International Journal of Engineering, Science and Mathematics
Vol. 8 Issue 4, April 2019,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

Formulation and evaluation of *Garciniacambogia*and *Commiphoramukul*Herbal tablets used for Anti-Obesity.

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ABSTRACT

Obesity is a complex disorder involving an excessive amount of body fat. It increases the risk of other diseases and health problems such as heart diseases, diabetes and high blood pressure. Obesity is diagnosed when our BMI is 30kg/m^{2}or higher. Traditional medicines, mainly herbal in nature are now a day used for treating many diseases than allopathic drug. The allopathic drug more side effect than herbal drug. The aim of this present work is to formulate and evaluate the herbal tablet of *Garciniacambogia*and *Commiphoramukul.* The formulation was prepared by wet granulation method. The prepared formulation was evaluated for pre-compression parameter and post- compression parameter. The formulation showed acceptable pre-compression and post-compression parameter is in limit. The result of assay of *Garciniacambogia* (HCA) content was 49.78 % and 49.69%, before and after stability study by IEC (Ion Exchange Chromatography). The *Commiphoramukul* (guggulsterones) content was 12.38% and 12.26%, before and after stability study by UV Spectrophotometer. From the result it was concluded that the formulation was relatively stable.

Key Word:- *Garciniacambogia,Commiphoramukul,* Obesity and Herbal table.

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INTRODUCTION

Obesity is chronic metabolic disorders which occurs as a result by increase the energy intake and decrease the exercise expenditure. Obesity can be defined as excess accumulation of fat rather than normal fat in the body. It is worldwide health problem. Obesity is a major risk for formed various disease such as cardiovascular disease, diabetes mellitus, cancer, high blood pressure, dyslipidemia[1]. In November 26th, 2010 is celebrated as Anti-Obesity Day in India[2]. If person is considered obese is body weight is more than 20%. If the BMI between 25 and 29.9 is considered as overweight. If your BMI is 30 or over you are considered obese person.BMI is defined as a person's weight in kilograms (kg) divided by height in meters squared (kg/m^2)[3].

The formulated herbal tablet for treating obesity contained combination drug *Garciniacambogia*(Vilaytiimlli)and *Commiphoramukul* (Guggul).[1] A herbal drug of GarciniaCambogia is mainly used as antiobesity drug and till today available in marketed in capsule dosage forms therefore we were formulated of tablet dosage forms. Now a days herbal drug more used because it lesser side effect as compared to synthetic drug.[2]The oral route of drug administration is the most popular and successfully used for conventional delivery of drugs. It offers the advantages of convenience, ease of administration, greater flexibility in dosage form design, ease of production and low cost[4].

Tablets are solid preparations each containing a single dose of one or more active substances and usually obtained by compressing uniform volumes of particles. Tablets are intended for oral administration[5,]The compressed tablet is refers to standard uncoated tablet made by compression and employing of the basic methods or manufacture: wet granulation, double compaction or direct compression. Tablets in the category are usually intended to provide rapid disintegration and drug release.Most tablets containing drug intended to exert a local effect in the GIT are of this type[4].

Herbalplant in pharmaceuticals have been used in the management of various diseasesaffecting humans. The fruit of *Garciniacambogia*has been mostly used in food preparation and cooking, having a sour taste. The fruit extract of *GarcinaCambogia*containing Hydroxycitricacidis mainactive constituent which affect on weight loss. Hydroxycitricacid is reducing the food intake or suppresses the appetite. It is traditionally used in various other activities such as astringent, constipating, cardio tonic, anti-fungal effect, ant diabetic effect, antineoplastic effect and lowering lipid effect[6].

Commiphoramukul contains Z and E Guggulsterone, Guggulsterol I, II, III, IV, V, Guggul lipid. The oleoresin part of guggul is mostly used for hypolipidemic activity. The traditionally used of guggul is anti-inflammatory, antispasmodic, carminative, hypoglycemic, antiseptic, astringent, anthelmintic[7].

MATERIALS AND METHOD

Materials:

The dried pure crude extract of Garciniacambogia was purchased from Ambephytoextracts, Delhi. The Commiphoramukul was purchased form local market ayurvedic store Aarti Product, Nagpur.

Methods:

Preparation ofCompressed Tablet

General Procedure:

Hydroxy Propyl Methyl Cellulose paste was prepared by adding a required quantity of HPMC powder to the water under use of mechanical stirrer it gives paste consistency. This mixture was then used as binder solution in the preparation of granules. Weighed accurately quantities of GarciniaCambogia, Microcrystalline cellulose, and starch are properly mixed together and added slowly HPMC slurry and then added guggul extract powder are mix together. Formulated the granules are passed in sieve no. 18 and dried at 50°C in hot air oven for 30 min.The dried granular mass was passed through a sieve no. 12 to obtain uniform sized granules. The different batches of the granules were then mixed with calculated equal quantities of magnesium stearate and aerosil and then were compressed into tablets compression machine double rotary.

Table no. 1:- Composition of F4 Formula

Sr. No.	Ingredients	F4
1.	GaarciniaCamboga	500 mg
2.	CommiphoraMukul	50 mg
3.	Microcrystalline cellulose	170 mg
4.	Starch	20 mg
5.	Croscarmellose	25 mg
6.	Hydroxy propyl methyl cellulose	20 mg
7.	Magnesium Stearate	10 mg
8.	Areosil	5 mg

Evaluation Parameter:

I. Pre-compression parameter[8]

1. Angle of repose:

The angle of repose of powder was determined by funnel method. The powder was passed through a funnel fixed to a burette stand at a height of 2.5 cm. A graph paper was placed below the funnel on the table. The height and radius of the pile was measured. Angle of repose of the powder was calculated using the formula:

$$\textbf{Angle of repose } (\theta) = \textbf{Tan}^{-1}\textbf{h / r}$$

Where,

h = Height of the pile

r = Radius of the pile

2. Bulk density:

It is the ratio of total mass of powder and the bulk volume of powder. It was measured by pouring the weighed powder into a measuring cylinder and initial weight was noted. From this the bulk density was calculatedaccording to the formula mentioned below.

Bulk density (Db) = Mass (M) / Bulk volume (Vb)

Where,

M is the mass of powder,

Vbis the bulk volume of the powder

3. Tapped density:

It is the ratio of total mass of the powder to the tapped volume of the powder. Volume was measured by tapping the powder for 100 times and the tapped volume was noted. It is expressed in gm/ml and is given by

Tapped density (Dt) = Mass (M) / Tapped volume (Vt)

Where,

M is the mass of powder;

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Vt is the tapped volume of the powder.

4. Compressibility index:

It is based on theapparent bulk density and the tapped density, the percentage compressibility index of the bulkdrug was calculated by the following formula.

$$\textbf{\% compressibility index} = \frac{\textbf{Tapped density} - \textbf{Bulk density}}{\textbf{Tapped density}} \textbf{x 100}$$

5. Hausner's ratio

The ratio of tapped density and bulk density is called as hausner's ratio. It is an indirect index of ease of powder flow. It was calculated by the following formula.

Hausner ratio = Tapped density (Dt) / Bulk density (Db)

II. Post-compression[8]

1. Weight variation:

Weighed 20 tablet individually and then calculate the average weight. Then percentage deviation was also calculated by using average weight and individual weight. It was calculated by the following formula:

$$\%\, deviation = \frac{Average\, weight - Individual\, weight}{Average\, weight}$$

2. Dimensions:

Thickness and diameter of the tablet was measured by using Digital Verniercaliper. 3 tablets of the formulation were picked randomly and measured individually.

3. Friability:

The friability is measure of tablet toughness. Roche Fraibilator was used to measure the friability of tablet. 10 tablets were weighed accurately and placed in the friabilator chamber that revolves 25 rpm for 4 min falling the tablets through a distance 6 inch with each rotation. After 4 min 100 rotations was completed then tablets were reweighed. The calculated % friabilator by using formula:

% Friability = [ ] **100**

4. Hardness:

The force of required to tablet crush in compression test. The method used for tablet hardness testing if the tablet placed between two jaws and crushed the tablet. The hardness of tablet measured by using Pfizer tester. The unit of hardness is kg/cm^2.

5. Disintegration Time:

Disintegration time is the time taken by a to break down a tablet into small particles or granules is called as disintegration. The disintegration test is carried out in an apparatus containing basket rack assembly with six glass tubes of 7.75 cm length and 2.15mm in diameter the bottom of which consists of 10 mesh sieve. The basket is raised and lowered 28-32 times per minute in the medium of 900 ml which is maintained at 37º C. six tablets were placed in each of the tubes and the time required for complete passage of tablet fragments through the sieve (# 10) was considered as the disintegration time of the tablet.

6. Estimation of (-) HCA[9]

6.1 By Ion Exchange Chromatography:-

Preparation of Sample:-

Taken 250 mg sample in a beaker was added 100 ml distilled water, heated to 75°C for 30-45 minutes under stirring. When most of the material goes into solution, cool and filter. To the residue added 50 ml distilled water, further heated it to 70° C, cool and filter. Repeat once more taking 50 ml water and heated it to 70° C, cool and filter. Collected and combined the filtrate.

Procedure:-

Charge the sample solution in the column maintaining a flow rate of 2.5 ml/min, when the whole solution is well circulated over packed resin column wash with distilled water until the eluate gives constant pH of 4 to 4.5. Combine the solution and water washing and adjust the volume to 500 ml with distilled water. A blank is also run by eluting 500 ml distilled water through the cation exchange resin. Titrate sample and blank separately against 0.1 N sodium hydroxide using phenolphthalein as indicator.

Calculation:-

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7. Estimation of Guggulsterones[9]

7.1 By spectrophotometer

Taken 2.5 g sample of guggul extract was transferred in 250 ml in round bottom flask. Added 35 ml of 0.5 N alcoholic potassium hydroxide and reflux for 90 min. on a water bath. Then were transferred the contents of flask to a separator, rinse the flask with 50 ml luke warm water. Extract while the liquid is warm by shaking vigourously with three successive quantities of 50 ml of petroleum ether 60-80°. Combine the petroleum-ether washings and wash with 20 ml water. Evaporated the petroleum ether and weighed the residue. Weighed accurately 0.1 g of above residue and make it to 10 ml by spectrophotometric grade methanol. Dil. 1 ml of the above solution to 10 ml with methanol and measured the absorbance at 327 nm using methanol as blank.E max 1% cm Path at 327 nm is 160

Calculation:-

8. Stability study:-

The stability studies were carried out most satisfactory formulation as per ICH guidelines. In the present study, stability studies were carried out at 40 °C and 75% RH for a specific time period up to 3 month for optimized formulations. For stability study, the tablets were sealed in aluminium packaging coated inside with polyethylene. These sample containers were placed in desiccators' maintained at 75% RH.

Animals Model:-

Albino wistar rats either sex between 150-200 g of weight of rat were used for the present study. Healthy adult male and female Wistar strain albino rats 4 months old and weight 150-200 gm. The animals were divided in 5 group comprising 6 rats in each group under standard laboratory condition of temperature (16 C) and 12/12 hrs. light /dark cycle.Animals were fed with rodent chow pellet diet and water *ad libitum.*The experimental

protocol was approved by Institutional Animal Ethics Committee (Reg. No. 731/PO/Re/S/2002/CPCSEA) and the laboratory animals were taken care according to the guideline of CPCSEA, ministry of Forest and Environment, Government of India.

Anti-obesity Activity[9]

Induction of Obesity

High Fat Diet:-

Corn starch-15%, Lard oil-17.6%, Mineral mixture-3.5%, Sugar-27.5%, Casein-20%, Vitamin mixture-1%, Cellulose powder-5%, Choline bitartrate- 0.2% and Methionine-3g. Above all high fat diet ingredients were mixed together and prepared the pellet and administered to everyday at morning to animal with water ad libitum. The diet was administered uptofourweek. After four week increase the weight of rats therefore conforming to induced the obesity in rat.

Experimental Design:-

Group I: Non obese control group fed with normal diet

Group II: Obese control group fed with High Fat Diet (HFD)

Group III: (Ob + Orl.) Obese standard treated with 10 mg/kg of orlistat

Group IV: (Ob + Comb.) Obese C. Mukul and G. Cambogiatablet 550 mg/kg

Group V: (Ob + Comb. + std.) Obese Combination tablet and standard (orlistat) 280 mg/kg

The above treatment was followed for the respective group of animals for 28 days. Daily all the animals were given high fat diet with drug treatment of herbal suspension formulation. Combination drug G. Cambogia and C. mukul tablet were triturated in mortar pastel added 0. 5% CMC, 0.1% Tween 80 and added sufficient quantity of water and mixed properly suspension was prepared. Fresh drug suspension were prepared for each day . The solution was kept in air tight coloured bottle and stored at room temperature till use. The volume of drug suspension was calculated based upon the body weight of the animal.

Pharmacological Studies:-

Body Weight:-The body weight (g) was recorded on day one and then every week for 30 days by using digital weighing balance.

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Food Intake:-Daily food intake for 5 group of 6 rats was measured daily for 30 days and calculated means of daily food intake 5 group of 6 rats.

Organ Weight:-The rats were sacrified by cervical decapitation on 28th days. Various organs such as liver and heart were isolated washed with formaline, then dried on filter paper and weighed. The organ to body weight (mg/g) ratio was recorded.

Biochemical Parameter: - Blood was withdrawal form retro-orbital route after overnight fasted animal were anesthetized by using ether. Blood was withdrawn on 28th day (after treatment) of study. Collected blood was centrifuged to separate for evaluation of lipid profile were estimated of Total Cholesterol (TC), Triglyceride (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDP) by using standard biochemical kits purchased from Ambica Diagnostic kit pvt. Parbhani.

Statistical Analysis:-

RESULTS AND DISCUSSIONS

Preformulation studies:

Preformulation studies are the first step in the rational development of dosage form of a drug substance. The objective of Preformulation studies is to develop a portfolio of information about the drug substance, so that this information useful to develop different dosage form. Preformulation can be defined as investigation of physical and chemical properties of drug substance alone and when combined with excipients.

1. Organoleptic Properties:

Table no 2:- Organoleptic properties of GarciniaCambogia and Commiphora Mukul

Sr. No.	Active Pharmaceutical Ingredients	Test	Observation	Result
1.	GarciniaCambogia	Colour	Light yellowish brown	Complies
		Odour	Charcteristics	Complies
		Taste	Sour taste	Complies

2.	Commiphora Mukul	Colour	White to off white powder	Complies
		Odour	Characteristics	Complies
		Taste	Acrid taste	Complies

2. Moisture content determination:-

Table no.3:-Results of Loss on Drying (LOD)

Sr. No.	**Active Pharmaceutical Ingredients**	**Standard LOD**	**Observed LOD**
1.	GarciniaCambogia	5.0%	3.9%
2.	Commiphora Mukul	5.0%	3.8%

Physicochemical Characterization:-
Pre and Post ccompression parameter:-
Table no.4:- Pre and post Compression parameter of optimized formula F4

Formula	**Angle of repose** (Θ)	**Bulk density gm/ ml**	**Tapped density gm/ml**	**Compressibility index %**	**Hausner's Ratio**
F4	24.61±0.099	0.476±0.112	0.555±0.108	14.23±0.034	1.16
Formula	**Avg. Weight (mg)**	**Thickness (mm)**	**Hardness kg/cm**	**Friability %**	**Disintegration Time (min.)**
F4	807.4±0.12	6.41±0.04	3.8±0.03	0.21	12 min

Table no.5:-Estimation of Guggulsterones in Extract and Tablet

Commiphora Mukul Extract (Gugglusterones %)	**In Tablet (Gugglusterones %)**
The percentage of guggulsterones in pure extract was 28 %	The percentage of guggulsterones in tablet was 12.17 %

Table no.6:-Estimation of (-)- Hydroxy Citric Acid in Extract and Tablet

GarciniaCambogia Extract (HCA %)	**In tablet (HCA %)**
% of (-)-Hdroxy Citric Acid of Pure GarciniaCambogia is 50%	% of (-)- Hydroxy Citric Acid in Tablet is 48.59%

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Stability studies:-

Physical and chemical parameters Anti-obesity herbal tablet (F4) after 3month at 40ºC ± 2ºC/75 %RH ± 5 % RH (packing: blister pack)

Table no.7:- Results of stability studies

Parameter	Initial month	After 3 month
Description	Off white or creamish colour	No change
Avg. weight (mg)	807.2 mg	807.1 mg
Hardness kg/cm2	3.8 kg/cm2	3.6 kg/cm2
Thickness (mm)	6.41 mm	6.43 mm
Friability (%)	0.21 %	0.20 %
Disintegration Time (min)	12 min.	13 min.
Estimation of guggulsterones in tablet	11.17%	11.15%
Estimation of (HCA) in tablet	48.59%	48.50%

Preliminary Study:-

Effect on Body weight:-

There was significant of ($p<0.05$) increase the body weight HFD group animals as compared to control group. Orlistat group animals were significant ($p<0.01$) decrease the body weight as compared to HFD group animals. Combination + orlistat produced significant decrease in body weight as compared the Orlistat group. Combination group animals also significant ($p<0.001$) decrease in body weight as compared to orlistat group.

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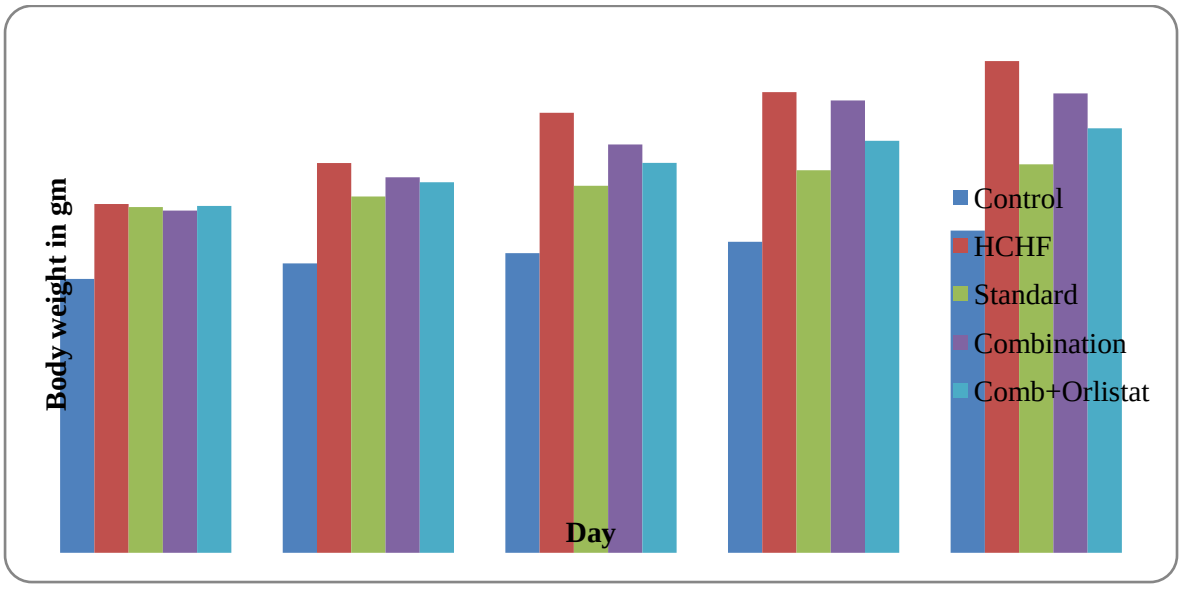

Figure no. 1: Effect HCHFD on body weight

Effect of food Intake:-

The food intake of high fat diet group shows significant ($p<0.05$) increase in daily food intake as compared to control group I. Treatment group of Orlistat show significant ($p<0.01$) decrease in daily food intake as compared to group II HFD. Combination+ orlistat group caused significant in daily food intake as compared to Orlistat group.

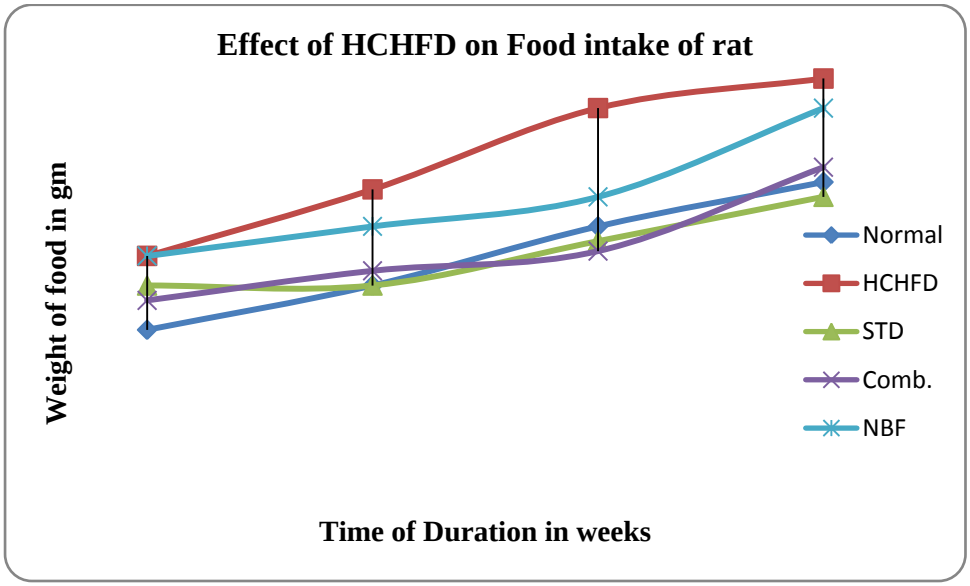

Figure no. 2: Effect of HCHFD on food intake of rat

Effect of Lipid Profile:-

The group II high fat diet animals were significant ($p<0.05$) increase the cholesterol level, triglycerides and Low Density Lipoprotein while decreased the High Density Lipoprotein as

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compared to control group I. Orlistatgroup animals were significant ($p<0.005$) decrease the cholesterol level, triglycerides and Low Density Lipoprotein while increased the High Density Lipoprotein as compared to HFD II group. Combination + Orlistat group were produced significant slightly decrease cholesterol level, triglycerides and Low Density Lipoprotein while slightly increased High Density Lipoprotein as compared to Orlistat group.

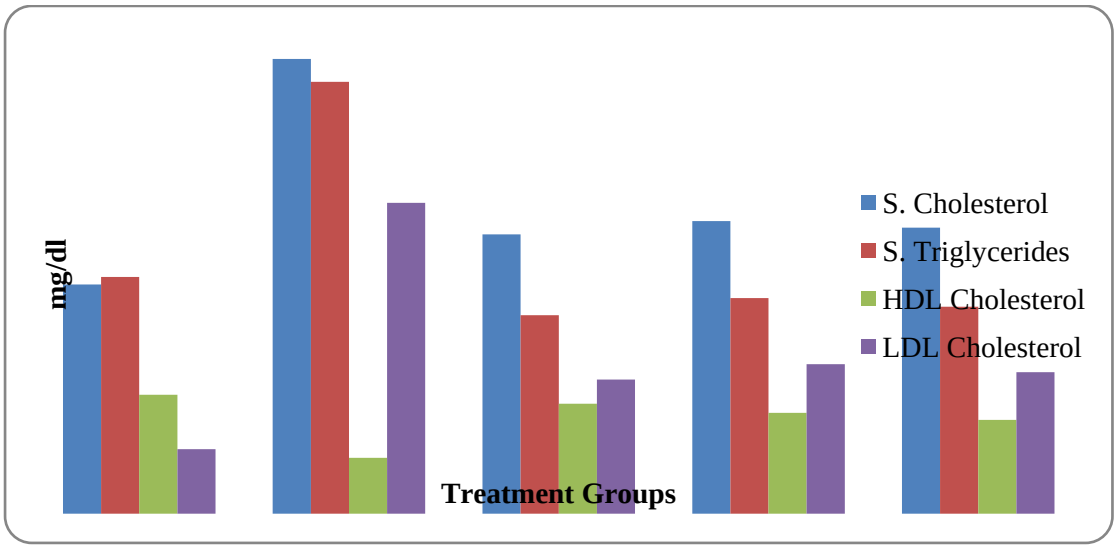

Figure no.3: Effect of Lipid Profile on rat

Effect on Organ Weight:-

There were significant ($p<0.05$) increase the organ weight HFD group as compared to control. Orlistat group animals were significant ($p<0.01$) decrease the organ weight as compared to HFD group animals. Combination + orlistat produced significant decrease in organ weight as compared the Orlistat group. Combination group animals also significant ($p<0.001$) decrease in organ weight as compared to orlistat group.

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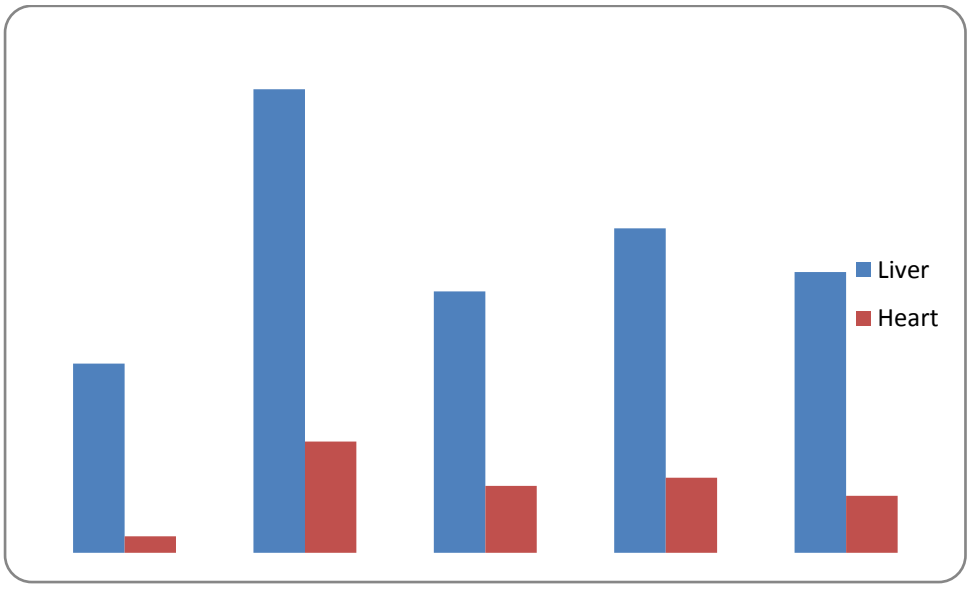

Figure no. 4: Effect on Organ Weight

DISCUSSION

In tablet pre-compression parameter the blends was analysed for the such as angle of repose, bulk density, tapped density, compressibility index, hausner`s ratio were found to be within limits. Then further studies were taken.

Post-compression parameter of tablet

- The total weight of each formulation was not maintained constant, but the weight variation was within limits of ±5%.
- Friability were found to be less than 5% and considered to be satisfactory in the range of 0.21% to 0.35%.
- Tablets thickness was almost uniform in all formulation and was found to be in the range of 6.32mm to 6.43mm
- Tablets hardness of each formulation was analyzed and found to be good in the range of 3.8 to 5.2 kg/cm^2.
- The prepared tablets were checked for disintegration time range within 15 min.

The rats feeding high fat diet causes stimulate the appetite resulting increase the body weight as compared to normal chow pellets therefore it is widely accepted model for obesity

In the present study the Combination tablet *C.Mukul*and*G. Cambogia* were found to be best combination when compared to other combination. Our results demonstrated that the combination has pronounced synergistic effect in High Fat Diet- induced obese rats. These effects were reflected in the body weight, organ weight, serum lipid profile of the rats in our various treatment groups.

These results suggest that combination tablet *C. Mukul* and *G. Cambogia* may reduce weight gain induced by a high fat diet it seemed low body weight in combined group due to loss of appetite. Treatment of Combination tablet group has decreasing concentration of serum TC, TG and LDL and increasing the ratio of HDL and Orlistat + combination is better effect of lipid serum as compared to Combination drug.

Organ weight such as Liver and Heart HFD diet increase the organ weight and effect of combination tablet lower organ weight but combination + orlistat is better effect than combination. During study the wistar rats not show mortality or the any side effect when the rats fed orally with combined the combination at the doses 275mg/kg/day. Therefore the garciniacambogia + commiphorawere good margin of protection.

CONCLUSION

Garciniacambogia and commiphora mukul these two combination drug used in treatment of obesity disease. This combination tablet was 800 mg formulated by using different excipients.

The pure extract identification test was performed such as description, solubility, pH, estimation of guggulsterones, and estimation of HCA. The Powder and blends were evaluated for tests such as bulk density, tapped density, compressibility index, hausner's ratio before being punched as tablets.

The formulation F1 to F4 was formulated by using wet granulation technique and this formulation is added superdisintegrant because the guggul was very slowly disintegrate the tablet they are binding to the tablet. Hence added superdintegrant then tablet wasdisintegrating within time limit. F4 formula was passed to the overall batches. Then F4 Formulation accelerated stability study was carried out.

The optimized batch (F4) tablets were packed in Blister packs and are further studied for stability at 400C ± 20C/ 75% RH ± 5%RH for a period of three month. Tablets were evaluated for assay but there was no significant change during the stability study period.

Result of this study shows that tablet of *G. Cambogia*and*C.Mukul* produced a favourable effect on body weight, it reduce body weight gain. Hence, finding of this study provides some biochemical basis for the use of*G. Cambogia*and*C.Mukul*as antihyperlipidemic agent with preventive and curative effects against hyperlipidemia; however more studies were required to gain insight into the possible mechanism of action.

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