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ACUTE ORAL TOXICITY OF MADHUMALINI VASANT, ARSHAKUTHAR RASA & SARVATOBHADRA VATI, AN AYURVEDIC HERBO-MINERAL FORMULATIONS IN WISTAR RATS

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ABSTRACT

Background: Madhumalini Vasant, Arshakuthar Rasa & Sarvatobhadra Vati were used in ethno-medicine to treat various disease. These Ayurvedic formulation contain heavy metals, despite its therapeutic use no studies on its toxicity have been reported. **Objective:** The aim of the study is to evaluate acute oral toxicity of Madhumalini Vasant, Arshakuthar Rasa & Sarvatobhadra Vati for safety aspect in wistar rats according to OECD TG 423. **Material & Method:** Female wistar rats were randomly divided into four groups each group contain three rats. Group I served as normal control and remaining three groups receives three Ayurvedic formulations. After dose administration, animals were observed for 14 days for any alterations in clinical signs, behaviour pattern, body weight and mortality. **Result:** The female wistar rats did not produce any signs & symptoms of treatment related toxicity at high dose of 2000mg/kg. No mortality was observed during 14 days of observation. **Conclusion:** The data obtained from this study revealed that all the three Ayurvedic formulation is safer at higher dose. It can concluded that the LD_{50} is greater than 2000mg/kg (LD_{50} > 2000mg/kg).

KEYWORDS: Ayurveda, Herbo-mineral, toxicity, Madhumalini Vasant, Arshakuthar Rasa, Sarvatobhadra Vati.

1. INTRODUCTION

In rural areas, traditional use of herbals as well as herbal preparations was very common before the evolution of synthetic or semi-synthetic medicine for the treatment of various diseases.[1] The utilization of natural herbal remedies on large scale, tends the scientist to conduct experimental studies on efficacy and safety of therapeutic plants.[2] Therapeutically active plants also shows low toxicity because of their prolonged use in humans as well as different medicinal plants used in traditional medicines have been accounted to manifest toxic effects.[3,4] The Fundamental science of poison with Physico-chemical interaction is toxicity leads to injury or death of living tissue. Acute toxicity is the adverse effect which occurs within a short duration after oral administration of single or multiple doses of a substance given within 24 hours.[5] Father of Toxicology, Paracelsus gives a statement which is often quoted: "All substances are poison; there is nothing which is not a poison. It is the accurate dose which discriminate remedy from poison".[6] In recent years, curiosity towards traditional medicines is increasing. It is postulate that, the popular traditional Ayurvedic medicine in our country have minor side effects as compared to modern medicines. In Indian sub-continent almost 80% of population use Ayurveda and herbal medicinal plants to fulfill their primary health care needs. In an individual, Ayurveda maintain body's mind and spirit in perfect equilibrium with nature.[7-9] Herbal drugs and formulation shows various adverse effects due to its improper Purification. Detoxification or Shodhana techniques of Ayurvedic products gives its purity as well as potency and efficacy.[10] The main aim of these study is to determine the acute oral toxicity of three Herbo-mineral Ayurvedic formulations in animal models. The three formulations are Madhumalini Vasant, Arshakuthar Rasa & Sarvatobhadra Vati.

Madhumalini Vasant is an Ayurvedic herbo-mineral formulation. Madhumalini Vasant tablet contains Shodhit Hingula, Yashada Bhasma, Solids derived from Kukkutanda, Karchoora, Maricha, and Lakucha Kwath. Madhumalini Vasant helps to nourish all the body tissues. It provides necessary nourishment to foetus and children. It stabilize and sustain the foetus by promoting intra-uterine growth. It is useful in increasing strength of children and elder. Madhumalini Vasant is effective in debility associated with chronic fever.

Arshakuthar Rasa contains Shodhit Parada, Shodhit Gandhak, Loha Bhasma, Tamra Bhasma, Choorna of Danti root (Baliospermum montanum), Shunthi rhizome (Zingiber officinale), Maricha fruit (Piper nigrum), Pippali fruit (Piper longum), Soorana corm (Amorphophallus

campanulatus), Vamsha (Bambusa bambos), Shodhit Tankana, Yavakshara, Saindhava lavana, processed in Snuhi latex (Euphorbia neriifolia), Gomutra each quantity sufficient. A medicine which cut down Arsha (haemorrhoids) like an axe, hence the name Arshakuthar rasa. It is effective in reducing pain, burning, suppuration and bleeding associated with Arsha. It is beneficial Rasakalpa in reducing Agnimandya which is main cause of Arsha. It improves digestion and also provide relief in constipation.

Sarvatobhadra Vati is an Ayurvedic herbo-mineral formulation. It contains Suvarna Bhasma, Rajat Bhasma, Abhrak Bhasma, Loha Bhasma, Shodhit Shilajatu, Shodhit Gandhak, and Suvarnamakshik Bhasma all this ingredients processed in Varuna stem bark (Crataeva nurvala) Kwath quantity sufficient. It is mainly recommended for disorders associated with kidney and urinary bladder, other than Bright's disease, kidney & bladder stones.

The acute oral toxicity was carried on Female Wistar rats as per Organization for Economic Cooperation and Development (OECD-423) guidelines to determine the Lethal dose (LD50) of the drug.[11]

2. MATERIAL AND METHODS

2.1 Animals

Female Wistar rats weighing 150- 250 g was selected for the study. The animals were housed in CPCSEA approved animal house facility of Shree Dhootapapeshwar Ayurvedic Research Foundation, Panvel. The animals were maintained at 22 ± 03°C with constant humidity (30-70%) and 12 hrs day and night cycle. Animals were fed with Amrut brand rat pellet feed and water ad libitum. The experiments were carried out in accordance of the Institutional Animal Ethics Committee.

2.2 Chemicals

The tablets of Madhumalini Vasant, Arshakuthar Rasa & Sarvatobhadra Vati was collected from Shree Dhootapapeshwar Limited, Panvel. Carboxy Methyl Cellulose (CMC) was procured from Loba chemie.

2.3 Experimental Design

Rats were randomly assigned into group of four, each group contains three female animals. Group I served as normal control group received 1% CMC w/v. Group II received

Madhumalini Vasant. Group III received Arshakuthar Rasa. Group IV received Sarvatobhadra Vati.

2.4 Acute Oral Toxicity

The Acute oral toxicity study was performed according to the OECD Guidelines-423. Nulliparous and Non-pregnant female wistar rats of 8-12 weeks of age and 150-250g body weight (falling within ± 20% of the mean initial body weights of each sex) were used.[12] The animals were randomly divided in to four groups. Each group contain three animals.[13, 14, 15, 16] Madhumalini Vasant, Arshakuthar Rasa, Sarvatobhadra Vati Tablets were administered orally at a dose of 2000mg/kg dissolved in 1% CMC (Carboxy Methyl Cellulose) adjusted to 1ml/100g of animal. Animals should be kept for fasting prior to dosing (food but not water should be withheld for 3-4 hours).

2.5 Clinical Observations

All the animals were keenly observed three times i.e. pre-dosing, during dosing & post-dosing. After dosing, individual animal should be observed during first 30 minutes, periodically during first 24 hours also gives special attention for further 4 hours, and daily thereafter for complete 14 days.[17] The animals should be observed for behavioral changes, changes in skin, fur, eyes. Attention to be given to observed tremors, convulsions, salivation, diarrhea, sleep and coma.[16]

2.6 Mortality and Clinical signs

All the animals were monitored twice daily throughout the study period for the presence of moribund or mortality, if any. For individual animals, all clinical signs related to poor health, behavioral changes or reaction after dosing were recorded once daily.

2.7 Body Weight

Individual body weight of animal were monitored before experimental dose administration and thereafter weekly till the end of the experiment.

2.8 Statistical Analysis

The data was analyzed using Graph pad prism software. The result was analyzed by one way ANOVA followed by Dunnett's Multiple Comparison test. All results were expressed as Mean ± SD.

3. RESULT

The main aim of the present study was to investigate the acute oral toxicity of three Ayurvedic Herbo-mineral formulations such as Madhumalini Vasant, Arshakuthar Rasa & Sarvatobhadra Vati. All the animals of test groups was administered with tablets at the dose of 2000mg/kg according to their body weight. No morbidity or mortality as well as no sign of toxicity was observed in treated animals. All animals showed similar food consumption, gain in body weight, and general appearance as that of normal control group. The survived animals were observed for further 14 days, there was no changes observed in behavioral pattern.

3.1 Clinical Observations

All the animals were observed for their behaviour on daily basis from the initiation of dosing to the end of the study. The treated animals of all groups at the dose 2000mg/kg does not shows any significant alteration in behaviour before and after administration of an oral dose. Behavioral observation were summarized in Table 1.

Table 1: General appearance and behavioral observation of the survived animals (For 14 days) Normal control group (A) and Test group (B).

(A) Normal Control Group					
Parameters	**30 mins**	**2hrs**	**4 hrs**	**24hrs**	**14th Day**
Fur & Skin	NAD	NAD	NAD	NAD	NAD
Eyes	NAD	NAD	NAD	NAD	NAD
Salivation	NAD	NAD	NAD	NAD	NAD
Urine Colour	NAD	NAD	NAD	NAD	NAD
Faeces consistency	NAD	NAD	NAD	NAD	NAD
Sleep	NAD	NAD	NAD	NAD	NAD
Convulsions	NAD	NAD	NAD	NAD	NAD
Tremors	NAD	NAD	NAD	NAD	NAD
Itching	NAD	NAD	NAD	NAD	NAD
Behaviour pattern	NAD	NAD	NAD	NAD	NAD
Coma	N.F	N.F	N.F	N.F	N.F

(B) Madhumalini Vasant					
Parameters	**30 mins**	**2hrs**	**4 hrs**	**24hrs**	**14th Day**
Fur & Skin	NAD	NAD	NAD	NAD	NAD
Eyes	NAD	NAD	NAD	NAD	NAD
Salivation	NAD	NAD	NAD	NAD	NAD
Urine Colour	NAD	NAD	NAD	NAD	NAD
Faeces consistency	NAD	NAD	NAD	NAD	NAD
Sleep	NAD	NAD	NAD	NAD	NAD
Convulsions	NAD	NAD	NAD	NAD	NAD

Tremors	NAD	NAD	NAD	NAD	NAD
Itching	NAD	NAD	NAD	NAD	NAD
Behaviour pattern	NAD	NAD	NAD	NAD	NAD
Coma	N.F	N.F	N.F	N.F	N.F

(C) Arshakuthar Rasa					
Parameters	**30 mins**	**2hrs**	**4 hrs**	**24hrs**	**14th Day**
Fur & Skin	NAD	NAD	NAD	NAD	NAD
Eyes	NAD	NAD	NAD	NAD	NAD
Salivation	NAD	NAD	NAD	NAD	NAD
Urine Colour	NAD	NAD	NAD	NAD	NAD
Faeces consistency	NAD	NAD	NAD	NAD	NAD
Sleep	NAD	NAD	NAD	NAD	NAD
Convulsions	NAD	NAD	NAD	NAD	NAD
Tremors	NAD	NAD	NAD	NAD	NAD
Itching	NAD	NAD	NAD	NAD	NAD
Behaviour pattern	NAD	NAD	NAD	NAD	NAD
Coma	N.F	N.F	N.F	N.F	N.F

(D) Sarvatobhadra Vati					
Parameters	**30 mins**	**2hrs**	**4 hrs**	**24hrs**	**14th Day**
Fur & Skin	NAD	NAD	NAD	NAD	NAD
Eyes	NAD	NAD	NAD	NAD	NAD
Salivation	NAD	NAD	NAD	NAD	NAD
Urine Colour	NAD	NAD	NAD	NAD	NAD
Faeces consistency	NAD	NAD	NAD	NAD	NAD
Sleep	NAD	NAD	NAD	NAD	NAD
Convulsions	NAD	NAD	NAD	NAD	NAD
Tremors	NAD	NAD	NAD	NAD	NAD
Itching	NAD	NAD	NAD	NAD	NAD
Behaviour pattern	NAD	NAD	NAD	NAD	NAD
Coma	N.F	N.F	N.F	N.F	N.F

NAD= No abnormality detected, N.F= Not found

3.2 Mortality and Clinical signs

No treatment related mortality and clinical signs were observed in treated animals.

3.3 Body Weight

To monitor the health of an animal body weight is an important factor. Frequently, first indicator of the onset of an adverse effect is loss of body weight. All the animals from treated and normal control groups did not shows any significant decrease in the body weight for all 14 days as compared with the 0 day, thus indicates no signs of toxicity (Table 2).

Table 2: Effect on body weight in wistar rats.

Day	Normal Control	Madhumalini Vasant	Arshakuthar Rasa	Sarvatobhadra Vati
Zero Day	214.7 ± 14.05	225.3 ± 3.215	219.0 ± 15.52	231.0 ± 12.17
14th Day	219.3 ± 14.01	230.0 ± 2.0	224.0 ± 14.53	235.0 ± 13.23

Values are expressed as mean± SD; n = 3; Data analyzed by One-way ANOVA test followed by Dunnett's multiple test for comparison.

4. DISCUSSION

Different groups of population uses traditional medicines for the treatment of various disease with improper dosage considering that they have negligible side effects. Chronic use of some Ayurvedic medicines to treat disease like diabetes, arthritis, etc. may lead to toxic manifestation.[18] Some of the Ayurvedic medicines were reported for safety while most of them remain ignored. To estimate safe and effective doses of administration and possible toxicological effects, appropriate scientific data and documentation of toxicity studies of traditional medicines is required.

Madhumalini Vasant, Arshakuthar Rasa & Sarvatobhadra Vati are used in Ayurvedic clinical practices for the treatment of various diseases. Madhumalini Vasant increases strength of children and provide essential nourishment to foetus & children as well as stabilize and sustain the foetus by promoting intra-uterine growth. Arshakuthar rasa is used to treat haemorrhoids. Sarvatobhadra Vati is used in the treatment of Urolithiasis and other kidney related disorders. The present study evaluate the acute oral toxicity of these three Ayurvedic herbo-mineral formulations according to OECD 423 guideline.[11] These study is mandatory for the determination of safer dose range and for the management of clinical signs and other symptoms of the drug.[19]

No mortality or moribund stage was found in all groups treated with Ayurvedic formulations at high dose (2000mg/kg). During 14 days of acute toxicity assessment, it was observed that food and water consumption was normal without any significant difference in body weight. No alteration was observed in clinical signs and behaviour in all treated animals compared to normal control. This indicates that maximum tolerated dose of all three formulations was 2000mg/kg. Based on LD_{50} (Lethal Dose) chemicals are divided into five groups as per Globally Harmonized Classification system.[20] The three Ayurvedic formulations can be put in group 5 (LD_{50} > 2000mg/kg), falling in lower toxicity class.

5. CONCLUSION

Madhumalini Vasant, Arshakuthar Rasa, Sarvatobhadra Vati all three are Ayurvedic herbo-mineral formulations. The presence of metals and minerals in Ayurvedic medicines is a matter of great concern for human health.[21] The result suggests that, oral administration of all three formulations did not produce any significant toxicity in wistar rats. All the three formulations was found to be safe at high dose of 2000mg/kg in wistar rats. It can be concluded that all three Ayurvedic formulations has LD_{50} more than 2000mg/kg (LD_{50} > 2000mg/kg).

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