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Safety Evaluation of Sibutramine in Wistar Rats

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Abstract: Sibutramine is a novel anti-obesity drug, acts pharmacologically as both serotonin and nor-adrenalin reuptake inhibitor. Inhibitory effect of sibutramine on body weight and food intake was studied extensively and however, very sparse information available on toxicity concerns. Therefore the present study was designed to assess the toxicological properties of sibutramine. Male and female wistar rats were divided into groups of five rats each for main study and three rats each for toxicokinetic study and were gavaged with 0, 10, 30 and 100 mg/kg/day sibutramine for 7 days. During and at the end of the treatment period various toxicological parameters were studied. Toxicokinetic was performed on day 1 and 7. Significant reduction in body weight was observed in male and female Wistar rats at 30 and 100 mg/kg/day. Food intake was significantly decreased in both sexes at first 3 days in all dose levels and only in high dose at day 5. Reduction in reticulocytes count was observed in all treated rats and serum cholesterol was also decreased at 100 mg/kg/day sibutramine. Decreased peritoneal fat mass, small size spleen and thymus were observed on gross pathological examination during terminal sacrifice. No major treatment related histopathological changes were observed up to the high dose. In conclusion that there was no adverse effect of sibutramine up to 30 mg/kg/day in Wistar rats.

Key words: Sibutramine · Safety evaluation · Toxicity · Wistar rats

INTRODUCTION

Obesity is one of the most challenging life style diseases in both young and adult population. The incidence of this chronic disease increases with increasing weight (overweight), that often leads to serious health consequences like hypertension, hyperlipidaemia, type 2 diabetes, coronary artery diseases, stroke, gall bladder disease, musculoskeletal disorders and some cancers [1, 2]. The World Health Organization has recognized obesity as a global epidemic and estimates that, in 2005, there were more than 400 million obese adults and over 1.6 billion and 20 million overweight adults and children under the age of 5 years, respectively. This figure is projected to be almost double by the year 2015 [3]. USA leads the way with about two third of the population being obese, but the other countries are not far behind and it could reduce the life expectancy [4].

Several anti-obesity drugs like fenfluramine and dexfenfluramine had poor safety profiles led to withdrawl from market followed by FDA announcement [5]. Sibutramine was one of the few existing drugs used for treatment of obesity, which has also been taken out from market recently in light of clinical trial data pointing to an increased risk for stroke and myocardial infarction [6].

Sibutramine is a centrally acting anti-obesity drug, which combinedly inhibits serotonin (5-HT) and nor-adrenalin (NA) reuptake from synaptic clefts. Sibutramine predominantly work through its two pharmacologically active metabolites (i.e. primary and secondary amines), which induce marked weight loss by affecting both food intake and energy expenditure. It is able to enhance the physiological process of satiety [7, 8] and potentiates energy expenditure by stimulating thermogenesis [9-13].

There are adequate literatures available on efficacy of sibutramine in human beings, but paucity of information on toxicity and safety concerns in laboratory animals. In March 2002, Italian health ministry suspended marketing license for sibutramine because of reports of few death in human and after reviewing the risk benefits European Union regulatory authority reinstated the

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marketing authorization [14]. Hence, attempts have been made to explore short-term toxicity of sibutramine in Wistar rats. The main objective of this study is to determine effects of sibutramine on different parameters of toxicological importance following 7-day repeated dose oral administration.

MATERIALS AND METHODS

Test Substance: Sibutramine was supplied as a hydrochloride salt (purity 99.52 %) by Discovery Chemistry, Suven Life Sciences, Hyderabad, India.

Experimental Procedure: 7-week old healthy male (140-170 g) and female (120-160 g) Wistar rats were used for the study. After 5 days of acclimatization, they were divided into four groups of five rats each per sex for main study and satellite groups of three rats each per sex for toxicokinetic. The rats were gavaged with sibutramine hydrochloride dissolved in deionized water at 10, 30 and 100 mg/kg/day for 7 days and the control rats were administered deionized water. Rats were housed in individual cages kept in air-conditioned room at 22±3°C and 40- 70 % relative humidity on a 12 h light/dark cycle and were given food and water *ad libitum* through out the experimental period. This experiment was conducted in compliance with Institutional Animal Ethics Committee (IAEC), Suven Life Sciences, Hyderabad, India.

Cage side observations were made twice daily during acclimatization, thrice (prior to dosing, after dosing and once in the evening) during treatment period for any abnormal clinical signs and mortality. Body weight and food consumption were measured on day 1, 3, 5 during acclimatization and on day 1, 3, 5 and 7 during treatment period.

Overnight urine was collected from individual rat at termination and analysed for various urine analytes by using urinalyser (Clinitek, Siemens Diagnostics). At termination on day-8, rats were anesthetized with CO_2. Blood was withdrawn from retro-orbital sinuses for hematology and serum chemistry analysis. Hematological parameters v*iz.,* erythrocyte count, white blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, reticulocytes count and differential leukocyte counts were determined using Advia-120 hematology analyzer (Siemens Diagnostics). Blood and bonemarrow smears were prepared from each rat for cell morphological and M/E ratio examination respectively. Serum chemistry parameters v*iz.,* alanine amino transferase, aspartate amino transferase, alkaline phosphatase, gamma glutamyl transferase, creatine kinase, total bilirubin, glucose, total protein, albumin, globulin, high density lipoprotein, low density lipoprotein, cholesterol, triglyceride, urea, creatinine, calcium, phosphorus, sodium, potassium and chloride were analyzed using Randox Daytona auto analyzer (Randox Laboratories).

All the survived rats from main groups were sacrificed by using CO_2 anesthesia. Gross pathological examinations were carried out and organs were collected, fixed in 10% Neutral buffered formalin for histomorphological evaluation of tissue sections. Organs like liver, kidneys, heart, adrenals, thymus, spleen, brain, prostate, testes, ovaries, pituitary gland, thyroid (with parathyroid) were weighed and relative organ weights were calculated based on the fasting body weight on day-8.

Satellite groups kept for toxicokinetics were bled serially at 0.25, 0.5,1,3,5,7 and 24 h on day 1 and 7. Plasma samples were analysed for sibutramine by LC-MS/MS method.

Statistics: Data obtained from this study were subjected to one-way ANOVA and Dunnett's multiple comparison test using Graph pad prism software version 4, 2003 to identify groups were significantly different from the control at either $p<0.05$ or $p<0.01$ level.

ERESULTS

Animal Observation, Body Weight and Food Consumption: All rats treated with 10 and 30 mg/kg/day sibutramine survived during the experimental period and did not show any treatment related clinical signs. However, one male and three female rats treated with 100 mg/kg/day sibutramine died during day 3-5 of the treatment period. Clinical signs like increased / decreased motor activity, abdominal breathing, piloerection, gasping, emaciation, hunched back, hypothermia, tremors and self inflicted injuries on different parts of the body were also observed in rats treated with 100 mg/kg/day sibutramine.

On day 7, mean body weight and food consumption on sibutramine treated groups was lower than control (Fig.1, 2, 3 and 4). Low or no gain in body weight was observed in rats treated with 10 or 30 mg/kg/day sibutramine. There was body weight loss in males (18.09 g) and females (29.38 g) treated with 100 mg/kg/day

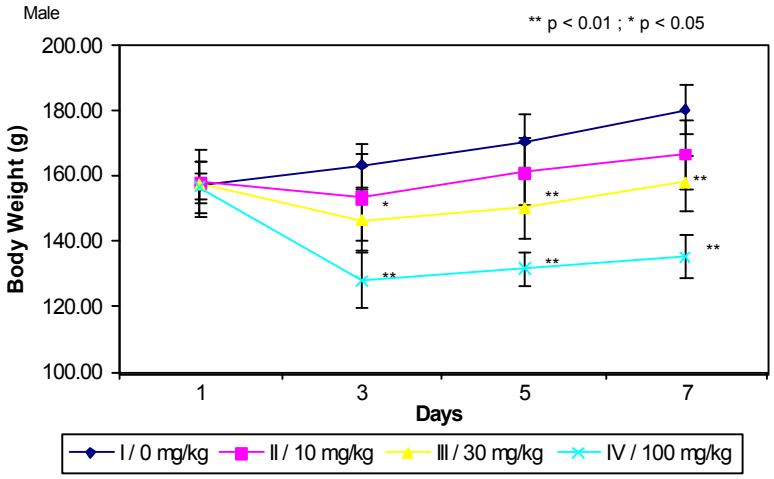

Fig. 1: Body weights of male Wistar rats following 7 day oral exposure of sibutramine. Values are mean ± SD. n= 5 /dose group except for 100 mg/kg/day n= 4-5.

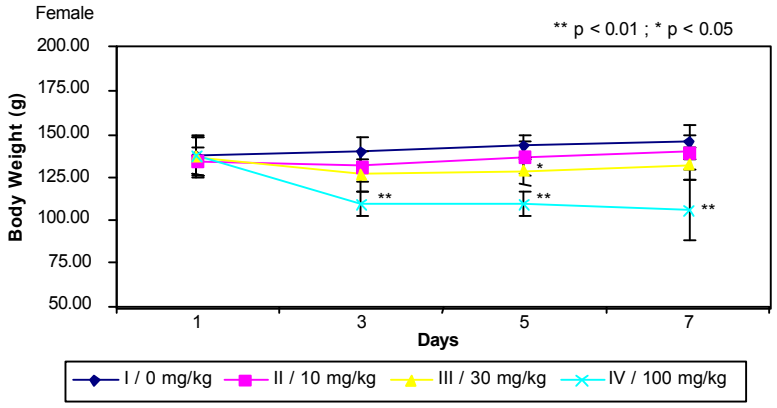

Fig. 2: Body weights of female Wistar rats following 7 day oral exposure of sibutramine. Values are mean ± SD. n= 5 /dose group except for 100 mg/kg/day n= 2-5.

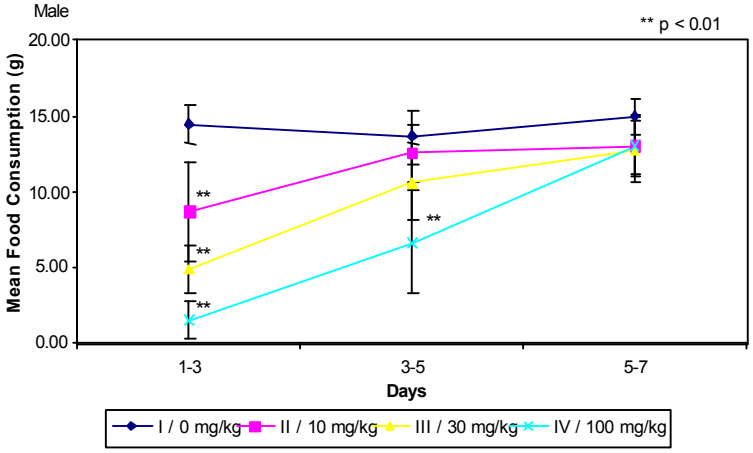

Fig. 3: Food consumption of male Wistar rats following 7 day oral exposure of sibutramine. Values are mean ± SD. n= 5 /dose group except for 100 mg/kg/day n= 4-5.

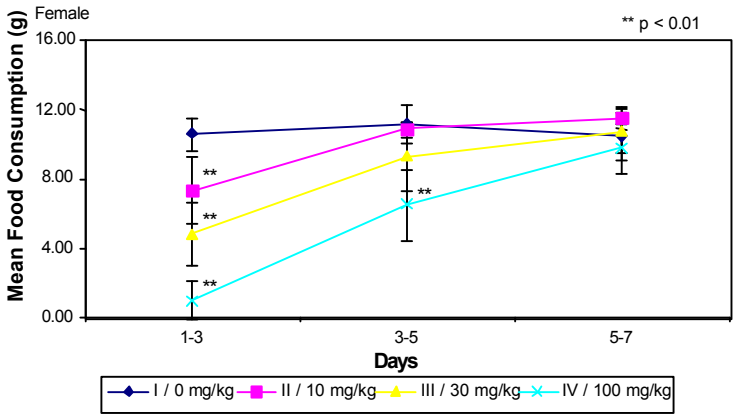

Fig. 4: Food consumption of male Wistar rats following 7 day oral exposure of sibutramine. Values are mean ± SD. n= 5 /dose group except for 100 mg/kg/day n= 2-5.

Table 1: Effect of Sibutramine on selected parameters in Wistar Rats following 7 day oral administration

		Dose			
Parameter	Sex	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Thymus: Body weight Ratio (g)	Male	0.2084±0.04	0.2057±0.0	0.1648±0.02	0.0635±0.04**
	Female	0.2268±0.02	0.2091±0.05	0.1912±0.02	0.0734±0.06**
Spleen: Body weight Ratio (g)	Male	0.2987±0.03	0.3163±0.08	0.2993±0.04	0.1811±0.05*
	Female	0.2688±0.04	0.2930±0.03	0.2329±0.02	0.1943±0.06
Serum Total Cholesterol (mg/dL)	Male	98.2±8.79	90.0±20.20	103.2±9.50	54.5±39.18*
	Female	83.8±12.05	82.4±12.99	90.2±18.38	61.0±14.14
Reticulocyte Count (%)	Male				
2.76±0.92	0.96±0.60*	1.23±0.68*	1.21±0.80*		
	Female	1.31±0.45	0.91±0.35	0.66±0.48	0.52±0.18

Note. Values are mean ± SD.

*Significantly different from control, $p \leq 0.05$; ** Significantly different from control, $p \leq 0.01$. n = 5 up to 30 mg/kg; for 100 mg/kg male: 4-5 & female: 2-5

Table 2: Toxicokinetic Profile of Sibutramine on day 1 and 7

	Day 1					
	10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Parameter	Male	Female	Male	Female	Male	Female
C_{max}[ng/ml]	22	144	143	425	170	759
t_{max}[h]	0.58	0.33	0.33	0.42	0.42	0.50
AUC_{0-t}[ng•h/ml]	49	292	222	1148	341	2035
$t_{1/2}$ [h]	(1.82)	1.54	1.25	4.38	(5.49)	4.39
CL [mL/kg/h]	172827	35561	148096	27991	253005	51190
	Day 7					
	10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Parameter	Male	Female	Male	Female	Male	Female
C_{max}[ng/ml]	3	36	24	15	61	NA
t_{max}[h]	0.58	0.33	0.42	0.33	0.50	
AUC_{0-t}[ng•h/ml]	5	42	58	48	167	
$t_{1/2}$ [h]	(0.92)	0.89	2.40	3.79	2.71	
CL [mL/kg/h]	1948246	395740	677238	653760	596645	

(NA)= Not available; Animals died on day 2 & 3.

sibutramine on day 7 as compared to body weight gain in control males (23.30 g) and females (8.25g). When compared to control, weight loss of 12 % in males of 30 mg/kg/day group and 25% and 27% in males and females respectively treated with 100 mg/kg/day sibutramine.

Significant decrease ($p<0.01$) in feed consumption was observed in sibutramine treated groups during day 1-3 of treatment period. Decrease was 40, 67, 90 % in male and 31, 54 and 91 % in female rats treated with 10, 30 and 100 mg/kg/day sibutramine respectively. However on day 7, food consumption of treated groups was compared to control.

Clinical Pathology: Decreased reticulocytes counts were observed in all sibutramine treated groups on hematology analysis. Among the clinical chemistry parameters analyzed, significant reduction in cholesterol (45 %) was observed in males treated at 100 mg/kg/day sibutramine (Table 1).

No abnormality was observed following blood and bone marrow smear examination. Further no treatment related changes observed in urine parameters.

Gross Pathology and Organ Weight: On gross pathology examination, decreased abdominal/peritoneal fat mass, small size spleen and thymus were observed at 100 mg/kg.

Significant decrease in thymus and spleen weight was recorded in rats treated with 100 mg/kg/day sibutramine (Table 1).

Histopathology: No treatment related findings were observed in examined tissues except varying degree of atrophic changes in thymus and spleen at 100 mg/kg/day sibutramine.

Toxicokinetics: Systemic exposure to sibutramine after single (Day 1) and repeated (Day 7) oral administration at all three dose levels were slightly higher in female than male rats, but there was no dose dependent increase in exposure at 100 mg/kg/day (Table 2). Exposure levels of sibutramine on day 7 were lesser than day 1 in both the sexes.

DISCUSSION

In the present study, rats dosed with 30 and 100 mg/kg/day sibutramine showed significant reduction in body weight and food intake. In accordance with the previous studies, administration of sibutramine reduces the body weight by suppressing the food intake in different rat models [7, 15-20].

Suppression of food intake could be due to the synergistic action of 5-HT and NA reuptake inhibition and was corroborated the findings of Jackson *et al*. [21] and Heal *et al*. [22], in which they found that administration of either 5-HT or NA reuptake inhibitor alone did not suppress the food intake, but combined administration of 5-HT and NA reuptake inhibitors did it noticeably. Additionally, increased activity observed in this study lead to loss in body weight through energy expenditure via monoamines reuptake inhibition in rat striatum and hypothalamus [23, 24]. Xu and Chen [25] suggested one more mechanism behind the reduced food intake by sibutramine in dogs i.e. increased gastric tone and impaired gastric accommodation.

Significant decrease in serum total cholesterol at 100 mg/kg/day sibutramine was well correlated with decreased intra-abdominal fat and the similar findings were observed by several researchers in human clinical trails even at much lower dose levels [26]. Levin and Dunn-Meynell [27] proposed that sibutramine treatment lowers the abdominal fat pad by loss of peripheral adipose tissue.

Decrease in reticulocytes count could be due to decrease in food consumption, which indirectly impairs haematopoiesis in bone marrow and was supported by a food restriction study in Sprague-Dawley rats [28].

No comparative literatures were available on organ weight and histopathological changes induced by sibutramine in animals. In the present study, we also could not find any drug related effect on organ weights and tissues. However, significant decrease in weights and atrophic changes observed in thymus could be because of stress related with highest dose (100 mg/kg/day) of sibutramine.

Toxicokinetic profiles of this study suggesting that exposure levels (AUC & Cmax) were higher in females when compared with males at all three dose levels and was well correlated with high mortality observed in females than males at 100mg/kg/day. Lesser concentration of sibutramine on day 7 could be attributed to either increased clearance from the system or increased biotransformation and which could be the reason for no major toxicity in this study.

In conclusion, there was no adverse effect of sibutramine in Wistar rats administered up to 30mg/kg/day. Further long-term studies will require for better understanding of pathogenesis and pathophysiology of sibutramine related safety concerns in laboratory animals.

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