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STUDY OF ANTIOBESITY ACTIVITY OF *WITHANIA SOMNIFERA* ROOT AND *TERMINALIA ARJUNA* BARK IN MSG-HFD INDUCED MICE

Kumari Sangita*, Pattnaik Ashok, Chopra M P, Mohanty Satyajit, Chakraborti Sanchari and K. B. Sulgana

Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra Ranchi, Jharkhand -835215 India.

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***Corresponding Author**
Kumari Sangita
Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra Ranchi, Jharkhand - 835215 India.

ABSTRACT

Obesity is a major health problem. It is known for the risk factor for the development of many vital disorder. In this study, the methanolic extract of T*erminalia arjuna* and Ethyl acetate extract of *Withania somnifera* have been tested for their anti-obesity property. Animals treated with both plants have reduced the increase in body weight, periepididymal, fat weight etc. Antiobesity activity produced by plants are may be also because of inhibition of amylase enzyme, antioxidant activity, presence of tannins and flavonoid content. Before carrying out pharmacological activity preliminary phytochemical analysis was carried out. The plants have shown presence of some common constituents like saponins, flavonoids steroids tri-terpinoids glycosides and anthocyanins In this study, both plants are found be beneficial for the suppression of obesity and associated complications.

KEYWORDS: Obesity, *Withania somnifera*, *Terminalia arjuna*, monosodium glutamate.

INTRODUCTION

Obesity is an important public health challenge in both developing and developed countries. The World Health Organization has defined obesity as a disease in 2007. Obesity has turned up as one of the major health concerns in the 21st century and is one of the leading causes of preventable death. Obesity is a term applied to excess body weight with an abnormally high proportion of body fat. Thermodynamically speaking, disparity between energy intake (feeding) and energy expenditure (physical activity) leads to obesity. Risk factors for

developing obesity are sedentary life style, genetic constitution, illness, microbiological aspects and neurobiological mechanisms. It has reached epidemic proportions worldwide and accounts for more than 1 billion adults overweight of which at least 300 million of them clinically obese and is a major contributor to the global burden of chronic disease and disability.[1]

OBJECTIVE

The present study was planned with the following objectives

1. To develop obesity animal model by monosodium glutamate (MSG) and high fat diet (HFD).
2. To perform phytochemical screening and in intro activities of *Withania somnifera* bark and *Terminalia Arjuna* bark extract.
3. To evaluate the effect *of Withania somnifera* bark extract on MSG –HFD induced obesity in swiss albino mice by assessing biochemical parameters and histopathological studies.
4. To evaluate the effect of *Terminalia Arjuna* bark on MSG –HFD induced obesity in Swiss albino mice by assessing bio-chemical parameters and histopathological studies.

MATERIAL AND METHOD

Experimental Animals

Inbred male and female adult Swiss albino mice were housed in the Institutional Animal House of Department of Pharmaceutical Sciences & Technology of Birla Institute of Technology, Meshra, Ranchi (Jharkhand). All experiments involving animal complies with the ethical standards of animal handling and was approved by the Institutional Animal Ethics Committee.

Extraction of plant material

The dried powdered material of plant of *Withania somnifera root and Terminalia arjuna bark* were extracted sequentially by maceration in n-butanol, methanol, chloroform, hydro-alchohlic and aqueous extract at room temperature and concentrated under by rotary evaporator under reduced pressure. The concentrated extracts were allowed to dry in petri plates at room temperature. The dried films which obtained were carefully collected and stored for further use.[2]

Identification of active constituents

Preliminary phytochemical investigation and *In-vitro* tests

All the extract of plants were individually analyzed for the various classes of phytoconsituent such as flavonoids, alkaloids glycosides tannins, steroids phenols, carbohydrates, tri-terpinoids using standard photochemical method. *In-vitro* tests like hydrogen peroxide scavenging assay DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity, alpha amylase inhibitory assay ,total flavonoid content and phenolic content were analyzed.[3,4,5,6,7]

***In-vivo* studies**

MSG- HFD induced obesity in rats is considered as reliable tool for evaluation of anti-obesity activity. The study comprises of 5 groups

1. GROUP I – Normal Control
2. GROUP II- Obese Group (MSG+ DIET INDUCED)
3. GROUP III-Standard Orlistat Group, 60mg/kg i. p for 30days
4. GROUP IV-Test Treatment Group 1 (*Terminalia arjuna*), 50mg/kg i.p for 30days
5. GROUP V-Test Treatment Group 2 (*Withania somnifera*), 20mg/kg i.p for 30 days.

Among all groups except normal control group, induction of neurotoxicity with MSG was performed.

Pharmacological Evalution

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

Biochemical estimations

Blood samples were collected from retro-orbital sinus of mice of different groups. The concentration of triglyceride, cholesterol, LDL, VLDL, HDL, AST, ALT were measured using commercial kit available in the market.[8-14]

Histopathological examination

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.[15]

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

Phytochemical screening

In preliminary phytochemical screening tests, ethyl acetate extract of *Withania somnifera* has shown significant presence of steroidal lactones, triterpenoids, alkaloids, flavonoids and methanolic extract of *Terminalia arjuna* has shown significant presence of flavonoids, tannins, carbohydrates, saponins, steroidal lactones, triterpenoids, glycosides.

***In-vitro* results**

It was found that the methanolic extract of *Terminalia arjuna* and Ethyl acetate extract of *Withania somnifera* respectively has shown maximum % scavenging activity compared to other fractions of sample.

Total Flavonoid content of *Withania somnifera* was found to be 133.64 mg/ g of Quercetin Equivalent and total Flavonoid content of *Terminalia arjuna* was found to be 266.96 mg/g of Quercetin Equivalent.

Total Phenolic content of *Withania somnifera* was found to be 20.52 mg/g of Gallic Acid Equivalent and total Phenolic content of *Terminalia arjuna* was found to be 48.88 mg/ g of Gallic Acid Equivalent.

From the study it was found that methanolic extract of *Terminalia arjuna* (META) and ethyl acetate extract of *Withania somnifera* (EAWS) has shown marked increase in DPPH scavenging activity with respect to increase in concentration, however META has shown greater scavenging activity than EAWS in all the concentration.

***In- vivo* studies**

Obesity activity

Changes in body wt from 5^{th} to 12 th week: Results showed significant increase in body weight gain in8 th week and massive wt. gain was observed by the end of 12 th week which indicates significant development of obesity in terms of body weight.

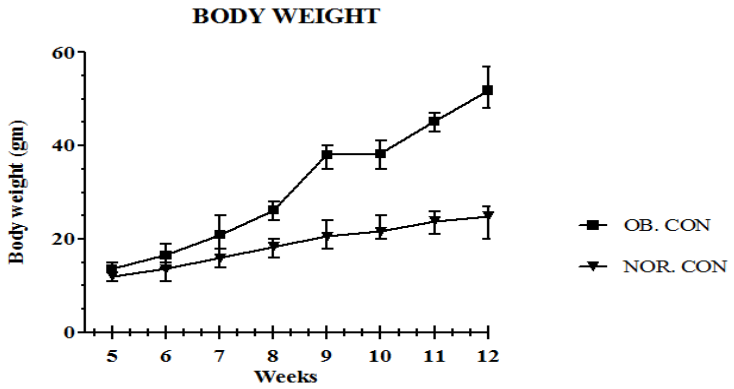

Fig. 1: Changes in body weight per week.

All values are expressed as mean ± S. E. M (n= 15). Statistically analysed by t-test where, *p <0.05, **p < 0.01, ***p < 0.001 compared with OB. CON group.

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

Parameters Measured after Drug Treatment (Anti-Obesity Activities)

Table 1: Changes in body weight.

No.of Weeks	Normal Group(g)	Obese Group (g)	Standard Treatment Group (g)	TA Treatment Group (g)	WS Treatment Group (g)
Initial Week	30.52±0.080***	42.26±0.050	42.26±0.011	42.08±0.007	42.17±0.023
After 1st week	31.28±0.030***	42.36±0.010	40.33±0.010	41.36±0.004	41.46±0.003
After 2nd Week	31.68±0.030***	42.46±0.003	37.14±0.003*	40.43±0.005	41.57±0.016
After 3rd Week	31.79±0.008***	42.62±0.001	35.44±0.080**	38.52±0.080*	40.31±0.020
After 4th Week	31.87±0.070***	43.00±0.050	33.14±0.030***	35.15±0.040***	38.72±0.240*

All values are expressed in mean±SEM; n=5 in each group. *p<0.05, **p<0.01,***p<0.001 when compared to obese group (one-way ANOVA followed by Dunnet's "t" test).

Table 2: Assessment of body parameters.

Parameter	Normal Control	Obese Control	Standard Treatment	*Terminalia arjuna* Treatment	*Withania somnifera* Treatment
Waist circumfernce (cm)	8.57±0.15***	14.73±0.20	9.70±0.05**	10.67±0.08**	11.40±0.12**
Hip circumfernce (cm)	9.40±0.17***	14.93±0.24	10.53±0.08**	11.23±0.07**	11.77±0.09**
Waist: Hip	0.911±0.002	0.987±0.003	0.921±0.003	0.950±0.003	0.969±0.003
Food Intake (g/day/mous)	4.30±0.12**	5.87±0.15	4.83±0.14*	4.93±0.08*	5.63±0.12
Liver Weight (g)	1.307±0.003**	1.410±0.005	1.350±0.005*	1.370±0.005*	1.380±0.005*
Brain Weight (g)	0.523±0.006**	0.460±0.005	0.470±0.001	0.473±0.003	0.486±0.003
Adipose Tissue Weight (g)	0.706±0.003**	1.713±0.003	1.510±0.005*	1.680±0.005*	1.690±0.005*

All values are expressed in mean±SEM; n=5 in each group. *p<0.05, **p<0.01, when compared to obese control group (one-way ANOVA followed by Dunnet's "t" test).

Table 3: Assessment of Biochemical Parameters.

Parameters Measured	Normal Group	Obese Control	Standard Treatment	*Terminalia arjuna* Treatment	*Withania somnifera* Treatment
SGOT (IU/L)	34.20±0.37c	48.60±0.40	37.40±0.41c	39.20±0.49c	42.60±0.24a
SGPT (IU/L)	27.60±0.59c	39.60±0.51	31.40±0.50b	33.00±0.31a	36.00±0.32
Blood Glucose (mg/d)	65.60±0.51c	99.40±0.50	71.00±0.32c	74.00±0.31c	79.40±0.50c
TCHO (mg/dl)	101.20±0.22c	146.80±0.17	105.60±0.34c	114.80±0.16c	118.00±0.11c
HDL (mg/dl)	38.52±0.17a	32.74±0.16	33.14±0.10	33.54±0.25	32.76±0.11
LDL (mg/dl)	34.98±0.11c	75.76±0.14	39.96±0.09c	45.90±0.10c	48.82±0.14c
VLDL (mg/dl)	28.74±0.25c	37.94±0.20	32.00±0.17a	34.60±0.14	36.14±0.09
TG (mg/dl)	102±0.31c	172±0.32	107.4±0.40c	112.6±0.24c	115.4±0.40c

All values are expressed in Mean±SEM; n=5 in each group. a=*p<0.05, b=**p<0.01, c=***p<0.001 when compared to obese control group (one-way ANOVA followed by Dunnet's "t" test).

There were body parameters measured such as changes in the body weight where it was observed that there was significant reduction in the body weight, as well as in waist and hip circumference. The organ weights were reduced significantly which may be due to presence of phenolic phytoconstituents such as ellagic acid which is responsible for loss of visceral fat

mass, and gallic acid which helps in lipid metabolism.[16] As the biochemical parameters were assessed there were significant reduction in Total cholesterol, Triglycerides, LDL and VLDL levels which may be due to presence of arjunolic acid (triterpenoids) responsible for lowering total cholesterol Ellagic acid (phenol) responsible for lowering Triglyceride level, luteolin (flavonoid) which improves lipid profile by lowering VLDL, LDL, Cholesterol.[17]

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. IP administration of 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 study suggested that the two chosen plant drugs *Terminalia arjuna* bark and *Withania somnifera* root have potential to treat MSG-HFD induced obesity. This study was carried out to identify possible leads from Ayurvedic herbs against obesity demonstrating that the treatment with the mentioned herbal drugs can efficiently attenuate metabolic dysfunction seen in the obesity induced mice in terms of controlling body weight and reduction in liver, adipose tissue weight due to low absorption of lipids and the non-deposition of visceral fat or hepatic steatosis improving blood lipid profile, together with improving antioxidant and α-amylase inhibitory status in the body.

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