ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

TO EVALUATE THE EFFECT OF *MARICH* (*PIPER NIGRUM* LINN.) ON EXPERIMENTAL MODEL OF HIGH FAT DIET INDUCED OBESITY

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ABSTRACT

As per today's modern world unhealthy and stressful lifestyle and wrong eating habits have lead to a deadly condition which effects our healthy well being and becomes a cause for various diseases which effects the longevity of life, yes it is nothing else than Obesity called as *Sthoulya* in Ayurved. *Marich* is responsible for destroying *Visha*, *Jantu*, *Kapha*, and *Vata* from body. It is useful in scraping *Kapha* from the body. Aim of the study is to evaluate the effect of *Marich Churna* in experimental models of high fat diet induced obesity. The experimental study was carried out for 42 days. In which for the first 21 days, 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 *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 weight was recorded on a weekly basis whereas 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 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. *Marich* has anti-obesity potential. According to variation of dose the results have significant changes.

KEYWORDS: *Maricha*, *Piper Nigrum* Linn, HFD, *Sthoulya,* Antiobesity, hyperlipidemia.

INTRODUCTION

According to Vedic literature (Upanishad Bhoj 2/2/77) Brahmans should not sell *Maricha* and *Pippali*. It means the utility of *Maricha* is very less compared to that of *Pippali* in the *Rugveda*. However, in the *Samhita* 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), and in *Aamrakosha*, (6th century A.D.) In *Gupt kala* some important spicy drugs were being transported from India to Arab country among them *Maricha* is one. Brihattraye 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*, *Shirovirechan* and further Vagbhatt has mentioned *Shirovirechana gana*, *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 *Katurasatmak* and taken in the weight of one Kola i.e., 10gms.

Nighantu- Classification *Maricha*

Dhanvantari	*Shatpushpadi varga*
Madanpala	*Shuntyadi varga*
Raj	*Pippalyadi varga*
Kaivyadev	*Oushadi varga*
Bhavaprakash	*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*

According to Ayurveda, *Sthoulya* is one of the most *Santarpanjanya rogas* which is regarded *Asninditha vishesha* and there is mention of eight various complications. This is one most effective disease which affects social physical and mental features. In, *Sthoulya Tikshagni* occurs and *Jatharagni* is found in excessive conditions where as *Medo dhatvagni* is found in *Manda* conditions is due to *Avama* of *Vayu* in *Koshta*. So if person indulge in more food, it nourishes only *Medodhatu*. 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 hyperlipedemia. Hyperlipidemia is a condition in which the levels of lipoproteins, i.e., is cholesterols, triglycerides or both are raided in plasma to the extent that it may have adverse effect in health leading to life expectancy. Activities have been studied on *Maricha* are Oxidative stress, Anti-oxident, Anti-thyroid, Hypercholesterimia, Dyslipidemia, Lipid lowering Antiamoebic Acardicidal Pharmacodynamics.

The effect of *Marich churna* on High Fat Diet induced obesity model in the male Wistar rats using following parameters, weight (weekly i.e. day 0,7,l4,21,28,35, 42), fasting blood sugar levels (day- 0,21,42) Histopathological changes: liver, adepocytes.

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 l80-200gm. They were divided into 6 groups as mentioned below.

Table 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 l (90 mg/Kg) - HFD + *Marich churna*– Day22 - Day 42
5	Formulation	6	Dose 2 (180 mg/Kg) - HFD + *Marich churna*- Day22 - Day 42
6	Formulation	6	Dose 3 (270 mg/Kg) - HFD + *Marich churna*- 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) 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, Maharasthra, further pharmacognostical study was carried out.

Description about Groups

1. Group 1 receives normal diet and served as normal control.
2. Group 2 receive 10ml/kg/ body weight of HFD (Coconut oil + Vanaspati ghee 2:3) throughout the study i.e. for 42 days.
3. Group 3 receive Atorvastatin (1.2mg/kg/day for 21 days) (i.e. from 21"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 150mg/Kg respectively for 21 days (i.e. from 21st day of the study till the end of the study).
5. Obesity got induced by the 21st day of the experiment, after 21st day these groups receive the treatments as mentioned above along with HFD till the Day 42.
6. Weight of animals was recorded weekly throughout the experimental period.

This is the period when 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 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. 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 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).
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.
3. Gold-Thio Glucose- In this method intraperitonial or intramascular injection of goldthio glucose induces obesity in mice.
4. Monosodium Glutonate - Monosodium Glutonate 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 degree Celsius in Remiscooling 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 weighed on S.Chaimdzu scale.

Observations

Physical properties of *Marich* show it is slightly soluble in *Jala*. Organoleptic observation shows that *Marich Churna* has Brown-blackish colour, pungent taste and odour. The changes in thin layer Chromatography shows that Yellow and Violet colour are seen which shows the drug are chemical components present in it. Weight of animals recorded on day 0, 7, 14, 21, 28, and 42 increase of weight. Weight of disease control group recorded; gradual changes have been seen in increase of weight. Weight of animals recorded in disease control group shows that 0, 7, 14, 21, 28 and 42 and increase in weight of disease control group compare to normal group are seen. Changes seen in standard control group till 21 days and after administration of standard drug is the gradual weight loss is seen. Changes seen in increase of weight till 21 days after administration the drug dose of Group A i.e., 90mg/kg the changes of weight loss is seen. Group A was more effective than others in High Fat Diet induced Obesity model in rats Percentage wise improvement parameter of weight shows group A is more effective weight.

Histopathological Observations

As seen in the hisotopathological reports the following observations are notes, Disease control group shows fatty infiltration of 75%, which when treated with standard drug atorvastatin the fatty infilteration is reduced to 25%,and with test drugs *Marich* group (C) with dose of 270mg/kg the fatty infiltration is seen upto 50% with test drugs *Marich* Group (B) with dose of 180mg/kg the fatty infiltration is seen upto 50% and with test drug *Marich* group (A) with 90mg/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.

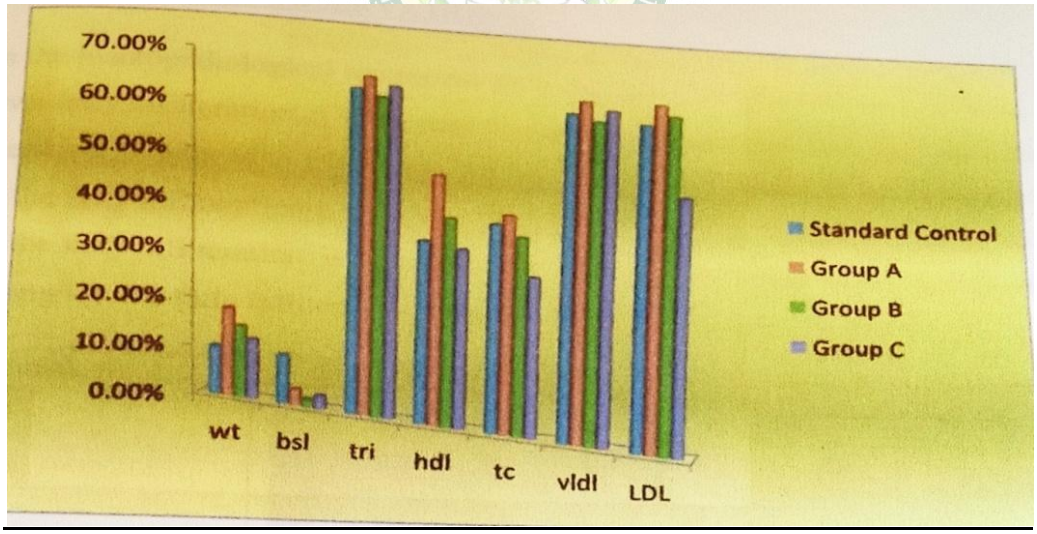
Comparison Standard drug With Group Disease control

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

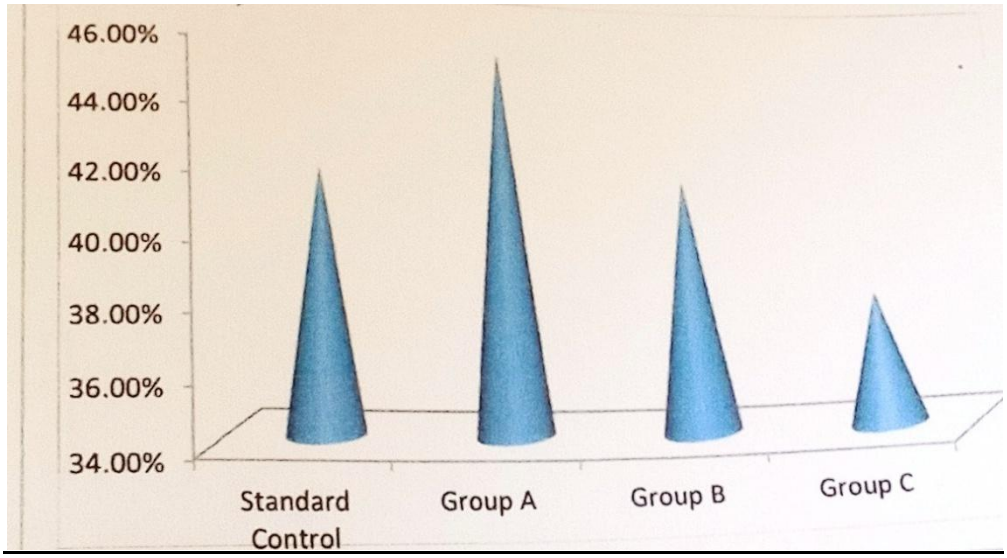
The standard control group shows significant changes with disease control group. The standard drug Atorvastatin shows good results.

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



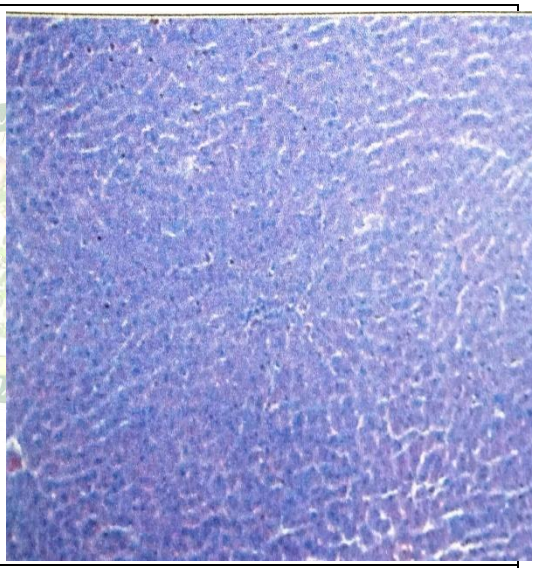
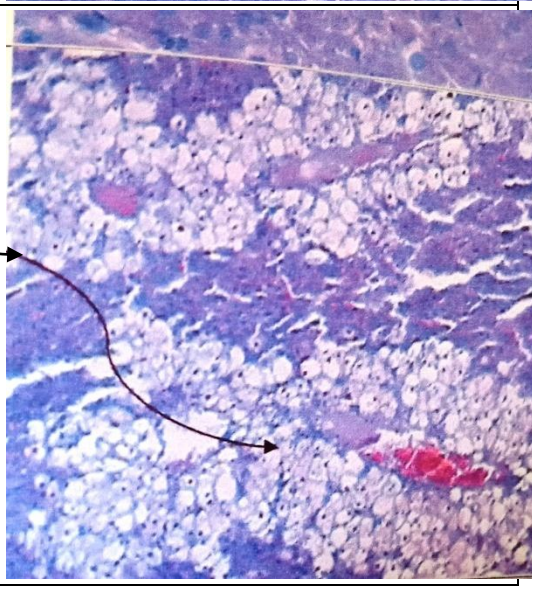
The standard control group shows significant changes with disease control group. The standard drug Atorvastatin shows good results.

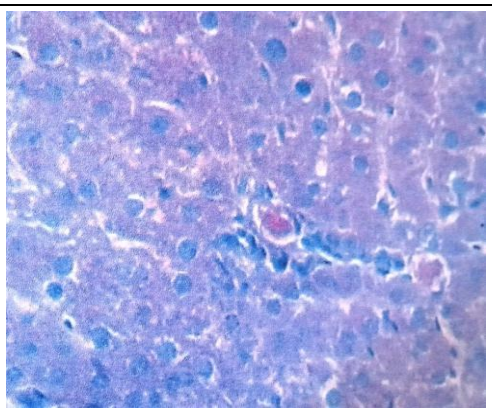
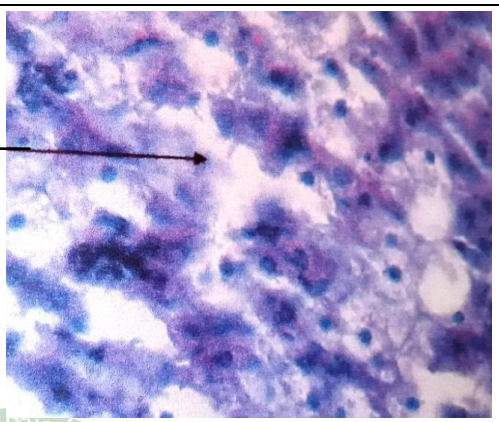
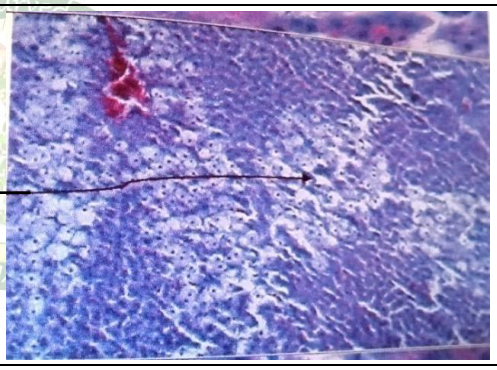
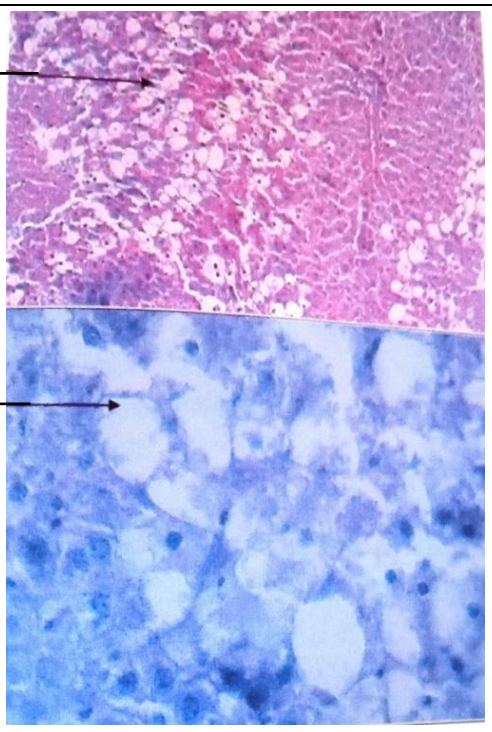
Graph 2: Comparison between Test drug groups and Standard drug



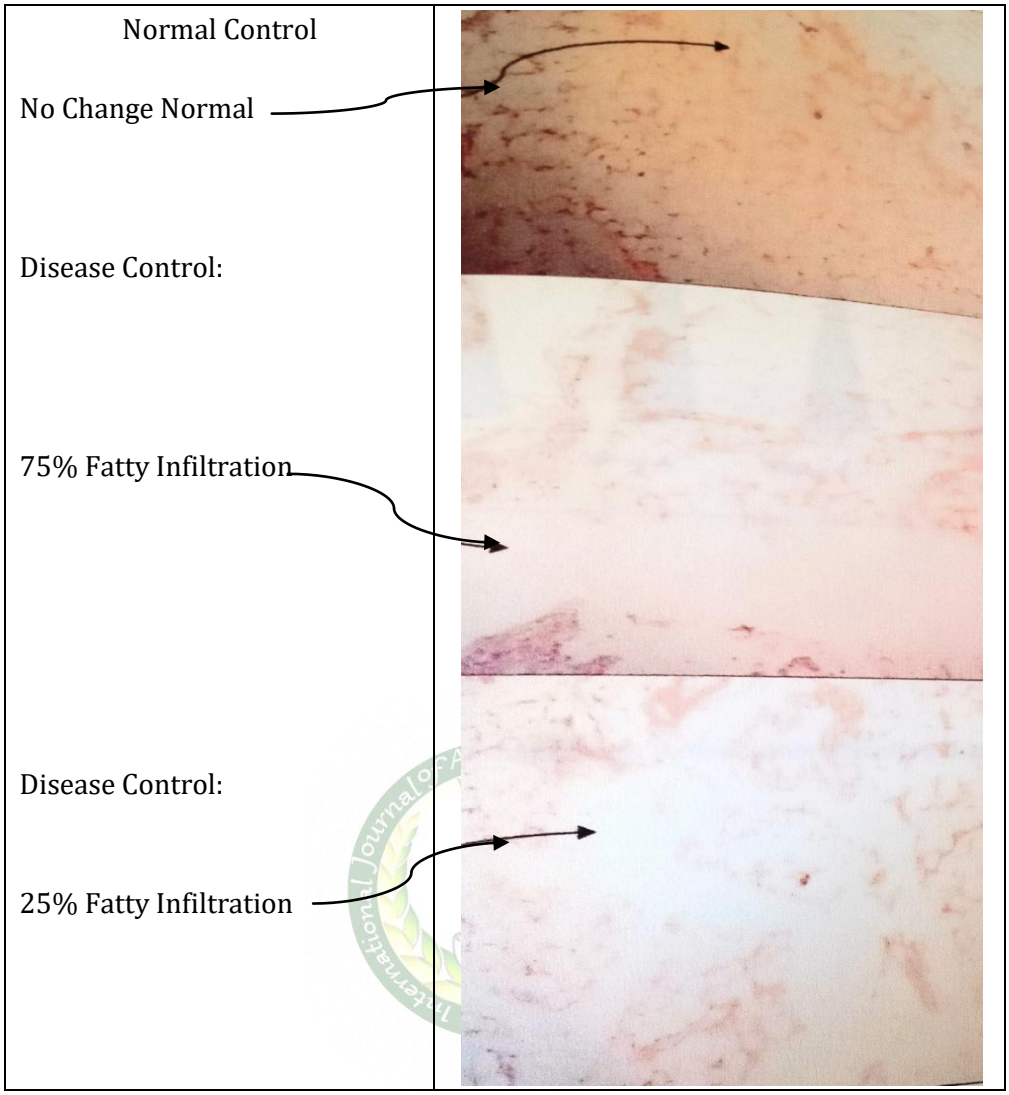
From above graph we found that more improvement was seen in Group A than others in High Fat Diet induced Obesity model in rats.

Histopathological Slides

Normal Control No Change Normal	
Disease Control: 75% Fatty Infiltration	

Disease Control: 75% Fatty Infiltration	
Marich churna-1. 50% Fatty Infiltration dose of 270mg/kg of	
Marich churna-II: 50% Fatty Infiltration dose of 180mg/kg of	
Marich churna-III: 25% Fatty Infiltration dose of 90mg/kg of *Marich churna*-III: 25% Fatty Infiltration dose of 90mg/kg of test drug	

Above Slides shows that the test drug of lower dose of 90mg/kg is more effective than compared to other two test drug doses.



DISCUSSION

Brihattraye extensively described this plant as Appetizer, Carminative and Antimicrobial. Sharangdhar quoted it as the example for *Chedana* and *Pramathi* group of drugs. *Maricha* is *Katu Rasatmak*, *Ushna Veerya*, and *Madhur* and *Katu Vipaka*, with *Laghu guna*, and with properties of *Kaphagna* and *Vataghna*. The Qualitative analysis by T.L.C. was carried out for the *Marich* drug. Yellow and Violet spots were obtained on various T.L.C plates. These spots indicate 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 impact on 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 rats fed 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 (Tripathi K.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 Band Group C. In comparison of Trilglyceride Group A was more effective but Group C showed more good results than Group B & C

CONCLUSION

The literary and experimental study reveals that *Marich* has anti-obesity potential. The *Marich Churna* dose of 90mg/kg shows maximum

satisfactory results comparing with Standard drug on High Fat Induced Obesity Experimental Model.

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Cite this article as:
Bomble Rajashri, Deshpande Mansi, Sawant Vidya. To Evaluate The Effect of Marich (Piper Nigrum Linn.,) on Experimental Model of High Fat Diet Induced Obesity. International Journal of Ayurveda and Pharma Research. 2020;8(8):7-14.
Source of support: Nil, Conflict of interest: None Declared

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