Regd. No. IPU-0152.07

STUDY CODE NO. 304(7)/1112

ADAPTATION SYSTEM WITH FUNCTIONAL CONDITION

AFTER 30 DAYS ADMINISTRATION OF

**"WATER FILTERED THROUGH HIGH REACTIVITY CARBON MIXTURE (HRCM)
ZF-FUNNEL (BUCKLET) FILTER"**

SUPPLIED BY

**WATER FREEDOM REVOLUTION (WFR), INDIA, IN COLLABORATION WITH OOO
"HOLDING "ZOLOTAYA FORMULA"**

(GOLDEN FORMULA), RUSSIA,

IN ALBINO RATS.

DATA REQUIREMENTS: FDA GUIDELINES

DR. (MISS) K.G.APTE, (Ph.D)

MANAGING DIRECTOR & STUDY DIRECTOR.

TESTING LABORATORY: NATIONAL TOXICOLOGY CENTRE,

S.N. 36/1/1, M.N. 199,

VADGAON KHURD,

PUNE 411 041.

Regd.No. IPU-0152.07

STATEMENT OF COMPLIANCE

Study Code No. : 304(7)/1112

Test Material   : "WATER FILTERED THROUGH HIGH REACTIVITY CARBON MIXTURE (HRCM) ZF-FUNNEL (BUCKLET) FILTER"

Test Title   : To Study Adaptation System with functional condition after 30 days administration of "WATER FILTERED THROUGH HRCM" in Rats

Study Director : Dr. (Miss) K.G.Apte

I hereby attest that this study was conducted in compliance with the FDA Guidelines following Good Laboratory Practice regulations.

Date: 06/April/2012.

Dr. (Miss) K.G.Apte,
Study Director.

Regd.No. IPU-0152.07

CONTENTS

STUDY PERSONNEL

The following personnel participated in conduct of the study.

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--------------------------------------------------------------
Name                              Responsibility and Function
Educational Qualification
Address
--------------------------------------------------------------
Dr. K. G. Apte                    Study Director: Overall
M.Sc.,Ph.D.,                      responsible to  conduct the
National Toxicology               study and report
preparation.
Centre



Mr. Amol More                     Technical Person
M. Pharm.
National Toxicology Centre



Mrs. Vaidehi R. Bhide             Technical Person
B. Sc.
National Toxicology Centre
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Dr. Dinesh M. Erlewad,            Principal Investigator
M.D.S.
Biological Scientific Head,
WFR, Pune.
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SUMMARY

The objective of the study was to determine the Recovery of the ability to positive effect upon the organism following 30 days administration of **"WATER FILTERED THROUGH HIGH REACTIVITY CARBON MIXTURE (HRCM) ZF-FUNNEL (BUCKLET) FILTER"** in rats as per FDA Guidelines.

Thirty six rats, 6 males and 6 females per group were assigned to the Control, Standard and Test groups. Control Group animals were administered the Pune Municipal corporation supplied water, Standard Group animals were administered **"Himalayan The natural Mineral Water"** and Test group animals were administered Pune Municipal corporation water filtered through **"HIGH REACTIVITY CARBON MIXTURE (HRCM) ZF-FUNNEL (BUCKLET) FILTER"** ad libitum for 30 days.

Group I Control: The control water caused no mortality. All the animals appeared normal and showed no clinical signs of intoxication after dosing till the end of the study.

Group II Standard: The standard water caused no mortality. All the animals appeared normal and showed no clinical signs of intoxication after dosing till the end of the study.

Group III Test: The test water caused no mortality. All the animals appeared normal and showed no clinical signs of intoxication after dosing till the end of the study.

Body Weight: There was no statistically significant change in the body weight gain in test group animals. Standard group females (106.2 gm) showed a statistically significant increase in body weight gain on day 7 (P = 0.0205) when compared to control group females (100.3 gm) analyzed by one way analysis of variance method of Prism Card 5 software.

Food Consumption: Food consumed was less in the test group male on day 0 (66.0 gm), on day 7 (67.0 gm), on day 14 (70.0 gm), on day 21 (72.0 gm) and on day 30 (75.0 gm) than the control group male on day 0 (74.0 gm), on day 7 (75.0 gm), on day 14 (75.5 gm), on day 21 (78.0 gm) and on day 30 (80.0 gm) respectively. The food consumption was less in standard females (64.5 gm) than the control females on day 0 (76.0 gm).

Water Consumption: Water consumed was more in test males on day 0 (145.5 ml), on day 7 (135.5 ml), on day 14 (157.7 ml) and on day 21 (162.9 ml), and was less on day 30 (170.6 ml) than control group male on day 0 (116.0 ml), on day 7 (105.9 ml), on day 14 (132.4 ml), on day 21 (151.5 ml), on day 30 (174.7 ml) respectively.
Water consumed was more in Standard group male on day 0 (141.0 ml) and was less on day 14 (127.0 ml), day 21 (137.4 ml) and day 30 (151.2 ml) than control males on day 0 (116.0 ml), on day 14 (132.4 ml), on day 21 (151.5 ml) and on day 30 (174.7 ml) respectively.
Water consumption was more in standard females on day 14

(165.6 ml), on day 21 (158.3 ml), on day 30 (164.9 ml) and in test females on day 14 (169.3 ml) and on day 30 (174.2 ml) than control females on day 14 (136.7 ml), on day 21 (151.2 ml) and on day 30 (152.8 ml) respectively.

Water consumption was less in standard females on day 0 (122.0 ml), on day 7 (123.1 ml) and in test females on day 0 (132.0 ml) and on day 7 (125.7 ml) than the control females on day 0 (139.0 ml) and on day 7 (135.6 ml) respectively.

Haematological Data: There was no statistically significant difference in the haematological parameters - RBC, Hb, PCV and TH count in standard and test group animals.

There was statistically significant increased ($P = 0.0199$) WBC count in Test males (9.917 THOUSAND/CMM) when compared with the control males (5.750 THOUSAND/CMM). Statistically significant Decrease ($P = 0.0005$) in Differential count of Neutrophils (N) was seen in Standard males (14.1 %) and Test males (20.0 %) when compared with control males (26.1 %).

There was statistically significant increased ($P = 0.0004$) in Differential count of Lymphocytes (L) in Standard Males (83.7 %) and Test males (77.2 %) when compared to control males (70.8 %) analyzed by one way analysis of variance method of Prism Card 5 software.

BIO CHEMICAL DATA

Serum Parameters: There was no statistically significant

difference in Total Bilirubin in all the animals when compared with the Control animals analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant decrease (*P = 0.0001*) in the serum glucose in the standard males (80.50 mg/dl) and test males (131.7 mg/dl) when compared to control males (136.3 mg/dl).

There was no statistically significant difference in serum glucose in the standard females (110.3 mg/dl) and test females (126.3 mg/dl) when compared with the Control females (129.7 mg/dl) analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant increased (*P = 0.0019*) serum Cholesterol in the standard males (64.33 mg/dl) when compared with the Control males (47.0 mg/dl). There was statistically

significant (*P = 0.0170*) increased serum Cholesterol in test females (52.33 mg/dl) when compared with the Control females (38.33 mg/dl) analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant decrease (*P = 0.0031*) in the serum triglycerides in the standard males (53.62 mg/dl) and test males (69.63 mg/dl) when compared to control males (93.02 mg/dl).

There was no statistically significant difference in serum triglycerides in the standard (92.03 mg/dl) and test females

(86.17 mg/dl) when compared with the Control group females (97.83 mg/dl) analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant increase ($P = 0.0003$) in the Total Protein in the test males (7.35 g/dl) and Standard males (6.22 g/dl) when compared with the Control males (5.83 g/dl). There was statistically significant increase ($P = 0.0001$) in the Total Protein in test females (7.55 g/dl) when compared to control females (4.98 g/dl) analyzed by one way analysis of variance method of Prism Card 5 software.

Tissue Parameters: There was no statistically significant increase or decrease in liver Glucose and liver glycogen in standard group animals when compared to the control group animals.

Test Males showed statistically significant increase ($P = 0.0001$) in liver glucose (62.54 mg/gm) and liver glycogen (56.34 mg/gm) when compared to the control males (32.78 mg/gm, 29.53 mg/gm) respectively.

Test females showed statistically significant decrease ($P = 0.0003$) in the liver glucose (32.61 mg/gm) and liver glycogen (29.38 mg/gm) when compared to the control females (53.20 mg/gm, 47.93 mg/gm) respectively analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant increase ($P = 0.0001$) in muscle glucose and muscle glycogen in standard males (17.47

mg/gm, 15.7 mg/gm) and in test males (15.22 mg/gm, 13.7 mg/gm) when compared to the control males (7.67 mg/gm, 6.91 mg/gm).

Standard females showed statistically significant increase (*P = 0.0001*) in muscle glucose and muscle glycogen in (18.61 mg/gm, 16.8 mg/gm) and in test females (17.23 mg/gm, 15.5 mg/gm) when compared to the control females (8.43 mg/gm, 7.59 mg/gm) analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant increase (*P = 0.0336*) in Reduced glutathione in test males (1.138 mg/gm) when compared to control males (1.044 mg/gm). There was statistically significant decrease (*P = 0.0060*) in Reduced glutathione in standard females (0.873 mg/gm) when compared to control females (1.024 mg/gm) analyzed by one way analysis of variance method of Prism Card 5 software.

<u>Organ Weights</u>: There was statistically significant decrease (*P = 0.0059*) in the absolute liver weight in standard males (4.660 gm) when compared to control males (8.439 gm). Relative Liver weight in standard males (2.927 gm) showed statistically significant decrease (*P = 0.0175*) when compared to control Males (5.096 gm).

There was statistically significant increase (*P = 0.0701*) absolute spleen weight in standard females (1.146 gm) when compared with the control females (0.714 gm) analyzed by one way analysis of variance method of Prism Card 5 software.

INTRODUCTION

Purpose

The purpose of this study is to determine the water biological activity of water following its filtration through HRCM (High reactivity capacity carbon mixture). In the course of study experimental investigation into water biological activity following its filtration was performed to determine its positive effect upon the organism.

The study permits the utilization of this information in Water treatment technology in installation of "The OOO "HOLDING "ZOLOTAYA FORMULA" (GOLDEN FORMULA), RUSSIA, HRCM Filter" which claims to remove the substances injurious and unsafe for health.

This test also enables the adaptation capacities and aid to recovery of disturbed functions in diseases.

This test enables to study Adaptation System with functional condition in Rats.

MATERIAL AND METHOD

Test Substance   : **"WATER FILTERED THROUGH HIGH REACTIVITY**
                          CARBON MIXTURE (HRCM) ZF-FUNNEL
(BUCKLET)

                          FILTER"

Name of the Company : **WFR IN COLLABORATION WITH OOO "HOLDING**

                      "ZOLOTAYA FORMULA"

                      (GOLDEN FORMULA), RUSSIA,

Testing Facility   : **NATIONAL TOXICOLOGY CENTRE,**

                          S.N. 36/1/1, M.N. 199,

                          Vadgaon Khurd,

                          Pune 411 041, India.

Date of receipt of sample   : 12/December/2011

Date of Initiation of the test: 16/January/2012.

Date of End of the test   : 17/February/2012.

Test Animal

1. Species   : Rats

2. Strain   : Wistar

3. Source   : National Toxicology Centre

4. Weight range   : 55 to 95 g approximately

5. Age   : 6 to 9 weeks

6. Sex   : male and female

7. Number   : 18 males and 18 females

8. Housing   : 6 of similar sex per cage

9. Diet   : Pelleted feed supplied by Nav

   Maharashtra Chakan Oil Mills Ltd., Pune.

10. Water   : Community tap water ad libitum.

11. Room temperature : 22 $\pm$ 3 degrees Celsius

12. Relative humidity : 55 $\pm$ 5 %

13. Light cycle   : 12 hours light & 12 hours dark

Experimental procedure

Thirty six rats, 18 male and 18 female were assigned to the Control, dose group. 6 males and 6 females per group were assigned to the Control, Standard and Test group. Control Group animals were administered the Pune Municipal corporation supplied water, standard Group animals were administered "Himalayan The natural Mineral Water" and Test group animals were administered Pune Municipal corporation water Filtered Through "HIGH REACTIVITY CARBON MIXTURE (HRCM) ZF-FUNNEL (BUCKLET) FILTER" ad libitum for 30 day. After completion of 30 days all animals were sacrificed and Bio chemical parameters were measured.

Observations:

<u>Clinical signs</u>: Toxic symptoms and mortality were recorded immediately, at ½, 1, 2, 4 and 24 hours and later twice a day thereafter upto 30 days to determine their general health, behavior and moribund condition. Any abnormality observed during this period was recorded.

<u>Body weight</u>: Animals were weighed individually on the day 0, 7, 14, 21, weekly upto day 30. The details of their individual body weight were given as per APPENDIX I Page 34 to 36 of this report.

Food Consumption: Food consumed by per cage was measured on the day 0, 7, 14, 21, weekly upto day 30. The details of their individual food consumption were given as per APPENDIX II Page 37 of this report.

Water Consumption: Water consumed by per cage was measured on daily basis upto day 30. For Analysis purpose the water consumption data were compiled as per weekly water consumption basis. The details of their individual weekly water consumption were given as per APPENDIX III Page 38 of this report.

Mortality: The circumstance of death was recorded and a macroscopic examination performed post mortem.

Necropsy: A gross necropsy was performed on all the animals that died during the course of the test and sacrificed at the termination of the test.

Haematology: Blood Samples were collected separately in tubes containing EDTA (dipotassium salt) on Day 30 before sacrifice the animals for Haematology. The estimations were performed using 'Coulter A^C.T diff' Hematology Analyzer. The details of their individual haematology were given as per APPENDIX IV Page 39 to 44 of this report.

Bio Chemical Parameters:

<u>Serum Parameters</u>: Blood Samples were collected separately in tubes containing Heparin, for clinical chemistry, as anticoagulants on Day 30 before sacrifice the animals. The analyses were performed using commercially available diagnostic kits manufactured by Crest Biosystems. The details of their individual serum parameters were given as per APPENDIX IV Page 45 to 47 of this report.

<u>Tissue Parameters</u>: Tissue Samples were collected for liver and muscle glucose and glycogen analysis after sacrificing the animals. The analysis was done according to the protocol described in Hawk's Physiological Chemistry (Page 224, 14th edition). The details of their individual tissue parameters were given as per APPENDIX V Page 48 to 50 of this report.

<u>ORGAN WEIGHTS</u>: At terminal necropsy, the following organs from all surviving animals were carefully dissected and trimmed to remove fat and other contiguous tissue and then were weighed immediately to minimize the effects of drying on organ weight: kidneys, liver, adrenals, testes, uterus, spleen, brain, heart, lung Values of these organs as percent of necropsy body weights were calculated (relative organ weights) The details of their individual organ weight were given as per APPENDIX V Page 51 to 56 of this report.

RESULTS

Clinical Signs

<u>Group I Control</u>: The control water caused no mortality. All the animals appeared normal and showed no clinical signs of intoxication after dosing till the end of the study.

<u>Group II Standard</u>: The standard water caused no mortality. All the animals appeared normal and showed no clinical signs of intoxication after dosing till the end of the study.

<u>Group III Test</u>: The test water caused no mortality. All the animals appeared normal and showed no clinical signs of intoxication after dosing till the end of the study.

<u>Body Weight</u>: There was no statistically significant change in the body weight gain in test group animals. Standard group females (106.2 gm) showed a statistically significant increase in body weight gain on day 7 (P = 0.0205) when compared to control group females (100.3 gm) analyzed by one way analysis of variance method of Prism Card 5 software.

<u>Food Consumption</u>: Food consumed was less in the test group male on day 0 (66.0 gm), on day 7 (67.0 gm), on day 14 (70.0 gm), on day 21 (72.0 gm) and on day 30 (75.0 gm) than the control group male on day 0 (74.0 gm), on day 7 (75.0 gm), on day 14 (75.5 gm), on day 21 (78.0 gm) and on day 30 (80.0 gm) respectively. The food consumption was less in standard

females (64.5 gm) than the control females on day 0 (76.0 gm).

Water Consumption: Water consumed was more in test males on day 0 (145.5 ml), on day 7 (135.5 ml), on day 14 (157.7 ml) and on day 21 (162.9 ml), and was less on day 30 (170.6 ml) than control group male on day 0 (116.0 ml), on day 7 (105.9 ml), on day 14 (132.4 ml), on day 21 (151.5 ml), on day 30 (174.7 ml) respectively.

Water consumed was more in Standard group male on day 0 (141.0 ml) and was less on day 14 (127.0 ml), day 21 (137.4 ml) and day 30 (151.2 ml) than control males on day 0 (116.0 ml), on day 14 (132.4 ml), on day 21 (151.5 ml) and on day 30 (174.7 ml) respectively.

Water consumption was more in standard females on day 14 (165.6 ml), on day 21 (158.3 ml), on day 30 (164.9 ml) and in test females on day 14 (169.3 ml) and on day 30 (174.2 ml) than control females on day 14 (136.7 ml), on day 21 (151.2 ml) and on day 30 (152.8 ml) respectively.

Water consumption was less in standard females on day 0 (122.0 ml), on day 7 (123.1 ml) and in test females on day 0 (132.0 ml) and on day 7 (125.7 ml) than the control females on day 0 (139.0 ml) and on day 7 (135.6 ml) respectively.

Haematological Data: There was no statistically significant difference in the haematological parameters - RBC, Hb, PCV and

PLT count in standard and test group animals.There was statistically significant increased (*P = 0.0199*) WBC count in Test males (9.917 THOUSAND/CMM) when compared with the control males (5.750 THOUSAND/CMM).

Statistically significant Decrease (*P = 0.0005*) in Differential count of Neutrophils (N) was seen in Standard males (14.1 %) and Test males (20.0 %) when compared with control males (26.1 %).

There was statistically significant increased (*P = 0.0004*) in Differential count of Lymphocytes (L) in Standard Males (83.7 %) and Test males (77.2 %) when compared to control males (70.8 %) analyzed by one way analysis of variance method of Prism Card 5 software.

BIO CHEMICAL DATA

<u>Serum Parameters</u>: There was no statistically significant difference in Total Bilirubin in all the animals when compared with the Control animals analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant decrease (*P = 0.0001*) in the serum glucose in the standard males (80.50 mg/dl) and test males (131.7 mg/dl) when compared to control males (136.3 mg/dl).

There was no statistically significant difference in serum glucose in the standard females (110.3 mg/dl) and test females (126.3 mg/dl) when compared with the Control females (129.7 mg/dl) analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant increased ($P = 0.0019$) serum Cholesterol in the standard males (64.33 mg/dl) when compared with the Control males (47.0 mg/dl).

There was statistically significant ($P = 0.0170$) increased serum Cholesterol in test females (52.33 mg/dl) when compared with the Control females (38.33 mg/dl) analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant decrease ($P = 0.0031$) in the serum triglycerides in the standard males (53.62 mg/dl) and test males (69.63 mg/dl) when compared to control males (93.02 mg/dl).

There was no statistically significant difference in serum triglycerides in the standard (92.03 mg/dl) and test females (86.17 mg/dl) when compared with the Control group females (97.83 mg/dl) analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant increase ($P = 0.0003$) in the

Total Protein in the test males (7.35 g/dl) and Standard males (6.22 g/dl) when compared with the Control males (5.83 g/dl). There was statistically significant increase (P = 0.0001) in the Total Protein in test females (7.55 g/dl) when compared to control females (4.98 g/dl) analyzed by one way analysis of variance method of Prism Card 5 software.

Tissue Parameters: There was no statistically significant increase or decrease in liver Glucose and liver glycogen in standard group animals when compared to the control group animals.

Test Males showed statistically significant increase (*P = 0.0001*) in liver glucose (62.54 mg/gm) and liver glycogen (56.34 mg/gm) when compared to the control males (32.78 mg/gm, 29.53 mg/gm) respectively.

Test females showed statistically significant decrease (*P = 0.0003*) in the liver glucose (32.61 mg/gm) and liver glycogen (29.38 mg/gm) when compared to the control females (53.20 mg/gm, 47.93 mg/gm) respectively analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant increase (*P = 0.0001*) in muscle glucose and muscle glycogen in standard males (17.47 mg/gm, 15.7 mg/gm) and in test males (15.22 mg/gm, 13.7 mg/gm) when compared to the control males (7.67 mg/gm, 6.91 mg/gm).

Standard females showed statistically significant increase (*P = 0.0001*) in muscle glucose and muscle glycogen in (18.61 mg/gm, 16.8 mg/gm) and in test females (17.23 mg/gm, 15.5 mg/gm) when compared to the control females (8.43 mg/gm, 7.59 mg/gm) analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant increase (*P = 0.0336*) in Reduced glutathione in test males (1.138 mg/gm) when compared to control males (1.044 mg/gm). There was statistically significant
decrease (*P = 0.0060*) in Reduced glutathione in standard females (0.873 mg/gm) when compared to control females (1.024 mg/gm) analyzed by one way analysis of variance method of Prism Card 5 software.

<u>Organ Weights</u>: There was statistically significant decrease (*P = 0.0059*) in the absolute liver weight in standard males (4.660 gm) when compared to control males (8.439 gm). Relative Liver weight in standard males (2.927 gm) showed statistically significant decrease (*P = 0.0175*) when compared to control Males (5.096 gm).
There was statistically significant increase (*P = 0.0701*) absolute spleen weight in standard females (1.146 gm) when compared with the control females (0.714 gm) analyzed by one way analysis of variance method of Prism Card 5 software.

TABLE 1

GROUP MEAN BODY WEIGHT DATA (gm)

Sex: Males

Group		0	7	14	21	30
				DAYS		
Control	Mean	77.08	100.6	136.0	149.8	171.4
	+-SD	10.41	19.12	23.31	21.68	29.12
Standard	Mean	76.17	105.9	134.7	149.7	160.1
	+-SD	5.939	9.656	12.68	21.12	21.56
Test	Mean	72.92	106.6	132.8	151.8	172.1
	+-SD	8.789	17.42	22.50	25.72	28.39

Sex: Females

Group		0	7	14	21	30
				DAYS		
Control	Mean	73.08	100.3	129.5	141.7	161.0
	+-SD	9.335	9.888	14.90	16.94	19.14
Standard	Mean	68.92	106.2	135.8	148.8	159.0
	+-SD	10.54	11.79	20.12	18.08	17.96
Test	Mean	66.75	97.33	129.7	140.3	169.0
	+-SD	8.993	10.41	8.406	8.710	9.757

TABLE 2

GROUP MEAN FOOD CONSUMPTION DATA (gm/cage)

Sex: Males

Group	Cage	0	7	14	21	30
Control	1	74.0	75.0	75.5	78.0	80.0
Standard	3	74.5	74.0	76.0	80.0	81.0
Test	5	66.0	67.0	70.0	72.0	75.0

Sex: Females

Group	Cage	0	7	14	21	30
Control	2	76.0	74.5	80.0	80.0	80.0
Standard	4	64.5	74.0	74.5	77.0	79.0
Test	6	76.0	75.5	76.0	77.0	79.5

TABLE 3

GROUP MEAN WATER CONSUMPTION DATA (ml/cage)

Sex: Males

Group	Cage	0	7	14	21	30
				DAYS		
Control	1	116.0	105.9	132.4	151.5	174.7
Standard	3	141.0	109.3	127.0	137.4	151.2
Test	5	145.5	135.5	157.7	162.9	170.6

Sex: Females

Group	Cage	0	7	14	21	30
				DAYS		
Control	2	139.0	135.6	136.7	151.2	152.8
Standard	4	122.0	123.1	165.6	158.3	164.9
Test	6	132.0	125.7	169.3	151.4	174.2

TABLE 4   MORTALITY DATA

MALES

Group	Dose	No. of animals died / No. of animals treated	% Mortality
I	Control	00/06	NIL
II	Standard	00/06	NIL
III	Test	00/06	NIL

FEMALES

Group	Dose	No. of animals died / No. of animals treated	% Mortality
I	Control	00/06	NIL
II	Standard	00/06	NIL
III	Test	00/06	NIL

TABLE 5a

GROUP MEAN HAEMATOLOGY DATA

Sex: Males

Gruop		HB	PCV	TH	RBC	WBC	DIFFERENTIAL COUNT			
							N	L	E	M
Control	Mean	6.45	21.2	292.7	3.700	5.750	26.1	70.8	1.0	2.13
	+ SD	2.01	6.33	54.15	1.090	2.032	4.65	4.71	0.0	0.51
Standard	Mean	8.27	25.7	278.2	4.778	6.467	14.1	83.7	1.0	1.27
	+ SD	2.52	7.62	105.7	1.436	2.556	3.71	4.07	0.0	0.62
Test	Mean	8.18	26.3	280.2	4.732	9.917	20.0	77.2	1.0	1.82
	+ SD	1.30	3.84	78.45	0.686	2.593	3.76	3.78	0.0	0.26

HB = Haemoglobin,   PCV = Packed Cell Volume

TH = Thrombocytes,   RBC = Red Blood Cells

WBC = White Blood Cells N = Neutrophils,

L = Lymphocyte   E = Eosinophils,

M = Monocyte

TABLE 5b

GROUP MEAN HAEMATOLOGY DATA

Sex: Females

Gruop		HB	PCV	TH	RBC	WBC	D I F F E R E N T I A L COUNT			
							N	L	E	M
Control	Mean	8.10	26.3	325.8	4.590	6.933	25.2	71.7	1.0	2.12
	+ SD	2.67	8.29	91.92	1.432	2.757	3.31	3.61	0.0	0.41
Standard	Mean	4.40	24.4	259.0	4.400	8.567	24.3	72.9	1.0	1.75
	+ SD	0.96	5.19	66.29	0.960	5.647	2.73	2.59	0.0	0.26
Test	Mean	8.62	27.9	316.3	4.913	6.000	23.0	74.5	1.0	1.43
	+ SD	1.58	4.94	79.81	0.808	2.747	4.52	4.80	0.0	0.45

HB = Haemoglobin,   PCV = Packed Cell Volume

TH = Thrombocytes,   RBC = Red Blood Cells

WBC = White Blood Cells   N = Neutrophils,

L = Lymphocyte   E = Eosinophils,

M = Monocyte

TABLE 6   GROUP MEAN BIO CHEMICAL SERUM PARAMETER DATA

Sex: Males

Group	Glucose (mg/dl)	Cholesterol (mg/dl)	Total Bilirubin (mg/dl)	TG (mg/dl)	TP (g/dl)
Control Mean	136.33	47.00	0.09	93.02	5.83
± SD	14.29	9.86	0.03	14.10	0.64
Standard Mean	80.50	64.33	0.11	53.62	6.22
± SD	14.95	5.50	0.02	16.75	0.49
Test Mean	131.67	50.67	0.09	69.63	7.35
± SD	12.58	5.16	0.01	18.19	0.34

Sex: Females

Group	Glucose (mg/dl)	Cholesterol (mg/dl)	Total Bilirubin (mg/dl)	TG (mg/dl)	TP (g/dl)
Control Mean	129.67	38.33	0.09	97.85	4.98
± SD	21.19	4.59	0.04	31.34	0.50
Standard Mean	110.33	46.00	0.10	92.03	6.75
± SD	19.23	6.57	0.02	31.38	0.85
Test Mean	126.33	52.33	0.09	86.17	7.55
± SD	25.86	9.95	0.01	38.12	0.23

TG = Triglyceride   TP = Protein (total)

TABLE 7   GROUP MEAN BIO CHEMICAL TISSUE PARAMETER DATA

Sex: Males

Group		liver Glucose (mg/gm)	liver glycogen (mg/gm)	muscle glucose (mg/gm)	muscle glycogen (mg/gm)	Reduce Glutathione (mg/gm)
Control	Mean	32.78	29.53	7.67	6.91	1.044
	± SD	1.577	1.420	1.09	0.99	0.032
Standard	Mean	29.02	26.15	17.47	15.7	1.055
	± SD	3.144	2.832	1.38	1.25	0.070
Test	Mean	62.54	56.34	15.22	13.7	1.138
	± SD	5.381	4.848	2.12	1.91	0.072

Sex: Females

Group		liver Glucose (mg/gm)	liver glycogen (mg/gm)	muscle glucose (mg/gm)	muscle glycogen (mg/gm)	Reduce Glutathione (mg/gm)
Control	Mean	53.20	47.93	8.43	7.59	1.024
	± SD	7.264	6.544	1.06	0.96	0.120
Standard	Mean	56.57	50.97	18.61	16.8	0.873
	± SD	12.28	11.07	1.07	0.97	0.106
Test	Mean	32.61	29.38	17.23	15.5	1.099
	± SD	2.664	2.400	0.74	0.67	0.083

TABLE 8

GROUP MEAN ORGAN WEIGHT DATA (gm)

ABSOLUTE VALUE

Sex: Male

Group		Adrenals	Heart	Kidney	Liver	Spleen	Testes
Control	Mean	0.047	0.614	1.249	8.439	1.012	2.252
	+ SD	0.006	0.127	0.261	2.055	0.314	0.323
Standard	Mean	0.035	0.624	1.144	4.660	0.915	2.149
	+ SD	0.005	0.081	0.147	0.599	0.167	0.389
Test	Mean	0.050	0.614	1.376	8.030	0.992	2.262
	+ SD	0.013	0.084	0.320	2.434	0.418	0.337

Sex: Female

Group		Adrenals	Heart	Kidney	Liver	Spleen	Ovaries
Control	Mean	0.049	0.632	1.058	6.783	0.714	0.129
	+ SD	0.015	0.073	0.215	1.968	0.066	0.017
Standard	Mean	0.053	0.639	1.206	7.533	1.146	0.106
	+ SD	0.010	0.093	0.171	1.821	0.424	0.021
Test	Mean	0.050	0.650	1.269	6.950	0.833	0.104
	+ SD	0.007	0.052	0.122	0.964	0.311	0.013

TABLE 9

GROUP MEAN ORGAN WEIGHT DATA (gm)

RELATIVE (%) VALUE

Sex: Male

Group		Adrenals	Heart	Kidney	Liver	Spleen	Testes
Control	Mean	0.0279	0.368	0.751	5.096	0.611	1.340
	+ SD	0.0069	0.113	0.241	1.857	0.241	0.281
Standard	Mean	0.0222	0.391	0.716	2.927	0.570	1.340
	+ SD	0.0030	0.032	0.039	0.343	0.062	0.154
Test	Mean	0.0292	0.359	0.798	4.614	0.565	1.323
	+ SD	0.0070	0.036	0.126	0.890	0.188	0.123

Sex: Females

Group		Adrenals	Heart	Kidney	Liver	Spleen	Ovaries
Control	Mean	0.0303	0.393	0.674	4.155	0.445	0.081
	+ SD	0.0060	0.020	0.175	0.675	0.034	0.011
Standard	Mean	0.0339	0.407	0.768	4.782	0.737	0.067
	+ SD	0.0080	0.081	0.152	1.280	0.312	0.015
Test	Mean	0.0297	0.385	0.753	4.119	0.498	0.062
	+ SD	0.0045	0.027	0.081	0.575	0.197	0.010

TABLE 10 GROSS NECROPSY DATA

MALES

```
--------------------------------------------------------------
Group     Observations
--------------------------------------------------------------
  I         No Gross Abnormalities Detected in any of the organs
--------------------------------------------------------------
  II        No Gross Abnormalities Detected in any of the organs
--------------------------------------------------------------
  III       No Gross Abnormalities Detected in any of the organs
--------------------------------------------------------------
```

FEMALES

```
--------------------------------------------------------------
Group     Observations
--------------------------------------------------------------
  I         No Gross Abnormalities Detected in any of the organs
--------------------------------------------------------------
  II        No Gross Abnormalities Detected in any of the organs
--------------------------------------------------------------
  III       No Gross Abnormalities Detected in any of the organs
--------------------------------------------------------------
```

APPENDIX Ia   INDIVIDUAL BODY WEIGHT DATA

Group I Control   Sex: Male

Animal No.	0	7	14	21	30
1	85.5	119.0	160.0	176.0	220.0
2	84.0	116.0	140.0	167.0	175.0
3	79.5	105.0	141.0	148.0	150.0
4	83.5	104.0	151.0	148.0	180.0
5	58.5	66.50	93.00	113.0	135.0
6	71.5	93.00	131.0	147.0	168.5

(Days)

Sex: Female

Animal No.	0	7	14	21	30
1	79.5	103.0	122.0	132.0	150.0
2	70.0	93.00	125.0	131.0	152.0
3	57.5	90.50	121.0	132.0	160.0
4	70.0	93.00	130.0	137.0	157.0
5	83.5	116.0	159.0	175.0	199.0
6	78.0	106.0	120.0	143.0	148.0

(Days)

APPENDIX Ib   INDIVIDUAL BODY WEIGHT DATA

Group II Standard   Sex: Male

| Animal No. | <---------------Days--------------> | | | | |
	0	7	14	21	30
1	76.0	102.0	130.0	144.0	148.0
2	85.5	121.0	150.0	164.0	169.5
3	67.5	95.50	120.0	127.0	140.0
4	78.0	109.0	133.0	152.0	168.0
5	77.0	97.00	125.0	129.0	140.0
6	73.0	111.0	150.0	182.0	195.0

Sex: Female

| Animal No. | <---------------Days--------------> | | | | |
	0	7	14	21	30
1	81.5	116.0	150.0	150.0	168.0
2	83.0	123.0	168.0	181.0	190.0
3	60.0	93.00	120.0	140.0	152.0
4	64.0	98.00	120.0	140.5	145.0
5	65.0	98.00	119.0	128.0	141.0
6	60.0	109.0	138.0	153.0	158.0

Regd.No. IPU-0152.07

APPENDIX Ic   INDIVIDUAL BODY WEIGHT DATA

Group III Test   Sex: Male

Animal No.	0	7	14	21	30
			Days		
1	88.0	122.0	146.0	162.0	179.0
2	76.0	132.0	165.0	184.0	205.0
3	68.0	98.00	119.0	122.0	132.0
4	74.0	103.0	123.0	130.0	157.0
5	69.0	100.5	142.0	176.0	201.5
6	62.5	84.00	102.0	137.0	158.0

Sex: Female

Animal No.	0	7	14	21	30
			Days		
1	84.0	113.0	140.0	142.0	170.0
2	66.0	103.0	139.0	149.0	174.0
3	63.0	84.00	122.0	130.0	157.0
4	59.5	93.00	127.0	147.0	184.0
5	60.5	90.00	120.0	129.0	160.0
6	67.5	101.0	130.0	145.0	169.0

APPENDIX II

INDIVIDUAL FOOD CONSUMPTION DATA (gm/Rat)

Sex: Male

Group	Cage	0	7	14	21	30
				DAYS		
I	1	12.3	12.5	12.6	13.0	13.3
II	3	12.4	12.3	12.7	13.3	13.5
III	5	11.0	11.2	11.7	12.0	12.5

Sex: Females

Group	Cage	0	7	14	21	30
				DAYS		
I	2	12.7	12.4	13.3	13.3	13.3
II	4	10.8	12.3	12.4	12.8	13.2
III	6	12.7	12.6	12.7	12.8	13.3

APPENDIX III

INDIVIDUAL WATER CONSUMPTION DATA (ml/Rat)

Sex: Male

Group	Cage	DAYS				
		0	7	14	21	30
I	1	19.3	17.6	22.1	25.3	29.1
II	3	23.5	18.2	21.2	22.9	25.2
III	5	24.3	22.6	26.3	27.1	28.4

Sex: Females

Group	Cage	DAYS				
		0	7	14	21	30
I	2	23.2	22.6	22.8	25.2	25.5
II	4	20.3	20.5	27.6	26.4	27.5
III	6	22.0	21.0	28.2	25.2	29.0

APPENDIX IVa

INDIVIDUAL HAEMATOLOGY DATA

Group I Control

Sex: Male

Animal No.	HB	PCV	TH	RBC	WBC	D I F F E R E N T I A L COUNT			
						N	L	E	M
1	9.2	29.5	382.0	5.30	7.1	26.9	70.1	1.0	2.0
2	7.0	22.7	323.0	4.24	8.1	26.1	70.4	1.0	2.5
3	7.3	23.8	299.0	3.38	4.5	23.0	73.5	1.0	2.5
4	5.9	19.4	264.0	3.38	6.8	19.0	78.4	1.0	1.6
5	3.1	10.3	253.0	1.99	2.5	29.5	66.8	1.0	2.7
6	6.2	21.7	235.0	3.72	5.5	32.1	65.4	1.0	1.5

HB = Haemoglobin,   PCV = Packed Cell Volume

TH = Thrombocytes,   RBC = Red Blood Cells

WBC = White Blood Cells N = Neutrophils,

L = Lymphocyte   E = Eosinophils,

M = Monocyte

Regd.No. IPU-0152.07

APPENDIX IVa

INDIVIDUAL HAEMATOLOGY DATA

Group I Control

Sex: Female

Animal No.						D I F F E R E N T I A L			
							COUNT		
	HB	PCV	TH	RBC	WBC	N	L	E	M
1	4.4	14.7	235.0	2.58	3.5	25.3	71.3	1.0	2.4
2	10.0	32.2	330.0	5.70	7.1	21.3	75.6	1.0	2.1
3	11.1	35.7	436.0	5.84	11.0	21.6	75.9	1.0	1.5
4	8.5	27.3	244.0	4.96	9.0	26.5	70.4	1.0	2.1
5	5.3	17.8	273.0	3.00	4.7	26.6	70.5	1.0	1.9
6	9.3	29.9	437.0	5.46	6.3	30.0	66.3	1.0	2.7

HB = Haemoglobin,   PCV = Packed Cell Volume

TH = Thrombocytes,   RBC = Red Blood Cells

WBC = White Blood Cells   N = Neutrophils,

L = Lymphocyte   E = Eosinophils,

M = Monocyte

APPENDIX IVb

INDIVIDUAL HAEMATOLOGY DATA

Group II Standard

Sex: Male

Animal No.						DIFFERENTIAL COUNT			
	HB	PCV	TH	RBC	WBC	N	L	E	M
1	10.1	31.9	371.0	5.76	7.5	17.4	80.2	1.0	1.4
2	10.8	32.7	317.0	5.99	9.1	12.0	85.7	1.0	1.3
3	4.0	12.5	132.0	2.27	3.3	14.5	84.1	1.0	0.4
4	7.5	23.4	268.0	4.73	7.6	14.9	82.5	1.0	1.9
5	9.8	30.0	400.0	5.83	8.1	17.9	79.2	1.0	1.9
6	7.4	23.5	181.0	4.09	3.2	7.9	90.4	1.0	0.7

HB = Haemoglobin,   PCV = Packed Cell Volume

TH = Thrombocytes,   RBC = Red Blood Cells

WBC = White Blood Cells   N = Neutrophils,

L = Lymphocyte   E = Eosinophils,

M = Monocyte

Regd.No. IPU-0152.07

APPENDIX IVb

INDIVIDUAL HAEMATOLOGY DATA

Group II Standard

Sex: Female

Animal No.	HB	PCV	TH	RBC	WBC	DIFFERENTIAL COUNT			
						N	L	E	M
1	5.3	17.3	137.0	3.23	4.8	24.3	72.9	1.0	1.8
2	7.4	23.1	295.0	3.97	6.3	27.9	69.5	1.0	1.6
3	7.3	24.0	291.0	4.25	8.7	21.9	75.6	1.0	1.5
4	8.1	26.3	232.0	4.65	19.4	23.3	73.7	1.0	2.0
5	10.4	33.1	319.0	6.11	8.4	21.3	75.6	1.0	2.1
6	7.0	22.7	280.0	4.19	3.8	27.2	70.3	1.0	1.5

HB = Haemoglobin,   PCV = Packed Cell Volume

TH = Thrombocytes,   RBC = Red Blood Cells

WBC = White Blood Cells   N = Neutrophils,

L = Lymphocyte   E = Eosinophils,

M = Monocyte

Regd.No. IPU-0152.07

APPENDIX IVc

INDIVIDUAL HAEMATOLOGY DATA

Group III Test

Sex: Male

Animal No.						D I F F E R E N T I A L COUNT			
	HB	PCV	TH	RBC	WBC	N	L	E	M
1	7.0	23.1	235	4.33	7.8	17.2	79.9	1.0	1.9
2	7.6	24.0	223	4.36	9.8	19.6	77.4	1.0	2.0
3	9.4	29.6	282	5.55	7.8	23.5	74.1	1.0	1.4
4	6.8	22.1	194	3.91	8.6	16.8	80.3	1.0	1.9
5	10.0	31.6	356.0	5.57	10.9	25.6	71.3	1.0	2.1
6	8.3	27.6	391.0	4.67	14.6	17.0	80.4	1.0	1.6

HB = Haemoglobin,   PCV = Packed Cell Volume

TH = Thrombocytes,   RBC = Red Blood Cells

WBC = White Blood Cells   N = Neutrophils,

L = Lymphocyte   E = Eosinophils,

M = Monocyte

APPENDIX IVc

INDIVIDUAL HAEMATOLOGY DATA

Group III Test

Sex: Female

Animal No.	HB	PCV	TH	RBC	WBC	D I F F E R E N T I A L COUNT			
	HB	PCV	TH	RBC	WBC	N	L	E	M
1	7.2	23.2	252.0	4.25	5.5	30.5	66.8	1.0	1.7
2	7.9	25.8	374.0	4.63	6.5	22.8	74.8	1.0	1.4
3	9.4	31.2	302.0	5.22	3.4	17.4	80.5	1.0	1.1
4	8.4	27.2	430.0	4.91	7.1	25.4	71.5	1.0	2.1
5	7.4	24.0	211.0	4.13	10.5	20.3	77.2	1.0	1.5
6	11.4	36.2	329.0	6.34	3.0	21.8	76.4	1.0	0.8

HB = Haemoglobin,   PCV = Packed Cell Volume

TH = Thrombocytes,   RBC = Red Blood Cells

WBC = White Blood Cells   N = Neutrophils,

L = Lymphocyte   E = Eosinophils,

M = Monocyte

APPENDIX Va   INDIVIDUAL BIO CHEMICAL SERUM PARAMETER DATA

Group I   Control   Sex: Males

Animal No.	Glucose (mg/dl)	Cholesterol (mg/dl)	Total Bilirubin (mg/dl)	TG (mg/dl)	TP (gm/dl)
1	134.0	57.0	0.084	89.8	6.6
2	156.0	51.0	0.085	106.8	6.1
3	150.0	32.0	0.099	80.3	4.8
4	117.0	47.0	0.085	111.2	5.9
5	130.0	56.0	0.120	94.3	6.2
6	131.0	39.0	0.039	75.7	5.4

Sex: Females

Animal No.	Glucose (mg/dl)	Cholesterol (mg/dl)	Total Bilirubin (mg/dl)	TG (mg/dl)	TP (gm/dl)
1	110.0	38.0	0.078	152.9	5.4
2	134.0	34.0	0.083	71.4	4.4
3	162.0	36.0	0.089	82.9	4.7
4	144.0	39.0	0.101	82.2	5.5
5	107.0	36.0	0.00	80	5.4
6	121.0	47.0	0.151	117.7	4.5

APPENDIX Vb   INDIVIDUAL BIO CHEMICAL SERUM PARAMETER DATA

Group II   Standard   **Sex: Males**

Animal No.	Glucose (mg/dl)	Cholesterol (mg/dl)	Total Bilirubin (mg/dl)	TG (mg/dl)	TP (gm/dl)
1	64.0	59.0	0.103	46.1	5.8
2	85.0	57.0	0.088	52	6.3
3	75.0	72.0	0.107	62	5.6
4	106.0	67.0	0.124	79.8	6.9
5	84.0	66.0	0.100	52.4	6.1
6	69.0	65.0	0.141	29.4	6.6

Sex: Females

Animal No.	Glucose (mg/dl)	Cholesterol (mg/dl)	Total Bilirubin (mg/dl)	TG (mg/dl)	TP (gm/dl)
1	91.0	51.0	0.100	105.7	5.3
2	128.0	49.0	0.105	80.1	6.6
3	102.0	55.0	0.077	123.9	6.7
4	90.0	42.0	0.110	123.6	7.6
5	115.0	40.0	0.113	74.6	6.7
6	136.0	39.0	0.121	44.3	7.6

APPENDIX Vc   INDIVIDUAL BIO CHEMICAL SERUM PARAMETER DATA

Group III Test   Sex: Males

Animal No.	Glucose (mg/dl)	Cholesterol (mg/dl)	Total Bilirubin (mg/dl)	TG (mg/dl)	TP (gm/dl)
1	112.0	47.0	0.0950	64.2	7.0
2	121.0	55.0	0.0890	105.4	7.7
3	133.0	42.0	0.0980	58.7	7.3
4	139.0	52.0	0.0920	57.9	7.8
5	142.0	53.0	0.0930	71.2	7.0
6	143.0	55.0	0.0600	60.4	7.3

Sex: Females

Animal No.	Glucose (mg/dl)	Cholesterol (mg/dl)	Total Bilirubin (mg/dl)	TG (mg/dl)	TP (gm/dl)
1	128.0	37.0	0.096	125.8	7.3
2	79.0	47.0	0.100	64.4	7.4
3	141.0	64.0	0.098	117	7.9
4	122.0	62.0	0.094	118	7.7
5	155.0	51.0	0.092	48.6	7.6
6	133.0	53.0	0.078	43.2	7.4

APPENDIX VIa   INDIVIDUAL BIO CHEMICAL TISSUE PARAMETER DATA

Group I   Control   Sex: Males

Animal No.	liver Glucose (mg/gm)	liver glycogen (mg/gm)	muscle glucose (mg/gm)	muscle glycogen (mg/gm)	Reduce Glutathione (mg/gm)
1	34.21	30.82	5.82	5.24	1.088
2	34.43	31.02	7.46	6.72	1.080
3	32.96	29.70	7.50	6.76	1.030
4	33.21	29.92	7.86	7.08	1.038
5	31.46	28.35	9.14	8.24	1.013
6	30.39	27.38	8.21	7.40	1.018

Sex: Females

Animal No.	liver Glucose (mg/gm)	liver glycogen (mg/gm)	muscle glucose (mg/gm)	muscle glycogen (mg/gm)	Reduce Glutathione (mg/gm)
1	56.96	51.32	8.93	8.04	1.240
2	53.61	48.29	9.57	8.62	1.033
3	64.18	57.82	8.86	7.98	0.905
4	47.61	42.89	6.71	6.05	0.925
5	43.32	39.03	7.57	6.82	1.040
6	53.54	48.23	8.93	8.04	1.000

APPENDIX VIb   INDIVIDUAL BIO CHEMICAL TISSUE PARAMETER DATA

Group II   Standard   Sex: Males

Animal No.	liver Glucose (mg/gm)	liver glycogen (mg/gm)	muscle glucose (mg/gm)	muscle glycogen (mg/gm)	Reduce Glutathione (mg/gm)
1	28.68	25.84	19.64	17.70	1.180
2	29.07	26.19	17.79	16.02	0.995
3	30.71	27.67	18.04	16.25	0.988
4	31.43	28.31	17.39	15.67	1.070
5	31.21	28.12	15.89	14.32	1.055
6	23.04	20.75	16.07	14.48	1.043

Sex: Females

Animal No.	liver Glucose (mg/gm)	liver glycogen (mg/gm)	muscle glucose (mg/gm)	muscle glycogen (mg/gm)	Reduce Glutathione (mg/gm)
1	47.14	42.47	19.29	17.37	0.823
2	43.68	39.35	18.43	16.60	1.080
3	67.54	60.84	19.29	17.37	0.790
4	68.07	61.33	19.86	17.89	0.850
5	45.39	40.89	17.86	16.09	0.880
6	67.61	60.91	16.96	15.28	0.818

APPENDIX VIc   INDIVIDUAL BIO CHEMICAL TISSUE PARAMETER DATA

Group III   Test   Sex: Males

Animal No.	liver Glucose (mg/gm)	liver glycogen (mg/gm)	muscle glucose (mg/gm)	muscle glycogen (mg/gm)	Reduce Glutathione (mg/gm)
1	55.32	49.84	15.71	14.16	1.123
2	68.68	61.87	16.79	15.12	1.080
3	59.93	53.99	13.11	11.81	1.280
4	59.43	53.54	12.14	10.94	1.100
5	68.71	61.90	16.07	14.48	1.130
6	63.14	56.89	17.50	15.77	1.115

Sex: Females

Animal No.	liver Glucose (mg/gm)	liver glycogen (mg/gm)	muscle glucose (mg/gm)	muscle glycogen (mg/gm)	Reduce Glutathione (mg/gm)
1	31.82	28.67	17.93	16.15	1.083
2	32.64	29.41	17.68	15.93	0.970
3	32.14	28.96	17.86	16.09	1.228
4	37.50	33.78	17.21	15.51	1.125
5	29.36	26.45	16.07	14.48	1.095
6	32.21	29.02	16.64	14.99	1.093

APPENDIX VIIa   INDIVIDUAL ORGAN WEIGHT DATA (gm)

ABSOLUTE VALUE

Group I Control   Sex: Male

Animal No.	Adrenals	Heart	Kidney	Liver	Spleen	Testes
1	0.049	0.727	1.456	9.731	1.048	2.711
2	0.049	0.616	1.159	7.39	1.088	2.146
3	0.044	0.513	1.139	7.446	0.764	1.993
4	0.035	0.452	0.884	5.789	0.529	1.85
5	0.053	0.790	1.627	11.65	1.310	2.514
6	0.049	0.588	1.226	8.632	1.334	2.296

Sex: Female

Animal No.	Adrenals	Heart	Kidney	Liver	Spleen	Ovaries
1	0.040	0.611	1.120	6.332	0.753	0.1220
2	0.038	0.643	1.142	5.751	0.658	0.1540
3	0.038	0.636	1.255	7.190	0.681	0.1270
4	0.052	0.578	1.125	5.685	0.664	0.1070
5	0.076	0.766	0.638	10.55	0.828	0.1440
6	0.052	0.559	1.068	5.191	0.699	0.1220

APPENDIX VIIb   INDIVIDUAL ORGAN WEIGHT DATA (gm)

ABSOLUTE VALUE

Group II Standard   Sex: Male

Animal No.	Adrenals	Heart	Kidney	Liver	Spleen	Testes
1	0.030	0.620	1.141	4.599	0.764	2.168
2	0.038	0.634	1.139	4.513	1.055	2.449
3	0.029	0.545	1.004	4.955	0.922	2.014
4	0.042	0.569	1.130	4.599	0.898	2.198
5	0.037	0.600	1.032	3.730	0.705	1.476
6	0.036	0.776	1.420	5.561	1.145	2.589

Sex: Female

Animal No.	Adrenals	Heart	Kidney	Liver	Spleen	Ovaries
1	0.051	0.637	1.158	7.151	1.055	0.1170
2	0.049	0.546	1.181	8.055	0.937	0.1120
3	0.068	0.796	1.478	10.27	1.679	0.1070
4	0.061	0.675	1.268	8.441	1.619	0.1360
5	0.048	0.547	1.199	6.227	0.999	0.0860
6	0.042	0.630	0.950	5.055	0.584	0.0770

APPENDIX VIIc   INDIVIDUAL ORGAN WEIGHT DATA (gm)

ABSOLUTE VALUE

Group III Test   Sex: Male

Animal No.	Adrenals	Heart	Kidney	Liver	Spleen	Testes
1	0.075	0.727	1.761	10.26	1.512	2.389
2	0.045	0.634	1.332	7.856	0.910	2.266
3	0.036	0.498	0.933	5.207	0.550	1.872
4	0.047	0.601	1.137	6.791	0.888	1.977
5	0.048	0.675	1.705	11.59	1.484	2.826
6	0.048	0.547	1.387	6.473	0.605	2.242

Sex: Female

Animal No.	Adrenals	Heart	Kidney	Liver	Spleen	Ovaries
1	0.062	0.653	1.499	7.732	1.236	0.1100
2	0.043	0.612	1.274	7.573	0.696	0.0940
3	0.051	0.568	1.200	6.708	0.636	0.1270
4	0.046	0.698	1.216	7.313	0.534	0.1010
5	0.049	0.671	1.275	7.260	1.220	0.0890
6	0.049	0.700	1.149	5.116	0.678	0.1010

APPENDIX VIIIa INDIVIDUAL ORGAN WEIGHT DATA (gm)

RELATIVE (%) VALUE

Group I Control   Sex: Male

Animal No.	Adrenals	Heart	Kidney	Liver	Spleen	Testes
1	0.0223	0.330	0.662	4.423	0.476	1.232
2	0.0280	0.352	0.662	4.223	0.622	1.226
3	0.0293	0.342	0.759	4.964	0.509	1.329
4	0.0194	0.251	0.491	3.216	0.294	1.028
5	0.0393	0.585	1.205	8.628	0.970	1.862
6	0.0291	0.349	0.728	5.123	0.792	1.363

Sex: Female

Animal No.	Adrenals	Heart	Kidney	Liver	Spleen	Ovaries
1	0.0267	0.407	0.747	4.221	0.502	0.0813
2	0.0250	0.423	0.751	3.784	0.433	0.1013
3	0.0238	0.398	0.784	4.494	0.426	0.0794
4	0.0331	0.368	0.717	3.621	0.423	0.0682
5	0.0382	0.385	0.321	5.302	0.416	0.0724
6	0.0351	0.378	0.722	3.507	0.472	0.0824

APPENDIX VIIIb INDIVIDUAL ORGAN WEIGHT DATA (gm)

RELATIVE (%) VALUE

Group II Standard   Sex: Male

Animal No.	Adrenals	Heart	Kidney	Liver	Spleen	Testes
1	0.0203	0.419	0.771	3.107	0.516	1.465
2	0.0224	0.374	0.672	2.663	0.622	1.445
3	0.0207	0.389	0.717	3.539	0.659	1.439
4	0.0250	0.339	0.673	2.738	0.535	1.308
5	0.0264	0.429	0.737	2.664	0.504	1.054
6	0.0185	0.398	0.728	2.852	0.587	1.328

Sex: Female

Animal No.	Adrenals	Heart	Kidney	Liver	Spleen	Ovaries
1	0.0304	0.379	0.689	4.257	0.628	0.0696
2	0.0258	0.287	0.622	4.239	0.493	0.0589
3	0.0447	0.524	0.972	6.757	1.105	0.0704
4	0.0421	0.466	0.874	5.821	1.117	0.0938
5	0.0340	0.388	0.850	4.416	0.709	0.0610
6	0.0266	0.399	0.601	3.199	0.370	0.0487

APPENDIX VIIIc INDIVIDUAL ORGAN WEIGHT DATA (gm)

RELATIVE (%) VALUE

Group III Test   Sex: Male

Animal No.	Adrenals	Heart	Kidney	Liver	Spleen	Testes
1	0.0419	0.406	0.984	5.732	0.845	1.335
2	0.0220	0.309	0.650	3.832	0.444	1.105
3	0.0273	0.377	0.707	3.945	0.417	1.418
4	0.0299	0.383	0.724	4.325	0.566	1.259
5	0.0238	0.335	0.846	5.752	0.736	1.402
6	0.0304	0.346	0.878	4.097	0.383	1.419

Sex: Female

Animal No.	Adrenals	Heart	Kidney	Liver	Spleen	Ovaries
1	0.0365	0.384	0.882	4.548	0.727	0.0647
2	0.0247	0.352	0.732	4.352	0.400	0.0540
3	0.0325	0.362	0.764	4.273	0.405	0.0809
4	0.0250	0.379	0.661	3.974	0.290	0.0549
5	0.0306	0.419	0.797	4.538	0.763	0.0556
6	0.0290	0.414	0.680	3.027	0.401	0.0598

CONCLUSION

Clinical Signs

<u>Group I Control</u>: The control water caused no mortality. All the animals appeared normal and showed no clinical signs of intoxication after dosing till the end of the study.

<u>Group II Standard</u>: The standard water caused no mortality. All the animals appeared normal and showed no clinical signs of intoxication after dosing till the end of the study.

<u>Group III Test</u>: The test water caused no mortality. All the animals appeared normal and showed no clinical signs of intoxication after dosing till the end of the study.

Body Weight: There was no statistically significant change in the body weight gain in test group animals. Standard group females (106.2 gm) showed a statistically significant increase in body weight gain on day 7 (P = 0.0205) when compared to control group females (100.3 gm) analyzed by one way analysis of variance method of Prism Card 5 software.

Food Consumption: Food consumed was less in the test group male on day 0 (66.0 gm), on day 7 (67.0 gm), on day 14 (70.0 gm), on day 21 (72.0 gm) and on day 30 (75.0 gm) than the control group male on day 0 (74.0 gm), on day 7 (75.0 gm), on day 14 (75.5 gm), on day 21 (78.0 gm) and on day 30 (80.0 gm) respectively.

The food consumption was less in standard females (64.5 gm) than

the control females on day 0 (76.0 gm).

Water Consumption: Water consumed was more in test males on day 0 (145.5 ml), on day 7 (135.5 ml), on day 14 (157.7 ml) and on day 21 (162.9 ml), and was less on day 30 (170.6 ml) than control group male on day 0 (116.0 ml), on day 7 (105.9 ml), on day 14 (132.4 ml), on day 21 (151.5 ml), on day 30 (174.7 ml) respectively.

Water consumed was more in Standard male on day 0 (141.0 ml) and was less on day 14 (127.0 ml), day 21 (137.4 ml) and day 30 (151.2 ml) than control males on day 0 (116.0 ml), on day 14 (132.4 ml), on day 21 (151.5 ml) and on day 30 (174.7 ml) respectively.

Water consumption was more in standard females on day 14 (165.6 ml), on day 21 (158.3 ml), on day 30 (164.9 ml) and in test females on day 14 (169.3 ml) and on day 30 (174.2 ml) than control females on day 14 (136.7 ml), on day 21 (151.2 ml) and on day 30 (152.8 ml) respectively.

Water consumption was less in standard females on day 0 (122.0 ml), on day 7 (123.1 ml) and in test females on day 0 (132.0 ml) and on day 7 (125.7 ml) than the control females on day 0 (139.0 ml) and on day 7 (135.6 ml) respectively.

Haematological Data: There was no statistically significant difference in the haematological parameters - RBC, Hb, PCV and PLT count in standard and test group animals.

There was statistically significant increased (P = 0.0199) WBC count in Test males (9.917 THOUSAND/CMM) when compared with the control males (5.750 THOUSAND/CMM).

Statistically significant Decrease (P = 0.0005) in Differential count of Neutrophils (N) was seen in Standard males (14.1 %) and Test males (20.0 %) when compared with control males (26.1 %).

There was statistically significant increased (P = 0.0004) in Differential count of Lymphocytes (L) in Standard Males (83.7 %) and Test males (77.2 %) when compared to control males (70.8 %) analyzed by one way analysis of variance method of Prism Card 5 software.

BIO CHEMICAL DATA

Serum Parameters: There was no statistically significant difference in Total Bilirubin in all the animals when compared with the Control animals analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant decrease (P = 0.0001) in the serum glucose in the standard males (80.50 mg/dl) and test males (131.7 mg/dl) when compared to control males (136.3 mg/dl).

There was no statistically significant difference in serum glucose in the standard females (110.3 mg/dl) and test females (126.3 mg/dl) when compared with the Control females (129.7mg/dl) analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant increased (*P = 0.0019*) serum Cholesterol in the standard males (64.33 mg/dl) when compared with the Control males (47.0 mg/dl).

There was statistically significant (*P = 0.0170*) increased serum Cholesterol in test females (52.33 mg/dl) when compared with the Control females (38.33 mg/dl) analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant decrease (*P = 0.0031*) in the serum triglycerides in the standard males (53.62 mg/dl) and test males (69.63 mg/dl) when compared to control males (93.02 mg/dl).

There was no statistically significant difference in serum triglycerides in the standard (92.03 mg/dl) and test females (86.17 mg/dl) when compared with the Control group females (97.83 mg/dl) analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant increase (*P = 0.0003*) in the Total Protein in the test males (7.35 g/dl) and Standard males (6.22 g/dl) when compared with the Control males (5.83 g/dl).

There was statistically significant increase (P = 0.0001) in the Total Protein in test females (7.55 g/dl) when compared to control females (4.98 g/dl) analyzed by one way analysis of variance method of Prism Card 5 software.

<u>Tissue Parameters</u>: There was no statistically significant increase or decrease in liver Glucose and liver glycogen in standard group animals when compared to the control group animals. Test Males showed statistically significant increase (*P = 0.0001*) in liver glucose (62.54 mg/gm) and liver glycogen (56.34 mg/gm) when compared to the control males (32.78 mg/gm, 29.53 mg/gm) respectively.

Test females showed statistically significant decrease (*P = 0.0003*) in the liver glucose (32.61 mg/gm) and liver glycogen (29.38 mg/gm) when compared to the control females (53.20 mg/gm, 47.93 mg/gm) respectively analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant increase (*P = 0.0001*) in muscle glucose and muscle glycogen in standard males (17.47 mg/gm, 15.7 mg/gm) and in test males (15.22 mg/gm, 13.7 mg/gm) when compared to the control males (7.67 mg/gm, 6.91 mg/gm).

Standard females showed statistically significant increase (*P = 0.0001*) in muscle glucose and muscle glycogen in (18.61 mg/gm, 16.8 mg/gm) and in test females (17.23 mg/gm, 15.5 mg/gm) when compared to the control females (8.43 mg/gm, 7.59 mg/gm) analyzed by one way analysis of variance method of Prism Card 5 software.

There was statistically significant increase ($P = 0.0336$) in Reduced glutathione in test males (1.138 mg/gm) when compared to control males (1.044 mg/gm).

There was statistically significant decrease ($P = 0.0060$) in Reduced glutathione in standard females (0.873 mg/gm) when compared to control females (1.024 mg/gm) analyzed by one way analysis of variance method of Prism Card 5 software.

Organ Weights: There was statistically significant decrease ($P = 0.0059$) in the absolute liver weight in standard males (4.660 gm) when compared to control males (8.439 gm).

Relative Liver weight in standard males (2.927 gm) showed statistically significant decrease ($P = 0.0175$) when compared to control Males (5.096 gm).

There was statistically significant increase ($P = 0.0701$) absolute spleen weight in standard females (1.146 gm) when compared with the control females (0.714 gm) analyzed by one way analysis of variance method of Prism Card 5 software.

Hence it can be concluded that the test material "WATER FILTERED THROUGH HIGH REACTIVITY CARBON MIXTURE (HRCM) ZF-FUNNEL (BUCKLET) FILTER" was safe to use.

Dr. K. G. Apte,
Study Director

ARCHIVES

On completion of the study, the raw data and other material, sample of the test substance and the study report would be retained for one year at National Toxicology Centre, Pune.