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Ayurveda in managing obesity: A preclinical study

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Abstract
Introduction: Obesity is a disorder with excessive body fat that increases the risk of health problems. There is a vast prevalence of obesity in both developed as well as developing countries, and now it is affecting children as well. The World Health Organization reported that more than 1 billion adults are overweight, and at least 300 million of them are obese. India hits by the number of more than 135 million. Obesity is associated with other serious complications, making its management of utmost importance. However, current management with allopathy and surgeries are associated with various adverse effects. Hence, we conducted this study for evaluating the safety and efficacy of herbal drugs in managing obesity.
Method: In the present study, an herbal formulation was evaluated for anti-obesity activity in Wistar rats (n=36). The anti-obesity potential of this formulation was studied on a high-fat diet (HFD)-induced obesity in male Wistar rats. In this study, chronic administration of HFD in rats produced hypercholesterolemia which led to an increase in the body weight, total cholesterol, triglycerides, and attenuation in the levels of HDL as well as changes in body temperature of animals. Then our formulation was tested for its efficacy to bring these values to normal.
Results: Our formulation was able to bring down these values towards normal range which was comparable with marketed formulations.
Conclusion: Hence, we can conclude that our polyherbal mixture possesses anti-obesity activity in preclinical study.

Keywords: obesity, preclinical evaluation, efficacy, herbal formulation, high fat diet induced model

Introduction

The human body is made up of water, fat, protein, carbohydrate, and lots of minerals as well as vitamin [1]. The people having too much fat, particularly around the waist, they are considered obese. They are at high risk of health problems related to heart disease, diabetes, certain cancers, sleep apnoea. Also, obesity affects human psychology, and leads to loss of self-confidence, physical fitness, and cause laziness. It is also a causative agent in many diseases like hypertension, cardiovascular disease, diabetes mellitus. etc. Obesity is not contained in any specific country and it is broadly spread all over the world, hence it is even hilted by WHO. Obesity is defined as body mass index (BMI), a measure of weight adjusted for height. BMI is a worldwide accepted tool for the population-level measure of obesity [2].

Table 1: Ideal BMI range

BMI range	Consideration
BMI<25 kg/m^2	Normal
BMI>25 kg/m^2	Overweight
BMI>30 kg/m^2	Obese
BMI>40 kg/m^2	Severe obesity

Problems associated with allopathic medicines and bariatric surgery:

Various management choices existing to treat obesity disorder. Some anti-obesity medications can have severe and even lethal side effects, fen-phen being a famous example. FDA reported that Fen-phen can cause heart valve problems, abnormal echocardiograms, and rare valvular diseases [3]. The commonly observed side effects of orlistat are gastrointestinal and include diarrhea, fecal incontinence, oily spotting, flatulence, bloating, and dyspepsia [4]. As per a result of the adverse effects, orlistat may not be sound tolerated.

All surgical procedures carry risks through Bariatric surgery is the most successful treatment option for weight loss. However, Bariatric surgery also carries nearly long-term risks for patients, such as Dumping syndrome (a condition that can lead to symptoms like nausea and dizziness), bowel obstruction, malnutrition, hypoglycemia, ulcers, vomiting, hernias [5]. It also has some side effects like acid reflux, chronic nausea as well as vomiting, inability to eat certain foods, obstruction of the stomach, etc [6].

As per the literature review, it has been found that *Garcinia, Coffea arabica, Commiphora Mukul, Chebulic myrobalan, Cyperus rotundus, Terminalia Chebula* has anti-obesity and anti-hyperlipidaemic action when tested individually in rats. They also possess potential therapeutic action to improve fat metabolism by acting on leptin [7]. The results of some studies show that each of the individual administrations of *Coffea arabica* significantly decreases the body weight and serum lipids including total lipid, triglycerides, and low-density lipoproteins when compared with the positive control group, while it significantly increases high-density lipoproteins when compared with positive control groups [8]. Various *in vivo* studies have contributed to the understanding of the anti-obesity effects of garcinia via regulation of serotonin level and glucose uptake [9]. Besides it also helps to enhance fat oxidation while reducing de novo lipogenesis. The results demonstrated that exudates of *Commiphora Mukul* possess significantly higher anti-hyperlipidemic activities compared with other resinoid [10].

Hence, considering the multiples aspects of actions of these herbs and need of complete solutions for obesity, the said study has been planned to evaluate the potential of test herbs in combination with other ingredients and excipients in the formulations. Here, we expect the synergistic and multiple actions in a single formulation and reduce the dose.

Materials and Methods

Chemicals and reagents

Orlistat was purchased from Ambika Medical Pimpri. Fat GO Capsule and Garcinia Tablet from Manikarnika Aushadhalaya Medical supply store, Pimpri-Chinchwad. Test product- PR/HC/1718/003 provided by Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune. Solvents like methanol, acetone, ketamine were all of the analytical grade (AR).

Serum cholesterol kit, serum triglyceride kit, serum HDL cholesterol kit, serum LDL-cholesterol kit was provided by Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune.

Animals

Male Wistar rats (180-230g) obtained from the National Institute of Biosciences, Pune were used in experiments. The animals had free access to standard food and water and were kept in separate rooms at 22±2°C with 55–65% humidity with a 12h light/ dark cycle. The animals were maintained in cages with raised wide mesh floors to prevent coprophagy.(Dauchy RT, *et al*, 2010) The experimental protocol (DYPIPSR/IAEC/18-19/P-32) was submitted to and approved by the Institutional Animal Ethics Committee.

Animals groups and methodology

The study comprised of 6 groups with 6 animals in each group, as provided in table no. 2.

Table 2: Groups of animal experiment

Group	Treatment	No. of animals
Group 1	Normal Control (0.5ml saline *p.o.*)	6
Group 2	Disease control (High fat diet + saline 0.1ml *p.o.*)	6
Group 3	Orlistat (HFD + Orlistat (37.0 mg/kg, *p*.o.)	6
Group 4	Garcinia Tablet (HFD+ Garcinia 123mg/kg, *p*.o.)	6
Group 5	Fat Go Capsule (HFD+ Fat Go 123mg/kg, *p*.o.)	6
Group 6	Product- PR/HC/1718/003 (HFD+ Product 72mg/kg, *p*.o.)	6
Total		36

The marketed formulation was suspended in 0.5% carboxymethyl cellulose (CMC) and administered orally using an oral gavage needle at the volume corresponding to 1 ml/100g body weight. The animals were administered freshly prepared drug solutions from the second week of HDF for 28 days. Various parameters like cholesterol (TC), high-density lipoproteins (HDL-C), triglycerides (TG), low-density lipoproteins (LDL-C) were recorded. The change in body weight of animals in all groups was also measured. At the end of the protocol, animals were sacrificed by cervical dislocation and liver, heart as well as kidney were removed surgically and were sent for histopathological evaluations.

High Fat Diet Inducer

High-fat diet (HFD) induced obesity in rats is considered to be a reliable tool for the evaluation of the anti-obesity activity. The HFD consist following combination given on 3 consecutive days for a total period of 28 days:

Table 3: High Fat Diet

Days: 0- 7th day	Animals on Diet/ Quarantine
8th day	Diet A: Condensed Milk (8g) + Bread (8 g)
9th day	Diet B: Chocolate (3 g) + Biscuits (6 g) + Dried Coconut (6 g)
10th day	Diet C: Cheese (8 g) + Boiled Potato (10 g)
Respectively repeated for 28 days	

Measurement of Calorie intake

The food intake of each animal was determined initially and then every week subsequently by computing the difference between the pre-weighed chows and the weight of the food that leftover after 24 hours, and the results were indicated as a mean energy intake for a group of six rats in kcal/week. The calculation of the daily caloric (kcal) intake was based on food intake separately multiplied by their caloric content.

Bodyweight

As animals divided into 12 groups (n=6) weight of each animal is taken on the first day of study with marking on the body using picric acid and during study daily body weight is taken after that sacrificing animal for histopathological evaluation.

Dose preparation

In the present study, 4 doses of Orlistat (37.0 mg/kg, *p*.o), Garcinia 123mg/kg, *p*.o, Fat Go 123mg/kg, *p*.o, Test Product 72mg/kg, *p*.o were selected. These doses were selected based on previous reports.

High-Fat Diet Formula

HFD that consists of 58% fat, 25% protein and 17% carbohydrate, lard (13%), cholesterol (1%), vitamin, and minerals (0.6%) as a percentage of total kcal ad libitum, respectively, was administered every day. Food intake was calculated every day and body weight was measured every alternative day.

Biochemical parameter

On the 42nd day, the experimental animals were kept on fasting, and on the 43rd day the experimental animals were anesthetized by ketamine, and blood was collected by retro-orbital puncture. The blood sample was allowed to clot for 45min and subjected to centrifugation at 4000rpm at 4˚C for15 min to obtain the serum. The serum sample was stored at -80˚C and used for the estimation of various biochemical parameters. (Pathozyme diagnostic kit) (Zhao M, *et al*, 2010)

Histopathology

All the preserved Liver, Kidneys, and Heart from all the groups were processed routinely and embedded in paraffin. The sections of 3-5 µ thickness were cut and stained with hematoxylin-eosin stain. Histopathology examination of all the organs was carried out by board-certified Toxicopathologist.

Results

1. Calorie Intake

Effect of Polyherbal Mixture on Calorie Intake

Calorie intake was found to be significantly increased in cafeteria diet control group (3177.483±210.428) as

compared to the normal control group (2063.93±43.087) while calorie intake is significantly decreased at PRHC (2279.05±321.14) as compared with cafeteria diet control and Orlistat control groups (1556.42±295.27). Garcinia (3062.143±235.842) showed no significant change as compared Orlistat control group.

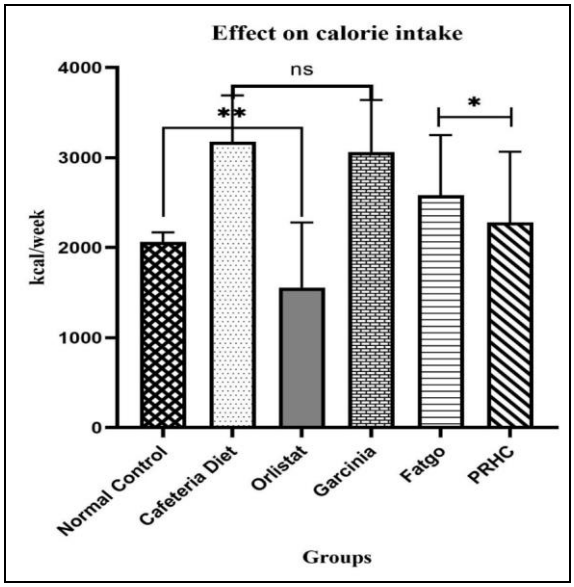


Fig 1: Effects on calorie intake

Data was expressed as Mean ± SEM, N=6, **P<0.0001, as compared to normal, *P<0.0001and ns as compared with cafeteria diet and orlistat by using One-way ANOVA followed by Sidak's multiple comparison Test.

2. Bodyweight

Effect of Polyherbal Mixture on Body Weight:

The body weight was found to be significant increases in all five groups as compared to normal control as it is fed with a cafeteria diet. On the 42nd day significant increase in the CD group (300±32.52) as compared to the normal control group (210±5.20). The PRHC showed a significant decrease in body weight as compared with the cafeteria diet.

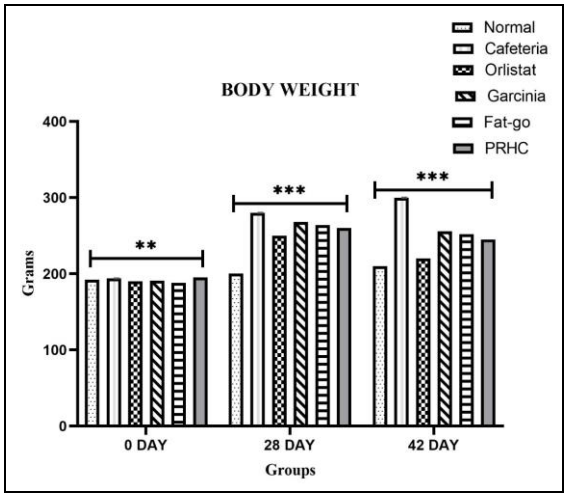


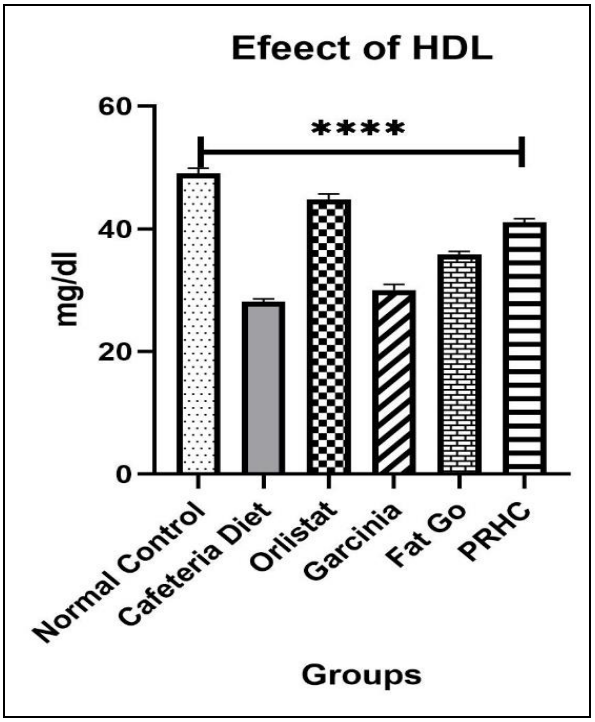
Fig 2: Effects on Body Weight

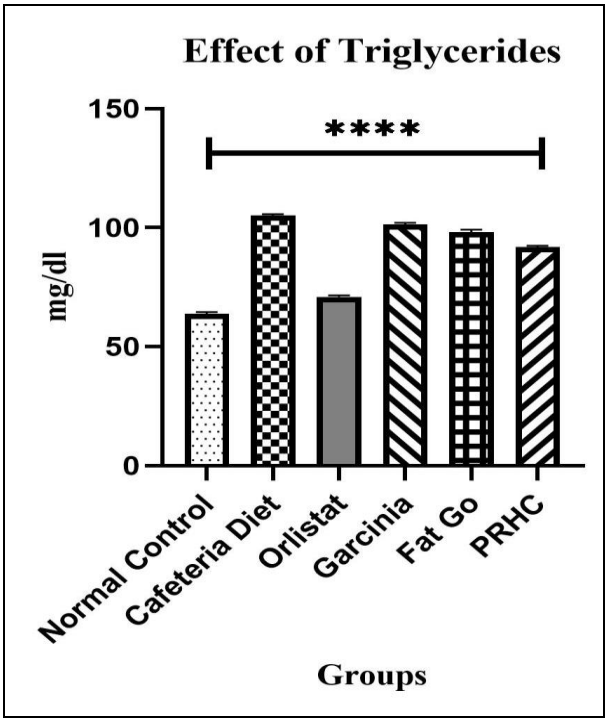
Data was expressed as Mean ± SEM, N=6, **P<0.0003, as compared to normal, ***P<0.0001 as compared with cafeteria diet and orlistat by using One-way ANOVA followed by Sidak's multiple comparison Test.

1. Biochemical Parameter

Effect of Polyherbal Mixture on Lipid profile parameters:

Cholesterol, triglycerides, and LDL level were found to be significantly increased in the CD control group (121.58±,105.02±0.21,55.41±0.17) as compared with the normal control group (65.44±0.58, 63.83±0.30, 35.76±0.20). Test doses PRHC (87.9±0.24, 91.85±0.25, 43.08±0.20) showed a significant decrease in the level of all three lipids as compared with CD control (121.58±,105.02±0.21,55.41±0.17) and positive control group.





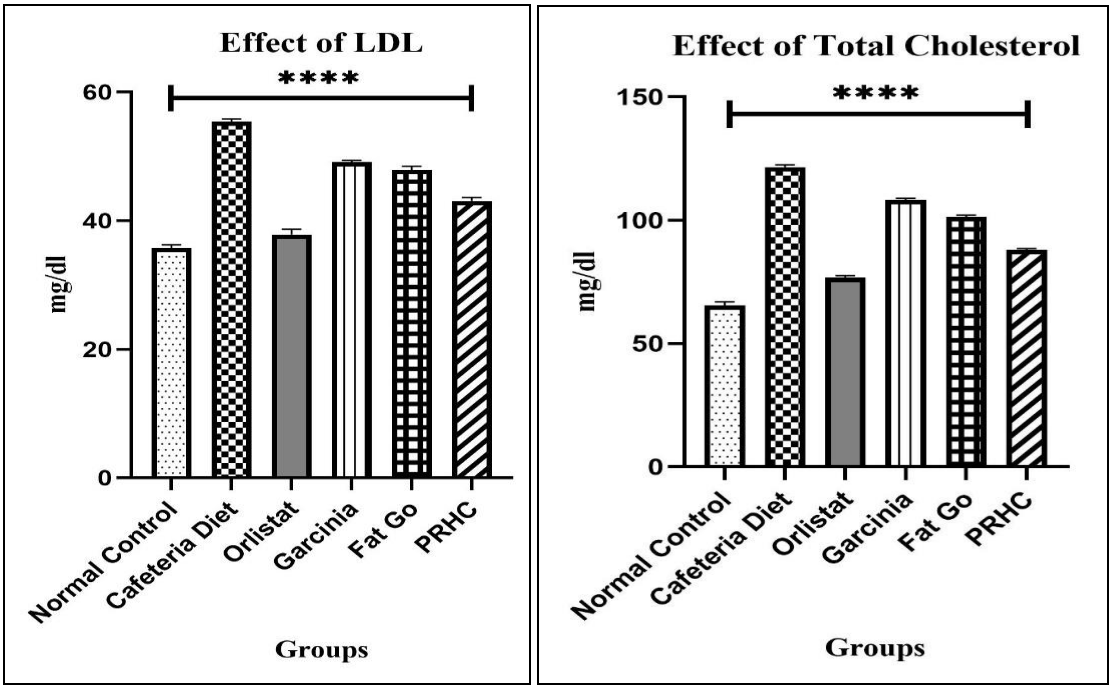


Fig 3

On the contrary HDL level was found to be significantly decreased in the CD control group (28.15±0.18) as compared to the normal control group (49.01±0.37). Test doses PRHC (41.08±0.23) showed a significant increase in HDL level as compared to CD control and the Orlistat control group (44.75±0.37).

Data was expressed as Mean ± SEM, N=6, **P<0.0001, as compared to normal, *P<0.0001and ns as compared with cafeteria diet and orlistat by using Two-way ANOVA followed by Sidak's multiple comparison Test.

Table 4: GP: Group, HFD: High-Fat diet, TG: Triglycerides, TC: Total Cholesterol, LDL: Low-Density Lipid, HDL: High-Density Lipid

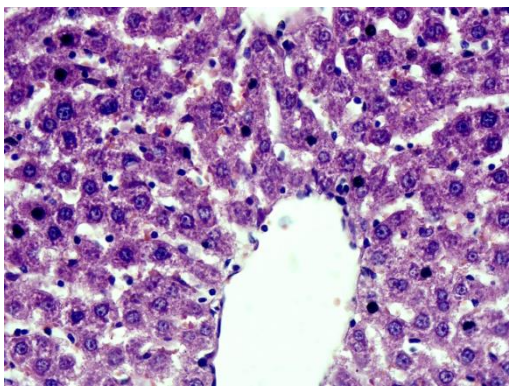
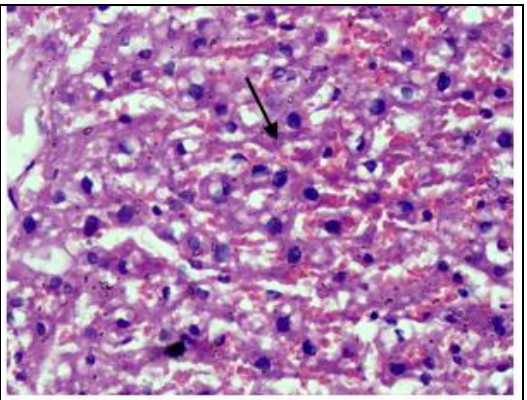
Sr.no	Treatment	TG (mg/dl)	TC (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
GP I	Normal Control (0.5ml saline) *p.o*	63.83±0.30	65.44±0.58	35.76±0.20	49.01±0.37
GP II	Disease control (High fat diet + saline 0.1ml p.o	105.2±0.21	121.58±0.35	55.41±0.17	28.15±0.18
GP III	Orlistat (HFD +Orlistat (37.0 mg/kg, *p*.o)	70.98±0.23	76.85±0.27	37.85±0.33	44.75±0.37
GP IV	Garcinia Tablet (HFD+ Garcinia 123mg/kg, *p*.o)	101.26±0.34	108.44±0.18	49.1±0.11	30.01±0.37
GP V	Fat Go Capsule (HFD+ Fat Go 123mg/kg, *p*.o)	98.23±0.38	101.38±0.29	47.9±0.23	35.8±0.22
GP VI	Product- PR/HC/1718/003(HFD+ Product 72mg/kg, *p*.o)	91.85±0.25	87.95±0.24	43.08±0.20	41.08±0.23

*Comparison of test and disease control with normal control, #Comparison test with the disease control

2. Histopathological Evaluation

The microscopic examination of kidneys and heart from all the groups did not show any abnormality of pathological significance. Microscopic examination of the liver from disease control animal showed hepatocellular vacuolation when compared with the control group. However, no such changes were recorded in other groups when compared with the control group animal.

Table 5: Images: Individual Animal- Liver

	
G1 (Normal Control); Liver; Showing normal centrilobular area and hepatic parenchyma. 40 X, H & E	**G2 (Disease Control): Liver;** Showing hepatocellular vacuolation (arrow). 40 X, H & E

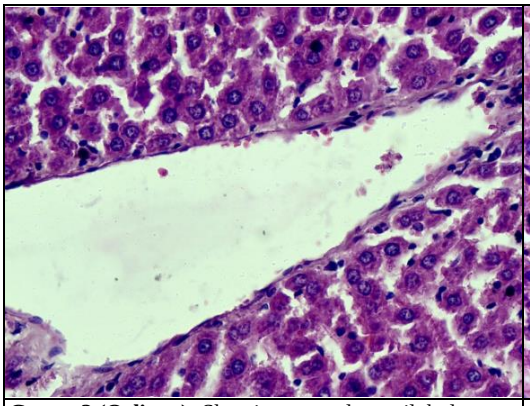
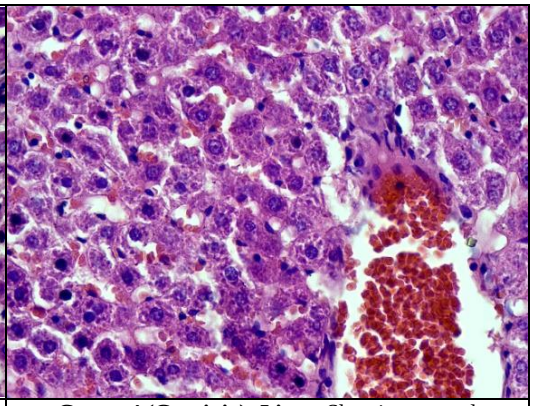
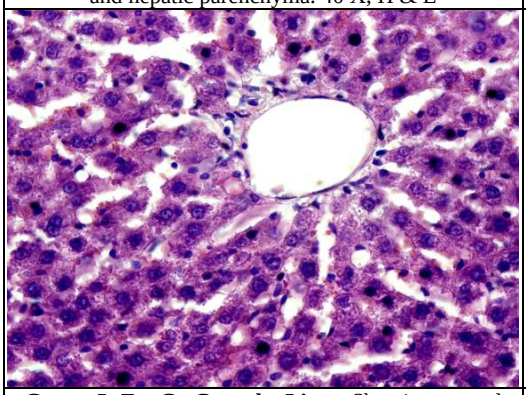
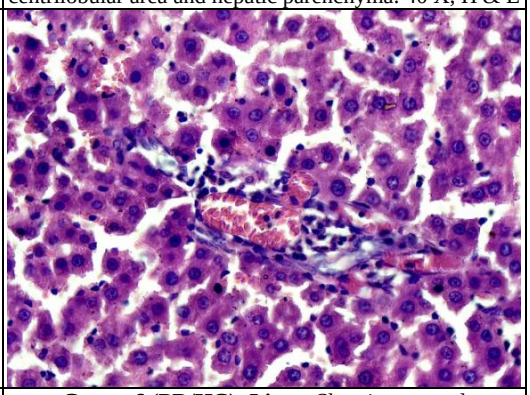
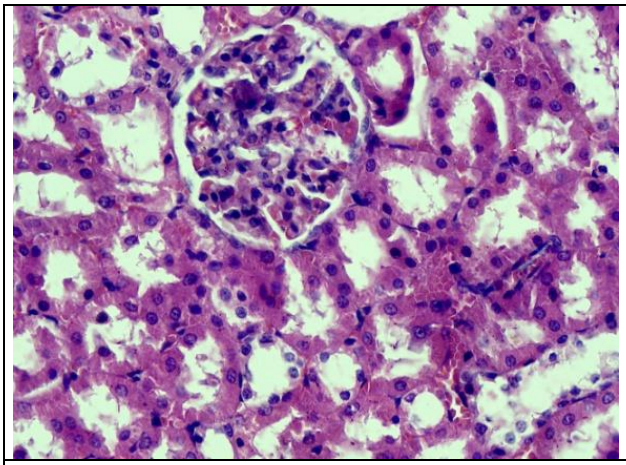
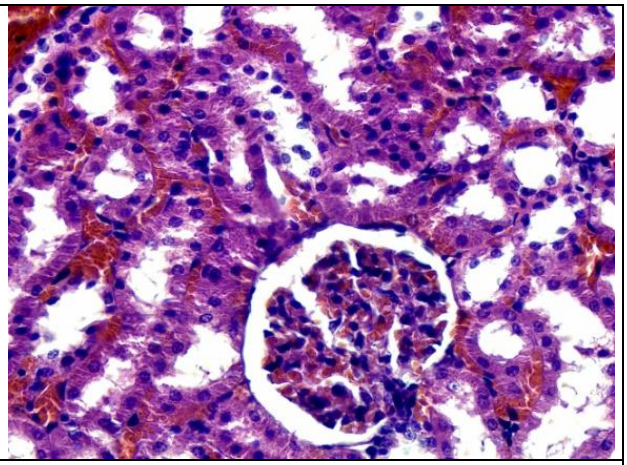
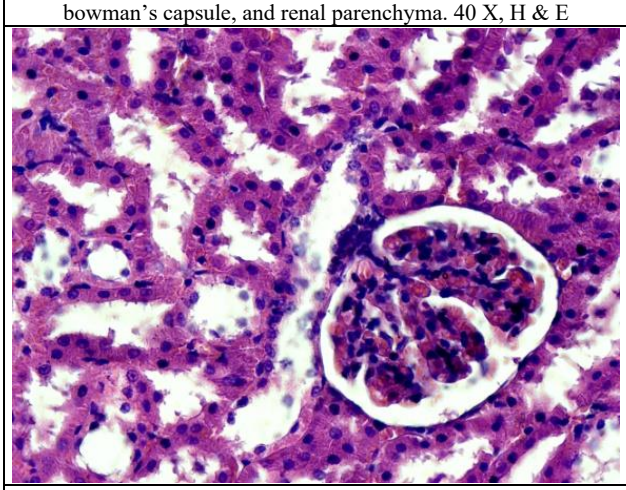
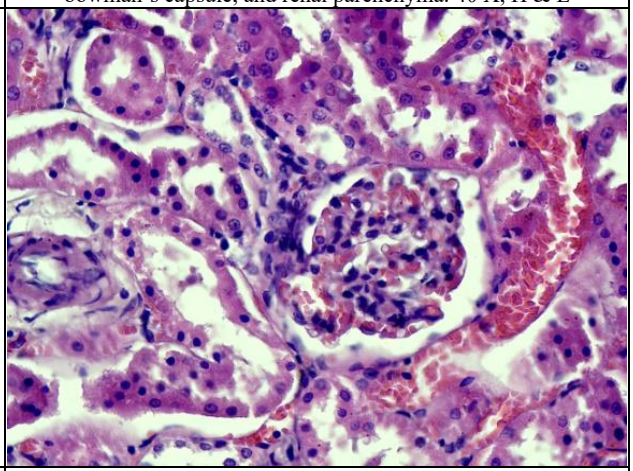
	
Group-3 (Orlistat); Showing normal centrilobular area and hepatic parenchyma. 40 X, H & E	**Group-4 (Garcinia): Liver;** Showing normal centrilobular area and hepatic parenchyma. 40 X, H & E
	
Group-5: Fat Go Capsule: Liver; Showing normal centrilobular area and hepatic parenchyma. 40 X, H & E	**Group-6 (PR/HC): Liver;** Showing normal centrilobular area and hepatic parenchyma. 40 X, H & E

Table 6: Images: Individual Animal-Kidneys

	
G1 (Normal Control); Kidneys; Showing normal renal tubules, bowman's capsule, and renal parenchyma. 40 X, H & E	**G2 (Disease Control): Kidneys;** Showing normal renal tubules, bowman's capsule, and renal parenchyma. 40 X, H & E
	
Group-3 (Orlistat); Kidneys; Showing normal renal tubules, bowman's capsule, and renal parenchyma. 40 X, H & E	**Group-4 (Garcinia): Kidneys;** Showing normal renal tubules, bowman's capsule, and renal parenchyma. 40 X, H & E

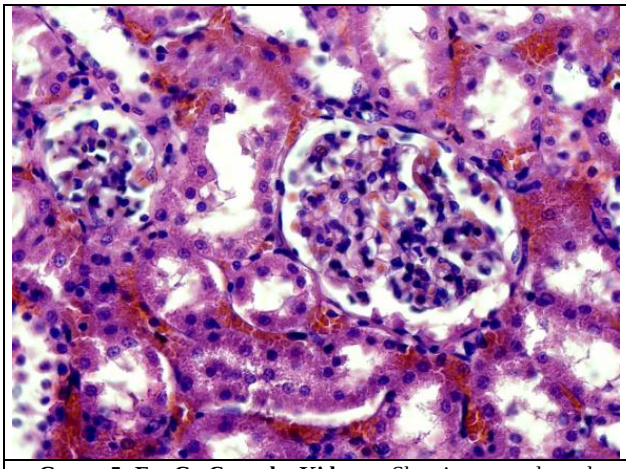
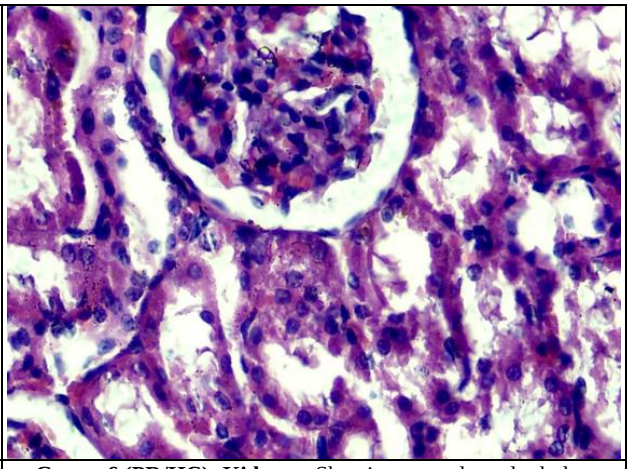
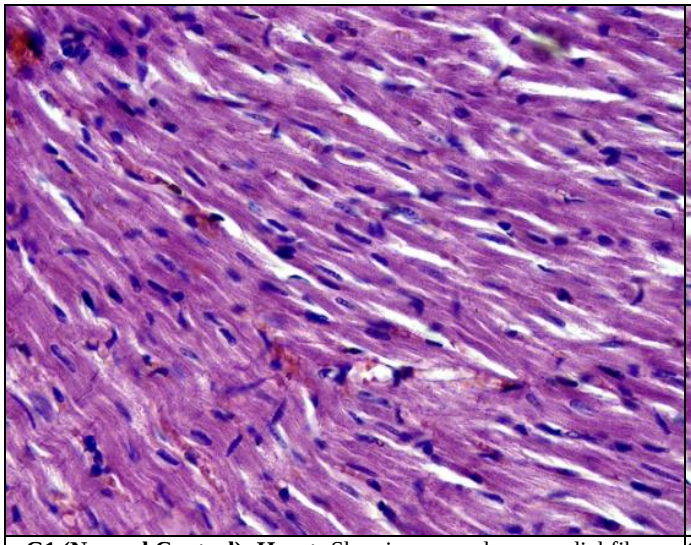
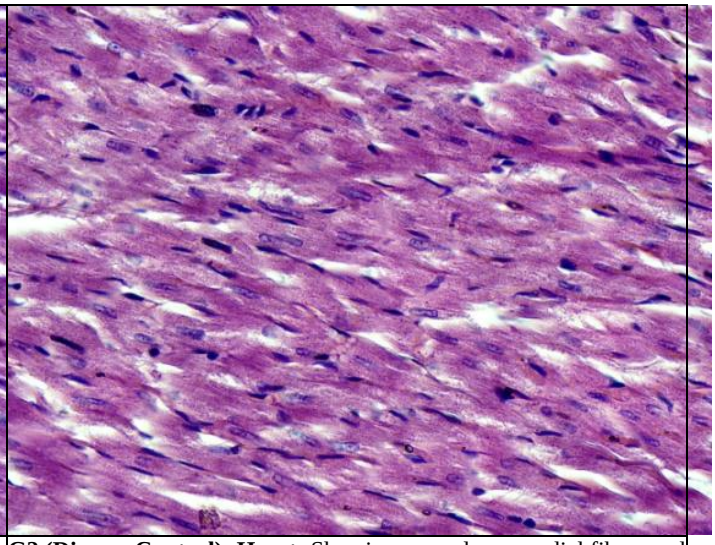
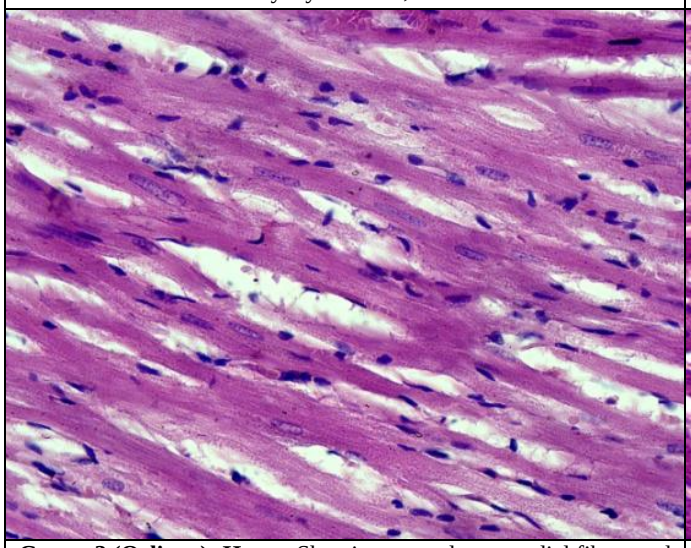
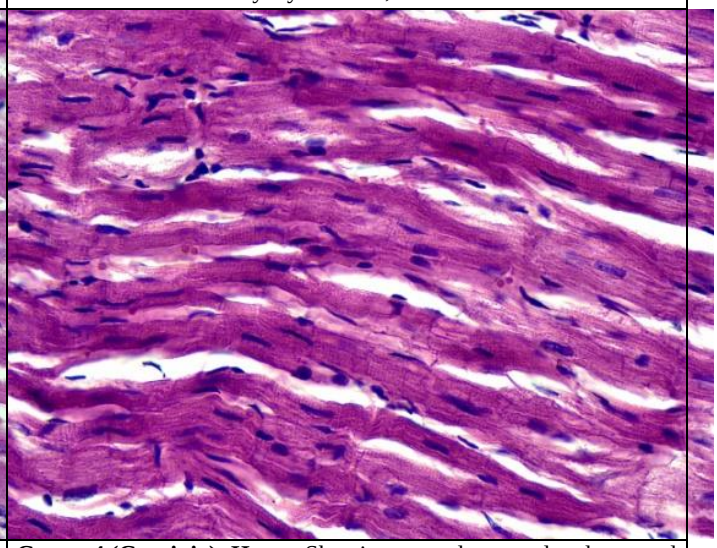
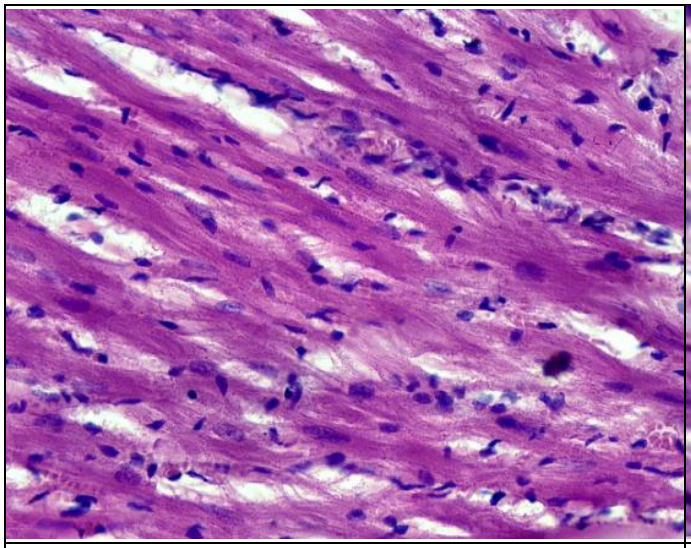
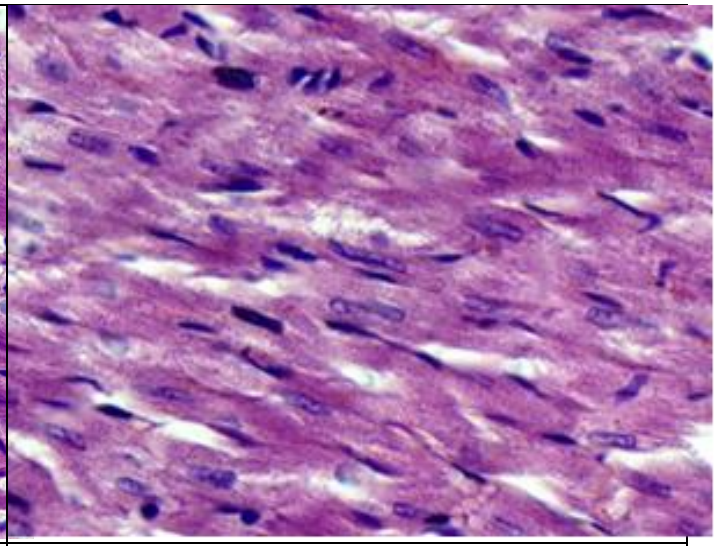
	
Group-5: Fat Go Capsule: Kidneys; Showing normal renal tubules, bowman's capsule, and renal parenchyma. 40 X, H & E	**Group-6 (PR/HC): Kidneys;** Showing normal renal tubules, bowman's capsule, and renal parenchyma. 40 X, H & E

Table 7: Images: Individual Animal- Heart

	
G1 (Normal Control); Heart; Showing normal myocardial fibers and myocytes. 40 X, H & E	**G2 (Disease Control): Heart;** Showing normal myocardial fibers and myocytes. 40 X, H & E
	
Group-3 (Orlistat); Heart; Showing normal myocardial fibers and myocytes. 40 X, H & E	**Group-4 (Garcinia): Heart;** Showing normal mucosal and mucosal glands. 40 X, H & E



Group-5: Fat Go Capsule; Heart; Showing normal myocardial fibers and myocytes. 40 X, H & E



Group-6 (PR/HC): Heart; Showing normal myocardial fibers and myocytes. 40 X, H & E

Based on histopathology findings, it can be concluded Kidneys and Heart did not show any effect due to the treatment of test items. The liver of disease control animal showed hepatocellular vacuolation however it was not noted in other treatment groups suggestive of ameliorating the effect of treatment.

Conclusion

The study evaluated the activity of polyherbal mixture in cafeteria diet-induced obesity in rats. Our test product (PRHC) revealed significant anti-obesity activity as compared to polyherbal standards. In a nutshell, our test polyherbal formulation consisting of a combination of *Garcinia, Coffea arabica, Commiphora Mukul, Chebulic myrobalan, Cyperus rotundus, Terminalia Chebula* was found to possess anti-obesity activity in experimental animals.

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Conflict of Interest

The authors confirm that there is no conflict of interest with respect to the current manuscript.

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