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VALIDATION OF A MARKETED POLY HERBAL FORMULATION FOR ANTI-OBESITY ACTIVITY ON MONOSODIUM GLUTAMATE WITH HIGH FAT DIET INDUCED OBESITY

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ABSTRACT

Obesity is a global epidemic and most common disorder in the world. It continues to be a growing problem worldwide with no suitable treatment options without side effects. The novel targets for anti-obesity activity includes 5-HT receptors, PPAR gamma receptor, PTP-1B and pancreatic lipases. Traditional herbal medicines have more acceptance than prescription drugs in many cultures with epidemics of obesity. The herbal formulation were examined for the presence of phytoconstituents, antioxidant activity, digestive enzyme activity, hypoglycaemic effect, hypophagic effect & histology of adipose tissue and fatty liver changes by using MSG-HFD induced obesity in Swiss albino mice. In this study animal models for obesity were developed by neonatal injections of monosodium L-glutamate and followed by high fat diet. Serotonin level of brain extract was estimated using HPLC ECD instrument. In obese animal the serotonin level was significantly less than the normal mice. After treatment both the test and standard drug decrease the elevated serotonin level of the obese group which was accounted for the decrease in food intake. Finally this report reveals the test drug (Medonil) showed anti-obesity activity but it is not so significant as compared to the standard drug (orlistat).However it was evident that food intake, adipose tissue weight reduced significantly on treating with test compound drug (Medonil).

KEYWORD: Anti-obesity, Monosodium glutamate (MSG), High fat diet (HFD), PPAR gamma, Orlistat.

INTRODUCTION[1,2]

Obesity is a leading preventable cause of death worldwide, with increasing rates in adults and children. In 2015, 600 million adults (12%) and 100 million children were obese. Obesity is more common in women than men. Authorities view it as one of the most serious public health problems of the 21st century. In 2013, the American Medical Association classified obesity as a disease. Obesity is most commonly caused by a combination of excessive food intake, lack of physical activity, and genetic susceptibility. According to WHO Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. Obesity and overweight a major risk for serious diet-related chronic diseases, including type 2diabetes, cardiovascular disease, hypertension and stroke, and certain forms of cancer. The health consequences range from increased risk of premature death, to serious chronic conditions that reduce the overall quality of life. It involves excess storage of food-derived calories as triglycerides in adipose tissue depots due to either excessive calorie intake, decreased calorie expenditure or both. Obesity is distinct as an overload of body fat. Being fat is different from being overweight. Overweight refers to excess body weight compared to normal standards. The excess weight may come from muscle, bone, fat, and/or body water. Anti-obesity tablets & anti-obesity drugs are used in the treatment of excessive obesity to reduce and control weight. These drugs are offered in a variety of compositions for different conditions of weight regulation process.

Objective

1. Two different obesity-treatment drugs are currently on the market: orlistat, which reduces intestinal fat absorption via inhibiting pancreatic lipase; and sibutramine, an anorectic or appetite suppressant. Both drugs have potential side-effects, including increased blood pressure, dry mouth, constipation, headache, and insomnia. For this reason, a wide variety of natural materials have been explored for their obesity treatment potential.
2. This study aim to survey the literature covering natural products with anti-obesity activity and to review the scientific data, including experimental methodologies, active components, and mechanisms of action against obesity.
3. To develop obese animal models for the study prevalent in larger population of human being.
4. To validate a marketed poly herbal formulation for anti-obesity properties and compare with a standard allopathic anti-obesity drug.

MATERIAL AND METHOD[3]

Experimental Animals

Swiss albino mice of (weighing between 20 and 25 g) were used for the breeding purpose. Animals were procured from laboratory animal house of Birla Institute of Technology, Mesra (Reg.no-621/02/ac/CPCSEA, Protocol no/PH/IAEC/29/2010/15.9.10). All animal experiments strictly compiled with the approval of the institution and ethical committee.

Induction of Obesity[4]

After delivery of pups received one subcutaneous (sc) dose of 2 mg/g MSG on the 2nd and 4th post natal days and 4 mg/g MSG on 6th , 8th and 10th postnatal days, each dose dissolved in 0.01ml/g of distilled water. Animals were provided with high fat diet after weaning period so as to develop better obese model. Daily food intake (24 hour food intake), body weight were determined regularly every week from 6 to 15-16 weeks of age in MSG mice and of their respective controls.

In-vitro study

Antioxidant activity[5,6]

The free radical scavenging activity of the extract was assessed on the basis of the radical-scavenging effect of the stable DPPH free radical. A series of extract concentration in the same extraction solvent was prepared (100, 200,300,400,500 mcg/ml). Then, 1ml of extract at different concentrations was mixed with 4ml of 0.004 % (0.1mM) DPPH in methanol. The disappearance of DPPH was read spectrophotometric ally at 517 nm after 30 min of incubation at room temperature in the dark. The same procedure was repeated with methanol solutions of synthetic antioxidant ascorbic acid as positive control. Methanol was used as blank. 50%of maximum scavenging activity was recorded) was calculated for each extract.

Lipase enzyme assay[6,7,8]

The ability of the compounds to inhibit porcine pancreatic lipase was measured using the method previously reported by, with modifications. Briefly, an enzyme buffer was prepared by the addition of 30µL (10 units) of a solution of porcine pancreatic lipase to 850µL of Tris buffer (100 mm Tris- HC1 and 5 mm CaCl2, pH 7.0). Then, 100µL of the compounds at the test concentration was mixed with 880µL of the enzyme-buffer, and incubated for 15 min at 37oC, with 20µL of the substrate solution [Triolein: (grade II; Sigma Chemical Co., 5000mcg/ml in pyridine)] added and the enzymatic reactions allowed to proceed for 15 min at 37oC. The lipase activity was determined by measuring the hydrolysis of trolling to

glycerol and fatty acid at 550 nm using spectrophotometer. Inhibition of the lipase activity was expressed as the percentage decrease in the OD when porcine pancreatic lipase was incubated with the test compounds.

In-vivo studies

The animals were divided into four groups for evaluation of anti-obesity activity of Poly herbal formulation (MEDONIL) with each group comprising of six mice (3 male And 3 female)

Group-i (Normal control): Normal treated mice

Group-ii (Obese control): HFD+MSG induced obese

Group-iii (Standard treatment): HFD+MSG induced obese administered with standard drug orlistat (50mg/kg/day, orally as 2% v/v tween 80 suspension).

Group-iv (Test treatment): HFD+MSG induced obese administered with test drug medonil (200mg/kg/day, orally as 2% v/v tween 80 suspension)

Pharmacological Evaluation

Body wt. and food intake: the body wt. was recorded on day 1 and then weekly consecutively up to 50 days using digital weighing balance. In addition to this, the daily food intake was recorded for each group.

Biochemical estimations[9,10,13]

Blood samples were collected from retro-orbital sinus of mice of different groups. The concentration of triglyceride, cholesterol, LDL, VLDL, HDL, AST, ALT, Body fat Pad, were measured using commercial kit available in the market. And Brain Serotonin level which is analysed by HPLC-ECD.

Histopathological Examination[11,12,14]

On completion of experimental work, mice from each group. Were sacrificed by ether overdose, then liver, brain, periepididymal fat tissues were collected to examine histopathological variations among group.

Statistical analysis

The results were expressed as mean ±SEM. Comparison for development of animal model between normal control and MSG-HFD obese control groups were performed by student t test and comparison done between all groups be ANOVA.

RESULT AND DISCUSSION

In-vitro results

In DPPH Free radical scavenging activity the IC50 values of Ascorbic acid and Extract in the scavenging of DPPH is 333.04 µg/ml & 3456.5 µg/ml respectively. The anti-oxidant potency of a compound is inversely proportional to the IC50 value. In the present study since the extract is having IC50 value greater than that of the standard. Therefore *in vitro* anti-oxidant potency of the extract is less compared to that of the standard.

In Lipase Enzyme Assay the IC50 values of orlistat and medonil extract in the lipase enzyme inhibition is 808.66 µg/ml & 1387.08 µg/ml respectively. The lipase enzyme inhibition of a compound is inversely proportional to the IC50 value.

In- vivo studies

Obesity activity: Changes in body wt. from 5th to 12th week: Results showed significant increase in body weight gain in 8th week and massive wt. gain was observed by the end of 12th week which indicates significant development of obesity in terms of body weight.

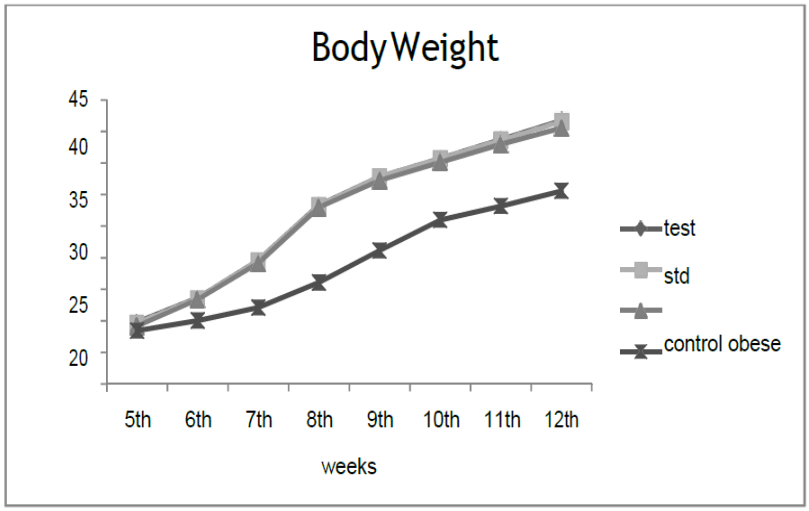


Fig. 1: Changes in Body Weight.

Data are expressed as the mean ± S.E.M; *n* = 6 in each group. *p<0.05, **p<0.01, ***p<0.001, when compared to normal control group (one-way ANOVA followed by Dunnet's't' test.).

Difference in fat mass: results shows highly significant increase in periepididyamal fat mass in obese mice control group as compared to animal of normal control group. Obese control group Shows reserve of white adipose tissue around different body parts.

Assessment of parameters after drug treatment

Table 1: Changes in body weight.

Groups	Normal Group	Obese Control Group	Standard treatment Group	Test treatment Group
Initial Weight (gm)	30.58±0.30**	40.53±0.23	41.48±0.09	41.7±0.15
Weight After 1st Week (gm)	31.08±0.08**	40.91±0.19	40.41±0.10	40.36±0.12
Weight After 2nd Week (gm)	31.18±0.11**	41.15±0.16	39.61±0.11*	39.76±0.11*
Weight after 3rd week (gm)	30.93±0.07***	41.48±0.14	37.95±0.15**	39.05±0.20*
Weight after 4th				
week (gm)	30.58±0.05***	41.8±0.14	35.76±0.19***	38.75±0.15*

All value expressed in mean ± S.E.M; *n* = 6 in each group. *p<0.05, **p<0.01, p<0.001, when compared to obese control group (one way ANOVA followed by Dunnet's't' test.).

Changes in body parameters

Table 2: Changes in Body Parameter.

Parameters	Normal Control Group	Control Obese Group	Standard treatment Group	Test treatment Mice Group
Food Intake (gm/day/mice)	4.09±0.09	6.09±0.11*	6.10±0.12*	6.02±0.14*
Blood Glucose Level (mg/dl)	62.65±0.24	99.89±0.49*	100.09±0.49*	99.94±0.42*
Blood Cholesterol Level	66.59±0.39	100.80±0.21**	100.87±0.48**	101.17±0.34**
Blood Triglyceride Level	36.89±0.49	95.71±0.38**	95.86±0.17**	95.92±0.50**

All values expressed in mean ± S.E.M; *n* = 6 in each group. *p<0.05, **p<0.01, ***p<0.001, when compared to normal control group (one-way ANOVA followed by Dunnet's't' test.).

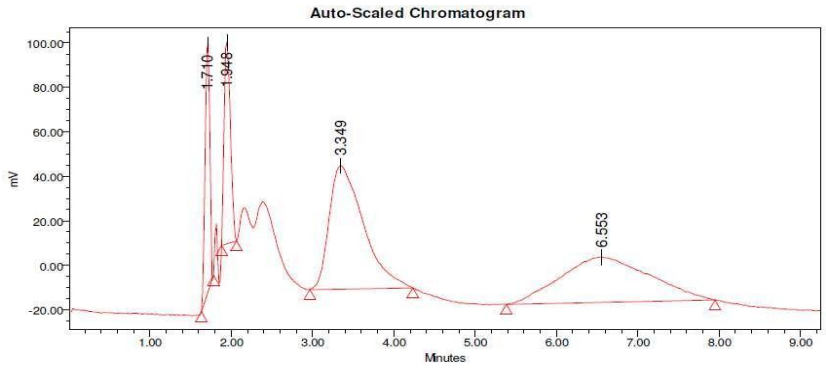
Fat pad (abdominal fat) and organ weight

Table 3: Fat Pad and Organ Weight.

Parameters	Normal mice	Control obese	Standard treatment	Test treatment
Fat pad weight (g)	1.19±0.07***	13.33±0.58	7.72±0.18**	10.27±0.33
Liver weight (g)	1.76±0.04*	2.31±0.06	1.83±0.07*	2.23±0.06
Brain weight (mg)	434.11±4.00*	393.63±4.67	392.36±1.70	391.41±0.60
Heart weight (mg)	178.38±0.55*	169.01±0.50	170.78±0.60	172.88±0.43*
Kidney weight (mg)	381.45±0.44**	421.46±0.85	395.06±1.25*	404.93±0.85

All values expressed in mean ± S.E.M; *n* = 6 in each group. *p<0.05, **p<0.01 ***p<0.001, When compared to control group (one-way ANOVA followed by Dunnet's't' test.).

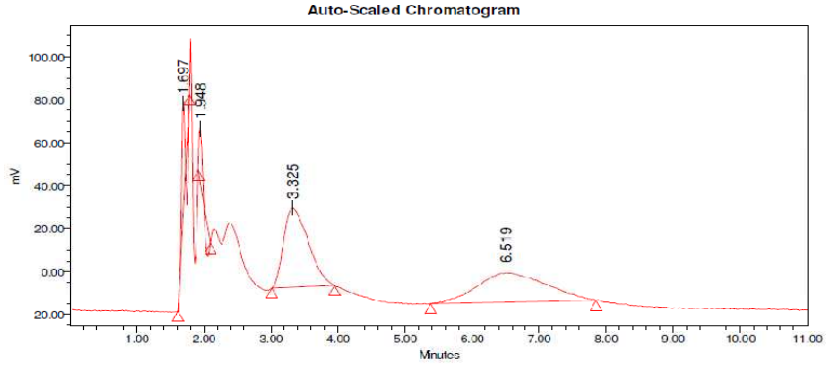
Estimation of brain amines (5-HT)



Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.710	452479	112713		
2		1.948	488927	90705		
3		3.349	1622991	55449		
4		6.553	1553838	20308		

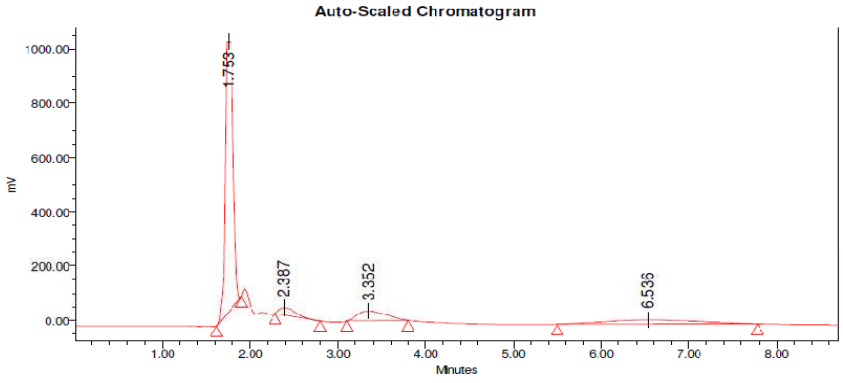
Fig. 2: HPLC Chromatogram of Brain Extract of Normal Mouse.



Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.697	224350	47972		
2		1.948	134766	25239		
3		3.325	941277	30572		
4		6.519	970907	10688		

Fig. 3: HPLC Chromatogram of Brain Extract of Obese Mouse.



Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.753	6437738	1050746		
2		2.387	344968	25643		
3		3.352	818712	35572		
4		6.536	1154143	15947		

Fig. 4: HPLC Chromatogram of Brain Extract of Mouse Treated With Test Compound.

Table 4: Brain serotonin Level from Peak Area of HPLC Chromatogram.

Group	Peak Area	%Peak Area
Normal	1553838	100
Obese	978907	62.99
Orlistat	1249151	80.39
Test	1154143	74.27

In obese mice brain, there is significant decrease in brain serotonin level as compared to normal mice brain. The serotonin level in standard treatment (orlistat) and test treatment (medonil) group mice also decreased as compared to the normal mice but it is less than the obese mice. The serotonin level in test treatment group is less as compared to the standard treatment group. As the serotonin level or function affects the food intake, therefore from this study, there is increase in food intake in the obese mice. After drug treatment there is decrease in food intake as there is increase in the serotonin level as compared to the obese mice group. The standard drug treatment group shows more serotonin level than the test group.

CONCLUSION

This experimental work strongly indicated their great potential as anti-obese. The mice model designed for conducting the study was developed by SC administration of MSG in neonatal pups was further continued by feeding HFD to facilitate obesity. Orally administration of 200mg/kg of both extract reduced the level of circulating lipids as well as amount of adipose tissues, resulting in remarkable improvement in obese animal model bearing close resemblance to human obesity. The test drug extract is having moderate lipase enzyme inhibition activity as compared to the orlistat which showed significant lipase enzyme inhibition activity. But the test drug extract is having very less antioxidant activity. The periepidydimal adipose tissue weight was found to decrease significantly in case of animals treated with test drug (Medonil) however the decrease in fat mass was more prominent in Orlistat treated group. The hypoglycemic effect of test compound caused decrease in blood glucose level but it was not significant as compared to standard group. The decrease in triglyceride and cholesterol level was not significant as compared to standard compound. Serotonin level of brain extract was estimated using HPLC-ECD instrument. In obese animal the serotonin level was significantly less than the normal mice. After treatment both the test and standard drug decrease the elevated serotonin level of the obese group which was accounted for the decrease in food intake. Finally this report though the test drug (Medonil) shows anti-obesity activity but it is not so significant as compared to the standard drug

(orlistat). However it was evident that food intake, adipose tissue weight reduced significantly on treating with test compound drug (Medonil).

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