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Acute and Subacute Toxicity Studies of Cell Wall Contents of Probiotic *(Lactobacillus Casei)* in Wistar Rats and Swiss Albino Mice

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ABSTRACT

The present study was carried out to assess the toxicological profile of the cell wall contents of probiotics and provide information on the possible health hazards likely to arise from single (acute) and repeated (subacute) exposure over a relatively limited period of time. In-vivo toxicity studies of test item were performed in rats and mice of each sex by subcutaneous administration. Acute study was carried out at high dose of cell wall contents of L.casei obtained from 10^{12} CFU/mL and single dose of test item was injected in each animal. In subacute toxicity study, cell wall contents of L.casei obtained from 10^{6}, 10^{9} and 10^{12} CFU/mL was injected in each animal for 28 days. Animals were observed periodically for any signs and symptoms. Change in body weight, food intake and water intake were measured weekly. At the completion of study, animals were sacrificed; their hematological and biochemical parameters were estimated and gross morphology with histopathology of vital organs was done. In result, no mortality and clinical signs of toxicity were found in test item administered group of animals. No significant alterations in hematological and biochemical parameters were observed. Gross morphological and histopathological analysis of vital organs showed normal architecture in all groups injected with cell wall contents of L.casei. The data obtained indicate no toxicity of cell wall contents of L.casei up to highest dose studied and indicate the clinical usefulness of test item.

Keywords: Acute and subacute toxicity, Cell wall contents, Lactobacillus casei, Probiotic, IBD

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INTRODUCTION

Probiotics are most popular, effective and safe remedies against variety of diseases and are being used widely world wide as food supplements. Probiotics are live organisms, when ingested in adequate amounts; confer a health benefit to the host [1]. The most commonly used Probiotics are Lactobacilli and Bifidobacterium**.** Examples of Lactobacillus species include L.acidophilus, L.casei, L.fermentum, L.jhonsonii, L.plantarum, L.rhamnosus and Bifidobacterium species include B.bifidum, B.breves, B.lactis, B.longum [2]. A set of lactobacillus species were shown to suppress transcription of IL-1β, TNF-α, NF-κB, as well as the translation of IL-1β and IL-6 in experimental colitis and other digestive disorders in rats [3]. Lactobacillus species regulate immune responses by enhancing innate immunity & modulating pathogen induced inflammation [4]. Further, many types of pathogens are involved in pathogenesis of IBD, but predominant level of potential harmful bacteria like E.coli [5] & decrease of beneficial bacterial species such as Lactobacillus & Bifidobacterium [6] have been identified in intestinal microbiota in patients with IBD. Thus, suggesting that manipulation of intestinal bacteria may provide an alternative therapy for IBD prevention and/or treatment. Additionally, cell wall contents (Lipoteichoic acid, Peptidoglycan and techoic acid) of Lactobacilli have been reported to inflammation in animal models of experimental colitis [7]. Other mechanisms of Probiotics include immunomodulation, enhancement of barrier function & anti-microbial activity may play an important role in treatment of IBD. It is interesting to note that spores of Lactobacillus casei has been tested in the IBD [8] as well as the relevance of inhibition by "cell wall contents of lactobacillus casei" has also been tested in our laboratory using experimental model of colitis by Chauhan and Chorawala, 2012 [9]. However, the toxicity study of cell wall contents of such probiotic has never been done. Therefore, we made an attempt to assess the possible health hazards likely to arise from repeated exposure over a relatively limited period of time. The results of this study should provide information on target organs for establishing safety criteria for human exposure.

MATERIALS AND METHODS

Experimental animals

Healthy Male and female Wistar rats weighing 180-220 gm and healthy male and female Swiss albino mice weighing 20-25 gm were used for the present study (Schedule Y, 2005). The experimental protocol (KBIPER/2011/287) of present study was approved by Institutional Animal Ethical Committee under the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India before carrying out the experiment. All animals were housed in polypropylene cage (6 rats per cage) under controlled conditions of temperature (22 $\pm$ 2°C), humidity (55 ± 5%) and 12hrs/12hrs light-dark cycle. Animals were acclimatized for one week prior to experiment. Animals had free access to conventional laboratory diet (normal pellet diet) (Pranav Agro, Baroda) and water ad libitum.

Preparation of Test Item or Isolation of Cell Wall Contents of L.casei

The method described by Roberson & Cromatte, 1962 [10] and Knox & Wicken, 1973 [11] was used with slight modification. Briefly, 20gm of bacteria (by weighing wet colonies) was suspended in 350ml of hot water (65-68^{0}C). To that, 350ml of 90% phenol (65-68^{0}c) was added and stirred for 1hr.at 65-68^{0}C. Then, it was cooled in an ice bath to 2-8^{0}C, or left overnight in refrigerator. Then, it was centrifuged at 6000-7000rpm for 45min. Upper water layer was preserved (hot phase) and residual phenol & interphase was further treated, if required, with equal volume of hot water and preceded as described above. The Phenol layer consists of lipids and insoluble residue of cell wall proteins whereas, aqueous phase consist of Lipoteichoic acid, Lipoic acid, Polysaccharides, amino acids, Teichoic acid and Peptidoglycans etc. The aqueous phase was used for treatment purpose.

Study Design

Acute Toxicity Study

The selected animals were randomly divided into two groups containing minimum 10 animals per group, each 5 males and 5 females for rats and mice as summarized in table 1. The animals were fasted overnight and single dose of cell wall contents of L.casei obtained from 10^{12} CFU/mL was administered subcutaneously to group IIA and IIB of rats and mice. Group IA and IB of rats and mice were served as vehicle control and received WFI subcutaneously. Animals were observed individually after dosing for a total of 14 days to assess any clinical sign of toxicity and mortality.

Cage Side Observation

Observations included changes in skin, fur, eyes, mucus membranes, respiratory, circulatory, autonomic functions, central nervous system, motor activity and behavior pattern. Attention was also directed toward observations of tremors, convulsion, salivation, diarrhoea, lethargy, sleep, and response to handling, tonic and clonic movements, walking backward or any other bizarre reaction.

Changes in Body Weight, Food Intake and Water Intake

Any changes in body weight, food and water intake were recorded weekly.

Gross Morphology

Overnight fasted surviving animals were weighed and humanely sacrificed on day 14 using ether overdose followed by cervical dislocation. Vital organs (lungs, liver, kidney, heart, spleen, brain, stomach, testis, uterine horn) were removed and subjected to gross necropsy.

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Table 1: Grouping of animals for acute toxicity study

Species	Group No.	Sex	Treatments	No. of animals per group
Rat	IA	Male	vehicle, s.c.	05
	IB	Female	vehicle, s.c.	05
	IIA	Male	Lactobacillus casei (10^{12} CFU/animal, s.c.)	05
	IIB	Female	Lactobacillus casei (10^{12} CFU/animal, s.c.)	05
Mice	IA	Male	vehicle, s.c.	05
	IB	Female	vehicle, s.c.	05
	IIA	Male	Lactobacillus casei (10^{12} CFU/animal, s.c.)	05
	IIB	Female	Lactobacillus casei (10^{12} CFU/animal, s.c.)	05

Subacute Toxicity Study

One day before the initiation of treatment, the selected animals were randomly divided into four different groups containing minimum 12 animals per group, each of 6 males and 6 females for rats and similar for mice as summarized in table 2.

Table 2: Grouping of animals for subacute toxicity study

Species	Group No.	Sex	Treatments	No. of animals per group
Rat	IA	Male	vehicle, s.c.	06
	IB	Female	vehicle, s.c.	06
	IIA	Male	Lactobacillus casei (10^{6} CFU/animal, s.c.)	06
	IIB	Female	Lactobacillus casei (10^{6} CFU/animal, s.c.)	06
	IIIA	Male	Lactobacillus casei (10^{9} CFU/animal, s.c.)	06
	IIIB	Female	Lactobacillus casei (10^{9} CFU/animal, s.c.)	06
	IVA	Male	Lactobacillus casei (10^{12} CFU/animal, s.c.)	06
	IVB	Female	Lactobacillus casei (10^{12} CFU/animal, s.c.)	06
Mice	IA	Male	vehicle, s.c.	06
	IB	Female	vehicle, s.c.	06
	IIA	Male	Lactobacillus casei (10^{6} CFU/animal, s.c.)	06
	IIB	Female	Lactobacillus casei (10^{6} CFU/animal, s.c.)	06
	IIIA	Male	Lactobacillus casei (10^{9} CFU/animal, s.c.)	06
	IIIB	Female	Lactobacillus casei (10^{9} CFU/animal, s.c.)	06
	IVA	Male	Lactobacillus casei (10^{12} CFU/animal, s.c.)	06
	IVB	Female	Lactobacillus casei (10^{12} CFU/animal, s.c.)	06

The test item was administered once daily for 28 days. Toxic manifestation (diarrhea, tremor, salivation, convulsion, changes in color of eyes, skin or fur, lethargy, sleep etc…), behavioral changes and mortality were monitored daily, while changes in body weight, food intake and water intake were observed weekly. At the end of study period, animals were fasted

for 12 hrs and blood samples were collected from all animals under anesthetic ether. The blood samples were collected by cardiac puncture method, transferred into 1.5 mL capacity microcentrifuge tube containing sodium citrate solution as an anti-coagulant and clinically evaluated for hematological and biochemical parameters. After blood collection, all the animals were sacrificed; vital organs (lungs, liver, heart and kidney) were removed, observed for gross morphology, freed of extraneous material, weighed and preserved in 10% buffered formalin for histopathology.

Changes in Body Weight, Food Intake and Water Intake

Body weights were measured before the treatment and weekly thereafter, and on the day of sacrifice. Similarly food and water intake were recorded weekly and on the day of sacrifice.

Hematological Studies

All the animals were fasted overnight prior to blood collection. Blood samples were collected by cardiac puncture under anesthetic ether into 1.5 mL capacity microcentrifuge tube containing sodium citrate as an anti-coagulant and clinically evaluated for hematological parameters. After that blood samples were centrifuged at 4000 RPM at 4 °C for 10 minutes to obtain plasma for biochemical analysis. Various hematological parameters (Hb%, Total RBC, Total WBC and Differential WBC) were determined by standard clinical procedure using automatic hematological analyzer (Roches Integra, 400 Plus, Diagnostic system).

Biochemical Studies

Plasma samples obtained after centrifugation were used to estimate biochemical parameters such as glucose, total cholesterol, triglyceride, albumin, total protein, SGPT, SGOT, alkaline phosphatase, total bilirubin, creatinine and urea. Biochemistry was done with commercially available standard kit of Span diagnostic limited, India using an automated biochemical analyzer (Reflotron plus, Roches, USA).

Gross Morphology, Organ Weight and Histopathology

After blood collection, all the animals were sacrificed; vital organs (lungs, liver, heart and kidney) were removed, observed for gross morphology, freed of extraneous material, weighed and preserved in 10% buffered formalin for histopathology. Standard histological procedures were followed to observe any microscopic changes in any above mentioned organs.

Statistical Analysis

Numerical data were expressed as mean ± SEM of six observations. Differences between the groups were analyzed using analysis of variance (ANOVA) followed by Dunnett's multiple

comparisons test and student unpaired t-test. Minimum criteria for statistical significant was set at p less than 5% ($p<0.05$) for all the comparisons.

RESULTS

Acute Toxicity Study

No mortality and morbidity or any signs of behavior changes or toxicity were observed throughout 14 days of study period after single subcutaneous administration of cell wall contents obtained from 10^{12} CFU/mL to rats and mice. Morphological characteristics (fur, eye, skin, nose, tongue) appeared normal. No tremors, convulsion, salivation, lethargy, diarrhoea or unusual behaviors such as self mutilation, walking backward, circling behavior and stereotype behavior were observed; gait and posture, response to handling or sensory stimuli and grip strength were normal. There were no significant changes in body weight, food and water intake (not mentioned) between control and treatment groups.

Subacute Toxicity Study

The animals were healthy with no difference being noted with respect to control group. No significant changes were observed in body weight, food and water intake of repeatedly treated group as compared to vehicle control groups (table 3a, 3b, 3c, 3d, 3e, 3f) and no mortality was observed during entire toxicity study period. The weight of vital organs (lungs, liver, kidney, heart) was not significantly altered by cell wall contents of L.casei as compared to vehicle control group (table 4a, 4b).

TABLE 3(a): Effect of cell wall contents of probiotics on body weight in rats.

SEX	GROUPS	BODY WEIGHT IN GMS (WEEKLY)				
		DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
MALE RATS	GROUP IA	263.33±3.33	269.17±3.96	275.83±3.27	284±4.64	293.5±3.36
	GROUP IIA	270±2.58	280.17±3.13	275.5±3.33	269±3.25	266.17±3.9
	GROUP IIIA	261.67±6.01	274.83±5.64	272±5.87	269.5±5.63	263.17±6.05
	GROUP IVA	251.67±3.57	264±2.79	256±6.59	250.17±6.17	243.33±5.85
FEMALE RATS	GROUP IB	230±2.89	236.83±3.08	244±3.88	251.67±3.21	256.67±3.52
	GROUP IIB	220±2.89	229.83±2.73	226±2.97	221±3.02	218.5±2.84
	GROUP IIIB	211.67±3.33	225.67±3.94	222±4.61	217.33±3.81	213.5±4.05
	GROUP IVB	219.17±3.52	232±2.86	228.5±3.75	225.17±3.75	221.17±4.25

Each observation represents value in mean ± SEM, n=6. No significant difference between group I, II, III and IV during entire study period.

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TABLE 3(b): Effect of cell wall contents of probiotics on food intake in rats.

SEX	GROUPS	FOOD CONSUMPTION IN GMS (WEEKLY)				
		DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
MALE RATS	GROUP IA	135	139	137	142	142
	GROUP IIA	128	132	136	139	142
	GROUP IIIA	121	120	123	128	129
	GROUP IVA	124	136	139	143	145
FEMALE RATS	GROUP IB	143	145	148	152	159
	GROUP IIB	117	124	124	125	127
	GROUP IIIB	153	155	162	165	157
	GROUP IVB	109	118	122	135	124

Each observation represents value in mean, n=6. No significant difference between group I, II, III and IV during entire study period.

TABLE 3(c): Effect of cell wall contents of probiotics on water intake in rats.

SEX	GROUPS	WATER INTAKE IN mL (WEEKLY)				
		DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
MALE RATS	GROUP IA	96	96	100	98	108
	GROUP IIA	104	104	112	114	116
	GROUP IIIA	113	111	118	124	135
	GROUP IVA	110	119	128	135	143
FEMALE RATS	GROUP IB	84	80	84	88	95
	GROUP IIB	98	97	98	108	101
	GROUP IIIB	97	100	100	107	112
	GROUP IVB	81	91	95	94	95

Each observation represents value in mean, n=6. No significant difference between group I, II, III and IV during entire study period.

TABLE 3(d): Effect of cell wall contents of probiotics on body weight in mice.

SEX	GROUPS	BODY WEIGHT IN GMS (WEEKLY)				
		DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
MALE MICE	GROUP IA	27.5±2.14	29.17±2.01	30.83±1.54	32.5±1.71	34.17±0.83
	GROUP IIA	32.5±2.14	30±1.83	29.17±2.39	31.67±3.07	32.5±1.71
	GROUP IIIA	32.5±3.1	30±2.58	30.83±2.39	30±2.24	33.33±2.47
	GROUP IVA	29.17±1.54	26.67±1.67	26.67±1.05	28.33±2.11	29.17±2.01
FEMALE MICE	GROUP IB	30±1.83	30.83±0.83	32.5±1.12	34.17±1.54	35.83±0.83
	GROUP IIB	34.17±0.83	32.5±1.12	30±1.29	33.33±2.11	32.5±2.14
	GROUP IIIB	40.83±0.83	39.17±1.54	35±1.83	35±1.83	33.33±1.05
	GROUP IVB	40±1.83	34.17±2.39	30.83±0.83	30.83±0.83	34.17±1.54

Each observation represents value in mean ± SEM, n=6. No significant difference between group I, II, III and IV during entire study period.

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TABLE 3(e): Effect of cell wall contents of probiotics on food intake in mice.

SEX	GROUPS	FOOD CONSUMPTION IN GMS (WEEKLY)				
		DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
MALE MICE	GROUP IA	10	30	30	25	20
	GROUP IIA	50	50	45	40	35
	GROUP IIIA	40	40	35	35	37
	GROUP IVA	20	40	40	35	32
FEMALE MICE	GROUP IB	20	30	20	20	25
	GROUP IIB	20	30	30	30	25
	GROUP IIIB	10	30	30	30	25
	GROUP IVB	20	20	20	25	25

Each observation represents value in mean, n=6. No significant difference between group I, II, III and IV during entire study period.

TABLE 3(f): Effect of cell wall contents of probiotics on water intake in mice.

SEX	GROUPS	WATER INTAKE IN mL (WEEKLY)				
		DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
MALE MICE	GROUP IA	75	70	70	65	65
	GROUP IIA	45	50	45	40	40
	GROUP IIIA	60	70	65	40	30
	GROUP IVA	60	65	50	50	50
FEMALE MICE	GROUP IB	40	20	25	20	20
	GROUP IIB	45	50	45	45	40
	GROUP IIIB	50	50	45	30	30
	GROUP IVB	30	20	35	40	35

Each observation represents value in mean, n=6. No significant difference between group I, II, III and IV during entire study period.

TABLE 4(a): Effect of cell wall contents of probiotics on organ weight in rats

SEX	GROUPS	ORGAN WEIGHT IN GMS			
		LIVER	KIDNEY	HEART	LUNGS
MALE RATS	GROUP IA	6.73±0.17	2.46±0.11	1.18±0.04	2.18±0.08
	GROUP IIA	6.66±0.18	2.38±0.09	1.2±0.04	2.33±0.04
	GROUP IIIA	7.05±0.08	2.51±0.04	1.31±0.06	2.2±0.06
	GROUP IVA	7.18±0.09	2.35±0.1	1.33±0.09	2.12±0.06
FEMALE RATS	GROUP IB	5.31±0.33	1.63±0.07	0.79±0.05	1.66±0.09
	GROUP IIB	5.14±0.1	1.48±0.09	0.72±0.02	1.55±0.08
	GROUP IIIB	5.59±0.26	1.32±0.03	0.7±0.04	1.65±0.05
	GROUP IVB	5.34±0.29	1.45±0.09	0.74±0.03	1.57±0.08

Each observation represents value in mean, n=6. No significant difference between group I, II, III and IV during entire study period.

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TABLE 4(b): Effect of cell wall contents of probiotics on organ weight in mice

SEX	GROUPS	ORGAN WEIGHT IN GMS			
		LIVER	KIDNEY	HEART	LUNGS
MALE MICE	GROUP IA	1.78±0.17	0.59±0.05	0.22±0.02	0.27±0.02
	GROUP IIA	1.53±0.08	0.53±0.04	0.2±0.02	0.21±0.01
	GROUP IIIA	1.9±0.13	0.52±0.04	0.2±0.01	0.22±0.01
	GROUP IVA	2.01±0.12	0.58±0.03	0.27±0.02	0.23±0.01
FEMALE MICE	GROUP IB	1.9±0.05	0.53±0.02	0.23±0.02	0.27±0.01
	GROUP IIB	1.86±0.07	0.46±0.03	0.23±0.02	0.25±0.02
	GROUP IIIB	1.72±0.1	0.48±0.03	0.19±0.01	0.27±0.01
	GROUP IVB	1.84±0.06	0.54±0.04	0.24±0.01	0.24±0.02

Each observation represents value in mean, n=6. No significant difference between group I, II, III and IV during entire study period.

Hematological Studies

The effect of repeated dose of subcutaneously administered cell wall contents on hematological parameters is presented in table 5 (a, b, c, d). Hematological analysis showed no significant changes in test item groups as compared to control groups.

TABLE 5(a): Effect of cell wall contents of probiotics on Hb %, Total RBC and Total WBC in rats

SEX	GROUPS	HAEMATOLOGICAL PARAMETERS		
		HAEMOGLOBIN (gm/dL)	TOTAL RBC (COUNT x 10^6 /cmm)	TOTAL WBC COUNT (CELLS/cmm)
MALE RATS	GROUP IA	14.83±0.44	6.71±0.59	9151.83±730.42
	GROUP IIA	15.33±0.76	8.09±0.37	10019.67±639.53
	GROUP IIIA	14.67±1.24	7.39±0.58	9211.17±218.98
	GROUP IVA	13.92±1.25	8.25±0.39	9388.5±265.46
FEMALE RATS	GROUP IB	15.67±0.75	7.25±0.52	9613.83±916.71
	GROUP IIB	14.5±0.29	7.73±0.24	9428.17±594.04
	GROUP IIIB	14.5±1.02	7.88±0.45	9576.67±671.06
	GROUP IVB	15.17±1	7.57±0.24	10100.5±566.82

Each observation represents value in mean, n=6. No significant difference between group I, II, III and IV during entire study period.

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TABLE 5(b): Effect of cell wall contents of probiotics on Differential WBC in rats

SEX	GROUPS	DIFFERENTIAL WBC				
		LYMPHOCYTES	MONOCYTES	NEUTROPHILS	EOSINOPHILS	BASOPHILS
MALE RATS	GROUP IA	66.83±2.33	6.67±0.71	25.33±2.3	1.17±0.31	0±0
	GROUP IIA	66.5±3.39	6.83±1.01	25±2.89	1.67±0.21	0±0
	GROUP IIIA	70.5±1.61	5.83±0.83	23±1.59	0.67±0.33	0±0
	GROUP IVA	71±3.22	7.17±0.87	20.5±3.15	1.33±0.33	0±0
FEMALE RATS	GROUP IB	68±1.46	6±0.52	24.67±1.58	1.33±0.21	0±0
	GROUP IIB	69.67±0.92	7.33±0.88	22±0.82	1±0.37	0±0
	GROUP IIIB	68.33±1.17	8±0.63	22.33±1.58	1.33±0.33	0±0
	GROUP IVB	69.67±2.64	6.17±0.83	23±3.17	1.17±0.31	0±0

Each observation represents value in mean, n=6. No significant difference between group I, II, III and IV during entire study period.

TABLE 5(c): Effect of cell wall contents of probiotics on Hb %, Total RBC and Total WBC in mice

SEX	GROUPS	HAEMATOLOGICAL PARAMETERS		
		HAEMOGLOBIN (gm/dL)	TOTAL RBC (COUNT x 10^6 /cmm)	TOTAL WBC COUNT (CELLS/cmm)
MALE MICE	GROUP IA	13.17±0.36	7.75±0.23	7830±258.7
	GROUP IIA	12.33±0.6	7.99±0.43	7714.5±390.7
	GROUP IIIA	11.5±0.9	8.11±0.41	7241±345.26
	GROUP IVA	10.67±1.22	8.49±0.33	7185.5±362.35
FEMALE MICE	GROUP IB	12.67±0.36	7.86±0.23	8200.17±133.38
	GROUP IIB	12.92±0.7	8.19±0.36	7350.83±305.25
	GROUP IIIB	11.92±0.76	8.42±0.43	7531.5±451.53
	GROUP IVB	11.67±0.78	7.8±0.28	7545.83±498.23

Each observation represents value in mean, n=6. No significant difference between group I, II, III and IV during entire study period.

TABLE 5(d): Effect of cell wall contents of probiotics on Differential WBC in mice

SEX	GROUPS	DIFFERENTIAL WBC				
		LYMPHOCYTES	MONOCYTES	NEUTROPHILS	EOSINOPHILS	BASOPHILS
MALE MICE	GROUP IA	72.67±2.78	5.67±1.05	20.5±3.55	1.17±0.31	0±0
	GROUP IIA	68.67±4.48	6±1.32	24.83±3.55	0.5±0.22	0±0
	GROUP IIIA	72.17±3.83	6.83±2.2	20.17±2.8	0.83±0.31	0±0
	GROUP IVA	72±3.45	5±0.58	22±3.14	1±0	0±0
FEMALE MICE	GROUP IB	64±3.6	7.83±1.22	26.83±3.32	1.33±0.33	0±0
	GROUP IIB	74±4.2	6.17±1.58	19±3.12	0.83±0.4	0±0
	GROUP IIIB	67.17±2.55	7±1.15	25±1.95	0.83±0.4	0±0
	GROUP IVB	71.67±1.89	6±1.44	21.5±1.38	0.83±0.31	0±0

Each observation represents value in mean, n=6. No significant difference between group I, II, III and IV during entire study period.

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Biochemical Studies

The effect of repeated dose of cell wall contents on biochemical markers (glucose, total cholesterol, triglyceride, albumin, total protein, SGPT, SGOT, alkaline phosphatase, total bilirubin, creatinine and urea) is summarized in table 6 (a, b, c, d). Results show that there were no significant changes in biochemical markers values of treated animals as compared to vehicle control group animals.

TABLE 6(a): Effect of cell wall contents of probiotics on various biochemical parameters in rats

SEX	GROUPS	BIOCHEMICAL PARAMETERS IN SERUM				
		GLUCOSE (mg/dL)	TOTAL CHOLESTEROL (mg/dL)	TRIGLYCERIDE (mg/dL)	ALBUMIN (gm/dL)	TOTAL PROTEIN (gm/dL)
MALE RATS	GROUP IA	91.89±2.82	117.05±4.85	102.57±2.33	3.59±0.17	5.44±0.31
	GROUP IIA	79.67±2.86	96.37±3.06	82.36±2.75	2.99±0.21	5.76±0.25
	GROUP IIIA	79.21±2.06	102.31±3.86	100.25±1.9	2.65±0.19	5.98±0.25
	GROUP IVA	73.17±4.49	89.35±2.71	102.07±2.06	3.47±0.24	5.38±0.41
FEMALE RATS	GROUP IB	97.94±2.26	112.96±2.55	104.02±2.42	2.55±0.34	5.82±0.28
	GROUP IIB	81.85±4.44	100.23±3.95	89.08±1.56	2.95±0.31	5.64±0.21
	GROUP IIIB	78.51±4.65	106.94±4.26	99.31±3.16	2.9±0.08	5.74±0.17
	GROUP IVB	82.43±2.68	94.98±3.9	96.86±3.63	3.38±0.24	5.05±0.08

Each observation represents value in mean, n=6. No significant difference between group I, II, III and IV during entire study period.

TABLE 6(b): Effect of cell wall contents of probiotics on various biochemical parameters in rats

SEX	GROUPS	BIOCHEMICAL PARAMETERS IN SERUM					
		SGPT ACTIVITY (IU/L)	SGOT ACTIVITY (IU/L)	ALKALINE PHOSPHATASE (IU/L)	TOTAL BILIRUBIN (mg/dL)	CREATININE (mg/dL)	UREA (mg/dL)
MALE RATS	GROUP IA	24.56±2.44	29.86±2.61	64.18±4.85	0.53±0.06	0.62±0.14	17.05±1.74
	GROUP IIA	21.41±2.91	19.25±1.48	64.79±5.93	0.56±0.05	0.73±0.13	18.56±1.8
	GROUP IIIA	23.38±3.91	25.73±2.81	45.8±10.18	0.53±0.08	0.64±0.08	16.67±1.52
	GROUP IVA	23.97±2.82	20.82±2.73	44.6±4.19	0.54±0.06	0.69±0.11	16.29±1.23
FEMALE RATS	GROUP IB	21.22±1.88	25.54±2.78	65.99±5.04	0.37±0.03	0.73±0.11	12.12±2.25
	GROUP IIB	20.82±2.25	22±2.72	45.2±4.62	0.46±0.08	0.69±0.09	16.29±2.23
	GROUP IIIB	21.61±2.73	20.63±1.74	30.13±3.19	0.43±0.07	0.67±0.12	10.61±0.96
	GROUP IVB	23.18±1.96	25.14±2.93	51.23±7.55	0.47±0.06	0.73±0.12	8.71±1.6

Each observation represents value in mean, n=6. No significant difference between group I, II, III and IV during entire study period.

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TABLE 6(c): Effect of cell wall contents of probiotics on various biochemical parameters in mice

SEX	GROUPS	BIOCHEMICAL PARAMETERS IN SERUM				
		GLUCOSE (mg/dL)	TOTAL CHOLESTEROL (mg/dL)	TRIGLYCERIDE (mg/dL)	ALBUMIN (gm/dL)	TOTAL PROTEIN (gm/dL)
MALE RATS	GROUP IA	90.38±5.81	107.38±6.31	107.9±4.82	4.48±0.24	4.69±0.43
	GROUP IIA	84.52±4.9	109.18±3.13	109.17±5.69	3.87±0.38	4.01±0.18
	GROUP IIIA	88.68±4.5	113.18±5.98	110.54±4.97	2.77±0.2	4.57±0.43
	GROUP IVA	85.6±5.2	106.16±3.67	115.89±6.63	3.6±0.29	3.74±0.29
FEMALE RATS	GROUP IB	91.15±7.05	109.23±4.24	116.8±8.22	3.21±0.46	4.09±0.21
	GROUP IIB	85.96±4.57	115.85±5.8	103.18±4.76	3.9±0.47	3.78±0.12
	GROUP IIIB	78.45±2.87	110.98±4.13	120.16±7.24	3.2±0.2	3.99±0.13
	GROUP IVB	82.41±5	115.39±4.5	106.36±4.9	3.32±0.27	3.52±0.06

Each observation represents value in mean, n=6. No significant difference between group I, II, III and IV during entire study period.

TABLE 6(d): Effect of cell wall contents of probiotics on various biochemical parameters in mice

SEX	GROUPS	BIOCHEMICAL PARAMETERS IN SERUM					
		SGPT ACTIVITY (IU/L)	SGOT ACTIVITY (IU/L)	ALKALINE PHOSPHATASE (IU/L)	TOTAL BILIRUBIN (mg/dL)	CREATININE (mg/dL)	UREA (mg/dL)
MALE RATS	GROUP IA	23.57±1.8	18.86±2.47	57.86±3.49	0.4±0.06	1±0.1	17.8±4
	GROUP IIA	22±2.52	18.66±3.74	65.69±4.71	0.45±0.04	1.09±0.11	15.53±0.91
	GROUP IIIA	23.38±3.91	20.23±3.15	47.01±3.17	0.42±0.1	0.98±0.09	14.02±3.78
	GROUP IVA	21.61±3.17	19.64±3.56	60.87±4.37	0.33±0.06	0.96±0.07	15.53±3.44
FEMALE RATS	GROUP IB	24.16±3.02	15.91±4.19	68.7±7.47	0.18±0.04	0.71±0.13	19.7±2.4
	GROUP IIB	21.61±3.74	16.11±2.59	31.94±3.12	0.21±0.03	0.96±0.13	22.35±3.29
	GROUP IIIB	17.09±2.01	14.54±2.01	41.28±9.07	0.22±0.04	0.62±0.06	15.91±3.05
	GROUP IVB	16.5±2.92	18.66±2.35	51.23±6.89	0.22±0.03	0.51±0.09	14.39±1.92

Each observation represents value in mean, n=6. No significant difference between group I, II, III and IV during entire study period.

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Histopathology

No abnormalities were detected in pathological examinations of tissues during microscopic examination of vital organs in comparative histology of tissues of control and test animals. Treatment of cell wall contents of L.casei did not affect the histology of vital organs, viz., lungs, liver, kidney and heart.

DISCUSSION

In acute toxicity study, no mortality was observed at highest dose of cell wall contents of L.casei obtained from 10^{12} CFU/mL after single dose administration in rats and mice. The changes in body weight have been used as a marker of adverse effect of test item [12]. Since no remarkable changes were observed in animal behavior, body weight, food and water intake at highest dose level in treated animals as compared to control groups, it can be inferred that cell wall contents of L.casei is non-toxic at the dose administered. Similar results were also observed in subacute toxicity study. Further, data analyses animals' blood parameters can be translated for risk evaluation in human, since changes in hematological system have a higher predictive value for human toxicity [13,14]. Subacute toxicity studies conducted in our laboratory also showed no significant changes in hematological parameters between control and tested item groups. There was a transient increase in total WBC counts. An increase in WBC counts may indicate impact of cell wall contents of L.casei on immune system of treated groups. The results indicate that cell wall contents of L.casei are neither toxic to circulating RBC nor it interferes with their production.

GPT, GOT, albumin and total bilirubin are generally used as markers of liver damage [13,14]. No significant changes were found in level of GPT, GOT, albumin and total bilirubin post cell wall contents administration. Therefore, cell wall contents of L.casei did not provoke any detrimental effect on liver. Moreover, activity of alkaline phosphatase enzymes in addition to levels of creatinine and urea were found normal suggest no toxic effect exerted on repeated administration of cell wall contents of L.casei. The non-toxicity of cell wall contents of L.casei on specific organ was further confirmed by histopathological assessment. Histopathological examination of selected vital organs (lungs, liver, kidney and heart) from both treated and control animals showed normal architecture, suggesting no microscopic changes and morphological disturbances were caused due to subcutaneous administration of cell wall contents of L.casei at all dose levels.

CONCLUSION

The results strongly suggest that the cell wall contents of L.casei is safe and well tolerated at tested subcutaneous doses since no deleterious changes were observed in animal macro-parameters, behavior, hematological and biochemical parameters and histopathology. Further, the isolated cell wall contents of L.casei were found to be nontoxic in acute and repeated dose toxicity studies. Animal toxicity study along with efficacy studies of cell wall

contents of L.casei conducted in our laboratory have shown very encouraging results, suggesting a long term, therapeutic/nutritive potential of cell wall contents of L.casei.

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