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ACUTE AND SUB-ACUTE TOXICITY STUDIES OF STAY-ON POWER CAPSULES IN RATS

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ABSTRACT

The objective of the study is to evaluate the acute and sub-acute toxicity of Stay-On Power Capsules in rats administered by oral gavage in accordance with the schedule Y of D & C Act (2005). The acute toxicity study of Stay-On Power Capsules was done by up and down method. The animals were fasted for 18 hours with water *ad libitum*. The Stay-On Power Capsules was administered in six different doses: 250, 500, 750, 1000, 1500, and 2000 mg/kg body weight. Sub-Acute toxicity studies were conducted in rats (n=5/sex/group) by administering single dose of 300, 900, and 1500 mg/kg Stay-On Power Capsules or vehicle (0.5% carboxy-methylcellulose). No mortalities/morbidities and gross lesions observed at necropsy on Day 7 in both sexes. Although there were no effects on body weight, food consumption was increased. The NOAEL was identified as 6 mg/kg/day at all dose levels for both sexes and changes in albumin levels observed at 15 mg/kg/day. Serum enzymes ALT, AST, ALP levels and serum total and direct bilirubin, serum protein, albumin, glucose, haemoglobin, urea, uric acid and creatinine levels were not significantly altered by the treatment. However, the treatment for 28 days decreased LDL, Total cholesterol and triglycerides levels whereas no significant influence on HDL levels were observed at the dose of 5 and 10 mg/kg. In sub-acute toxicity dose was investigated as 10 mg/kg. which didn't show any toxic effect. Therefore, this study concluded that the Stay-On Power Capsules, at doses investigated, did not provoke toxic effects to the animals.

KEYWORDS: Stay on power capsules, acute and sub-acute toxicity, oral gavage, mortality and morbidity, NOAEL.

INTRODUCTION

Herbs are alternative medicines for treatment of various diseases due to their assumed acceptability, effectiveness, affordability, safety and low cost.[1] There is also an emerging increase in the consumption of herbal formulations by the public because of the strong belief that these products are natural; hence, they are safe for the treatment of ailments.[2] However, herbal preparations assumed to be safe may contain contaminants such as heavy metals.[3] aflatoxins and pathogenic microbes due to the manner in which they are prepared or as a result of acquisition of metals (*e.g.* cadmium) from the soil.[4,5] There is also the belief that because herbal remedies are derived from nature, they are devoid of adverse or toxic side effects often associated with synthetic drugs used in conventional medicine.[6] However, for proper and documented herbal medicinal products, the toxicity should be explored as in the case with conventional orthodox drugs that are properly researched and developed; the toxicity of traditional herbal medications is not often assessed.[7] As such, the users often look at the medicinal benefit of the herbal drugs and neglect their toxic effects to various organs. The present investigation deals with Toxicity Studies (Both acute and sub-acute toxicity) of Stay-On Power Capsules in Rodents administered orally by gavage to rats in accordance with the schedule Y of drugs and Cosmetic Act (2005). The acute toxicity study of Stay-On Power Capsules was done by up and down method.[8] Stay-On formulations of rare herbs include the highest quality Ginseng and Kesar which have proven benefits for rejuvenation. The ingredients of Stay-on capsules consist of Ashwagandha, Ginseng, kesar, Safed Musli, Salam and Shilajeet.[9]

MATERIALS AND METHODS

Acute oral toxicity study/single dose toxicity study

Materials

The test item was furnished from Shree Maruti herbals 401, Kapurwala Bldg, Samuel Street, Nxt. to Bank of Baroda, Masjid Bandar (West), Mumbai, Maharashtra 400003 named as Stay-On Power Capsules. The physical

appearances of the capsules were found to be brown powder in packets. Manufacture date 09/2016 and expiry date 08/2019. 3 bottles containing 30 capsules of Stay-On Power Capsules and two packets containing 25 gm of the powder were given by the supplier. The test drug was administered by dissolving in Normal water considered as vehicle for the test items. The standard was selected as 0.5% Carboxy-methylcellulose a white crystalline solid soluble in water stored at 15-30 degree centigrade room temperature. The reference item is any item used to provide a basis for comparison with the test item. In this experiment carboxymethyl cellulose is used. As it can be easily administered orally by making a solution of 5% in water and is well tolerated in rats

Method

Randomization, Numbering and Grouping of Animals

Acute toxicity/ single dose toxicity study

Wistar albino rats of both the sex were procured from the animal house of Royal College of pharmacy and health sciences male rats weighing between 191-262g and females weighing between 164-201g. Sixty rats i.e. 30 males and 30 female healthy rats were divided into six groups of 5 rats per sex i.e. six groups receiving the dose of 250, 500, 750, 1000, 1500 and 2000 mg/kg body weight, per oral. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of dosing with access to laboratory diet and aqua guard pure water dispensed in plastic bottles *ad libitum*. The rats were housed 5 each of the same sex in polycarbonate cages provided with bedding of husk. The temperature was maintained in between 20 to 24°C and relative humidity between 30 to 70%; 12 hours each of dark and light cycle was maintained.[11] The individual animal was fur marked with picric acid. The females were nulliparous and not pregnant.

Sub-acute toxicity/repeated dose toxicity study

Wistar albino rats of both the sex were procured from the animal house of Royal College of pharmacy and health sciences male rats weighing between 191-262g and females weighing between 164-201g. Sixty rats i.e. 30 males and 30 female healthy rats were divided into six groups of 5 rats per sex i.e. six groups receiving the dose of 250, 500, 750, 1000, 1500 and 2000 mg/kg body weight, per oral. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of dosing with access to laboratory diet and aqua guard pure water dispensed in plastic bottles *ad libitum*. The rats were housed sex in polycarbonate cages provided with bedding of husk. Forty rats i.e. 20 males and 20 female healthy rats were divided into four groups of 5 rats per sex i.e. three test groups receiving the dose of 300, 900 and 1500 mg/kg /animal/day and one control receiving 0.5% carboxy-methylcellulose. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of dosing. Rats were assigned to five per cage sex wise and the individual animal was fur marked with picric acid. The females were nulliparous and not pregnant. The route of administration was selected as oral. The purpose behind selecting wistar albino rats for the study is one of the rodent species is recommended as test system for the use in toxicity studies which has been demonstrated to be sensitive to toxins and as this system is widely used throughout industry for the evaluation of acute and sub chronic toxicity testing of orally administered drugs as historical data and evidence at the facility suggests.[12]

Dose administration

In Acute Toxicity Studies rats (n=5/sex/group) were administered a single dose of 250, 500, 750, 1000, 1500, and 2000 mg/kg body weight/ animal of Stay-On Power Capsules or vehicle (0.5% carboxy-methylcellulose) by oral gavage

In Sub-Acute Toxicity Studies rats (n=5/sex/group) were administered a repeated dose of 300, 900 and 1500 mg/kg /animal/day of Stay-On Power Capsules or vehicle (0.5% carboxy-methylcellulose) by oral gavage.[13]

Examination of major parameters prior to test

All the animals selected for testing, were examined within 24 h before testing started by the same procedure to be used during the test examination. Animals have no abnormal hematology and serum chemistry parameters.[14]

RESULTS AND DISCUSSION

A. Acute Toxicity Study

Symptoms

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Food consumption

The quantity of food consumed by groups consisting of forty rats each was recorded for 7 days and the food consumption per Rat was calculated for all groups.

TABLE -I: GROUP MEAN FOOD CONSUMPTION (g/animal)

Sex - Male

Animal No	Dose mg/kg/animal	Mean Food consumption	
		Day 0	Day 7
1-5	250	5.19	5.32
6-10	500	5.20	5.34
11-15	750	5.19	5.33
16-20	1000	5.21	5.35
21-25	1500	5.20	5.34
26-30	2000	5.19	5.35

Sex- Female

Animal No	Dose mg/kg/animal	Mean Food consumption	
		Day	Day 7

		0	
31-35	250	5.02	5.32
36-40	500	5.01	5.30
41-45	750	5.01	5.31
46-50	1000	5.03	5.32
51-55	1500	5.02	5.33
56-60	2000	5.01	5.31

TABLE -II: GROUP MEAN BODY WEIGHT (gm)

Sex-Male

Animal No	Dose mg/kg/animal	Mean Body Weight	
		Day 0	Day 7
1-5	250	198.8	201.5
6-10	500	200.7	203.2
11-15	750	202.6	204.1
16-20	1000	201.5	202.9
21-25	1500	202.2	204.2
26-30	2000	202.5	204.3

Sex-Female

Animal No	Dose mg/kg/animal	Mean Body Weight	
		Day 0	Day 7
31-35	250	167.2	171.8
36-40	500	168.7	171.6
41-45	750	170.2	172.1
46-50	1000	170.9	172.2
51-55	1500	170.5	172.3
56-60	2000	170.7	172.5

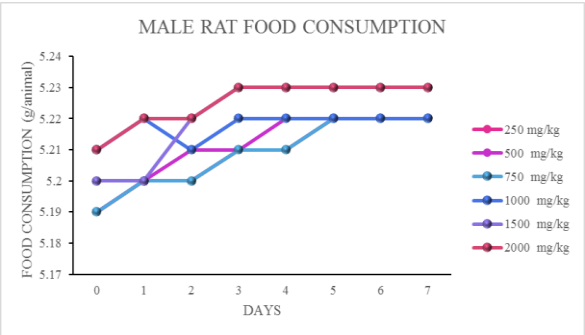

Fig. 1: MEAN FOOD CONSUMPTION OF RATS (MALE)

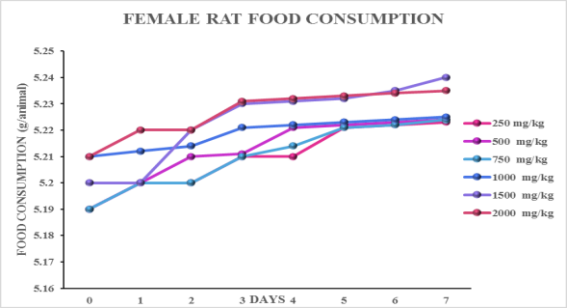

Fig. 2: MEAN FOOD CONSUMPTION OF RATS (FEMALE)

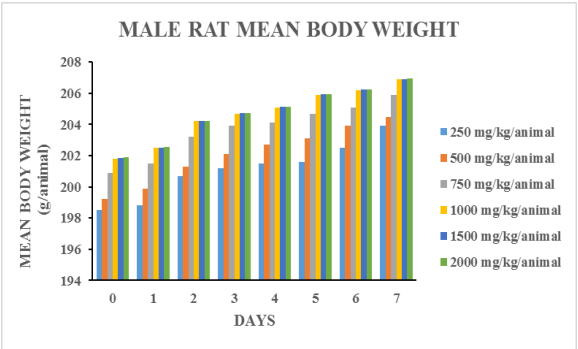

Fig. 3: MALE RAT GROUP MEAN BODY WEIGHT (g/animal)

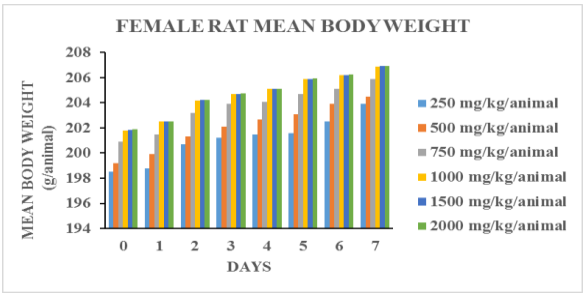

Fig. 4: FEMALE RAT GROUP MEAN BODY WEIGHT (g/animal)

Clinical examination

The rats were fasted overnight prior to blood collection. All blood was collected from the abdominal aorta. Hematology evaluations were performed with the aid of a Bayer ADVIA 120 analyzer and ACL-Advance instrument while a Hitachi P800 analyzer was used for serum chemistry determinations prior to the administration and 7 days after the administration. There is no evidence of toxicity at 7 days.

Fig. 5: Housing and acclimatization of rats (Rats in cage showing water bottles and food pellets)

Fig. 6: Oral Gavage in Rats (Oral administration of Stay- On Power Capsules)

RESULTS

Acute toxicity studies did not reveal any toxic signs and symptoms in albino rats treated with Stay-On Capsules (up to 2000 mg/kg, orally). No mortality and CNS abnormality was detected in experimental animals, the animals did not show convulsion, tremor ataxia depression, hyperesthesia motor incoordination or any allergic reaction.

Table III: Hematology Data of Rats – Males

TEST	UNIT	MEAN					
		250 mg/kg/ animal	500 mg/kg/animal	750 mg/kg/ animal	1000 mg/kg/ animal	1500 mg/kg/ animal	2000 mg/kg/ animal
MPV	fL (μm^3)	8.0	8.0	8.1	8.1	8.1	8.2
Platelets	$10^3/\mu L$	904	919	924	929	931	935
RDW	%	12.7	13.0	13.0	13.1	13.1	13.2
MCHC	g/dL	34.9	35.3	35.9	36.2	36.4	36.6
MCH	pg	18.6	18.6	18.7	18.7	18.9	18.9
Hematocrit	%	45	45	46	46	47	47
Hemoglobin	g/dL	15.7	15.9	16.1	16.2	16.2	16.2
Red Blood Cells	$10^6/\mu L$	8.39	8.46	8.51	8.52	8.52	8.55
White Blood Cells	$10^3/\mu L$	4.52	5.01	5.07	5.11	5.14	5.18
MCV	fL (μm^3)	53.5	54.51	54.53	54.58	54.6	54.8
Neutrophils	$10^3/\mu L$	0.68	0.70	0.70	0.71	0.71	0.71
Lymphocytes	$10^3/\mu L$	3.63	3.79	3.98	4.0	4.1	4.2
Monocytes	$10^3/\mu L$	0.08	0.08	0.08	0.09	0.09	0.09
Eosinophils	$10^3/\mu L$	0.06	0.06	0.06	0.08	0.08	0.08
Basophils	$10^3/\mu L$	0.02	0.02	0.03	0.03	0.03	0.03
Reticulocytes	$10^9/\mu L$	238	240	242	244	245	245
CHCM	g/dL	35.3	35.5	35.6	35.7	35.8	35.9
CH	pg	18.9	19.0	19.0	19.1	19.1	19.1
HDW	g/dL	2.7	2.7	2.8	2.8	2.9	2.9
PDW	%	52.9	53.5	53.9	54.4	54.9	55.1
% Neutrophils	%	15.5	16.0	16.2	16.4	16.5	16.7
% Lymphocytes	%	80.2	81.3	81.6	81.9	82.1	82.3
% Monocytes	%	1.9	2.0	2.0	2.1	2.2	2.2
% Eosinophils	%	1.3	1.7	1.9	2.1	2.3	2.5
% Basophils	%	0.3	0.3	0.3	0.4	0.4	0.4
% Large unstained cells	%	0.5	0.5	0.6	0.6	0.7	0.7
Large unstained cells	$10^3/\mu L$	0.02	0.03	0.03	0.03	0.04	0.04
% Reticulocytes	%	2.9	2.9	3.0	3.0	3.1	3.1

Rats – Females

TEST	UNIT	MEAN					
		250 mg/kg/ animal	500 mg/kg/ animal	750 mg/kg/ animal	1000 mg/kg/ animal	1500 mg/kg/ animal	2000 mg/kg/ animal
MPV	fL (μm^3)	7.8	8.2	8.24	8.24	8.3	8.24
Platelets	$10^3/\mu L$	929	931	933	935	937	937
RDW	%	12.2	12.8	13.3	13.5	13.7	13.8
MCHC	g/dL	35.3	35.8	36.1	36.5	36.7	36.9
MCH	pg	19	18.5	18.6	18.6	18.7	18.7
Hematocrit	%	43.3	44	45	46	47	48
Hemoglobin	g/dL	15.2	15.4	15.6	15.7	15.7	15.94
Red Blood Cells	$10^6/\mu L$	8.02	8.15	8.24	8.36	8.43	8.54
White Blood Cells	$10^3/\mu L$	3.12	3.85	4.38	4.75	5.26	5.74
MCV	fL (μm^3)	53.8	54.80	54.80	54.81	54.82	54.82
Neutrophils	$10^3/\mu L$	0.46	0.57	0.61	0.68	0.75	0.81
Lymphocytes	$10^3/\mu L$	2.5	3.6	3.9	4.2	4.5	4.8

Monocytes	10^3/µL	0.06	0.06	0.06	0.07	0.08	0.08
Eosinophils	10^3/µL	0.05	0.06	0.07	0.07	0.08	0.08
Basophils	10^3/µL	0.01	0.02	0.02	0.02	0.02	0.02
Reticulocytes	10^9/µL	216.6	227	234	242	249	252
CHCM	g/dL	35.7	35.7	35.7	35.7	35.7	35.7
CH	pg	19.2	19.2	19.2	19.2	19.1	19.2
HDW	g/dL	2.31	2.4	2.42	2.45	2.5	2.6
PDW	%	52.7	53.5	53.9	54.2	54.8	55.5
% Neutrophils	%	15.4	16.1	16.5	16.5	16.7	16.9
% Lymphocytes	%	80	80.7	81.3	81.8	82.4	82.7
% Monocytes	%	2	2.1	2.3	2.4	2.5	2.6
% Eosinophils	%	1.7	2.1	2.5	2.7	2.8	2.9
% Basophils	%	0.3	0.35	0.4	0.44	0.48	0.5
% Large unstained cells	%	0.5	0.5	0.56	0.58	0.6	0.6
Large unstained cells	10^3/µL	0.01	0.01	0.02	0.02	0.03	0.03
% Reticulocytes	%	2.7	3.5	3.5	3.5	3.5	3.5

TEST	**UNIT**	**MEAN**					
		250 mg/kg/ animal	**500 mg/kg/ animal**	**750 mg/kg/ animal**	**1000 mg/kg/ animal**	**1500 mg/kg/ animal**	**2000 mg/kg/ animal**
MPV	fL (µm^3)	7.8	8.2	8.24	8.24	8.3	8.24
Platelets	10^3/µL	929	931	933	935	937	937
RDW	%	12.2	12.8	13.3	13.5	13.7	13.8
MCHC	g/dL	35.3	35.8	36.1	36.5	36.7	36.9
MCH	pg	19	18.5	18.6	18.6	18.7	18.7
Hematocrit	%	43.3	44	45	46	47	48
Hemoglobin	g/dL	15.2	15.4	15.6	15.7	15.7	15.94
Red Blood Cells	10^6/µL	8.02	8.15	8.24	8.36	8.43	8.54
White Blood Cells	10^3/µL	3.12	3.85	4.38	4.75	5.26	5.74
MCV	fL (µm^3)	53.8	54.80	54.80	54.81	54.82	54.82
Neutrophils	10^3/µL	0.46	0.57	0.61	0.68	0.75	0.81
Lymphocytes	10^3/µL	2.5	3.6	3.9	4.2	4.5	4.8
Monocytes	10^3/µL	0.06	0.06	0.06	0.07	0.08	0.08
Eosinophils	10^3/µL	0.05	0.06	0.07	0.07	0.08	0.08
Basophils	10^3/µL	0.01	0.02	0.02	0.02	0.02	0.02
Reticulocytes	10^9/µL	216.6	227	234	242	249	252
CHCM	g/dL	35.7	35.7	35.7	35.7	35.7	35.7
CH	pg	19.2	19.2	19.2	19.2	19.1	19.2
HDW	g/dL	2.31	2.4	2.42	2.45	2.5	2.6
PDW	%	52.7	53.5	53.9	54.2	54.8	55.5
% Neutrophils	%	15.4	16.1	16.5	16.5	16.7	16.9
% Lymphocytes	%	80	80.7	81.3	81.8	82.4	82.7
% Monocytes	%	2	2.1	2.3	2.4	2.5	2.6
% Eosinophils	%	1.7	2.1	2.5	2.7	2.8	2.9
% Basophils	%	0.3	0.35	0.4	0.44	0.48	0.5
% Large unstained cells	%	0.5	0.5	0.56	0.58	0.6	0.6
Large unstained cells	10^3/µL	0.01	0.01	0.02	0.02	0.03	0.03
% Reticulocytes	%	2.7	3.5	3.5	3.5	3.5	3.5

Table-IV-Serum Chemistry data-Rats-Males

TEST	**UNIT**	**MEAN**					
		250 mg/kg/ animal	**500 mg/kg/ animal**	**750 mg/kg/ animal**	**1000 mg/kg/ animal**	**1500 mg/kg/ animal**	**2000 mg/kg/ animal**
Phosphorous	mg/dL	8.04	9.03	9.03	9.04	9.04	9.04
Calcium	mg/dL	10.4	10.8	10.9	10.88	10.9	10.88

Total protein	g/dL	6	6.1	6.2	6.2	6.2	6.2
Triglycerides	mg/dL	44	47	48	49	48	49
Cholesterol	mg/dL	58	59	60	61	62	62
Glucose	mg/dL	123	134	135	136	137	137
Creatinine	mg/dL	0.3	0.4	0.4	0.4	0.4	0.4
Indirect bilirubin	mg/dL	0.06	0.08	0.08	0.08	0.08	0.08
Direct bilirubin	mg/dL	0.04	0.04	0.04	0.04	0.04	0.04
Total bilirubin	mg/dL	0.09	0.10	0.10	0.10	0.10	0.10
Alkaline phosphatase	U/L	113	121	122	123	125	125
Aspartate aminotransferase	U/L	105	112	120	123	127	127
Alanine aminotransferase	U/L	28	30	31	32	32	32
Creatine kinase	U/L	658	667	669	670	671	671
Albumin	g/dL	4	4.1	4.1	4.2	4.2	4.2
Globulin	g/dL	2	2.1	2.1	2.1	2.2	2.2
A/G ratio	ratio	1.99	1.97	1.97	1.98	1.98	1.99
Urea	mg/dL	17.1	17.9	18.1	18.5	18.9	19.5
Sodium	mmol/L	146	146	147	147	148	148
Potassium	mmol/L	4.48	4.56	4.58	4.61	4.63	4.65
Chloride	mmol/L	103	104	104	104	105	105

TEST	**UNIT**	**MEAN**					
		250 mg/kg/ animal	**500 mg/kg/ animal**	**750 mg/kg/ animal**	**1000 mg/kg/ animal**	**1500 mg/kg/ animal**	**2000 mg/kg/ animal**
Phosphorous	mg/dL	8.04	9.03	9.03	9.04	9.04	9.04
Calcium	mg/dL	10.4	10.8	10.9	10.88	10.9	10.88
Total protein	g/dL	6	6.1	6.2	6.2	6.2	6.2
Triglycerides	mg/dL	44	47	48	49	48	49
Cholesterol	mg/dL	58	59	60	61	62	62
Glucose	mg/dL	123	134	135	136	137	137
Creatinine	mg/dL	0.3	0.4	0.4	0.4	0.4	0.4
Indirect bilirubin	mg/dL	0.06	0.08	0.08	0.08	0.08	0.08
Direct bilirubin	mg/dL	0.04	0.04	0.04	0.04	0.04	0.04
Total bilirubin	mg/dL	0.09	0.10	0.10	0.10	0.10	0.10
Alkaline phosphatase	U/L	113	121	122	123	125	125
Aspartate aminotransferase	U/L	105	112	120	123	127	127
Alanine aminotransferase	U/L	28	30	31	32	32	32
Creatine kinase	U/L	658	667	669	670	671	671
Albumin	g/dL	4	4.1	4.1	4.2	4.2	4.2
Globulin	g/dL	2	2.1	2.1	2.1	2.2	2.2
A/G ratio	ratio	1.99	1.97	1.97	1.98	1.98	1.99
Urea	mg/dL	17.1	17.9	18.1	18.5	18.9	19.5
Sodium	mmol/L	146	146	147	147	148	148
Potassium	mmol/L	4.48	4.56	4.58	4.61	4.63	4.65
Chloride	mmol/L	103	104	104	104	105	105

Rats-females

TEST	**UNIT**	**MEAN**					
		250 mg/kg/ animal	**500 mg/kg/ animal**	**750 mg/kg/ animal**	**1000 mg/kg/ animal**	**1500 mg/kg/ animal**	**2000 mg/kg/ animal**
Phosphorous	mg/dL	7.92	8.20	8.21	8.21	8.22	8.23
Calcium	mg/dL	10.5	10.6	10.7	10.8	10.9	10.9
Total protein	g/dL	6.3	6.5	6.6	6.6	6.7	6.8
Triglycerides	mg/dL	28	31	33	33	34	35
Cholesterol	mg/dL	48	53	54	54	55	56

Glucose	mg/dL	117	125	126	126	128	128
Creatinine	mg/dL	0.4	0.4	0.5	0.5	0.5	0.5
Indirect bilirubin	mg/dL	0.08	0.08	0.09	0.09	0.09	0.09
Direct bilirubin	mg/dL	0.04	0.04	0.05	0.05	0.05	0.05
Total bilirubin	mg/dL	0.11	0.13	0.14	0.15	0.15	0.15
Alkaline phosphatase	U/L	59	65	66	66	67	67
Aspartate aminotransferase	U/L	102	122	123	123	124	124
Alanine aminotransferase	U/L	25	26	27	28	29	29
Creatine kinase	U/L	575	580	582	582	583	584
Albumin	g/dL	4.4	4.6	4.6	4.7	4.8	4.8
Globulin	g/dL	2	2.1	2.1	2.1	2.1	2.2
A/G ratio	ratio	2.2	2.3	2.3	2.4	2.5	2.5
Urea	mg/dL	19.3	19.5	19.5	19.6	19.7	19.7
Sodium	mmol/L	144	144	144	144	144	144
Potassium	mmol/L	4.07	4.07	4.07	4.08	4.08	4.08
Chloride	mmol/L	103	104	104	105	105	105

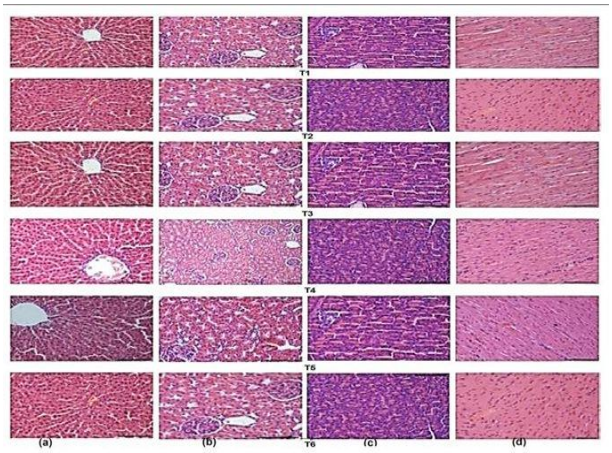

Fig. 7: Photomicrographs (reading from left to right) of the liver (a), kidneys (b), pancreas (c) and heart (d) of Wistar rats subjected to the treatments: T1 – 250 mg/kg /rat/day; T2 – 500 mg/kg /rat/day; T3 – 750 mg/ kg/ rat/day; T4 – 1000 mg /kg rat/day; T5– 1500 mg /kg rat/day; T6 – 2000 mg /kg rat/day

In Figure 7, images of the histological sections obtained from different organs are shown. No macroscopic changes were observed in the organs analysed, and neither were injuries, or significant microscopic changes.

B. Sub-Acute Toxicity Study

Mortality, Clinical Signs and Feed Consumption

Viability checks were conducted twice daily. The Stay-On Power Capsules produced no mortality even at 1500 mg/kg/day. Main study animals were observed for clinical signs at 1 and 2 h post-dosing. Detailed clinical evaluations were conducted once weekly. Treatment with Stay-On Power Capsules for 28 days at the doses of 300, 900 and 1500 mg/kg body weight, p.o., did not cause any obvious toxic symptoms. The general behaviour of animals was not changed. Similarly, food and water intake of animals and body weight gain were not significantly altered in the treatment groups, compared to control. State of the fecal droppings was not changed by the 28-day treatment in rats of all groups.

TABLE V: GROUP MEAN FOOD CONSUMPTION (g/animal)

Sex-Male

Animal No	**Dose mg/kg/animal**	**Mean Food Consumption**				
		Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
1-5	Vehicle	5.19	5.32	5.39	5.61	6.02
6-10	300	5.20	5.34	5.48	5.67	6.03
11-15	900	5.19	5.33	5.51	5.73	6.09
16-20	1500	5.21	5.35	5.6	5.83	6.07

Sex-Female

Animal No	**Dose mg/kg/animal**	**Mean Food Consumption**				
		Day 0	**Day 0**	**Day 0**	**Day 0**	**Day 0**
21-25	Vehicle	5.02	5.32	5.51	5.57	5.65
26-30	300	5.01	5.30	5.53	5.58	5.77
31-35	900	5.01	5.31	5.56	5.62	5.87
36-40	1500	5.03	5.32	5.58	5.71	5.98

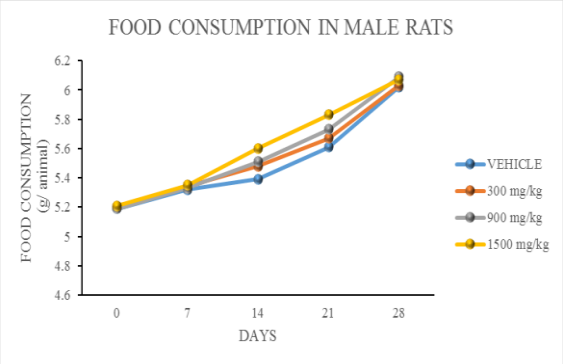

Fig. 8: Graphical Representation of Food Consumption of Male Rats

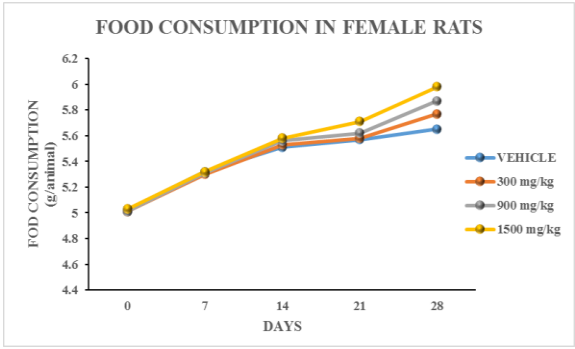

Fig.9: Graphical Representation of Food Consumption of Female Rats

TABLE -VI: GROUP MEAN BODY WEIGHT (gm)

Sex-Male

Animal No	Dose mg/kg/animal	Mean Body Weight				
		Day 0	Day 7	Day 14	Day 21	Day 28
1-5	Vehicle	198.8	201.5	203.1	203.7	204.9
6-10	300	200.7	203.2	206.2	207.1	207.5
11-15	900	202.6	204.1	205.3	205.9	206.1
16-20	1500	201.5	202.9	204.2	204.8	205.9

Sex-Female

Animal No	Dose mg/kg/animal	Mean Body Weight				
		Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
21-25	Vehicle	167.2	171.8	172.6	173.1	173.8
26-30	300	168.7	171.6	172.6	173.5	174.2
31-35	900	170.2	172.1	173.7	173.7	174.7
36-40	1500	170.9	172.2	173.2	174.2	175.2

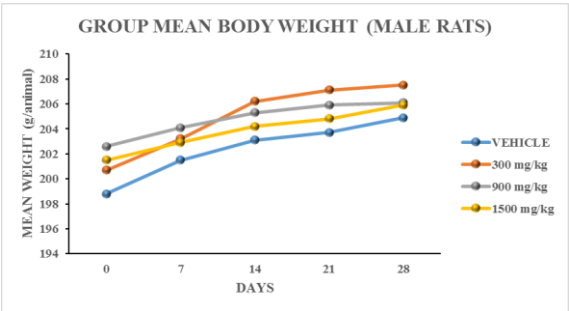

Fig. 10: MALE RAT GROUP MEAN BODY WEIGHT (g/animal)

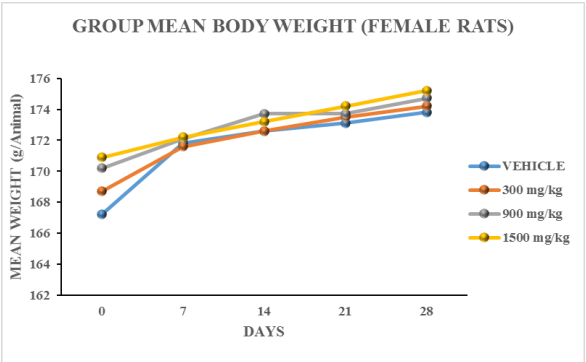

Fig. 11: FEMALE RAT GROUP MEAN BODY WEIGHT (g/animal)

Ophthalmoscopy

An ophthalmic examination (indirect) were conducted on main study animal's pre-test and during the last week of dosing. There were no treatment-related findings.[15]

Hematology

Blood samples were collected from fasted animals prior to scheduled terminal necropsy (main study only). Samples were analyzed for standard hematological parameters at Dr. Lal Path Labs, Bowmikhal Cuttack Road. Bhubaneswar-75014

In high dose males, there were statistically significant changes in numerous parameters compared to controls (RBC +6%, MCV -4%, MCH -5%, platelets +26%). There were also statistically significant increases (compared to controls) in WBC (+53%) and absolute lymphocytes (+44%), monocytes (+159%), eosinophils (+100%), and leucocytes (+150%). At the end of the recovery period, several changes persisted (RBC +4%, MCV -8%, MCH -8%, compared to controls) at 1500 mg/kg/day.

In females, there were statistically significant differences at all dose levels but changes occurred primarily at the high dose. Prothrombin time was decreased at all doses by 10 to 12%, compared to controls. MCHC was decreased by 6% at 900 and 1500 mg/kg/day. HGB and

MCH were decreased by 4% and 6%, respectively, at 1500 mg/kg/day (compared to controls). Additional statistically significant changes (compared to controls) in high dose females included increased WBCs (+40%) and absolute neutrophils (+88%), lymphocytes (+31%), and large unclassified cells (+67%). While not statistically significant, the increase in percent neutrophils (+34% compared to controls) and decrease in percent lymphocytes (-6% compared to controls) were considered consistent with increased inflammation in the liver. Following the recovery period, MCH and MCHC were decreased by 4% and prothrombin time was increased by 14% at 1500 mg/kg/day.

Table VII: Hematology Data
Rats – Males

TEST	UNIT	MEAN			
		Vehicle	300 mg/kg/animal	900 mg/kg/animal	1500 mg/kg/animal
MPV	fL (μm^3)	7.7	8.1	8.12	8.14
Platelets	$10^3/\mu L$	904	934	935	936
RDW	%	12.7	13.1	13.2	13.1
MCHC	g/dL	34.9	36.49	36.57	36.6
MCH	pg	18.7	18.8	18.8	18.9
Hematocrit	%	45	46.8	47	47.3
Hemoglobin	g/dL	15.7	16.1	16.2	16.3
Red Blood Cells	$10^6/\mu L$	8.39	8.51	8.52	8.53
White Blood Cells	$10^3/\mu L$	4.52	5.13	5.15	5.14
MCV	fL (μm^3)	53.5	54.84	54.85	54.86
Neutrophils	$10^3/\mu L$	0.68	0.70	0.71	0.72
Lymphocytes	$10^3/\mu L$	3.63	4.1	4.2	4.3
Monocytes	$10^3/\mu L$	0.08	0.085	0.09	0.091
Eosinophils	$10^3/\mu L$	0.06	0.086	0.089	0.09
Basophils	$10^3/\mu L$	0.02	0.03	0.04	0.03
Reticulocytes	$10^9/\mu L$	238	244	245	246
CHCM	g/dL	35.3	35.7	35.8	35.9
CH	pg	18.9	19.0	19.1	19.2
HDW	g/dL	2.7	2.91	2.93	2.94
PDW	%	52.9	55.1	55.11	55.14
% Neutrophils	%	15.5	16.5	16.6	16.7
% Lymphocytes	%	80.2	82.1	82.2	82.3
% Monocytes	%	1.9	2.1	2.2	2.3
% Eosinophils	%	1.3	2.5	2.5	2.5
% Basophils	%	0.3	0.4	0.44	0.45
% Large unstained cells	%	0.5	0.67	0.69	0.7
Large unstained cells	$10^3/\mu L$	0.02	0.031	0.033	0.035
% Reticulocytes	%	2.9	3.1	3.1	3.1

Rats – Females

TEST	UNIT	MEAN			
		Vehicle	300 mg/kg/animal	900 mg/kg/animal	1500 mg/kg/animal
MPV	fL (μm^3)	7.8	8.2	8.3	8.24
Platelets	$10^3/\mu L$	929	935	936	937
RDW	%	12.2	13.8	13.9	13.88
MCHC	g/dL	35.3	36.7	36.8	36.9
MCH	pg	19	18.5	18.6	18.7
Hematocrit	%	43.3	47	48	48
Hemoglobin	g/dL	15.2	15.9	15.91	15.94
Red Blood Cells	$10^6/\mu L$	8.02	8.52	8.53	8.54
White Blood Cells	$10^3/\mu L$	3.12	5.74	5.75	5.74
MCV	fL (μm^3)	53.8	54.80	54.81	54.80
Neutrophils	$10^3/\mu L$	0.46	0.81	0.82	0.83
Lymphocytes	$10^3/\mu L$	2.5	4.6	4.7	4.8
Monocytes	$10^3/\mu L$	0.06	0.07	0.08	0.08

Eosinophils	10^3/µL	0.05	0.06	0.07	0.08
Basophils	10^3/µL	0.01	0.02	0.024	0.029
Reticulocytes	10^9/µL	216.6	251	252	253
CHCM	g/dL	35.5	35.6	35.7	35.8
CH	pg	19.1	19.0	19.1	19.2
HDW	g/dL	2.31	2.51	2.55	2.6
PDW	%	52.7	55.76	55.78	55.8
% Neutrophils	%	15.4	16.3	16.7	16.9
% Lymphocytes	%	80	82.4	82.6	82.7
% Monocytes	%	2	2.4	2.5	2.6
% Eosinophils	%	1.7	2.5	2.8	2.9
% Basophils	%	0.3	0.4	0.5	0.5
% Large unstained cells	%	0.5	0.5	0.57	0.6
Large unstained cells	10^3/µL	0.01	0.031	0.03	0.03
% Reticulocytes	%	2.7	3.2	3.3	3.5

Clinical Chemistry

Samples collected at necropsy were evaluated for standard serum chemistry parameters. In males, chloride, phosphorous and uric acid were increased at all dose levels (up to +5%, +24% and +157%, respectively, compared to controls). At 900 mg/kg/day, there were statistically significant increases in ALT (+34%), ALP (+27%) and albumin (+5%). At 1500 mg/kg/day, ALT, AST, ALP and LDH were increased by 478%, 526%, 193% and 603%, respectively, compared to controls. There were also increases in GGT (1 IU/L compared to 0 IU/L in controls), total protein (+6%), albumin (+8%), A/G ratio (+7%), and cholesterol (+31%). While not significant, other parameters such as total bilirubin and creatine kinase were increased compared to controls. In recovery males, cholesterol was increased by 55%, compared to controls.

In females, chloride was increased at all dose levels (up to +5%, compared to controls). LDH was also increased in all groups (+211%, +206%, and +91%, at 300, 900, and 1500 mg/kg/day, respectively; only statistically significant at mid- and high-dose). At 900 mg/kg/day, statistically significant changes compared to controls included increased creatinine (+14%), ALP (+32%), and uric acid (+36%). At 1500 mg/kg/day, there were the following statistically significant changes relative to controls: creatinine (+14%), AST (+46%), ALP (+40%), uric acid (+55%), sodium (+1%), calcium (+5%), albumin (+8%). At the end of the recovery period, cholesterol and triglycerides were increased by 68% and 123%, respectively, compared to controls.

Table VIII: Serum Chemistry Data
Rats – Males

TEST	UNIT	MEAN			
		Vehicle	300 mg/kg/animal	900 mg/kg/animal	1500 mg/kg/animal
Phosphorous	mg/dL	8.04	9.04	9.03	9.04
Calcium	mg/dL	10.4	10.8	10.9	10.88
Total protein	g/dL	6	6.1	6.2	6.2
Triglycerides	mg/dL	44	47	48	49
Cholesterol	mg/dL	58	60	61	62
Glucose	mg/dL	123	134	136	137
Creatinine	mg/dL	0.3	0.4	0.4	0.4
Indirect bilirubin	mg/dL	0.06	0.08	0.08	0.08
Direct bilirubin	mg/dL	0.04	0.04	0.04	0.04
Total bilirubin	mg/dL	0.09	0.10	0.10	0.10
Alkaline phosphatase	U/L	113	121	123	125
Aspartate aminotransferase	U/L	105	125	126	127
Alanine aminotransferase	U/L	28	31	32	32
Creatine kinase	U/L	658	667	670	671
Albumin	g/dL	4	4.1	4.2	4.2
Globulin	g/dL	2	2.1	2.1	2.2
A/G ratio	ratio	1.99	1.97	1.97	1.97
Urea	mg/dL	17.1	19.1	19.3	19.5
Sodium	mmol/L	146	147	148	148
Potassium	mmol/L	4.48	4.59	4.61	4.65
Chloride	mmol/L	103	104	105	105

Rats-Females

TEST	UNIT	MEAN			
		Vehicle	300 mg/kg/animal	900 mg/kg/animal	1500 mg/kg/animal
Phosphorous	mg/dL	7.92	8.20	8.21	8.22
Calcium	mg/dL	10.5	10.7	10.8	10.9
Total protein	g/dL	6.3	6.5	6.6	6.7
Triglycerides	mg/dL	28	31	33	34
Cholesterol	mg/dL	48	53	54	55
Glucose	mg/dL	117	125	126	128
Creatinine	mg/dL	0.4	0.5	0.5	0.5
Indirect bilirubin	mg/dL	0.08	0.09	0.09	0.09
Direct bilirubin	mg/dL	0.04	0.05	0.053	0.055
Total bilirubin	mg/dL	0.11	0.13	0.14	0.15
Alkaline phosphatase	U/L	59	65	66	67
Aspartate aminotransferase	U/L	102	122	123	124
Alanine aminotransferase	U/L	25	27	29	29
Creatine kinase	U/L	575	580	582	584
Albumin	g/dL	4.4	4.6	4.7	4.8
Globulin	g/dL	2	2.1	2.1	2.1
A/G ratio	ratio	2.2	2.4	2.5	2.5
Urea	mg/dL	19.3	19.5	19.6	19.7
Sodium	mmol/L	144	144	144	144
Potassium	mmol/L	4.07	4.08	4.08	4.08
Chloride	mmol/L	103	105	105	106

Gross Pathology

At terminal sacrifice following 28 days of dosing or the recovery period, full necropsies were performed on main study animals. At necropsy, yellow discoloration of tissues and distended bile duct were observed at 1500 mg/kg/day. In addition, discoloration (red, black, or dark) was observed in numerous tissues of animals that died and this correlated with histopathological findings of congestion or haemorrhage.

Organ Weights

At scheduled necropsy, all the organs were weighed and organ/body weight and organ/brain weight ratios were calculated.

In males, there were statistically significant increases in absolute (+13%) and relative (+15%) liver weights at the high dose compared to controls. Absolute and relative pituitary weights were increased by up to +33%, compared to controls. Relative (to body weight) heart weights were increased by 10%. In recovery males, liver weights remained increased by up to 11%.

In females, statistically significant increases in absolute and relative liver weights occurred at all doses. Absolute liver weights were increased by 19%, 26%, and 34% at 25, 65, and 900 mg/kg/day, respectively. Absolute and relative weights of thyroid/parathyroid were decreased by up to 40% at 300 mg/kg/day and 48% at the higher doses. At the high dose, absolute and relative spleen weights were increased by up to 37% and relative thymus weights were increased by up to 32%. In recovery females, liver weights remained increased by up to 20% and absolute kidney weights were increased by 15%, compared to controls.

Table IX: Relative organ weights of rats after 28 days of treatment with Stay-On Power Capsules

Organs	Control	Treatments		
	5% carboxy methyl cellulose	300 mg/kg/rat/day	900 mg/kg/rat/day	1500 mg/kg/rat/day
Male				
Heart	0.30 ± 0.04	0.30 ± 0.05	0.28 ± 0.04	0.28 ± 0.02
Liver	2.79 ± 0.41	2.59 ± 0.30	2.58 ± 0.34	2.50 ± 0.10
Spleen	0.18 ± 0.02	0.18 ± 0.03	0.16 ± 0.03	0.17 ± 0.02
Lungs	0.36 ± 0.05	0.34 ± 0.03	0.36 ± 0.04	0.32 ± 0.04
Kidneys	0.65 ± 0.06	0.62 ± 0.04	0.63 ± 0.06	0.63 ± 0.05
Brain	0.56 ± 0.05	0.54 ± 0.05	0.51 ± 0.06	0.53 ± 0.04
Thymus	0.08 ± 0.01	0.08 ± 0.02	0.08 ± 0.03	0.08 ± 0.01
Paranephros	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Testis	0.81 ± 0.07	0.78 ± 0.08	0.80 ± 0.09	0.78 ± 0.09
Epididymis	0.28 ± 0.05	0.28 ± 0.04	0.26 ± 0.04	0.27 ± 0.03

Females				
Heart	0.28 ± 0.03	0.25 ± 0.05	0.28 ± 0.03	0.29 ± 0.04
Liver	2.79 ± 0.41	2.59 ± 0.30	2.58 ± 0.34	2.50 ± 0.10
Spleen	0.18 ± 0.02	0.18 ± 0.03	0.16 ± 0.03	0.17 ± 0.02
Lungs	0.36 ± 0.05	0.34 ± 0.03	0.36 ± 0.04	0.32 ± 0.04
Kidneys	0.65 ± 0.06	0.62 ± 0.04	0.63 ± 0.06	0.63 ± 0.05
Brain	0.72 ± 0.06	0.71 ± 0.04	0.69 ± 0.05	0.61 ± 0.11
Thymus	0.11 ± 0.01	0.11 ± 0.02	0.10 ± 0.01	0.11 ± 0.01
Paranephros	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00
Uterus	0.21 ± 0.04	0.17 ± 0.06	0.20 ± 0.06	0.21 ± 0.07
Ovary	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01

Relative organ weight was calculated as (organ weight/body weight) × 100%. The values are presented as means ± standard deviation of mean (5 rats/sex/group).

Histopathology

The tissue samples from the liver, kidney and heart for histological examination were passed through the process of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining. To ensure good fixation, the tissues were trimmed to about 5 mm thickness, so as to obtain good fixation. The tissues were then fixed in 10 % formol saline and were then transferred to 50 % alcohol (70 %, 80 %, 85 %, 95 and 100 %), for two hours.

Alcohol was removed from the treated tissues by titrating them through first an equal mixture 100 % (absolute) alcohol and xylene for one hour each in that order. Infiltration was carried out twice by passing each tissue through molten paraffin wax in an oven at a temperature of 30 °C for one and a half hours each. The tissues so embedded in molten paraffin wax were later placed on a wooden block and trimmed to size. Serial sections 10µm thick were made using a rotatory microtome. The cut sections were then floated in a warm water bath at a temperature of 30 - 40 °C and were placed slides. Eight sections were obtained from each treated organ from each animal. Four samples were placed on each slide.

Microscopic examination was done by using varying magnifications of 10, 40, 100 and 400 to determine if the samples were properly fixed on the slides. Following staining, mounting of sections was carried out using dimethyl paraffinate xylene (DPX) as a mounting agent, after which microscopic examination was done.

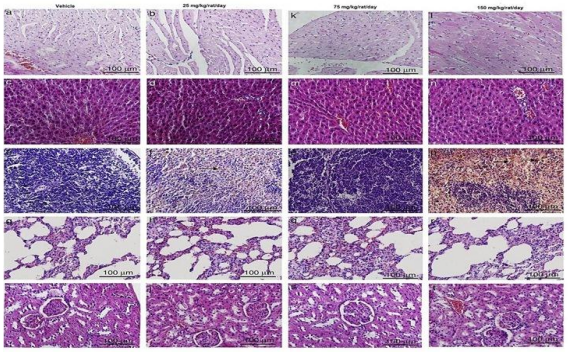

Fig. 12: Effects of treatment on the microstructures of various rat organ after 28 days (HE 400×). (a), (b), (k) and (l): heart; (c), (d), (m) and (n): liver; (e), (f), (o) and (p): spleen; (g), (h), (q) and (r): lung; (i), (j), (s) and (t): kidney.

In the macroscopic examination, no significant difference was observed in gross pathology. As shown in Fig. XII, in addition to bleeding, there were no remarkable histological changes observed in the heart, liver, lungs and kidneys. However, plenty of macrophages phagocytosed amounts of hemosiderin granules in the spleen was observed compared to the organs of the control group.

DISCUSSION

In the above study the acute and sub-acute toxicity was screened for the stay-on capsules. The feed and water intake, body weight measurements, hematology tests biochemical tests and finally necropsy and organ weight measurements were done. It was found that in all the tests there were no significant changes so that the test drug can be reported as majorly toxic. But In case of sub-acute toxicity The liver and kidney weight in some cases were found to be increased. While not significant, other parameters such as total bilirubin and creatine kinase were increased compared to controls. In recovery males, cholesterol was increased by 55%, compared to controls. At necropsy, yellow discoloration of tissues and distended bile duct were observed at 1500 mg/kg/day. In addition, discoloration (red, black, or dark) was observed in numerous tissues of animals that died and this correlated with histopathological findings of congestion or haemorrhage.

Acute toxicity studies did not reveal any toxic signs and symptoms in albino rats treated with Stay-On Power Capsules (up to 2000 mg/kg, orally). No mortality and CNS abnormality was detected in experimental animals, the animals did not show convulsion, tremor ataxia depression, hyperesthesia motor incoordination or any allergic reaction.

CONCLUSION

Hence from the above discussion it can be concluded that repeated doses continuously for 28 days at a dose of 1500mg/kg/day can be toxic and affect the metabolism bilirubin of liver and creatinine levels in kidney. The

dose of 1500mg/kg/day is very high for daily intake. Hence dose of 300-900mg/kg/day is found to be safe for normal intake in rodents at least for 28 days daily.

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