

"To Evaluate the effect of *Trikatu* on the 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.. The *Trikatu churna* makes this traditional drug more stable for long term storage and hence, easier

to prepare. The *Trikatu churna* is a reputed drug mentioned in the ancient books of *Ayurveda* used for the treatment of various diseases. 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 wistar rats male, which was further treated for next 21 days with three different test drug dosage of *Trikatu* (A), of 90mg/kg., *Trikatu* (B) of 180mg/kg and *Trikatu* (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 sent for *histopathological* results and the statistical analysis was done with Anova method. Obesity was induced till day 21 and again was reduced satisfactorily by *Trikatu* group (A) and group (C) showed maximum satisfactory results with *histopathological* changes.

KEYWORDS: *Trikatu*, HFD, hyperlipidemia, Lipids.

INTRODUCTION:

Hyperlipidemia is an elevation of lipids (fats) in the bloodstream. These lipids include cholesterol, cholesterol esters (compounds), phospholipids and Triglycerides. *Hyperlipidaemia* is a condition in which the levels of lipids in Plasma are increased. It is of utmost significance because it leads to *Artherosclerosis* 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 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 $LDL = \text{Total cholesterol} - HDL - VLDL$.

$VLDL = \frac{\text{triglycerides}}{5}$

Where all the values are measured in milligrams per deciliter.

Trikatu comes under the group of Mishrak gana of as first mentioned by Rajnighantu, before the Samhittakars have grouped the drug together and used but the gana was firstly mentioned by Rajnighantu, so the drug *Trikatu* group of three pungents is selected for the study as no such group of drug was studied on HFD (high fat induced obesity) model. I

The *Trikatu churna* makes this traditional drug more stable for long term storage and hence, easier to prepare. The *Trikatu churna* is a reputed drug mentioned in the ancient books of *Ayurveda* used for the treatment of fever, asthma, cold and cough, diabetes, nasal diseases, obesity, anorexia, digestive, respiratory system and normal urinary tract function. Taking this drug for study the main aim is to study the various factors associated with *Trikatu churna* and its researches done previously. To evaluate the effect of *Trikatu 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.

MATERIALS AND METHODS:

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, for 42 days (Ref : M.P. Shyamala, Antioxidant potential of the *Syzgium 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

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	Atorvatiation 1.2mg/kg/day
4	Formulation	6	Dose 1 (90 mg/Kg) — HFD + <i>Trikatu churna</i> — Day22 - Day 42
5	Formulation	6	Dose 2 (180 mg/Kg) — HFD + <i>Trikatu churna</i> — — Day22 - Day 42
6	Formulation	6	Dose 3 (270 mg/Kg) — HFD + <i>Trikatu churna</i> — — Day 22- Day 42

EXPRIMENTAL 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. Authentification,
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 (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 *Trikatu churna* 50, 100 and 150 mg/Kg respectively for 21 days (i.e. from 21st day of the study till the end of the study).
5. Obesity get induced by the 21st day of the experiment, to reveal hyperlipidemic changes Blood samples taken, after 21st day 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 25 degree celcius, 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 today's world major reason of obesity induction is heavy intake of starchy and fatty food like oilcorns, 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 intramuscular injection of goldthio glucose induces obesity in mice.
4. Monosodium Glutamate - Monosodium *Glutamate* 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 day 42. The serum was separated at 3800rpm for 15 min at 25 degree Celcius in Remiscooling microfuge and samples are stored at -20degree celcius 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:

It has been observed that following activities have been studied on Sunthi, namely Pharmacognostic study Phytochemical Study, Pharmacological Study, Clinical study Antimicrobial, Cytotoxic, Antioxidant, Antibacterial, Antidiabetic, Prediabetic, Antiinflammatory, Neuroprotective. For Maricha, Following activities have been studied on Maricha. Obesity on HFD model, Dyslipidemia, Oxidative stress, Antioxidant, Antithyroid, Hypercholesterolemia, Lipid lowering, Antiamebic, Acardicidal, Pharmacodynamics For Pippali, following activities have been studied, Pharmacodynamics Clinical Antidiabetics, Antitussive, Antiinflammatory, Lipid lowering, Antiobesity, Antihelminthic, Immunomodulatory. Following activities have been studied on *Trikatu* Etiopathological, Pharmacodynamic

Pharmacognostical, Antihelminthic, Hepatoprotective, Standardisation, Appetitestimulant, Obesity in aartavakshaya, Pharmacokinetics Apettite stimulant. *Trikatu* is a formulation of three drugs in equal proportion. It comes under category of Mishrak Gana. As the name suggest *Trikatu* means three peppers or three Pungents.- TABLE-Table showing Karm of Trikatu on Dosha Dhatu, Strotas & Vyadhi

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

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 *Trikatu* group (C) with dose of 270 mg/kg the fatty infiltration is seen upto 50% with test drugs *Trikatu* Group (B) with dose of 180 mg/kg the fatty infiltration is seen upto 50% and with test drug *Trikatu* 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.

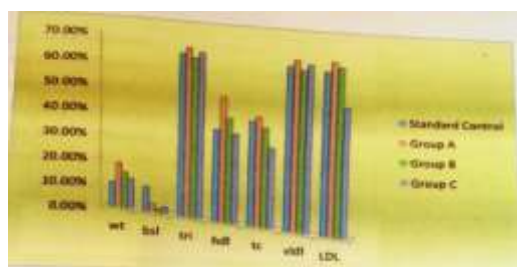
Comparison Standard drug With Group Disease control

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

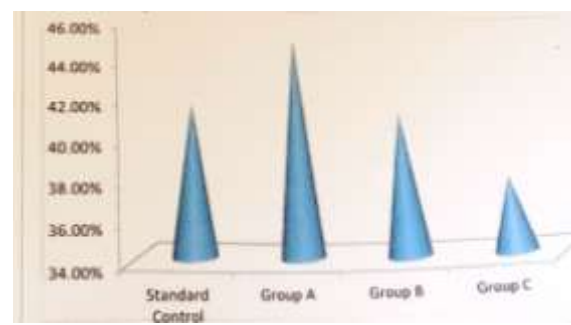
Parameter	Mean		SD		t value	p value
	Disease Control	Standard Control	Disease Control	Standard Control		
WT	347.8333	258	27.70138	14.49138	7.038569	3.55E-05
BSL	115.77	101.72	5.539213	5.431968	4.436013	0.001262
TRI	186.3833	127.8517	1.525787	13.75609	10.35896	1.15E-06
HDL	16.355	20.395	0.853387	1.094454	-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.

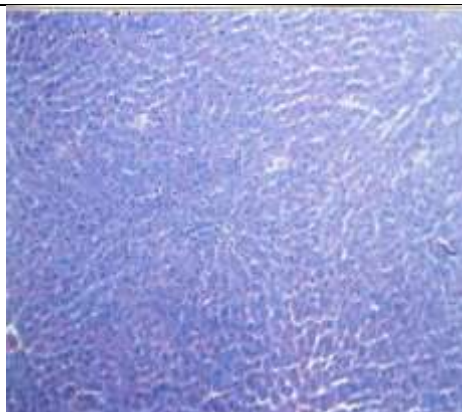


From above graph we found that more improvement was seen in Group A than others in High Fat Diet induced Obesity model in rats.

HISTOPATHOLOGICAL SLIDES

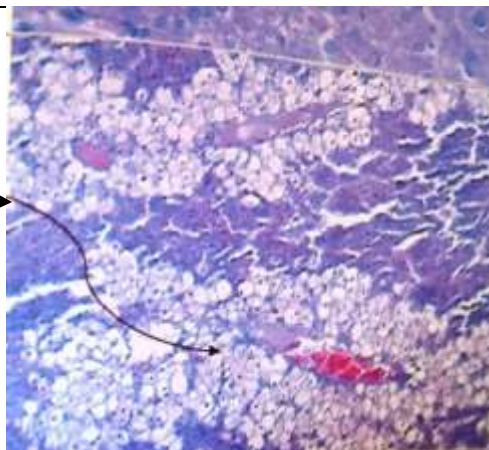
Normal Control

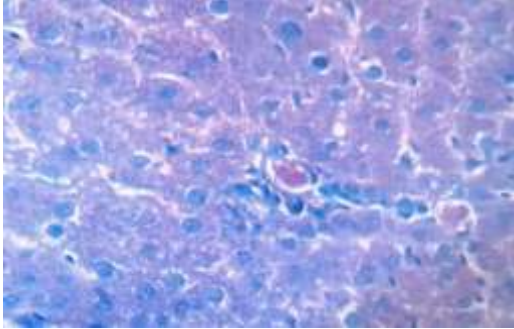
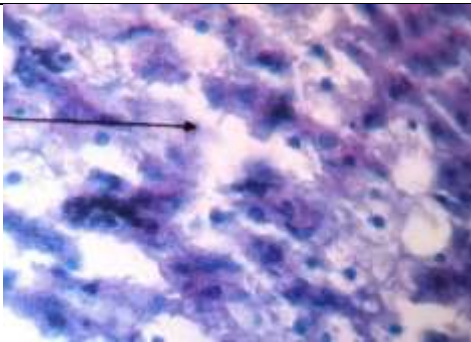
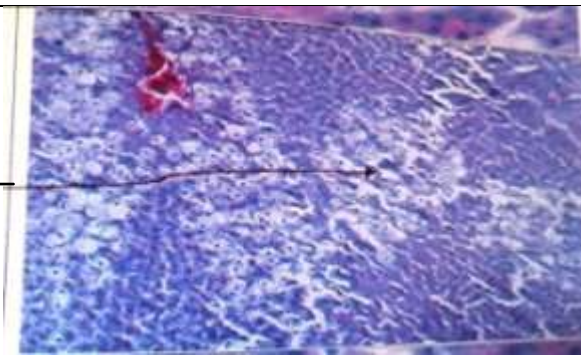
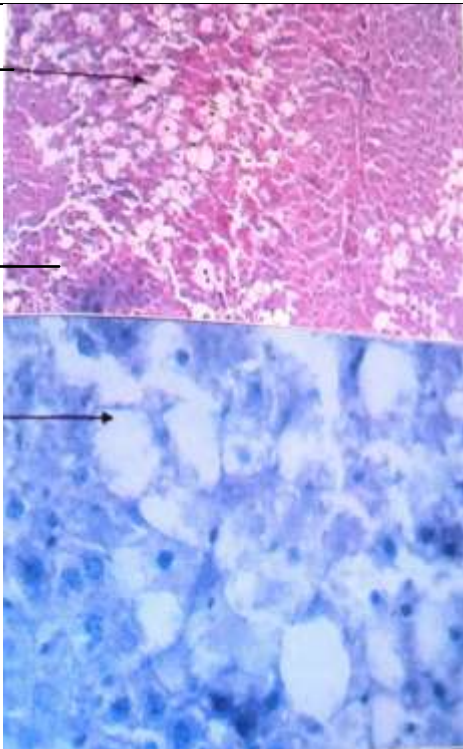
No Change Normal

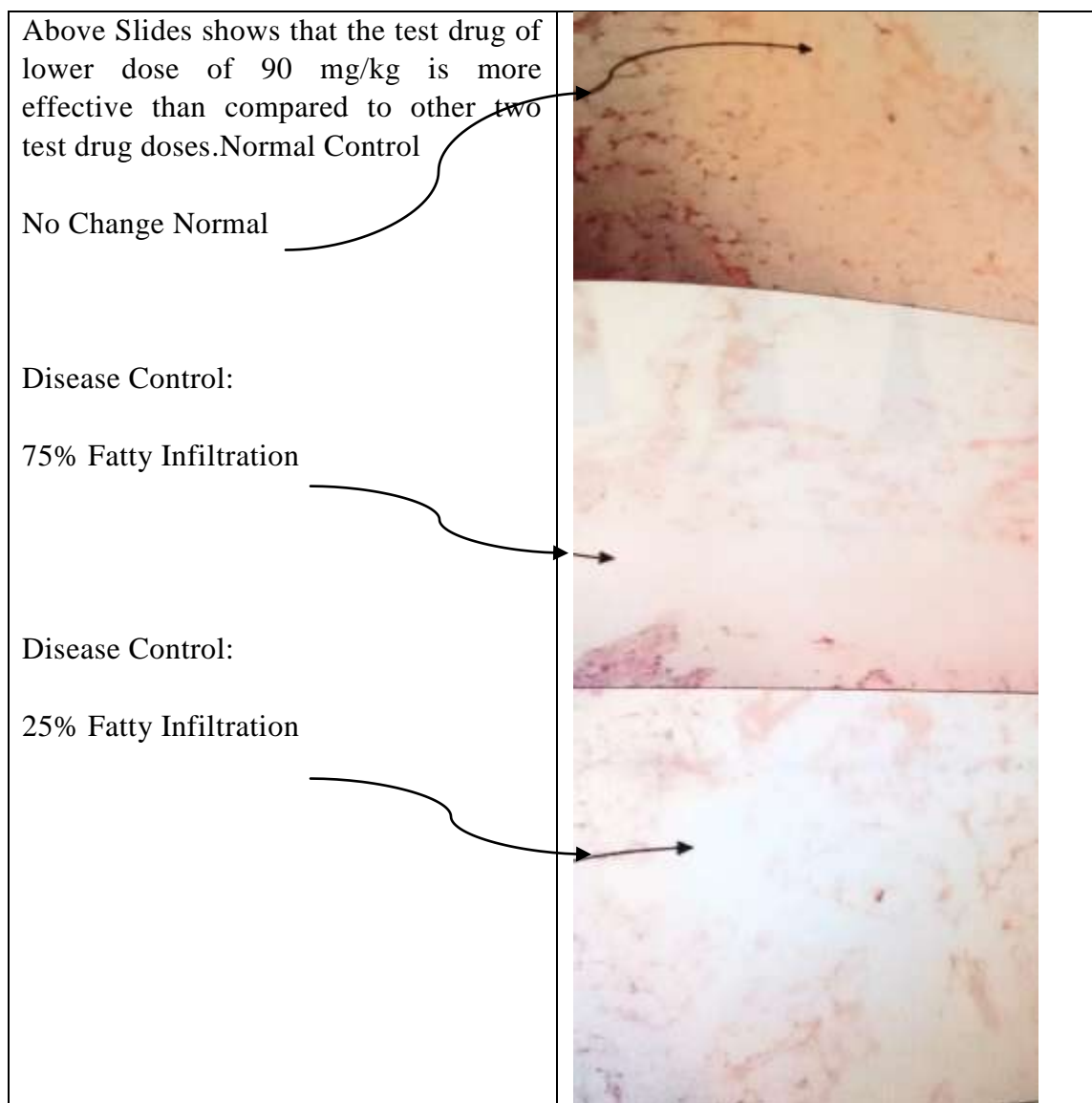


Disease Control:

75% Fatty Infiltration



<p>Disease Control:</p> <p>75% Fatty Infiltration</p>	
<p><i>Trikatu churna</i>-1.</p> <p>50% Fatty Infiltration dose of 270 mg/kg of</p>	
<p><i>Trikatu churna</i>-II:</p> <p>50% Fatty Infiltration dose of 180 mg/kg of</p>	
<p><i>Trikatu churna</i>-III:</p> <p>25% Fatty Infiltration dose of 90 mg/kg of <i>Trikatu churna</i>-III:</p> <p>25% Fatty Infiltration dose of 90 mg/kg of test drug</p>	



DISCUSSION

The Qualitative analysis by .T.L.C. was carried out for the Triaktu drug. Yellow and Violet spots were obtained on various T.L.C plates. These spots indicates the presence of various chemical components present in the drug. The study was performed on wistar rats male, female species not taken to avoid hormonal impacton results., HFD model was prepared i.e high fat induced obesity model was build up by soaking feed of animals in *Vanaspati Ghee* and coconut oil, as per reference 1 Ref:M.P.Shyamala, Antioxidant potential

of the *Syzgium aromaticum* (gaertn.) Linn. (Cloves) in ratsfed with high fat diet, Indian Journal of Pharmacology 2003;35; 99-103.

Atorvastatin is the most widely used, well tolerated drug for lowering cholesterols and LDL levels .It is potent at low doses and also has long plasma half life of 18-24 hrs (TripathiK.D., 2008). There are a lot of evidences of beneficial effects of atorvastatin in cardiovascular diseases and stroke.

When weight of groups was compared it was found, As p value >0.05 accept null

hypothesis, hence we conclude that there was no significant difference in Group A, Group B and Group C. Further in comparison of HDL, VLDL, LDL Total cholesterol Group A was more effective than Group B and Group C. In comparison of Triglyceride Group A was more effective but Group C showed more good results than Group B & C

CONCLUSION

The literary and experimental study reveals that *Trikatu* has antihyperlipidemic potential. The *Trikatu* dose of 90mg/kg shows maximum satisfactory results comparing with Standard drug on High Fat Induced Obesity Experimental Model in Hyperlipidemia

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