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## Research Article

### Evaluation of Safety and Anti-obese Activity of a Polyherbal Formulation – Simlim Capsule

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#### Abstract

Obesity is one the most common and now considered as global health problem which triggers many other metabolic disorders. In the present study, a polyherbal formulation - Simlim Capsule was evaluated for its anti-obesity activity in high fat diet (HFD) induced male Wistar albino rats. It was also compared with modern medicine available in the market i.e. Orlistat. It was revealed that HFD elicited significant increase in body weight, daily food intake, and serum levels of glucose, total cholesterol, LDL Cholesterol, VLDL cholesterol, Triglycerides, ALT and AST level. Treatment with Simlim Capsule in dose of 180mg/kg (p.o) inhibited rise in body weight and daily food intake which shows its hypophagic activity. It also reduced lipid level such as triglyceride, LDL, VLDL and serum glucose levels while high density lipoproteins (HDL) was found increased which indicates its cardio protective activity. It has also significantly reduced ALT and AST level in serum. Histopathological changes of liver like hepatic steatosis induced by high fat diet was overcome by Simlim capsule and results were found comparable with Orlistat. Hence, it was concluded that Simlim Capsule is having anti-obesity activity by reducing cholesterol and triglyceride content, thus helping to reduce weight.

#### 1.0 Introduction

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. A crude population measure of obesity is the body mass index (BMI=weight in kg/height in m<sup>2</sup>). A person with a BMI of 30 or more is generally considered obese. A person with a BMI equal to or more than 25 is considered overweight.<sup>1</sup> Obesity is global problem and is a complex, multi-factorial disease that develops from the interaction between genotype and the environment. It involves the integration of social, behavioral, cultural, physiological, metabolic and genetic factors.<sup>2</sup> Excessive accumulation of fat in the body, general hypertrophy of adipose tissue etc., is the main reasons. The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended.<sup>3</sup> The estimate of the current global prevalence of obesity provided by the International Obesity Task Force (IOTF, 2007) states that there are over 300 million people worldwide who would be classified as clinically obese.<sup>4</sup> It is a major risk factor for a number of chronic diseases, including diabetes, cardiovascular diseases and cancer. Once considered a problem only in high income countries, overweight and obesity are now dramatically on the rise in low- and middle-income countries, particularly in urban settings.<sup>1</sup> There are limited numbers of allopathic drugs are available for treating obesity such as Sibutramine, Orlistat, Ribonobanat etc, but they have severe side effects on Cardiovascular and Central nervous system.<sup>5</sup> Whereas plant drugs and herbal formulations are considered to be less toxic and free from side effects than synthetic one and also complementing antiobese effect of each other by different mechanism of antiobese activity. Hence for obesity, many traditional plant treatments are used throughout the world.

Based on the WHO recommendations antiobese agents of plant origin used in traditional medicine are much important and the attributed antiobese effects of these plants are due to their ability to decrease the accumulation of adipose tissue in the body and increases in thermogenesis, also by reducing cholesterol and triglyceride content, thus help to reduce weight.<sup>6</sup> In Indian traditional system of medicine, herb base formulations are used as drug of choice rather than individual. Various herbal formulations are well known for their antiobese effects and the present investigation is undertaken to study the anti-obesity effect of one of such polyherbal formulation namely Simlim Capsule manufactured by "Vasu Healthcare Pvt. Ltd." in high fat diet induced obese animal model. The effects produced by this polyherbal formulation on different parameters were compared with a reference drug Orlistat.

#### 2.0 Materials and Method

##### 2.1 Experimental Animals

Albino Wistar rats (160–180 g) were procured from the Flair Lab, Surat, India. The animals were housed in standard polypropylene cages (three rats/cage) and maintained under controlled room temperature (22±2°C) and humidity (55±5%) with 12:12 h light and dark cycle. All the rats were provided with commercially available rat normal pellets diet (NPD) and water *ad libitum*, prior to the dietary manipulation. The guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India were followed and prior permission was sought from the institutional animal ethics committee IAEC. Protocol No. 984/11/07 for conducting the study.

##### 2.2 Drugs and Diagnostic Kits

Polyherbal formulation (Simlim capsule) was obtained from Vasu Healthcare, Baroda, Gujarat, India. Standard drug Orlistat was obtained from Intas Pharmaceuticals, Ahmedabad. The food

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ingredients such as cholesterol (Sigma Eldrich Lab, Mumbai, India), dl-methionine (Vadodara, India), vitamin and mineral mix (Sarabhai chemicals, Vadodara, India) and yeast powder (India) were procured from the commercial sources. Lard (U.K), and sodium carboxy methyl cellulose (Na-CMC) were also obtained from commercial sources. The compounds were administered orally as suspension by mixing with vehicle 1% Na-CMC. AST, ALT, glucose kits were procured from Span Diagnostics, Surat, India.

### 2.3 Acute Toxicity Study

Healthy female Wistar albino rats (210 – 240g) were divided into 2 groups of 3 animals each. The animals had free access to water and food throughout the experiment, except for the fasting period before the oral administration of the single dose of the Formulation. The formulation was suspended in distilled water and administered by gavages (orally) at single doses of 2000 mg/kg followed by single doses of 5000mg/kg. The general behaviour of the rats was continuously monitored for 1hr after dosing periodically during first 24 hr (with special attention given during the first 4 hrs.) and then daily for a total of the 14 days. Changes in the normal activity of rats, sign and symptoms of toxicity and mortality were monitored and recorded. Acute toxicity study was done as per OECD Guidelines 423<sup>7</sup>.

### 2.4 High fat diet Induced obesity in experimental rats

#### 2.4.1 Preparation of diet

High fat diet is a hyper caloric diet and was prepared by mixing the below constituents in fixed strength. The below mentioned weight is in g/kg of given substance. The food was prepared, dried, powdered and will be administered every day in morning to animals with water. Diet was administered and weight gain was observed in rats on alternate days, therefore confirming the development of obesity in rats. Study was continued for 40 days.<sup>8</sup>

**Table 1:** Composition of High Fat Diet

Ingredients Diet	g/kg
Powdered NPD	365
Lard	310
Cholesterol	10
Vitamin and mineral mix	60
dl-Methionine	03
Yeast powder	01
Sodium chloride	01

#### 2.4.2 Treatment protocol

Albino Wistar rats (160-180g) were given high fat diet for 40 days. Twenty four rats were randomly divided into 4 groups of six animals each. The following schedule of dose and diet administration in experimental groups was followed:<sup>9</sup>

- **Group I-** The animals were given CMC (carboxy methyl cellulose) solution as vehicle.
- **Group II-** The animals were given only high fat diet.
- **Group III-** The animals were given high fat diet and Suspension of Simlim capsule (180 mg/kg/day in CMC) by p.o route.
- **Group IV-** The animals were given high fat diet and Suspension of Orlistat (45mg/kg/day in CMC) standard drug by p.o. route.

#### 2.4.3 Collection of blood

On day 41 of the experiment the animals were anaesthetized by ether and blood was collected from retro orbital plexus in eppendorf tubes. The blood samples were allowed to clot for 45 minutes at room temperature. Serum was separated by centrifugation at 2500 rpm at 4°C for 15 min. and used for estimation of various biochemical estimations. After collection of blood samples, the rats were sacrificed by over dose of ether anesthesia and their liver, perinephric fat was excised, rinsed in ice-cold normal saline and preserved in 10% formalin solution for histopathological studies.

### 2.4.4 Observation Parameters

#### 2.4.4.1 Physical parameters

##### Body Weight

The body weight in (g) was recorded on day one and then on alternate days for 40 days using digital weighing balance.<sup>9</sup>

##### Food Intake

The daily food intake for group of 6 rats was measured daily for 40 days and expressed as mean daily food intake for group of 6 rats.<sup>9</sup>

#### 2.4.4.2 Biochemical parameters

##### Lipid Profile

- Serum total cholesterol (by CHOD-PAP method)<sup>10</sup>
- Serum triglyceride (by GPO-PAP method)<sup>11</sup>
- LDL Cholesterol (by the Friedewald formula)<sup>12,13</sup>
- VLDL Cholesterol (by the Friedewald formula)<sup>12,13</sup>
- HDL Cholesterol (by PEGCHOD- PAP method)<sup>14</sup>

##### Blood Glucose Level (by GOD POD method)<sup>15</sup>

##### Liver Function Tests

- Aspartate Aminotransferase –AST (Reitman and Frankel method)<sup>16</sup>
- Alanine Aminotransferase –ALT(Reitman and Frankel method)<sup>16</sup>

#### 2.4.4.3 Histopathology

Animals were dissected. Samples of Liver tissue were excised and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin and then with bovine solution. They were processed for paraffin embedding following the microtome technique. The sections were taken at 5µ thickness processed in alcohol-xylene series and were stained with alum-haematoxylin and eosin. The sections were examined microscopically for the evaluation of histopathological changes related to adipose tissue.<sup>9</sup>

### 2.5 Statistical analysis

The results were expressed as Mean ± S.E.M. The Statistical Analysis was carried out using analysis of variance (ANOVA) followed by Dunnet's test. p values <0.05 were considered as significant. The entire statistical analysis was performed using statistical package called Graph pad Instate Software.<sup>9</sup>

## 3.0 Results and Discussion

### 3.1 Acute Toxicity Study

#### 3.1.1 Clinical Signs of Intoxication and Mortality

In the preliminary acute toxicity study, Simlim Capsule seems to be safe at 2000 mg/kg and 5000 mg/kg dose. 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. Cage side observation and mortality record was represented in Table 2 and 3.

**Table 2:** Cage side observations of animals

Sr. No.	Parameters	Observations	
		2000 mg/kg	5000 mg/kg
1	Condition of fur	Normal	Normal
2	Skin	Normal	Normal
3	Subcutaneous swelling	Nil	Nil
4	Eyes dullness	Nil	Nil
5	Eyes opacities	Nil	Nil
6	Color and consistency of faeces	Normal	Normal
7	Condition of teeth	Normal	Normal
8	Breathing abnormalities	Nil	Nil

**Table 3: Mortality Record**

Group	Dose (mg/kg body wt)	Mortality
I	2000	0/3
II	5000	0/3

3.2 Evaluation of Anti-obese Activity of Simlim Capsule

3.2.1 Physical parameters

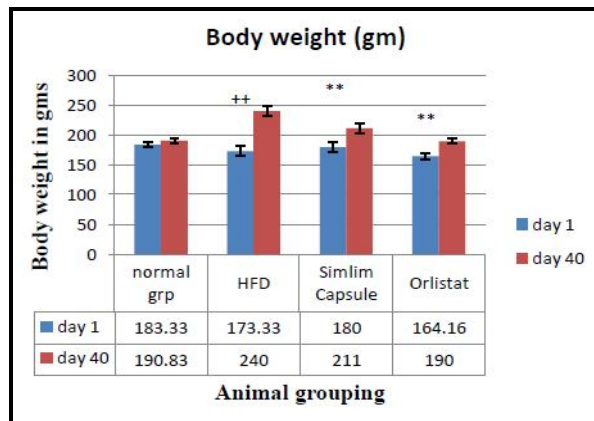
3.2.1.1 Effect of Simlim Capsule on Body Weight

A gain in body weight is a common index of obesity. High-fat diet food for 40 days to all animals except control group resulted in increase in body weight. Significant weight gain was observed in high fat diet treated group (group-II) as compared to normal group (group I). In Simlim capsule (group III) treated group and Orlistat treated group, significant inhibition of weight gain was observed as compared to group II. This result indicates that Simlim treated groups significantly reduces the increase in body weight induced by the high-fat diet, a clear sign of an anti-obesity effect. Results are shown in Table 4 and Fig 1.

**Table 4: Effect of Simlim capsule on Body Weight in rats**

Sr. No.	Groups	Treatment	Weight on day 1 (g)	Weight on day 40 (g)	Weight gain (g)
1	I	Normal control	183.33±4.410	190.83±4.549	7.5±1.118
2	II	High fat Diet	173.33±7.710	240.00±8.367	66.67±1.667 <sup>++</sup>
3	III	Simlim capsule	180.00±7.718	211.00±8.029	31.66±1.667 <sup>**</sup>
4	IV	Orlistat (Std)	164.16±4.729	190.09±4.472	28.53±1.537 <sup>**</sup>

All the values are expressed as mean ± SEM (n=6) in each group. Statistical significance test for comparisons was done by ANOVA, followed by Dunnet's test. + p< 0.05, ++p<0.01 considered statistically significant compared to Normal control (group I) while \*p< 0.05, \*\*p<0.01 considered statistically significant compared to HFD group (group II).



**Fig 1: Effect of Simlim capsule on Body Weight in rats**

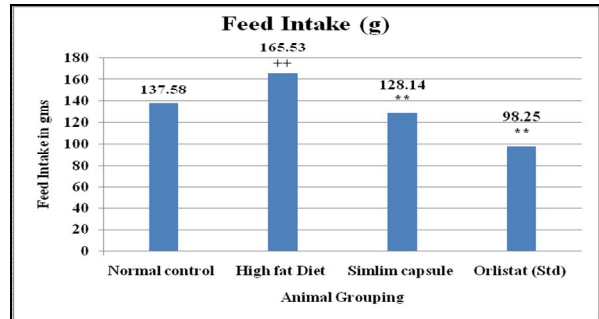
3.2.1.2 Effect of Simlim Capsule on Food intake

Whether or not Simlim capsule influenced food intake in rats as compared to the high-fat diet only was investigated by the measurement of food intake. Group II animals fed on HFD showed significant (p<0.01) increase in daily food intake when compared with group I (normal control) animals. Treatment with Simlim Capsule (180mg/kg/day) showed significant (p<0.01) decrease in daily food intake as compared with group II animals. Results are shown in Table 5 and figure 2.

**Table 5: Effect of Simlim capsule on daily Food Intake in rats**

Sr. No.	Group	Treatment	Food Intake (g)
1	I	Normal control	137.58±0.6982
2	II	High fat Diet	165.53±2.294 <sup>++</sup>
3	III	Simlim capsule	128.14±1.087 <sup>**</sup>
4	IV	Orlistat (Std)	98.25±2.428 <sup>**</sup>

All the values are expressed as mean ± SEM (n=6) in each group. Statistical significance test for comparisons was done by ANOVA, followed by Dunnet's test. + p< 0.05, ++p<0.01 considered statistically significant compared to Normal control (group I) while \*p< 0.05, \*\*p<0.01 considered statistically significant compared to HFD group (group II).



**Fig 2: Effect of Simlim capsule on daily Food Intake in rats**

3.2.2 Biochemical parameters

3.2.2.1 Effect of Simlim Capsule on Serum lipid profile

Group II animals fed with HFD exhibited significant (p<0.01) increase in total cholesterol, TG, LDL and VLDL when compared with group I animals. Group III and Group IV animals exhibited a significant (p<0.01) decrease in total cholesterol, TG, LDL and VLDL level when compared with Group II animals. The Group II animals exhibited significant (p<0.01) reduction in HDL cholesterol when compared with Group I animals. Group III and IV animals exhibited significant (p<0.01) increase of HDL when compared to Group II animals. Results are shown Table 6 and Figure 3 to Figure 7.

**Table 6: Effect of Simlim Capsule on Serum lipid profile in rats**

Groups	Treatment	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)
I	Normal control	66.62±1.16	170.43±0.79	26.73±0.59	32.70±0.49	42.18±0.68
II	High fat Diet	156.66±1.26 <sup>++</sup>	252.33±3.74 <sup>+</sup>	45.69±0.74 <sup>+</sup>	53.90±0.99 <sup>++</sup>	31.51±0.78 <sup>++</sup>
III	Simlim capsule	100.71±1.57 <sup>*</sup>	202.40±1.67 <sup>*</sup>	35.99±0.68 <sup>*</sup>	43.92±0.87 <sup>*</sup>	37.11±0.73 <sup>*</sup>
IV	Orlistat (Std)	77.75±1.24 <sup>**</sup>	182.40±4.03 <sup>**</sup>	30.03±0.43 <sup>**</sup>	36.09±0.84 <sup>**</sup>	43.53±0.74 <sup>**</sup>

All the values are expressed as mean ± SEM (n=6) in each group. Statistical significance test for comparisons was done by ANOVA, followed by Dunnet's test. + p< 0.05, ++p<0.01 considered statistically significant compared to Normal control (group I) while \*p< 0.05, \*\*p<0.01 considered statistically significant compared to HFD group (group II).

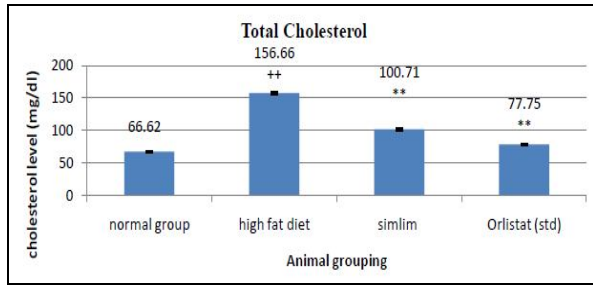


Fig 3: Effect of Simlim Capsule on Serum Cholesterol level in rats

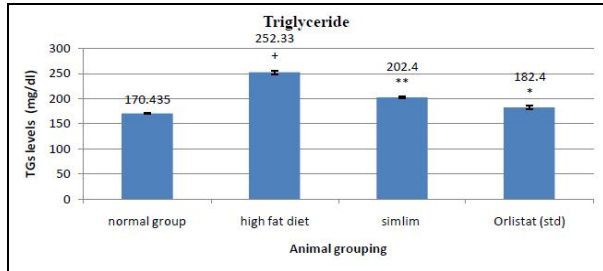


Fig 4: Effect of Simlim Capsule on Serum triglyceride level in rats

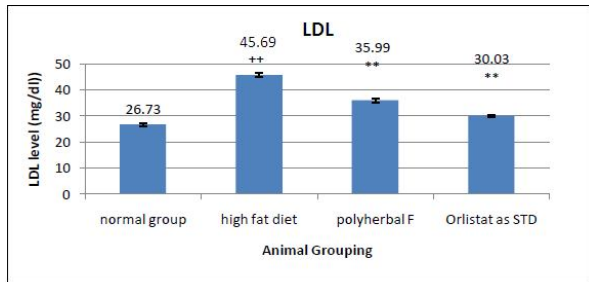


Fig 5: Effect of Simlim Capsule on Serum Low density lipoprotein (LDL) level in rats

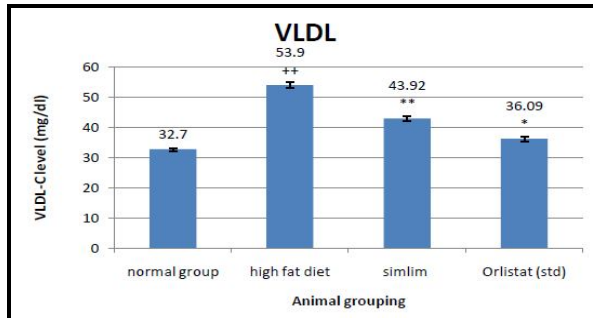


Fig 6: Effect of Simlim Capsule on Serum very low density lipoprotein (VLDL) levels in rats

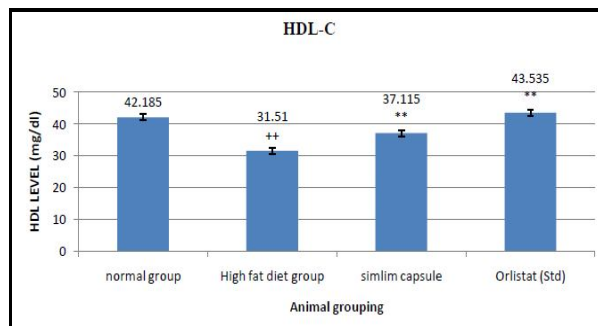


Fig 7: Effect of Simlim Capsule on Serum high density lipoprotein (HDL) level in rats

### 3.2.2.2 Effect of Simlim Capsule on Liver Function Tests and Blood Glucose

The levels of ALT, AST and Blood Glucose in Group II animals were significantly ( $p < 0.01$ ) increased when compared with group I animals. Group III and group IV animals significantly ( $p < 0.01$ ) inhibited the rise in AST and ALT level as compared to group II animals. Results are shown in Table 7 and Figure 8 to 10.

Table 7: Effects of Simlim capsule on Liver function test and blood glucose in rats

Groups	Treatment	AST Levels (IU/l)	ALT Levels (IU/l)	Blood glucose (mg/dl)
I	Normal control	36.31 ± 0.47	26.34 ± 0.66	94.45 ± 0.78
II	High fat Diet	65.27 ± 0.85 <sup>++</sup>	45.99 ± 0.38 <sup>++</sup>	194.36 ± 1.92 <sup>++</sup>
III	Simlim capsule	51.14 ± 1.11 <sup>*</sup>	37.26 ± 0.60 <sup>*</sup>	145.28 ± 9.4 <sup>*</sup>
IV	Orlistat (Std)	57.09 ± 1.00 <sup>ns</sup>	44.35 ± 0.10 <sup>ns</sup>	122.78 ± 6.54 <sup>ns</sup>

All the values are expressed as mean ± SEM (n=6) in each group. Statistical significance test for comparisons was done by ANOVA, followed by Dunnet's test. <sup>\*</sup>  $p < 0.05$ , <sup>++</sup>  $p < 0.01$  considered statistically significant compared to Normal control (group I) while <sup>\*</sup>  $p < 0.05$ , <sup>++</sup>  $p < 0.01$ , considered statistically significant compared to HFD group (group II) and <sup>ns</sup> is considered non significant i.e.  $p > 0.05$ .

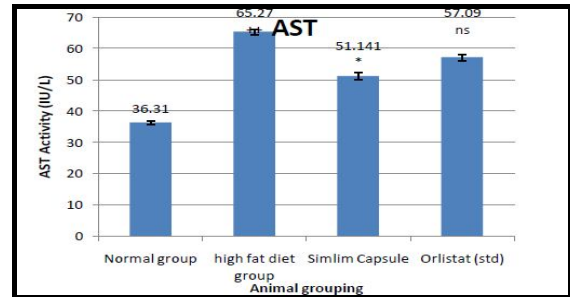


Fig 8: Effect of Simlim Capsule on AST

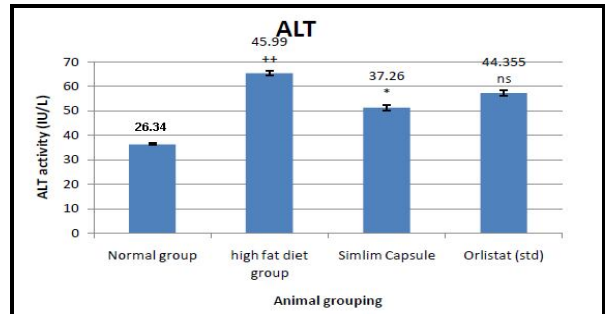


Fig 9: Effect of Simlim Capsule on ALT

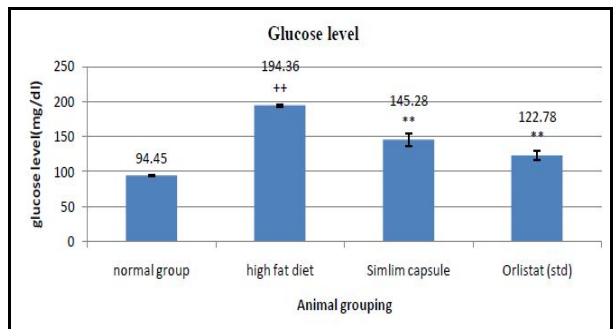
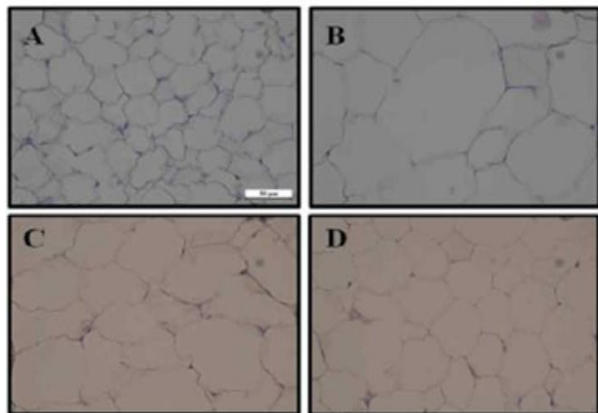


Fig 10: Effects of Simlim capsule on blood glucose level in rats

### 3.2.2.3 Histopathological Studies of Liver

In control group, section studied from the liver showed parenchyma with intact architecture. Most of the perivenular hepatocytes and periportal hepatocytes appear normal. In high fat diet treated group section studied from liver showed hepatic steatosis. Most of the hepatocytes showed apoptotic changes (B of Fig 11) while some show cytoplasmic vacuolations. In Simlim treated and Orlistat treated group, section studied from liver showed decrease in hepatic steatosis as compared to high fat diet group. (C of Fig 11)



**Fig 11:** Histopathology of liver showing adipose tissues

Here A=Normal control group, B=High Fat Diet group, C=Simlim Capsule treated group, D=Orlistat treated group

In the present study, the anti-obese activity of a polyherbal formulation Simlim Capsule manufactured by Vasu Healthcare Pvt. Ltd., Vadodara was studied using dietary animal's model of Obesity. The present pharmacological investigation revealed that HFD elicited significant increase in body weight, food intake, serum levels of glucose, total cholesterol, LDL Cholesterol, VLDL cholesterol, Triglycerides, ALT and AST level. Treatment with Simlim Capsule in dose of 180mg/kg inhibited increase in body weight resulted in reduction of body weight in HFD fed rats indicating that this poly herbal formulation possesses weight reducing property. Since obesity is associated with hyperphagia, HFD fed rats consumed more food than normal diet fed rats. Simlim Capsule is effective in decreasing daily food intake in HFD fed rats, indicating that it possesses hypophagic property.

Lipids are mostly consumed in the form of neutral fats, which are also known as triglycerides. The triglycerides are made up of a glycerol nucleus and free fatty acids and form major constituents in food of animal origin and much less in food of plant origin. Saturated fats increase blood cholesterol and thereby increase risk of atherosclerosis and coronary heart disease. Monounsaturated and polyunsaturated fats decrease blood cholesterol and reduce blood pressure. If there is risk of obesity, Trans fats increase LDL and increase risk of atherosclerosis and coronary heart disease.

In the present study Simlim Capsule showed significant reduction in serum total cholesterol levels, LDL, VLDL, triglycerides along with significant increase in serum HDL levels in HFD fed rats. Considering the increase of cardioprotective lipid HDL<sup>17</sup> and decrease in LDL, VLDL and TGs levels it can be concluded that Simlim capsule can act as a potent cardioprotective agent. Blood glucose levels were also significantly decreased in Simlim capsule treated groups as compared to high fat Diet group. The Enzymes ALT, AST were increased in group of animals treated with HFD and decreases the ALT, AST levels in serum were observed in Simlim and Orlistat treated groups. Histopathological changes of liver like hepatic steatosis induced by high fat diet was overcome by Simlim capsule and results were comparable with Orlistat.

Thus, observations from present study indicate that ingredients added in Simlim capsule are effective in decreasing food intake, lipid levels and liver enzymes.

### 4.0 Conclusion

The result of the acute toxicity test, for oral preparation of Simlim capsule formulation indicates that it is relatively safe and non-toxic to rats.

From the observations of the study performed, it could be predicted that Simlim capsule exerted significant anti-obese activity due to its hypophagic, hypoglycemic and hypolipidemic effect in rats fed on high fat diet. The long history of use of various ingredients of Simlim capsule may have therapeutic and protective applications in the treatment of cardiovascular disorders and also metabolic disorders. Further investigation involving measurement of enzymes in lipid pathways and hormones would ascertain the exact mechanism of anti-obese effect and can figure out the therapeutic potential of Simlim Capsule in the treatment of obesity.

### 5.0 Acknowledgement

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