Research Article

Preparation and Development of Poly-herbal Extract and its Evaluation for its Anti-obesity Property in Modified Diet Induce Obese Model in Rats

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ABSTRACT

Obesity has emerged as a major health problem and risk factor for various disorders like ischemic heart diseases, atherosclerosis, diabetes, and hypertension. Currently no pharmacological treatment provides sustained weight loss with minimal adverse effects. Hence, there is a need for research in this discipline to discover drugs with better therapeutic index and least or no side effects. In this study Polyherbal extract were prepared. Prepared extract was responsible for the anti-obese action and produces synergetic activity. The Polyherbal extract of *Nigella sativa, Garcinia cambogia*, *Coffea Arabica* were separately defatted followed by Soxhlet extraction. Thymoquinone present in *Nigella sativa*, Hydroxycitric acid (HCA) present in *garcinia cambogia* and Caffeic acid and chlorogenic acid present in *Coffea having the anti-obesity action*. High fat feed containing both animal derived fat and plant oils were prepared and fed to the rats for 40 days to induce obesity. Obese rats were treated with Polyherbal extract *Nigella sativa* (100mg/kg p.o), *Garcinia cambogia* (200mg/kg p.o), *Coffea Arabica* (400mg/kg p.o) for 21 days. At the end of the study, lipid and serum parameters were measured and histopathology of the liver was done. Animal groups were treated with standard drug (Orlistat) and Polyherbal extract (mid dose and high dose) were showing significant change in the body weight of the animal. There was significant decrease in lipid profile (LDL, VLDL, TG and TC) and serum parameters (SGOT, SGPT) of standard and test drug as compared to the diseased control group, HDL levels of the test drug and standard group showed significant increase as compared to the diseased control. Group were treated with high dose which is showing potential effect on the normal architecture of liver tissues. Group were treated with mid dose which is not showing significant change in the liver tissues considered as a best dose for the treatment.

Keywords: Anti-obesity, Thymoquinone, Hydroxycitric Acid, Caffenic Acid, Polyherbal extract, modified diet, Lipid profile.

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INTRODUCTION

Obesity is a lifestyle disease and according to the WHO, obesity is one of the commonest and neglected conditions among both developed and developing countries[1]. Over the past two decades there is a drastic rise in the prevalence of obesity throughout the globe. By 2014 almost 30% of the worldwide population was overweight and 5% of the deaths worldwide were owing to obesity. 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[2]. The development of obesity is dependent on an imbalance between energy intake and energy expenditure during an extended period of time. The cause may be viewed as excess energy intake relative to daily energy expenditure, or as low energy expenditure relative to daily energy intake[3]. In this context the anti-obesity effect of polyherbal extract is studied. Polyherbal extract of *Coffea arabica, Garcinia cambogia* and *Nigella sativa* in treating obesity are studied.

MATERIALS AND METHODS

The seeds of *nigella sativa, coffea Arabica, garcinia cambogia* were collected from market.

Preparation of Alcohol extract

Extraction of Nigella Sativa

The seeds of *nigella sativa* were cleaned and dried in shadow and powdered using a grinder then the 100g seed powders were defatted with petroleum ether at 40-60 degree Celsius using the Soxhlet apparatus. Then the powder was extracted in 800ml ethanol in a Soxhlet apparatus for 72 hours and the mixture was subsequently filtered and concentrated at 40 degree Celsius[4].

Extraction of Garcinia Cambogia

The fruit of *Garcinia Cambogia* were cleaned and dried in shadow and the 100g fruit were defatted with petroleum ether at 40-60 degree Celsius using the Soxhlet apparatus. Then the powder was extracted in 800ml ethanol in a Soxhlet apparatus for 72 hours and the mixture was subsequently filtered and concentrated at 40-degree Celsius[5].

Extraction of raw Coffee Arabica

The seeds of *Coffee Arabica* were cleaned and dried in shadow and powdered using a grinder then the 100g seed powders were defatted with petroleum ether at 40-60 degree Celsius using the Soxhlet apparatus. Then the powder was extracted in 800ml ethanol in a Soxhlet apparatus for 72 hours and the mixture was subsequently filtered and concentrated at 40 degree Celsius[6].

Experimental animals

Inbred albino wistar rats, weighed 130-200g belonging to either sex 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 Institutional ethics committee under the supervision of CPCSEA[7,8].

Preliminary phytochemical studies were carried out for the obtained extract by using standard procedures. The seeds collected were dried completely to remove all the moisture from it to perform phytochemical analysis and to identify the chemical constituent present[9].

Identification of Chemical Constituents

Alkaloid Identification

Small portion of extract approximately 5g was dissolved in dilute hydrochloride and filtered, this filtrate is used in different test for determining alkaloids.

Mayer's test

To 1ml of filtrate, potassium mercuric iodide (Mayer's reagent) was added. Cream coloured precipitate observed then alkaloids are identified in the sample.

Dragendroff's test

To 1ml of the filtrate, potassium bismuth iodide (Dragendroff's reagent) was added. Reddish brown precipitate observed then alkaloids are identified in the sample.

Wagner's test

To 1ml of the filtrate, solution of iodine in potassium iodide (wagner reagent) was added. Brown precipitates observed then alkaloids are identified in the sample.

Hager's test

To 1ml of the filtrate, picric acid solution (hager's reagent) was added. Yellow coloured precipitate observed then alkaloids are identified in the sample[10].

Carbohydrates and Glycosides Identification

Small portion of extract approximately 5g was dissolved in distilled water and filtered; this filtrate is used in different test for determining carbohydrates and glycosides

Molisch test

To 2ml of the filtrate, alcoholic solution of α-naphtha (molisch reagent) is added. Then concentrated sulphuric acid is added dropwise on the sides of the test tube, violet ring appeared on the layer of two liquids then carbohydrates are identified in the extract[11].

Preclinical Studies

The study was approved by the Institutional Animal Ethics Committee (IAEC) wistar albino rats, weighing 120-150g were taken and acclimatized under standard laboratory conditions at 28±2°C, relative humidity 50±15%. They were kept in polypropylene cages, under standard laboratory conditions, 12 hours light (day) and 12 hours (night cycle). The normal group rats were provided with normal rat chow. Tap water and dietary regimen was given for 21 days[12].

Acute Oral Toxicity Studies

Acute oral toxicity studies were carried out as per OECD guidelines and female albino rats were used for toxicity studies. No results of toxic symptoms such as behavioural changes or death were seen in rats. Therefore, this Polyherbal extract was safe and further used for treatment in anti-obesity.

Induction of Obesity Using High Fat Diet

The rats were weighed before inducing high fat diet. The high fat diet was induced to all the five groups except normal control using peanut butter, cheese, dalda ghee, fish oil and egg yolk. The high fat consisted of different quantities of all these products. The three different combinations was given on 3 consecutive days and was repeated for 28 days. This diet was given in animal pellet chow. Cholesterol was given along with the high fat diet[13,14].

Preparation of feed

2000g of normal rat chow pellets were weighed and it was initially grounded into coarse powder using a pestle mortar, and further ground into a fine powder using an electric mixer grinder. Cholesterol was weighed using a standard weighing balance in the required quantity (60g) and then mixed with the weighed rat chow powder. 200g of peanut butter was heated and mixed along with 100g of cheese and 200g of egg yolk and was added to the above mixture followed by 200g of fish oil and mixed well. To the above mixture 400g of ghee was added to mask the odour and taste of peanut butter and egg yolk and mixed well. The consistency of the above mixture is to be maintained such that it can be made into spherical pellets each weighing around 40g. The pellets were allowed to dried for 30minutes feeding it to the rats. Each cage containing 6 rats were provided with 200g of high fat diet every day[15].

Animals weighing between 120-150g were acclimatized to room temperature. High fat diet(rat chow, cholesterol, peanut butter, dalda ghee, egg yolk, fish oil) was fed for 14 days to all the rats. Rats weighing 280g and above were considered as obese and were divided into disease control, test group I, test group II, test group III and standard group[16].

Experimental design

The rats were divided into six different groups containing six animals each. In this experiment, 36 animals were used.

Group (n=6)	Treatment and dose
Group I	Normal control
Group II	Diseased control
Group III	Low dose (alcohol extract-100mg/kg,p.o)
Group IV	Mid dose (alcohol extract-200mg/kg,p.o)
Group V	High dose (alcohol extract-400mg/kg,p.o)
Group VI	Standard (Orlistat)

After induction of obesity using high fat diet for 21 days, standard and test drugs were administered using oral gavage, once a day for next 14 days. During the overall period of 35 days, parameters such as daily food intake, body weight were measured.

Body weight

The body weights of animals were recorded on day 1 and throughout the experimental period in each group daily using electrical balance.

Daily food intake to measure the food intake animals were fed with 200g/day of high fat diet. Any spilled food was collected and the total food consumed was calculated. The total consumption of high fat diet by each group was recorded daily[17,18].

Post treatment

After 21days, animals were starved for period of 16 hours and on the 35th day the overnight fasted animals were anesthetized using ether and blood samples were collected using retro orbital plexus by use of micro capillary tubes and the serum was separated to analyse triglycerides, total cholesterol, HDL, LDL AND VLDL. The animals were sacrificed, and liver was removed for histopathological study[19,20].

Statistical study the statistical analysis data of each group for different parameters were performed using ANNOVA.

RESULT AND DISCUSSION

Individual alcohol extract of defatted *coffee Arabica, Nigella sativa and Garcinia cambogia* were prepared by using Soxhlet extractor. The extracts obtained were sticky mass of various colour dark green, blackish brown, yellowish white accordingly and Percentage yield was found to be 2.2 %w/w, 0.8% w/w,2.3%w/w accordingly.

The high fat feeding to the rodents leads to the induction of obesity and metabolic disorders which was similar to the human metabolic syndrome. Common animal fats and the plant oils contain omega -6, omega-9 used induce obesity. This high fat diets induces metabolic syndrome in rodent model with insulin resistance and significantly reduce the function of the β-cells. High fat diet increases the levels of triglycerides (TG), low density lipoprotein (LDL), very low density lipoproteins (vLDL) and also reduces the levels of high density lipoprotein (HDL). Variation in these lipid profile leads to increases the chance of atherosclerosis and cardiovascular diseases(CVD). For the induction of the obesity in rodents different type of high fat diet have been used with various amount of fat (energy level between20-60%).

In this study high fat diet was the combination of both animal derived fats and plant derived fat. For inducing obesity in rats fat from both animal source (cholesterol) as well as plant source (coconut oil, peanut butter, ghee, dalda etc..) was used, Increasing in the body weight was considered as obesity index. The body weight of all the animal group receiving the high fat feed was increased gradually during the period of study significant change in the body weight was observed only after one week.

Effect of Polyherbal extract (coffee Arabica, Nigella sativa and Garcinia cambogia) on obese rat

The preparation of the Polyherbal extract by combining the individual extracts of *coffee Arabica (3%), Nigella sativa (5%)* and *Garcinia cambogia (2%).* The effect of the combined extracts was studied against high fat induced rats. The animals were treated with high fat diet for a period of 21 days and the weight of each animals in each group were recorded a period of every 7days the body weight of 200 and above were considered obese. The animals which are obese was treated with standard drug (Orlistat 60mg/kg) and test drug for a period of 7 days during which the changes in the body weight were recorded and biochemical parameters were measured at the end of the study by sacrificing the animal.

Body weight variation in Rats

The body weights of the animals were increased gradually during the period of high fat diet. The significant increases in the body weight were observed after 7 days of high fat diet. Consumption of high concentration of the lipids lead to the deposition of TG in adipose tissues hence the body weight of the animal receiving high fat diet was increased. Both groups were treated with orlistat and Test drug, showed significant effect on reducing the body weight of animal during the treatment period comparing with disease control.

Serum parameters

After the completion of the studies the blood was collected from animals and the serum was separated for measuring various serum parameters and lipid levels. Obesity is the state positive energy balance, mean energy intake exceeds energy expenditure. According to many studies indicating that obesity is related with increased concentration of triglycerides, changes in HDL,LDl and other type of lipid proteins.

Increased levels of Total cholesterol (TC), VLDL, TG and LDL which lead to atherosclerosis. In this study the levels of these parameters are significantly higher in diseases group. The groups (treatment) where treated with the poly

herbal extract and the group which was treated with standard drug (orlistat) significantly reduce these levels. According to many studies benefit of HDL cholesterol was lowering the risk of coronary diseases. This study which underlined the significant effect on increasing the levels of HDL cholesterol which have the positive effect on hypercholesterolemia treatment. Especially in case of patients having altered HDL cholesterol. They were prone to ipoprotein abnormality.

Table 1: Variation in body weight of animals during the study period of 21 days

Normal control	Disease control (high fat diet)	Standard (high fat diet + Orlistat 60mg/kg)	Low dose (high fat diet + 100mg/kg)	Mid dose (high fat diet + 200mg/kg	High dose(high fat diet + 400mg/kg)
137.5±4.425	182.5±2.141	165.333±1.667	173.333±1.745	172.5±2.141	177.5±2.141
172.5±3.354	197.5±3.096	189.163±2.713	185.16±1.537**	181.66±2.108**	182.15±1.537***
171.66±2.472	220.01±9.52	197.51±3.096***	180.02±2.236***	180.166±1.667***	173.33±2.472***
176.66±2.472	240.02±8.165	202.83±3.745***	206.51±1.893***	210.85±2.386***	205.33±2.789***
177.5±2.045	250.83±7.743	192.02±3.765***	180.83±2.088***	171.16±2.400***	169.833±3.497***

Values are expressed as mean ± SEM: n=6.one way ANOVA: p value was found to be <0.0001, considered extremely significant. Tukey-Kramer test: ap<0.001 in comparison with normal control; ***p<0.001 in comparsion with disease control; **p<0.01 in comparison with disease control; *p<0.05 in comparison with disease control

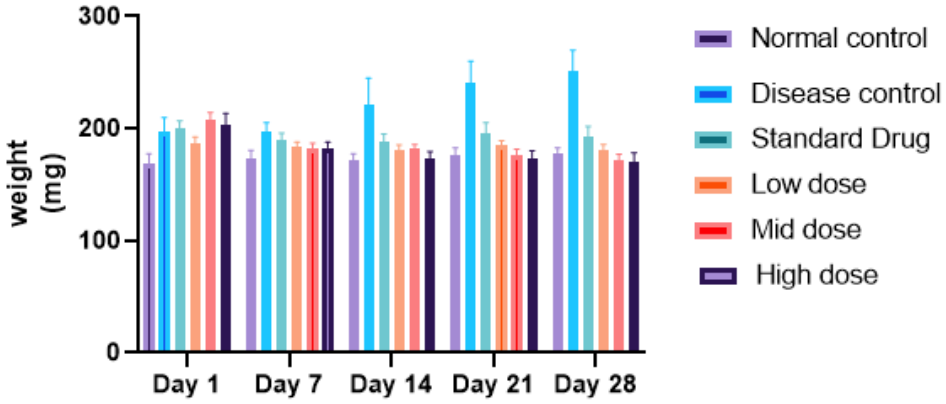


Figure 1: Graphical representation of variation in body weight of animals during the study period of 28 days

Table 2: Effect of alcoholic poly herbal extract on lipid profile of rats

Parameter	Normal control	Disease control (high fat diet)	Standard (high fat diet + Orlistat 60mg/70kg)	Low dose (high fat diet + 100mg/kg)	Mid dose (high fat diet + 200mg/kg)	High dose(high fat diet + 400mg/kg)
HDL (mg/dl)	57.22± 1.155	30.166± 1.195a	41.666± 0.8819***	36.5± 0.7638**	42 ± 0.9661***	48.5± 1.746***
LDL (mg/dl)	13.5± 0.7638	17.667± 0.7149a	12.2833± 0.1905***	14.033± 0.1801***	12.1833 ± 0.2428***	10.6833 ± 0.3772***
VLDL (mg/dl)	12.93± 0.6009	16.766± 0.7145a	11.766± 0.1801***	13± 0.3022***	11.3± 0.1983***	9.8± 0.2933***
Total cholesterol (mg/dl)	55± 0.9309	74.333± 1.202a	37.166± 0.9458***	44.333±1.054***	37± 1.065***	34.33± 1.229***
Triglycerides (mg/dl)	105.833± 0.9458	187.666± 2.290a	104.5± 17.183***	134.166±1. 167***	122.166± 1.740***	86.833± 2.151***

Values are expressed as mean ± SEM: n=6.one way ANOVA: p value was found to be <0.0001, considered extremely significant. Tukey-Kramer test: ap<0.001 in comparison with normal control; ***p<0.001 in comparison with disease control; **p<0.01 in comparison with disease control; *p<0.05 in comparison with disease control

Table 3: Effect of Polyherbal alcohol extract on serum parameters of rat

	Normal control	Disease control (high fat diet)	Standard (high fat diet + Orlistat 60mg/70kg)	Low dose (high fat diet + 100mg/kg)	Mid dose (high fat diet + 200mg/kg)	High dose(high fat diet + 400mg/kg)
SGOT IU/L	1.8333±0.9458	69.166±1.740a	45.02±1.065***	56.33±0.8028***	47.16±0.9458***	39.52±0.7638**
SGPT (IU/L)	51.1666±1.515	77.1656±0.945a	40.512±0.9916***	48.062±1.238	39.512±0.7638***	34.1666±0.9458***

Values are expressed as mean ± SEM: n=6.one way ANOVA: p value was found to be <0.0001, considered extremely significant. Tukey-Kramer test: ap<0.001 in comparison with normal control ;***p<0.001 in comparison with disease control; **p<0.01 in comparison with disease control; *p<0.05 in comparison with disease control.

Histopathology of liver

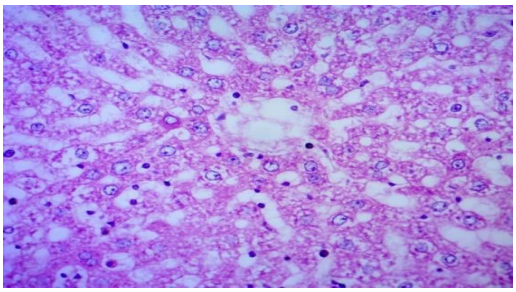


Figure 2: Standard control

The current tissue showed normal hepatic Architecture with normal sinusoidal space

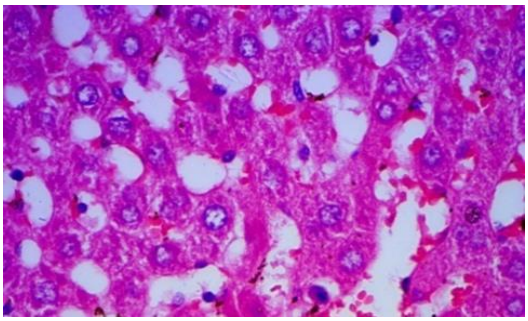
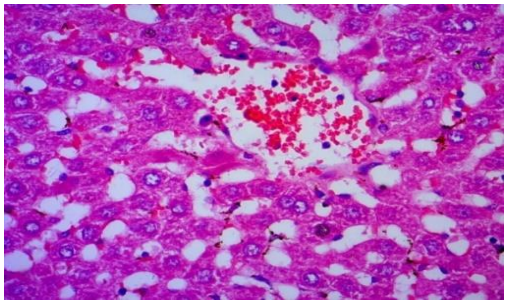


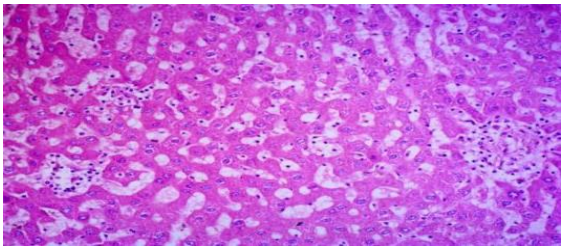
Figure 3: Diseased control

Disease control showed periportal fatty infiltration with focal fat necrosis, lymphocyte infiltration and dilated sinusoids



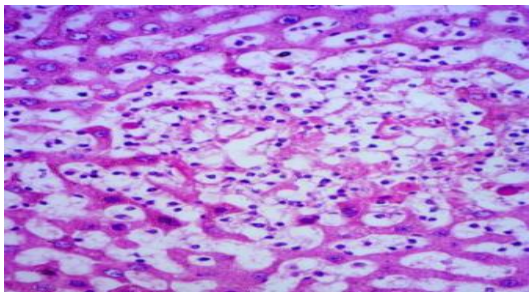
Low dose

The lesional tissue showed for the presence of normal architecture of the liver parenchyma



Mid dose

Fatty infiltration was less and there was no lesions seen in mid dose in comparison to low and high dose.



High dose

The lesional tissues showed for the presence of moderate to severe multifocal necrotic foci with MNC infiltration. Certain areas also showed for the presence of bile duct hyperplasia. Organ toxicity was seen**.**

CONCLUSIONS

The present study revealed that alcoholic poly-herbal extract showed significant in vivo Anti-obesity activity. The treatment group and the standard showed decrease in body weight as com the present study revealed that alcoholic poly-herbal extract showed significant in vivo Anti-obesity activity. The treatment group and the standard showed decrease in body weight as compared to diseased group. There was significant decrease in lipid profile (LDL, VLDL, TG and TC) and serum parameters (SGOT, SGPT) of standard and test drug as compared to the diseased control group. HDL levels of the test drug and standard group showed significant increase as compared to the diseased control.

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