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Preclinical evaluation of *Sesbanian grandiflora* flower extract for antihyperlipidimic and antiobesity activity on experimental rats

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ABSTRACT

Obesity is a chronic disorder of global prevalence and associated with morbidity and mortality. So the attention is being focused on the investigation of plant based drug used in the traditional medicine for the treatement of obesity .The present study is undertaken to evaluate the anti- hyperlipidimic and anti-obesity activity of *Sesbania grandiflora* flower extract in High Fatty Diet induced Obesity in rats. Female wistar rat weighing 150-200 g were divided into different groups i.e. normal control, Negative control [Hfd control], orlistat [STD control], extract of sesbania grandiflora flower contain 200mg/kg and 400mg/kg group. .Obesity was assessed by measuring biochemical parameters such as glucose, triglyceride, serum cholesterol, HDL [High Density Lipoprotein], LDL [Low Density Lipoprotein] level. The results of the present investigation demonstrated that, the extract of sesbania grandiflora flower at 200mg/kg and 400 mg/kg shows significant protective effects on biochemical parameters such as body weight, BMI, obesity index, and adiposity index respectively as compared to HFD [High Fatty Diet] control group. Similarly, serum glucose, triglyceride, total cholesterol HDL, LDL was found to be attenuated as compare to HFD control group. The ethanolic extract of sesbania grandiflora flower exhibit significant anti-hyperlipidemia and anti- obesity activity in High fatty diet induced in obese rat.

Keywords: HFD [High fatty diet], sesbania grandiflora flower extract, anti- obesity, Anti –Hyperlipidimic Activity.

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INTRODUCTION

Obesity is the condition in which fat accumulates the body, which exhibits adverse effect on health and life expectance. According to the WHO, obesity is one of the five leading causes of death all around the world. It is chronic disorder caused by genetic and environmental factors.[1] It characterized by high cholesterol level indicated by increase in level of fatty acid; alteration in metabolism; insulin resistence;lethargy ,stone in gall bladder , hypertension, breathing deficiency; cancer , liver disorder arthritis. Overweight and obesity are the most common nutritional disorders in developed countries. Individuals having BMI between 25 and 30/ kg/m^2 are defined as obese [2]. Obesity accompanied by hyperlipidemia which is indicated by abnormally high concentration of lipid in blood. Nowadays [3,4] , change in human lifestyle and high energy diet have been increased the incidence of obesity and even have become risk factor to the population. There are several pharmacological substances available as antiobesity drugs, however they have several hazardous effect and hence natural products have been used for treating obesity in many Asian countries.[5] The potential of natural products for the treatement of obesity is still largly unexplored and can be excellent for the safe and effective development of antiobesity drug.[6]

Currently the drug available in the market for treatement of obesity can be divided into two measure classes one being orlistat which reduces the fat absorption of [7,8] pancreatic lipase and second is subutramine which is an appetite suppresment. The synthetic drug having high cost, and it show the potentially hazardous side effect, So the need of natural products against the obesity is under exploration [9] which may be an alternative strategy for developing effective, safe antiobesity drug. The antiobesity effect of natural products from more diverse source. Sesbania grandiflora scientifically reported to wound healing activity, Antimicrobial activity, Antioxidant, Antiulcer Activity[10]. .posses; there is also reported paper for its hypolipidemic

activity. Hyperlipidemia is one of the causes for the development of obesity and Sesbania grandiflora has reported for its hypolipidemic purpose while there is no any scientific report available for its antiobesity activity. Thus in the present investigation, the plant extract of Sesbania grandiflora has been used to evaluate its antihyperlipidemic and antiobesity activities in high fatty diet induce obese rats.

Materials and methods

Plant Material :

Fresh flowers of Sesbania grandiflora were collected from the local area of Pune and the collected in the month of October and November and authenticated by Dr Gautam, Botanist , Pune, India .The sample was identified at the national herbarium where some specimen was already available with the number B/II/293/0206776.

Preparation of plant extract:

A weighed quantity (50g) of the air-dried powdered flowers of sesbania grandiflora was drawn and then it was extracted with 90% ethanol in a Soxhelt extractor. The hydroalcoholic extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50˚ C to get a solid residue. Different concentration (200mg/kg and 400 mg/kg p.o.) of hydroalcoholic extract of flowers of *Sesbania grandiflora* was given according to body weight of animals.

Experimental Animals

The present study, wistar rat of female 200-250mg/kg body weight were used. Under the standard environmental condition of the temperature 25± °C, with a 12 h light/dark cycle and humidity around (50 ± 5) %. During acclimatization, the rats were randomized into experimental and control groups and housed individually in sanitized cages housed with sterile husk as bedding. The animals had free access to food and water ad libitum throughout study. All experimental procedures were carried out between 9-16 hours with the Institutional Animal Ethical Committee approved protocol (CPCSEA/IAEC/PC-06/01-2K18) for the study.

High fat diet induced obesity in rat

In the present investigation, obesity in experimental animal was induced by daily use of high fatty diet (Table 1). HFD is reported to result in obesity and its associated condition such as metabolic alterations, hyperphagia reduced lipolytic activity in fat tissue , reduction in leptin secretion and or sensitivity, hypothalamic neuron apotosis impairement of mitochondrial metabolism and insulin resistance.

Table 1: Composition of High Fatty Diet

S.N.	Ingrediant	Quntity
1	Cholesterol	10 G/KG
2	Casine	280G/KG
3	Lard/Tallo Oil	300G/KG
4	Starch	260 G/KG
5	Cellulose	50 G/KG
6	Soya Oil	50 G/KG
7	Vit + Minrals	50 G/KG

Experimental induction of obesity in rat:-

For induction of obesity, the animals were fed with HFD for 6 week followed by drug treatments (*S.Grandiflora* flower extract, std orlistat). After confirmation of significant obesity, the animals were group into different group each contains 6 rats. In the second phase, the animals were treated with Sesbania grandiflora (200 and 400 mg/kg p.o.) and standard Orlistat IR capsule (30 mg/kg) for further 6 week along with HFD. While the normal control animals were fed with standard plain diet. Antiobesity and antihyperlipidemic activity was confirmed by evaluating the parameters such as lipid profile, daily food intake and body weight respectively. At the end of the study all the animals were sacrificed and liver was isolated for histopathological examination.

Body weight:

The body weight (gm) was recorded on day one and then weekly consecutively for 49 days using a digital weighing balance. In addition to this, the daily food intake for each group was measured Weekly for 49 days.

Biochemical Estimations

On the 49th day of the experiment all the animals were sacrificed by cervical dislocation, and blood samples were collected by carotid bleeding separately into sterilized dry centrifugation tubes and allowed to stand for 30 minutes at 20–25°C. The clear serum was separated at 2500 rpm for 10 min using a centrifuge. The levels of serum glucose, total TC, HDL-C, TG, LDL-C, were determined to analyze using Chariot Prince Biochemistry Analyzer.

Relative organ weight

The above-mentioned organs were quickly removed and weighed individually. Each organ to body weight ratio (relative organ weight) was calculated as (weight of organ/body weight of rat on the day of sacrifice) *100%.

Histopathological Examination

Liver, excised from each treatment group were subjected to histopathological examinations. After fixing the tissues in 10 % formalin, they were dehydrated and mounted in paraffin blocks. The sections of 3-5 µ thickness were cut and stained with hematoxylin-eosin stain.

Statistical Analysis:

Statistical analysis was carried out using the GraphPad Instat 3. All of the data are shown as the mean ± standard error of the mean (S.E.M) and were analyzed using one-way analysis of variance (ANOVA). Significant differences between theHFD control and experimental groups were determined using Dunnet multiple comparison test, $P<0.001$ was considered significant.

Result

Effects of High-Fat Diet and *S. Grandiflora* flower extract on Food Intake and Body Weight

There was no significant difference in all of the treatment groups at the commencement of the study. However, animals feed with high-fat diet showed significant increase in body weight compared to those feed with normal pellet diet (NPD) . Similarly, the average daily feed intake of all the groups was the same at the start of the study; however, 28-day treatment with HFD+ treatment drug resulted in a slight decrease in body weight shown in table 2

Table 2: Body weight

Body weight of rats fed a high-fat diet and treated with S. Grandiflora flower extracts for 7 weeks; all values are expressed as Mean ± SEM, ($p<0.001$). (a) Significant difference compared to control; (b) significant difference compared to HFD, (one-way ANOVA, Dunnets multiple comparision test, per group). Following 2 week treatment with S Grandiflora supplementation, low food intake was observed in the S. Grandiflora 200 mg/kg and S .Grandiflora

400 mg/kg groups () when compared to the HFD group. Subsequently, the body weight gain (at days 0, 7, 14, 21, 28,] [29, 35, 42, and 49) in these groups was significantly lower than the rats feed with HFD+ treatement drug.

Table 3: food intake

Food consumption (kcal) in rats fed a high-fat diet and treated withS.Grandiflora flower extract for 7 weeks; all values are expressed as Mean ± SEM, ($p<0.001$) (a) significant difference compared to control; (b) significant difference compared to HFD (one-way ANOVA, dunnets multiple comparision test.

Table No 4: Effect of S.Grandiflora flower extract on liver parameters and blood glucose in obese rats.

As mentioned in Table 4, it has been observed that rats feed on high-fat diet consecutively for 28 days resulted in a marked increase in the level of lipids, characterized by elevated levels of total cholesterol, triglycerides, LDL , and levels of HDL when compared to normal control, that is, rats receiving the normal feed. (Table 4) . No significant increase was found on the 7th and 14th days. However, treatment with S. Grandiflora flower extract for 3 weeks reversed the hyperlipidimic effect produced by high-fat diet significantly. Similar results were obtained with the standard drug [orlistat]. Further. Treatment with S. Grandiflora (200 mg/kg and 400 mg/kg) significantly reduced the body weight Effect of S. Grandiflora Extract on Blood Glucose It has been observed that rats receiving high-fat diet show a remarkable increase in blood glucose level evaluated on the 49th day . Treatment with extract 200 mg/kg and 400 mg/kg dose dependently decreased the blood glucose level (Table 4)

Effect of S. Grandiflora Extract on Organ Weight[Table no 5]

The untreated obese group showed significantly higher organ weight (liver) in comparison to the HFD group($p<0.05$). Conversely, groups treated with S. Grandiflora flower extract at 200 mg/kg and 400 mg/kg showed a significantly lower organ/body weight[Table 5]

Histopathology of Liver in HFD Treated Rats

Rats of control group fed with plain diet did not reveal any lesion of pathological significant at liver. Negative control group[HFD] rats fed with high fat diet showed multifocal moderate hepatocellular vacuolation at liver .Treatment of standard drug in rats fed with high fat diet showed multifocal minimal hepatocellular vacuolation at liver .Treatment of Test drug A in rats fed with high fat diet showed multifocal minimal hepatocellular vacuolation at liver. Treatment of Test drug B in rats fed with high fat diet showed multifocal mild hepatocellular vacuolation at liver. Data presented in this report suggestive of treatment at negative control group produces degenerative lesions in hepatocytes of liver. Treatment oftest drug at Standard, Drug A and Drug B group mitigates the lesions liver

Table 2: Effect On body weight and food intake hfd induced rat.

Treatement group	Parameter	0day	28 day	49 day
Control	Body weight	167.75±4.60**	234±2.34	218±6.64*
Hfd control	Body weight	166.75±1.75	279.25±1.65*	226±27.85
Std orlistat	Body weight	170.5±5.14	263.75±9.06**	231.25±5.83*
200 mg/kg extract	Body weight	168.5±4.66	260.75±3.83**	233.5±4.17*
400mg/kg extract	Body weight	174±7.49	277.75±3.49**	227.75±2.016**

Result are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnet multiple comparison test. *p<0.05 and **p<0.01 when obesity control compaird with different treated group

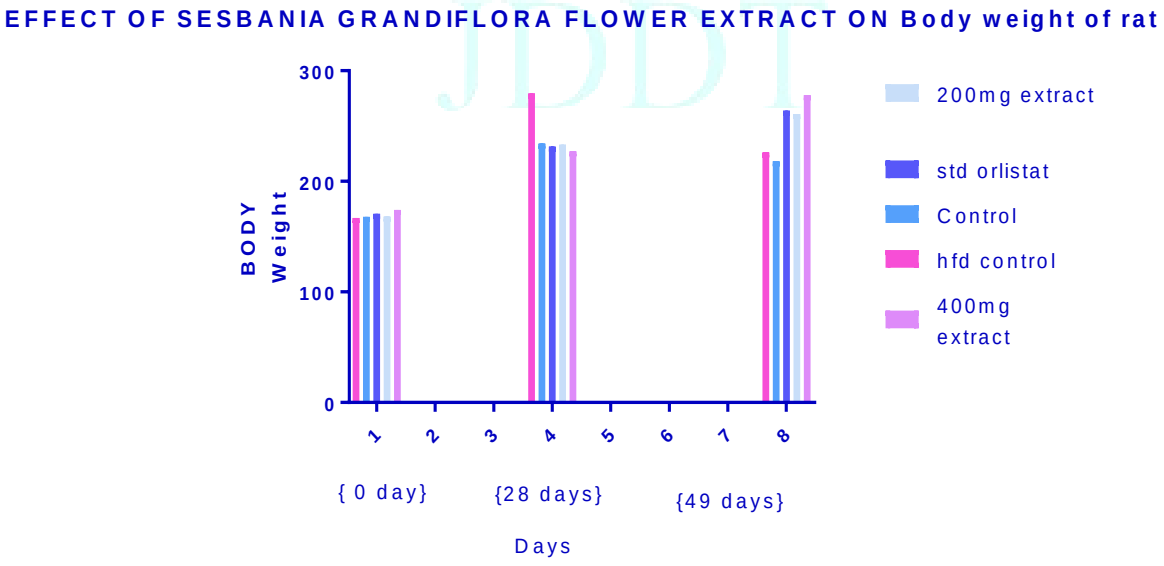


Figure 1: Body weight of rats fed a high-fat diet and treated with S.Grandiflora flower extracts for 7 weeks; all values are expressed as Mean ± SEM, ($p<0.001$). (a) Significant difference compared to control; (b) significant difference compared to HFD $P<0.001$, (one-way analysis of variance (ANOVA),Dunnets multiple comparision test, per group

Table 3: Food intake

Treatement group	Parameter	0day	28 day	49 day
Control	Food intake	17.65±0.15	16±0.34	18.18±0.92
Hfd control	Food intake	18.56±0.43	18.62±0.65*	20.23±0.63
Std orlistat	Food intake	16.70±0.27	13.48±0.92	17.95±0.98
200 mg/kg extract	Food intake	16.13±0.34	14±0.78	17.20±38
400mg/kg extract	Food intake	15.76±0.36	12.40±0.75	16.44±0.70

Result are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnet multiple comparison test. *p<0.05 and p>0.05when obesity control compaird with different treated group.

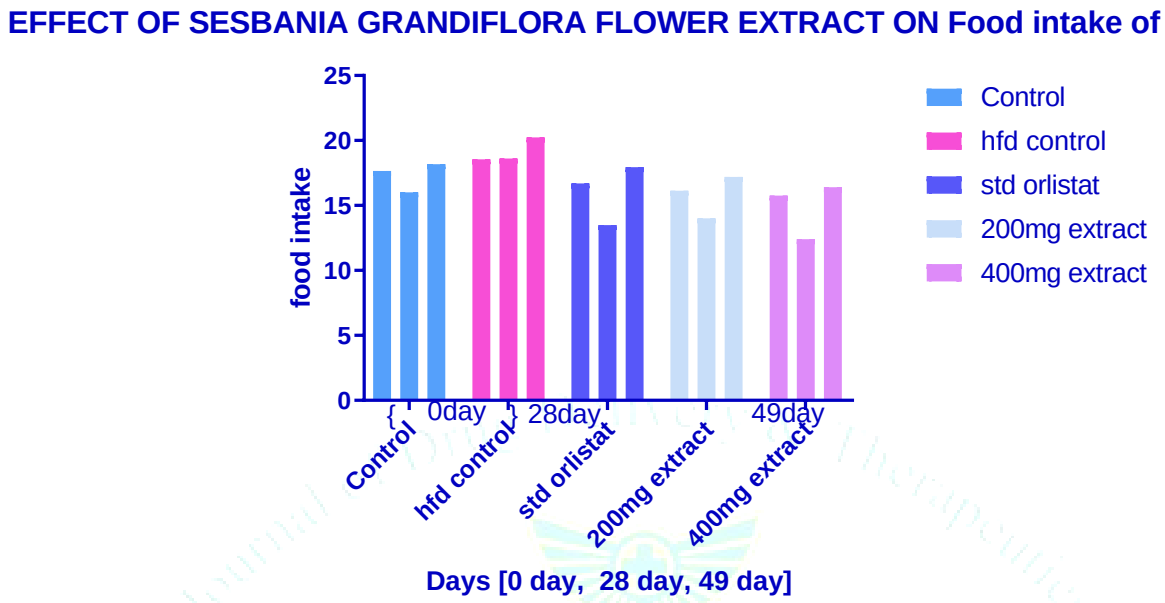


Figure 2: Food intake

Table 4: Effect of *S. Grandiflora* flower extract on liver parameters and blood glucose in obese rats

SERUM PARAMETERS	CONTROL	HFD CONTROL	STD ORLISTST	200MG/KG EXTRACT	400MG/KG EXTRACT
TOTAL CHOLESTEROL	** 50.7± 3.107	95.73±1.83	** 60.16±1.72	ns 88±1.45	** 60±4.57
TRIGLYCERIDE	** 86.13±2.78	222±29.66	** 104.9±1.704	** 127.33±2.839	** 161.23±2.40
HIGH DENSITY LIPOPROTEIN [HDL]	* 43.84±4.73	69.03±8.20	* 51.95±3.45	** 50.59±2.90	** 25.96±3.34
LOW DENSITY LIPOPROTEIN	** 9.66±0.29	19.64±0.98	* 11.93±0.48	** 17.12±1.17	ns 12.91±0.97
Blood glucose mg/dl	78.9±3.3**	94.1±4.2	83±7*	79.01±3.3ns	73.2±5.2***

Result are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnet multiple comparison test.** *p<0.001.** p<0.01 and p>0.05 ns when obesity control compaird with different treated group.

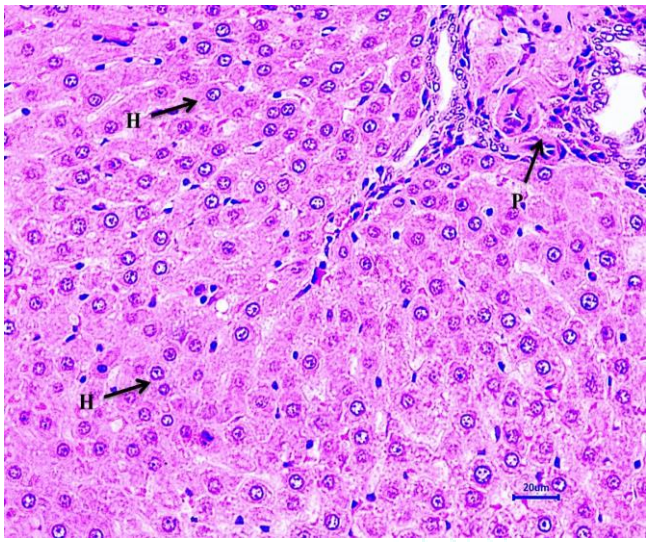
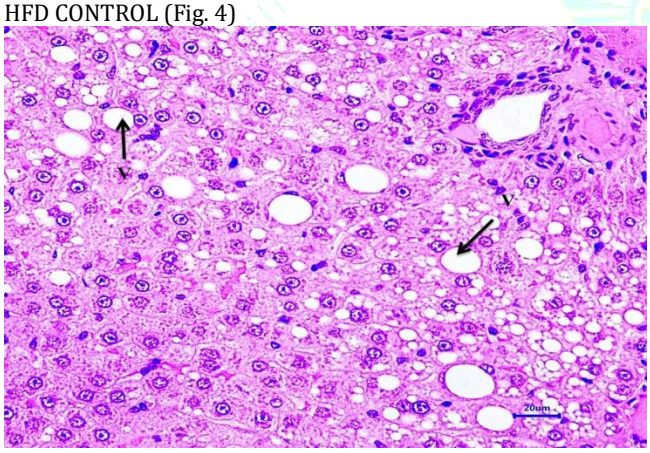
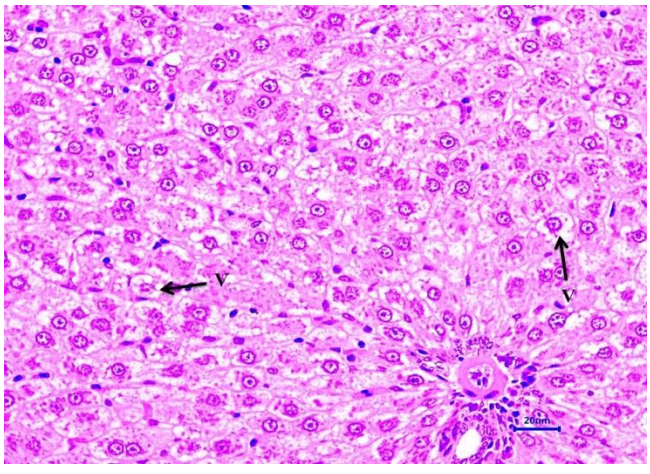
Table 5: Effect of sesbania grandiflora extract on organ weight

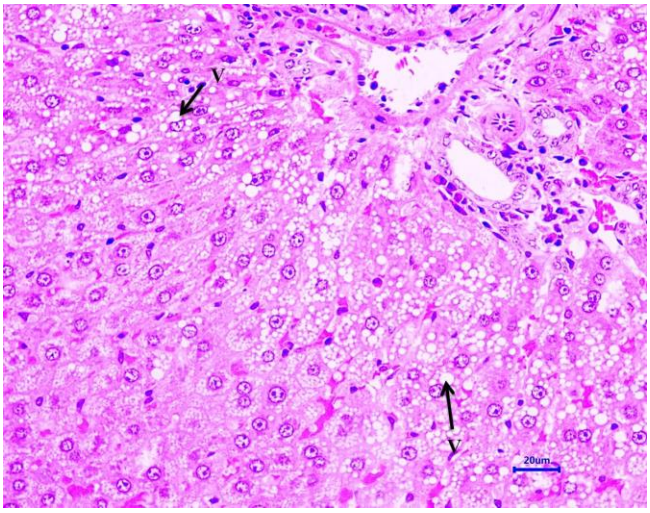
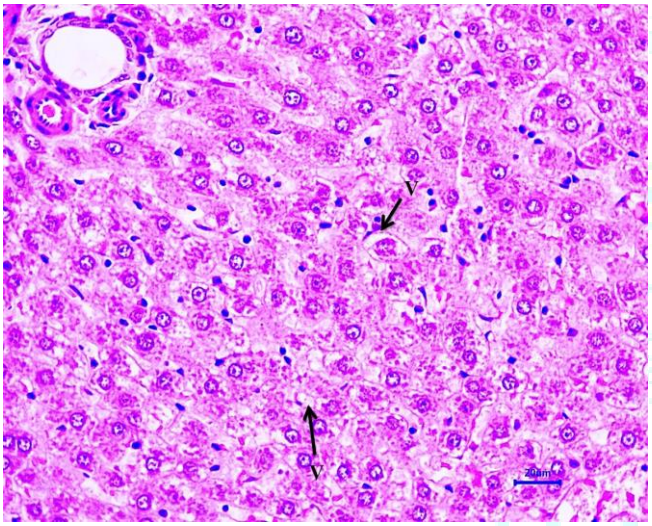
ORGAN WEIGHT	NORMAL CONTROL	HFD CONTROL	STD ORLISTAT	200MG/KG EXTRACT	400MG/KG EXTRACT
LIVER	5.79±0.088	8.89±0.92***	6.15±0.89**	7.56±0.77*	6.97±1.89**

Result are expressed as mean ± SEM. (n=6) Data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnet multiple comparison test.control* p<0.05.** p<0.01 and p>0.05 ns when obesity control compaird with different treated group.

Histopathological Examination

Microscopic examination of liver showed minimal to mild focal hepatocellular infiltration of inflammatory cells in female animals treated with extract at 200 and 400 mg./kg body weight when compared with control group (Figure 3).and standard group.[figure 7]

CONTROL (Fig. 3) 	Control liver showing normal histology Report P= portal triad H=Hepatocyte (H & E 400X)
HFD CONTROL (Fig. 4) 	HFD control liver shows the hepatocellular vacuolation (v){H & E , 400 X}
200MG/KG S. Grandiflora flower Extract (Fig.5) 	200MG/KG extract liver showing hepatocellular vacuolation {v} {H &E , 400X}

400mg/kg extract S. Grandiflora flower Extract (Fig.6)  	400MG/KG extract showing hepatocellular vacuolation {v} (H & E 400X)
STANDARD- Orlistat capsule (Fig. 7)  	Standard liver showing hepatocellular vacuolation {v}(H & E 400X)

On the basis of histopathology findings, it can be concluded that animals treated with extract *S. grandiflora* flower. Data presented in this report suggestive of treatment at negative control group produces degenerative lesions in hepatocytes of liver. Treatment of test drug at Standard [orlistat 30mg capsule], Drug A [*S. Grandiflora* flower extract 200mg/kg] and Drug B[*S. Grandiflora* flower extract 400mg/kg] group mitigates the lesions incurred in liver.

DISCUSSION

Obesity is a major risk factor for augmenteObesity is a major risk factor for augmented morbidity and mortality and is associated with various medical ailments [11]. High fat diet-induced obesity has been considered as the most popular model among researchers due to its high similarity of mimicking the usual route of obesity episodes in human [12] for studying obesity as they will readily gain weight when feed high-fat diets [13]. Human studies have revealed that increased fat intake is associated with body weight gain, which can lead to obesity and other related metabolic diseases. This study thus proved that rats exposed to high-fat diet for 2 weeks cause a significant increase of animals' body weight, thus verifying the obese status [14]. Although there was a significant difference in the body weights between the high-fat and normal diet groups, no significant difference was observed in the daily food intake of animals. This observation provides us with the fact that an increase in body weight is independent of the amount of food consumed by the animals. Treatment of HFD rats with S.Grandiflora flower extract at 200 mg/kg and 400 mg/kg p.o conversely causes a remarkable reduction of body weights was observed [15]. Further, treatment with s. grandiflora flower extract remarkably decreases the organ weight of rats feed on high-fat diet. Thus it proved the weight reducing potential ofS.Grandiflora flower extract. Further, dyslipidemia is another important hallmark in the pathogenesis of obesity characterized by hypertriglyceridemia with decreased level of LDL and VLDL [16]. Chronic dyslipidemia has been characterized as a major risk factor for cardiovascular risk, including atherosclerosis [17]. In the present study apart from reduction in weight, supplementation with S.Grandiflora flower extract and std orlistat, was observed to attenuate significantly the levels of total cholesterol and LDL and increased the level. of HDL level in rats feed with HFD. The increase in the level of HDL was found to be in a dose dependent manner; that is, supplementation with S.Grandiflora flower extract at a dose of 400 mg/kg shows a better effect in comparison to 200 mg/kg serum HDL level

and decreased levels of total cholesterol, LDL, and triglyceride. [18]. the histopathological studies were also performed. The literature review revealed that high fat diet-induced obesity and abnormal lipid metabolism consumption of high-fat diet may play a crucial role in the pathogenesis of fatty liver or hepatic steatosis associated with obesity depicted via ballooning degeneration. Elevated levels of liver enzymes are a monitor of hepatocellular damage and correlate with increased liver weight [19]. Histological studies are used as benchmarks for determining pathological changes in tissues and organs. Histological analysis of liver (Fig. 1) revealed no abnormalities in cellular architecture of these vital organs in the morphology of vital organs. This also supports our results that liver biomarkers were not elevated in groups treated with S .Grandiflora extract. S ..Grandiflora flowers have been widely used for the treatment of many ailments. Many studies have demonstrated their utility, including their biological activities, in vitro and their therapeutic benefits in rodents. The present study thus concludes that the extract of flowers of S.Grandiflora possess Anti-hyperlipidimic and antiobesity activity potential that protects the body against adverse effects of high fat diet-induced obesity. Further, we demonstrated that the daily supplementation of S.Grandiflora flower extract may reverse the formation of hepatic steatosis and nonalcoholic fatty liver disorder. The results in the present study established that high-fat diet causes elevation in body weight and reduces lipid metabolism as clearly seen by the marked elevation of liver enzymes and lipid level. However, supplementation with S.Grandiflora flower extract reverses all the parameters thus suggesting its weight reducing potential.

CONCLUSION

Thus, from the present study it can be concluded that the Ethanolic Extract of the S.Grandiflora flower extract is beneficial to the weight management, which supports its traditional claim. Further, studies are carried out in order to determine the active principle of this plant, followed by the identification of the mechanistic approach of S.Grandiflora that helps in weight management. This study showed that the administration of the S.Grandiflora flower extract to Wistar rats was not toxic in any of the tested doses. The extract did not have a direct impact on the liver and kidney functions as corroborated by results from hematological and blood sample of both sexes. Also the extract did not change in food and water intake. The histology examination revealed no remarkable changes in the internal organs, like liver, of the rats, in both control and treated groups. Furthermore, the data of acute and sub-acute toxicity studies on this plant were obtained in order to increase the confidence in its safety to humans for the use in the development of pharmaceuticals. The studies also need further experimental activities, like sub-chronic toxicity study, the effect of the extract on pregnant rats, foetuses, and their reproductive capacity to complete the safety profile of this plant.

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