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SUB-ACUTE TOXICITY STUDIES OF ACETAMINOPHEN IN WISTAR RATS

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ABSTRACT

The aim of the present study was to evaluate the sub-acute oral toxicity of acetaminophen in Wistar rats at doses of 250 to 1000 mg/kg body weight. The following observations were noticed during the study. No mortality, no incidence of any abnormal clinical signs, no significant effect on body weight gain, no effect on the daily feed consumption, no toxicologically significant effect on the haematological and biochemical parameter in either sex, at and up to the dose of 1000 mg/kg body weight. No toxicologically significant effect on the urinalysis parameters, absolute and relative organ weights, gross pathological alterations and histopathological findings were observed in male and female rats treated at and up to the dose of 1000 mg/kg body weight. Based on the findings of this study, the No Observed Adverse Effect Level (NOAEL) of acetaminophen in Wistar rats, following oral administration at the doses of 250, 500 and 1000 mg/kg on daily basis was found to be 1000 mg/kg body weight.

KEYWORDS: **Paracetamol, Toxicity, Rat, Haematology and Biochemistry**

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INTRODUCTION

Acetaminophen (paracetamol) is a widely consumed analgesic found in several non-prescription pharmaceuticals. Acetaminophen was first evaluated for pharmacologic activity in 1893 by Von Mehring, who discovered its analgesic and antipyretic properties; however, it was not until the work of Brodie in the 1940's that serious consideration was given to its use in humans.[1] Acetaminophen was first introduced as a prescription drug in the United States in 1955 and was approved by the Food and Drug Administration for sale as a non prescription drug in 1960. [2] Toxicity from acetaminophen is not from the drug itself but from one of its metabolites, N-acetyl-p-benzoquinoneimine (NAPQ1). Acetaminophen biotransformation involves conjugation with glucoronide and sulphate. A small amount of acetaminophen is metabolised by mixed function oxidase enzymes to form highly reactive compound NAPQ1, which is immediately conjugated with glutathione and subsequently excreted as cysteine and mercapturic conjugates. In overdoses, large amounts of acetaminophen are metabolised by oxidation because of saturation of the sulphate conjugation pathway [3-4], but once the protective intracellular glutathione stores are depleted hepatic and renal damage may ensue. Hepatotoxicity is the most remarkable feature of acetaminophen overdose.[5] Acute overdoses of acetaminophen can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same; the risk is heightened by alcohol consumption. Acetaminophen toxicity is the foremost cause of acute liver failure.[6] Renal effects of acetaminophen overdose are less commonly seen than hepatic effects. However, renal impairment may be more common than previously recognised. There are extensive toxicity studies presently available on acetaminophen. However, there are only few reports available on the toxicity of acetaminophen in India. Therefore the present study was designed to evaluate the sub-acute toxicity of acetaminophen in order to find out no observed effect level, target organ toxicity and reversibility of signs of toxicity after the recovery period in Wistar Rats.

MATERIALS AND METHODS

The methods and test procedure were followed as per the Organisation for Economic Co-operation and Development test guideline No. 407.[7]

(i) Test System

Total seventy two healthy Wistar rats (36 male and 36 female rats, weight 147-228 g) of age 6-8 weeks were selected for the present study. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. Prior to final assignment to the study, it was ensured that the selected rats were in a good state of health, females were nulliparous and non-pregnant. Rats were housed in sterilized suspended polycarbonate cages, with stainless steel top grills having facilities for holding pellet feed and drinking water in bottle with stainless steel sipper tube. All rats were freely accessible to reverse osmosis (RO) generated potable water and standard pellet laboratory animal diet *ad libitum* (Tetragon Vetcare, Bangalore). Autoclaved corn cob was used as bedding material. Throughout the acclimatization and treatment period, animal room temperature and relative humidity were maintained at 19 to 25^{o}C and 30 % to 70 % respectively. Illumination was controlled to give 12 hours light and 12 hours dark cycle during the 24-hour period. The study protocol was approved by Institutional animal ethics committee.

(ii) Study Design

The rats were distributed into 6 groups each consisting of 12 rats/group (6 male + 6 female / group). In groups I, II, III and IV rats were received doses of 0, 250, 500 and 1000 mg/kg body weight of acetaminophen respectively. Groups V and VI were administered doses of 0 and 1000 mg/kg body weight of control and acetaminophen respectively as a recovery group. The rats were examined twice daily for signs of toxicity, moribund status and mortality. Further they were also subjected to detailed clinical examination before initiation of the study and daily thereafter during the exposure period. Body weights and food

consumption were recorded daily. Laboratory investigations were performed on day 29 for main and on day 43 for recovery groups. All rats in main and recovery groups were sacrificed at termination on day 29 and 43 respectively and subjected to a detailed gross pathology and organ weights were recorded.

(iii) Observation

General clinical observations were carried out daily once during acclimatization and twice daily, throughout the study and recovery period. Detailed clinical examination was carried out prior to initiation of treatment and daily intervals during treatment and recovery period. During detailed clinical examination, all animals were observed for changes in skin and fur, eyes, mucous membrane, occurrence of secretions and excretions, autonomic activity, changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypes and bizarre behaviour. Throughout the study, all animals were observed twice daily once in the morning and once in the afternoon, for moribund condition and mortality. Individual animal body weights were recorded prior to the test item administration on day 1 and at daily intervals on day 8, 15, 22 and 28 during treatment and on day 35 and 42 of recovery period. Fasting body weights were recorded prior to necropsy. The quantity of feed consumed by rats in each cage from each group was recorded on day 8, 15, 22 and 28 during treatment and recovery period (day 35 and 42). Feed intake per rat was calculated using the amount of feed offered to and left out in the cage on day 8, 15, 22 and 28 during treatment and recovery period (day 35 and 42). Clinical laboratory investigations were performed at termination of treatment (day 29) and at end of recovery (day 43) period. Blood samples were collected from the over-night fasted (water allowed) rats through the retro-orbital sinus under isoflurane anaesthesia. Aliquots of blood were collected in tubes containing sodium citrate for determination of prothrombin time, tubes containing 10% K_2EDTA anticoagulant for hematology and without anticoagulant for clinical chemistry parameters were used. Serum was separated within 30 minutes of collection by centrifugation (10 minutes at 1000 x g, 2 to 8^{o}C) respectively. The following haematological parameters were determined; Red blood cell count, White blood cell differential count (absolute and relative), Hemoglobin, Platelets count, Packed cell volume (PCV/HCT), and Red blood cell distribution width (RDW). The following biochemical parameters were analyzed with the help of automatic biochemical analyzer, using standard reagent kits (Siemens) and standard laboratory methodology; Alanine aminotransferase (ALT), Albumin (Alb), Creatinine (Creat), Aspartate aminotransferase (AST), Blood Urea Nitrogen (BUN), Glucose (Glu), Total plasma protein (T.Pro), Alkaline Phosphatase (ALP), GGT (Gamma Glutamate transferase), Triglycerides (TG), Cholesterol, Bilirubin, Globulin (Glob) , Albumin/Globulin ratio (A/G ratio)

The serum electrolytes viz., Sodium, Potassium, Calcium, Phosphorus were analyzed with the help of automatic biochemical analyzer, using standard reagent kits. Urine samples were collected from animals for main and control groups at the end of the treatment (day 29) and for recovery groups after recovery period (day 43) by using metabolic cages. Urine samples were analyzed for approximate color, appearance, specific gravity, pH, Albumin, glucose, ketone bodies, urobilinogen, bilirubin and erythrocytes using uriscan pro+, Merck India, diagnostic strips as qualitative indicators of analyte concentration. An aliquot of the urine samples were centrifuged at approximately 1500 RPM for 10 minutes and the resulting deposit was spread on to a microscope slide. The deposit was examined for the presence of the following: Epithelial cells (E), Polymorphonuclear leucocytes (P), Casts/Crystals (Cr) and other abnormal constituents (A). All rats of main, control and recovery groups were sacrificed on day 29 and 43 respectively. Rats were fasted overnight (water allowed), weighed and euthanized using CO_2. The animals were subjected to detailed gross pathological observation during necropsy (Adrenal glands, Aorta, Brain, Epididymides, Esophagus, Eyes (with optic nerve), Heart, Kidneys, Large intestine (cecum, colon, rectum), Liver, Lung, Mammary gland, Ovaries, Skin, Small intestine (duodenum, jejunum, ileum), Spleen, Stomach (cardia, fundus, pylorus), Testis, Thymus, Thyroid gland, Urinary Bladder,

Uterus, Thymus, Skeletal muscle, Mesentric Lymph Nodes). The following organs (adrenals, brain, heart, lung, kidneys, liver, spleen, testes/uterus) from main, control and recovery group rats were sacrificed as scheduled, dissected free of fat and weighed wet as soon as possible to avoid drying. The absolute organ weights were used to estimate the organ-body weight ratio (relative) by using the terminal body weights. Group mean and standard deviation of mean were calculated for all generated data using IBM SPSS 2.0 version for windows software. Dunnett's paired t-test was employed to confirm significance difference between control and treated groups. All statistical analysis and comparisons will be determined at $P < 0.05$ level.

RESULTS

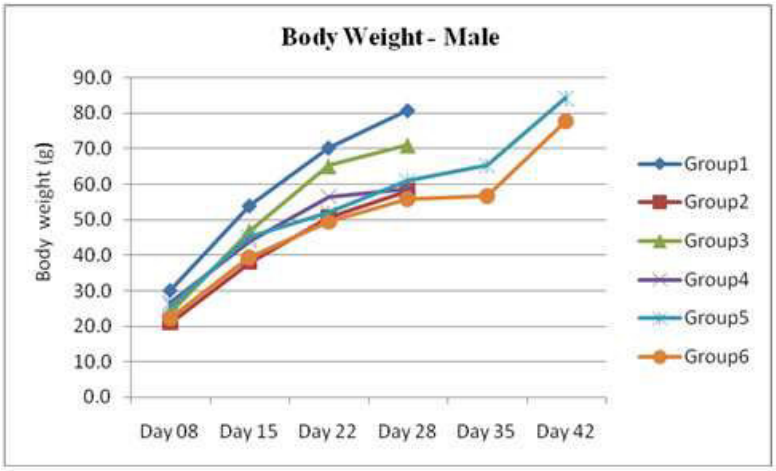
(i) Survival and Clinical Signs

There was no incidence of mortality among the male and female rats exposed to acetaminophen at and up to 1000 mg/kg body weight did not induce any abnormal clinical signs in both male and female rats during the study and reversal period.

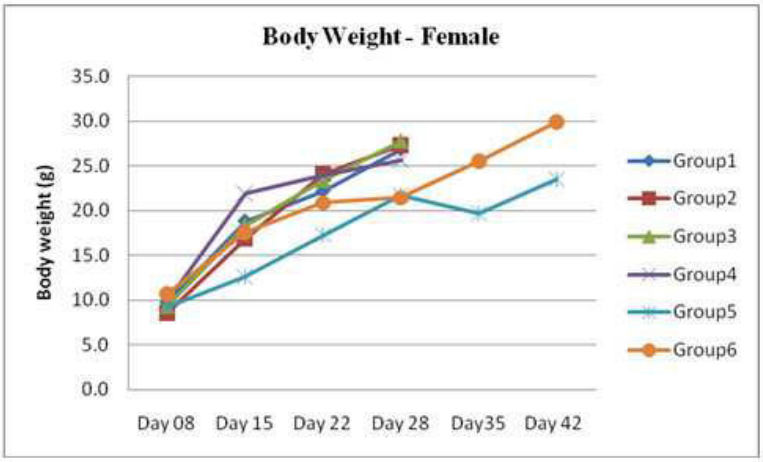
(ii) Body Weight

Acetaminophen at and up to 1000 mg/kg body weight dosage did not result in any significant change in the group mean body weight and mean cumulative net body weight gain when compared to control and treatment groups. (Graph 1 and 2)

Graph 1
***Mean cumulative body weight gain in male* rats**



Graph 2
***Mean cumulative body weight gain in female* rats**

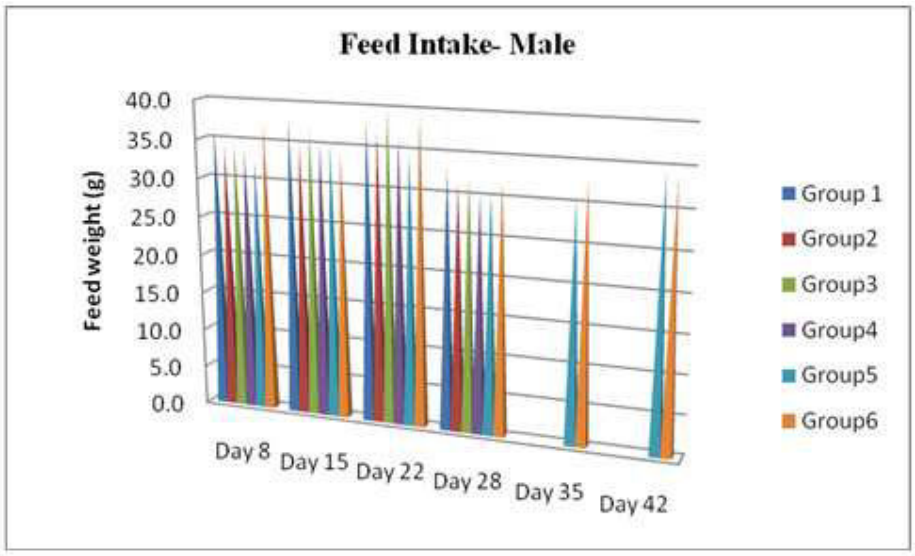


(iii)Food Consumption

The food consumption in rats exposed to acetaminophen at and up to the dose of 1000 mg/kg body weight was found to be comparable to the control groups. (Graph 3 & 4)

Graph 3

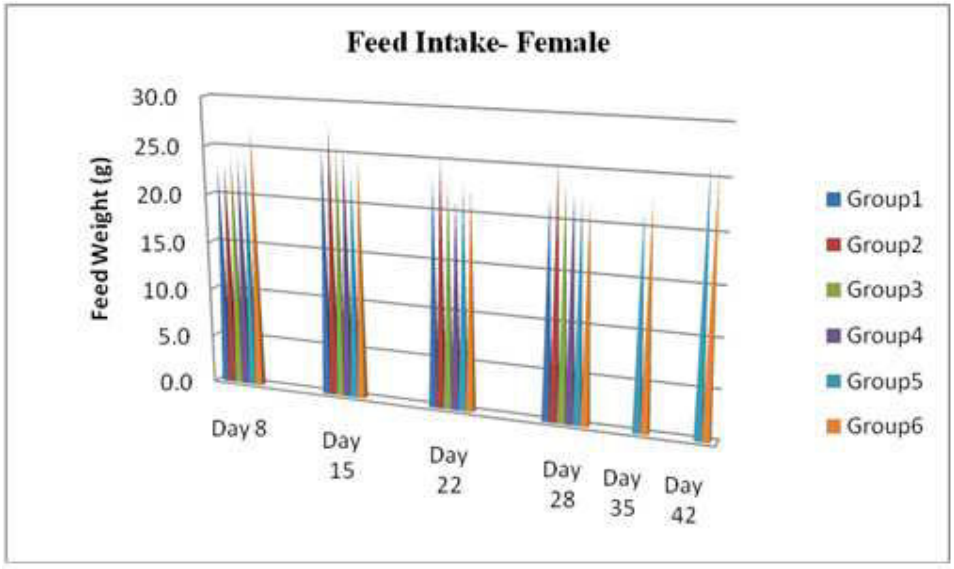
Comparative feed intake between groups on different weeks



On day 35 and day 42 only two groups recovery groups available (5&6)

Graph 4

Comparative feed intake between groups on different weeks



On day 35 and day 42 only two groups recovery groups available (5&6)

(iv)Laboratory Investigations

Rats treated with acetaminophen up to 1000 mg/kg body weight did not exhibit any significant treatment related effects, on haematology, clinical chemistry and urine analysis parameters and the results of the treatment groups were found to be comparable to the control groups. (Table 1 to 6)

Table 1
Mean Values of Haematological Parameters – Males

Group No. Dose (mg/kg)	Mean & Standard Deviation	WBC 10^3 cells/µL	RBC 10^6 cells/µL	Hb g/dl	Hct %	RDW %	Plat 10^3 cells/µL
GI 0	Mean	12.74	6.99	12.60	40.80	13.57	905.17
	SD	2.02	0.47	0.55	1.30	0.72	76.21
GII 250	Mean	14.23	7.56	12.57	41.07	11.27	1021.50
	SD	4.77	0.16	0.24	0.78	0.59	327.91
GIII 500	Mean	12.95	7.52	13.53	41.98	13.57	929.33
	SD	2.72	0.24	0.51	1.38	0.85	51.60
GIV 1000	Mean	09.34	7.24	12.07	41.38	13.63	852.33
	SD	2.46	0.36	0.61	1.79	0.47	73.11
GV 0	Mean	19.17	8.27	13.53	42.98	11.17	833.00
	SD	5.39	0.46	0.70	1.68	0.82	136.37
GVI	Mean	19.35	8.54	13.32	41.70	11.22	939.33
1000	SD	2.21	0.20	0.08	0.41	0.35	29.78

Table 2
Mean Values of Haematological Parameters –Females

Group No. Dose (mg/kg)	Mean & Standard Deviation	WBC 10^3 cells/µL	RBC 10^6 cells/µL	Hb g/dl	Hct %	RDW %	Plat 10^3 cells/µL
GI 0	Mean	6.10	8.26	11.28	41.03	10.98	816.00
	SD	1.38	0.59	1.01	2.81	0.78	108.56
GII 250	Mean	8.83	8.61	11.57	40.22	11.38	855.83
	SD	3.25	0.54	0.92	2.03	0.90	94.03
GIII 500	Mean	8.81	8.93	11.90	40.85	12.62	919.00
	SD	2.56	0.56	0.40	1.24	0.79	83.02
GIV 1000	Mean	9.48	8.97	12.98	41.85	11.13	1025.83
	SD	1.46	0.50	0.48	1.55	0.85	49.39
GV 0	Mean	8.24	8.77	11.02	39.80	11.32	846.00
	SD	1.88	0.49	0.57	1.55	0.59	86.81
GVI	Mean	8.68	8.97	12.38	41.50	11.35	931.00
1000	SD	4.68	0.49	0.88	1.57	0.38	84.12

Table 3
Mean Values of Clinical Chemistry Parameters– Males

Group No. Dose mg/kg		Glu mg/dl	T.Pro g/dL	Alb g/dL	Glo g/dL	A/G	T.Cho mg/dl	Trig mg/dL	BUN mg/dl	Creat mg/dL
G I 0	**Mean**	111.33	6.21	2.45	3.27	0.85	93	94.17	14.83	0.58
	SD	18.6	0.22	0.07	0.2	0.06	8.15	15.99	2.4	0.08
G II 250	**Mean**	121.67	5.9	2.38	3.23	0.89	87.5	130.5	14.67	0.48
	SD	13.25	0.16	0.13	0.16	0.07	9.5	40.11	1.37	0.04
G III 500	**Mean**	125	5.9	2.82	3.08	0.92	86	140.5	14.33	0.48
	SD	15.24	0.18	0.08	0.12	0.03	10.73	28.47	0.52	0.04
G IV 1000	**Mean**	121.17	6.4	3.06	3.25	0.91	95.33	97.33	14.67	0.52
	SD	14.03	0.25	0.08	0.19	0.04	4.89	17.94	1.51	0.08
G V 0	**Mean**	102.33	6.44	3.01	3.22	1.01	94.17	131.83	15.17	0.45
	SD	23.98	0.33	0.11	0.3	0.1	20.65	37.15	2.23	0.05
G VI 1000	**Mean**	116.17	6.33	3.13	3.196	0.98	91.17	92.83	14.33	0.5
	SD	11.96	0.32	0.15	0.196	0.04	14.2	8.45	1.21	0

Group No. Dose (mg/kg)		ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	T.Bil (mg/dL)	Na^+ (mmol/L)	K^+ (mmol/L)	Ca^{++} (mg/dl)	PO_4^- (mg/dl)
G I 0	Mean	41.67	182	313.17	<5.0	<0.1	140.83	4.93	10.02	6.32
	SD	2.42	13.74	70.51			1.47	0.14	0.25	0.18
G II 250	Mean	45.5	195.17	371.67	<5.0	<0.1	141.67	5.03	10.02	5.97
	SD	5.82	33.01	47.99			0.82	0.48	0.23	0.16
G III 500	Mean	43.67	181	360.67	<5.0	<0.1	142.17	4.97	10.15	6.12
	SD	2.94	11.4	39.06			1.47	0.36	0.24	0.55
G IV 1000	Mean	51.7	221.2	358.2	<5.0	<0.1	144.83	5.42	9.9	5.87
	SD	12.42	25.04	40.85			1.72	0.26	0.11	0.43
G V 0	Mean	55.5	128	285.67	<5.0	0.22	145.67	5.07	10.87	6.27
	SD	6.75	15.58	27.89		0.04	1.03	0.23	0.45	0.31
G VI 1000	Mean	53.3	114.33	281.17	<5.0	0.2	149.17	4.87	10.82	6.02
	SD	2.16	17.5	69.54		0.01	1.47	0.19	0.33	0.4

Table 4
Mean Values of Clinical Chemistry Parameters – Females

Group No. Dose mg/kg		Glu mg/dl	T.Pro g/dL	Alb g/dL	Glo g/dL	A/G	T.Cho mg/dl	Trig mg/dL	BUN mg/dl	Creat mg/dL
G I 0	Mean	100.11	6.09	3.17	3.42	0.93	104.17	51.17	14.5	0.48
	SD	15.85	0.26	0.14	0.18	0.06	6.77	3.79	2.07	0.08
G II 250	Mean	107.67	6.06	3.21	3.05	1.05	105.5	44.17	17	0.43
	SD	16.9	0.25	0.22	0.11	0.08	13.13	11.55	1.55	0.05
G III 500	Mean	110	6.85	3.21	3.03	1.06	98.67	44.5	17.83	0.48
	SD	23.31	0.3	0.25	0.11	0.08	8.02	11.95	2.4	0.085
G IV 1000	Mean	101.5	6.82	3.47	3.35	1.05	105.5	52.5	14.83	0.47
	SD	6.06	0.49	0.37	0.28	0.13	11.88	13.4	2.14	0.08
G V 0	Mean	100.67	6.6	3.16	3.45	0.93	99.83	42.5	14.83	0.45
	SD	16.08	0.285	0.28	0.33	0.15	11.69	6.06	2.04	0.05
G VI 1000	Mean	85	6.56	3.23	3.33	0.97	89.83	43.83	15.5	0.5
	SD	8.25	0.14	0.19	0.14	0.09	9.54	5.6	2.26	0.06

Group No. Dose (mg/kg)		ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	T.Bil (mg/dL)	Na^+ (mmol/L)	K^+ (mmol/L)	Ca^{++} (mg/dl)	PO_4^- (mg/dl)
G I 0	Mean	47.33	110.33	240.83	<5.0	<0.1	140.5	4.57	9.85	5.4
	SD	2.88	12.45	81.85			1.05	0.19	0.19	0.32
G II 250	Mean	47.33	111.83	212.33	<5.0	<0.1	140.67	4.6	10.05	5.88
	SD	5.68	16.39	41.44			1.51	0.25	0.2	0.4
G III 500	Mean	49.5	113.83	197.83	<5.0	<0.1	141.67	4.75	10	5.78
	SD	5.54	20.71	28.81			1.21	0.541	0.09	0.82
G IV 1000	Mean	49.5	124.83	268	<5.0	<0.1	149.33	5.1	10.42	6.03
	SD	7.74	10.53	83.24			1.86	0.45	0.31	0.96
G V 0	Mean	53	113.33	190.83	<5.0	0.23	144.33	4.7	10.23	5.45
	SD	5.97	19.62	58.24		0.02	0.52	0.28	0.32	0.63
G VI 1000	Mean	53.33	114.33	194.67	<5.0	0.26	145.33	4.77	10.08	5.5
	SD	3.27	16.31	34.62		0.03	2.25	0.14	0.25	0.37

Table 5
Results of urine analysis – Males

Group No. Dose (mg/kg)	Colour	Appearance	Occult Blood	Bilirubin	Urobil	Ketones	Protein	Nitrite
G I 0	Yellow(6/6)	Clear (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)
G II 250	Yellow(6/6)	Clear (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)
G III 500	Yellow(6/6)	Clear (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)
G IV 1000	Yellow(6/6)	Clear (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)
G V	Yellow(6/6)	Clear (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	Pos (2/6)	Pos (1/6)	neg (6/6)
0						neg (4/6)	neg (5/6)	
G VI 1000	Light Yellow(6/6)	Clear (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)

Grading of cell frequency in the centrifuged deposit:
Nil = none found in any field examined; Urobil: Urobilinogen; Tr: Trace; neg: Negative; Pos: Positive

Table 6
Results of urine analysis – Females

Group No. Dose (mg/kg)	Colour	Appearance	Occult Blood	Bilirubin	Urobil	Ketones	Protein	Nitrite
G I 0	Yellow(3/6) Light Yellow(3/6)	Clear (6/6)	neg (6/6)	neg (6/6)	Neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)
G II 250	Light Yellow(6/6)	Clear (6/6)	neg (6/6)	neg (6/6)	Neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)
G III 500	Yellow(3/6) Light Yellow(3/6)	Clear (6/6)	neg (6/6) Pos(1/6)	neg (6/6)	Neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)
G IV 1000	Yellow(1/6) Light Yellow(5/6)	Clear (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)
G V 0	Light Yellow(6/6)	Clear (6/6)	neg (6/6)	neg (5/6) Pos(1/6)	neg (6/6)	Neg(6/6)	Pos (1/6) neg (5/6)	neg (6/6)
G VI 1000	Light Yellow(6/6)	Clear (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)	neg (6/6)

Grading of cell frequency in the centrifuged deposit:
Nil = none found in any field examined; Urobil: Urobilinogen; Tr: Trace; neg: Negative; Pos: Positive

(v) Organ Weights and Organ Weight Ratios

There was no statistically significant change in terminal absolute and relative organ weights in male and female rats treated at and up to the dose of 1000 mg/kg body weight when compared to control groups.

Table 7
Mean relative organ weight (g) values – Males

Group No. Dose (mg/kg)		Liver	Kidneys	Brain	Testes	Spleen	Heart	Adrenals	Lung
G I	**Mean**	3.70	0.90	0.72	1.13	0.43	0.42	0.019	0.62
0	**SD**	0.45	0.10	0.05	0.11	0.12	0.04	0.002	0.07
G II	**Mean**	3.31	0.87	0.73	1.15	0.43	0.43	0.016	0.72
250	**SD**	0.20	0.02	0.08	0.09	0.06	0.02	0.004	0.08
G III	**Mean**	3.43	0.86	0.71	1.15	0.44	0.43	0.019	0.72
500	**SD**	0.16	0.06	0.03	0.08	0.04	0.04	0.003	0.16
G IV	**Mean**	3.64	0.87	0.74	1.11	0.47	0.44	0.017	0.74
1000	**SD**	0.43	0.06	0.05	0.07	0.06	0.03	0.001	0.07
G V	**Mean**	3.43	0.86	0.70	1.11	0.38	0.39	0.017	0.62
0	**SD**	0.18	0.03	0.04	0.09	0.04	0.02	0.001	0.10
G VI	**Mean**	3.31	0.90	0.69	1.11	0.35	0.43	0.020	0.58
1000	**SD**	0.12	0.04	0.02	0.11	0.04	0.02	0.004	0.04

Table 8
Mean relative organ weight (g) values – Females

Group No. Dose (mg/kg)		Liver	Kidneys	Brain	Testes	Spleen	Heart	Adrenals	Lung
G I	Mean	3.37	0.90	1.06	0.25	0.46	0.46	0.034	0.72
0	SD	0.29	0.02	0.22	0.08	0.06	0.02	0.003	0.06
G II	Mean	3.48	0.91	1.02	0.26	0.42	0.43	0.035	0.73
250	SD	0.27	0.06	0.09	0.05	0.06	0.03	0.004	0.05
G III	Mean	3.38	0.89	0.99	0.25	0.41	0.44	0.034	0.73
500	SD	0.43	0.06	0.08	0.09	0.05	0.03	0.005	0.07
G IV	Mean	3.40	0.89	0.98	0.23	0.45	0.53	0.036	0.78
1000	SD	0.28	0.09	0.04	0.03	0.08	0.11	0.005	0.10
G V	Mean	3.35	0.85	0.95	0.23	0.35	0.48	0.034	0.77
0	SD	0.43	0.05	0.05	0.09	0.05	0.04	0.003	0.20
G VI	Mean	3.26	0.79	0.94	0.24	0.35	0.43	0.030	0.75
1000	SD	0.21	0.06	0.05	0.04	0.06	0.03	0.003	0.09

(vi) Gross findings and Histopathology

Acetaminophen up to the level of 1000 mg/kg body weight did not induce any treatment related gross pathological alterations in any of the organs / tissues. In thymus (males and females), there was an increase in the numbers of tangible body macrophages in groups II, III and IV treated with the test item. The lesion was also present in high incidence in group VI in females. The histological change observed was of mild severity and there was no dose related increase. The lesion did not progress to atrophy of the thymus and there were no lesions in spleen or mesenteric lymph nodes. Hence the lesion in thymus is considered to be less adverse and without much pathological significance. All the other changes observed were considered incidental and not treatment related as the incidences at high dose were similar to or lower than that in the control animals.

(vii) No Observed Adverse Effect Level (NOAEL)

Based on the findings of this study, the No Observed Adverse Effect Level (NOAEL) of Acetaminophen in Wistar rats, following oral administration daily at doses of 250 mg/kg body weight to 1000 mg/kg body weight of male and female rat was found to be 1000mg/kg body weight.

DISCUSSION

Acetaminophen is a widely used over the counter analgesic and antipyretic drug.[8] Oral administration of acetaminophen has been shown to be at least as effective as intravenous administration of an equivalent dose of acetaminophen, and the target concentration achieved more rapidly and with less variability in plasma concentrations compared with enteral formulations.[9-10] In the present investigation, there were no signs of behavioural changes were observed during the study period in all the treatment groups. Increase in body weights and growth of treated animals of either sex were of similar pattern as in control groups. Blood was evaluated for haematological toxicity of acetaminophen administration. Haemogram was estimated and results showed no deleterious effect on blood cell count, haemoglobin and other related parameters. The liver is the vital organ of paramount importance involved in the maintenance of metabolic function and detoxification of drugs.[11-12] After the intake of toxic dose of acetaminophen causes P_{450}- dependent hepatotoxicity in man and various laboratory animals as observed by the release of serum alanine aminotransferase (ALT) into the serum.[13-14] Liver damage is always associated with cellular necrosis, increase in tissue liquid peroxidation and depletion in the tissue GSH levels. In addition, serum levels of many biochemical markers like SGOT, SGPT, ALP and bilirubin are elevated.[14-16] The laboratory features of hepatotoxicity induced by paracetamol resemble other kinds of acute inflammatory liver disease with prominent increase in levels of SGOT, SGPT, and ALP. Hepatotoxicity is the most remarkable feature of paracetamol overdose.[5] However, in the present study, there was no significant change in the levels of hepatic enzymes AST, ALT, GGT and ALP in acetaminophen treated groups of either sex as compared to the

respective control group. Studies in the human and animals reports that the overall incidence of acute renal failure with acetaminophen toxicity.[17-18,13] In the present study, biochemical parameters related to kidney function were evaluated and no significant differences were observed in blood urea, creatinine, glucose and proteins with respect to control. However, it has been reported that certain strains of rats that have high concentrations of microsomal cytochrome $P\text{-}_{450}$ in their kidneys developed acute tubular necrosis after a single, nonlethal dose of paracetamol.[19] It has been observed that conditions that are associated with glutathione depletion or increased activity of $P\text{-}_{450}$ microsomal oxidase enzymes enhance acetaminophen toxicity even at the therapeutic dosages. Examples include chronic alcohol use, starvation, fasting or ingestion of drugs that induce these enzymes, such as anticonvulsants. It has been reported that the proximal tubules are the target of APAP toxicity because of their active absorptive and secretory activities. [20-22] There was no signs of toxicity were observed in any of the organ in histopathological analysis. Thus histopathological studies provide supports to the safety data of other physiological, biochemical and haematological parameters of acetaminophen treatment. In summary, our data suggest that acetaminophen oral administration upto 1000 mg/kg body weight in Wistar rat of either sex did not show any impact on the feeding, body weight gain, behaviour, physiological and biochemical parameters and suspected target organ liver and kidney found to be normal on histopathological analysis. The above results and discussion confers that the No Observed Adverse Effect Level (NOAEL) of acetaminophen in Wistar rats, following oral administration at the doses of 250, 500 and 1000 mg/kg on daily basis for 28 days found to be 1000 mg/kg body weight and there is no reversibility of toxicity observed after 14 day recovery period.

CONCLUSION

Based on the findings of this study, the No Observed Adverse Effect Level (NOAEL) of acetaminophen in Wistar rats, following oral administration at the doses of 250, 500 and 1000 mg/kg on daily basis for 28 days found to be 1000 mg/kg body weight and there is no reversibility of toxicity observed after 14 day recovery period in the target organ of liver and kidney. These findings may be useful for preclinical study reference on acetaminophen before marketing of generic version as per the regulatory requirement in countries like India.

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