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Sub chronic oral toxicity study of Janma Ghunti Honey in Wistar rats

S Kumar[a],*, P Singh[b], R K Rai[a], A Gupta[a] & J L N Sastry[a]

[a]Dabur Research and Development Centre, Dabur India Limited, Ghaziabad,

[b]Dabur Research Foundation, Ghaziabad

E-mail: Satyendra.kumar@dabur.com

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The present study was conducted to evaluate the safety profile of Janma Ghunti Honey in Wistar rats on oral administration for 28 consecutive days. Animals were randomized on the basis of body weight into 6 groups. Three groups received test item (TI) at three different dose levels (3 mL/k g, 6 mL/kg and 12 mL/kg body weight). One group served as high dose satellite reversal group (12 mL/kg). One group each served as the control and satellite control group. Animals were observed for clinical signs of toxicity and mortality at least once daily. Animals from satellite groups were observed for further 14 days without treatment to evaluate delayed occurrence or reversibility of any signs/toxicity. At the end of the study, animals were studied through clinical pathology and necropsy examination. No treatment related mortality was observed in any group. Except in female low dose and female high dose satellite groups, all the treated groups exhibited weight gain and no statistically significant alterations were observed. No TI related toxicity was found on hematological investigation, blood biochemistry parameters and absolute and relative organ weights among treated groups. 'No Observed Adverse Effect Level' of TI in male and female Wistar rats was found to be above 12 mL/kg body weight.

Keywords: Janma Ghunti Honey, Sub chronic toxicity, Wistar rats

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Janma Ghunti Honey is a Poly-herbal Ayurvedic formulation for maintenance of the overall digestive health of babies. It comprises ingredients like Phalgu (*Ficus carica*), Draksha (*Vitis vinifera*), Vidanga (*Embelia ribes* Burm.f.), Amaltas (*Cassia fistula* L.), Senna (*Cassia angustifolia*) and Vacha (*Acorus calamus*) etc. that are traditionally used in Ayurveda with added benefits of honey. By virtue of its ingredients, Janma Ghunti acts as an effective remedy for stomach ailments such as flatulence, constipation and stomach ache and helps improve digestion (Table 1). The antihistaminic and intestinal motility efficacy of the Janma Ghunti Honey has been established in in pre-clinical models[1].

In the current study, the sub-chronic safety profile of Janma Ghunti was investigated via., 28-day repeated dose oral administration to Wistar rats. The study followed "OECD Guideline for testing of chemicals, Guideline 407- "Repeated Dose 28 Day Oral Toxicity Study in Rodents" adopted in October 2008".[2]

*Corresponding Author

Methodology

Study compliance and ethical approvals

This study was conducted in compliance with OECD Environmental Health and Safety Publications, Series on Principles of Good Laboratory Practice and Compliance Monitoring No. 1. ENV/MC/CHEM (98)17 at the animal facility registered and compliant with CPCSEA [Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA registration no. 64/PO/Br/s/99/CPCSEA)], Ministry of Environment and Forest, Govt. of India. Study was prior approved by the Institutional Animal Ethics Committee (Approval No. IAEC/27/274 dated 26JUN2013).

Chemicals/reagent/media

Formaldehyde (Rankem), isofluorine (Raman & Weil Pvt. Ltd.), sodium phosphate (Diabasic) (SDFCI), sodium phosphate (Monobasic) (Rankem/ RFCL Ltd.), isopropyl alcohol (Rankem/RFCL Ltd.), xylene (Finar Limited), Paraffin Wax (D K Lab Solutions), hematoxylin (Merck) & eosin (Fischer Scientific).

Table 1 — Summary of Mortality

GROUP	DOSE (ML/KG B. WT.)	NO. OF ANIMAL		MORTALITY			
		MALE	FEMALE	MALE	FEMALE	TOTAL	% MORTALITY
G1	CONTROL	5	5	0	0	0	0
G2	3	5	5	1	0	1	10
G3	6	5	5	1	0	1	10
G4	12	5	5	0	1	1	10
G1S	CONTROL SATELLITE	5	5	1	0	1	10
G4S	12	5	5	1	1	2	20

Experimental animals

Wistar rats, male and female, less than 9 weeks of age at time of dosing in weight ranges of 96.22 to 154.00 g (male) and 97.68 to 147.52 g (Female) obtained from Animal Facility, LUVAS, Hisar, were used in this study.

Husbandry practices

Animals up to five per cage were housed in polypropylene cages and acclimatized (6 days male & 7 days for female, temperature 21.3-24.2°C, relative humidity: 42%-61%, 12h/D cycle). The room temperature and relative humidity were recorded daily using a temperature data logger. Throughout the study, the rats were given unlimited supply of Reverse Osmosis (RO) water and fed conventional pellet diet (Golden Feeds, New Delhi).

Test item

Janma Ghunti Honey (DRDC/2014/028) was obtained from Dabur India Ltd. as brown coloured syrup; Batch No. 61025, Mfd: August 2014 with expiry 3 years from the date of manufacture was used in this study. It was stored at room temperature.

Preparation of test item

Considering the strength 300 mg was present in 1 Ml of Janma Ghunti Honey (TI). The TI was in ready to use form and used as such.

Dose levels

Three dose levels: namely, low dose (3 mL/kg bwt.), mid dose (6 mL/kg b.wt.) and high dose (12 mL/kg b.wt.) were used. The dosage was based on human therapeutic dose (1.5 g/5 mL day) converted to rat dose applying standard conversion factor considering human body weight (60 kg) by allometric method. +

Randomization & dose administration

Animals were randomized to six (G1-G4, G1S & G4S) groups [5 male & 5 females in G1-G4 & 10 animals each (5 male & 5 female) in G1S & G4S] before initiation of experiment, on the basis of body weight. The test item was administered orally to animals of the groups G2, G3, G4 and G4S once daily for 28 consecutive days using disposable syringes (2 mL /5 mL) with an oral gavage needle (18 gauge). Rats in the control group received only Milli-Q water in G1 and G1S at a dose volume of 20 mL/kg BW.

Observations

Clinical sign and mortality

Daily observation of study animals was done for any clinical signs of toxicity and twice for morbidity and mortality. Feed consumption and behavioral examination such as posture, convulsion (home cage observation), ease of removing and handling reactivity/skin/piloerection/palpebral closure/ lacrymation/ salivation, etc. (handling observation) and open field observation like arousal, respiration, movements, etc were performed at weekly intervals.

Ophthalmic examination was done before and after the study. Before termination during the fourth week for the main group and on the sixth week for recovery satellite group, they were tested for various sensory reactivity and motor activity assessment.

Laboratory parameters

Hematological and biochemical estimations were performed for animals in treatment groups (G2 to G4) while animals of satellite groups (G1S & G4S) were tested after the end of reversal period. Blood samples were collected from anesthetized animals after overnight fasting from the retro-orbital sinus using capillaries.

Hematology and biochemistry

Hematological tests included complete hemogram with hemoglobin, cell counts and blood clotting time. Biochemical testing included tests like blood glucose, liver function tests, renal function tests, lipid and total proteins.

Necropsy

Test and control animals were sacrificed on Day 29 of study. Animals in satellite groups were sacrificed 14 days later. External surface, orifices and cavities of body were examined under necropsy. Content organs and tissues of cavities viz., eye, brain, adrenal, heart, testes/ovaries, epididymis/uterus, kidneys, liver, lungs, spleen, spinal cord, trachea, thymus, stomach, duodenum, jejunum, colon, thigh muscle, lymph node, urinary bladder, prostate & seminal vesicles were examined. Adrenal, brain, heart, testes/ovaries, epididymis/uterus, kidneys, liver, thymus and spleen were weighed and recorded. The relative organ weights were calculated using the formula = {(organ weight/body weight) x100}. The organs collected were preserved using 10% formalin solution. The modified Davidson's fixative was used to preserve testes and eyes.

Histopathological examination

The organs and tissues preserved during necropsy were processed by paraffin embedding. Sample sections of 6 µM thickness were examined after hematoxylin staining and eosin dyeing as per standard methodology. Except for satellite group animals, histopathologic analysis was carried out for all groups.

Statistical analysis

Data on feed consumption, body and organ weights, hematologic and biochemical analyses were analyzed for differences among treated/control groups using One-way ANOVA Dennett multiple and Two-way ANOVA Bonferroni post-tests and *t* tests (and nonparametric tests) with the help of the software GraphPad Prism, version 4.01. Significance was considered at $p<0.05$ with 5% significance level.

Results and Discussion

General health status and mortality

Out of 60 animals, total six mortalities (G2-1/10; G3-1/10; G4-1/10; G1S-1/10 and G4S-2/1 0) were observed during the study. One male (rat no. 13) from G2 and one male (rat no. 25) from G3 were found dead on day 1 of dosing. One female (rat no. 39) from G4 and one male (rat no. 53) from G4S were found dead on day 4 and 2 of dosing, respectively. These animals were necropsied immediately post death and the mortalities were found to be due to inadvertent dose administration of TI in trachea instead of esophagus.

Rat No. 41 male from satellite control group treated with vehicle (Milli Q water) exhibited clinical sign such as nostril discharge (from days 24 to 26) and swelling in upper thoracic region (from day 25 to 26) and was found dead on day 27.

Animal No. 56 female from high dose satellite group treated with TI at 12 mL/kg body weight showed clinical signs such as swelling in left forelimb, abdominal breathing (on day 08), kyphosis (animal sitting in a position of humpback) and morbidity on day 9. The animal was found dead on day 9 of dosing. Necropsy examination (gross pathology) of both these animals revealed pus in the thoracic region and esophagus which might be due to inadvertent dose administration procedure. Based on the above findings, all the six mortalities were not considered as test item related and might be considered as accidental death and not attributed to treatment of test item.

Rest of the animals from all the groups were found normal throughout the study period except one female from G2 (rat no. 16) which showed ptosis from days 22 to 29. Since this clinical sign was observed in one animal only from low dose, it may not be considered as a treatment related effect (Table 1).

Feed consumption

Mean values of the feed consumption in treatment groups including satellite groups were not statistically significant when compared with their controls except males from high dose group (G4) showed significant increase during week 2. However, this was considered incidental since this finding was inconsistent and absent in high dose satellite group (Fig. 1a-d).

Body weights

Male and female animals from all the treated groups including the satellite group exhibited body weight gain during the study. No statistically significant alterations were observed except in females from low dose group which showed significant ($p<0.05$) decrease on week 0 (day of dosing prior to administration of Tl) and week 1 and 2 when compared with control group. Female rats from high dose satellite (G4S) also showed significant decrease ($p<0.05$) in body weight during week 6 when compared with satellite control group G1S. The change in body weight was not considered an effect of TI as the alteration was not in dose dependent manner or absence in another sex (Fig. 2a-2d).

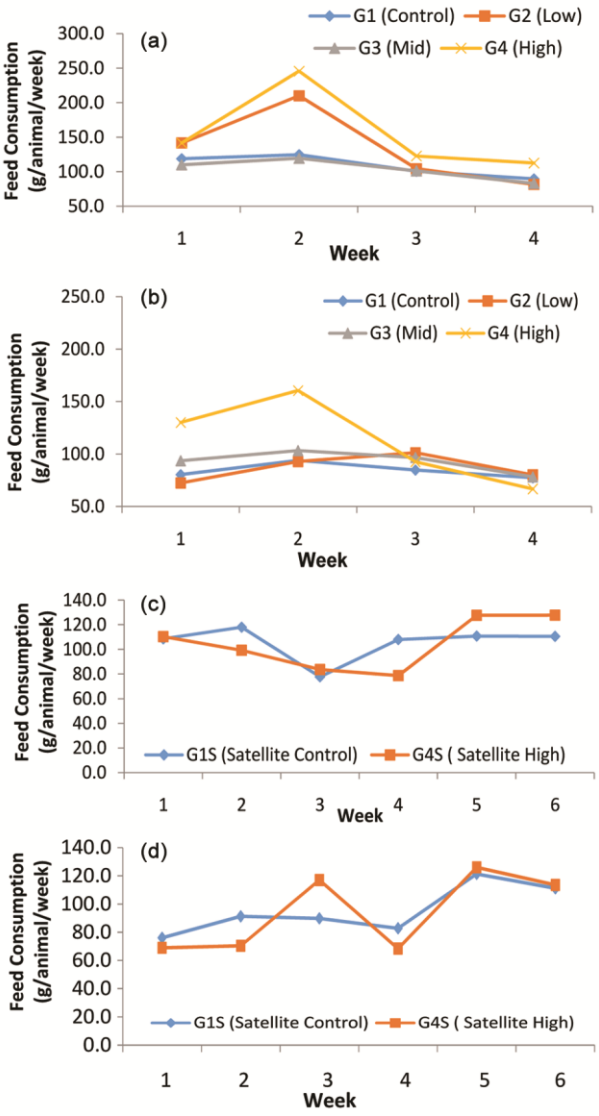

Fig. 1 a-d — Effect of sub-acute administration of Janma Ghunti for 28 days on feed consumption of male and female rats. Data are expressed as Mean±Standard Deviation (*Significant value ($p<0.05$). (a) Feed Consumption – Males, (b) Feed Consumption – Females, (c) Feed Consumption (g/animal/week) - Satellite Male & (d) Feed Consumption (g/animal/week) - Satellite Female

Hematological investigations

Evaluation of hematological parameters in terminal sacrificed male rats showed increase in neutrophils (G4) and decrease in lymphocytes (G4), hemoglobin (G2 and G4), PCV (G2), MCH (G2 and G4), MCHC (G2, G3 and G4) and platelet count (G2). Female rats revealed decrease in TLC and MCH value (G4). Above changes were observed to be statistically significant at $p<0.05$ or at $p<0.01$ level. Changes observed were inconsistent, not in a dose dependent manner or absent in satellite groups, hence considered as incidental finding (Table 2a-d).

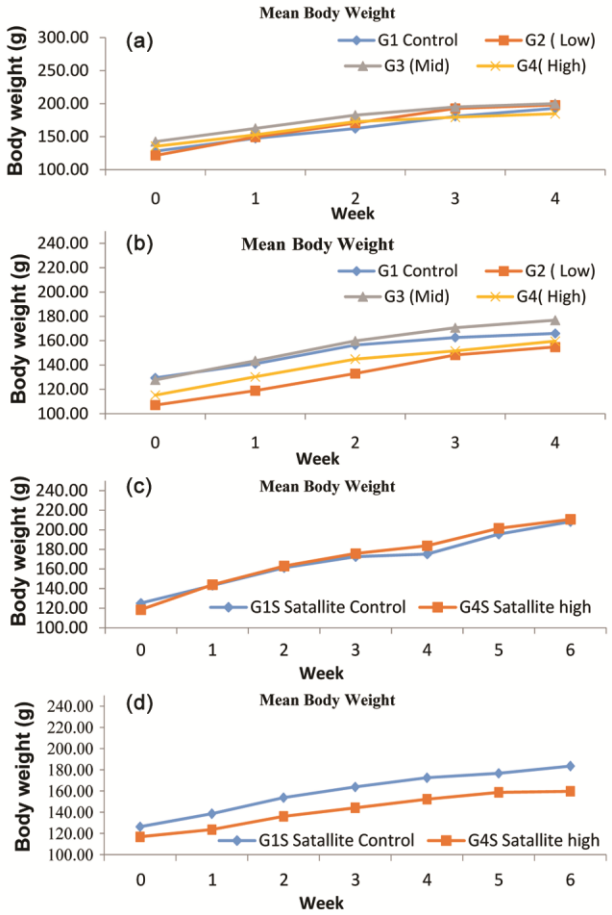

Fig. 2a-d — Effect on body weight. T(a)– Male, (b)– Female, (c)- Satellite Male & (d)- Satellite Female

Blood biochemistry

Evaluation of blood biochemistry in male rats showed statistical increase in ALP (G3, G4 and G4S) and decrease total cholesterol (G4), BUN (G2 and G4), phosphorous (G2 and G4) and triglycerides (G4S) group. Female rats revealed statistically significant increase in ALP (G4), chloride (G3) and total Bilirubin (G4S) and decrease total cholesterol (G4S). The above changes were observed to be statistically significant at $p<0.05$, $p<0.01$ level. The changes observed in the above parameters were inconsistent or not in a dose dependent manner and hence were considered incidental findings. The significant levels are expressed in (Table 3a-d).

Organ weights and relative organ weights

Absolute organ weights and relative organ weights of animals in treated and satellite groups were comparable with respective control groups and found to be nonsignificant except absolute male organ that revealed statistical increase in weight of heart and spleen from low dose (G2) whereas in females, it

Table 2a — Summary of Hematology Parameters- MALE

PARAMETER	GROUP MEAN VALUE G1 CONTROL		G2 3 ML/KG B. WT.		G3 6 ML/KG B. WT.		G4 12 ML/KG B. WT.	
	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
TLC (K/UL)	6.53	0.23	6.29	0.32	6.50	1.68	4.00	0.34
NEUTROPHIL (%)	17.34	1.58	18.62	3.29	25.85	5.12	36.14 ↑	6.24
LYMPHOCYTE (%)	66.08	1.72	62.05	6.93	57.20	5.03	43.28↓	5.60
MONOCYTE (%)	8.30	0.48	10.71	2.50	9.31	1.51	13.10	1.70
EOSINOPHIL (%)	3.11	0.62	2.71	0.54	2.59	0.60	2.60	0.70
BASOPHIL (%)	5.18	0.57	5.89	1.61	5.05	0.79	4.87	0.47
RBC (M/UL)	7.59	0.11	6.98	0.35	7.27	0.17	7.16	0.16
HEMOGLOBIN (G/DL)	14.26	0.21	12.55↓↓	0.58	13.25	0.28	12.86↓	0.24
PCV (%)	41.32	0.49	37.25↓	1.65	39.23	0.79	38.26	0.62
MCV (FL)	54.46	0.30	53.43	0.65	53.98	0.84	53.42	0.37
MCH(PG)	18.78	0.10	17.98↓↓	0.17	18.23	0.25	17.96↓↓	0.13
MCHC (G/DL)	34.50	0.09	33.63↓↓	0.17	33.78↓↓	0.17	33.60↓↓	0.13
PLATELET COUNT (K/UL)	935.20	44.32	558.75↓↓	108.36	907.75	87.07	967.60	31.11

VALUES ARE IN MEAN ± SEM, ↑=INCREASED SIGNIFICANT AT P<0.05, ↓=DECREASED SIGNIFICANT AT P<0.05, ↓↓=DECREASED SIGNIFICANT AT P<0.01

Table 2b — Summary of Hematology Parameters-SATELLITE GROUPS-MALE

PARAMETER	GROUP MEAN VALUE - MALE G1S CONTROL		G4S 12 ML/KG B. WT.	
	MEAN	SEM	MEAN	SEM
TLC (K/UL)	7.37	0.73	7.15	0.43
NEUTROPHIL (%)	32.00	6.13	20.98	2.08
LYMPHOCYTE (%)	47.08	4.20	55.78	2.60
MONOCYTE (%)	11.11	2.02	11.38	2.05
EOSINOPHIL (%)	3.71	0.91	4.11	0.84
BASOPHIL (%)	6.13	1.36	7.76	0.34
RBC (M/UL)	8.07	0.24	7.93	0.12
HEMOGLOBIN (G/DL)	14.38	0.40	14.08	0.23
PCV (%)	42.68	0.98	41.55	0.64
MCV (FL)	52.90	0.62	52.40	0.98
MCH(PG)	17.83	0.21	17.75	0.32
MCHC (GM/DL)	33.68	0.19	33.90	0.13
PLATELET COUNT (K/UL)	825.50	33.00	899.75	71.28

VALUES ARE IN MEAN ± SEM

Table 2c — Summary of Hematology Parameters-FEMALE

PARAMETER	GROUP MEAN VALUE – FEMALE G1 CONTROL		G2 3 ML/KG B. WT.		G3 6 ML/KG B. WT.		G4 12 ML/KG B. WT.	
	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
TLC (K/UL)	7.34	0.69	5.65	0.49	5.55	0.49	4.66↓	0.50
NEUTROPHIL (%)	25.10	2.32	27.10	1.63	33.32	9.20	26.45	6.56
LYMPHOCYTE (%)	52.94	2.05	51.92	2.64	44.40	9.70	53.83	6.72
MONOCYTE (%)	9.16	0.39	8.70	1.42	9.64	0.70	8.65	1.76
EOSINOPHIL (%)	6.58	0.71	7.20	0.94	7.31	1.21	5.45	2.00
BASOPHIL (%)	6.20	0.14	5.08	0.81	5.31	0.59	5.66	0.59
RBC (M/UL)	7.03	0.18	6.84	0.19	6.81	0.14	6.85	0.14
HEMOGLOBIN (G/DL)	13.20	0.28	12.66	0.23	12.46	0.34	12.35	0.20
PCV (%)	38.46	0.81	37.26	0.71	36.92	0.94	36.33	0.45
MCV (FL)	54.80	0.65	54.50	0.70	54.20	0.63	53.10	0.63
MCH(PG)	18.78	0.16	18.50	0.20	18.28	0.17	18.08↓	0.14
MCHC (GM/DL)	34.28	0.14	33.98	0.15	33.78	0.16	34.05	0.22
PLATELET COUNT (K/UL)	908.40	29.56	908.40	19.88	904.20	56.87	1021.50	57.20

VALUES ARE IN MEAN ± SEM, ↓=DECREASED SIGNIFICANT AT P<0.05,

Table 2d — Summary of Hematology Parameters-SATELLITE GROUPS-FEMALE

PARAMETER	GROUP MEAN VALUE - FEMALE G1S CONTROL		G4S 12 ML/KG B. WT.	
	MEAN	SEM	MEAN	SEM
TLC (K/UL)	7.33	0.50	9.14	0.87
NEUTROPHIL (%)	29.14	2.24	31.03	2.94
LYMPHOCYTE (%)	47.04	1.80	44.85	3.78
MONOCYTE (%)	10.92	1.21	10.41	0.76
EOSINOPHIL (%)	7.44	1.00	8.04	1.01
BASOPHIL (%)	5.46	0.78	5.69	0.91
RBC (M/UL)	7.59	0.20	7.05	0.38
HEMOGLOBIN (G/DL)	13.66	0.40	12.68	0.49
PCV (%)	39.94	1.13	37.68	1.58
MCV (FL)	52.62	0.64	53.48	0.78
MCH(PG)	17.98	0.16	18.05	0.32
MCHC (GM/DL)	34.24	0.14	33.73	0.17
PLATELET COUNT (K/UL)	932.00	40.16	880.25	25.47

VALUES ARE IN MEAN ± SEM

Table 3a — Summary of Biochemistry Parameters: Male Animals

Parameter	G1 Control		G2 3 mL/kg b. wt.		G3 6 mL/kg b. wt.		G4 12 mL/kg b. wt.	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Blood Glucose (mg/dL)	105.47	5.67	120.08	8.74	114.65	14.04	131.10	5.69
Total Cholesterol (mg/dL)	61.67	8.58	44.31	4.13	42.55	3.13	40.06↓	1.85
Triglyceride (mg/dL)	64.20	11.15	60.01	4.32	38.35	7.02	45.59	12.09
BUN (mg/dL)	27.33	1.47	19.04↓↓	0.34	25.61	1.68	15.92↓↓	2.05
Creatinine (mg/dL)	0.61	0.03	0.55	0.01	0.60	0.01	0.57	0.02
Total Bilirubin (mg/dL)	0.20	0.07	0.31	0.05	0.23	0.06	0.27	0.01
SGOT (IU/L)	103.19	3.59	101.54	2.78	95.22	6.59	94.30	2.16
SGPT (IU/L)	40.19	2.55	35.61	2.70	44.21	3.78	36.14	1.05
ALP (U/L)	227.86	32.96	335.93	27.50	343.93↑	29.72	349.55↑	24.54
Total Protein (gm/dL)	6.55	0.20	6.41	0.11	6.59	0.16	6.18	0.10
Albumin (gm/dL)	4.12	0.09	3.99	0.07	4.10	0.04	3.97	0.06
Calcium (mg/dL)	11.25	0.20	11.28	0.19	11.42	0.17	11.28	0.25
Phosphorus (mg/dL)	6.83	0.30	5.33↓	0.17	6.67	0.19	4.82↓↓	0.41
Sodium (m Eq/l)	149.60	0.81	149.75	0.63	151.50	0.29	150.60	0.24
Potassium (m Eq/l)	4.58	0.12	4.63	0.15	5.30	0.49	5.10	0.23
Chloride (m Eq/l)	110.00	0.77	111.50	0.65	111.75	0.95	111.00	0.89

Values are in Mean ± SEM; ↑=Increased Significant at p<0.05, ↓↓=Decreased Significant at p<0.01, ↓=Decreased Significant at p<0.05

Table 3b — Summary of Biochemistry Parameters: Male Animals Satellite Group

Parameter	Group Mean Values - Male G1S Control		G4S 3 ml/kg bw	
	Mean	SEM	Mean	SEM
Blood Glucose (mg/dL)	132.19	7.87	109.56	10.06
Total Cholesterol (mg/dL)	50.07	12.65	69.19	7.61
Triglyceride (mg/dL)	66.55	2.90	37.06↓	3.60
BUN (mg/dL)	30.41	5.91	29.27	0.96
Creatinine (mg/dL)	0.59	0.02	0.59	0.01
Total Bilirubin (mg/dL)	0.79	0.08	0.59	0.02
SGOT (IU/L)	91.12	4.05	93.93	2.26
SGPT (IU/L)	47.27	1.76	41.49	2.02
ALP (U/L)	334.74	13.83	427.94↑	20.73
Total Protein (gm/dL)	6.87	0.15	6.71	0.16
Albumin (gm/dL)	4.13	0.10	4.15	0.06
Calcium (mg/dL)	12.03	0.20	11.88	0.15
Phosphorus (mg/dL)	7.22	0.69	7.08	0.18
Sodium (m Eq/l)	151.75	0.63	152.00	0.82
Potassium (m Eq/l)	4.30	0.27	4.45	0.19
Chloride (m Eq/l)	110.75	0.85	111.25	0.85

Values are in Mean ± SEM;↑=Increased Significant at p<0.05, ↓=Decreased Significant at p<0.05

Table 3c — Summary of Biochemistry Parameters: Female Animals

Parameter	Group Mean Values - Female							
	G1		G2		G3		G4	
	Control		3 mL/kg b. wt.		6 mL/kg b. wt.		12 mL/kg b. wt.	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Blood Glucose (mg/dL)	105.51	1.87	103.62	6.80	110.66	6.59	99.16	5.27
Total Cholesterol (mg/dL)	56.76	3.93	52.84	2.09	52.11	3.58	50.42	1.50
Triglyceride (mg/dL)	52.90	7.89	39.94	4.49	37.82	2.55	46.47	5.99
BUN (mg/dL)	29.53	0.80	31.32	2.66	25.14	2.22	28.61	0.80
Creatinine (mg/dL)	0.63	0.02	0.56	0.02	0.60	0.03	0.55	0.03
Total Bilirubin (mg/dL)	0.24	0.07	0.31	0.04	0.24	0.05	0.33	0.07
SGOT (IU/L)	100.21	3.00	108.32	3.67	102.82	3.20	103.53	4.54
SGPT (IU/L)	36.76	1.10	42.98	3.52	40.46	2.28	36.63	1.79
ALP (U/L)	182.37	9.61	212.99	12.25	200.51	13.86	258.58↑	25.86
Total Protein (gm/dL)	6.90	0.13	6.88	0.06	6.94	0.18	6.72	0.14
Albumin (gm/dL)	4.20	0.13	4.28	0.05	4.26	0.06	4.23	0.09
Calcium (mg/dL)	11.75	0.15	11.54	0.07	11.77	0.20	11.66	0.14
Phosphorus (mg/dL)	7.15	0.34	7.07	0.63	5.95	0.36	6.88	0.29
Sodium (m Eq/l)	150.80	1.77	149.60	0.68	151.00	0.63	152.00	0.41
Potassium (m Eq/l)	4.50	0.15	4.74	0.06	4.78	0.27	5.08	0.28
Chloride (m Eq/l)	109.80	0.37	110.40	0.24	111.60↑	0.60	109.50	0.50

Values are in Mean ± SEM, ↑=Increased Significant at $p<0.05$

Table 3d — Summary of Biochemistry Parameters: Female Animals Satellite Group

Parameter	Group Mean Values - Female			
	G1S		G4S	
	Control		3 mL/kg b. wt.	
	Mean	SEM	Mean	SEM
Blood Glucose (mg/dL)	97.80	3.71	104.92	6.18
Total Cholesterol (mg/dL)	72.26	2.78	62.50↓	1.38
Triglyceride (mg/dL)	52.48	5.77	37.62	5.75
BUN (mg/dL)	32.48	1.72	34.81	2.02
Creatinine (mg/dL)	0.59	0.01	0.58	0.02
Total Bilirubin (mg/dL)	0.71	0.02	0.89↑	0.08
SGOT (IU/L)	96.00	2.71	95.64	5.22
SGPT (IU/L)	49.36	2.83	45.33	2.54
ALP (U/L)	201.63	10.42	193.34	30.53
Total Protein (gm/dL)	6.91	0.18	6.84	0.04
Albumin (gm/dL)	4.23	0.07	4.18	0.04
Calcium (mg/dL)	11.71	0.12	11.51	0.22
Phosphorus (mg/dL)	7.97	0.36	7.56	0.28
Sodium (m Eq/l)	152.00	0.71	153.25	1.70
Potassium (m Eq/l)	4.16	0.17	4.30	0.13
Chloride (m Eq/l)	111.60	0.87	114.50	1.44

Values are in Mean ± SEM; ↑=Increased Significant at $p<0.05$, ↓=Decreased Significant at $p<0.05$

revealed statistical increase in kidney weight (G3). The changes were significant either at $p<0.05$, $p<0.01$ levels (Table 4a-b).

Relative male organ weight revealed statistical increase in liver and spleen low dose (G2) group and in relative female organ weight adrenal showed increase in (G3) and brain (G4S). The changes were at $p<0.05$, $p<0.01$ levels. Above changes were not in a dose dependent manner, hence considered incidental (Table 5a-b).

Gross pathology

External gross pathological examination of moribund sacrificed dead animals revealed swelling

Table 4a — Summary of Organ Weight (g): Male Animals

Organ		Mean Organ Weight (g)					
	Sex	Male					
	Group	G1	G2	G3	G4	G1S	G4S
	Dose (mL/kg b. wt.)	Control	3	6	12	Control Satellite	12
Mean Fasting Body weight	Mean	182.146	192.453	195.485	183.242	198.325	205.298
	SEM	10.871	12.401	9.976	4.863	5.925	8.626
Brain	Mean	1.577	1.600	1.569	1.518	1.587	1.550
	SEM	0.024	0.100	0.068	0.075	0.021	0.021
Heart	Mean	0.734	0.863↑	0.733	0.715	0.670	0.707
	SEM	0.046	0.026	0.016	0.030	0.037	0.012
Adrenal	Mean	0.034	0.034	0.030	0.036	0.040	0.030
	SEM	0.003	0.002	0.006	0.004	0.004	0.002
Testis	Mean	2.266	2.570	1.928	2.116	2.208	2.461
	SEM	0.146	0.199	0.376	0.046	0.165	0.073
Epididymis	Mean	0.715	0.763	0.646	0.642	0.766	0.787
	SEM	0.067	0.078	0.088	0.017	0.067	0.018
Kidneys	Mean	1.251	1.384	1.296	1.279	1.177	1.222
	SEM	0.111	0.076	0.081	0.043	0.130	0.041
Liver	Mean	7.317	9.403	7.032	7.052	7.098	7.094
	SEM	0.744	0.678	0.571	0.355	0.425	0.193
Thymus	Mean	0.313	0.331	0.297	0.281	0.310	0.365
	SEM	0.023	0.028	0.015	0.037	0.015	0.038
Spleen	Mean	0.573	0.966↑↑	0.398	0.406	0.394	0.468
	SEM	0.066	0.087	0.034	0.027	0.044	0.020

Values are in Mean ± SEM, ↑=Increased Significant at p<0.05, ↑↑=Increased Significant at p<0.01

Table 4b — Summary of Organ Weight (g): Female Animals

Organ		Mean Organ Weight (g)					
	Sex	Female					
	Group	G1	G2	G3	G4	G1S	G4S
	Dose (mL/kg b. wt.)	Control	3	6	12	Control Satellite	12
Mean Fasting Body weight	Mean	160.860	150.542	173.378	154.695	176.242	155.640
	SEM	4.121	3.674	4.660	14.104	6.241	7.219
Brain	Mean	1.501	1.479	1.616	1.496	1.693	1.719
	SEM	0.041	0.038	0.065	0.043	0.051	0.050
Heart	Mean	0.638	0.636	0.649	0.585	0.749	0.705
	SEM	0.013	0.021	0.027	0.039	0.024	0.059
Adrenal	Mean	0.049	0.053	0.035	0.042	0.050	0.046
	SEM	0.006	0.004	0.004	0.005	0.005	0.004
Ovaries	Mean	0.077	0.081	0.068	0.069	0.099	0.117
	SEM	0.016	0.012	0.004	0.007	0.018	0.006
Uterus	Mean	0.456	0.373	0.534	0.398	0.420	0.613
	SEM	0.059	0.038	0.064	0.044	0.050	0.161
Kidneys	Mean	0.968	1.023	1.161↑	1.053	1.289	1.195
	SEM	0.026	0.059	0.040	0.082	0.049	0.086
Liver	Mean	6.851	6.483	7.041	6.402	8.411	7.676
	SEM	0.204	0.421	0.345	0.534	0.510	0.501
Thymus	Mean	0.366	0.337	0.382	0.295	0.453	0.377
	SEM	0.024	0.041	0.024	0.044	0.041	0.050
Spleen	Mean	0.416	0.366	0.414	0.341	0.489	0.424
	SEM	0.016	0.025	0.013	0.038	0.033	0.035

Values are in Mean ± SEM, ↑=Increased Significant at p<0.05

Table 5a — Summary of Relative Organ Weight (%): Male Animals

Organ	Relative Organ Weight (%)						
	Sex	Male					
	Group	G1	G2	G3	G4	G1S	G4S
	Dose (mL/kg b. wt.)	Control	3	6	12	Control Satellite	12
Brain	Mean	0.882	0.848	0.809	0.829	0.803	0.759
	SEM	0.071	0.095	0.055	0.041	0.028	0.035
Heart	Mean	0.404	0.452	0.376	0.392	0.337	0.347
	SEM	0.016	0.020	0.011	0.024	0.013	0.018
Adrenal	Mean	0.019	0.018	0.016	0.020	0.020	0.015
	SEM	0.001	0.002	0.003	0.002	0.002	0.001
Testis	Mean	1.250	1.333	0.974	1.157	1.115	1.207
	SEM	0.061	0.030	0.178	0.030	0.081	0.072
Epididymis	Mean	0.391	0.395	0.327	0.351	0.388	0.386
	SEM	0.020	0.021	0.034	0.013	0.039	0.020
Kidneys	Mean	0.683	0.721	0.662	0.699	0.590	0.597
	SEM	0.030	0.020	0.010	0.023	0.051	0.024
Liver	Mean	3.972	4.920↑	3.582	3.855	3.586	3.464
	SEM	0.232	0.390	0.130	0.203	0.225	0.080
Thymus	Mean	0.172	0.175	0.152	0.154	0.156	0.179
	SEM	0.011	0.022	0.005	0.020	0.005	0.021
Spleen	Mean	0.312	0.510↑↑	0.203	0.222	0.197	0.229
	SEM	0.023	0.060	0.011	0.015	0.016	0.014

Values are in Mean ± SEM; ↑↑=Increased Significant at $p<0.01$, ↑=Increased Significant at $p<0.05$

Table 5b — Summary of Relative Organ Weight (%): Female Animals

Organ	Relative Organ Weight (%)						
	Sex	Female					
	Group	G1	G2	G3	G4	G1S	G4S
	Dose (mL/kg b. wt.)	Control	3	6	12	Control Satellite	12
Brain	Mean	0.936	0.984	0.938	0.986	0.963	1.108↑
	SEM	0.035	0.030	0.060	0.069	0.022	0.036
Heart	Mean	0.397	0.423	0.376	0.382	0.425	0.456
	SEM	0.007	0.016	0.019	0.021	0.009	0.043
Adrenal	Mean	0.030	0.035	0.020↑	0.027	0.028	0.030
	SEM	0.004	0.002	0.002	0.002	0.003	0.004
Ovaries	Mean	0.047	0.053	0.040	0.047	0.057	0.076
	SEM	0.010	0.008	0.003	0.009	0.011	0.005
Uterus	Mean	0.285	0.247	0.307	0.268	0.241	0.398
	SEM	0.038	0.022	0.034	0.046	0.033	0.108
Kidneys	Mean	0.602	0.680	0.670	0.686	0.731	0.766
	SEM	0.016	0.036	0.021	0.037	0.008	0.035
Liver	Mean	4.274	4.300	4.068	4.150	4.762	4.923
	SEM	0.195	0.233	0.208	0.063	0.178	0.149
Thymus	Mean	0.228	0.222	0.220	0.189	0.256	0.241
	SEM	0.017	0.024	0.014	0.016	0.018	0.027
Spleen	Mean	0.259	0.242	0.240	0.219	0.278	0.272
	SEM	0.011	0.012	0.009	0.009	0.016	0.019

Values are in Mean ± SEM; ↑=Increased Significant at $p<0.05$ when compared with G1S

in left forelimb in one female from high dose satellite group (animal no. 56) and nasal discharge, swelling in upper thoracic region found in one male (animal no. 41) from control satellite group. Terminally sacrificed animals did not reveal any external gross pathological findings. Internal (visceral) gross pathological examination did not show any abnormalities in both the groups, except in following animals: pus formation was seen at left forelimb and esophagus (animal No 56 female, G4S group) and in upper thoracic region of (animal No. 41 male, G1 S group) of dead animal along with enlarged adrenal and small spleen, diminished testes was observed in animal no 24 male (G3 group) and animal no 42 (male, G1S

Table 6 — Summary of Gross Pathological Findings

Organs/ Lesions	Group	G1		G2		G3		G4		G1S		G4S	
	Dose (mL/kg)	Control		3		6		12		Satellite Control		12	
	Sex	M	F	M	F	M	F	M	F	M	F	M	F
	No. of Animals	5	5	4	5	4	5	5	4	5	5	4	5
EXTERNAL													
No abnormalities detected		5	5	4	5	4	5	5	4	4	5	4	4
Swelling in left forelimb		0	0	0	0	0	0	0	0	0	0	0	1
Nasal Discharge, Swelling in upper thoracic region		0	0	0	0	0	0	0	0	1	0	0	0
INTERNAL													
No abnormalities detected		5	5	4	5	3	5	5	4	3	5	4	4
Pus found under left forlimb and in esophagus		0	0	0	0	0	0	0	0	0	0	0	1
Pus found in upper thoracic region, enlarge adrenal, small spleen		0	0	0	0	0	0	0	0	1	0	0	0
Dminished testes		0	0	0	0	1	0	0	0	0	0	0	0
One kidney shrunken, Stone in urinary bladder		0	0	0	0	0	0	0	0	1	0	0	0

group), one shrunken kidney and stone were found in urinary bladder and all other terminally sacrificed animals did not revealed any internal gross pathological findings (Table 6).

Histopathological evaluation

Histopathological investigation carried out in organs from all the terminally sacrificed animals (both sexes) from control and high dose groups did not reveal any evidence of test item related toxicity in any of the organs examined except bronchus associated lymphoid tissue in lung was observed in one female from control group (animal no. 10) which is considered as an incidental finding.

Histopathological evaluation of animals (No. 41, 56) found moribund/dead animals and tissue sample from animals with gross lesions did not show abnormal histological features.

The changes observed in various parameters such as body weight, feed consumption, biochemical parameters, hematological parameters, organ weight and pathology were found inconsistent or not in dose dependent manner, hence are not attributed to TI administration.

Janma Ghunti is a traditional polyherbal formulation for overall digestive health of babies. The therapeutic benefits of individual ingredients contribute to the product efficacy synergistically. Anjeer (*Ficus carica*) is a nutritive fruit as per Ayurveda. It provides energy as it is a source of essential elements like calcium, phosphorus, iron and dietary fibers. It also works in constipation as a mild laxative, to treat dysentery and enteritis[3]. Vidanga (*Embelia ribes*) is a well-known vermifuge as per Ayurvedic literature. It also acts as carminative, appetizer and relieves flatulence. *E. ribes* showed potent anthelmintic activity at a concentration of 10-200 Ug/mL[4]. Palash (*Butea monosperma*) is an appetizer and digestive astringent. It has flavonoids, phenolics and alkaloids that possess antioxidant activity and nephroprotective activity[5]. Vaca (*Acorus calamus*) is a *Medhya rasayana*, digestive and promotes physical strength and immunity in children. It is used traditionally in gastrointestinal disorders such as colic pain and diarrhea[6]. Badara (*Zizyphus jujuba*) is good for respiratory health and is a digestive stimulant. It has shown anti-fatigue and antioxidant activity[7]. Draksha (*Vitis vinifera*) has good nutritional value and also acts as mild laxative, appetizer and relieves thirst. It has known anti-inflammatory and anti-spasmodic effect[8,9]. Madhu (honey) improves immunity in children, is good for respiratory health and potentiates action and absorption of other herbs due to its *Yogavahi* property[10].

Conclusions

Janma Ghunti on oral administration for 28 days was found to be nontoxic at the doses of 3, 6 & 12 mL/kg body weight in both the sexes of wistar rats. Based on the study outcome, it may be noted that the No Observed Adverse Effect Level (NOAEL) of

Janma Ghunti in male and female Wistar rats was found to be greater than 12 mL/kg body weight.

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Conflict of interest

Authors declare there is no conflict of interest.

Author(s) contributions

K S, G A, R R K and S J L N designed the study and prepared/reviewed the manuscript. SP lead the study activities and reviewed the manuscript.

References

1 Bhattacharya S K, Final report of the research on Dabur Janamghunti and Janamghunti Extract, Department of Pharmacology, Institute of Medical Science, Banaras Hindu University-Varanasi, Data on Files, Dabur India Limited.

2 OECD , Test No. 407: Repeated dose 28-day oral toxicity study in rodents, OECD guidelines for the testing of chemicals, Section 4, OECD Publishing, (2008) Paris, https://doi.org/10.1787/9789264070684-en.

3 Borolo M I, Mostacero N L & Lopez S N, Ficus carica L, (Moraceae): An ancient source of food and health, *Food Chem*, 164 (2014) 119-127. http://dx.doi.org/10.1016/j.foodchem.2014.04.112

4 Bist M & Prasad S B, *Embelia ribes*: A valuable medicinal plant, *J Chem Pharm Res*, 8 (4) (2016) 1229-1233

5 Sonkar N, Ganeshpurkar A, Yadav P, Dubey S & Bansal D *et al*., An experimental evaluation of nephroprotective potential of *Butea monosperma* extract in albino rats, *Ind J Pharmacol*, 46 (1) (2014) 109-112

6 Imam H, Riaz Z, Azhar M, Sofi G & Hussain A, Sweet flag (*Acorus calamus* Linn.): An incredible medicinal herb, *Int J Green Pharm*, (2013) 288-296.

7 Kumar G P, Sekhar Y C, Ashok G, Anand K & Anilakumar K, Functional benefits of *Ziziphus jujuba* fruits: Anti-fatigue activity and antioxidant enzyme activities in experimental animal models, *EC Nutrition*, 13 (5) (2018) 288-298.

8 Garg N & Jain A, Therapeutic and medicinal uses of Draksha - A review, 6 (3) (2017) 2365-2369.

9 Anonymous, Assessment report on *Vitis vinifera* L., folium, EMA/HMPC/464682/2016 Committee on Herbal Medicinal Products (HMPC), (2016).

10 Sharma K, Goyal C & Prajapati D, Critical review on madhu w.s.r. to honey, *Int J Ayu Pharm Res*, 3 (9) (2015) 75-82.