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Single dose oral toxicity study of nano formulated extract of *Picrorhiza kurroa* in wistar rats

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ABSTRACT

Single dose oral toxicity study of Nano formulation made from extract of *Picrorhiza kurroa* was carried out in Wistar Rats. The objective of this study was to assess the single dose oral toxicity of nano formulation of *Picrorhiza Kurroa extract* in Wistar Rats, at a dosage of 200, 1000 or 2000 mg/kg body weight. Forty Wistar rats were distributed to 4 dose groups, consisting of 10 rats (5 male + 5 female) each. Three groups were orally administered with *Picrorhiza Kurroa extract* at doses of 200 mg/kg, 1000 mg/kg or 2000 mg/kg body weight and observed daily for 14 days. Animals were sacrificed on Day 15. The control (0 mg/kg) group received the vehicle (0.5 % carboxy methylcellulose). The dose volume was given at the rate of 20 ml/kg body weight. All the rats were examined daily for signs of toxicity and mortality, and were subjected to detailed clinical examination before initiation of the study and weekly thereafter during the exposure period. Body weights and food consumption were recorded twice weekly. All animals were sacrificed at termination on day 15 and were subjected to detailed gross pathology. In the present study, nano formulation of *Picrorhiza Kurroa extract* has not caused any mortality or any symptoms of toxicity at and up to a dose of 2000 mg/kg body weight (Limit Dose), under the conditions of the study.

Keywords: Nano formulation, *Picrorhiza kurroa* , Single dose oral toxicity

1. Introduction

As people become more interested in the food safety and well-being in recent years, so the demand for functional food from natural sources is increased [1]. Therefore, medicinal plants have provided high opportunities for the development of herbal food products, dietary supplements, and functional foods Toxicological aspect of natural origin-functional foods has been neglected because they have been used for a long time on various purposes. However, medicinal plants have also undesirable side effects as patients on herbal medicine therapy experienced some side effects[2].Since last many years, plants have beneficial activity in different type of diseases producing in human beings. As per WHO calculate that about 80% of the world's inhabitants problem should treated by medicinal herbal drug for their primary health care [3-4]. Natural products remain an important source of new drugs, new drug leads and new chemical entities. The plant based drug discovery resulted mainly in the development of anticancer agents including plants (vincristine, vinblastine, etoposide, paclitaxel, camptothecin, topotecan and irinotecan), marine organisms (citarabine, aplidine and dolastatin 10) and micro-organisms (dactinomycin, bleomycin and doxorubicin) [5-6]. Till Current research on Picrorhiza kurroa has focused on its hepatoprotective, anticholestatic, antioxidant, and immune-modulating activity [7-8]. Kutkin is the active principal of Picrorhiza kurroa and is comprised of kutkoside and the Iridoid glycoside picrosides I, II, and III. Other identified active constituents are apocynin, drosin, and nine cucurbitacin glycosides [7-8]. Apocynin is a catechol that has been shown to inhibit neutrophil oxidative burst in addition to being a powerful anti-inflammatory agent [9] while the curcubitacins have been shown to be highly cytotoxic and possess antitumor effects [10]. The present study is carried out to find the single dose oral toxicity of the nano formulated *Picrorhiza kurroa* extract on wistar rats.

2. Materials

2.1 Preparation of 0.5% aqueous carboxy methylcellulose

30 ml of water was heated up to 80 ^{o}C on hot plate. Carboxy methylcellulose (1 g) was added to the hot water and stirred continuously for 15 minutes, to create a uniform dispersion of 0.5% aqueous carboxy methylcellulose. Chilled water (2-8^{o}C) was added to make the final volume up to 200mL. The container was then placed in wet ice and stirred for 2 hours and stored at 4^{o}C.

2.2 Preparation of dose formulation

The concentrations of the test substance prepared for dosing were 10, 50 and 100 mg/mL, to administer doses of Active Ingredient (AI) equivalent to 200, 1000 and 2000 mg/kg to animals of Group 2, Group 3 and Group 4, respectively. Group 1 animals were treated with vehicle (0.5% aqueous carboxy methylcellulose)

The test substance was weighed on an analytical balance and transferred to a mortar. Initially, volume of vehicle was added to wet the test substance and triturated with a pestle to homogenize the test substance. The processed volumes were then poured into a 100 ml graduated glass measuring cylinder. The mortar and pestle were rinsed with vehicle and each rinse was poured into the graduated cylinder to make up the final volume and mixed thoroughly. Similar procedure was used for formulation of all three doses.

2.3 Stability, Homogeneity and Dose Formulation Analysis

Stability and homogeneity analyses were not conducted by the testing laboratory. However, two (2) quantitative 1.0-mL aliquots was collected from all dosing formulations (middle of container) at the time of each preparation and stored at -20° C for possible verification of test substance concentration and will be maintained for 2 months from study initiation and may be discarded if the sponsor does not desire to carry out further analytical analysis of the formulation.

2.4 Test System

30 male and 30 female Wistar rats between 4 - 6 weeks of age were obtained from RCC, laboratories, Hyderabad. The animals was sexually mature and females was nulliparous and non-pregnant. At study initiation the age of the rats was 6 - 8 weeks and the body weight of male and female rats ranged between 156 – 184 g and 128 – 143 g respectively.

2.5 Acclimatization:

The animals were acclimatization for a period of 5 days. During the acclimatization period, individual rats were subjected to physical examinations and assessed for their health condition and suitability to be included into the study. During the acclimatization period, each animal were observed at least once a day, to determine any abnormalities, infighting inflicted injuries or disease. Only those animals certified by the in-house veterinarian as healthy, were used for the study.

3. Methods

3.1 Randomization and Grouping:

Prior to treatment initiation, 20 male and 20 female rats were randomly selected and allocated to different groups, so that each group contained a similar population of rats. Group mean body weight was assessed by computerized random number generation on day of randomization. The initial group mean body weight within and across the groups were approximately equal and did not vary beyond ± 20%. After randomization rats were housed in 2 and 3 rats per cage of same sex and within each group. Animals were identified by a unique fur (body) marking using 10% picric acid. Further each cage was identified by color coded labels according to group and each label stated the study number, cage number, species, strain, sex, dose, animal identification numbers, dosing details and important dates like date of receipt, date of acclimatization, date of grouping, date of experiment initiation and date of experiment termination as documented in table 1.

Table 1: Description about randomization and grouping

Group	Dose (mg/kg)	Concentration (mg/ml)	Dose Volume (mL/kg)	Number of Rats	Sex	Animal Number	
						From	To
Control (G1)	0	0	20	5	M	A501	A505
				5	F	A506	A510
Low Dose (G2)	200	10	20	5	M	A511	A515
				5	F	A516	A520
Mid Dose (G3)	1000	50	20	5	M	A521	A525
				5	F	A526	A530
High Dose (G4)	2000	100	20	5	M	A531	A535
				5	F	A536	A540

3.2 Administration of Test Substance

Before dosing, the animals chosen for the study were fasted overnight. On day of dosing (Day 1), the test substance, was administered orally, as a suspension in aqueous 0.5 % carboxy methylcellulose, as two fractional gavage doses given approximately one hour apart using a ball tipped stainless steel gavage needle attached to a 2 mL graded syringe. The dose volume was calculated for each individual animal, on the basis of their body weights. Control animals received the vehicle (0.5% methylcellulose) alone at the same dose volume. After the second administration of the test substance, feed was provided to all rats.

3.3 Observations

The following observations were made during the course of the study.

3.5 Mortality

The rats were observed for any mortality/moribund conditions, at 10, 30 minutes and, 1.0, 3.0 and 6.0 hours post-dose on day 1, and, twice daily (morning and afternoon) thereafter, for 14 days.

3.5 Clinical Signs

All signs of ill health, together with any behavioral changes or reaction to treatment were recorded for individual animals. These cage side observations of individual animals were carried out before dosing, on Day 1 (pre-dose) and post-dose, at 10 and 30 minutes and further, at 1.0, 3.0 and 6.0 hour intervals, and, then twice daily (morning and afternoon) thereafter, for 14 days. Animals were observed for onset of clinical signs and recovery. During Holidays, animals were observed at least once daily.

Detailed clinical examination of all animals was carried out at initiation of the study and on days 1, 8 and 14. During detailed examination, animals were removed from their cage and observed for test substance related or spontaneous changes in skin, fur, eyes, mucous membranes, and musculature or skeletal, CNS, and respiratory, or gastrointestinal signs.

3.6 Body Weight

The weights of individual rat were recorded at the time of allocation to groups, on the day of commencement of treatment (Day 1) and subsequently, on Day 3, 6, 9, 12 and 14. Fasted weights were recorded before necropsy (Day 15).

3.7 Feed Consumption

The quantity of feed consumed by rats in each cage from each group was recorded on Day 3, 6, 9 12 and 14. Feed intake per rat was calculated from the feed left over in each cage on Day 3, 6, 9 12 and 14, Feed consumption per rat was calculated by feed left over/ No. of rats in cage/Number of days.

3.8 Necropsy

On Day 15 of the treatment period, rats from all dose groups were fasted overnight and euthanized using deep over-dose of isoflurane anesthesia and subjected to necropsy. Detailed gross pathological observation.

3.9 Statistical Analysis

Group mean and standard deviation were calculated for all generated data using Barlett's test for intra group variances. The data with homogeneous intra-group variances was subjected to one-way analysis of variance (ANOVA – Scedecor and Cochran, 1980) and Dunnett's pair-wise comparison (Scheffe 1953), to confirm significance in the ANOVA test.

All statistical analysis and comparisons was determined at $P < or = 0.05$ level. Statistically significant differences $P < or = 0.05$, indicated in the aforementioned test are designated by superscripts in the report as below: +/- : Significantly higher (+)/lower (-) than control.

4. Results and discussion

4.1 Mortality and Morbidity

No mortality/morbidity was observed in any of male and female rats, at and up to dose of 2000 mg/kg body weight after the oral administration of nano formulation of extract of *Picrorhiza kurroa* for 14 days of study period. (Table 3)

Table 2: Summary of Clinical signs data - males and females

Observation Day	Male				Female			
	G1	G2	G3	G4	G1	G2	G3	G4
1	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5
2	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5
3	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5
4	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5
5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5
6	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5
7	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5
8	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5
9	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5
10	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5
11	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5
12	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5
13	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5
14	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5
15	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5	NAD/5

NAD: No abnormality detected, 5=Number of animals per group

Table 3: Summary of Mortality Data - males and females

Observation Day	Male				Female			
	G1	G2	G3	G4	G1	G2	G3	G4
1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
3	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
6	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
7	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
8	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
9	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
11	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
12	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
13	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
14	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
15	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

5=Number of animals per group

4.2 Clinical signs

General and Detailed Clinical Examinations (Table 2) test substance, at and up to the dose of 2000 mg/kg did not induce any remarkable abnormal clinical signs, in any of the treated male or female rats.

4.3 Body Weight

The group means body weights of male and female rats treated with nano formulation of *Picrorhiza kurroa* at and up to the dose of 2000 mg/kg did not differ significantly from those of the controls during the 14 day treatment period. Ref (Table 4, 5, 6 and 7)

Table 4: Summary of mean body weights (g) males

Group No. Dose (mg/kg)		Days					
		1	3	6	9	12	14
G1	Mean	171	191	214	231	253	263
0	SD	9.55	11.14	12.98	12.12	10.87	10.26
	N	5	5	5	5	5	5
G2	Mean	168	188	212	232	247	261
500	SD	8.16	8.92	8.30	11.35	11.87	11.23
	N	5	5	5	5	5	5
G3	Mean	167	187	209	232	247	258
1000	SD	6.72	7.94	8.94	8.42	6.27	2.26
	N	5	5	5	5	5	5
G4	Mean	170	191	213	238	254	269
2000	SD	8.66	9.07	9.81	11.91	14.68	15.56
	N	5	5	5	5	5	5

N=number of animals; +/- significantly greater/lesser then control

Table 5: Summary of mean body weights (g) Females

Group No. Dose (mg/kg)		Days					
		1	3	6	9	12	14
G1	Mean	135	148	162	175	181	187
0	SD	5.08	1.98	4.12	4.33	4.98	2.88
	N	5	5	5	5	5	5
G2	Mean	136	147	159	169	175	183
500	SD	4.78	4.81	3.82	4.37	3.14	6.29
	N	5	5	5	5	5	5
G3	Mean	137	150	150	171	179	185
1000	SD	5.79	5.79	9.46	7.00	7.68	7.46
	N	5	5	5	5	5	5
G4	Mean	132	147	157	169	177	184
2000	SD	3.67	3.09	3.65	2.97	4.72	5.44
	N	5	5	5	5	5	5

N=number of animals; +/- significantly greater/lesser then control

Table 6: Summary of Cumulative net Body Weights gain (g) – Males

Group No. Dose (mg/kg)		Days				
		3	6	9	12	14
G1	**Mean**	20	44	61	82	92
0	SD	3.03	5.08	7.77	3.89	5.51
	N	5	5	5	5	5
G2	**Mean**	20	44	64	79	93
500	SD	9.55	4.41	5.74	6.18	6.42
	N	5	5	5	5	5
G3	**Mean**	21	42	65	80	92
1000	SD	2.05	4.78	4.93	3.87	7.29
	N	5	5	5	5	5
G4	**Mean**	21	43	68	84	99
2000	SD	2.43	4.98	6.03	8.18	9.53
	N	5	5	5	5	5

N=number of animals; +/- significantly greater/lesser then control

Table 7: Summary of Cumulative net Body Weights gain (g) – Females

Group No. Dose (mg/kg)		Days				
		3	6	9	12	14
G1	Mean	13	27	40	46	52
0	SD	3.67	1.70	2.00	3.55	4.59
	N	5	5	5	5	5
G2	Mean	11	24	33	40	47
500	SD	1.50	3.09	3.82	2.54	4.60
	N	5	5	5	5	5
G3	Mean	13	14	34	43	48
1000	SD	2.48	10.43	3.86	5.97	7.22
	N	5	5	5	5	5
G4	Mean	14	24	37	44	52
2000	SD	2.12	3.45	4.11	4.96	5.57
	N	5	5	5	5	5

N=number of animals; +/- significantly greater/lesser then control

Males: The cumulative net body weight gain, computed over the period of 14 days, in male rats receiving nano formulation of *Picrorhiza kurroa* at 500, 1000 or 2000 mg/kg was 101%, 100% and 108% respectively similar to that of control group.

Females: Similarly, the cumulative net body weight gain by female rats receiving nano formulation of *Picrorhiza kurroa* at 500, 1000 or 2000 mg/kg was 90 %, 92 % and 100% respectively similar to that of control group.

4.4 Feed consumption

The values of average daily food consumption by male and female rats exposed to nano formulation of *Picrorhiza kurroa* at and up to the dose of 2000 mg/kg were found to be comparable to those of the concurrent control groups. Ref (Table 8, 9)

Table 8: Summary of Feed Consumption/ Rat/ day: 2/3 animals/cage (g) – Males

Group No. Dose (mg/kg)		Days				
		3	6	9	12	14
G1	Mean	14.2	18.9	20.8	21.8	14.6
0	SD	0.9	0.2	1.4	0.7	0.3
	N	2	2	2	2	2
G2	Mean	13.4	19.9	20.0	20.4	14.5
500	SD	0.3	0.6	1.1	1.6	0.5
	N	2	2	2	2	2
G3	Mean	13.5	19.4	20.8	21.1	14.9
1000	SD	0.2	0.4	1.3	0.8	0.72
	N	2	2	2	2	2
G4	Mean	13.0	19.4	21.3	21.5	15.1
2000	SD	0.9	0.3	1.3	1.8	0.7
	N	2	2	2	2	2

N=number of cages/group; +/- significantly greater/lesser then control

Table 9: Summary of Feed Consumption/ Rat/ day (g) – – Females

Group No. Dose (mg/kg)		Days				
		3	6	9	12	14
G1	Mean	10.2	14.5	15.4	14.9	9.7
0	SD	0.9	0.2	0.4	0.4	0.1
	N	2	2	2	2	2
G2	Mean	9.6	14.0	13.6	14.5	9.6
500	SD	0.4	1.1	0.7	0.1	0.0
	N	2	2	2	2	2
G3	Mean	10.0	13.5	15.0	15.0	9.8
1000	SD	0.1	1.6	1.1	0.3	0.2
	N	2	2	2	2	2
G4	Mean	10.3	14.5	14.8	15.0	9.9
2000	SD	0.3	0.0	0.0	0.3	0.6
	N	2	2	2	2	2

N=number of cages/group; +/- significantly greater/lesser then control

Males: The average daily food consumption per rat per day, computed over the period of 4 weeks, by male rats receiving nano formulation of *Picrorhiza kurroa* at 500, 1000 or 2000 mg/kg was 98%, 91% and 100 % respectively similar to that of control group.

Females: Similarly, the average daily food consumption by female rats receiving the test substance at 500, 1000 or 2000 mg/kg was 95%, 98% and 100% respectively of that by control rats.

4.5 Gross Pathology

Nano formulation of *Picrorhiza kurroa*, at and up to the level of 2000 mg/kg, did not induce any treatment related gross pathological alterations in any of the vital organs / tissues of treated rats.

5. Conclusion

Following exposure of nano formulation of *Picrorhiza kurroa* two fractional gavage doses of 200 mg/kg, 1000 mg/kg or 2000 mg/kg, on a single day to groups of 5 male and 5 female Wistar rats, the observations made were as under:

- No mortality was observed in any of the male or female rats, at and up to a dose of 2000 mg/kg
- No incidence of any abnormal clinical signs were observed in any of the exposed rats in either sex, at and up to a dose of 2000 mg/kg No significant effect on the net body weight gain of treated male and female rats were observed, at and up to a dose of 2000 mg/kg. None of the male and female rats exposed to nano formulation of Picrorhiza *kurroa* at and up to a dose of 2000 mg/kg body weight, showed any significant effect on their average daily food consumption. Nano formulation of Picrorhiza *kurroa* did not elicit any remarkable gross pathological changes in any of the male or female rats exposed at and up to a dose of 2000 mg/kg body weight.

Based on the findings of this study, the Maximum Tolerated dose of nano formulation of Picrorhiza *kurroa* in Wistar rats following two fractional gavage doses on a single day was found to be > 2000 mg/kg body weight, under the conditions of this study.

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