Research Article

Anti-Obesity Activity of Leaves of Methanolic Extract of *Mentha spicata* in High Fat Diet-Induced Wister Rats

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ABSTRACT

Medicinal Plants are the replacements of synthetic drugs. Traditional medicine offers us knowledge on biological activity of Medicinal plants. Most of the Nutraceutical's have become popular these days. People started believing herbal medications than synthetic drugs due to their low or none side effects. Obesity is the prevailing situation now-a-days. WHO is expecting about 27million population may become obese until 2030. Therefore, the research of the present study aimed at anti-obesity activity of Methanolic extract of leaves of *Mentha spicata* (MEMS). *In-vivo* pancreatic lipase inhibitory activity was studied for MEMS at different doses (100mg/kg, 200mg/kg, 400mg/kg) and compared with activity of standard drug Orlistat (2mg/kg). MEMS at dose of 400mg/kg showed highest weight loss and decrease in lipid levels. This study is possibly advantageous as the bottom line for further study on *Mentha spicata* for anti-obesity activity.

Keywords: Nutraceutical's, Anti-Obesity, *Mentha spicata*, Orlistat.

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INTRODUCTION

Traditional medicine over the past few decades, eco-friendly, bio-friendly, affordable, and generally safe herbal medications have transitioned from the fringe to the mainstream. The use of medicinal plants is essential in alternative medicine. Indians have historically had a significant impact on the management of biological resources for millennia and have been the guardians of relevant knowledge for generations through trial and error. India has an abundance of medicinal plants and the capacity to rise to the task of satisfying the demand for them on a worldwide scale. The primary healthcare systems in Indian society include Ayurveda, Naturopathy, Unani, Siddha, and Folk medicine, all of which are completely reliant on natural resources[1].

Accumulation of abnormal or excess fat in the body leads to obesity. For a common individual, obesity is the difference between calories intake and calories burnt. Consuming high-calorie diet without any physical activity leads to weight gain and energy imbalance.

Overweight and obesity are evolving health problems in India[2].

According to data 2005-2006:

- 13 % of women of 15-49 years are obese
- 9 % of men of 15-49 years are obese

Obesity is caused by Health conditions Genetics, Sex, Family history, Lack of sleep, Medicines, Smoking, Unhealthy diet, Age, Pregnancy, Emotional factors. Complications of Obesity Neurological disease, Respiratory, Gut microbiome, Diabetes, Cardio vascular disease, Inflammation, Fertility, Cancer Kidney disease, Gastrointestinal, Musculoskeletal, Psychosocial, Immune system[3]. Symptoms of Obesity may be Breathlessness, Increased sweating, Snoring, Back and joint pains, Feeling tired even with routine activities, Inability to cope with sudden physical activity, Psychological problems such as low self-esteem, low confidence level.

Mentha spicata is a creeping rhizome belonging to the family Lamiaceae. *Mentha spicata* has historically been used as a strewing herb. It has been demonstrated that it deters rats, mice, ticks, and acts as a larvicide for a number of mosquito species. The use of essential oils as anti-fungal agents is shown to be successful[4]. Since ancient times, mint has been used for everything from medical wraps to talismans that frighten away devils. Spearmint has been used to treat colic, digestive issues, headaches, and fever in conventional medicine. Today, one of the most popular therapeutic uses of spearmint extracts is menthol (found in cough syrups and drops). *Mentha spicata* extracts may be used to treat cancer, gout, hirsutism, and as an antiemetic, according to recent studies[5]. *Mentha spicata*, native to Europe and southern temperate Asia, extending from France in the west to southern China in the east. It is naturalized in many other temperate parts of the world,

including northern and southern Africa, North America and South America. In India, Gujarat, Maharashtra[5]. Numerous theories were put out to explain the weight-reduction benefits of *Mentha spicata*, including limiting nutrition absorption, boosting lipolysis, blocking pancreatic lipase activity, and diminishing adipocytes[5].

MATERIALS AND METHODS

Collection and identification of plant materials

Mentha spicata were collected from Sri Venkateshwara University Tirupati, A.P., India. The plant was morphologically identified and Authenticated by Dr.K. Madhava cheety(Rtd.) Plant taxonomist, assistant professor, Department of Botany bearing voucher number 0779 belonging to the family Lamiaceae. This sample was shade dried as it contains volatile oils for 2-3 weeks. Then size reduced to fine powder and stored in a airtight container for further use.

Chemicals and kits used

Methanol, Water, Petroleum ether, Orlistat, Casein, Cysteine, Starch, Sucrose, Cellulose, Groundnut oil, Mineral oil, Vitamin mix, Cholesterol, Vanaspati ghee, CHOD-PAP kit (**cholesterol oxidase phenol 4-aminoantipyrine peroxidase**.), GPO-PAP kit (**glycerine phosphate oxidase peroxidase**.)

Extraction Procedure

100gm of powder of leaves of *Mentha spicata* were transferred to 500ml conical flask containing 400ml of Methanol for simple Maceration technique. Intermittent shaking is done for about 1 week. Further extract is filtered and filtrate is kept for evaporation in Rota evaporator.

Institutional Ethical Committee Approval

The Institutional Animal Committee (IAEC) of CMR College of Pharmacy has approved the experimental protocols for evaluation of Anti-Obesity Activity of *Mentha spicata* and approval number is CPCSEA/1657/IAEC/CMRCP/COL-21/104.

Experimental animals

The animals were kept in a continuous environment with 12/12 hours of light and darkness, a humidity of 55%, and a temperature of 22±2 c. They were kept in polypropylene cages and provided with a regular pellet feed as well as unlimited access to water. The CMR College of Pharmacy's (Hyderabad) institutional animal ethics committee gave its approval for the tests and methods employed in the study. Healthy Male Wister albino rats weighing (200-250 g) were divided into 6 groups containing 6 animals each as mentioned in the table 1. Normal group received standard pellet diet which serves as control. All other groups received a high-fat-diet along with respective treatments. Animals were treated for 45 days. Normal group received standard chew diet and all other groups received high-fat-diet consisting of standard pellet diet, casein, cysteine, starch, cellulose, ground nut oil, mineral oil, vitamin mix, Vanaspati ghee, Cholesterol and coated with sucrose water for 45 days.

Table 1: Experimental design of Methanolic extract of leaves of *Mentha spicata*.

Groups (N=6)	Treatment and route of administration	Dose and duration
Normal control	Normal water	45 days
Obesity control	Normal water	45 days
High fat diet + Standard	Orlistat	2mg/kg. p.o (45 days)
High fat diet +Low dose	Methanolic extract of leaves of *Mentha spicata*	100mg/kg. p.o (45 days)
High fat diet + Medium Dose	Methanolic extract of leaves of *Mentha spicata*	200mg/kg. p.o (45 days)
High fat diet + Maximum Dose	Methanolic extract of leaves of *Mentha spicata*	400mg/kg. p.o (45 days)

Evaluation of Anti-Obesity Activity

Collection of Blood

Blood was taken via retro-orbital puncture after dosage for every seven days. The blood samples in heparinized tubes were immediately centrifuged for the separation of serum. For the purpose of estimating serum parameters such as TC, HDL, LDL, VLDL, TGs and body weight. serum was centrifuged at 3000 rpm for 15 minutes. The clear supernatant was separated and analysed by using CHOD-PAP kit (cholesterol oxidase phenol 4-aminoantipyrine peroxidase.), GPO-PAP kit (glycerine phosphate oxidase peroxidase.) and semi-auto analyser.

Statistical Analysis

Results were expressed as Mean ± standard error of the mean (SEM). Differences between the control and treatment groups in the experiments were tested for significance using unpaired student's 't' test. Values of $P<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Extractive Values

Following table 2 determines the extractive values of leaves of *Mentha spicata* in different solvents.

The extract was prepared with leaves of *Mentha spicata* powder by simple Maceration technique for 7 days with three different solvents. The obtained crude extract 24.976%w/w, 21.08%w/w, 7.36%w/w of Water, Methanol, and Pet.ether respectively. As a result, it was found that

the water and methanol solvent quantities were higher, at 24.976g and 21.08g, respectively. However, as water solvent produced an excessive amount of mould, methanolic extract was suggested for future usage.

Table 2: Below table 2 describes the values of *Mentha spicata* leaves extracted using various solvents.

Nature of extract	Colour	Consistency	Extractive values (gm)
Water soluble	Yellowish brown 	Semi solid	24.976 %w/w
Methanol soluble	Greenish yellow 	Semi solid	21.08%w/w
Petroleum ether soluble	Dark green 	Thick	7.36 %w/w

Preliminary Phytochemical Analysis

The Preliminary Phytochemical investigation were estimated by standard analytical procedures. Methanolic extract, aqueous extract, pet. Ether extract of leaves of *Mentha spicata* showed the presence of alkaloids, carbohydrates, saponin glycosides, tannins, steroids. Below Table 3 determines the presence of various constituents in following solvents Water, Petroleum Ether and Methanol.

Table 3: Below Table 3 Determines Phytoconstituents in various extracts of *Mentha spicata*

S.NO	Test		Water	Methanol	Petroleum ether
1	Alkaloids				
		a) Dragendoff's test	+	+	+
		b) Mayer's test	+	+	-
2	Carbohydrates	Molisch's test	-	-	+
3	Proteins and Amino acids		-	-	-
4	Saponin glycoside	Frothing test	+	+	-
5	Tannins	Ferric chloride test	+	+	-
6	Steroids	liebermann–burchard's test	+	+	+
7	Proteins	Biurette test	-	-	-

Note :- (+) Indicates presence ; (-) Indicates absence in Leaves of *Mentha spicata*

Water extract revealed the presence of alkaloids, saponin glycosides, tannins, and steroids. Methanolic extract revealed the presence of alkaloids, saponin glycosides, tannins, and steroids. petroleum ether extract revealed the presence of alkaloids, saponin glycosides, tannins, and steroids.

***In-Vivo* Anti-Obesity Activity**

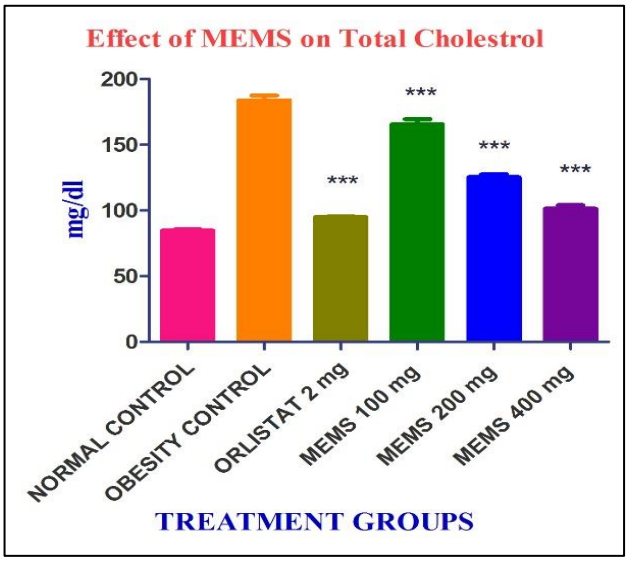
Methanolic extract of leaves of *Mentha spicata* was explored for its anti-obesity activity in high-fat-diet induced male Wister rats. After the treatment with Methanolic Extract of Leaves of *Mentha spicata* for 45 days, Obesity was evaluated by Biochemical parameters and Body weights. All the results obtained in this study were represented in the table 4.

The total cholesterol level, TG, HDL, LDL and VLDL were measured by using plasma serum and are depicted in figures (1-5). Below table 5.3 describes the concentrations of total cholesterol, TG's, HDL, LDL and VLDL were markedly elevated while HDL level decreased in experimental obese rats when compared to normal control rats. Oral administration of MEMS reversed these alterations in a dose-dependent manner, the profound effect being noted at a dose of 200mg/kg and 400mg/kg.

Table 4: Effects of MEMS treatment on Lipid profile in Normal and Obese Animals.

Name of the Group	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Normal Control	84.75±1.02	62.98±0.87	52.69±1.95	19.50±2.57	12.60±0.17
Obesity Control	184.0±3.61	99.70±2.07	22.94±1.03	139.7±2.81	19.94±0.42
Orlistat 2 mg	95.08±0.50***	66.09±0.48***	49.57±0.40***	48.22±1.54***	13.60±0.31***
MEMS 100 mg	165.7±3.86***	93.19±1.11*	30.32±2.02*	116.7±4.50***	18.64±0.22*
MEMS 200 mg	125.4±2.23***	79.28±1.56***	39.13±1.47***	93.12±2.32***	15.86±0.31***
MEMS 400 mg	101.5±2.67***	68.76±1.34***	47.22±1.26***	68.86±1.64***	13.75±0.27***

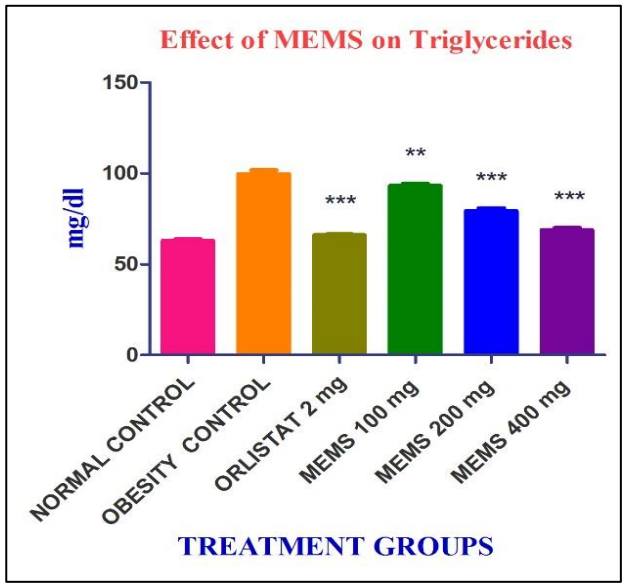
Values are represented as Mean ± SEM (n=6). Statistical analysis performed using one way ANOVA followed by post hoc Dunnett's test, ***p< 0.001, **p< 0.01, *p< 0.05Vs Obesity group.



Y axis= Total cholesterol; X axis= Treatment group

Figure 1: Effect of MEMS on lipid profile-Total cholestrol (TC)

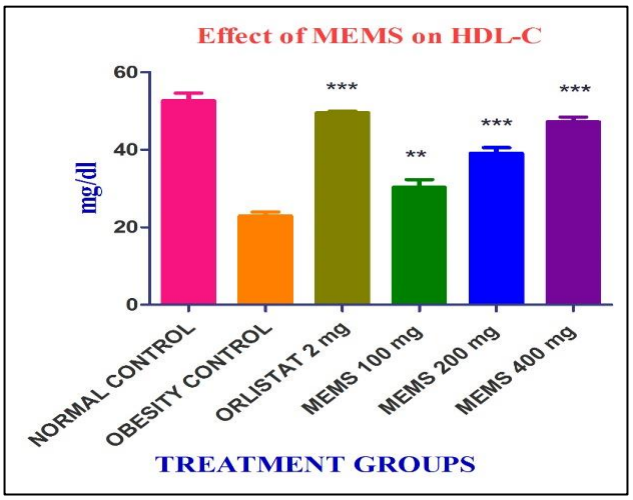
Values are represented as Mean ± SEM (n=6). Statistical analysis performed using one way ANOVA followed by post hoc Dunnett's test, ***p< 0.001, **p< 0.01, *p< 0.05Vs Obesity group.



Y axis= Triglycerides; X axis= Treatment group

Figure 2: Effect of MEMS on lipid profile- Triglycerides (TG)

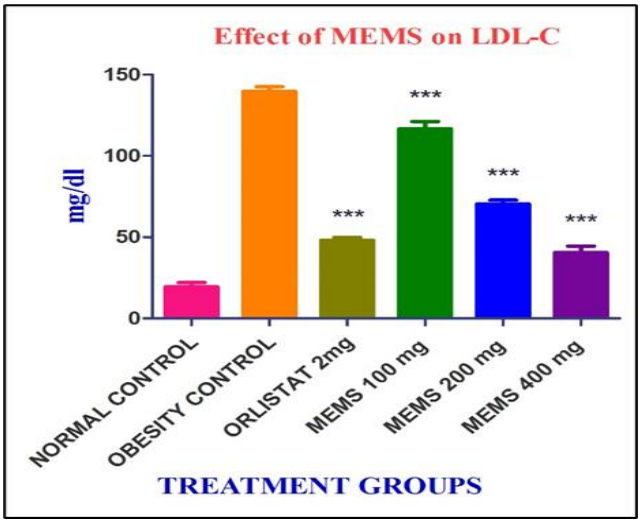
Values are represented as Mean ± SEM (n=6). Statistical analysis performed using one way ANOVA followed by post hoc Dunnett's test, ***p< 0.001, **p< 0.01, *p< 0.05Vs Obesity group.



Y axis= HDL cholesterol; X axis= Treatment group

Figure 3: Effect of MEMS on lipid profile-HDL

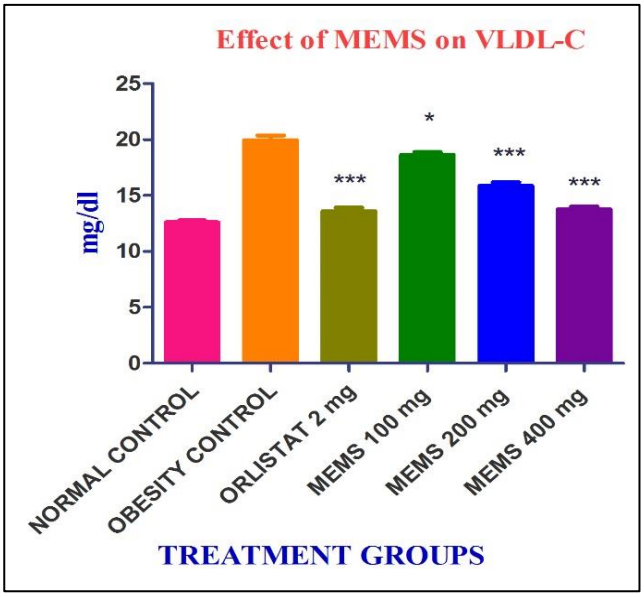
Values are represented as Mean ± SEM (n=6). Statistical analysis performed using one way ANOVA followed by post hoc Dunnett's test, ***p< 0.001, **p< 0.01, *p< 0.05Vs Obesity group.



Y axis= LDL- cholesterol; X axis= Treatment group

Figure 4: Effect of MEMS on lipid profile-LDL

Values are represented as Mean ± SEM (n=6). Statistical analysis performed using one way ANOVA followed by post hoc Dunnett's test, ***p< 0.001, **p< 0.01, *p< 0.05Vs Obesity group.



Y axis= VLDL- cholesterol; X axis= Treatment group

Figure 5: Effect of MEMS on lipid profile-VLDL

Values are represented as Mean ± SEM (n=6). Statistical analysis performed using one way ANOVA followed by post hoc Dunnett's test, ***p< 0.001, **p< 0.01, *p< 0.05Vs Obesity group.

Effects of MEMS treatment on Body weight.

The changes in body weight in different groups of animals during the experimental period were shown in table 6 and graph is drawn in fig 6. When normal control group rats compared with obesity control group, there was a substantial gain in body weight in obesity group. However oral supplementation with MEMS (200 and 400mg/kg) and orlistat significantly reduced body weight as compared to the obesity control group but there was less action with a low dose of MEMS (100mg/kg).

Table 6: Bodyweights of High fat diet induced Wister rats during Treatment period.

Name of the Group	1st day	7th day	14th day	21st day	30th day
Normal Control	246±0.431	252±0.346	263±0.333	269±0.627	278±0.440 **(↑32 gm) (+13%)**
Obesity Control	298±0.366	315±0.330	325±0.388	339±0.286	351±0.406 **(↑53 gm) (+17.7%)**
Orlistat 2 mg	288±1.57	285±0.313	263±0.482	251±1.48	238±0.553 **(↓50 gm) (-17%)**
MEMS 100 mg	292±0.378	289±0.423	283±1.11	276±1.15	263±0.911 **(↓29 gm) (-9.93%)**
MEMS 200 mg	284±0.596	278±0.816	268±1.28	255±2.74	240±2.58 **(↓44 gm) (-15.49%)**
MEMS 400 mg	286±1.19	281±1.18	272±1.01	265±1.02	239±4.02 **(↓47 gm) (-16.43%)**

Values are represented as Mean ± SEM (n=6). Statistical analysis performed using one way ANOVA followed by post hoc Dunnett's test, ***p< 0.001, **p< 0.01, *p< 0.05Vs Obesity group. Note:- ↑ = Indicates increase in body weight ; ↓ = Indicates decrease in body weight.

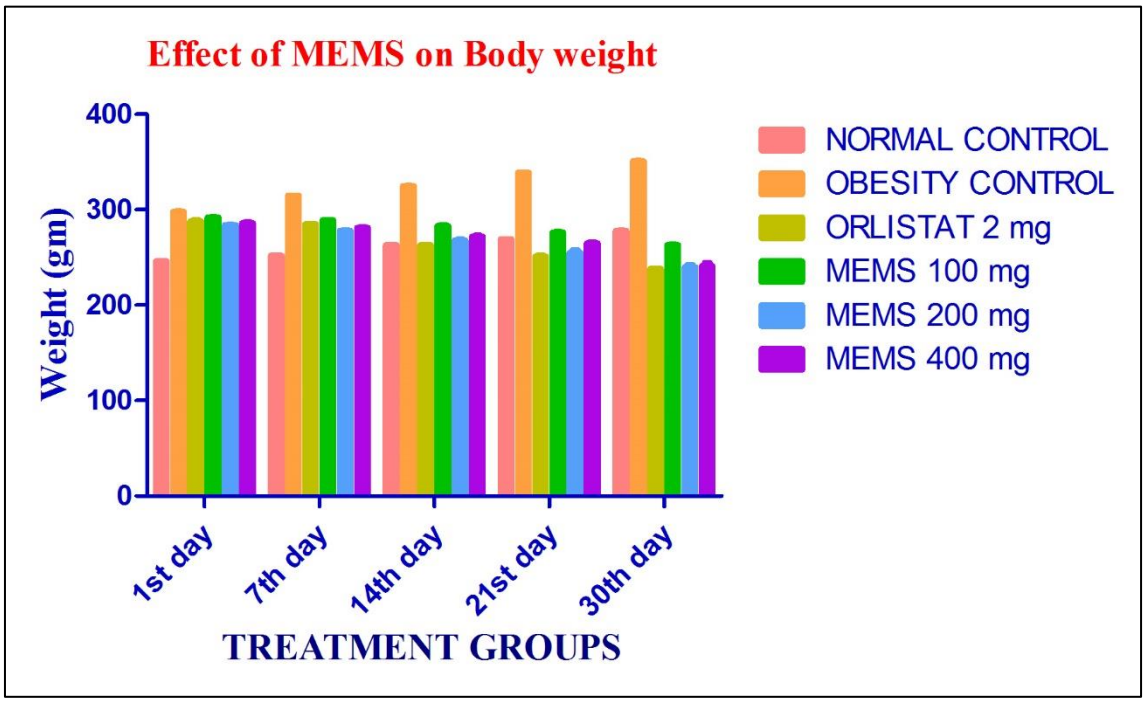


Figure 6: Effect of MEMS treatment on Body weight.

DISCUSSION

Permanent weight loss is challenging to attain and weight control is a lifelong endeavor. An imbalance between calorie intake and energy expenditure brought on by complicated interactions between numerous hereditary and environmental variables is the underlying cause of obesity. Millions of individuals worldwide suffer from the chronic disease of obesity, which significantly increases morbidity and mortality. Calorie intake and energy expenditure must be balanced for a weight loss programme to be effective. The foundations of weight management have traditionally been diet and exercise. With regard to obesity, natural products, especially those that contain fiber, polyphenols, sterols, and alkaloids, can be both safe and effective. They also serve as a rich source of vitamins and minerals. Generally speaking, natural substances that have the ability to treat obesity serve as a general body cleanser, control metabolism, dissolve fat in the body, aid in the reduction of food cravings, stimulate glandular secretions, lessen water retention, boost energy, and aid in constipation. They should, however, be used in conjunction with consistent exercise, dietary changes, and behavioral adjustments. Multiple phytochemicals may have synergistic and increased benefits when used together. Increasing natural drug demand for pharmaceutical uses has encouraged Scientifics all over the world to explore medicinal plants recognized as efficient remedies. In this context, *Mentha spicata* is concentrated for prominent pharmacological properties.

CONCLUSION

In this study, an attempt was made to find the potentiality of MEMS against high fat diet induced male Wister rats. For the studies Methanolic extract of leaves of *Mentha spicata* were selected from SRI VENKATESWARA UNIVERSITY TIRUPATI and stored for further use.

Extractive values of *Mentha spicata* were determined in different solvents (Water, Methanol, Pet-ether), among the 3 solvents Methanol is found to show better yield 21.08%w/w.

The Preliminary Phytochemical investigation were estimated by standard analytical procedures. Excess phytochemical constituents were observed in Methanolic extract, revealed the presence of alkaloids, saponin glycosides, tannins, and steroids.

Further the extract was prepared by simple Maceration technique using Methanol as solvent in 100gms of *Mentha spicata* powder to obtain Methanolic Extract of *Mentha spicata*. 14gm of yield is achieved after drying and evaporation.

On feeding the animals with high fat diet for 45days, there was increase in levels of lipid (LDL, HDL, VLDL, TC, TGL) and decrease in (HDL) levels in disease controlled rats, indicating fat deposits on tissue. The levels of TC, TGL, HDL, LDL and VLDL in standard groups were compared with test groups.

Treatment with medium dose (200mg/kg) and high dose (400mg/kg) have shown decrease in body weights and lipid parameters from 21st day to 45th day.

The above results are the clear evidence for the potent anti obesity effect of Methanolic extract of *Mentha spicata* and the effect is completely dose dependent in nature. The another important benefit possessed by the plant is, It did not shown any effect on animals feed with normal diet which were treated with the same doses used in high fat diet treated animals, where the high dose of drug shown

predominant effect on blood parameters except HDL levels of normal diet fed animals which levels were increased.

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