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Investigation of Effect of Polyherbal Mixtures on Cafeteria Diet Induced Obesity in Wistar rats

Komal V Umbarkar, Vaishali R Undalea*, Abhijeet S Kulkarni, Suchita Gupta, Sagar S Jadhav and Roshani R Pagar

Department of Pharmacology, Dr. DY Patil Institute of Pharmaceutical Sciences & Research, India

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***Corresponding author:** Vaishali R Undalea, Department of Pharmacology, Dr. DY Patil Institute of Pharmaceutical Sciences & Research, India

Abstract

Background: To evaluate the anti-obesity activity of polyherbal mixture of selected herbs in cafeteria diet induced obesity in Wistar rats.

Method: Hydro alcoholic extracts of herbal plant *Annona muricatta*, *Momordica charantia*, *Vaccinium uliginosum* were purchased and authenticated from Green Heaven India. The Acute Oral Toxicity study of polyherbal mixture was performed according to OECD guideline 423.The antiobesity effect of polyherbal mixture was evaluated in cafeteria diet (CD) induced obesity. The polyherbal mixture (PHM) at two different dose level (200 mg/kg and400 mg/kg BW) was administered orally for a period of 35 days and various parameters of anti-obesity were estimated.

Result: The half maximal inhibitory concentration (IC50) values were estimated for combination of PHM in *in vitro* for antilipase activity. Oral administration of PHM for 35 days significantly decreases in calorie intake, bodyweight, cholesterol level and fasting glucose level. It showed that there is significant increase in parameters of liver function, BMI and Nasoanal length and level of white adipose tissue.

Conclusion: The dose of 200 mg/kg and 400 mg/kg of PHM significantly reduced body weight and calorie intake indicating its anti-obesity effect. The mixture of PHM at both doses effectively improved serum lipids level and decreased serum blood glucose level suggesting its effect on carbohydrate and lipid metabolism. The decreased AST and ALT level signifies that the PHM do not interferes in liver function. The histopathological studies of liver tissue from PHM treated animals showed improvement in fatty liver and hepato-steatosis as compared to control group. From all above findings it is concluded that the PHM produces significant antiobesity effect in CFD induced obesity in Wistar rats at doses 200 & 400 mg/kg.

Keywords: Obesity; Cafeteria diet; Acute oral toxicity; Hepato-steatosis; Antilipase activity

Abbreviations: CD: Cafeteria Diet; PHM: Polyherbal Mixture; IC50: Half Maximal Inhibitory Concentration; NAFLD: Non- Alcoholic Fatty Liver Disease; IEAC: Institutional Animal Ethics Committee; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; CMC: Carboxy Methyl Cellulose; WAT: White Adipose Tissue; SEM; Standard Error of the Mean; BT: Bonferroni Test; P: Probability

Introduction

The major cause of morbidity and mortality is obesity worldwide in the 21st Century [1]. Over the last 3 decades the morbidity rate of obesity has tripled worldwide. About 1.4 billion of people suffering from obesity and among them 200millions of men and 300millions of women were overweight [2]. In India, first four positions occupied in obesity states like Punjab, Kerala, Goa and Tamil Nadu (35%, 30%, 25% and 20%) [3]. The worldwide increase in obesity is because of changes in eating patterns and the consumption of high caloric diet. Stress, combined with overindulges and lethargy, can lead to obesity and this condition increases the frequency of most deadly diseases including diabetes mellitus (Type II), high blood pressure, coronary heart diseases, osteoarthritis, obstructive sleep disorders, non- alcoholic fatty liver disease (NAFLD) and certain cancer [1]. The occurrences of all these co morbidities are on rise in developed and developing countries stinging beyond the age and sex. Obesity builds up when there is irregular development of adipose tissue due to increase in fat cell size or fat cell number or combination thereof.. This is happened because of imbalance between energy input and energy output.

The unparallel evolution has recently experienced in drug therapy of obesity. However, the considerable hurdles that erode ever-lasting obesity management approach involve absence of individual obesity discipline of health expertise, mind-sets, faith, along with report age with accessibility of antiobesity drugs. Presently, existing condition of souk for anti-obesity drugs is developing fast although very less drugs are clinically available in proportion to their need.

Hence, there is need to investigate new anti-obesity drug to solve the obesity troubles [3]. Natural products/ dietary photochemical have instigated extensive scrutiny in recent years as prospective therapeutics to restrain obesity. These composites employ the anti-obesity potential primarily via harmonization of numerous mechanisms, along with increasing lipolysis, intake and output of energy lipid absorption and decrease lipogenesis, and distinction and escalation of preadipocytes.

Multifarious bioactive constituent's saponins, glycosides and flavonoid (apigenin, luteolin, Kaempferol, myricetin, quercetin, genistein) from nature are potentially used in obesity treatments. Most Ayurvedic preparations are polyherbal in nature to take care of the various constituents of disease conditions [4]. Graviola (*Annona muricatta*, family: Annonaceae), Bitter melon (*Momordica charantia*, family: Cucurbitaceae) [5], Bog blueberry (*Vaccinium uliginosum*, family: Ericaceae) are widely distributed in India. According to the chemical constituents like Flavonoid, polyphenols, glycosides, antho-cyanine, saponins etc. present in the plants and based on traditional claim the polyherbal mixture reduces the obesity and lessen their co morbidities by acting on different pathways.

However, the mixture of these herbs has not been scrutinized so far for its antiobesity potential. Hence, this study objective was to evaluate *in-vitro* and *in-vivo* anti-obesity effect of polyherbal mixture (Graviola: -*Annona muricatta* - family: Annonaceae, Bitter Melon: - *Momordica charantia* – family: Cucurbitaceae and Blueberry: -*Vaccinium uliginosum* – Family: Ericaceae) on cafeteria diet induced obesity in Wistar rat model. The various experimental studies conducted in cafeteria diet or high fat diet induced obesity in rat have illustrated those diets high in fat and high in calorie are known to elevate body weight and fat mass, produce alterations in metabolism of carbohydrate and lipid, results to insulin impedance, production and release of leptin increases and the role of lipase in humans, rodents, and other animals also get increased.

Materials and Methods

Plant extract

The herbal plant hydro alcoholic extracts were purchased and authenticated separately from Green Heaven India (An Herbal Manufacturing Unit), Nagpur, Maharashtra, India. (Batch no. GHCO1017, FG/BMG/130417/1, TRG170214A).

Phytochemical estimation

The phytochemical estimation was performed separately for each extract for various phytoconstituents [6].

Anti-lipase assay

Extraction and purification of lipase enzyme: The lipase enzyme was extracted from peanut seeds. 20gm of seeds was germinated for 24hrs and remove the seed coats. The seeds were homogenized with chilled acetone to obtain a suspension, centrifuge at 3000rpm for 15min then the residue dissolved in water and again centrifuge at 7500rpm for 20min. The supernatant was used as source of crude enzyme. Obtained supernatant was partially purified by precipitated with ammonium sulphate then centrifuged at 10000rpm for 20min, the precipitated was dissolved in tris buffer (pH 8.5) followed by dialysis for overnight in same buffer [7].

***In-vitro* anti-lipase assay:** Lipase assay was performed by using olive oil as a substrate. Prepared different concentration of standard and samples starting from 5mg/ml up to 50mg/ml. take 100µl from each concentration and mixed with olive oil emulsion (18ml distilled water+ 5ml olive oil+ 0.2gm sodium benzoate + 2.5gm gum acacia), 0.4ml phosphate buffer and 1ml freshly prepared seed lipase. This mixture was incubated for 60min at 30°C then add 10ml acetone and 95% ethanol mixture (1:1) to stop the reaction. By using phenolphthalein as an indicator, and titrating the solution against 0.02M NaOH, the free fatty acids were determined (standardized by 0.01M oxalic acid). The following formula used to calculate the percent inhibition of lipase:

$$\text{Percent Lipase inhibition} = X - Y/X \times 100$$

Where, X-Activity of lipase, Y-Lipase activity when the extract gets incubated [7]. IC50 was calculated by using graph plot and from equation-y=mx+c.

Experimental method

The experimental protocol was approved (Protocol no. DYPIPSR/IAEC/17-18/P-07) by the Institutional Animal Ethics Committee (IEAC) formed by the norms of The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute oral toxicity: The AOT study was accomplished according to OECD guideline 423. Three healthy female Wistar albino rats (150-180g) were taken for AOT study. The animals were fasted for 12 hours before the administration of test compounds, while the animals had free water access and food during whole of the experiment. The polyherbal mixture was prepared by mixing the selected herb in 1:1:1 to 2000 mg/kg. The mixture was dangling in 0.1% Carboxy methyl cellulose (CMC) and given orally at single doses of 2000 mg/kg. The normal behavior of the rats was continuously monitored for 1hr after dosing during first 24 hrs. periodically. (The first 4 hrs. special attention is needed) and then daily for a total of the 14 days. Variation in the normal

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activity, sign and symptoms of toxicity and mortality of rats, were observed and recorded.

Cafeteria diet induced obesity: The cafeteria diet included administration of following components on consecutive days:

Day 1: Bread (8 g) + Condensed Milk (8g).

Day 2: Biscuits (6 g) + Dried Coconut (6 g) + Chocolate (3 g)

Day 3: Boiled Potato (10 g) + Cheese (8 g).

The diet mentioned above was given to each rat individually on days first, second, third, respectively, and for 42 days repeated the diet in the same manner.

Except Group I the animals were administered with cafeteria diet and treated with standard or test drugs for the evaluation of antiobesity activity as follows.

a) Group I (Normal Control): Rats fed with rodent chow diet and administered with saline solution as per body weight (BW)for the period of 42 days.

b) Group II (Negative Control): Rats fed with CD for the period of 42 days).

c) Group III (Positive control): Rats fed with CD for the period of 42 days + from 8thday, treated with Orlistat (45mg/kg, BW per orally) for period 35 days (Positive Control).

d) Group IV (Test Group I): Rats fed with CD for the period of 42 days + from 8th day, treated with polyherbal mixture (100mg/kg, BW per orally) for period 35 days.

e) Group V (Test Group 2): Rats fed with CD for the period of 42 days + from 8th day, treated with polyherbal mixture (200mg/kg, BW per orally) for period 35 days.

f) Group VI (Test Group 3): Rats fed with CD for the period of 42 days + from 8th day, treated with polyherbal mixture (400mg/kg, BWper orally) for period 35 days.

All the drugs were administered by oral gavage once a day. For each animal food intake was decided initially and then every week. The intake of food and water was measured for each cage and the daily caloric (kcal) intake was calculated depends on food consumed individually by multiplying with their caloric content. When the experimental period was ended (on 43rd day), by using ether the animals were anesthetized, ensuing overnight fasting. For the evaluation of biochemical parameters by retro-orbital method blood was withdrawn and collected and by centrifugation the serum was obtained. Rats were sacrificed; after collection of blood, all organs and other tissues like perirenal, epididymal and mesenteric white adipose tissue (WAT), were removed and then immediately rinsed with distilled water and weighed. For further biochemical and histopathological estimation the serum and liver samples were stored at -70°C.

Statistical analysis

Data is expressed as the mean±standard error of the mean (SEM). The statistical data was calculated by analysis of variance either by One way or two-way ANOVA followed by Bonferroni test (BT) using updated software Graph pad prism - 7. Values of Probability (P) is less than 0.05 were considered as significant.

Results

Antilipase assay

Acute oral toxicity study

In the preliminary acute toxicity study, polyherbal mixture seems to be Safe at 2000 mg/kg. No toxic or deleterious effects were observed immediately in 24 hours or during 48 hours and up to 14 days of observation period. There was no mortality found in any animal (Table 1).

Table 1: IC50 values of Orlistat, *Annona muricatta*, *Momordica charantia*, *Vaccinium uliginosum* and Combination of AM+MC+VU.

Sr. No.	Name of Drug	IC50	% Inhibition
1	Annona muricatta	25.48477886	57.44%
2	*Momordica charantia*	29.40350877	56.23%
3	*Vaccinium uliginosum*	20.58289537	51.57%
4	Orlistat	16.59397715	63.36%
5	Combination of AM+MC+VU (1:1:1)	18.59013092	58.03%

Calorie intake

Calorie intake was shown to be significantly increased in contrast to normal control group in cafeteria diet control group, while calorie intake is significantly decreased at dose of 200mg/kg and 400mg/kg as compared with cafeteria diet control and Orlistat control groups. The dose of 100mg/kg showed no significant change as compared Orlistat control group (Figure 1).

Data was signified by Mean±SEM, N=6, d P<0.0001 in contrast to normal control, *P<0.05, ***P<0.001 as contrast to negative group and NS, ### P<0.001 as compared to orlistat by using ONA followed by BT.

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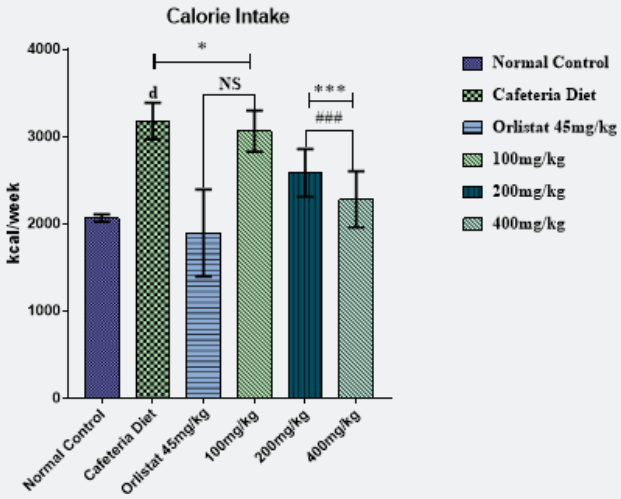

Figure 1: Effect of polyherbal mixture on calorie intake.

Effect on bodyweight

In all five groups administered with cafeteria diet as compared to normal group the body weight was found to be significantly increased. The CD group as compared to normal group was significantly increased on day 42. The test doses of 200mg/kg and 400mg/kg indicate significantly drop down the body weight as distinguished with negative and positive control group. Test dose of 100mg/kg shows significant decrease in body weight, but it was less effective as compared to doses at 200mg/kg and400mg/kg (Figure 2).

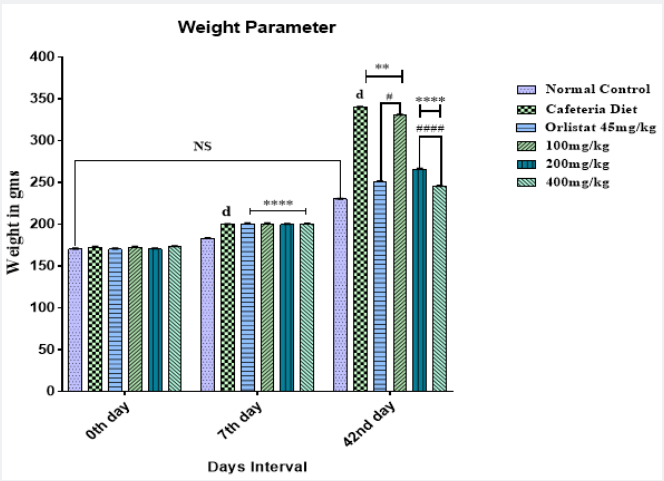

Figure 2: Effect of polyherbal mixture on body weight.

Data was signified MEAN±SEM, N=6, **P<0.01, ****P<0.0001 related to negative control and # P<0.05, #### P<0.0001 as contrast to positive control by using TWA accompanied by BT.

Effect on nasonal length and BMI

The significantly increased of Nasoanal length and BMI in CD as contrast to control group while it was found to decreased significantly at the test doses of200mg/kg and 400mg/kg as distinguished to CD and standard group (Figure 3).

Data was expressed as Mean±SEM, N=6, dP<0.0001 as related to control group, **P<0.001, ****P<0.0001 related to CD and # P<0.05, #### P<0.0001 as distinguished to +ve control by using OW- ANOVA followed by BT.

Biochemical estimation

Lipid profile test: Cholesterol, triglycerides and LDL level was found to be significantly increased in CD group as related with normal control group. Test doses 200mg/kg and 400mg/kg

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found to be substantially decreased the level of all three lipids as compared with CD control and positive control group. On contrary HDL level was shown to be significantly decreased in CD as related to control group. Test doses at 200mg/kg and 400mg/kg showed significantly increased in HDL level as related to CD and Orlistat group. Test dose 100mg/kg showed less significant result (Figure 4). Data was revealed as mean plus minus Standard error mean (SEM), N=6, d P<0.0001 in relation to normal control, **P<0.01, ****P<0.0001 as related to negative control and # P<0.05, #### P<0.0001 as distinguished to +ve control by using OW-ANOVA followed by BT.

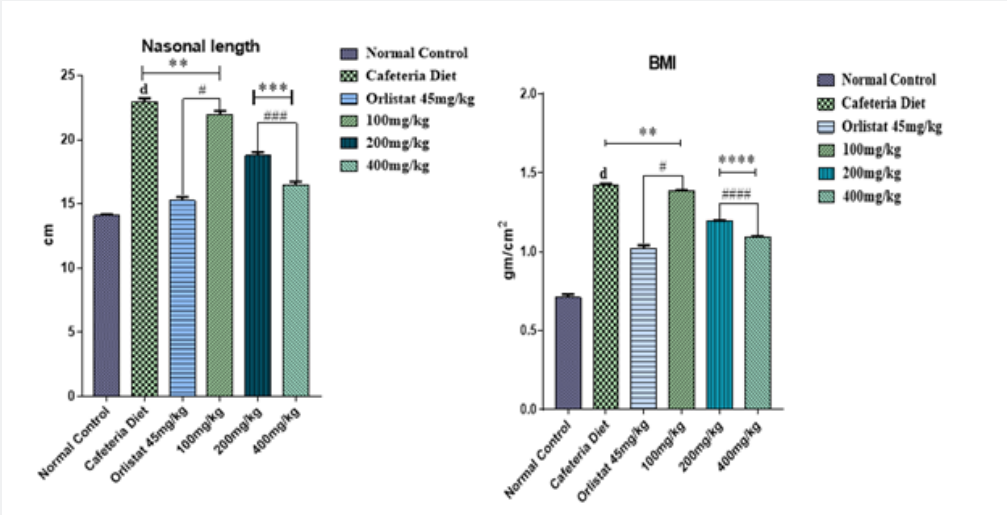

Figure 3: Effect of polyherbal mixture on nasoanal length and BMI.

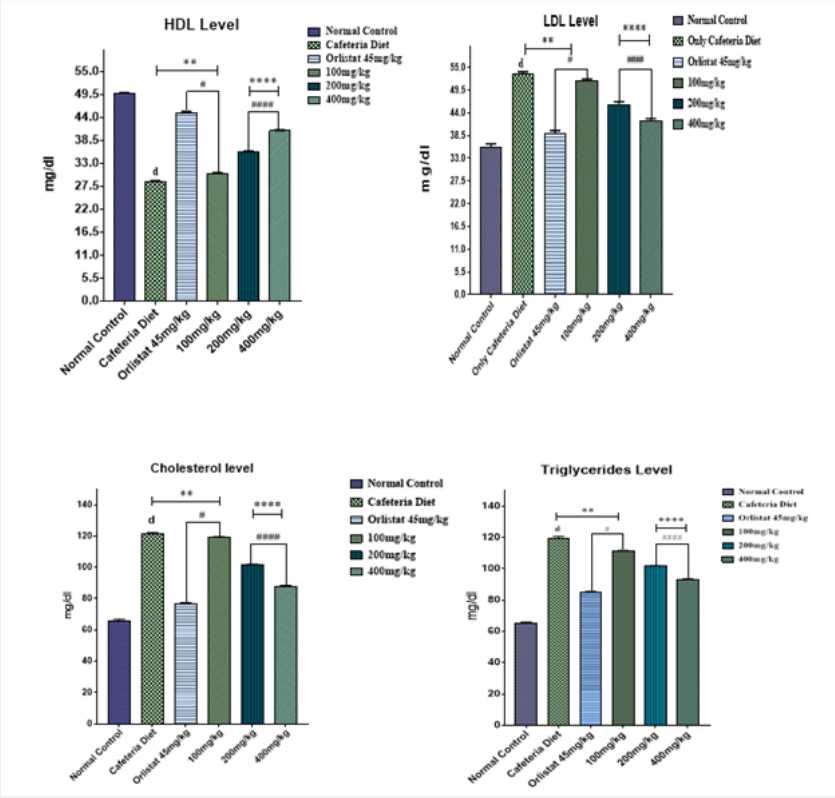

Figure 4: Effect of polyherbal mixture on lipid profile parameters.

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Effect on fasting glucose level: The serum glucose level was shown to be significantly increased in CD in relation to normal group while test doses at 200mg/kg and 400mg/kg was significantly decreased serum glucose level as compared to CD control and positive control group. Test dose 100mg/kg showed less significant result (Figure 5). Data was revealed as MEAN plus minus SEM, no of animals (N)=6, d P<0.0001 as compared to normal control, **P<0.01, ****P<0.0001 as distinguished to negative control and #P<0.05, #### P<0.0001 in relation to +ve control by using OW-ANOVA followed by BT.

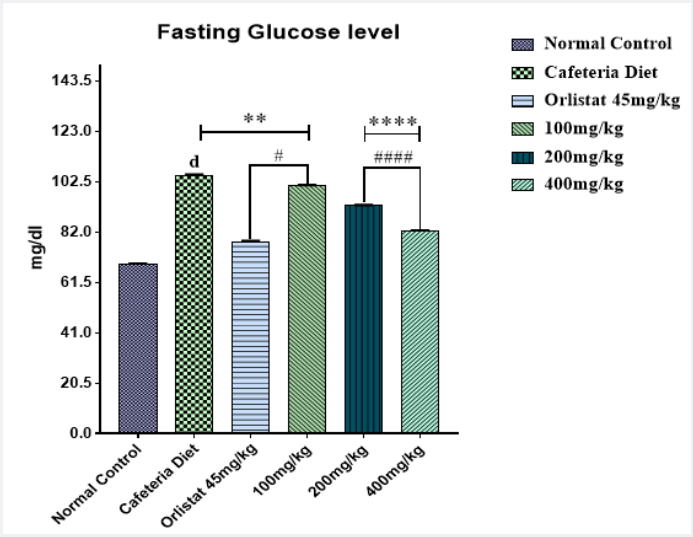

Figure 5: Effect of polyherbal mixture on lipid profile parameters.

Effect on liver function parameters: ALT and AST level was shown to be substantially increased in CD as related to normal group while test doses at 200mg/kg and 400mg/kg showed significant decrease in ALT and AST level as related to CD and positive control (Figure 6). Data revealed as Mean±SEM, N=6, d P<0.0001 as related to normal control, **P<0.01, ****P<0.0001 as related to negative control and # P<0.05, #### P<0.0001 as related to positive control followed by Bonferroni Test by using One-way ANOVA.

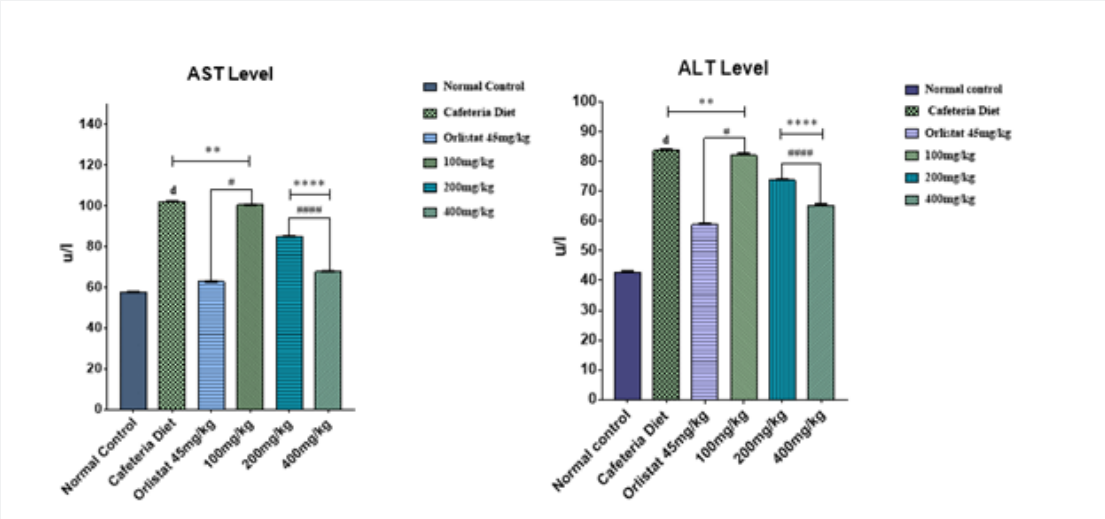

Figure 6: Effect of polyherbal mixture on liver function parameters.

Effect on white adipose tissue (WAT): In CD control, NC and drug treated group were evaluated the effect on fat pad after treatment period. The weight of WAT was found to be significantly increased (epididymal WAT by 1.59-fold, perirenal WAT by 1.70-fold, and mesenteric WAT by 1.89-fold higher) in CD control as compared to NC while test dose 200mg/kg and 400mg/kg

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found significant decrease in weight of white adipose tissues as compared to CD control and Orlistat control group (Figure 7). Data revealed as Mean ± SEM, N=6, d P<0.0001 as related to normal control, **P<0.01, ****P<0.0001 as compared to negative control and # P<0.05, #### P<0.0001 in comparison to positive control followed by BT by using TW- ANOVA.

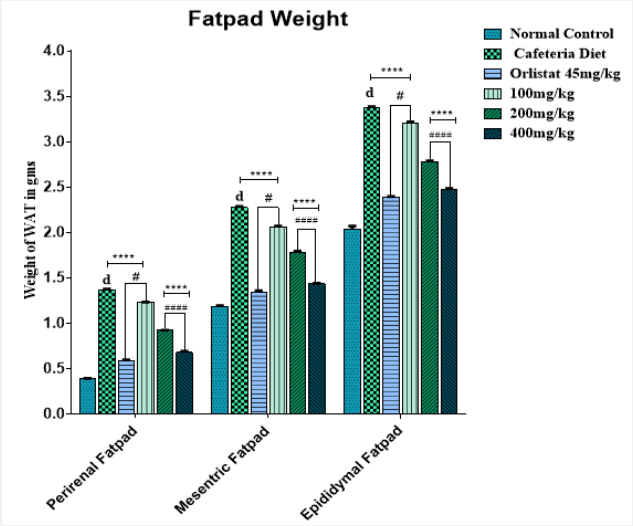

Figure 7: Effect of polyherbal mixture on white adipose tissue (WAT).

Effect on organs weight

The CD control group found to be significant increase in the weight of organs in comparison to normal group while test doses at 200mg/kg and 400mg/kg significantly decreased organ weight as compared with CD control and positive control group. The test dose 100mg/kg showed less significant effect (Figure 8).

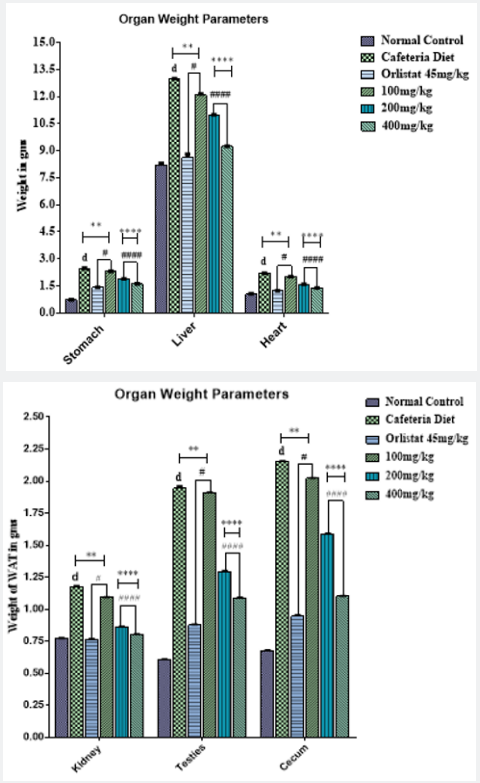

Figure 8: Effect of polyherbal mixture on organs weight.

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Data was revealed as Mean plus minus SEM, no of animals (N)=6, d P<0.0001 as related to normal control, **P<0.01, ****P<0.0001 in comparison to negative control and # P<0.05, #### P<0.0001 as related to positive control by using TW-ANOVA followed by Bonferroni Test.

Histopathological analysis

After microscopic examination of liver, the normal control rats did not show any lesion of pathological significance on the contrary the negative control rats fed with cafeteria diet were observed with multifocal mild cytoplasmic vacuolation at hepatocyte, sinusoidal dilation i.e., abnormal accumulation of lipids (steatosis), hepatocyte degeneration and lymphocytic infiltration. Treatment of standard drug and Test drug at 200 and 400 mg/kg to rats fed with cafeteria diet did not report any lesion of pathological significance. Treatment of Test drug at 100 mg/kg to the rat fed with cafeteria diet showed multifocal mild cytoplasmic vacuolation i.e., less lipid accumulation and focal mild lymphocytic infiltration (Figure 9).

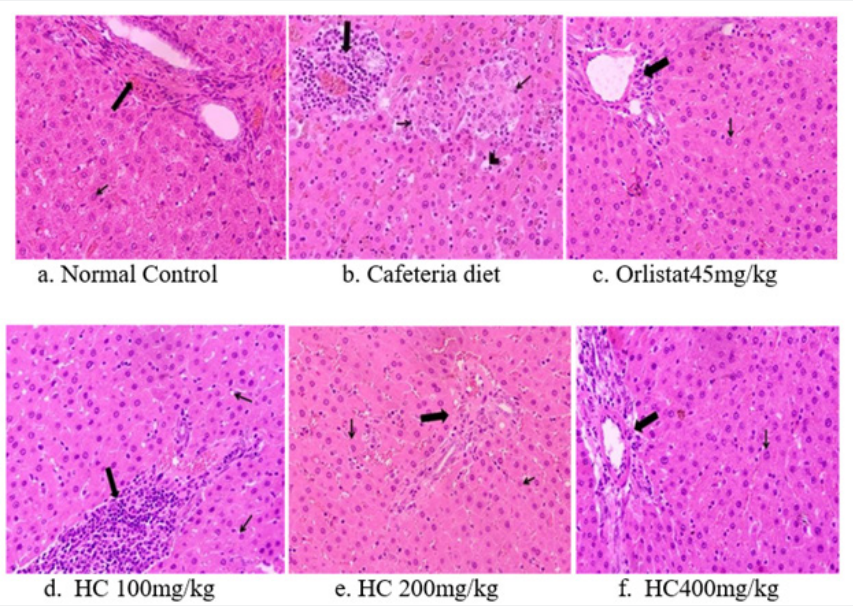

Figure 9: a. Normal Control: Showing normal histology, portal triad (big arrow), hepatocyte (short arrow) {H & E, 400X}, b. Negative control: Showing degeneration of Parenchymal cells (hepatocytes) (small arrow), sinusoidal obstruction (arrow head), lymphocytic infiltration (large arrow) {H & E, 400X}, c. Positive control: Showing normal histology, portal triad (large arrow), hepatocyte (small arrow) {H & E, 400X}, d. Test 1(100mg/kg):Showing cytoplasmic vacuolization (small arrow), lymphocytic infiltration (large arrow) {H & E, 400X}, e. Test 2(200mg/kg):Showing normal histology, portal triad (large arrow), hepatocyte (small arrow) {H & E, 400X}, f. Test 3(400mg/kg):Showing normal histology, portal triad (large arrow), hepatocyte (small arrow) {H & E, 400X}.

Discussion

The worldwide increase in obesity is because of changes in eating patterns and the intake of consumption of high caloric diet. Additionally, nuisances cause anatomical and neuroendocrine changes that are associated with increased food intake and adipogenesis. Stress, combined with overindulges and lethargy, can lead to obesity. It increases the incidence of life-threatening diseases including type II diabetes mellitus, hypertension, coronary heart diseases, osteoarthritis, obstructive sleep disorders, non-alcoholic fatty liver disease (NAFLD) and certain cancers.

In the present study, anti-obesity effect of polyherbal mixture (containing hydroalcoholic extracts of *Annona muricatta* leaves, *Momordica charantia* leaves and *Vaccinium uliginosum* berry) was evaluated in a cafeteria diet-induced obesity in Wistar rats. Decrease in body weight in CD-fed rats was reported to be due to depletion of body fat stores and reduction in obesity parameters studied [26]. Rashmi Shivannaet.al. observed that hydrolytic action of pancreatic lipase enzyme on dietary lipid is necessary for the direct absorption of lipids from the intestine. The end products fatty acids and 2-monoacylglycerols, formed due to hydrolysis in presence of pancreatic enzyme are absorbable though the intestine. For the treatment and management of obesity the inhibition of the absorption of the dietary lipids through inhibition of lipase enzyme can be targeted.

They proved that herbal plant extracts have played an imperative role in inhibition of pancreatic lipase and reduction in obesity, like *Eleusine indica*, *Myristica fragrans*, *Melastoma*

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candidum and *Phyla nodiflora*, *Punica granatum*, *Citrullus lanatus*, *Abroma augusta* etc. because of the presence of potential phytoconstituents. They found the % inhibition & IC50 of these plants individually and in combination [8] In the present study inhibition of lipase enzyme was evaluated by titrimetric assay method [8,9]. The IC50 value was calculated for individual herbal extracts as well as combination of the herbal extracts from concentration verses % inhibition graph plotted. The inhibition of lipase enzyme was found to be concentration dependent. The combination of three herbs and blueberry extract showed IC50 value similar to Orlistat.

After the in-vitro assay the safe dose of polyherbal mixture was determined by conducting acute oral toxicity as per OECD guideline 423. From previous study reports the proportion of these herbs to form 2000mg dose was calculated (Annona muricata-814.10mg/kg, Momordica charantia-814.10mg/kg and *Vaccinium uliginosum*- 315.78mg/kg to make a concentration of 2000mg/kg). The mixture was found to be safe at 2000mg/kg. So, for the further evaluation of anti-obesity activity the doses of 100mg/kg, 200mg/kg and 400mg/kg were selected. Undale et. al [10] reported acute toxicity studies of polyherbal formulation BSL-150. The formulation was reported to be safe at 2000mg/kg.

In the present study, obesity was induced by feeding rats with cafeteria diet for 42 days, to evaluate anti-obesity potential of polyherbal mixture. Shiv Kumar et.al. have reported cafeteria diet induced obesity in rats as one of the potential models to induce obesity based on the observations from various experimental studies. The study reports elevation in body weight and fat mass, alterations in carbohydrate and lipid metabolism, causing insulin resistance, increased production and release of leptin and increased lipase activity in high fat and high calorie diets in humans, rodents, and other animals [11-16].

Alexander & Shikov et al. investigated the anti-obesity potential of extracts of fermented and black leaves of *B. crassifolia* in cafeteria diet induced obesity in rats. The study reported significantly elevated body weight over 23 days in rat fed with cafeteria diet as compared to rats fed with normal diet. And later seven days consecutive oral administration of the 50mg/kg extracts of fermented and black leaves of *B. crassifolia* in rats decreased the body weight significantly. In the present study after administration of polyherbal mixture from day 7 to end of the study at the dose of 200mg/kg and 400mg/kg showed similar decrease in body weight [17-21].

Shiv Kumar et al. reported significant reduction in body weight and other obesity parameters in cafeteria diet induced obesity in rats after treatment with 500mg/kg of ASE (*Argyreiaspeciosa* root extract). The study also reported induction of fatty liver and accumulation of hepatic triglycerides. This result suggests that the decline in the body weight and calorie reduction due to ASE in cafeteria diet-fed rats might be due to its anorexic property. In the present study, body weight, nasoanal length, calorie intake and BMI were found to be decreased at a dose of 200mg/kg and 400mg/kg. The decrease in obesity parameters due to polyherbal mixture in cafeteria diet-fed rats may be due to its hypophagic property phytoconstituents. The mass of white adipose tissue, organ weight and lipid parameters increase in obesity. The study conducted by Mei-Yin Chien et al. on herbal mixture extracts reported significant decrease in organ weight, mass of white adipose tissue, lipid parameters and liver function test. The present study revealed that polyherbal mixture at a dose of 200mg/kg and 400mg/kg showed significant decrease in the organ weight and white adipose tissue weight (fat pad weight) in treated animals. These observed results may be because of the bioactive constituents such as flavonoids, quercetin, kaempferol, antho-cyanins, polyphenols, etc. present in plant materials. The decrease in the lipid parameters and liver parameters by a dose of 200 mg/kg and 400 mg/kg confers to their antiobesity potential [22-26].

Sanaa R Galaly et al. evaluated that cafeteria diet showed induction hepatostetosis hepatic triglycerides and total cholesterol accumulation (Lipids cytoplasmic vacuoles), and hepatic vacuoles (lymphocytes infiltration) in rats. In their study they reported treatment with T1 & T2 complex mixture significantly normalizes abnormal hepatic changes induced due to cafeteria diet. In the present study, from histopathological reports microscopically liver of negative control group rat fed with cafeteria diet revealed multifocal mild cytoplasmic vacuolation at hepatocyte, sinusoidal dilation, hepatocyte degeneration and lymphocytic infiltration. Treatment of standard drug (Orlistat 45mg/kg) and test drug at 200 and 400 mg/kg to rats fed with cafeteria diet dose not revealed any lesion of pathological significance. Treatment of test drug at 100 mg/kg to the rat fed with cafeteria diet showed multifocal mild cytoplasmic vacuolation i.e. less lipid accumulation and focal mild lymphocytic infiltration [26,27].

In total the present study suggests the antiobesity activity of combination of hydroalcoholic extracts of *Annona muricatta, Momordica charantia* and *Vaccinium uliginosum* in *in-vitro* as well as *in-vivo* studies. Further evaluation of exact mechanism of action and for herb interaction needs to be evaluated.

Conclusion

From the result and discussion treatment with Polyherbal mixture especially at the dose of 200mg/kg and 400mg/kg significantly reduced in body weight gain and calorie intake which is a clear sign of an antiobesity effect. This dose effectively improves all lipids level, decreases blood glucose level, decreases AST and ALT level. The histopathological finding of liver tissue also suggested improved fatty liver and hepatostetosis in animals treated with herbal mixture. It is concluded that the herbal composite of *Annona muricatta*, *Momordica charantia*, *Vaccinium uliginosum* may prove as potential antiobesity formula after details mechanistic studies to explore the action at molecular level.

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