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Development of an Anti-obesity Polyherbal Formulation containing *Terminalia arjuna, Lagenaria siceraria* and *Piper nigrum*.

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Abstract
Obesity is one of the most common health problems and has become an epidemic on the global scale. In the present study, new anti-obesity polyherbal formulations were developed and their effect was studied in female rats fed on high fat diet. Obesity was induced in wistar albino rats by feeding them with high fat diet for 28 days. Group-I served as normal control (1% Carboxy Methyl Cellulose (CMC)) and Group-II as obese control (1% CMC) fed on high fat diet, Group- III, IV, V, VI were treated with various polyherbal tablet formulations of *Lagenaria siceraria* alone or in combination with other plant extracts of *Terminalia arjuna* and *Piper nigrum* (400 mg/kg body wt) and Group-VII served as marketed formulation (400 mg/kg body wt), Group-VIII served as positive standard (Orlistat 45 mg/kg body wt). All the animals except normal control were fed on high fat diet. The animals were treated for 14 days. On 14th day, 2 hrs after drug administration, the body weight, organ weights, locomotor activity and various biochemical parameters like Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) levels were determined. There was a significant reduction in food intake, body weight, organ weights, TC, TG, LDL, locomotor activity and an increase in HDL levels in high fat diet fed rats treated formulation containing *Lagenaria siceraria*, *Terminalia arjuna* and *Piper longum* as compared to the Positive Standard, Marketed and Normal treated animals.

Keywords: Antiobesity effect, *Lagenaria siceraria*, *Terminalia arjuna*, *Piper longum*, Orlistat.

1. Introduction
Obesity is becoming one of the most prevalent health concerns among all populations and age groups worldwide, resulting in a significant increase in mortality and morbidity related to Coronary heart diseases, Diabetes Type 2, Metabolic syndrome, Stroke and Cancer. Obesity is the condition of abnormal body weight resulting from accumulation of extra adipose tissue, generally in response to a state of positive energy balance that occurs when energy intake exceeds energy expenditure. Obesity has been viewed as a chronic disease, much like hypertension and diabetes. Contributing factors include genetics, metabolism and appetite regulation, along with environmental, psycho-social, and cultural factors. Due to obscure etiology, the treatment of obesity is difficult and challenging. Further, the cause of concern is the non-availability of drugs for its treatment and the short-term efficacy and limiting side effects of the available drugs [1-4].

Plant-based pharmaceuticals have been employed in the management of various diseases affecting humans. *Lagenaria siceraria* (LS) fruit is a common vegetable known as bottle gourd which was traditionally used for its cardio-protective, cardio-tonic, anti-diabetic and diuretic properties [5]. LS have also been reported as a lipid lowering agent by preventing the fat absorption in the body [6].

Terminalia arjuna (TA) contains ellagic acid, arjunolic acid, arjunine, Triterpene glycosides like Arjunetosides I, II, III, IV. The bark is rich in Saponins, natural anti-oxidants (flavonoids - arjunone, arjunolone) which have been reported to reduce cholesterol level; TA improves cardiac muscle function and pumping actions of the heart [7-9]. *Piper longum* (PL) contains an alkaloid piperine (5%), essential oils like piperlongumine, piperlonguminine. Piperine is well known for its bioavailability enhancing property of a number of drugs. It also has a thermogenic effect which may prove to be helpful in weight loss [10].

The literature survey reveals that LS has weight reducing activity, TA has cholesterol lowering property while PL is a well-known antioxidant, bioavailability enhancer and thermogenic agent [13-14]. Thus, in the present study, an attempt has been made to evaluate the anti-obesity potential of a polyherbal tablet formulation containing LS, TA and PL in various

combinations.

2. Materials and Methods

2.1 Animals

Albino rats of Wistar strain, belonging to either sex, weighing between 250-280 g were used in the study. They were housed under standard environmental conditions and fed with commercial diet and water *ad libitum*. All experiments were carried out as per the guidelines of the Institutional ethics committee, 1370/ac/10/CPCSEA.

2.2 Chemicals and Reagents

Total Cholesterol estimation kit (enzymatic method), HDL, cholesterol (precipitation and enzymatic method) and triglycerides (enzymatic method) estimation kits (manufactured by Sigma diagnostics (India) PVT. Ltd, Baroda) were procured from Qualigens Fine Chemicals, Mumbai, India. Orlistat (LIPOPHASE) was obtained as gift sample from Franco-Indians, Mumbai. Ayurvedic Marketed formulation was purchased from the local market of Amravati, Maharashtra.

2.3 Plant material

Lagenaria siceraria (LS) fruits and *Terminalia arjuna* (TA) bark were collected from the local gardens of Amravati and *Piper longum* (PL) was purchased from a local crude drug store in Amravati. All the plant materials were authenticated by Dr. P.Y. Bhogaokar, Department of Botany Vidarbh Mahavidyalya, Amravati, Maharashtra. The voucher specimens have been deposited in the Department of Pharmacognosy, Government College of Pharmacy, Amravati, Maharashtra.

2.4 Extraction

Dried fruits of LS were extracted in water by maceration; TA and PL were extracted with 90% alcohol by soxhlet apparatus. The crude extracts were concentrated and dried under reduced pressure. The dry extracts were stored in airtight containers and used for further studies.

2.5 Preparation of formulation

The dry extracts were mixed with the excipients, granulated and finally punched into tablets using Multi-station Rotary Tablet Punching machine (Agile Machineries Pvt. Ltd.) The basic formula of the tablets prepared was as shown in Table-1. Formulation F-1 was prepared using LS and the other three formulations (Table-2) were prepared by the same procedure and stored in well closed containers at room temperature.

2.6 High Fat Diet [11-13]

Groups (II-VIII) were considered as model group and fed with high fat diet (HFD). The HFD consisted of 40 g of condensed milk and 40 g of bread, 15 g of chocolate and 30 g of biscuit and 30 g of dried coconut, and 40 g of cheese and 50 g of boiled potatoes. The three different combinations were given on 3 consecutive days and repeated for 28 days. This diet was provided in addition to normal pellet chow.

2.7 Study design

The rats were divided into eight different groups, containing six animals each.

Group-I served as control kept under normal diet as normal control administered with vehicle (1% CMC) orally, Group-II-Group-VIII rats were fed HFD. Group-II served as the obese control administered with vehicle (1% CMC) orally. Group-III was treated with F-1 (400 mg/kg orally), Group-IV was treated with F-2 (400 mg/kg orally), Group-V was treated with F-3 (400 mg/kg orally), Group-VI was treated with F-4 (400 mg/kg orally), Group-VII was treated with marketed formulation (400 mg/kg orally) and Group-VIII was treated with 45 mg/kg Orlistat orally in 1% CMC. The treatment was given for 14 days [14-17].

Table 1: Formula of the Tablet Formulation F-3.

Sr. no.	Ingredients	Quantity Taken for 100 Tablet in gms
1	LS extract	10.0
2	TA extract	10.0
3	PL extract	5.0
4	Lactose	5.0
5	Maize starch	2.5
6	PVPK 30	3.5
7	Sodium Methyl Paraben	0.40
8	Sodium propyl paraben	0.1
9	Talc	1.5
10	Magnesium Stearate	1.0
11	Sodium Starch Glycolate	1.0

Table 2: Formula of the Tablet

S. No.	Tablet Formulation	Ingredients
1	F-1	LS extract
2	F-2	LS extract + TA extract
3	F-3	LS extract + TA extract +PL extract
4	F-4	TA extract +PL extract

2.8 Body Weight

The body weights of the animals were recorded on Day 1 and then throughout the experimental period in each group daily.

2.9 Daily Food Intake

To measure the food intake the animals were fed with 100 gm/day of HFD daily from 8.00 am to 9.00 am. Any spilled food was collected and the total food consumed was calculated. The total consumption of HFD by each group was recorded daily.

2.10 Locomotor activity [17-18]

The Locomotor activity was recorded on 14th day, using open field behavior test apparatus and 30 min after dose administration to treatment groups. The apparatus consisted of a circular wooden arena of 75 cm diameter and wall with a height of 25 cm. Open field test was performed by placing the rat in the center circle and recording the ambulatory activity, the frequency of rearing and grooming for a 5 minute test period.

2.11 Biochemical parameter [19]

After 14 days, animals were starved for period of 16 hrs and blood samples were collected from recto orbital plexus by using micro capillary tubes and the serum was separated to analyze TG, TC, LDL and HDL. The animals were scarified and different organs like kidney, liver, heart and spleen were removed, weighed sent for histopathology study.

2.12 Statistical study
All findings were made in Triplicates and the results expressed as mean ± SEM. Comparison between treatment groups with normal group or obese groups animals were performed using Tukey Test, Dunnett's Test, at 95% confidence interval, the criterion for statistical significance was p <0.001.

3. Results
3.1 Effect on body weight
There was a significant (p<0.05) increase in body weight of HFD group animals when compared to control group. Orlistat produced significant decrease in body weight of rats on HFD. Treatment with F-3 and F-4 (400 mg/kg, p.o.) caused significant decrease in body weight of animals on HFD when compared to control group animals. The marketed formulation was also significantly effective in reducing weight in animals (Figures 1). The groups treated with F-1 and F-2 did not show much reduction in body weight.

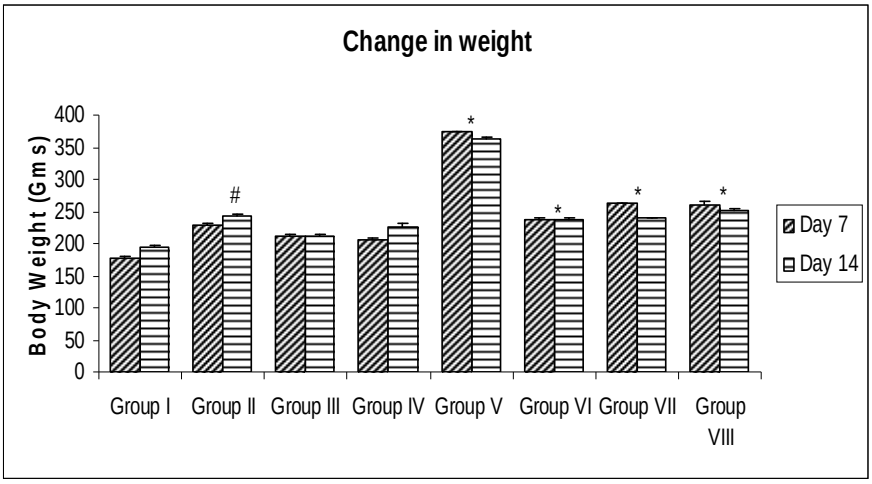

Fig 1: Effect on Body Weight

3.2 Effect on food intake
The food intake of normal group and obese group did not change during the 14 days study. F-1 (400 mg/kg, p.o.) decreased the food intake slightly, while F-2, F-3 and F-4 (400 mg/kg, p.o.) showed significant decrease in the food intake. The groups treated with Orlistat and marketed formulation also showed the significant decrease in the food intake. (Figure-2)

3.3 Effect on Locomotor activity
The results as depicted in Table-3 suggested that the Open Field Locomotor activity was increased significantly in case of F-3 and F-4 formulations. The Locomotor activity in the control group decreased significantly when compared to the Normal group. While the animals treated with F-1 showed slight increase in the activity. The formulations F-3 and F-4 showed significant increase in ambulation, rearing and grooming when compared with Orlistat and Control Group. The marketed ayurvedic formulation also showed significant increase in the activity when compared with control group.

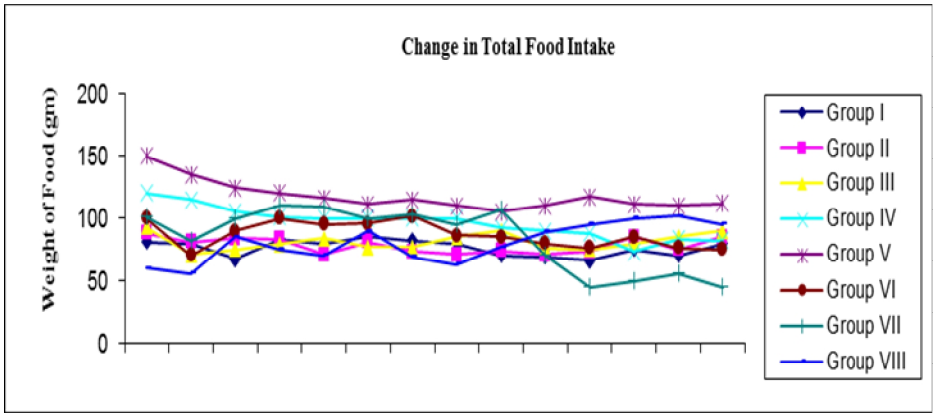

Fig 2: Effect on Total Food Intake

Table 3: Effect on Locomotor activities.

Groups	Frequency of Open Field Locomotor Activity*		
	Ambulation	Rearing	Grooming
Normal	65.0 ± 6.23	32.0 ± 2.12	7.0 ± 0.8
Control on HFD	28.0 ± 6.88[a]	16.0 ± 1.8 [a]	3.0 ± 1.1 [a]
F-1 Treated (400 mg/kg b.w. p.o.)	35.0 ± 7.11	20.0 ± 3.1	5.0 ± 0.6
F-2 Treated (400 mg/kg b.w. p.o.)	46.0 ± 5.44 [b]	24.0 ± 2.4 [b]	5.0 ± 0.9 [b]
F-3 Treated (400 mg/kg b.w. p.o.)	52.0 ± 8.24 [b,c]	27.0 ± 2.9 [b,c]	6.0 ± 1.2 [b,c]
F-4 Treated (400 mg/kg b.w. p.o.)	48.0 ± 5.28 [b,c]	26.0 ± 1.6 [b,c]	6.0 ± 0.9 [b,c]
Marketed Ayurvedic Formulation (400 mg/kg b.w. p.o.)	36.0 ± 6.55	19.0 ± 4.3	4.0 ± 0.8 [b]
Orlistat (45 mg/kg b.w. p.o.)	61.0 ± 4.67 [b]	30.0 ± 2.2 [b]	7.0 ± 0.7 [b]

*Tested for 5 minutes duration

Values represents Mean ± SEM (n = 6), *aP<0.05*, significant as compared to Normal group, *bP<0.05*, significant as compared to the Control on HFD group, c*P<0.05*, significant as compared to the Standard group

3.4 Effect on biochemical parameters

High fat diet containing group showed significantly increased TC, TG and LDL, while decreased HDL levels as compared to control groups. The animals treated with F-1 (400 mg/kg, p.o.) showed significant reduction in TC and increase in HDL level, while no significant effect was observed on LDL and TG. The Group-IV animals treated with F-2 (400 mg/kg, p.o.) showed significant decrease in TG and no significant changes were observed in TC, LDL and HDL levels. The F-3 treated (400 mg/kg, p.o.) animals had significant reduction in TC, TG, LDL and significant increase in HDL level. The Group-VI animals treated with F-4 (400 mg/kg, p.o.) had similar effects like F-3 treated group, except that no reduction in TC was observed (Fig. 3 - Fig. 6). The Marketed Ayurvedic formulation showed significant decrease in the LDL and TC levels without much effect on HDL and Triglycerides.

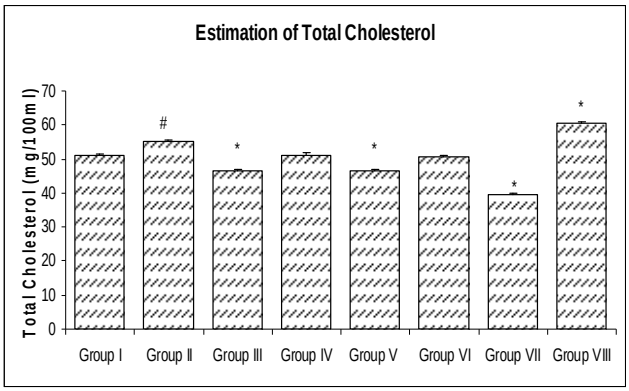

Fig 3: Effect on Total Cholesterol

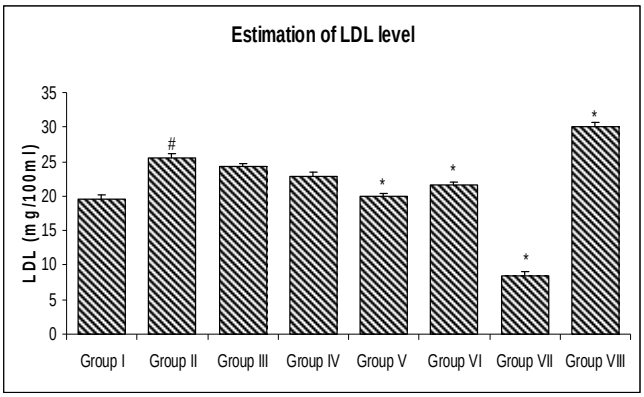

Fig 4: Effect on LDL level

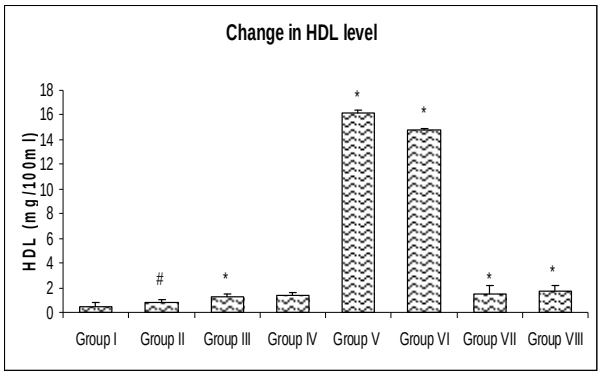

Fig 5: Effect on HDL Level

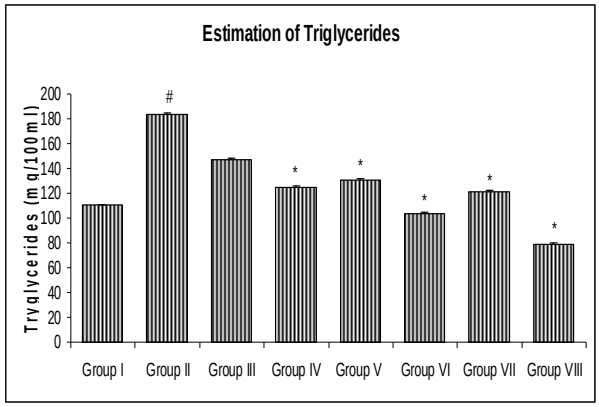

Fig 6: Effect on Triglycerides

4. Discussion

The results of our study showed that rats fed with a variety of highly palatable, energy rich, high carbohydrate foods elicited significant increase in body weights. High fat diet has been previously reported to increase energy intake and cause obesity in humans as well as animals. In this study, high fat diet fed rats exhibited an increased body weight along with a corresponding rise in cholesterol levels.

The various formulations were prepared by using LA extract alone and in combination with alcoholic extracts of TA and PL. The major constituent of TA was found to be present in alcoholic extract. However, not much weight loss has been observed when administered alone. LS showed significant decrease in cholesterol and increase in HDL levels. LS, TA and PL in combination, together showed significant anti-obesity effects as compared to LS alone. However, LS and PL

showed significant anti-obesity potential when compared to normal, obese and standard groups.

LS has been reported previously as an anti-hyperlipidemic drug [20]. TA is reported to have cholesterol reducing property [21]. The preliminary phyto-chemical evaluation of LS and TA showed that various chemical constituents like saponins, pectin, ellagic acid required for reducing body weight and cholesterol levels were present in the Aqueous extract. The LS fruits are considered as a good source of Triterpenoid saponins, cucurbitacins B, D, G, H and pectin, which have been claimed to have lipid reducing property [20]. The bark of TA is rich in saponins, natural anti-oxidants (flavonoids - arjunone, arjunolone) which have been reported to reduce cholesterol levels [21]. Piper longum (pippali) has heat generating property and has been referred by Charak as the best digestive stimulant [22]. The Ayurvedic marketed formulation purchased from local Amravati market was showed five times greater weight loss compared to standard group, while other parameters were not affected to a great extent. It has been observed that this formulation had drastically reduced the body weight, which may be due to toxic materials or adulterants present in the formulation. Thus, it proves that standardization of such formulations is mandatory for the safe and effective utilization of the Ayurvedic drugs.

The changes in the food intake, body weights, biochemical parameters ambulatory and other locomotor activities by F-3 may be attributed to the overall hypolipidemic, cholesterol lowering and thermogenic property of the ingredients of the formulation. Thus, on the basis of results the present study, it can be concluded that polyherbal formulation have definite potential as reported for several traditional anti-hyperlipidemic drugs. Further researches on the fractionation of extracts, isolations, purifications responsible for the anti-hyperlipidemic activity and their mechanisms should be performed.

5. Conclusion

From the overall result of the biochemical and behavioral results, it could be inferred that F-3 showed anti-obesity activity at 400 mg/kg dose. Present studies reveal that LS, TA and PL can be used in combination as anti-obesity agents. Further experiments are required to prove the mechanism and advantages of the present findings.

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