



Evaluation of Anti-Obesity Activity of Methanolic Extract of *Sapindus emarginatus* by Progesterone Induced Obesity on Albino Mice

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ABSTRACT

Obesity is the serious problem now a day. From the ancient days investigation is going on many drugs which are employing to treat not only obesity but also the disorders arises due to the obesity. Many drugs and treatments are available in the market to treat obesity now. But no drug is ideal to treat all sort problems of obesity. Still the research is going on for the ideal drug. As a part of research work on drug development process for obesity the preclinical research is mandatory to evaluate the drug activity. Normally rodents are the organisms for first stage of drug evaluation and the obesity could be induce by using chemicals and high fat diet. These two methods are ideal methods for induction of obesity in rodents. In the present study of evaluation of anti obesity activity the organisms used were albino mice. The standard drug used was orlistat. The two test doses of methanolic extract of pericarps of flowers of *Sapindus emarginatus* were prepared. The total evaluation period was 28 days.

Keywords: Anti-obesity activity, Orlistat, Progesterone, *Sapindus emarginatus*.

INTRODUCTION

However, the ancient and traditional practitioners reported that the plant based products beneficial for the treatment of obesity conditions including diabetes and cardiovascular diseases. So on this basis, it is required that scientific validation of traditional claim of methanolic extract of pericarps of flowers of *Sapindus emarginatus* for its anti-obesity property was selected for the present study. At present most often used drug to treat obesity is Orlistat and at present it's been considered as ideal drug for present scenario. Several trails have been going on for anti-obesity drugs and some new approaches are for some drugs such as in phase-I clinical trials like Mazindol which was adrenergic agonist.¹ A new approach was finding herbal based drugs have been used for obesity and other associated obesity disorders.^{2,3} From the literature survey the plant *Sapindus emarginatus* is used to treat anti microbial⁴, CNS activity⁵, anti oxidant⁶, anti-fertility and anti-androgenic activity⁷, anti diabetic⁸ and anti larvicidal activity.^{9,10} The total time period for the anti obesity activity screening was 28 days. All the animals used in this screening were female albino mice weighed in between 20-25 gm. Before conducting the screening for anti obesity activity of methanolic extract of pericarps of flowers of *Sapindus emarginatus* was subjected to phytochemical screening. The drug used to induce obesity to female mice was female reproductive hormone and neuro steroid.¹¹ Some reports have suggested that being female reproductive hormone progesterone changes pathophysiology of organism and behaviour of the organism too. It helps in the excessive fat deposition in the body also. So for the present study it was taken as control drug to induce obesity in the mice. Body weight, rearing, grooming, ambulatory moments,

food consumption behaviour in mice and all biochemical parameters were studied.

MATERIALS AND METHODS

Plant Material Collection

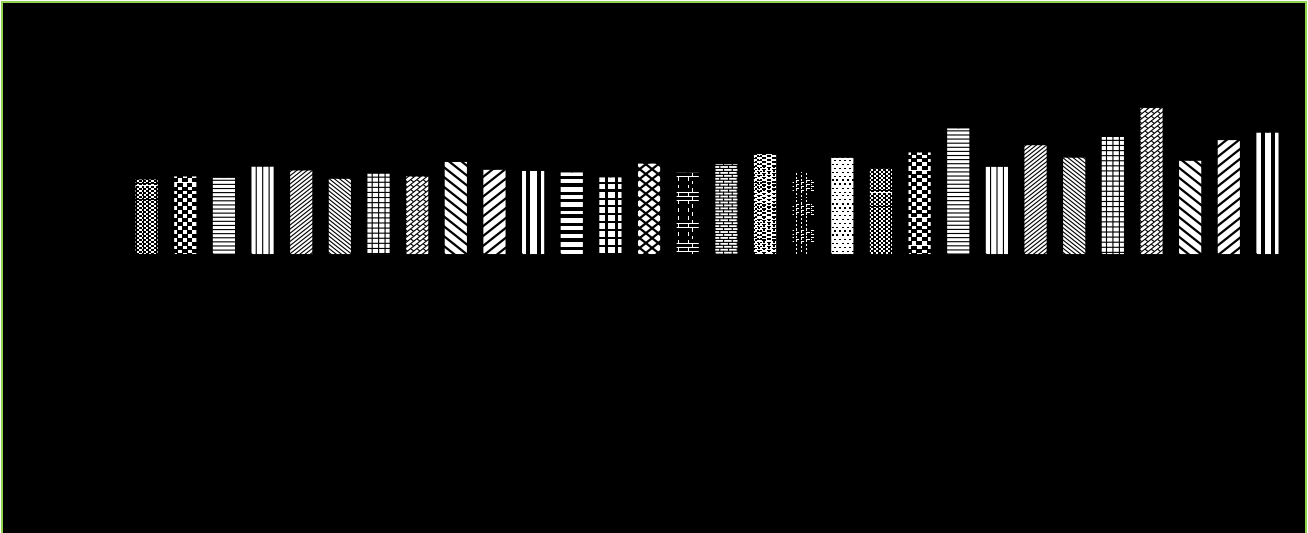
Pericarps of flowers of *Sapindus emarginatus* were collected from Tirupathi. The plant authentication was done by Department of Botany, Sri Venkateswara University, Tirupathi dist. Chittoor, Andhra Pradesh.

Preparation of the Extract

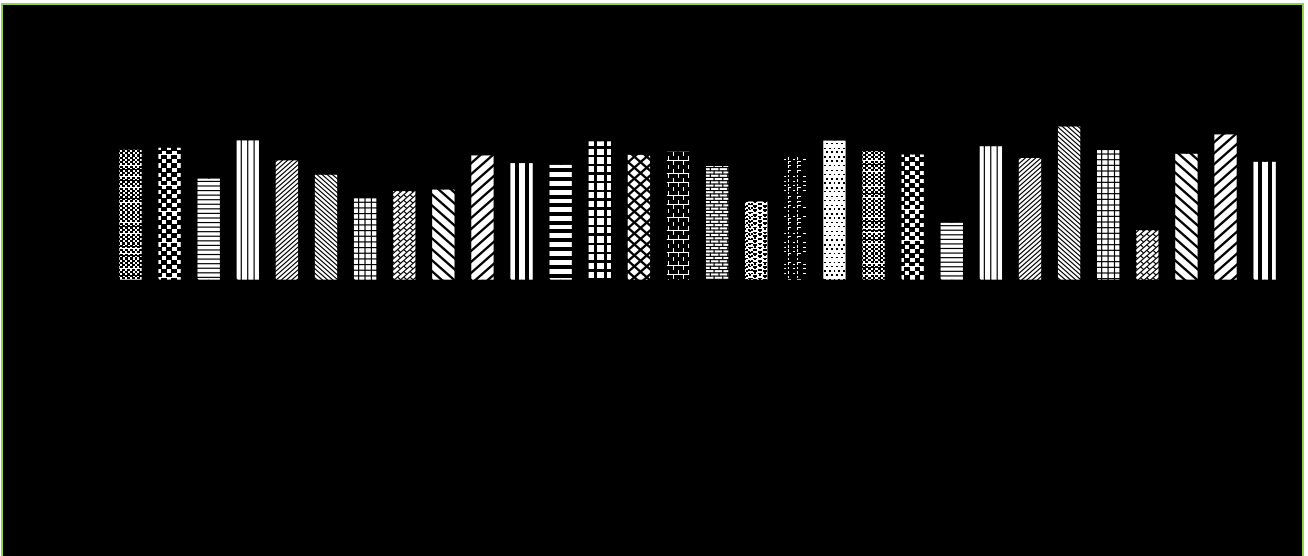
Pericarps of flowers of *Sapindus emarginatus* were dried at room temperature for 2-3 days. The dried pericarps of flowers of *Sapindus emarginatus* powdered in a mixture. The extraction process was done in a Soxhlet extractor. From the total powder 100gms of fine powder was taken and suspended in 200ml of methanol for 24 hours at 72 degrees of temperature. After 24hours the extract is taken and residue was dried.¹²

Experimental Animals

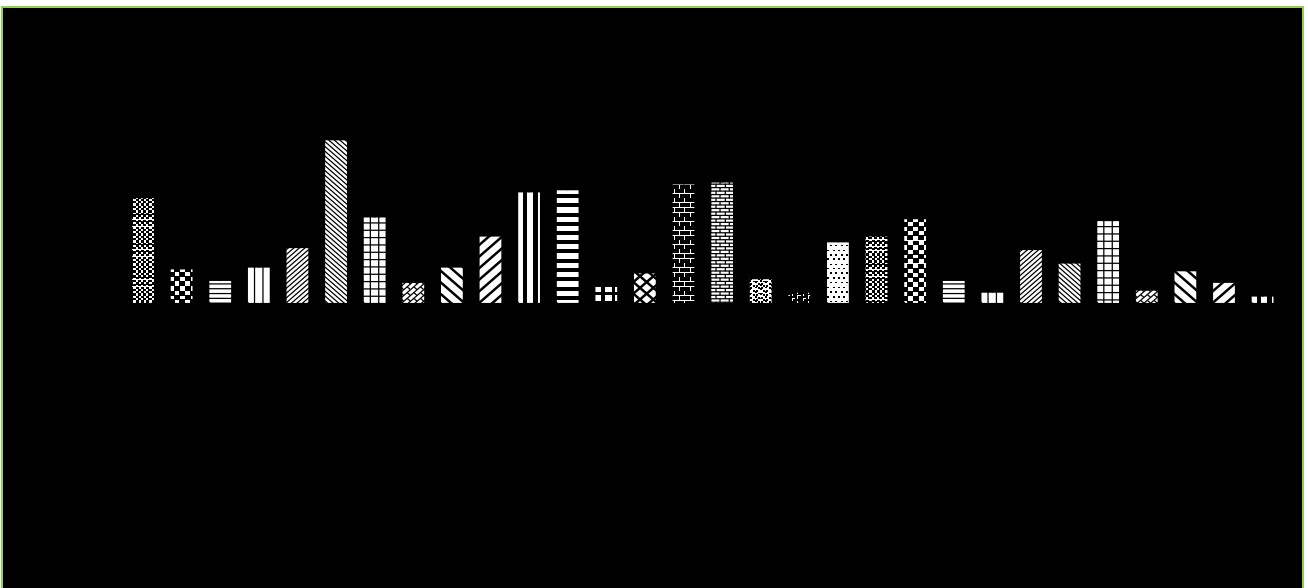
Healthy female albino mice of 20-25 gms of weighed and age of 2-3 months were used for the present study. Total 25 female mice were used for the present study and the animals were housed 5mice per each polypropylene cage, maintained under standard conditions (12 hours light and 12 hours dark cycle, 23±5°C and 40-60% humidity). They were fed with standard rat pellet diet (National Institute for Nutrition, Hyderabad) and provided water ad libitum. All the animals are collected from central animal house sicra labs pvt.ltd, ida-kukatpally, hyderabad and all experiments were conducted according to the ethical norms approved by CPCSEA, Ethical committee IAEC reg. no. (769/2011/CPCSEA).



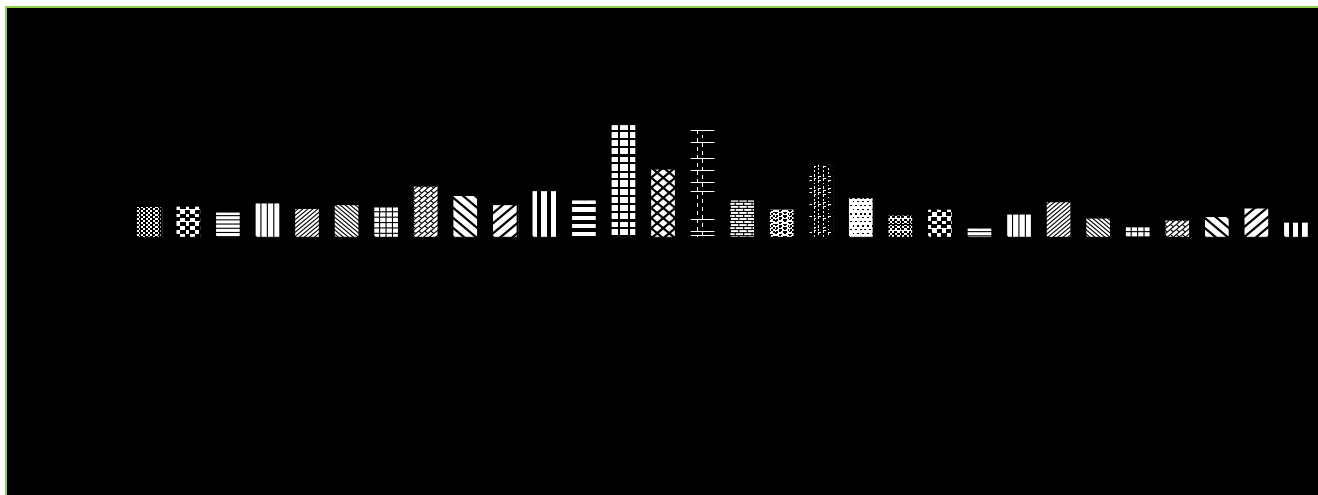
Graph 1: Effect of methanolic extract of pericarps of flowers of *Sapindus emarginatus* on Body Weight in progesterone induced Obesity albino mice.



Graph 2: Effect of methanolic extract of pericarps of flowers of *Sapindus emarginatus* on ambulatory movement in progesterone induced Obesity albino mice.



Graph 3: Effect of methanolic extract of pericarps of flowers of *Sapindus emarginatus* on rearing in progesterone induced Obesity albino mice.



Graph 4: Effect of methanolic extract of pericarps of flowers of *Sapindus emarginatus* on grooming in progesterone induced Obesity albinomice.

Preliminary Phytochemical Screening

The methanolic extract of Pericarps of the flowers of *Sapindus emarginatus* were found large percentage of saponin¹³. Normally the plant *Sapindus emarginatus* consists of various phytochemical constituents such as flavonoids, Triterpenoids, glycosides, carbohydrates, fatty acids, phenols, fixed oil, and saponins.¹⁴

Experimental Procedure

Induction of Progesterone to Produce Obesity

Progesterone is the obesity control used to induce obesity. The dose of obesity control is 10mg/kg of body weight. It was prepared by dissolving in arachis vial contents were dissolved in arachis oil and a dose of 10 mg/kg was administered subcutaneously in the dorsal neck region to mice for 28 days. The test drugs were injected 30 min before progesterone administration.¹⁵

Preparation of the Test Drug

The extract and standard Orlistat were soluble in distilled water. For progesterone, arachis oil was used as a vehicle and diluents for appropriate doses. All drugs were given at a dose of 0.4 ml/100 g body weight. All the drug concentrations were prepared freshly just before administration. All the test drugs, including the standard were given by oral gavages by p.o. route.

Experimental Procedure

The rats were divided into five groups with five mice each:

Group I: Normal Diet Fed or Normal

Group II: Obesity control (10mg/kg Progesterone)

Group III (standard): Progesterone + Standard Drug Orlistat (10mg/kg b.w.).

Group IV (test-1): 10mg/kg Progesterone +200mg/kg MESM

Group V (test-2): 10mg/kg Progesterone +400mg/kg MESM.

The total time period for the evaluation of anti-obesity activity was 28days. Daily body weight and behavioural changes were examined and recorded. After completion of 28 studies the animals were sacrificed and before that the blood is collected from each rat from all the five groups for biochemical estimation.

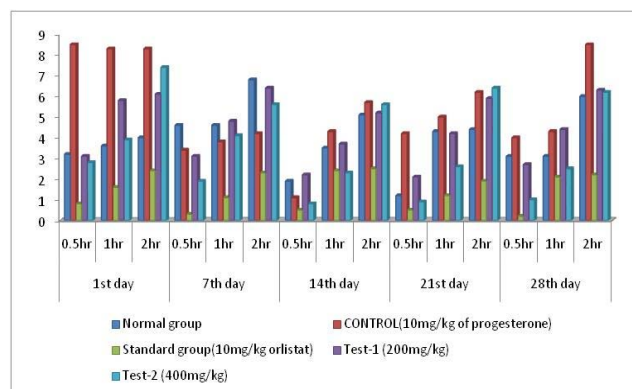
Evaluation

Body weight

The body weights every mouse (g) were recorded for every week for 28 days in each group just before dosing by using electronic weighing balance.

Assessment of food consumption behaviour in mice

It is very important to assess the food consumption behaviour of all organisms so the consumption behaviour was studied on days 1, 7, 14, 21, and 28. On experimental days, 30 min after last drug administration, 10 g of sweetened chow was presented to groups of mice in glass Petri dishes and food intake was recorded at 0.5, 1, and 2 h time intervals. Nearest to 0.1 g with correction for spillage and the amount of food consumed/20 g body weight was calculated.¹⁶



Graph 5: Effect of methanolic extract of pericarps of flowers of *Sapindus emarginatus* on food consumption behaviour in progesterone induced Obesity albino mice.

Table 1: Effect of methanolic extract of pericarps of flowers of *Sapindus emarginatus* on food consumption behaviour in progesterone induced Obesity albino mice.

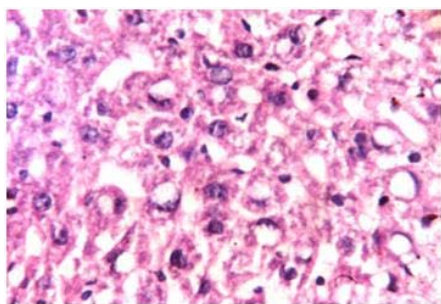
Group	1 st day			7 th day			14 th day			21 st day			28 th day		
	0.5hr	1hr	2hr	0.5hr	1hr	2hr	0.5hr	1hr	2hr	0.5hr	1hr	2hr	0.5hr	1hr	2hr
Normal group	3.2±0.12	3.6±0.24	4±0.21	4.6±0.08	4.6±0.15	6.8±0.23	1.9±0.16	3.5±0.15	5.1±0.2	1.2±0.34	4.3±0.15	4.4±0.4	3.1±0.41	3.1±0.15	6±0.12
CONTROL (10mg/kg of progesterone)	8.5±0.16	8.3±0.11	8.3±0.23	3.4±0.12	3.8±0.05	4.2±0.12	1.1±0.21	4.3±0.15	5.7±0.24	4.2±0.26	5±0.15	6.2±0.37	4±0.12	4.3±0.16	8.5±0.28
Standard group (10mg/kg orlistat)	0.8±0.21	1.6±0.16	2.4±0.22	0.3±0.13	1.1±0.16	2.3±0.34	0.5±0.3	2.4±0.12	2.5±0.16	0.5±0.12	1.2±0.14***	1.9±0.15***	0.2±0.16**	2.1±0.16***	2.2±0.38** *
Test-1 (200mg/kg)	3.1±0.22	5.8±0.15	6.1±0.14	3.1±0.16	4.8±0.11	6.4±0.21	2.2±0.12	3.7±0.19	5.2±0.11	2.1±0.41	4.2±0.16	5.9±0.27	2.7±0.24	4.4±0.33	6.3±0.41*
Test-2 (400mg/kg)	2.8±0.14	3.9±0.19	7.4±0.09	1.9±0.14	4.1±0.12	5.6±0.14	0.8±0.27	2.3±0.16	5.6±0.04	0.9±0.35	2.6±0.12**	6.4±0.15	1.0±0.18	2.5±0.35	6.2±0.12*

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01 and * P<0.05.

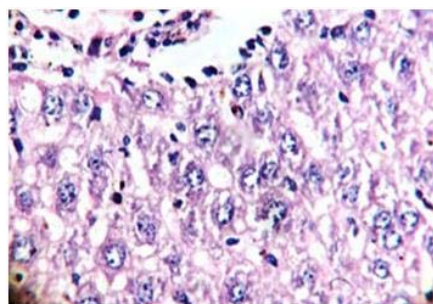
Table 2: Effect of methanolic extract of pericarps of flowers of *Sapindus emarginatus* on Biochemical parameters in progesterone induced Obesity albino mice.

Treatment	Glucose(mg/dl)	TC(mg/dl)	HDL-C	LDL-C	VLDLC	TG(mg/dl)	SGOT(U/L)	SGPT(U/L)
Normal	118.31±3.6	105.6±4.3	29.61±2.00	58.24±0.8	14.2 ± 0.9	79.30±1.30	128.54±1.25	65.±1.5
Control(10mg/kg of progesterone)	186.0±4.3	142.6±2.5	16.75±1.2	74.04±1.5	22.23 ± 0.18	152.9±1.6	190.0±0.7	90.21±1.45
Standard group(10mg/kg orlistat)	125.8±4.4***	120.6±1.5***	24.05±2.62**	40.2±2.8***	11.11 ± 0.12	90.6±2.0***	134.7±1.65***	52.04±2.1***
Test-1 (200mg/kg MESM)	150.0±4.0***	128.9±2.43**	27.14±0.4	60.4±1.08**	18.02 ± 1.5	120.2±2.4**	150.5±4.15***	65.9±1.11**
Test-2 (400mg/kgMESM)	135.3±2.6***	122.6±2.13**	25.14±1.09**	50.32±1.81**	14.15 ± 1.02*	110.9±1.55**	139.8±2.20***	54.4±2.0**

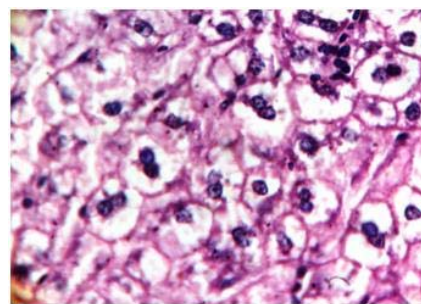
Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01 and * P<0.05, MESM=Methanolic extract of pericarps of flowers of *Sapindus emarginatus*.



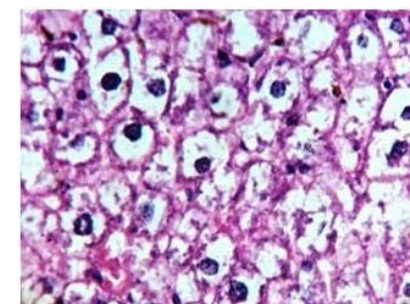
A) Control (10mg/kg of progesterone)



B) Standard group (10mg/kg orlistat)



C) Test-1 (200mg/kg MESM)



D) Test-2 (400mg/kg MESM)

Histopathology Reports: a) Presence of the fatty changes in the liver cells and induction of obesity; b) In the case of standard drug treatment there was mild necrosis was found; c) In the case of test drug sample -1 focal necrosis and slight hepatic toxicity; d) In the case of test drug sample-2 there was a significant and increased focal necrosis and total absence of fat globules and this extract MESM had perfect decrease in the fat cells.

Rearing, grooming and ambulatory moments were studied.

Biochemical parameters

On 29th day the blood is collected from all the mice by retro orbital puncture method, and subjected for centrifugation at 3000 rpm immediately. The serum samples of all the blood samples were carefully collected and preserved for bio chemical estimation. Serum glucose levels, total cholesterol¹², LDL-C, HDL-C, VLDL-C, Triglycerides¹⁷, SGOT, and SGPT were estimated by using standard chemicals and ROBONIK biochemical analyser.

Histopathology of Liver

After the biochemical examination was over the animals were sacrificed for histopathology studies. The liver of every organism from all 5 groups were isolated for histopathology studies. The isolated livers were carefully kept in 10% formalin solution in order to prevent the damage.

Statistical analysis

The obtained results were expressed as mean±SEM. Comparisons between the treatment groups and control groups were performed by one-way analysis of variance (ANOVA) followed by Dunnett's test. In all the tests the criterion for statistical significance was $P < 0.05$ (95% level). P value < 0.05 is considered as significant ($*P < 0.05$, $**P < 0.01$).

RESULTS

In the study of anti obesity activity of methanolic extract of pericarps of flowers of *Sapindus emarginatus* we were studied food consumption behaviour, body weight, grooming, rearing, ambulatory moments and examined serum glucose, total cholesterol(TC), triglycerides(TG), low density lipoproteins(LDL), very low density lipo proteins(VLDL), high density lipoproteins(HDL), SGOT, SGPT and these results were compared with obesity control group and statistical analysis was done by using graph pad prism software and found that the results were significant.

DISCUSSION

The body weights of all animals were recorded from the first day to 28th day. From the graph-1 the control group mice body were increased from day 1 to day 28, but the test and standard drug using groups' mice were sustained and decreasing gradually. Along with body weight the grooming, rearing, ambulatory moments and food consumption behaviour were also examined from day 1 to day 28 to test the test drug activity and these test and standard drug results were compared with the obesity control group and the readings were mentioned in the graph-2,3,4 and table-1. As I discussed earlier the Progesterone which was used in the screening of anti obesity activity as an obesity control is not only showed weight gain by hyperphagia but also exceeded the deposition of fat too. That was the main reason why we

have choose this as a obesity control. The serum glucose, total cholesterol, triglycerides, LDL-C, HDL-C, VLDL-C, SGOT, SGPT were examined by using standard reagents and ROBONIK biochemical analyser and the results were tabulated in table-6. All the results were compared with the control group to find significance. Orlistat of 10mg/kg body weight was used as a standard drug in the present study. The obtained results had shown the significant results. The histopathology of liver was conducted by using H&E stain and it clearly showed the excessive fat deposition.

CONCLUSION

The present study is the evaluation of anti-obesity activity of methanolic extract of pericarps of flowers *Sapindus emarginatus* shows significant anti obesity property by the obtained significant results. So we suggest it for the further level of examination.

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