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Acute and Repeated Dose (28 Day) Oral Toxicity Study of a Polyherbal Drug (Smartlean[®]) In Wistar Rats.

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ABSTRACT

The study was conducted to evaluate the acute and *repeated dose 28-day* oral toxicity study of Smartlean[®]. According to OECD Test No. 420 guideline the acute oral toxicity study was conducted in four groups of five animals each. Control group received 2% gum acacia 10ml/kg; second, third & fourth groups received one dose each of 54, 540 & 1080mg/kg smartlean[®]. The animals were observed for clinical signs of toxicity and/or mortality at 10-15min, 30-40min, 1, 2, 4 and 6hours on day one and thereafter once daily for 14 days. Four groups of animals with ten animals each were used for *repeated dose 28-day oral toxicity study*. The groups received 2% gum acacia 10ml/kg, 54, 540 & 1080mg/kg of smartlean[®] respectively, daily for 28 days. On 29th day blood was sent for biochemical, haematological investigation and histopathological examination of organs was done. None of the animals revealed any clinical signs of toxicity/mortality nor biochemical, haematopoetic or histopathological abnormalities in both acute and repeated dose 28-day oral toxicity study. The test item smartlean[®] is non-toxic up to and at 1080 mg/kg body weight which is 20 times higher than the recommended dose, in acute and *repeated dose 28-day* oral toxicity study.

Keywords: Smartlean[®], Acute oral toxicity study, *Repeated dose 28-day* oral toxicity study, wistar rats

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INTRODUCTION

Plant products are commonly utilized in developing countries for the treatment of various diseases especially chronic diseases, as an alternative to compensate for some apparent paucities in established pharmacotherapy. But there is only partial scientific evidence regarding safety and efficacy of herbal remedies to authenticate the sustained clinical use of these therapies. The rationale for their use in therapy is based largely on long-term clinical experience [1]. But now, with the upsurge in the use of herbal medicines, a thorough scientific investigation of these plants will go a long way in validating their folkloric usage [2].

Obesity is a chronic disorder due to majoretiological factors like sedentary life style, white collar jobs, lack of exercise, psychological stress and the consumption of energy rich diets [3, 4]. Prevalence of obesity is rising across the world, with three hundred million overweight people worldwide [5]. Research in this field has projected the evidence for the promising role of natural products in conjunction with regular exercise, dietary and behavioral modifications in treatment of obesity [6].

Smartlean[®] is a capsule, a poly herbal product produced by Sreedhareeyam Ayurvedic Medicine private limited, claimed to produce anti-obesity effects. However, its preclinical toxicity has not been studied yet. Earlier studies have been done to establish the safety profile of *Embelia ribes* [7], *Ficus racemosa* [8], *Ficus religiosa* [9] and *Terminalia chebula* [10], but the safety profile of the combination product is yet to be established.

The objective of this study was to assess the acute oral toxicity and repeated dose 28-day oral toxicity of Smartlean[®] when administered to wistar rats at defined doses.

MATERIALS AND METHODS

Study drug

The study drug, containing many herbal products in different concentrations was supplied by the manufacturer Sreedhareeyam Ayurvedic Medicine private limited. The botanical names of plant products used are as follows: *Embelia ribes*, *Ficus racemosa*, *Ficus lacor*, *Ficus bengalensis*, *Ficus religiosa*, *Zingiber officinalis*, *Piper longum*, *Piper nigrum*, *Emblica officinalis*, *Terminalia bellerica*, *Terminalia chebula*, *Pterocarpus marsupium*, *Acacia catechu*, *Curcuma longa*, *Plumbago zeylanica* and mineral Ferrous fumarate. The required quantity of smartlean[®] was suspended in 2% Gum acacia solution, shortly before dosing.

In-house bred wistar rats aged 10 – 12 weeks, weighing 150 – 200g were used. Animals were acclimatized for 7 days before initiation of study, housed in clean polypropylene cages covered with stainless steel top grill mesh having facilities for holding pelleted food and drinking water in water bottles fitted with stainless steel sipper tube. Animals were housed under standard laboratory conditions, room temperature $25 \pm 2^{\circ}\text{C}$, with 12 hours light and dark cycle. A maximum of three animals were housed in each cage. Water and feeds were provided *ad libitum* throughout the acclimatization and study period except two hours before & after dosing of the test drug, when only feeds were withdrawn. The study was conducted according to CPCSEA guidelines and after the approval of the Institutional Animal Ethics Committee.

Acute Oral Toxicity Study

For acute oral toxicity, Fixed Dose test procedure [11] was followed. In the sighting study, effects of various doses to a single animal of one sex was investigated in a sequential manner, and a fixed dose level of 54, 540 and 1080 mg/kg was chosen for the main study because of unavailability of sufficient toxicological data on the test item. Dosing was sequential, allowing at least 24 hours before dosing the next animal. Five female rats were allocated in each group of the three doses of smartlean[®] and in the control group which received 2% gum acacia solution. A single dose of smartlean[®] was given to each animal in a group and was observed for signs of toxicity and /or death at 10-15min, 30-40min, 1hr ($\pm 10\text{min}$), 2hr ($\pm 10\text{min}$), 4hr ($\pm 10\text{min}$) and 6hr ($\pm 10\text{min}$) following dosing on day 1 and thereafter once daily for 14 days. All the physical signs of toxicity were observed throughout the study period. The body weight of the animals was determined prior to dosing, and weekly thereafter. On the 15th day, all rats were fasted for 12 hours and then sacrificed by over dosage of

intraperitoneal sodium pentobarbitone (100mg/kg body weight) for necropsy examination. All rats in the study were subjected to a complete necropsy and the gross pathological changes were recorded.

Repeated Dose Oral Toxicity Study

A 28 day repeated dose oral toxicity study was conducted as per OECD guidelines for the testing of chemicals 407 (adopted October 3, 2008 with modifications)[12]. Forty adult wistar rats of both the sexes were allocated equally into three groups, ten in each group. Group 1 (control) received vehicle, Group 2, 3 and 4 received smartlean[®] 54 mg/kg, 540 mg/kg and 1080 mg/kg body weight orally for 28 days.

All the animals were observed for physical signs of toxicity, morbidity and mortality twice daily for 28 days. Body weights of the animals were recorded once a week and the amounts of daily food and water consumption were recorded. Haematological and biochemical parameters were done at the end of 28 days; all rats were fasted overnight and anesthetized for blood collection by cardiac puncture. Heparinized blood samples were taken for determining the haematological parameters which included haemoglobin, total RBC count, platelet count and total WBC count. Serum from non heparinized blood was used for assessment of biochemical parameters which included total serum protein, blood urea nitrogen, creatinine, ALT, AST, bilirubin and blood glucose.

On the 29th day, all rats were fasted for 12 hours and then sacrificed by over dosage of intraperitoneal sodium pentobarbitone (100mg/kg body weight) for necropsy examination. All rats in the study were subjected to a complete necropsy and the gross pathological changes were recorded. The internal organs namely liver, stomach, heart, lung, kidney were excised and sent for histopathological examination after preserving in 10% neutral buffered formalin. These tissues were embedded in paraffin wax, sectioned at five micrometres and stained with haematoxylin and eosin.

Statistical analysis

The results were expressed as mean \pm SEM. SPSS version 16 was used to analyze the data. Statistical tests One-way ANOVA followed by Dunnett test was used, a value of $P < 0.05$ was considered statistically significant.

RESULTS

Acute toxicity study

There was no significant sign of toxicity or mortality in both treatment groups and control group. The weight in all the groups increased steadily when compared to baseline values but without any significant difference in treated groups and control. At the dose of 1080 mg/kg body weight which is 50 times the therapeutic human dose when converted to rat dose there were no signs of toxicity nor mortality.

Repeated dose oral toxicity study

There were no treatment related signs of toxicity and mortality observed in both sexes of rats treated at 54, 540 and 1080 mg/kg orally for a period of 28 days. No significant difference in body weight gain was observed between control and treated groups during the study (Table 1). Feed and water consumption of treated groups were found to be insignificant in both the sexes when compared to the control. Hematological parameters such as haemoglobin, red blood cells, white blood cells, platelet count were found to be well within the physiological range of rats, table 2. The plasma biochemical profile is shown in table 3. There was no significant difference among different groups.

Table 1: Body weight of rats in different groups after 28 days of treatment

Duration of treatment in weeks	Mean body weight in grams \pm SEM			
	Control (G1)	Treatment Groups		
		G2 (54mg/kg)	G3 (540 mg/kg)	G4 (1080mg/kg)
0	150 \pm 2	150 \pm 2.6	150 \pm 5.3	154 \pm 1
1	155 \pm 1	154 \pm 2.3	155 \pm 4.9	159 \pm 0
2	160 \pm 1.5	159 \pm 2.9	159 \pm 3.8	165 \pm 1
3	163 \pm 0.5	164 \pm 2.3	162 \pm 3.8	169 \pm 1.5
4	167 \pm 0.5	169 \pm 1.9	169 \pm 1.5	173 \pm 1.5

Table 2: Haematological parameters in different groups after 28 days of treatment

Parameters	G ₁	G ₂	G ₃	G ₄
Hb (g %)	14.16 \pm 1.8	14.16 \pm 1.8	14.16 \pm 1.8	13.6 \pm 0.54
Total RBC(millions/cu mm) \pm SEM	7.85 \pm 0.55	7.2 \pm 0.06	8.32 \pm 0.46	7.02 \pm 0.37
Platelets (Cells/cu.mm)	766000 \pm 1	743670 \pm 1.41	771000 \pm 5	760900 \pm 5.35
Total WBC(Cells/cu.mm)	7400 \pm 1	7300 \pm 1.73	7833 \pm 2.03	7200 \pm 1

Table 3: Biochemical parameters in different groups after 28 days of treatment

Parameters	G ₁	G ₂	G ₃	G ₄
Total Serum Protein (g %)	6.0 \pm 0.7	6.4 \pm 0.3	6.6 \pm 0.23	6.8 \pm 0.6
BUN (mg %)	44.67 \pm 2.5	46 \pm 2.6	54.67 \pm 2.0	45.0 \pm 0.7
Creatinine (mg/dL)	0.43 \pm 0.03	0.37 \pm 0.67	0.37 \pm 0.33	0.43 \pm 0.33
ALT (IU/L)	60 \pm 3	69.3 \pm 3.3	75.3 \pm 10.3	79 \pm 4.7
AST (IU/L)	181.67 \pm 12.5	228.67 \pm 6.5	192.67 \pm 4.1	218 \pm 2.5
Bilirubin (mg/dL)	0.683 \pm 0.33	0.616 \pm 0.318	0.35 \pm 0.23	0.566 \pm 0.32
ALP (IU/L)	127.33 \pm 3.3	148.33 \pm 3.9	143.67 \pm 3.8	123 \pm 4.2
Glucose (mg%)	139.33 \pm 3.2	147.66 \pm 4.6	133.66 \pm 1.9	143.33 \pm 2.1

Necropsy and Histopathology: Gross pathology and histopathology of the tissues embedded in paraffin wax, sectioned at five micrometres and stained with haematoxylin and eosin did not show any significant changes.

DISCUSSION

Obesity is one of the non-communicable disease which is a health problem seen early in childhood itself. Various plant products are promoted for treatment of obesity, which are available for consumers in drug stores and online. One such polyherbal product available for treatment of obesity is smartlean[®]. In acute toxicity study, smartlean[®] at three different doses did not produce any mortality or behavioral changes. smartlean[®] did not show any signs of acute toxicity at high dose 1080 mg per kg body weight, double the converted therapeutic dose used in man. In 28 day repeated dose oral toxicity study, there was comparable increase in body weight measured at weekly interval in all groups. Food and water intake did not alter through the study period, or between control and treated groups. Biochemical parameters measuring the liver function like alanine amino transaminase (ALT), aspartate amino transaminase (AST) are serum marker enzymes to assess the injury to liver especially by drugs [13]. In the present study there is no significant difference in ALT, AST or alkaline phosphatase (ALP) enzyme levels in all three smartlean[®] treated groups when compared to control which suggests normal functioning of liver in smartlean[®] treated groups on repeated dosing. Parameters like serum urea, creatinine and total protein are indicative of proper functioning of kidneys. The values of serum urea, creatinine and total protein in different doses of study drug treated group was comparable to that of control group suggesting that there was no damage caused to the kidneys [14]. There was no significant change in different haematological parameters, suggesting the non-toxic nature of smartlean[®] to the circulating red cells, white blood cells and with production of RBC and platelets at different doses in repeated doses [15]. The gross weight and appearance and histopathology of all organs did not significantly differ from control at all dosage levels.

CONCLUSION

The test item smartlean® is non-toxic up to and at 1080 mg/kg body weight when administered in single dose and in daily for 28 days by oral gavage in wistar rats.

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