AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

90 Day Repeat DOSE Oral Toxicity Study of GM Derived Cotton Seed In Wistar Rats

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ABSTRACT

GM derived seed is one type of powered test item. It is used in 90 day repeat oral toxicity studies. Powered is administered orally to wistar rats for a period of 90 days. This study also compared the profile of the GM derived cotton seed with the non GM derived cotton seed at the highest does tested. The study was performed to generate information on major systemic toxic effects (if any), target organs and an estimate of the No Observed Effect Level (NOEL) / No Observed Adverse Effect Level (NOAEL) and also reversibility of observed changes, if any, after discontinuation of treatment with the Test Items.

Keywords: GM Derived Cotton Seed CMC (carboxy methyl cellulose), oral dose, NOAEL No Observed Adverse Effect Level.

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Received 04 April 2019, Accepted 18 April 2019

Please cite this article as: Tiwari P**.,** 90 Day Repeat DOST Oral Toxicity Study Of GM Derived Cotton Seed In Wistar Rats American Journal of PharmTech Research 2019.

INTRODUCTION

The oral route of administration is the most preferred route because of its numerous advantages. Tablets and capsules are the most preferred dosage form of pharmaceutical scientists and clinicians because of its convenience in the term of self- administration, compactness, ease in manufacturing, high precision dosing, and relatively low cost production. Gliclazide is structurally classified as a sulphonyl urea second generation analogue. The mean absolute bioavailability of gliclazide sustained release was 97%. It shows the linear pharmacokinetic .The objective of present work was to prepare sustained release tablet of GL by wet granulation method and study to effect on IR

Toxicology

The adverse events of drugs, chemicals or any poison on environment and living organisms including humans and animals with their detection, symptoms, pathogenesis, mechanism and treatment can be studied by a branch of science called as toxicology**.** Poison is any chemical which is toxic and can cause serious health hazards when used by any means either intentionally or un-intentionally (Shahwal *et al* (2011)).

Scope of toxicology

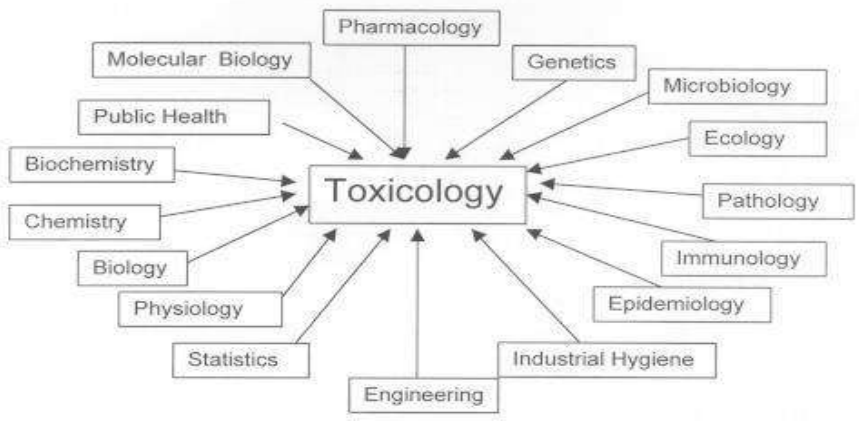


Figure 1: Scope of toxicology

MATERIAL AND METHOD

Selection of Animals

Species	**Rats**
Strain	Wistar rats: conventionally bred (In-house random bred)
Source	Animal Facility, Gheru Campus, CSIR-Indian Institute of Toxicology Research.
Justification for the selection of	Rat is one of the standard rodent test system recommended by various regulatory authorities for toxicity assessment.
Justification for route of administration	The oral route was chosen because it is the probable route of exposure in humans, if it happens.

No. of group	6 Main Groups: • Control (G1) • Low dose GM (GM2) • Mid dose GM (GM3) • High dose GM(GM4) • High Dose Non-GM (NGM5) Recovery Groups: • Control recovery (Vehicle G1R) • High dose GM recovery(GM4R) • High Dose Non recovery (NGM5R)
No. of animals/sex/ group	• Main groups: 10 males + 10 females • Recovery groups: 5 males + 5 females

Total number of rats	65 males + 65 females
Age at beginning of treatment	6 to 8 weeks
Body weight range	At the commencement of the treatment, the weight variation of animals was minimal and within the acceptable range of ± 20 % of the mean body weight in each sex
Identification	By rat accession number, cage card and picric acid body marking
acid body marking	After physical examination to ensure that rats are clinically healthy and suitable for the study, they were acclimatized for six days before start of the treatment. During the After physical examination to ensure that rats are clinically healthy and suitable for the study, they were acclimatized for six days before start of the treatment. During the acclimatization period, all rats were observed once daily (except on closed holidays) and only healthy and suitable rats were assigned to the study. Females used in this study were nulliparous and non-pregnant.

METHODS

Husbandry Conditions

Rats were housed in an environment controlled room at 22.2 to 24.9 °C and relative humidity of 40 to 68 per cent. There were two occasions each when the room temperature and relative humidity was marginally above the acceptable range. However this increase was very minimal and did not affect the health of the animals in any appreciable manner. The photoperiod was 12 hours light and 12 hours darkness. Adequate fresh air supply with regular air changes was maintained in the experimental room.

Housing

Five rat per sex per cage were housed in sterilized standard polycarbonate cages (Size: approximately L 445 x B 290 x H 210 mm) with stainless steel top grill having facilities for

pelleted food and drinking water in polycarbonate bottle with stainless steel sipper tube. Cages were placed on 5/6 tier racks.

Bedding

Steam sterilized clean paddy husk was used and changed along with the cage twice a week.

Feed: ad libitum

Gamma Irradiated Altromin 1324 Diet – Rats was provided to the animals.

Figure 2 Altromin of Feed

Water: ad libitum

Deep bore-well water filtered through Aquaguard on-line water.

Homogeneity of Dose Formulations

Homogeneity of the test item formulation during dosing was ensured by constantly stirring the formulation using a magnetic stirrer. The homogeneity and active ingredient concentration analysis was not conducted as part of this.

Dose Selection and Justification

Based on preliminary data from the acute toxicity study of GM derived cotton seeds (No mortality at the highest dose tested in the acute toxicity study i.e. 2000 mg/kg) the doses chosen for the 90 day repeat dose toxicity study in rats were 100 mg/kg, 200 mg/kg and 400 mg/kg for low, middle and high dose groups respectively. The non GM Cotton seed was only administered at the highest dose of 400 mg/kg body weight. In addition, a concurrent vehicle control group was used.

Grouping

Rats were randomly distributed to different groups by the body weight stratification method. Grouping was done 1 day prior to initiation of treatment.

Group Allocation, Dose levels, Number of Animals and Animal numbers

The selected male and female rats were assigned to control and various treatment groups as shown below:

Control

Low Dose GM

Mid Dose GM

High Dose GM

NON GM High Dose

Control Recovery

High Dose Recovery **NON GM High Dose Recovery**

Figure 3 Groups of Animals

GROUPS

Main Groups

Table 1. Main groups of animals

Group No.	Treatment Groups	No. of rats	Sex	Rat Numbers	
				From	To
G1	Vehicle control	10	M	R0201	R0210
		10	F	R0211	R0220
GM2	100 mg/kg	10	M	R0221	R0230
		10	F	R0231	R0240
GM3	200 mg/kg	10	M	R0241	R0250
		10	F	R0251	R0260
GM4	400 mg/kg	10	M	R0261	R0270
		10	F	R0271	R0280
NGM5	Non GM Cotton Seed 400 mg/kg	10	M	R0281	R0290
		10	F	R0291	R0300

Recovery Groups

Table 2= Recovery group of animal

Group No.	Treatment Groups	No. of rats	Sex	Rat Numbers	
				From	To
G1R	Vehicle control	5	M	R0301	R0305
		5	F	R0306	R0310
GM4R	400 mg/kg	5	M	R0311	R0315
		5	F	R0316	R0320
NGM5	Non GM Cotton Seed 400 mg/kg	5	M	R0321	R0325
		5	F	R0326	R0330

M= Male, F=Female

Preparation and Characterization of Doses

Selections of Vehicle

As per information provided by the sponsor, the test item was administered as a suspension in 0.5 % Carboxy Methyl Cellulose.

Test Item Preparation, Dose Volume and Administration

The test item suspension in 0.5 % Carboxy Methyl Cellulose was prepared twice a week and stored under refrigeration between dosing. Homogeneity of the Test Item (T.I) suspension was maintained during dose administration by constant stirring using a magnetic stirrer.

Test Item Formulation Preparation

Table 3 DOSE FORMULATION

Dose Group	**Dose**	**Concentration**	**Amt of T.I Required**	**Volume made up to(with vehicle)**
GM Low Dose	100 mg/kg	10 mg/ml	1.5 g	150.0 ml
GM Mid Dose	200 mg/kg	20 mg/ml	3.0 g	150.0 ml
GM High Dose	400 mg/kg	40 mg/ml	8.0 g	200.0 ml
Non GM High Dose	400 mg/kg	40 mg/ml	8.0 g	200.0 ml

Table 4 DOSE FORMULATION

Dose Group	Dose	Concentration	Amount of T.I	Volume made up
GM Low Dose	100mg/kg	10mg/ml	2.0g	200.0 ml
GM Mid Dose	200mg/kg	20mg/ml	4.0g	200.0 ml
GM High Dose	400mg/kg	40mg/ml	10.0g	250.0 ml
Non GM Dose	400mg/kg	40mg/ml	10.0g	250.0 ml

Table 5 DOSE FORMULATION

Dose Group	Dose	Concentration	Amount of T.I Required	Volume made up to (with vehicle)
GM Low Dose	100 mg/kg	10 mg/ml	2.0 g	200.0 ml
GM Mid Dose	200 mg/kg	20 mg/ml	4.0 g	200.0 ml
GM High Dose	400 mg/kg	40 mg/ml	12.0 g	300.0 ml
Non GM High Dose	400 mg/kg	40 mg/ml	12.0 g	300.0 ml

Table 6 DOSE FORMULATION

Dose Group	Dose	Concentration	Amount of T.I Required	Volume made up to(with vehicle)
GM Low Dose	100 mg/kg	10 mg/ml	2.5 g	250.0 ml
GM Mid Dose	200 mg/kg	20 mg/ml	5.0 g	250.0 ml
GM High Dose	400 mg/kg	40 mg/ml	14.0 g	350.0 ml
Non GM High Dose	400 mg/kg	40 mg/ml	14.0 g	350.0 ml

Table 7 DOSE FORMULATION

Dose Group	Dose	Concentration	Amount of T.I Required	Volume made up to (with vehicle)
GM Low Dose	100 mg/kg	10 mg/ml	2.5 g	250.0 ml
GM Mid Dose	200 mg/kg	20 mg/ml	5.0 g	250.0 ml

GM High Dose	400 mg/kg	40 mg/ml	16.0 g	400.0 ml
NonGM High Dose	400 mg/kg	40 mg/ml	16.0 g	400.0 ml

The dose formulations and vehicle was administered at the dose volume of 10 mL/kg body weight. The dose volume was calculated for individual animals on the first day of the treatment and was thereafter adjusted according to the body weights recorded twice weekly during the treatment period.

Treatment

Dose formulations or vehicle was administered orally to the respective groups once daily, 6 days a week (Monday to Saturday) at approximately the same time each day (varying by ± 1 hour) for a period of 90 days. The intended use of the Test item is as a feed additive and hence 6 days per week dose regimen was followed.

Figure 4 Image of oral route

OBSERVATION

Clinical Examinations and Mortality

General observation of the rats in study was carried out once daily (except on closed holidays). Rats were observed for changes in skin and fur, eyes, mucous membrane, occurrence of secretions and excretions, changes in gait, posture and presence of any abnormal behaviour. All rats were observed for morbidity and mortality once daily (except on closed holidays).

Body Weights

Individual body weights were recorded before the administration of test item (day 1) and twice weekly thereafter. Fasting body weights were recorded prior to sacrifice. Body weights were recorded from the recovery animals after cessation of dosing, during the recovery period. Fasted body weights were used for calculation of organ: body weight ratios.

Food Intake

The food consumption was measured at weekly intervals. The cage-wise average food intake (g/rat/day) is presented in the report. Food consumption was measured during in-life phase of the experiment.

Clinical Pathology Investigations (hematology, clinical chemistry)

Blood collection:

Blood samples were collected at the end of the treatment period from main groups and at the end of recovery period from recovery groups. All rats were fasted overnight (water allowed) before blood collection. The blood samples were collected by retro-orbital sinus and heart puncture, under chloroform anaesthesia, with the help of a fine capillary tube. Blood was collected into K2EDTA coated tubes for haematology and tubes without anticoagulant for clinical chemistry. After haematology analysis, whole blood samples were discarded. The clinical chemistry samples were also discarded after data review.

Haematology:

The following haematological parameters were determined using Sysmex XT 1800 iv haematology analyzer

S. No.	Parameter
1	Haemoglobin (Hb)
2	Red Blood Corpuscles (RBC)
3	White Blood Corpuscles (WBC)
4	Platelets (PLT)
5	Haematocrit (HCT)
6	Mean Corpuscular Volume (MCV)
7	Mean Corpuscular Haemoglobin (MCH)
8	Mean Corpuscular Haemoglobin Concentration (MCHC)
9	Differential Leucocyte Count (DLC)- absolute counts

Biochemical Analysis:

Serum was separated at room temperature followed by centrifugation in a refrigerated centrifuge at approximately 3000 rpm for 10 minutes and analyzed using Rx Daytona (Randox) automatic analyzer for the following parameters:

S. No.	Parameter	Abbreviations	Units
	Alanine Amino Transferase	ALT	U/L
	Albumin	Alb	mg/dL
	Alkaline Phosphatase	ALP	U/L
	Aspartate Amino Transferase	AST	U/L
	Urea	BUN	mg/dL
	Creatinine	Creat	mg/dL
	Glucose	Glu	mg/dL
	Sodium	Na	mmol/L

Potassium	K	mmol/L
Total Cholesterol	T.Chol	mg/dL
Total Protein	T.Pro	mg/dL

Pathology

Necropsy

Rats from all groups in the study were subjected to detailed necropsy and findings were recorded. The rats to be sacrificed at term were fasted overnight, weighed, anaesthetized with chloroform and exsanguinated. At necropsy, the animals were examined visually for external abnormalities including palpable masses. The abdominal, thoracic, and cranial cavities and their contents were examined for abnormalities and the organs removed, examined, and where specified in the table below, placed in 10% formal saline. The five rats that died during the course of the study were noticed after autolysis had set in. Hence, though the rats were necropsy organs were not collected from them.

Tissue collection

On completion of the gross pathology examination the tissues and organs noted below were collected from all animals and preserved in 10% formal saline:

Table 8: Pathology examination the tissues and organs

S. No.	Tissue	Organ Weights	Collection Preservation	Microscopic Examination
1	Adrenal glands	√	√	√
2	Brain	√	√	√
3	Oesophagus	X	√	√
4	Duodenum	X	√	√
5	Epididymides	√	√	√
6	Heart	√	√	√
7	Ileum with patches	X	√	√
8	Jejunum		√	√
9	Kidneys	√	√	√
10	Liver	√	√	√
11	Lungs (with bronchi and bronchiole	X	√	√
12	Ovaries	√	√	√
13	Pancreas	X	√	√
14	Prostate with Seminal vesicles and coagulating glands	X	√	√
15	Spleen	√	√	√
16	Stomach	√	√	√
17	Testes	√	√	√
18	Uterus with Cervix	√	√	√

√ : Done, X: Not done

Figure 5

$: Prostate + Seminal vesicles with coagulating glands will be collected as a whole.

Figure Organ Collection of Animals

The organs were weighed as mentioned in above table from all rats except from those found moribund and/or dead and presented in the report. The paired organs were weighed together and combined weight is presented in the report.

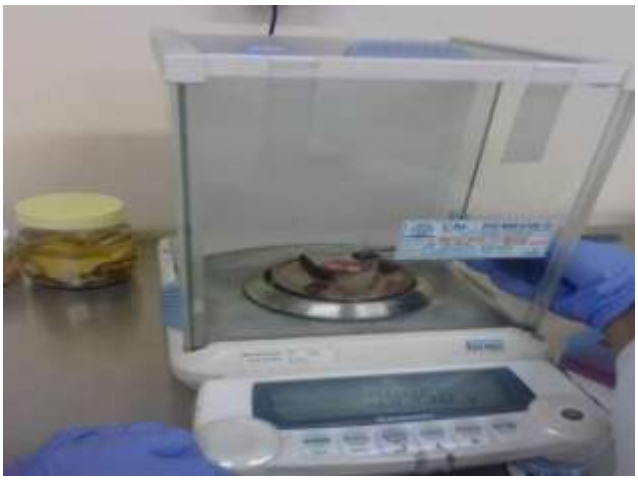


Figure 6 Weight of Organs

Histopathology

Histopathological examination was carried out on the preserved organs of vehicle control (V1), Test item high dose (TIAH) and Test item high dose (TIBH) group rats. Since no organ/tissue showed test item related histopathological changes in the high dose groups as compared to the control animals tissues from the lower dose groups (TIAH and TIAL) were not examined

Figure 7 Preserved Organs

The tissues were processed for routine paraffin embedding and sections were stain with Haematoxylin and Eosin stain.

Statistical Analyses

The statistical analyses of the experimental data was carried out by the statistician the test facility all quantitative variables like laboratory investigations (haematology and clinical chemistry) were subjected to one-way Anova test. In the case of recovery groups also, data was analyzed using the methods stated above comparison of means between treatment recovery group(s) and control recovery group was performed. All analyses and comparisons were evaluated at the 5 % (p <0.05) level. Statistically significant differences (p <0.05), indicated by the aforementioned tests have been designated by the following superscripts: significantly higher / lower than the vehicle control group.

RESULTS AND DISCUSSION

Clinical Examinations and Mortality

No abnormal clinical signs or change in behavior was observed in any animal of the control. GM High Dose treated or Non GM High Dose treated groups, at the doses tested, during the course of the study. A total of 5 mortalities occurred during the course of the study. Spontaneous occurrence of skin lesions (hair loss, mild swelling, discolouration over the skin etc) were also observed in a few rats during the course of the study. However these lesions were mild in nature and recovered spontaneously

Body Weights

Rats in all the groups, exhibited a comparable body weight gain during the course of the study. The extent of change in body weight was similar in both the Test item A treated and Test item B treated groups.

Food Intake

Feed consumption behaviour of rats from all the groups was similar during the course of the study. However the recovery group animals exhibited a slightly reduced feed consumption from the beginning of the study as compared to the main group rats.

Clinical Pathology Investigations (hematology, biochemical analysis)

No abnormal clinical signs or change in behavior was observed in any animal of the control, GM Cotton treated or Non-GM Cotton treated groups, at the doses tested, during the course of the study. A total of 5 mortalities occurred during the course of the study. One rat (number R0222) was found dead on two rats (number R0256 and number R0290) were found dead.one rat (number R0277) was found dead and the last rat (number R0330) was found dead. Out of these five, two rats exhibited sluggishness and reduced activity 1-2 days prior to being found dead. Spontaneous occurrence of skin lesions (hair loss, mild swelling, discolouration over the skin etc) were also observed in a few rats during the course of the study. However these lesions were mild in nature and recovered spontaneously.

Pathology

Control Group

Liver showed mild congestion in two animals and foci of mono nuclear cellular infiltrations (MNCs) in one animal. Kidney revealed mild congestion in two rats. Lung showed mild congestion along with increased bronchus associated lymphatic tissue (BALT) in one rat , foci of MNCs in one rat and mild alveolar rupture along with increase in BALT in one rat.

Non GM Cotton High Dose Group

Liver showed mild to moderate congestion in two rats foci of MNCs in one animal and necrotic foci in one animal. Kidney showed mild congestion in three rats. Lung revealed mild congestion and increased BALT in two animals and mild thickening of alveolar septa in one animal. In one rat adrenal showed mild congestion. Spleen of one animal showed mild depletion of lymphoid follicle.

GM Cotton High Dose Group

Liver showed mild congestion in one animal and foci of MNCs in two rats. Mild congestion of kidney noticed in one rat. Lung showed mild thickening of alveolar septa in two animals, mild congestion in one animal and increase in BALT in three rats. Adrenal of one rat showed mild congestion.

Inference

Lesions observed in high dose groups of Test item B and Test item A treated groups appears to be spontaneous and incidental in nature and are also comparable in nature with the Control group and hence cannot be related with the treatment with the test item.

90 Day Repeat Dose Oral Toxicity Study in Wistar Rats–Weekly Body Weight (g, Mean ± SD)–Males

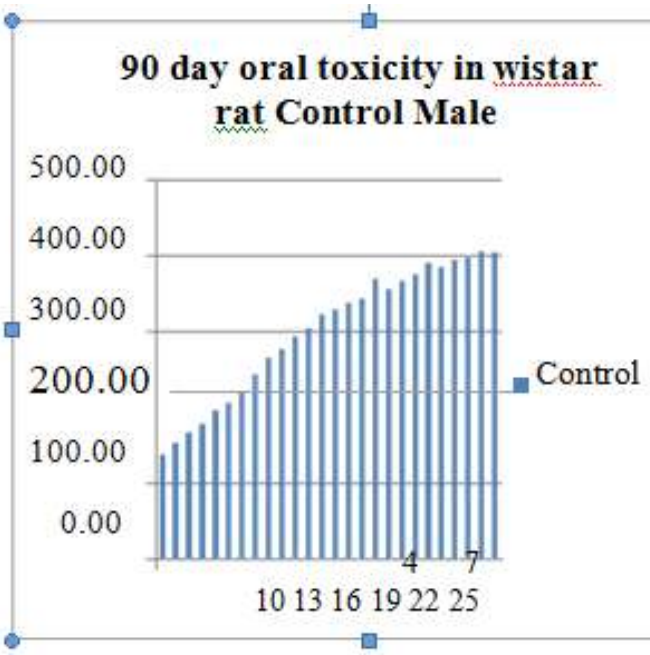
Group	1DAY	2DAY	3 DAY	4 DAY	5 DAY	6 DAY	7 DAY	8 DAY
Control	138.56	153.27	167.50	178.01	196.07	206.21	220.38	242.94
	16.29	17.74	22.14	23.51	25.91	30.82	31.78	32.56
Low Dose GM 100 mg/kg	140.86	150.40	152.40	190.19	209.01	220.61	245.42	264.35
	11.61	13.21	51.11	15.90	17.88	17.30	24.90	30.25
Mid Dose GM200 mg/kg	145.39	159.04	172.84	181.25	206.08	217.47	231.82	255.60
	13.14	15.07	14.31	15.79	16.31	19.97	26.50	32.08
High DoseGM, 400mg/kg	143.88	159.60	174.66	191.46	209.79	219.04	243.45	265.67
	13.31	11.39	12.57	14.69	15.42	16.32	17.24	23.15
NON GM High Dose ,400mg/kg	137.35	151.99	165.39	172.40	200.35	211.59	239.48	264.58
	12.64	14.25	16.22	16.47	17.13	19.26	17.92	16.95
Control- Recovery	138.30	147.46	169.82	182.22	194.60	212.42	220.90	231.00
	6.94	8.30	11.45	13.18	20.24	24.77	25.10	36.39
High Dose GM 400 mg/kg- Recovery	140.48	151.52	169.86	171.70	207.96	227.14	253.86	273.54
	13.47	12.12	15.25	11.61	15.09	16.69	15.38	15.08
NON GM HIGH DOSE 400 mg/kg- Recovery	133.96	143.46	155.78	172.14	185.96	201.80	220.76	243.14
	6.91	6.78	12.96	5.88	17.45	16.51	23.53	28.10
Low Dose GM 100 mg/kg	140.86	150.40	152.40	190.19	209.01	220.61	245.42	264.35
	11.61	13.21	51.11	15.90	17.88	17.30	24.90	30.25
Mid Dose GM200 mg/kg	145.39	159.04	172.84	181.25	206.08	217.47	231.82	255.60
	13.14	15.07	14.31	15.79	16.31	19.97	26.50	32.08
High Dose GM, 400 mg/kg	143.88	159.60	174.66	191.46	209.79	219.04	243.45	265.67
	13.31	11.39	12.57	14.69	15.42	16.32	17.24	23.15
NON GM High Dose,400mg/kg	137.35	151.99	165.39	172.40	200.35	211.59	239.48	264.58
	12.64	14.25	16.22	16.47	17.13	19.26	17.92	16.95
Control- Recovery	138.30	147.46	169.82	182.22	194.60	212.42	220.90	231.00
	6.94	8.30	11.45	13.18	20.24	24.77	25.10	36.39
High Dose GM 400 mg/kg- Recovery	140.48	151.52	169.86	171.70	207.96	227.14	253.86	273.54
	13.47	12.12	15.25	11.61	15.09	16.69	15.38	15.08
NON GM HIGH DOSE 400 mg/kg- Recovery	133.96	143.46	155.78	172.14	185.96	201.80	220.76	243.14
	6.91	6.78	12.96	5.88	17.45	16.51	23.53	28.10

Group	9DAY	1ODAY	11DAY	12DAY	13DAY	14 DAY	15 DAY	16 DAY
Control	265.39	276.41	293.01	304.07	321.80	329.07	338.09	343.96
	35.84	37.76	39.54	43.53	44.32	52.95	55.05	60.26
LOW DOSE GM 100 mg/kg	280.85	290.48	316.09	332.51	340.98	352.38	360.40	369.90
	43.70	52.48	29.89	33.71	35.95	38.19	41.67	42.69
MID DOSE GM 200 mg/kg	274.49	282.80	300.92	313.38	308.86	326.04	325.78	343.77
	34.55	39.19	38.90	42.98	51.04	48.25	54.89	52.75
HIGH DOSE GM, 400 mg/kg	277.03	300.15	307.68	326.32	334.63	341.14	356.72	367.84
	31.53	30.09	29.94	30.77	32.80	34.15	34.35	34.19
NON GM HIGH DOSE,400mg/kg	280.11	294.60	313.10	328.78	342.52	356.55	359.46	369.46
	14.13	11.41	22.69	20.87	22.33	21.13	19.82	18.33
Control-Recovery	230.10	233.70	290.08	306.76	320.14	307.16	335.56	345.40
	206.40	215.70	51.01	55.57	60.89	59.63	70.50	73.11
HIGH DOSE GM 400 mg/kg-Recovery	299.46	307.24	331.92	350.10	364.46	377.68	380.04	357.76
	16.69	18.88	14.62	14.51	11.69	15.22	15.31	17.15
NON GM HIGH DOSE, 400 mg/kg- Recovery	262.26	274.00	286.08	294.04	308.88	318.36	328.76	334.22
	27.45	30.00	30.63	33.97	38.12	41.95	44.67	46.24

Group	17DAY	18 DAY	19 DAY	20 DAY	21 DAY	22 DAY	23DAY	24DAY
Control	369.53	356.31	367.24	375.02	391.10	384.21	394.21	397.61
	31.20	58.89	57.07	58.99	45.33	64.48	62.80	65.78
LOW DOSE GM 100 mg/kg	377.40	382.80	395.91	399.08	400.84	411.39	414.79	422.23
	43.67	45.96	44.77	50.80	48.07	51.52	53.25	51.96
MID DOSE GM, 200 mg/kg	353.00	350.81	372.42	378.15	381.35	393.48	399.84	403.40
	51.68	61.81	56.17	57.32	67.70	59.63	58.23	61.70
HIGH DOSE GM, 400 mg/kg	372.66	372.70	377.82	395.53	395.24	405.75	408.78	412.80
	35.76	39.17	42.39	41.56	44.74	44.37	44.31	45.59
NON GM HIGH DOSE,400mg/kg	381.43	389.19	393.02	404.84	411.96	417.66	420.06	426.73
	21.05	22.97	22.27	24.65	26.58	30.46	29.69	31.45
Control- Recovery	354.02	357.64	370.24	374.74	379.20	386.74	387.16	391.66
	78.86	77.83	82.76	83.67	86.59	88.80	88.37	92.26
HIGH DOSE GM,	392.18	401.98	246.40	426.60	432.78	439.06	446.94	453.54

400 mg/kg- Recovery								
	19.04	18.26	251.60	18.07	18.84	19.26	20.63	21.71
NON GM HIGH DOSE, 400 mg/kg- Recovery	333.48	324.84	350.76	362.60	368.98	373.74	379.66	386.68
	64.19	52.78	54.65	53.96	56.19	59.27	56.94	63.44

Group	**25 DAY**	**26 DAY**	**27 DAY**	**28 DAY**	**29 DAY**	**30 DAY**	**31 DAY**
Control	405.60	404.33	-	-	-	-	-
	69.94	71.24	-	-	-	-	-
LOW DOSE GM 100 mg/kg	428.86	433.37	-	-	-	-	
	51.68	53.38	-	-	-	-	-
MID DOSE GM 200 mg/kg	411.00	414.50	-	-	-	-	-
	61.93	64.08	-	-	-	-	-
HIGH DOSE GM, 400 mg/kg	418.99	424.51	-	-	-	-	-
	47.99	48.42	-	-	-	-	-
NON GM HIGH DOSE,400mg/kg	434.17	434.84	-	-	-	-	
	33.62	35.61	-	-	-	-	-
Control- Recovery	400.50	406.34	410.56	415.10	421.60	428.06	430.28
	95.15	97.69	95.68	99.03	102.33	106.24	106.11
HIGH DOSE GM, 400 mg/kg- Recovery	461.54	464.12	469.06	471.58	475.02	484.80	491.40
	22.59	26.10	31.95	29.14	24.87	26.93	29.15
NON GM HIGH DOSE, 400 mg/kg- Recovery	394.74	399.12	403.20	408.68	414.24	419.62	422.08
	63.71	62.30	63.84	64.87	67.74	66.86	67.60


90 day oral toxicity in wistar rat Control Male
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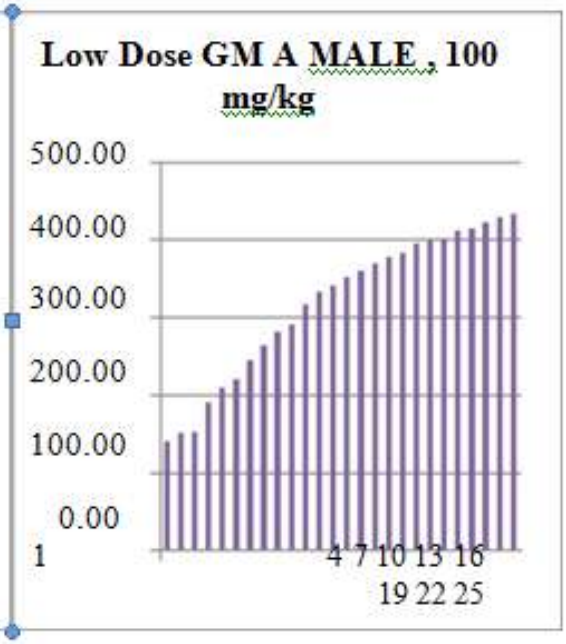
300.00
200.00
100.00
0.00
Control
4 7
10 13 16 19 22 25


Low Dose GM A MALE , 100 mg/kg
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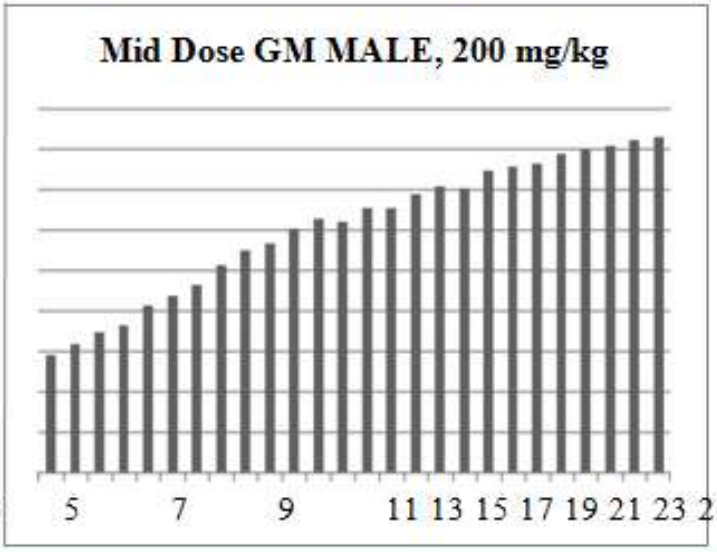
200.00
100.00
0.00
1
4 7 10 13 16
19 22 25


Mid Dose GM MALE, 200 mg/kg
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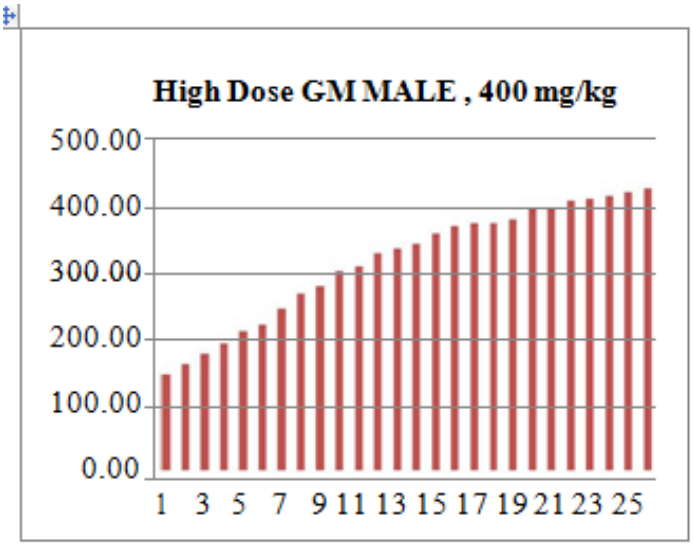

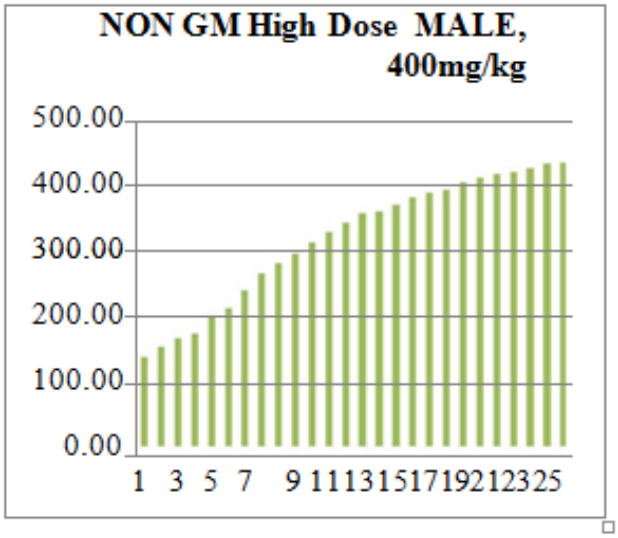

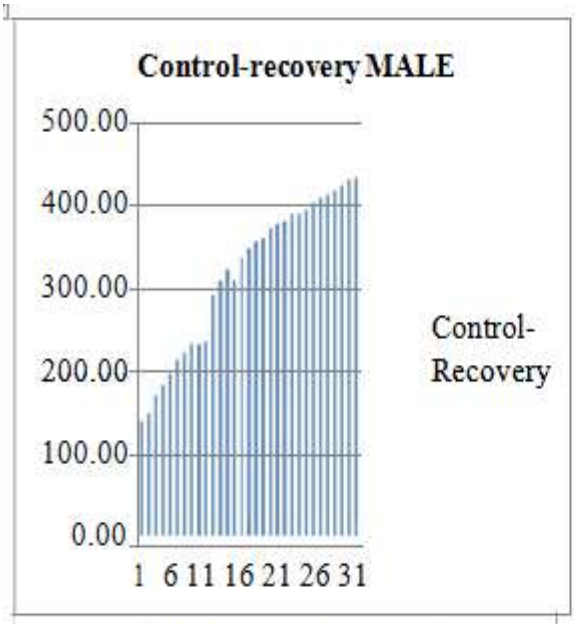

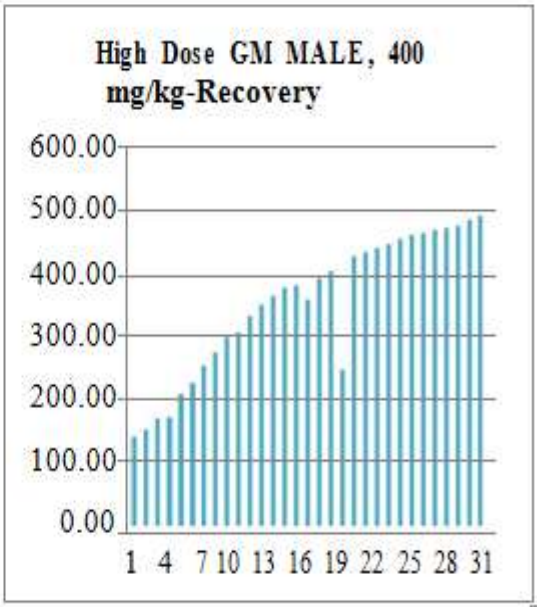

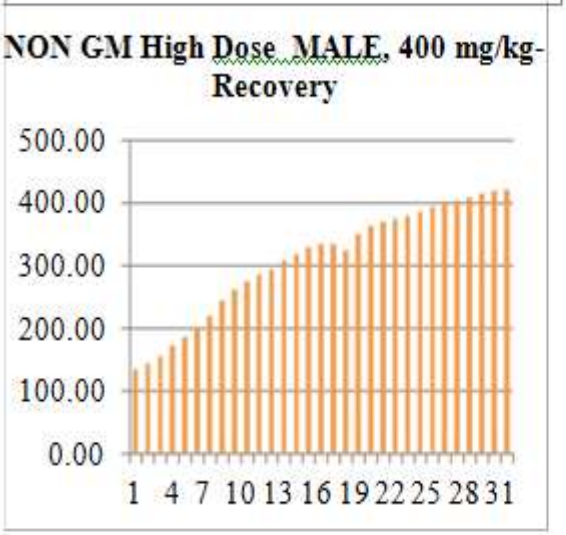

Graph in weakly body weight of male

90 Day Repeat Dose Oral Toxicity Study of Wistar Rats–Weekly Body Weight (g, Mean ± SD)–Females

GROUP	1 DAY	2 DAY	4 DAY	5 DAY	6 DAY	7 DAY	8 DAY
Control	145.51	156.23	171.48	182.45	192.02	202.62	209.06
	10.61	12.14	9.36	11.50	15.88	17.23	19.52
LOW DOSE GM,100 mg/kg	145.66	155.73	177.59	188.71	196.24	201.49	209.65
	11.96	11.06	10.04	10.26	10.46	12.10	12.43
MID DOSE GM,200 mg/kg	140.41	157.84	174.61	182.94	380.99	201.47	207.56
	15.86	11.25	13.98	11.62	611.49	16.87	16.08
HIGH DOSE,400 mg/kg	144.48	154.91	171.59	175.81	174.83	195.74	204.03
	9.41	7.51	10.04	13.03	19.15	15.02	14.16
NON GMHIGH DOSE,400mg/kg	145.59	154.12	170.83	179.32	182.33	191.19	197.02
	8.77	9.91	9.20	11.38	12.90	13.99	13.92
Control- Recovery	134.08	140.62	185.26	207.96	227.14	253.86	273.54
	6.49	5.08	16.25	15.09	16.69	15.38	15.08
HIGH DOSE GM,400mg/kg-Recovery	137.66	151.90	159.80	184.82	191.46	199.06	205.14
	8.02	7.89	17.75	8.54	8.37	8.24	9.08
NON GM HIGH DOSE,400mg/kg-Recovery	141.84	148.46	172.14	176.12	185.82	193.64	198.64
	8.26	5.75	5.88	8.49	4.82	9.63	9.98

Group	9 day	10 day	11 day	12 day	13 day	14 day	15 day	16 day
Control	224.64	228.59	230.18	233.76	236.72	240.12	248.80	254.63
	22.86	25.49	29.20	31.22	33.41	30.47	32.49	34.08
LOW DOSE GM 100 mg/kg	222.35	224.80	228.11	227.46	232.82	236.26	237.98	245.06
	15.30	19.07	19.27	20.22	21.04	21.21	20.84	20.67
MID DOSE GM 200 mg/kg	219.20	227.80	226.27	232.78	228.01	244.30	237.20	243.51
	17.45	19.72	17.57	17.92	18.13	37.84	21.93	19.23
HIGH DOSE GM, 400 mg/kg	217.58	222.54	226.70	230.73	233.80	227.37	237.39	242.72
	13.56	14.58	14.63	13.49	13.74	17.85	13.45	13.57
NON HIGH DOSE GM,400mg/kg	207.23	208.70	214.09	220.02	222.21	225.01	226.05	231.92
	15.44	19.00	17.19	17.12	18.70	16.86	17.11	17.43
Control- Recovery	215.78	216.12	221.72	227.18	228.50	234.98	236.32	247.96
	20.94	17.90	19.63	20.69	21.73	22.51	21.58	27.50
HIGH DOSE GM, 400 mg/kg-Recovery	216.08	218.86	223.06	226.50	227.98	231.38	234.78	239.84
	12.29	11.57	10.37	9.69	9.61	11.67	12.67	16.31
NON HIGH DOSE GM, 400 mg/kg- Recovery	209.56	210.36	213.46	219.60	219.28	222.82	224.26	231.76
	6.86	4.63	6.78	11.79	11.75	8.01	8.78	10.86

Group	17DAY	18DAY	19DAY	20DAY	21DAY	22DAY	23DAY	24DAY
Control	257.44	256.84	260.85	260.95	262.19	265.84	266.04	270.93
	34.14	35.78	37.54	38.51	36.00	34.47	37.95	39.94
LOW DOSEGM,100mg/kg	244.45	248.91	247.58	252.09	254.85	254.97	256.18	259.24
	21.05	21.17	22.27	21.03	25.03	22.68	23.27	26.09
MID DOSE GM, 200 mg/kg	243.06	240.63	245.32	250.12	251.79	254.07	257.19	261.74
	22.07	19.43	20.60	23.58	23.20	27.79	29.75	30.21
HIGH DOSE GM, 400 mg/kg	241.82	239.81	246.72	250.80	254.27	255.57	256.89	258.30
	13.53	17.11	16.74	12.27	15.01	15.41	15.56	15.62
NON GM HIGH DOSE,400mg/kg	232.63	235.67	236.55	242.58	242.90	244.22	243.26	241.93
	15.35	16.62	15.61	17.19	16.17	15.39	13.99	16.97
Control- Recovery	245.76	246.56	247.88	245.20	251.48	256.36	258.22	258.80
	26.46	25.07	26.79	27.25	27.92	27.92	27.22	30.58
HIGH DOSE GM, 400 mg/kg- Recovery	240.08	240.18	238.46	241.72	245.58	250.70	248.86	248.38
	17.86	10.65	14.50	15.40	15.35	16.74	18.09	21.36
NON GM HIGH DOSE, 400 mg/kg- Recovery	228.36	232.68	233.34	233.54	237.30	240.18	240.54	239.74
	9.62	14.20	9.41	15.55	16.65	17.43	13.74	9.87

GROUP	25 DAY	26 DAY	27 DAY	28 DAY	29 DAY	30 DAY	31 DAY
Control	273.65	274.13	-	-	-	-	-
	40.09	42.89	-	-	-	-	-
LOW DOSE GM 100 mg/kg	261.29	264.60	-	-	-	-	-
	25.91	26.70	-	-	-	-	-
MID DOSE GM, 200 mg/kg	263.54	265.43	-	-	-	-	-
	30.23	27.26	-	-	-	-	-
HIGH DOSE GM, 400 mg/kg	260.64	260.40	-	-	-	-	-
	16.50	18.61	-	-	-	-	-
NON GM HIGH DOSE,400mg/kg	243.92	246.68	-	-	-	-	-
	15.33	16.14	-	-	-	-	-
Control- Recovery	261.36	263.96	264.18	264.44	264.30	267.54	270.28
	31.17	30.00	30.09	28.43	27.93	29.08	30.36
HIGH DOSE GM, 400 mg/kg- Recovery	251.34	252.10	258.44	260.20	257.10	260.22	260.60
	19.26	12.24	13.20	11.24	10.22	17.24	16.85
NON GM HIGH DOSE, 400 mg/kg- Recovery	234.44	245.68	246.15	249.70	247.85	248.70	251.83
	15.56	14.36	17.24	21.80	20.63	18.44	15.12

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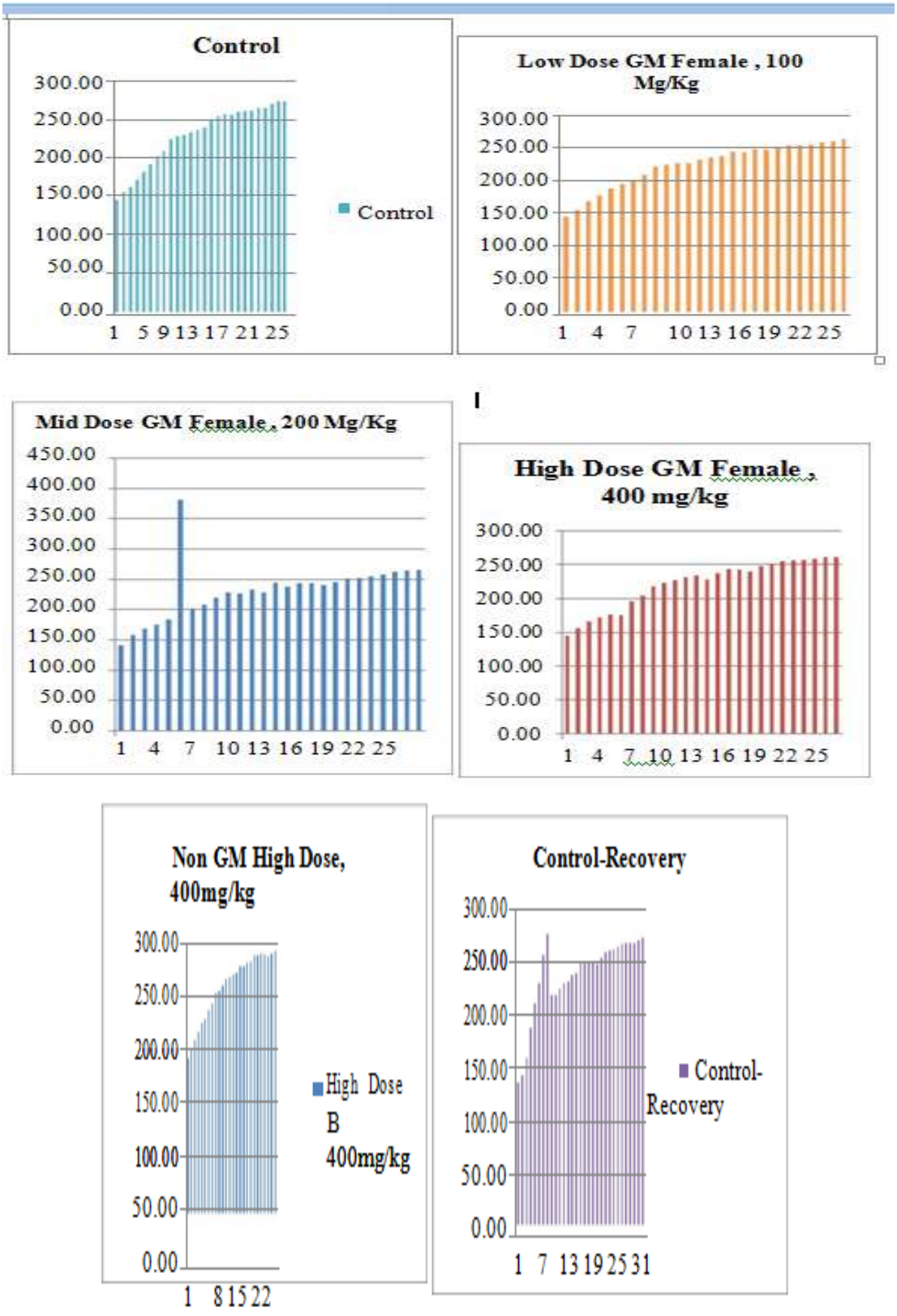
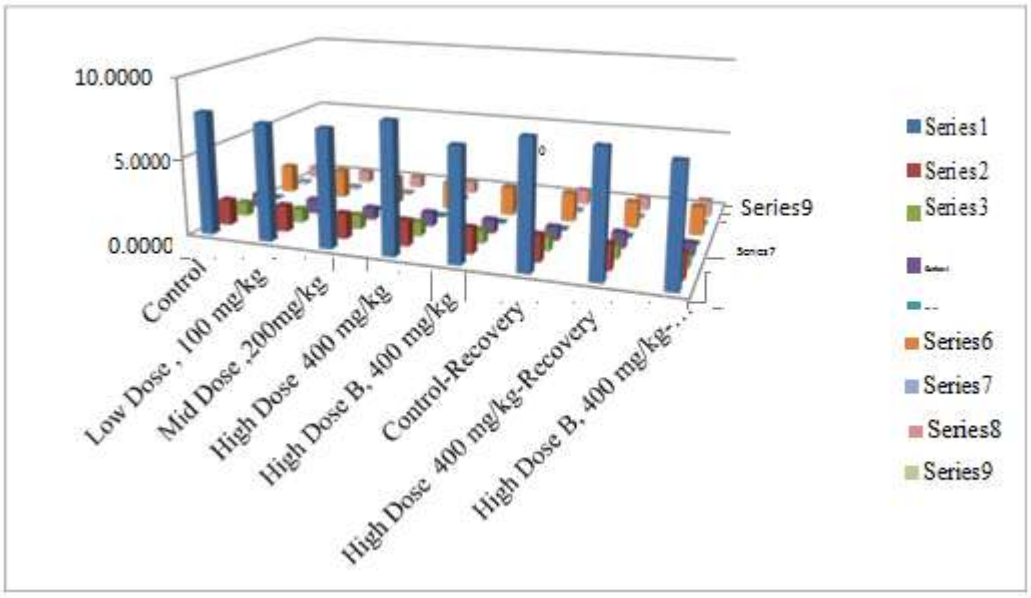


Graph in weakly body weight of female

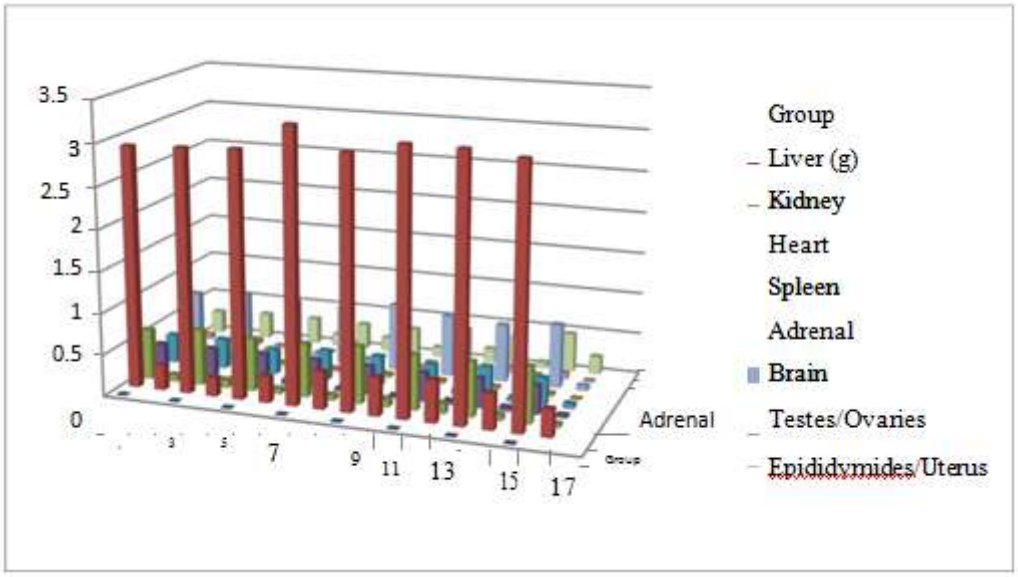
90 Day Repeat Dose Oral Toxicity Study of Wistar Rats–Organ Weights (gm per 100 gm body weight)–Males

Group	Liver(g)	Kidney	Heart	Spleen	Adrenal	Brain	Testes	Epididymides
Control	3.0170	0.6790	0.3254	0.2940	0.0175	0.5078	0.8342	0.3816
	0.4124	0.0827	0.0472	0.0368	0.0072	0.1283	0.2225	0.0808
LOW DOSE GM 100 mg/kg	3.0166	0.6071	0.3046	0.2779	0.0156	0.4538	0.8710	0.3795
	0.4348	0.0809	0.0448	0.0353	0.0065	0.0587	0.2494	0.0816
MID DOSE GM 200 mg/kg	2.9836	0.6498	0.3157	0.3280	0.0186	0.4975	0.7911	0.3786
	0.5779	0.1083	0.0456	0.0678	0.0064	0.0706	0.1027	0.0439
HIGH DOSE GM 400 mg/kg	2.9180	0.6007	0.3068	0.2914	0.0166	0.4917	0.8574	0.3803
	0.3650	0.0454	0.0339	0.0549	0.0056	0.0572	0.1229	0.0867
NON GM HIGH DOSE, 400 mg/kg	2.7782	0.5994	0.3137	0.3022	0.0168	0.4723	0.8583	0.3789
	0.2780	0.0761	0.0275	0.0377	0.0063	0.0363	0.1027	0.0456
Control Recovery	2.9724	0.6225	0.3150	0.3074	0.0163	0.5163	0.9357	0.3791
	0.5216	0.0538	0.0243	0.0213	0.0059	0.1207	0.1774	0.1057
HIGH DOSE GM, 400 mg/kg- Recovery	2.6663	0.6142	0.3080	0.2731	0.0167	0.4059	0.7370	0.3128
	0.1332	0.0445	0.0299	0.0535	0.0051	0.0254	0.0900	0.0529
NON GM HIGH DOSE, 400 mg/kg-Recovery	2.6175	0.6086	0.3211	0.3085	0.0197	0.4623	0.8403	0.3612
	0.2935	0.0252	0.0228	0.0676	0.0041	0.0732	0.0907	0.0268

N=10



Graph in organ weight of male



Graph in organ weight of female

90 Day Repeat Dose Oral Toxicity Study of Wistar Rats–Organ Weights (gm per 100 gm body weight)–Females

Group	Liver(g)	Kidney	Heart	Spleen	Adrenal	Brain	Ovaries	Uterus
Control	2.9125	0.6059	0.3262	0.3424	0.0284	0.6933	0.0558	0.2663
	0.2974	0.0768	0.0446	0.0425	0.0052	0.1242	0.0165	0.0649
LOW DOSE GM 100 mg/kg	2.9371	0.6698	0.3406	0.3543	0.0653	0.7559	0.0594	0.2996
	0.2273	0.0914	0.0225	0.1112	0.1026	0.0634	0.0094	0.0707
MID DOSE GM,200 mg/kg	2.9658	0.6357	0.3481	0.3009	0.0290	0.7146	0.0529	0.3081
	0.3184	0.0568	0.0618	0.0466	0.0065	0.0872	0.0082	0.1346
HIGH DOSE GM, 400 mg/kg	3.2886	0.6483	0.3456	0.3594	0.0298	0.7189	0.0664	0.3022
	0.4567	0.0617	0.0309	0.0465	0.0079	0.0837	0.0190	0.1591
NON GM HIGH DOSE, 400 mg/kg	3.0199	0.6917	0.3446	0.3626	0.0368	0.8067	0.0628	0.3104
	0.4672	0.0941	0.0231	0.0641	0.0102	0.0571	0.0116	0.1032
Control- Recovery	3.1600	0.6633	0.3421	0.3336	0.0356	0.7434	0.0621	0.3786
	0.5083	0.1193	0.0413	0.0688	0.0069	0.1027	0.0138	0.1588
HIGH DOSE GM, 400 mg/kg-Recovery	3.1528	0.6586	0.3538	0.3885	0.0344	0.6964	0.0622	0.3515
	0.4321	0.0649	0.0443	0.0456	0.0086	0.0985	0.0141	0.0622
NON GM HIGH DOSE 400 mg/kg-Recovery	3.0968	0.6780	0.3456	0.3282	0.0324	0.7718	0.0713	0.4604
	0.3328	0.0444	0.0299	0.0548	0.0034	0.0527	0.0067	0.2080

N=10

90 Day Repeat Dose Oral Toxicity Study of Wistar Rats– Biochemical Analysis–Females

Group	Glucose	Creatinine	AST	Urea	Total Protein	Triglycerides	Albumin	ALP
Control	103.00	0.65	137.00	39.00	6.47	83.20	2.95	110.50
	40.98	0.07	68.31	8.56	1.24	32.87	0.46	58.00
LOW DOSE GM, 100 mg/kg	108.30	0.70	144.80	38.90	6.74	66.50	2.97	168.30
	32.23	0.13	42.79	10.05	1.51	23.74	0.55	136.20
MID DOSE GM, 200 mg/kg	124.20	0.68	128.30	42.60	7.17	78.00	3.06	161.50
	56.57	0.15	42.83	9.89	1.94	33.86	0.82	98.56
HIGH DOSE GM, 400 mg/kg	131.56	0.77	145.22	43.78	7.88	64.11	3.48	154.00
	26.99	0.09	29.14	9.24	1.68	33.66	0.64	74.74
HIGH DOSEGM, 400 mg/kg	117.80	0.74	158.80	43.70	7.97	76.00	3.38	166.00
	38.18	0.15	68.10	6.13	1.74	33.33	0.60	77.20
Control-Recovery	155.80	0.82	159.40	49.40	8.60	80.60	4.32	96.20
	53.21	0.23	54.08	13.80	2.27	24.18	1.13	57.76
HIGH DOSE GM, 400 mg/kg Recovery	98.20	0.88	185.40	41.20	11.30	95.80	4.42	111.00
	48.02	0.22	46.21	12.18	2.74	32.26	1.07	39.94
NON GM HIGH DOSE, 400 mg/kg- Recovery	91.98	0.74	175.28	42.52	9.42	96.23	3.59	106.15
	24.36	0.26	66.60	14.36	3.19	39.92	1.24	56.83

Biochemical Analysis Contd. – Females

Group	Cholesterol	Globulin	ALT	Total Bilirubin	Na+	K+	Cl-
Control	28.50	3.52	33.20	0.16	160.90	6.30	116.10
	7.50	0.81	10.46	0.05	6.94	1.03	5.11
LOW DOSE GM 100 mg/kg	31.60	3.77	35.60	0.18	158.10	6.52	115.10
	8.26	0.99	13.16	0.09	5.99	0.95	4.77
MID DOSE GM, 200 mg/kg	32.70	4.11	31.90	0.20	159.67	6.05	115.67
	17.63	1.17	9.42	0.07	5.87	0.95	5.22
HIGH DOSE GM, 400 mg/kg	36.67	4.40	41.56	0.23	156.22	6.05	113.67
	9.14	1.07	8.65	0.05	4.41	0.76	3.54
NON GM HIGH DOSE 400 mg/kg	35.10	4.59	40.40	0.22	159.10	6.65	115.30

	12.42	1.21	11.54	0.08	9.39	1.51	7.27
Control-Recovery	54.20	4.28	52.00	0.24	171.00	5.80	123.60
	15.77	1.19	31.50	0.06	44.06	1.63	31.51
HIGH DOSE GM, 400 mg/kg-Recovery	42.00	#DIV/0!	50.00	0.20	177.67	6.82	134.50
	13.77	1.22	9.07	0.07	43.49	1.63	31.80
NON GM HIGH DOSE, 400 mg/kg-Recovery	32.63	#DIV/0!	48.07	0.17	150.73	5.98	109.47
	11.43	#DIV/0!	21.39	0.07	53.40	2.17	38.55

N=10

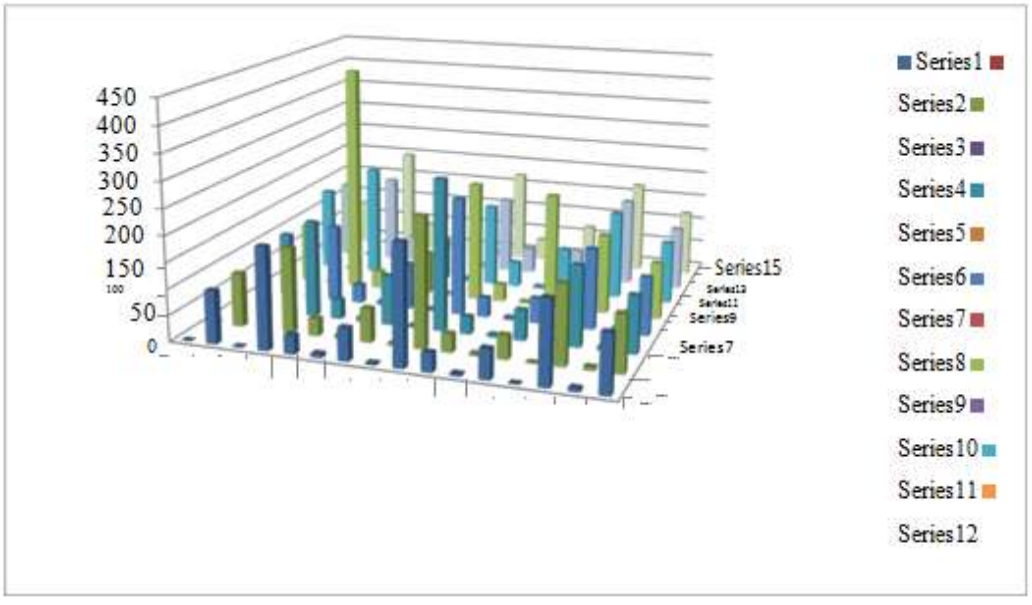
90 Day Repeat Dose Oral Toxicity Study of Wistar Rats–Biochemical Analysis–Males

Group	Glucose	Creatinine	AST	Urea	Total Protein	Triglycerides	Albumin	ALP
Control	101.60	0.69	194.70	35.90	7.49	62.40	3.10	229.20
	34.59	0.07	56.06	8.88	1.05	14.99	0.34	100.32
LOW DOSE GM, 100 mg/kg	103.22	0.64	163.22	33.67	6.88	65.11	3.09	247.89
	29.58	0.14	43.41	6.96	1.11	22.13	0.37	86.56
MID DOSE GM, 200 mg/kg	99.40	0.65	184.60	37.20	7.36	95.90	3.13	290.30
	50.76	0.11	36.99	5.57	1.09	39.90	0.37	197.32
HIGH DOSE GM, 400 mg/kg	122.20	0.59	148.20	35.70	7.08	89.30	3.09	227.40
	29.90	0.19	30.77	4.45	1.38	26.54	0.40	61.26
NON GM HIGH DOSE, 400 mg/kg	113.75	0.60	439.75	33.63	6.74	83.13	3.01	231.25
	45.41	0.11	744.82	5.63	1.23	26.84	0.47	113.52
Control-Recovery	161.80	0.80	216.80	54.00	7.98	83.20	3.88	161.00
	49.72	0.23	155.25	14.68	2.39	30.13	1.06	63.97
HIGH DOSE GM, 400 mg/kg-Recovery	152.20	0.76	171.40	49.60	7.78	92.60	3.90	149.60
	51.12	0.20	43.68	12.75	2.00	24.55	0.99	44.41
NON GM HIGH DOSE, 400 mg/kg- Recovery	86.50	0.85	206.00	51.25	10.16	67.80	3.68	180.75
	42.92	0.22	53.41	12.59	2.78	34.16	1.04	51.75

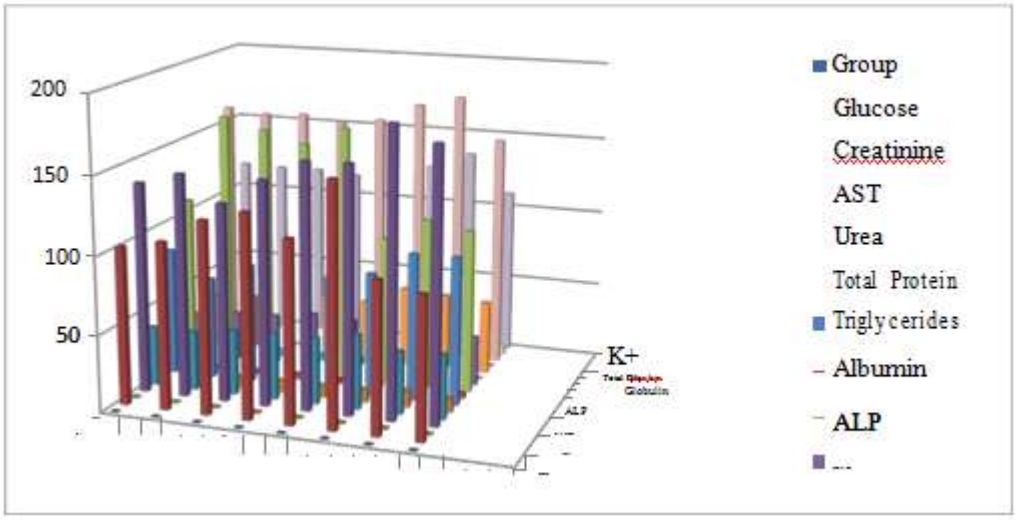
Table 9 Biochemical Analysis Contd. – Males

Group	Cholest erol	Globulin	ALT	Total Bilirubin	Na+	K+	Cl-
Control	36.70	4.39	56.90	0.09	158.78	6.71	111.44
	6.09	0.84	14.56	0.03	4.89	1.32	3.05
LOW DOSE GM, 100 mg/kg	35.67	3.79	45.44	0.16	152.3	6.14	109.67
	11.76	0.83	6.64	0.07	4.66	0.61	3.00
MID DOSE GM, 200 mg/kg	33.89	4.23	59.07	6.00	154.9	6.58	110.50
	14.97	0.84	25.87	18.62	6.74	1.01	4.77
HIGH DOSE GM, 400 mg/kg	38.40	3.99	48.40	0.12	155.7	5.78	112.80
	11.00	1.02	13.43	0.06	9.66	0.84	6.86
NON GM HIGH DOSE , 400 mg/kg	33.63	3.73	222.50	0.21	153.2	5.65	109.22
	5.63	0.81	485.70	0.44	5.93	0.53	3.63
Control- Recovery	49.00	4.10	87.00	0.18	170.6	6.76	121.00
	14.77	1.40	108.84	0.09	49.48	2.13	35.57
HIGH DOSE GM, 400 mg/kg-Recovery	49.00	3.88	56.00	0.12	169.6	6.24	121.80
	13.57	1.01	26.76	0.07	39.10	1.55	28.11
NON GM HIGH DOSE, 400 mg/kg-Recovery	41.25	#DIV/0!	78.25	0.12	178.8	7.21	127.80
	12.17	#DIV/0!	18.33	0.05	44.67	1.87	32.34

N=10



Graph in Biochemical Analysis of Female



Graph in Biochemical Analysis of Male

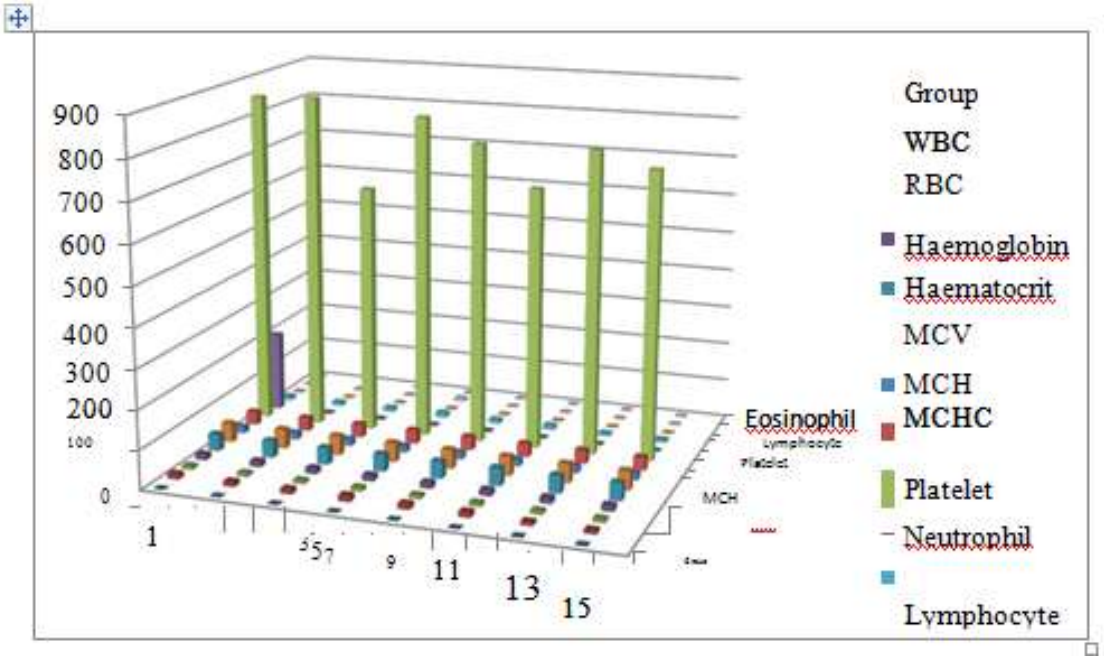
90 Day Repeat Dose Oral Toxicity Study of Wistar Rats–Haematology– Males

Group	WBC	RBC	Haemoglobin	Haematocritn	MCV	MCH	MCHC
Control	13.68	9.00	14.71	43.51	48.39	16.36	33.82
	5.03	0.50	0.52	1.61	2.05	0.66	0.64
LOW DOSE GM, 100 mg/kg	12.36	8.77	14.68	43.13	49.30	16.77	34.04
	4.11	0.57	0.58	1.58	2.51	0.70	0.55
MID DOSE GM, 200 mg/kg	12.48	8.56	14.36	42.34	49.54	16.79	33.90
	3.95	0.77	1.23	3.62	2.47	0.71	0.39
HIGH DOSE GM, 400 mg/kg	15.36	9.02	15.01	43.78	48.60	16.66	34.29
	7.35	0.28	0.46	0.83	1.65	0.62	0.75
NON GM HIGH DOSE, 400 mg/kg	13.95	8.91	14.89	43.27	48.57	16.69	34.41
	4.86	0.35	0.58	1.87	1.86	0.47	0.52
Control- Recovery	13.70	9.02	15.04	44.80	49.68	16.68	33.56
	7.52	0.36	0.98	2.10	1.12	0.65	0.74
HIGH DOSE GM, 400 mg/kg- Recovery	9.17	8.97	14.96	44.02	49.02	16.68	34.00
	4.46	0.41	0.81	2.83	1.68	0.53	0.41
NON GM HIGH DOSE, 400 mg/kg- Recovery	9.21	9.18	15.08	44.84	48.84	16.40	33.66
	5.85	0.16	0.58	2.31	2.05	0.51	0.47

Group	Platelet	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Control	854.70	203.96	10.20	0.63	0.36	0.01
	127.84	637.04	4.54	0.30	0.19	0.01
LOW DOSE GM, 100 mg/kg	859.22	2.30	9.11	0.56	0.38	0.01
	78.18	1.14	3.30	0.24	0.15	0.01
MID DOSE GM, 200 mg/kg	634.90	1.93	9.46	0.64	0.44	0.01

	229.07	0.56	3.65	0.18	0.21	0.01
HIGH DOSE GM, 400 mg/kg	827.40	1.97	12.21	0.68	7.41	0.02
	120.40	0.45	6.91	0.36	21.99	0.01
NON GM HIGH DOSE , 400 mg/kg	768.89	2.76	9.95	0.71	0.50	0.03
	132.77	2.11	4.11	0.29	0.41	0.05
Control- Recovery	667.20	2.09	10.85	0.51	0.24	0.02
	63.61	0.48	7.13	0.25	0.16	0.01
HIGH DOSE GM, 400mg/kg Recovery	773.20	1.71	6.76	0.37	0.32	0.01
	115.02	0.77	3.65	0.19	0.14	0.01
NON GM HIGH DOSE, 400 mg/kg-Recovery	735.20	1.42	7.09	0.42	0.26	0.02
	76.40	0.44	5.39	0.30	0.08	0.02

N=10



Graphs in Haematology of Male

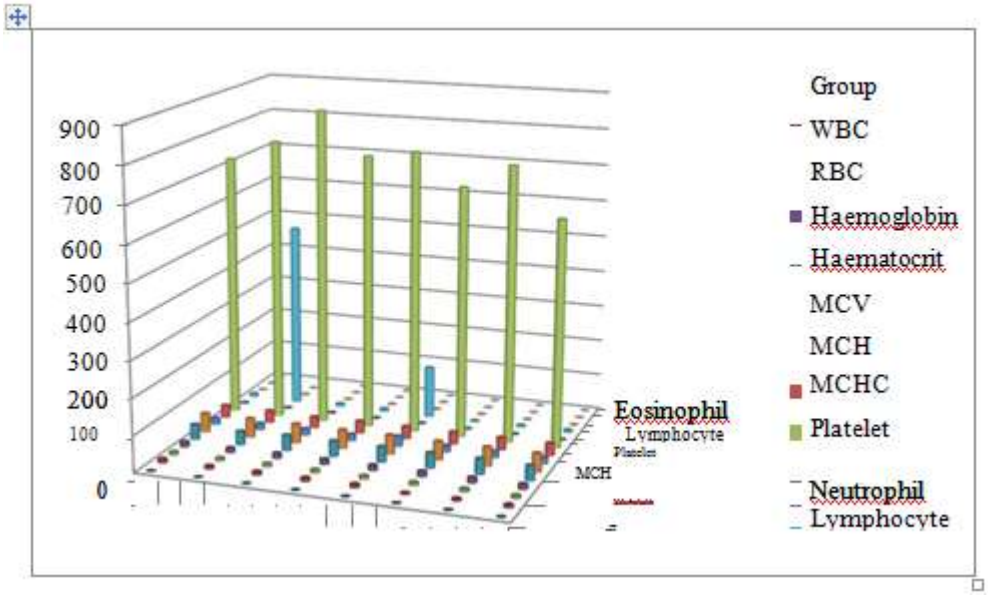
90 Day Repeat Dose Oral Toxicity Study of Wistar Rats–Haematology– Females

Group	WBC	RBC	Haemoglobin	Haematocrit	MCV	MCH	MCHC
Control	11.17	7.97	14.06	41.53	52.22	17.53	33.62
	4.46	0.43	0.72	1.45	2.51	0.71	0.55
LOW DOSE GM, 100 mg/kg	8.37	7.22	12.72	38.02	52.78	17.60	33.33
	3.84	1.23	2.32	6.29	2.01	0.63	0.93
MID DOSE GM, 200 mg/kg	9.43	8.10	14.21	42.37	52.38	17.57	30.21
	4.11	0.53	0.68	1.83	2.23	0.61	10.11

HIGH DOSE GM, 400 mg/kg	9.27	8.36	14.44	42.71	51.27	17.32	33.81
	4.21	0.72	0.78	1.82	3.11	0.73	0.73
NON GM HIGH DOSE, 400 mg/kg	9.58	8.16	14.35	42.67	52.39	27.60	33.61
	5.29	0.56	0.77	1.75	2.14	31.38	0.62
Control- Recovery	3.21	8.18	14.14	41.94	51.30	17.30	33.72
	1.39	0.19	0.32	0.97	1.60	0.28	0.64
HIGH DOSE GM, 400 mg/kg- Recovery	5.73	8.25	14.36	42.54	51.66	17.44	33.78
	2.31	0.35	0.26	1.08	2.70	0.77	0.34
NON GM HIGH DOSE, 400 mg/kg- Recovery	7.68	7.89	13.73	40.38	51.25	17.40	34.00
	2.52	0.48	0.54	1.16	2.14	0.45	0.89

Group	Platelet	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Control	720.30	1.57	8.41	0.48	0.39	0.02
	240.06	0.72	4.14	0.18	0.26	0.02
LOW DOSE GM, 100 mg/kg	775.40	1.51	507.17	0.40	0.26	0.01
	298.34	1.10	1583.89	0.23	0.12	0.01
MID DOSE GM, 200 mg/kg	867.56	1.60	7.00	0.47	0.34	0.01
	149.92	0.54	3.66	0.23	0.18	0.01
HIGH DOSE GM, 400 mg/kg	752.78	1.18	7.37	0.34	0.36	0.01
	153.90	0.36	3.66	0.18	0.17	0.01
NON GM HIGH DOSE, 400 mg/kg	772.60	1.09	142.06	0.40	0.35	0.02
	178.49	0.39	426.91	0.22	0.19	0.02
Control- Recovery	687.60	0.60	2.34	0.13	0.13	0.01
	59.98	0.25	1.07	0.06	0.08	0.01
HIGH DOSE GM, 400 mg/kg- Recovery	754.20	0.83	4.49	0.22	0.17	0.02
	80.91	0.41	1.70	0.16	0.14	0.01
NON GM HIGH DOSE, 400 mg/kg- Recovery	622.75	0.61	6.45	0.35	0.20	0.08
	112.52	0.22	2.20	0.14	0.11	0.09

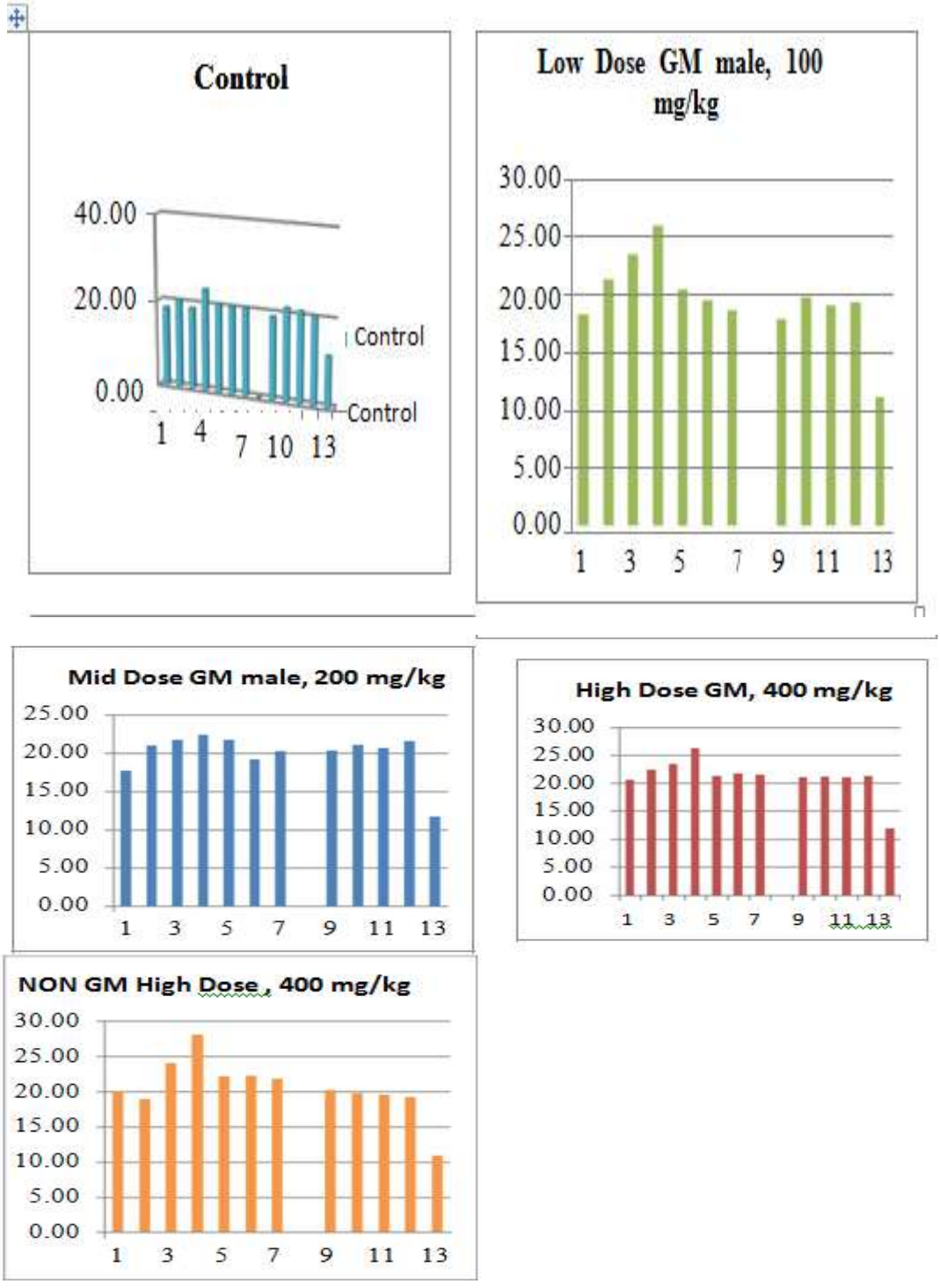
N=10



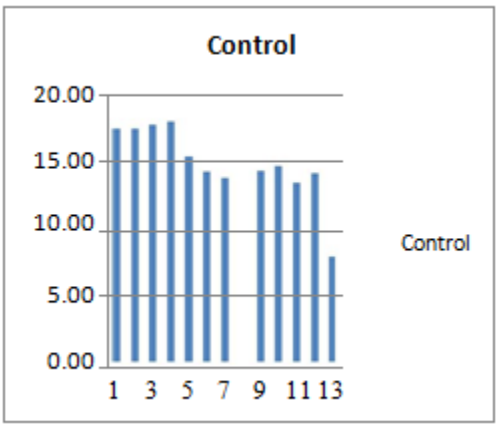
90ay Repeat Dose Oral Toxicity Study of Wistar Rats–Feed Consumption (g/rat/day)– Males

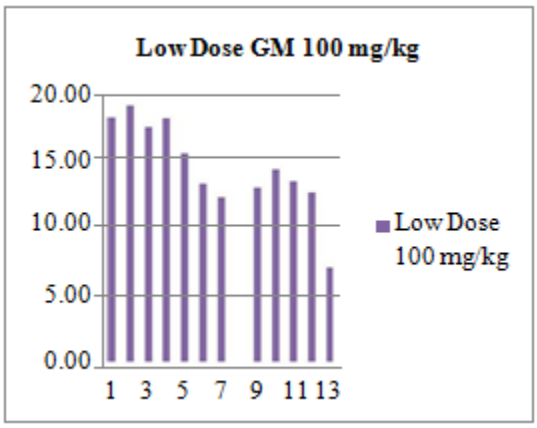
Group	1 DAY	2 DAY	3 DAY	4 DAY	5 DAY	6 DAY	7 DAY
Control	18.44	20.48	18.91	23.63	20.66	20.23	20.41
LOW DOSE GM, 100 mg/kg	18.36	21.41	23.53	26.03	20.54	19.54	18.67
MID DOSE GM, 200 mg/kg	17.66	20.98	21.73	22.35	21.70	19.15	20.24
HIGH DOSE GM, 400 mg/kg	20.61	22.39	23.44	26.18	21.22	21.72	21.52
NON GM HIGH DOSE, 400 mg/kg	19.99	18.95	23.96	27.99	22.16	22.23	21.77
Control- Recovery	18.57	22.25	21.48	19.25	23.59	21.14	18.87
HIGH DOSE GM, 400 mg/kg- Recovery	18.95	22.55	25.04	26.51	24.84	23.84	22.57
NON GM HIGH DOSE, 400 mg/kg	18.27	17.36	24.57	25.37	20.50	18.74	20.28

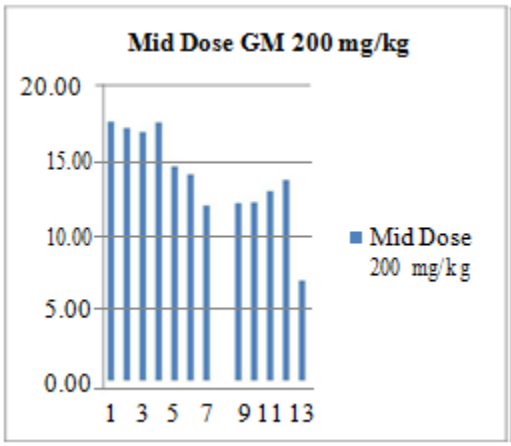
Group	8 DAY	9 DAY	10 DAY	11 DAY	12 DAY	13 DAY	14 DAY
Control	19.23	21.52	21.14	20.31	11.86	-	-
LOW DOSE GM, 100 mg/kg	17.93	19.80	19.14	19.36	11.14	-	-
MID DOSE GM, 200 mg/kg	20.32	21.08	20.70	21.55	11.65	-	-
HIGH DOSE GM, 400 mg/kg	21.03	21.17	21.03	21.20	11.91	-	-
NON GM HIGH DOSE, 400 mg/kg	20.18	19.76	19.53	19.17	10.83	-	-
Control- Recovery	21.11	20.01	19.87	20.67	11.94	21.82	21.54
HIGH DOSE GM, 400 mg/kg- Recovery	22.70	22.71	22.51	22.55	12.78	25.06	23.88
NON GM HIGH DOSE, 400 mg/kg	19.37	21.61	20.59	20.80	11.69	22.70	21.54

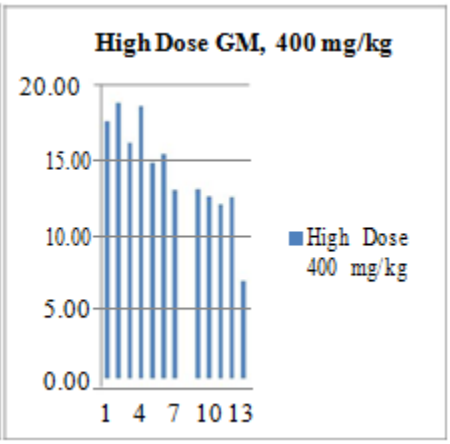


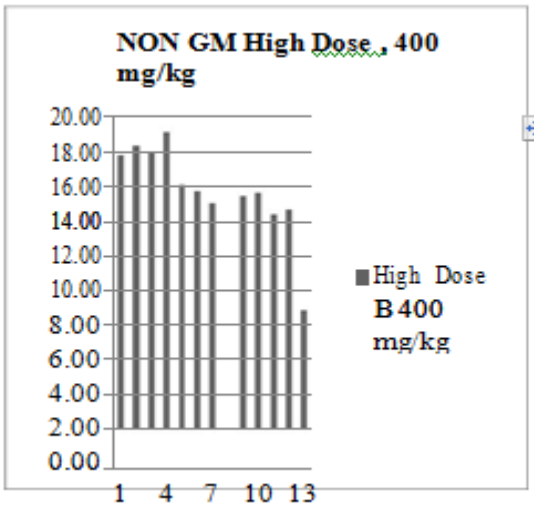
Graph in feed of males

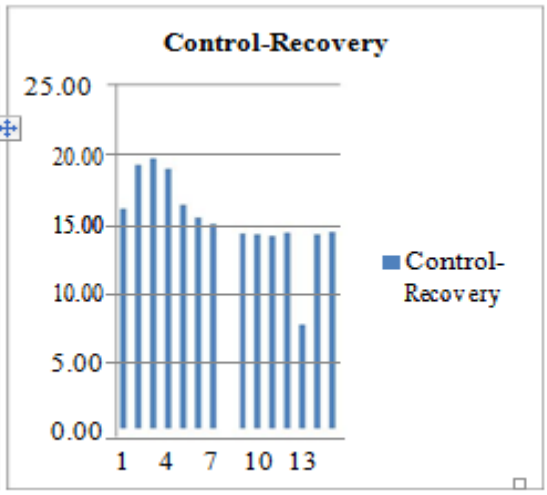

Control
20.00
15.00
10.00
5.00
0.00
1 3 5 7 9 11 13
Control

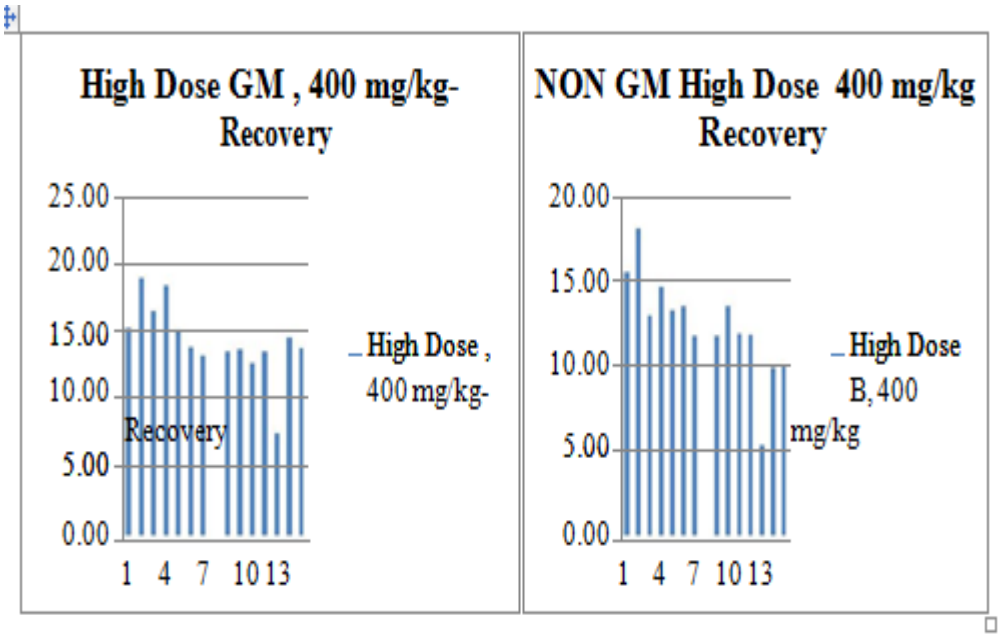

Low Dose GM 100 mg/kg
20.00
15.00
10.00
5.00
0.00
1 3 5 7 9 11 13
Low Dose 100 mg/kg


Mid Dose GM 200 mg/kg
20.00
15.00
10.00
5.00
0.00
1 3 5 7 9 11 13
Mid Dose 200 mg/kg


High Dose GM, 400 mg/kg
20.00
15.00
10.00
5.00
0.00
1 4 7 10 13
High Dose 400 mg/kg


NON GM High Dose , 400 mg/kg
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
1 4 7 10 13
High Dose B 400 mg/kg


Control-Recovery
25.00
20.00
15.00
10.00
5.00
0.00
1 4 7 10 13
Control-Recovery



Graph in feed of female

90 Day Repeat Dose Oral Toxicity Study of Wistar Rats–Feed Consumption (g/rat/day)– Females

GROUP	1 DAY	2 DAY	3 DAY	4 DAY	5 DAY	6 DAY	7 DAY
Control	17.55	17.56	17.88	18.07	15.44	14.33	13.82
LOW DOSE GM, 100 mg/kg	17.89	18.79	17.23	17.84	15.24	13.05	12.07
MID DOSE GM, 200 mg/kg	17.51	17.06	16.79	17.47	14.47	13.93	11.85
HIGH DOSE GM, 400 mg/kg	17.48	18.74	16.04	18.51	14.67	15.28	12.83
NON GM HIGH DOSE, 400 mg/kg	15.87	16.43	16.02	17.22	14.13	13.81	13.11
Control- Recovery	15.96	19.15	19.61	18.87	16.28	15.33	14.83
HIGH DOSE GM, 400 mg/kg- Recovery	15.49	19.17	16.75	18.59	15.24	14.01	13.37
NON GM HIGH DOSE, 400 mg/kg	15.71	18.36	13.14	14.83	13.41	13.68	11.91

GROUP	8 DAY	9 DAY	10 DAY	11 DAY	12 DAY	13 DAY	14 DAY
Control	14.40	14.75	13.48	14.20	7.89	-	-
LOW DOSE GM, 100 mg/kg	12.78	14.11	13.26	12.42	6.93	-	-
MID DOSE GM, 200 mg/kg	11.99	12.01	12.81	13.53	6.75	-	-
HIGH DOSE GM, 400 mg/kg	12.87	12.39	11.84	12.34	6.65	-	-
NON GM HIGH DOSE, 400 mg/kg	13.52	13.70	12.43	12.73	6.86	-	-
Control- Recovery	14.19	14.08	14.00	14.23	7.55	14.09	14.25
HIGH DOSE GM, 400 mg/kg- Recovery	13.67	13.85	12.80	13.69	7.61	14.77	13.96
NON GM HIGH DOSE, 400 mg/kg	11.91	13.71	12.02	11.97	5.40	10.00	10.07

CONCLUSION-

Based on a collective review of all the findings in this study, during which the test item A was administered at three dose levels (100 mg/kg, 200 mg/kg and 400 mg/kg body weight), the "No Observed Adverse Effect Level (NOAEL)" of Test item B in this study is determined to be above 400 mg/kg body weight. Also there was no appreciable difference in the parameters under study, between Test item A and Test item B treated rats.

ACKNOWEDGEMENT

The process of writing a dissertation is long and arduous—and it is certainly not done Single handedly. Owing a great gratitude to the most Merciful and Benevolent ALMIGHTY who was and will always be with me in my life, I would also like to thank a few other people who stood by me throughout. I am indebted to my parents for their moral support and personal sacrifice throughout this dissertation work. I am deeply indebted to my respected guide Prof. Mayank Thakur, Advance Institute of Biotech and Paramedical sciences, for taking very keen interest and giving his Valuable guidance, constructive criticisms, stimulating discussions and suggestions and constant encouragement throughout my project work and made this work successful. I am very much indebted to C.S Bhargava Director of "Advance Institute of Biotech &Paramedical Sciences, Kanpur for providing me this opportunity and for his constant encouragement throughout the project work. I consider myself lucky to work under the guidance of Dr. Akshay Dwarakanath "CSIR-Indian Institute of Toxicology Research Lucknow" and Yatendra Singh, Assistance Professor, Department of Pharmacology, "Advance Institute of Biotech &Paramedical Sciences, Kanpur" as their continuous guidance and support have always propelled me to perform better and paved the way for the completion of this research work. I am thankful to Dr. Alok Dhawan, Director, CSIR-Indian Institute of Toxicology Research Lucknow for providing all the necessary infrastructure and facilities which has enabled the successful completion of the dissertation. I would like to thanks Dr. Dhirendra Singh, Dr. M.K. Srivastava, Dr. Mahadeo Kumar, Dr. Anjaneya Ayanur for allowing me to use laboratory instruments under their guidance.

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