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EFFECT OF POLYHERBAL FORMULATIONS MEF-4 AND MEF-8 ON HIGH FAT DIET INDUCED OBESITY IN SD RATS

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Abstract*:* Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicine have made large contribution to human health and well-being. In this study, two polyherbal formulations (MEF-4 and MEF-8) were prepared using methanolic extracts of two medicinal plants such as *Camelia sinensis* and *Citrus Aurantium*. The present study is undertaken to evaluate Anti-obesity potentials through *in-vivo* high fat diet induced obesity of the polyherbal formulations prepared in-house. The rats were fed with high fat diet for the induction of hyperlipidemia for two weeks. Upon confirmation of disease induction the animal experiments were conducted as per standard protocols. The hyperlipidemic rats were administered with both the formulation at a dose of 250 mg/kg and the control groups received standard diet & high fat diet orally for 30 days. After treatment for 30 days, blood samples from the MEF-4/MEF-8/standard/vehicle were collected and effect on body weight, hematology and lipid profile was determined. The formulations MEF-4 and MEF-8 was associated with a significant ($p<0.01$) decline in total cholesterol (20-22%), LDL (48-52%), triglyceride (36-40%) and phospholipids (24-27%) respectively. It was also observed that the formulation MEF-4 was more effective than MEF-8 at the same dose.

Keywords: - High fat diet, Hyperlipidemia, Lipid profile, Polyherbal formulation.

Introduction*:* Obesity is a common metabolic disease that is growing worldwide. With increased body weight gain, obesity is a common risk factor for diseases such as diabetes, hypertension, and cardiovascular disease due to the excessive deposition of adipose tissues. Increased energy intake (calories) with the decline of physical activity promotes weight gain, body fat storage and adiposity growth in a pathologic direction[1]. Adipocyte, so called the fat cell, is the major component of adipose tissue that is known as loose connective tissue or fat tissue that function as an energy storage site in the form of triglyceride[2]. Adipose tissue plays an important role in maintaining the free fatty acid levels and triglycerides in circulation. Research suggests that it is possible to inhibit adipose tissue mass by decreasing the adipose tissue mass as well as adipocyte number[3]. Lipid lowering drugs, mostly statins and fibric acid derivatives have been widely used to manage the elevated levels of various forms of lipids in hyperlipidemia patients. Due to its serious complications, these drugs have to be used safely or avoided when possible[4]. Evidences show that green tea and its catechins, especially EGCG, reduce body weight as well as adipose tissue and blood lipid level[5]. Also other research report suggests that the PMF from orange named hesperetin can lower the risk of coronary heart disease by the effect of hypolipidemia[6].

In recent years, the development of lipid lowering drug or formulation from natural source has gained importance. Hence an attempt is made to develop a polyherbal formulation containing the extracts of *Camelia sinensis* and *Citrus Aurantium* and to evaluate its anti-obesity action.

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Materials and Methods

Plant materials collection and extraction: The two plant materials used in the formulation were collected from an authorized raw drug dealer, The plant materials were properly authenticated and shade dried, coarsely powdered using mechanical grinder. The *Camelia sinensis* leaves and *Citrus Aurantium* peel were extracted

with 70% methanol individually by soxhlet extraction process for three cycles. The extract were collected and concentrated under vacuum. The crude extract obtained was stored in refrigerator for further use. The two formulations were prepared by mixing both extracts with varying polymer as specified in Table 1.

Table 1: The ingredients of the polyherbal formulations MEF-4 and MEF-8 under study

MEF-4	MEF-8	Function	Part used	Extract	Quantity (mg)
Camelia sinensis	*Camelia sinensis*	Active	Leaves	Methanolic	125
Citrus Aurantium	*Citrus Aurantium*	Active	Peel	Methanolic	125
Eudragit S 100	Pectin	Polymer	-	-	98
Microcrystalline cellulose	Microcrystalline cellulose	Binder	-	-	5
Peppermint	Peppermint	Flavourent	-	-	4
Stevioside	Stevioside	Sweetner	-	-	2
Colloidal silicon dioxide	Colloidal silicon dioxide	Glident	-	-	1

Drugs and chemicals: The cholesterol, casein and coline were purchased from Himedia Laboratories, Mumbai. The biochemical kits used for the estimations were brought from Merk Diagnostics Pvt. Ltd., Mumbai, India.

Preparation of Polyherbal Formulation

Preliminary phytochemical analysis: A portion (2 g) of the polyherbal formulation was used for the preliminary phytochemical analysis, which includes tests for carbohydrates, glycosides, proteins, amino acids, alkaloids, phenols, flavonoids, phytosterols, fats, fixed oils, saponins, tannins and sugar in accordance with the methods of Trease and Harborne[7,8].

Experimental animals: Sprague-Dawley rats weighing 180±20 g were used as experimental animals and maintained as per standard guidelines in accordance with Institutional Animal Ethics Committee (IAEC) regulations and approved by CPCSEA.

Induction of hyperlipidemia: The high fat diet (HFD) was prepared as per Table 2 and administered for 2 weeks except for the normal control group which was fed with standard chow only. At the end of 2th week, total cholesterol level in serum was estimated and the animal with greater than 250 mg/dl level was selected and considered as hyperlipidemic rats[9].

Table 2: Composition of high fat diet (HFD)

S.No.	Ingredients	Quantity
1	Casein	240
2	Corn starch	299.6
3	Soybean	250
4	Cholestrol	10
5	Coline	0.4
6	Salt mixture*	100
7	Vitamin mixture#	20
8	Cellulose	80
	Total Calories (Kcal)	4538.4

*Composition (g/Kg of mixture): NaCl - 139.3 / KI - 0.79 / MgSO4.7H2O - 57.3 / CaCO3 - 381.4 / MnSO4. H2O - 4.01 / FeSO4.7H2O - 27.0 / ZnSO4.7H2O - 0.548 / CuSO4.5H2O - 0.477 / CoCl2.6H2O - 0.023 / KH2PO4 - 389.0.

#Composition (g/Kg of mixture): Retinol acetate - 2,000,000IU / Cholecalciferol - 200,000IU / P-aminobenzoic acid - 10.00 / I-Inositol - 10.00/ Niacin - 4.00 / Calcium pantotenate - 4.00 / Riboflavin - 0.80/ Tiamine HCL - 0.50 / Piridoxine HCL - 0.50 / Folic acid- 0.20 / Biotin - 0.04 / Vitamin B12 - 0.003 / Choline - 200.0 / a-Tocopherol - 10,000IU/ Sucrose qsp 1000

Experimental design: The animals were grouped into 4 groups each containing 10 rats.

Group I: Control group receiving high fat diet as vehicle.

Group II: Control group receiving standard diet as vehicle.

Group III: Hyperlipidemic rats treated with MEF-4 (250 mg/kg) orally for 4 weeks.

Group IV: Hyperlipidemic rats treated with MEF-8 (250 mg/kg) orally for 4 weeks.

Blood samples after 24 hours of last dose was collected from retro-orbital plexus and allowed to coagulate at room temperature which was then centrifuged at 3000 rpm for 10 minutes. The serum was separated and used for the biochemical estimations.

Estimation of serum lipid profile: Serum lipid profile was carried out using standard protocols[10,11].

Statistical Analysis: Statistical analysis was done using ANOVA[12].

Results: The data obtained on the preliminary phytochemical analysis was given in Table 3. The effect of the two formulations on body weight, hematology and

serum biochemistry was given in Table 4, 5 and 6 respectively.

Table 3: Preliminary phytochemical analysis of polyherbal formulations

TESTS	MEF-4	MEF-8
Carbohydrates (Fehling's test)	P	P
Glycosides (General test)	A	A
Protein and amino acids (Ninhydrin test)	A	A
Alkaloids(Hager's test)	P	P
Phenol (Lead acetate test)	P	P
Flavonoids (Alkaline reagent test)	P	P
Phytosterols (Libermann Burchard test)	P	P
Saponins (Foam test)	P	P
Tannins (Ferric chloride test)	P	P
Fats and fixed oils (spot test)	A	A

Present: P Absent: A

Table 4: Effects of the Formulation MEF-4 and MEF-8 on the body weight of diet-induced obese rats compared to the control groups receiving high fat (HFD) and diet.

Groups	Base Line	After One Month
HFD	180.22±2.24	225.14±2.42
STD	166.27±2.22	200.21±2.72
F4	165.20±2.01	173.11±2.12[a]
F8	164.23±2.32	175.11±2.32[b]

Values are expressed as mean ± SD, a, p<0.001; b, p< 0.01

Table 5: Effects of the Formulation MEF-4 and MEF-8 on the heamatology test of diet-induced obese rats compared to the control groups receiving high fat (HFD) and diet

GROUPS	RBC	HB	HCT	RDW	MCV	MCH	MCHC	PLT	MPV	WBC
HFD	8.02 ±0.11	14.79 ±0.32	42.16 ±0.42	11.83 ±0.03	52.20 ±0.44	18.02 ±0.40	33.62 ±0.22	570.20 ±22.80	7.00 ±0.22	3.50 ±0.31
STD	8.02 ±0.12[a]	14.27 ±0.31	41.79 ±0.93	11.79 ±0.04	52.14 ±0.36	17.56 ±0.42	33.52 ±0.22[a]	578.20 ±22.25	7.18 ±0.20	3.63 ±0.32[a]
F4	8.050 ±0.16[b]	14.43 ±0.33	41.72 ±0.84	11.76 ±0.04	52.15 ±0.48	16.87 ±0.18	33.52 ±0.22[b]	627.20 ±22.05	7.43 ±0.24	4.05 ±0.34
F8	8.10 ±0.11[a]	14.19 ±0.22	43.77 ±0.68	11.80 ±0.05	53.12 ±0.40	17.78 ±0.12	33.22 ±0.12	635.20 ±22.29	7.27 ±0.22	4.09 ±0.31

Values are expressed as mean ± SD
a, p<0.001; b, p< 0.01
Legends: RBC-Red blood Cells, Hb- Hemoglobin, HCT- Hematocrit, RDW- RBC distribution width, MCV- Mean corpuscular volume, MCH- Mean corpuscular hemoglobin, MCHC- Mean corpuscular hemoglobin concentration, PLT- Platelets, MPV-Mean platelet volume, WBC- White blood cells.

Table 6: Effects of the Formulation MEF-4 and MEF-8 on the serum biochemistry of diet-induced obese rats compared to the control groups receiving high fat (HFD) and diet.

GROUPS	GLU	AST	ALT	BIL	PRO	ALB	GLB	CHL	LDL	TG	PHL
HFD	190.20 ±10.21	140.28 ±12.02	29.44 ±2.14	0.20 ±0.02	5.81 ±0.02	3.10 ±0.04	2.69 ±0.07	102.50 ±3.20	50.4 ±2.34	90.2 ±2.42	47.14 ±0.14
STD	208.02 ±10.20	164.29 ±21.22	41.26 ±2.24	0.23 ±0.02	5.66 ±0.22	3.14 ±0.03	2.54 ±0.02	71.20 ±3.20	34.2 ±1.24	75.2 ±2.12	41.14 ±0.10
F4	204.29 ±12.21	186.22 ±22.24	38.22 ±2.28	0.26 ±0.02	5.88 ±0.22	3.21 ±0.04	2.57 ±0.04	80.10 ±2.12	25.2 ±1.42[a]	52.0 ±1.60[a]	35.22 ±0.10[a]
F8	206.10 ±12.29	178.27 ±22.28	32.27 ±2.23	0.24 ±0.02	5.70 ±0.20	3.18 ±0.05	2.46 ±0.03	80.20 ±2.05	27.2 ±2.41[b]	54.10 ±2.24[b]	37.14 ±0.10[b]

Values are expressed as mean ± SD, a, p<0.001; b, p< 0.01, Legends: GLU- Glucose, AST- Aspartate aminotransferase, ALT- Alanine aminotransferase, BIL-Bilirubin, PRO-Protein, ALB-Albumin, GLB-Globulin, CHL-Cholesterol, LDL-Low density lipoprotein, TG- Triglyceride, PHL-Phospholipids.

Results and Discussions: Obesity results from energy imbalance between energy intake and energy expenditure over a period of time. Increased energy intake (calories) with the decline of physical activity promotes weight gain, body fat storage and adiposity growth in a pathologic direction. Studies have shown that the obesity contributes to an increasing risk of major depression, emotional disorders, early death, disability, menstrual disorders, infertility, miscarriage, and poor pregnancy. It is also concluded that there is strong evidence that obesity is associated with increased morbidity and mortality. Several chronic diseases are demonstrated related to obesity which including the following hypertension, Dyslipidemias (high total cholesterol or high levels of triglycerides), Type 2 diabetes, Coronary heart disease, Metabolic syndrome, Stroke, Gallbladder disease, Osteoarthritis, Sleep apnea and respiratory problems and some cancers (endometrial, breast, and colon). In addition, obesity has been predicted to be the number one health problem globally by the year 2025 and thought to be overtaking cigarette smoking soon to become the leading cause of death in the USA[13]. The importance of medicinal plants in the treatment of hyperlipidemia was experimentally studied in recent years, where oxidative stress induced apoptosis in adipose tissue was noticed[14,15]. Medicinal plants have been used to control the lipid level and also used as an inhibitor to trigger oxidative stress which reduces adipose tissue apoptosis. For many centuries, tea has been the most wildly consumed beverages in Asian countries. Today, the health benefits of tea have gained more attention from consumers and scientists. Tea has been investigated for its ability to prevent several chronic diseases, including cancer, neurodegenerative diseases and obesity[16]. Reduction of serum cholesterol level, prevention of arteriosclerosis and effects such as protection of blood vessels, were reported as integrated pharmacological effects by tea[17,18]. Evidences show that green tea (*camellia seninsis*) and its catechins, especially EGCG, reduce body weight as well as adipose tissue and blood lipid level[5].The mechanism of action of EGCG involve certain pathways, including (1) decrease in the energy intake[19], (2) increase in energy expenditure[20] and (3) alterations in the activities of fat, liver, muscle, and intestinal cells[5]. The major component of orange peel is flavonoids, especially the polymethoxyflavones (PMFs). PMFs, especially tangeretin and nobiletin, have been found to have health benefits, including anti-inflammatory, anti-carcinogenic, anti-tumor, anti-viral, anti-oxidant, anti-thrombogenic and anti-atherogenic properties[21]. Other reported also suggested that the other PMF from orange named hesperetin can lower the risk of coronary heart disease by the effect of hypolipidemia[22]. Based on these informations, the polyherbal formulation was prepared using extracts of above mentioned two medicinal plants which are known to possess anti-obesity potentials and evaluated scientifically for these potentials using SD rats as experimental animal models. The high fat diet administered in present study includes HFD for effective hyperlipidemia/obesity induction. The significant ($p<0.01$) change in lipid profile noticed in the experimental animals confirmed the induction of obesity in HFD fed rats (Table 6).

High fat diet increased triglycerides level and leads to weight gain. The present study showed that HFD significantly ($p<0.01$) increased TG level when compared with standard pellet treated rats. Treatment with MEF-4 at 250 mg/kg for 30 days showed significant ($p<0.001$) decrease in triglyceride level. The decline in triglyceride ($p<0.01$) was also noted for MEF-8 when compared with control and standard. The polyherbal formulations at the dose level of 250 mg/kg, b.wt reduced significantly the serum TG level in hyperlipidemic rats compared to control animals on high fat diet. Increase in LDL level causes deposition of cholesterol in the arteries and aorta and hence is a leads to CHD. LDL transports cholesterol from the liver to the periphery[5,19]. The fortification of LDL from oxidation and decrease in oxidative stress might therefore be useful for prevention of atherosclerosis associated CVD[20]. In the present study administration of MEF-4 and MEF-8 at 250 mg.kg dose levels effectively reduced LDL cholesterol content of hyperlipidemic rats. For a good lipid lowering therapy, a drug should be able to significantly lower LDL and this appreciably decreases the fatty cytoplasmic vaculated cells in liver parenchyma and prevents hepatic necrosis and this correlates with the present study[20]. The body weight gain rates in the MEF-4 and MEF-8 groups were significantly lowered and maintained almost constantly at 173.11±2.12 and 175.11±2.32 respectively. The body weight gain of the MEF-8 group was comparable with the standard control group (Table 4). There were no treatment-related adverse effects observed based on the hematology (Table 5) and blood chemistry results (Table 6). To sum up, the effect of polyherbal formulation MEF-4 and MEF-8 was

studied in experimental rats, where obesity was induced through high fat diet. The administration of MEF-4 to the hyperlipidemic rats significantly reduced total cholesterol, TG, and LDL. The formulation MEF-4 revealed maximum protective effect at a dose of 250 mg/kg in comparison with MEF-8 at the same dose. Further in-depth studies can result in the development of an effective herbal anti-obesity drug.

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