that 0, 7,14,21,28 and 42 and increase in Lipids of disease control group compare to normal group in seen. Changes seen in standard control group till 21 days and after administration of standard drug is the gradual level loss is seen. Changes seen in increase of lipid levels till 21 days after administration the drug dose of Group A i.e90mg/kg the changes of decrease is seen. Group A was more effective than others in High Fat Diet induced hyperlipidemia⁽⁸⁾ model in rats Percentage wise improvement parameter of lipids shows group A is more effective levels.

TABLE-Table showing Karm of *Maricha*, on *Dosha Dhatu* , *Strotas* & *Vyadhi*

<i>SR. NO.</i>	<i>CATOGORY</i>	<i>ACTION</i>
1.	<i>On Dosha</i>	<i>Kapha and Vatadosha hara (ref.V.G.Desai)</i>
2.	<i>On Dhatu</i>	<i>Meda and Rakta dhatu</i>
3.	<i>On Strotas</i>	<i>Medovaha strotas</i>
4.	<i>On Vyadhi</i>	<i>Aruchi, Hridrog, Sthoulya,etc.</i>

HISTOPATHOLOGICAL OBSERVATIONS:

As seen in the histopathological reports the following observations are notes, Disease control group shows fatty *infiltration* of 75%, which when treated with standard drug atorvastatin the fatty *infiltration* is reduced to 25%, and with test drugs *Maricha*⁽¹²⁾. group (C) with

dose of 270 mg/kg the fatty *infiltration* is seen upto 50% with test drugs *Maricha*., Group (B) with dose of 180 mg/kg the fatty *infiltration* is seen upto 50% and with test drug *Maricha* group (A) with 90 mg/kg the fatty *infiltration* is seen upto 25%.

GROUP WISE IMPROVEMENT:

TABLE.2.-Table showing the group wise improvement compared with Standard control Group

GROUP	WT	BSL	TRI	HDL	TC	VLDL	LDL
Standard Control	9.69%	9.78%	64.92%	36.65%	41.63%	64.92%	64.17%
Group A	17.83%	3.14%	67.50%	49.99%	43.69%	67.50%	68.28%
Group B	14.39%	1.51%	63.85%	41.78%	39.69%	63.85%	66.35%
Group C	12.08%	2.62%	66.17%	35.88%	32.06%	66.17%	51.00%

Above table showed that Group A was more effective than Group B and Group C.

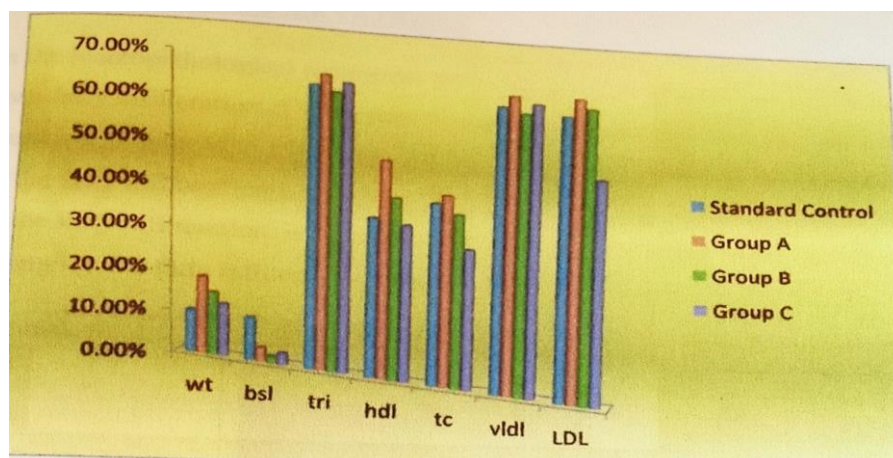
Comparison Standard drug With Group Disease control

TABLE: 3 Table showing parameter improvement by Standard Drug with Disease control Group

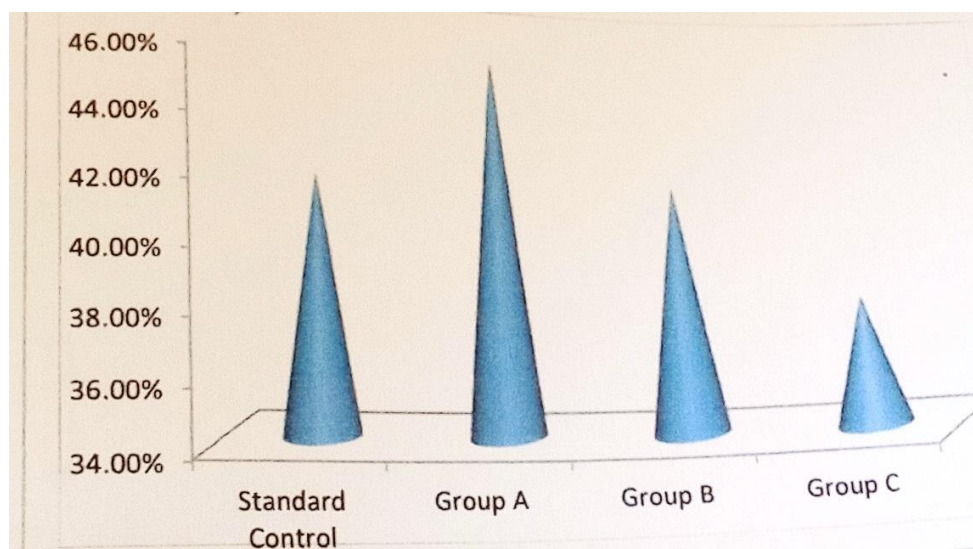
Parameter	Mean		SD		t value	p value
	Disease Control	Standard Control	Disease Control	Standard Control		
WT	347.8333	258	27.70138	14.49138	7.038569	3.55E-05
BSL	115.77	101.72	5.539213	5.431968	4.436013	0.001262
TRI	186.3833	127.8517	1.525787	13.75609	10.35896	1.15E-06
HDL	16.355	20.395	0.853387	1.094454	-7.13046	3.18E-05
TC	142.2217	90.38667	2.094463	1.332752	51.14492	1.97E-13
VLDL	37.27667	25.57033	0.305157	2.751218	10.35896	1.15E-06
LDL	88.59	44.42133	2.667341	3.378673	25.13336	2.28E-10

The standard control group shows significant changes with disease control group. The standard drug Atorvastatin shows good results.

Graph 1. Shows parameter improvement of standard drug and test drugs



Graph 2—Comparison between Test drug groups and Standard drug



From above graph we found that more improvement was seen in Group A than others in High Fat Diet induced Hyperlipidemia model in rats.

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Conflict of Interest: Non

Source of funding: Nil

Cite this article:

To Evaluate the effect of Marich (Piper nigrum., Linn.) on experimental model of high fat diet induced Hyperlipidemia.

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Ayurline: International Journal of Research In Indian Medicine 2023; 7(1):01-08