Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 11, 2014

Original Article

ACUTE AND SUB ACUTE SAFETY STUDIES OF HERBMED PLUS-A HERBAL FORMULATION IN LABORATORY ANIMALS

SURESH PATANKAR, ARVIND MUJUMDAR

ACE Hospital & Research Centre, 32/2a, Erandwana. Pune 411004
Email: Researchamai@Gmail.Com

Received: 03 Sep 2014 Revised and Accepted: 05 Oct 2014

ABSTRACT

Objective: To study Acute, sub-acute oral toxicity profile of Herbmed plus (HP) - A herbal formulation.

Methods: HP was derived from *Crataeva nurvala* Buch - Ham (*Varun)* bark, *Musa paradisiaca* Linn (*Kadali/Banana)* stem and roots, *Achyranthes aspera* Linn (*Aghada*) whole plant and *Hordeum vulgare* Linn (*Yav)* seeds. Above materials were converted into *Varun bhavit kadali bhavit, kshars of Kadali, Aghada* and *Yav* respectively and were mixed in certain proportions in GMP certified manufacturing facility to formulate HP capsules form. Acute and sub-acute safety profiles of HP was studied by OECD guidelines Number 423 and FDA guidelines in Swiss albino mice and Wistar rats respectively. For the acute study, HP was administered orally in a single dose of 2000mg/kg and for sub-acute study HP was administered orally using 90,180 and 450 mg/kg doses for 90 days.

Results: In an acute study there were no behavioral changes and mortality at 2000 mg/kg by the oral route in mice up to 14 days. In sub-acute study after administration of various doses of HP for 90 days to various groups; there was no significant difference in body weight, food consumption, hematology / enzyme profiles, relative organ weights and histological observations of vital organs in comparison to control animals.

Conclusion: The acute LD50 cut off for HP was found to be > 2000mg/kg in mice and No Observed Adverse Effect Level (NOAEL) for HP was found to be > 450 mg/kg by oral route for 90 days in Wistar rats.

Keywords: Herbmed plus, Safety profiles in animals, Swiss albino mice, Wistar rats, OECD and FDA guidelines.

INTRODUCTION

For the medical management of Urolithiasis, the surgical procedures are considered as treatment of choice due to development of advanced techniques [1]. In the traditional system of medicine number of herbs and their formulations are prescribed and used empirically for the treatment of Urolithiasis. However, majority of them are not evaluated systematically by using acceptable guidelines for their safety, efficacy, indications and limitations. Earlier, we have developed, a herbal formulation 'Herbmed' derived from bark of *Crataeva nurvala* Buch-Ham commonly known as *Varun* in vernacular and *Kadali / banana* roots, stem of *Musa paradisiaca* Linn based on traditional knowledge from Ayurved for the urinary tract disorders in general and for the treatment of Urolithiasis in particular [2-4]. This formulation was clinically evaluated in the prospective, randomized clinical studies which revealed promising activity for the management of upper urinary tract calculi, with reduction in pain, especially for renal calculi by reducing the size of calculi and facilitating their passage [5]. This formulation was modified to HP by supplementing with *Achyranthes aspera* Linn (*Aghada*) whole plant and seeds of *Hordeum vulgare* Linn (*Yav*). Based on traditional knowledge from Ayurved above plant materials were converted into *Varun bhavit kadali bhavit, Kshars of Kadali, Aghada* and *Yav* respectively [6] and formulated in certain proportion at GMP certified facility in capsule form. As per the Reverse Pharmacology principle it is the prerequisite to ensure safety of the formulation, hence, in the present communication preclinical acute and sub-acute safety profiles of HP were investigated in laboratory animals and the results of these studies are reported.

MATERIALS AND METHODS

Preparation of HP

The bark of *Crataeva nurvala* Buch- Ham (*Varun*), stem and roots of *Musa paradisiaca* Linn (*Kadali/Banana*), *Achyranthes aspera* Linn (*Aghada*) whole plant and seeds of *Hordeum vulgare* Linn (*Yav*) were procured and authenticated at Agharkar Research Institute, Pune. These materials were converted into *Varun bhavit Kadali bhavit, kadali kshar, Aghada kshar* and *Yav kshar* respectively using typical Ayurvedic protocols [7,8] at Vishvaranga Ayurved Pharmacy, Pune: which is a GMP certified organization having FDA Maharashtra state approval. These materials were ensured for safety parameters at Charak Testing Laboratory, Mumbai for heavy metals (Pb, As, Hg, Cd) and Microbial count (Total aerobic count, Yeast, Mould and pathogenic bacteria count) following Ayurvedic pharmacopeia [9] for determination of their limits.

Animals

Female Swiss albino mice of 18-22 g and Wistar rats of mean weight139-178 g of either sex were used for these studies. They were housed in the CPCSEA approved animal house facility of National Toxicological Centre, Pune (NTC). They were housed in polypropylene cages in an air-conditioned area at 22±2 ^{0}C and 12 hours light and dark cycle. They were provided with pellets of balanced animal food of Amrut brand of Nav Maharashatra Oil Mills, Pune and water was provided *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee of the NTC, Pune.

Methods

Acute oral toxicity study

The study was conducted in female Swiss albino mice using OECD guidelines Number 423 [10]. The animals were deprived of food for 3-4 hours before and two hours after administration of HP and water was provided *ad libitum*. Based on exploratory studies and directives in respective OECD guidelines, as per the limit dose protocol HP was administered orally in a dose of 2000mg/kg in six animals for the final experiment. They were observed initially for four hours and subsequently, twice a day for 14 days for any behavioral changes or mortality if any. At the end of the study, necropsy observations of all animals were recorded.

Sub acute toxicity study

This study was conducted using Wistar rats; 24 males and 24 females. They were divided into 4 equal groups, control and three

treatment groups. The treatment groups were given low, intermediate and higher oral doses of HP; 90, 180 and 450 mg/kg/day respectively for 90 days based on exploratory studies. Initially all the animals were observed continuously for four hours and monitored daily at least once for their health status and signs of any abnormalities and morbid condition or death, if any. The body weight and food intake of the rats was determined once every week. At the end of the experimental period, blood samples were withdrawn from the retro-orbital plexus. Blood and serum samples were used for determination of various hematological and biochemical parameters. The hematological parameters were Hb, PCV and PT, in addition to Erythrocytes, Leucocytes count as well as differential count by a fully automated blood cell counter ERMA PCE-210. The serum was separated from the blood by centrifugation and stored at −20°C for analysis of biochemical parameters viz., Glucose, SGPT, BUN, ALP using a semi automatic clinical chemistry analyzer AGD 400. The rats were mildly anaesthetized under pentobarbitone and sacrificed by cervical dislocation for necropsy observations of vital organs like liver, kidneys, lungs, spleen, adrenals, heart and ovaries/testis were dissected to determine their weights. They were expressed as relative % weight in g. kidneys, lungs, heart, testis/ovaries were preserved in 10% formalin solution. Subsequently, they were embedded in paraffin. They were sectioned and stained with hematoxylin and eosin to examine under microscope for histological observations.

Statistical analysis

All the results were expressed as Mean ± SD. The statistical analysis was carried out by using Prism card software.

The treatment group animals were compared with control using one way analysis of variance (ANOVA) and the results were express as statistically significant for value $p<0.05$

RESULTS AND DISCUSSION

Acute oral toxicity study

In acute toxicity study all the experimental animals well tolerated the dose of 2000 mg/kg of HP by oral route. They appeared normal and showed no abnormal clinical signs or symptoms and necropsy observations in comparison to control animals, with no mortality till 14 days as per OECD guidelines No 423.

Thus, according to the Global Harmonized Classification System (GHS) the LD50 cut off for HP was found to be > 2000 mg/kg body weight, which is GHS category- 5.

Sub acute toxicity study

In sub acute toxicity studies all rats appeared normal, without showing any signs or symptoms of abnormality in comparison to control animals at various doses like 90,180 and 450 mg/kg of HP by oral route of administration for 90 days. Usually alteration in the body weight is considered as an important parameter for the assessment of response of individual to the drugs [11] and may also indicates its side effects [12]. In the present study there was no significant change in body weight as well as food consumption in male and female rats in treatment group as compared to the respective control animals as shown in fig. 1 and 2.

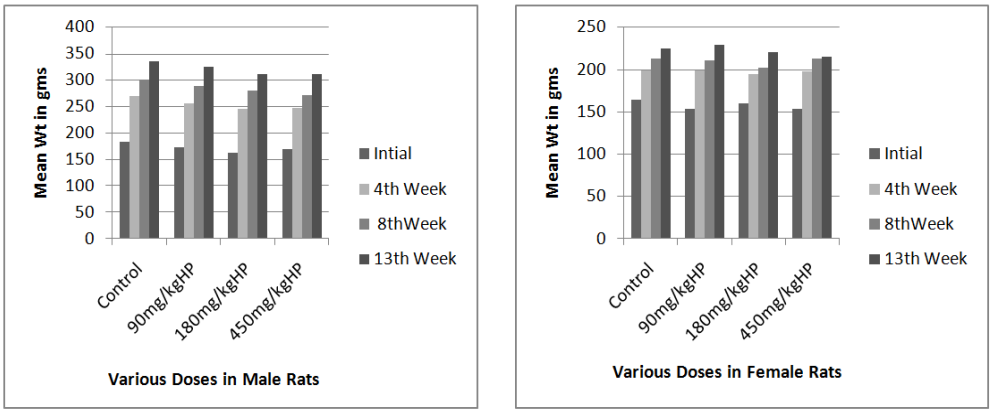

Fig. 1: Effect of treatment of various doses of HP for 90 days on Mean Body Weights

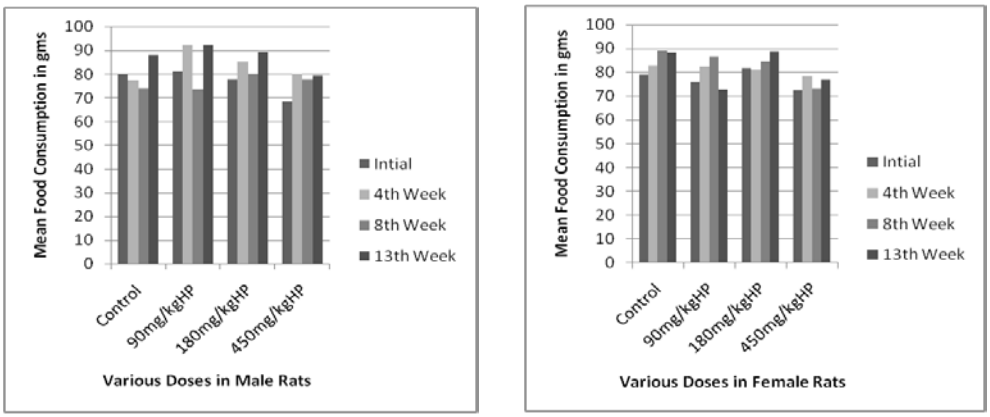

Fig. 2: Effect of various doses of HP for 90 days on Mean Food consumption

After the administration of various doses of HP to rats, there was no statistically significant effect on different hematological parameters like Erythrocytes, Leucocytes count and differential count, as well as, Hb, PCV PT in comparison to respective control animals as shown in Table1.

Table 1: Effect of HP treatment on Hematological parameters in Male / Female rats

Group	Hb (g/dl)	PCV %	PT Sec	Erythrocytes (x 10^6/ mm^3)	Leucocytes (x 10^3/mm^3)	N%	L%	E%	M%
Control(M)	12.48 ± 1.41	39.72 ± 4.34	584.8± 14.27	7.502 ± 0.67	5.817 ± 1.57	28.45 ± 4.87	67.80 ± 4.86	1.0 ± 0.0	2.75 ± 0.52
HP 90mg/kg(M)	12.00 ± 1.25	39.98 ± 3.31	583.7± 12.50	7.077 ± 0.71	6.783 ± 1.35	24.82 ± 4.01	70.80 ± 4.37	1.0 ± 0.0	3.38 ± 0.98
HP 180mg/kg(M)	11.97 ± 1.33	35.97 ± 5.91	588.0± 11.66	6.777 ± 1.15	5.60 ± 0.68	27.13 ± 1.75	68.77 ± 2.03	1.0 ± 0.0	3.10 ± 0.0
HP 450mg/kg(M)	12.52 ± 0.57	40.23 ± 2.22	593.3± 11.66	7.790 ± 0.53	6.067 ± 0.53	21.55 ± 2.73	74.97 ± 2.54	1.0 ± 0.0	2.48 ± 0.50
Control (F)	11.97 ± 0.85	38.07 ± 2.19	605.0± 13.22	7.22 ± 0.90	5.420 ± 4.00	27.42 ± 7.97	69.23 ± 8.67	1.0 ± 0.0	2.35 ± 0.97
HP 90mg/kg(F)	10.00 ± 0.85	38.04 ± 2.64	582.6± 11.91	5.54 ± 0.84	5.920 ± 0.34	19.32 ± 3.12	76.36 ± 2.72	1.0 ± 0.0	3.32 ± 0.74
HP 180mg/kg(F)	11.90 ± 1.33	40.02 ± 3.36	593.0± 13.71	6.57 ± 1.78	6.000 ± 0.71	23.22 ± 4.78	72.57 ± 5.84	1.0 ± 0.0	3.22 ± 1.18
HP 450mg/kg(F)	11.83 ± 0.69	39.00 ± 4.22	588.5± 11.48	7.497 ± 0.63	5.827 ± 0.46	26.80 ± 6.57	69.40 ± 6.10	1.0 ± 0.0	2.88 ± 0.87

Note: No statistical difference observed in control and treatment groups.

While conducting sub-acute study, the hematological observation is a direct reflection of the possible tissue injury caused by the test material under consideration. Blood biochemistry parameters constitute sensitive parameters of toxicity evaluation. For instance, changes in blood enzymes may be due to cellular/tissue injury leading to their systemic leakage from intracellular sites or target tissues [13]. As far as various blood chemistry parameters like, SGPT, BUN, ALP and total proteins were not significantly altered due to various doses of HP up to 450 mg/kg as compared to respective control group of animals as shown in Table 2.

Table 2: Effect of HP treatment on blood chemistry parameters in Male/ Female rats

Group	Plasma Glucose mg%	BUN mg%	Total Proteins g%	ALP U/L	SGPT U/L
Control(M)	91.50 ± 8.19	14.83 ± 2.56	5.10 ± 0.56	296.2 ± 9.33	88.00 ± 8.32
HP 90mg/kg(M)	92.83 ± 8.16	13.83 ± 2.14	5.18 ± 0.95	295.7 ± 9.59	77.50 ± 8.31
HP 180mg/kg(M)	99.50 ± 8.12	12.83 ± 2.56	5.93 ± 0.50	299.0 ± 9.32	85.83 ± 8.30
HP 450mg/kg(M)	99.67 ± 8.17	15.17 ± 2.56	5.25 ± 0.62	291.8 ± 9.30	62.83 ± 7.86
Control (F)	94.33 ± 8.17	13.50 ± 2.43	5.03 ± 0.94	182.2 ± 8.16	73.83 ± 8.38
HP 90mg/kg(F)	97.40 ± 8.47	12.40 ± 2.19	6.20 ± 0.79	199.8 ± 8.35	73.80 ± 8.32
HP 180mg/kg(F)	100.30 ± 8.38	13.67 ± 2.58	6.13 ± 0.89	197.3 ± 8.64	72.00 ± 8.17
HP 450mg/kg(F)	99.17 ± 8.28	13.50 ± 2.59	5.83 ± 0.85	195.7 ± 8.90	72.67 ± 8.36

Note: No statistical difference observed in control and treatment groups.

In addition, repeated administration of HP did not affect the plasma glucose levels as compared to control group animals, which indicate that the HP did not affect carbohydrate metabolism or blood glucose regulation system. While evaluating sub-acute toxicity profile of any formulation in animals, at the termination of the study usually absolute as well as relative weight of vital organs are determined. These are indicative for the changes resulted in the functioning of various vital organs due to metabolic changes, secretion of enzymes/hormones leading to hyper/ hypoplasia and possible necrosis of tissue architecture [14]. In the present sub-acute study at termination the weights of vital organs like adrenals, heart, kidneys, liver, spleen, lungs, testis/ ovaries were determined and were converted into relative % organ weight and these were compared with control rats as shown in table 3. There was no significant change in the weights of vital organs of various treatment groups and control animals, indicating no significant effect on vital organs due to treatment of HP orally for 90 days at maximum dose up to 450 mg/kg.

Table 3: Mean relative% organ weight data in g after HP Treatment in Male/Female rats

Group	Adrenals	Heart	Kidneys	Liver	Spleen	Lungs	Testis/ Ovaries
Control(M)	0.0281 ± 0.008	0.348 ± 0.09	0.666 ± 0.07	2.653 ± 0.55	0.350 ± 0.05	0.481 ± 0.07	0.810 ± 0.11
HP 90mg/kg(M)	0.0226 ± 0.006	0.318 ± 0.04	0.648 ± 0.10	2.794 ± 0.57	0.338 ± 0.07	0.486 ± 0.13	0.834 ± 0.15
HP 180mg/kg(M)	0.0245 ± 0.003	0.307 ± 0.03	0.699 ± 0.07	2.986 ± 0.71	0.343 ± 0.03	0.532 ± 0.09	0.880 ± 0.12
HP 450mg/kg(M)	0.0217 ± 0.003	0.321 ± 0.03	0.683 ± 0.06	2.762 ± 0.49	0.339 ± 0.07	0.532 ± 0.09	0.806 ± 0.09
Control(F)	0.0310 ± 0.009	0.349 ± 0.02	0.689 ± 0.04	3.428 ± 0.34	0.381 ± 0.07	0.606 ± 0.09	0.063 ± 0.01
HP 90mg/kg(F)	0.0312 ± 0.006	0.368 ± 0.05	0.636 ± 0.08	3.199 ± 0.35	0.426 ± 0.09	0.648 ± 0.19	0.063 ± 0.01
HP 180mg/kg(F)	0.0427 ± 0.015	0.396 ± 0.08	0.638 ± 0.04	3.275 ± 0.31	0.391 ± 0.06	0.592 ± 0.08	0.056 ± 0.01
HP 450mg/kg(F)	0.0321 ± 0.012	0.391 ± 0.06	0.649 ± 0.08	3.524 ± 0.59	0.392 ± 0.09	0.864 ± 0.50	0.058 ± 0.01

Note: No statistical difference observed in control and treatment groups.

In gross necropsy observations there was no difference in control and HP treatment groups. Moreover, there were no histo pathological changes observed in sections of heart, liver, kidneys, testis/ovaries in HP treatment animals as compared to control group. This is in consistent with observations recorded in blood chemistry parameters like SGPT, BUN, ALP, etc. Thus, in sub acute treatment with 90 mg/kg, 180 mg/kg and 450 mg/kg of HP for 90 days to various groups of rats, showed no significant effect on body weight, food consumption, hematological as well liver enzyme profiles, relative organ weight and histological observations of vital organs in comparison to control animals. The NOAEL for HP was found to be > 450 mg/kg by oral route for 90 days in Wistar rats.

CONCLUSION

The acute oral LD50 study cut off for HP by employing OECD guidelines in Swiss albino mice was found to be > 2000 mg/kg body weight after observing for 14 days. The sub acute oral treatment with 90 mg/kg, 180 mg/kg and 450 mg/kg of HP for 90 days to various groups of rats, showed no mortality no mortality and behavioral changes, no alteration in blood profile, biochemistry parameters and vital organs profiles in comparison to control animals. Thus, NOAEL for HP was found to be > 450 mg/kg by oral route for 90 days in Male and Female Wistar rats.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Gettman MT, Segura JW. Management of ureteric stones: issues and controversies. Brit J Urol Int 2005; 95 (Suppl 2): 85–93.
2. Singh PP, Hussain F, Ghosh R. Effect of simultaneous sodium oxalate and methionine feeding with and without *varuna* (*Crataeva nurvala* Hook and Frost) therapy on urolithiogenesis in guinea pigs. Indian J Clin Biochem 1992;**7**:23-26.
3. Anand R, Patnaik GK, Kulshreshtha DK. Antiurolithiatic activity of lupeol, the active constituent isolated from *Crataeva nurvala*. Phytother Res 1994;8:417-21.
4. Prasad KVSRG, Bharathi K, Srinivasan KK. Evaluation of (*Musa paradisiaca Linn*. Cultivar)- "Puttable" stem juice for antilithiatic activity in albino rats. Indian J Physiol Pharmacol 1993;33:337-41.
5. Patankar S, Dobhada S, Bhansali M, Khaladkar S, Modi J. A prospective, randomized, controlled study to evaluate the efficacy and tolerability of Ayurvedic formulation Varuna and Banana stem in the management of urinary stone. J Altern Complem Med 2008;14(10):1287-90.
6. Ananymous, Shushrut Samhita vol 2, Commentary by Anant Ram Sharma Choukhamba Surbharati Prakashan, Varanasi Chikitsasthan; 2004;7(22):236.
7. Ananymous, Abhinav Bhaishajya Kalpana Commentary by Acharya Sidhhinandan Mishra, Choukhamba Surbharati Prakashan, Varanasi; 2007;7:212-18.
8. Ananymous, Shushrut Samhita vol 1, Commentary by Anant Ram Sharma Choukhamba Surbharati Prakashan, Varanasi Sutrasthan; 2006;11:4.
9. Ayurvedic Pharmacopoeia of India, part II, vol I, Govt. of India, Ministry of Health & Family Welfare, Dept. AYUSH New Delhi. First Ed; 2007.
10. Organization for Economic Cooperation and Development, OECD Guidelines for testing of chemicals. Test guideline 423. Acute Oral Toxicity–Acute Toxic Class Method adopted; 2001.
11. Winder CV, Lembke LA, Stephens MD. Comparative bioassay of drugs in Adjuvant-induced arthritis in rats: Flufenamic acid, mefenamic acid and phenylbutazone. Arthritis Rheum 1969;12 (Suppl 5): 472-82.
12. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90 day oral gavage toxicity studies of D-methyl penidate and DL methyl penidate in Sprague Dawley Rats Toxicol 2002;179 (Suppl 3):183-96.
13. Organization for Economic Cooperation and Development, OECD Guidelines for the testing of chemicals, Test guideline 408, Repeated Dose 90-day Oral Toxicity Study in Rodents; 1995.
14. Dhumal R, Patil P, Selkar N, Chawda M, Vahlia M, Vanage G. Sub-chronic Safety evaluation of ayurvedic immunostimulant formulation immuforte in rats in reverse pharmacology. Toxicol Int 2013;20(1): 87–94.