Biomedical & Pharmacology Journal, June 2019. Vol. **12**(2), p. 677-682

Effect of Myristica Fragrans Extract on Lipid Profile, Glucose, Body Weight, Food Intake, Liver and Renal Functions in Experimental Obese Rats

Yakaiah Vangoori[1], Anusha Dakshinamoorthi[2]* and S. Kavimani[3]

[1]Asst. Prof of Pharmacology. Santhiram Medical College, Nandyal (AP). PhD Scholar- Sri Ramachandra Institute of Higher Education and Research (SRIHER) - Chennai, India.
[2]Associate Professor of Pharmacology, Sri Ramachandra Institute of Higher Education and Research. Chennai, India.
[3]Prof & HOD of Pharmacology- Mother Theresa Post Graduate and Research Institute of Health Sciences. Pondicherry, India.
*Corresponding author E-mail:drdanusha@gmail.com

http://dx.doi.org/10.13005/bpj/1688

(Received: 31 January 2019; accepted: 23 March 2019)

The effect of the ethanolic extract of Myristica fragrans was evaluated on cafeteria diet induced body weight, glucose and lipid elevations in albino rats. 30 rats were taken randomly and divided into five groups and six each. Group-1 normal control and Group 2-5 were give cafeteria diet for 6 weeks to induce obesity and treatment period was 10 weeks. After 70 days of treatment, the extract, at doses of 200 and 400mg/kg, significantly reduced the body weight, glucose and lipid levels ($p < 0.001$) dose dependently. The standard drug Orlistat at 50mg/kg effectively prevented the body weight, glucose and lipid levels when compared with control and test groups. With these observations and previous data, the study concludes that *Myristica fragrans* extract can stimulate AMP-Kinase enzyme system and can reduce glucose and lipid concentrations. This may be useful for obesity treatment..

Keywords: Mace, weight gain, Orlistat, Myristica fragrans, pancreatic lipase, Obese, Glucose, LFT, RFT.

As per previous research reports, hyperlipidemia can cause cardiovascular problems, hyperglycemia, obesity and have major role in pathogenesis of various tissues. Obesity can induce insulin resistance, there by hyperglycemia, increase in blood pressure, dyslipidemia, collectively called "metabolic syndrome"[1]. Obesity, which has been termed as "New World syndrome" is now considered by world health organisation as a global problem. According to 2014-WHO report, 1.9 billion adults are overweight, of which 600 million are obese. The obesity is spreading to not only developed countries, but also all over the world 4.5 million people were died in 2013 due to overweight and obesity[2]. Obesity can be treated by reducing lipid levels. Animal models are useful tools for obesity research as they readily gain weight in short period when fed with high-fat diet. Human obesity clinical features are almost similar to animal models of obesity physiologically[3]. Therefore diet induced hyperlipidemia model was selected to observe the hypolipidemic effect of Myristica fragrans extract in the present study. Plants contain different chemical compounds and can act on specific cell sites. Many new drugs have been produced from the plant source, and these find use among the most common complementary and alternative medicine systems[4]. Presence of multiple-Phytochemical combinations in plant drugs may result in synergistic effect

by their action on multiple molecular targets, thus offering advantages over treatments which use a single constituent[5]. The development of standardized, safe and effective drugs from plant origin can provide economical alternatives for the treatment of obesity. Therefore, there is a need to develop and screen large number of plant extracts and this approach can surely be a driving force for the discovery of anti-obesity drugs from medicinal plants. *Myristica fragrans* is an aromatic evergreen tree cultivated in South Africa, India, and other tropical countries. It is commonly used spice. It has been prescribed in Asia for many diseases like rheumatism, muscle spasm, to decrease appetite, and Hyperlipidemia[6, 7]. It was found hypolipidemic effects in rabbits in some previous research reports[8, 9] but there are no anti-obesity studies with *Myristica fragrans*. It has been reported that the spice can be toxic when ingested in large quantities (1-3 nutmegs) causing convulsions, hallucinations, and possibly death[10]. Some active compounds present in Nutmeg can also alter physiological functions of hepatic and renal systems. Hence, the objectives of the current study are to investigate the hypolipidemic and Antiobesity effect of MF in high fat-fed rats and to evaluate the pharmacological activities on hepatic and renal functions[11].

AIM

To evaluate the effect of ethanolic extract of *Myristica fragrans* on body weight, lipid profile, glucose, hepatic and renal systems in experimentally induced obese wistar albino rats.

MATERIALS AND METHODS

Fresh and dried Mace was purchased from wholesale grocery store for the preparation of extract. Authentication was done by Dr.K.Venkata Ratnam. M.Phil., PhD, Assistant Professor of Botany, Rayalaseema University. Kurnool. A.P. Dried mace was ground to a fine powder and extract was prepared by using Soxhlet apparatus with ethyl alcohol as solvent[12]

Research Design

Thirty (30) healthy albino rats weighing between 150-180 gm were taken from central animal house, Santhiram Medical College. The rats were housed under 22±2ºC temperature, 40-60% humidity and 12-12±1 h light-dark cycle and allowed food and water *ad libitum*. Rats were randomly divided in to five groups of six rats each (n= 6). To induce obesity, rats were fed on high fat diet (HFD/CD) for 6 weeks and to test the plant extract efficacy, rats were administered with MFE along with CD for 70 days. Before starting the study, Institutional Animal Ethics Committee (IAEC) permission was taken (IAEC/SRMC/2017/2). For all the rats, body weight, Food intake, normal lipid profile, Glucose levels was done before starting the study.

Induction of obesity

Normal control rats (Group-I) were fed with standard pellet diet of standard composition containing all the recommended macro and micronutrients prepared according to AIN-93 guidelines with water *ad libitum*. Group-II to Group-V rats were initially fed with cafeteria diet (CD) for 6 weeks to induce obesity (20 g daily) and from 7th week on wards, different doses of *Myristica fragrans* (200, 400 mg/kg b.wt) were supplemented for 70 days (10weeks) along with CD as mentioned. Total duration was 16 weeks.

Hyper calorie/cafeteria diet (CD): (It consisted of 3 variants) (13)

1) Condensed milk + bread + peanuts + pellet chow (4:1:4:1),
2) Chocolate + biscuits + dried coconut + pellet chow (3:2:4:1), and
3) Cheese + boiled potatoes + pellet chow (4:2:1).

The different variants were fed on alternate days throughout the treatment period (70days)

Experimental design

Group 1: Standard pellet diet- Normal control
Group 2: Cafeteria diet (CD)-Obese control
Group 3: Obese + Orlistat 50 mg/kg
Group 4: Obese + MFE 200 mg/kg
Group 5: Obese + MFE 400 mg/kg

Food intake

All the rats were fed with normal laboratory Pellet diet. Food was presented in the form of pellets in grams. FI was measured manually. A known amount of food was given to the individual rat in separate cage of a group. After 24 hours, the leftover food was weighed again and the amount consumed was calculated and then for every 10 days up to 70 days.

Consumption of food = Total quantity of food given to rat – leftover food

At the end of the study period, body weight, lipid profile-TC (Total cholesterol), TG (Triglycerides), LDL (Low density lipoprotein), VLDL (Very low density lipoprotein), HDL (High density lipoprotein), liver function test-SGPT (Serum glutamic puruvic transaminases), SGOT (Serum glutamic oxaloacetic transaminase), ALP (Alkaline phosphatase) and renal function test (CREATININE, UREA, URIC ACID) was done to examine MFE effects on body weight and lipid profile.

Statistical analysis

All the data was presented as mean ± SEM. The one way ANOVA was used to analyze the data, followed by Dunnett's test. The results were measured statistically using SPSS Statistics 20.0 (IBM software) for the analysis. The results considered significant if p values < 0.05.

RESULTS AND DISCUSSION

At the end of 70th day of treatment, Body weight was recorded and blood was withdrawn from retro-orbital sinus from all the groups and assessed for lipid profile and compared with control group. When plasma lipid levels were analyzed, CD (Cafeteria Diet) or high fat diet caused substantial elevation in TC, TD, LDL, VLDL, and reduced the levels of HDL when compared with normal control group (Group-1). Treatment with ethanolic extract of *Myristica fragrans* significantly ($p<0.05$) and dose dependently reduced the concentrations of TC, TD, LDL, VLDL, but increased the levels of HDL when compared to CD fed obese control rats (Group-2) depicted in table:1

MF-Extract effect on Body weight

At the end of 70 days, food intake increased in obese model but decreased in orlistat treated and extract treated groups. Body weight was recorded in all the groups. In group-I, there was no change in body weight but in group-II, there was tremendous gain in body weight due to high fat diet. In group-III, body weight gain was well prevented due to standard drug when compared with group-IV and V. In MF extract treated groups (IV, V) body weight gain controlled dose dependently and significantly ($p<0.001$) less compared with obese group (II) over 70 days. Effect of *Myristica fragrans* extract was less in treatment models when compared with preventive models. (Tab: 2)

Effect of MF extract on Glucose

There was a significant ($p<0.05$) rise in blood sugar levels in obese groups in comparison with untreated control. Treatment with MFE for 70 days reduced blood glucose levels (108.14±0.11) significantly ($p<0.01$) when compared to untreated obese group (194.62±2.18) results shown in (Tab: 3)

Effect of MFE on Liver Functions

High fat diet can accumulate more fat in liver and can lead to fatty liver in animals. This can increase liver enzymes SGPT and SGOT. If enzyme levels increased, that can be considered as marker of obesity, and decreased of these enzyme levels can be taken as an anti-obesity effect[14]. in the present study, there is increased SGPT, SGOT and ALP levels in obese groups and decreased when treatment with standard drug and MFE over 70 days of study period when compared with obese and control groups. In all this liver enzymes, much

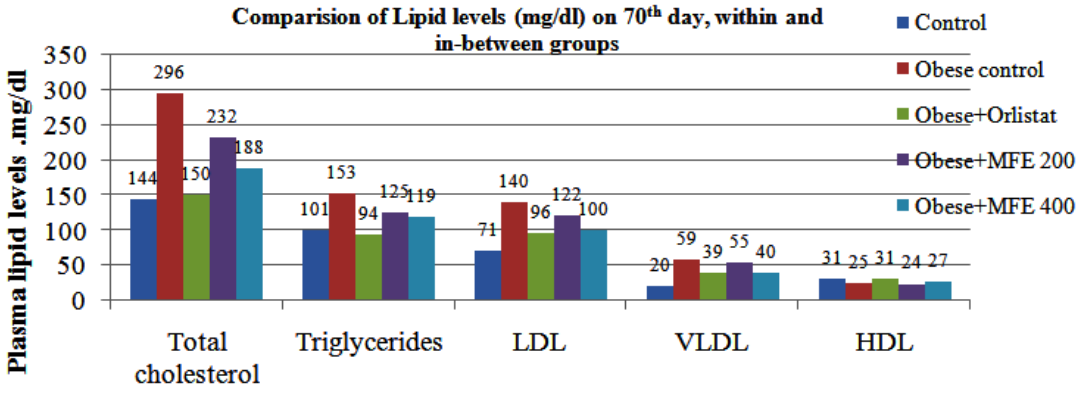

Fig. 1. Effect of *Myristica fragrans* on lipid profile

Table 1. Effect of *Myristica Fragrans* on lipid profile*:* (n=6, Mean±SEM)

Groups	T-Cholesterol		Triglycerides		LDL		VLDL		HDL	
	Day 1	Day 70	Day 1	Day 70	Day 1	Day 70	Day 1	Day 70	Day 1	Day 70
Group-1N-Control	136.7 ±5.37	144.81 ±3.8	86.6 ±0.25	101.66 ±04	69.33 ±0.12	71.83 ±2.12	18.66 ±0.15	20.16 ±5.23**	30.83 ± 5 . 1 2	31.16 ±4.14
Group-2Obese control	242.4 ±1.26	296.15 ±2.14	191.21 ±1.25	153.9 ±2.24	135.31 ±0.24	140.61 ±0.47	56.43 ±0.64	59.42 ±1.64	23.10 ±0.34	25.43 ±3.26
Group-3Obe+Orlistat 50mg/kg	256.4 ±6.17	150.8 ±5.26**	185.12 ±1.32	94.41 ±3.26**	147.11 ±0.36	96.24 ±5.32**	48.54 ±1.61	39.46 ±2.17**	27.01 ±1.32	31.52 ±1.39*
Group-4Obe+MEF 200mg/kg	243.8 ±1.31	232.1 ±4.11	205.14 ±3.51	125.12 ±0.12	139.51 ±0.52	122.46 ±0.81	72.57 ±1.35	55.64 ±1.12	26.4 ±0.42	24.04 ±1.61
Group-5Obe+MEF 400mg/kg	239.6 ±4.41	188.4 ±4.17**	184.9 ±1.36	119.4 ±0.31*	129.43 ±0.25	100.64 ±0.35*	44.61 ±0.86	40.64 ±0.80 *	24.41 ±6.57	27.66 ±0.52*

Comparison of Lipid profile within and in-between the groups. (n=6, Mean±SEM) *p<0.05, **p<0.01, ***p<0.001 compared to Control statistically analysed by one-way ANOVA followed by Dunnett's t-test

difference was not observed. Results shown in (Tab: 4).

Effect of MFE on Renal Functions

Significant rise in creatinine, urea, and uric acid were noticed in obese rat models. High fat deposition in renal system due to fat food can damage the kidneys and change the levels of urea and uric acid[15]. This can be considered as an index

Table 2. Effect of Myristica fragrans on Body weight & Food intake

Groups	Body weight (grams)		Food intake(grams/10days)	
	Day-1	Day-70	Day-10	Day-70
Group-1Normal control	171.83±4.81	256.00±5.11	152±3.01	161±5.06
Group-2Obese control	373.12±2.41	501.34±1.22**	173±2.13	220±4.16*
Group-3(Obese+Orlistat 50mg/kg	404.31±1.22	346.31±2.11**	144±3.154±4.41	147±6.12*
Group-4Obese+MEF 200mg/kg	398.51±1.26	463.25±6.31	144±3.170±1.20	175±4.16
Group-5Obese+MEF 400mg/kg	396.41±3.34	345.12±2.61**	144±3.161±2.11	154±8.02*

Comparison of Lipid profile within and in-between the groups. (n=6, Mean±SEM) *p<0.05, **p<0.01, ***p<0.001 compared to Control statistically analysed by one-way ANOVA followed by Dunnett's t-test

Table 3. Effect of Myristica fragrans on Blood glucose

Groups	Blood Glucose(Obese+treatment)	
	Day-1	Day-70
Group-1Normal control	65.12±2.14	86.32±1.24
Group-2Obese control	179.60±0.12	275.62±2.18 *
Group-3(Obe+Orlistat 50mg/kg)	162.11±3.54	85.64±1.21 **
Group-4Obese+MEF 200mg/kg	174.65±1.25	121.22±0.14
Group-5Obese+MEF 400mg/kg	166.42±6.18	108.14±0.11 **

Comparison of Lipid profile within and in-between the groups. (n=6, Mean±SEM) *p<0.05, **p<0.01, ***p<0.001 compared to Control statistically analysed by one-way ANOVA followed by Dunnett's t-test

Table 4. Effect of Myristica fragrans on Liver function

Obese groups	SGPT(IU/L)		SGOT(IU/L)		ALP(IU/L)	
	Day-1	Day-70	Day-1	Day-70	Day-1	Day-70
Group-1-NC	27.3±1.21	35.3±6.24	40.3±2.43	44.1±6.42	91.1±0.82	110.6±2.82
Group-2Obese control	35.2±1.23	51.3±4.54	26.4±9.00	45.2±4.84	81.2±0.31	104.2±5.20
Group-3(Obese+Orlistat 50mg/kg)	44.4±0.41	32.5±3.22	31.7±4.81	36.4±9.31	74.4±6.35	88.4±2.61
Group-4Obese+MEF 200mg/kg	29.5±0.64	43.6±5.91	47.6±0.21 NS	42.6±1.64	98.5±4.64	124.6±0.35 NS
Group-5Obese+MEF 400mg/kg	42.4±4.20	38.5±7.05	40.4±1.54	32.5±9.22	90.7±5.44	114.9±2.64NS

Comparison of Lipid profile within and in-between the groups. (n=6, Mean±SEM) *p<0.05, **p<0.01, ***p<0.001 compared to Control statistically analysed by one-way ANOVA followed by Dunnett's t-test

Table 5. Effect of Myristica fragrans on renal function. (Treatment model)

Obese groups	CREATININE(mg/dl)		UREA(mg/dl)		URIC ACID(mg/dl)	
	Day-1	Day-70	Day-1	Day-70	Day-1	Day-70
Group-1-NC	0.8±0.03	0.7±0.01	25.43±2.31	39.4±0.6	3.1±0.04	3.9±0.09
Group-2Obese control (CD)	0.9±0.04	1.2±0.08	24.25±0.24	28.5±1.41	3.1±0.04	6.4±0.09
Group-3(Orlistat 50mg/kg)	0.8±0.04	1.0±0.06 NS	35.14±6.51	41.9±4.26NS	1.8±0.06	2.2±0.06 NS
Group-4MEF 200mg/kg	1.5±0.01	1.9±0.07 NS	19.41±2.48	24.6±4.13	4.4±0.06	4.9±0.01 NS
Group-5MEF 400mg/kg	1.9±0.02	2.1±0.01 NS	36.17±0.48	39.6±4.11NS	5.4±0.07	4.9±0.06 NS

Comparison of Lipid profile within and in-between the groups. (n=6, Mean±SEM) *p<0.05, **p<0.01, ***p<0.001 compared to Control statistically analysed by one-way ANOVA followed by Dunnett's t-test

of obesity. If these levels reduced to normal, can be considered as an anti-obesity action. Based on this concept, the concentrations of creatinine, uric acid and urea levels were measured in the normal, obese control and MFE treated groups but there were no signiûcant changes observed in treatment models. (Tab: 5).

CONCLUSION

These results suggest that *Myristica fragrans* extract may have clinical value in the treatment of hyperlipidemia, and obesity. Based on the observations and previous research studies, the possible mechanism for hypolipidemic property may be due to presence of Saponins in mace extract. Saponins can inhibit the pancreatic lipase enzyme (16); thereby reduce the cholesterol levels (7). *Tetrahydrofuran* (Lignan) is one of the active chemical compound present in the Mace, also contributes to prevent weight gain by stimulating AMPK enzyme in differentiated C2, C12 cells. Its steroidal compounds have inhibitory effect on hunger sensory mechanism in hypothalamus (6). This could be beneficial to the obese persons and to treat hypercholestremic associated complications. With 400 mg/dl as maximum dose in the present study, does not change any physiological functions of the hepatic and renal systems. Further studies are needed to establish the safety.

ACKNOWLEDGEMENTS

The author Yakaiah Vangoori expresses his gratitude to his guide, Dr. D. Anusha and the staff of Santhiram Medical College, Nandyal, Andhra Pradesh for their support and guidance throughout this research work.

REFERENCES

1. Zhou YT, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unfger RH: Lipotoxic heart disease in obese rats: Implications for human obesity. *Proc Natl Acad Sci, USA* **97**: 1784–1789 (2000).
2. OECD obesity Update 2014: ww.oecd.org/health/obesity-update.html.
3. Buettner R, Scholmerich J. "High fat diets "modelling the metabolic disorders of human obesity in rodents. *Obesity:* (**5**)4: 798-808 (2007).
4. Bhutani KK, Gohil VM. Natural products drug discovery research in India: Status and appraisal. *Indian J Exp Biol.;* **48**: 199–207 (2010).
5. González-Castejón M, Rodriguez-Casado A. Dietary phytochemicals and their potential effects on obesity: A review. *Pharmacol Res.;* **64**: 438–55 (2011).
6. Nguyen PH, Le TV, Kaug HW, Chae J, Kim SK, Kwon KL, et al. AMP-activated protein kinase (AMPK) activators from Myristica fragrance and its anti obesity effect. *Bio Org Med Chem Lett.*; **20**(4):128-31 (2010).
7. Arulmozhi DK, Kurian R, Veeranjaneyulu A, Bodhankar SL. Antidiabetic and antihyperlipidemic effects of Myristica fragrans in animal models. *Pharmaceutical Biology.;* **45**(1):64-68 (2007).
8. Sharma A, Mathur R, Dixit VP. Prevention of hypercholesterolemia and atherosclerosis in rabbits after supplementation of Myristica fragrans seed extract. *Indian J Physiol Pharmacol.:* **4**: 407–410 (1995).
9. Yakaiah Vangoori, Anusha Dakshinamoorthi. Effect of Myristica fragrans Extract on Food Intake and Body Weight in Experimental Models. *Journal of Clinical and Diagnostic Research.* **12**(2): FF01-FF052 2 (2018).
10. Olaleye M.T, Afolabi C. Akinmoladun. Antioxidant properties of Myristica fragrans (Houtt) and its effect on selected organs of albino rats. *African Journal of Biotechnology.:* **5**(13). 1274-1278 (2006).
11. Andrew Osayame Eweka, Abieyuwa Eweka. Histological effects of oral administration of nutmeg on the kidneys of adult Wister rats. *N Am J Med Sci.;* **2**(4): 189–192 (2010).
12. Semiz A, Sen A. Antioxidant and chemoprotective properties of Momordica Charantia L (bitter melon) fruit extract. *African J Biotechnol.;* **6**(3): 273-77 (2007).
13. Harris RB. The impact of high or low-fat cafeteria foods on nutrient intake and growth of rats consuming a diet containing 30% energy as fat. *Int J Obes Relat Metab Disord.*; **17**(6):307-15 (1993).
14. Yamamoto M., Shimura S., Itoh Y., Ohsaka T. *Relat. Metab. Disord. Int. J. Obes.:* **24**; 758-64 (2000).
15. Cohen M. P., Clements R. S., *Hud E. Exp. Nephrol.:* **4**; 166-171 (1996).
16. Xu BJ, Han LK, Zheng YN. In vitro inhibitory effect of Saponins from Platycodi radix on Pancreatic lipase. *Arch Pharm Res.*: **28**(2): 180-185 (2005).