



**ACUTE TOXICITY STUDY OF RASAYANA YOGA- AN OPHTHALMIC  
POLYHERBOMINERAL FORMULATION IN EXPERIMENTAL RATS**

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**ABSTRACT**

*Rasayana Yoga* is an experience based compound herbomineral Ayurvedic formulation, administered orally in the management of Primary open angle glaucoma in Shalakyatantra Outpatient department of Institute for Post Graduate Teaching and Research in Ayurveda. It consists of herbomineral drugs like *Rasayana Churna* (combination of *Guduchi*, *Gokshura* and *Amalaki*), *Saptamrita Lauha* (classical formulation containing *Hareetaki*, *Vibheetaki*, *Amalaki*, *Yashtimadhu* and *Lauha Bhasma*), *Abhraka Bhasma* and *Swarnamakshika Bhasma*. All these, herbal and mineral drugs are extensively used in traditional system of medicine and by folk practitioners in India, because of their remarkable therapeutic potential. The aim of the present study was to evaluate acute toxicity of the formulation in female wistar rats. Acute toxicity test was evaluated as per the Organisation for Economic Co-operation and Development - 425 guidelines with 2000 mg/kg as limit test. The aqueous suspension of the powdered drug was administered once orally as a single dose of 2000mg/kg of body weight to female wistar rats in sequential manner. The general behaviors of animals were continuously monitored for 1 hour after dosing and periodically for 24 hours. The animals were also monitored for any mortality during the first 24 hours and daily thereafter for a total of 14 days. Acute toxicity test results showed that *Rasayana churna* at 2000 mg/kg did not produce any behavioral and physiological changes and observable toxic effects during entire duration of study and all animals survived for 14 days of observation.

**KEYWORDS:** Acute toxicity, Mortality, Primary open angle glaucoma, *Rasayana Yoga*.

**INTRODUCTION**

The Ayurveda system of medicine is based on the resources derived from herbs, metals, minerals and animal products. For global acceptance, this system of medicine should undergo scientific validation, i.e., upgrading the levels of quality, safety, reliability and efficacy.<sup>[1]</sup> *Rasayana Yoga* is an experience based Ayurvedic formulation with a combination of herbomineral drugs having the exclusive property of *Chakshushya* (beneficial to eyes) and *rasayana* (rejuvenating). It consists of formulations like *Rasayana Churna* (combination of *Guduchi*, *Gokshura* and *Amalaki*), *Saptamrita Louha* (classical formulation containing *Hareetaki*, *Vibheetaki*, *Amalaki*, *Yashtimadhu* and *Lauha Bhasma*) and mineral drugs like *Abhraka Bhasma* and *Swarnamakshika Bhasma*. All these, herbal and mineral drugs are extensively used in traditional system of medicine and by folk practitioners in India, because of their remarkable therapeutic potential. As a *Chakshushya Rasayana*, it is used in the management of degenerative diseases causing gradual loss of vision

especially in Primary open angle glaucoma. These drugs have separately been explored scientifically for the presence of many active principles for various pharmacological actions. However, till date, no safety profile of this unique combination has been reported; hence powdered form of the formulation was evaluated for acute toxicity.

**MATERIALS AND METHOD**

**Preparation of *Rasayana Yoga***

The individual herbal drugs of the formulation were collected from the Pharmacy of IPGT & RA, Gujarat Ayurved University, Jamnagar. The raw drugs were identified based on their morphological characteristics and authenticated at Pharmacognosy, IPGT & RA, Jamnagar. The collected herbal drugs were dried, powdered individually and passed through sieve number 120 to prepare a fine powder. These powders were then blended homogeneously and later *Swarnamakshika bhasma*, *Abhraka bhasma* and *Loha bhasma* were added

to make the formulation in the form of a homogenous powder.<sup>[2]</sup>

**Table 1: Ingredients of Rasayana Yoga.**

Sr. No.	Drug	Scientific/ Botanical Name	Parts used	Quantity
1	<i>Suvarna Makshika</i>	Chalcopyrite	<i>Bhasma</i>	60 mg
2	<i>Abhraka</i>	Mica	<i>Bhasma</i>	60 mg
3	<i>SaptamrutaLauha</i>	<i>Lauha</i> Formulation	<i>Choorna</i>	250 mg
4	<i>Rasayanachurna</i> a) <i>Guduchi</i> b) <i>Gokshura</i> c) <i>Amalaki</i>	<i>Tinospora cordifolia</i> Willd. <i>Tribulus terrestris</i> Linn. <i>Emblica officinalis</i> Gaertn.	<i>Choorna</i>	2 gm

## EXPERIMENTAL DESIGN

### Experimental animals

The wistar albino rats were obtained from the Animal house attached to Pharmacology Lab., I.P.G.T. & R.A., Gujarat Ayurved University, Jamnagar. They were kept in departmental animal house at 22 ± 03°C and relative humidity of 50% - 70% with light and dark cycle of 12 hour in polypropylene cages. Dry wheat (post hulled) waste was used as bedding material and was changed every morning. Animals were provided with standard rodent pellet diet and water ad libitum. All the selected animals were kept under acclimatization for 7 days before experimentation. All the procedures were reviewed and approved by IAEC (IAEC/19/2015/46) in accordance with the guideline formulated by CPCSE, India.

### Preparation of Test solution

Test dose for administration was prepared shortly prior to administration. The test drug was suspended in distilled water with suitable concentration 2000mg/10ml/kg body weight of rat, during the time of administration.

**Testing procedure:** Acute toxicity test was evaluated as per OECD 425 guidelines in Wistar strain albino rats.<sup>[3,4]</sup> Five healthy non pregnant female Wistar albino rats weighing 200 ± 20g were used for evaluation of acute toxicity test. Female sex was preferred because of their higher sensitivity. The limit test dose of 2000 mg/kg body weight of rat was used as stipulated in OECD guidelines. After acclimatization for 7 days, the animals were marked, weighed and put on fasting overnight. Food, but not water, was withheld for

overnight before the experiment and further, 1 hour after administration of test drug. The next morning the fasted animals were administered with test drug at 2000mg/kg as a single dose of suspension orally, in sequential manner.

The following observations were made in all the test animals.

- Physical, behavioral and clinical signs changes like increased or decreased motor activity, tremors, convulsions, sedation, excitation, straub's reactions, arching and rolling, aggressiveness, lacrimation, writhing, mode of respiration, changes in skin colour, salivation, diarrhoea, lethargy etc. were observed at about 30 mins, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr and 6 hr on day 1 and daily thereafter for 14 days.
- Mortality and morbidity during the first 24 hours and for 14 days.
- Body weight of each animal was recorded just prior to dosing on day one, 7<sup>th</sup> and 14<sup>th</sup> day.

**Statistical analysis:** The results on weight gain were presented as Mean ± SEM (standard error of the mean) for five rats tested. Statistical comparisons in weight gain before and after 14 days of drug administration were performed by paired 't' test with the level of significance set at  $P < 0.05$ .

## OBSERVATION AND RESULTS

All the animals of test group didn't show any toxic clinical signs throughout the study period. The changes in body weight of the treated rats have been shown in Table 2. A normal progressive weight gain was observed in test group.

**Table 2: Effect of test drug on body weight of female Wistar rats.**

Group	Initial body weight	Final body weight	Actual change	% difference
Drug	191.00 ± 8.39	224.40 ± 11.10*	33.40 ± 2.71	17.49%

\*when compared with initial value (Paired 't' test)

No mortality was observed during the course of study in drug treated group at dose level of 2000 mg/kg. Gross behavior of all the animals was found to be normal during the period of study.

## DISCUSSION

Acute toxicity studies present fast, significant information on the health risk that is possible to occur from the short-term experience to a drug and may specify whether additional toxicity studies must be conducted.<sup>[5]</sup> Determination of acute oral toxicity is usually an initial

screening step in the assessment and evaluation of the toxic characteristics of all compounds. The drugs intended to be used therapeutically should be subjected to toxicity evaluation before they are considered safe for use in the human beings. This is important because incomplete knowledge about the toxicity profile of a putative drug will entail certain amount of risk to the recipient. The experience based formulation *Rasayana Yoga* is used clinically to treat glaucoma; however, its acute toxicity profile is not reported till date. Hence, in the present study, acute toxicity profile of the formulation has been evaluated.

Change in body weight is an important factor to monitor the health of an animal. Frequent loss of body weight is the first indicator of the onset of an adverse effect and the dose, at which body weight loss is by 10% or more is considered to be a toxic dose, irrespective of whether or not it is accompanied by any other changes.<sup>[6,7]</sup> In the present study, in test drug administered group, gain in body weight was observed. It was observed that at 2000 mg/kg, sample did not produce any behavioral and physiological changes and observable toxic effects during entire duration of study and all animals survived for 14 days of observation. *Rasayana Yoga* can be classified under category-5 since the LD50<sup>[8]</sup> value was greater than 2000 mg/kg in accordance with GHS [Globally Harmonized System of Classification and Labelling of Chemicals]<sup>[9]</sup> and this provides us a direct relevance for protecting human and animal health.

## CONCLUSION

Acute toxicity test results showed that *Rasayana churna* did not affect any behavioral changes. It did not produce any signs or symptoms of toxicity or mortality up to a dose of 2000 mg/kg during the 14 days of observation, indicating that the formulation is unlikely to induce any drastic toxic effect in spite of containing mineral drugs like *Abhraka Bhasma*, *Swarnamakshika Bhasma* and *Lauha Bhasma*. Therefore, it can be concluded that *Rasayana Yoga*, when administered at single dose, is non-toxic and can be used safely as oral formulation.

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