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ACUTE TOXICITY STUDY OF AN ANTIPYRETIC HERBOMINERAL COMPOUND: AMRUTKALANIDHI RASA

[1*]Dr. Kaumudi Bagul and [2]Dr. Sheela Pargunde

[1]PG. Scholar, [2]Professor and Guide

Y.M.T Ayurvedic Medical College and Hospital, Kharghar, Navi Mumbai.

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***Corresponding Author**
Dr. Kaumudi Bagul
PG. Scholar, Y.M.T Ayurvedic Medical College and Hospital, Kharghar, Navi Mumbai.

ABSTRACT

Amrutkalanidhi rasa is an antipyretic medicine mentioned in *Yogaratnakara* in *Jwara chikitsa* chapter. Purified *Vatsanabha*, *Maricha* and *Kapardika bhasma* are the ingredients used in the preparation of this medicine. Now a days a lot of emphasis has been given to safety and toxicity studies of herbo mineral compounds. Hence aim of current study was to study acute toxicity of Amrutkalanidhi rasa on Wistar albino rats that was carried out according to general guidelines for safety and toxicity evaluation of Ayurvedic formulations by CCRAS. Therapeutic dose in rat was 5.40 mg/kg and its 10 times dose approximately 50 mg/kg was taken as limit dose. Animals were dosed individually and observed continuously for 24 hrs for 14 days to detect any changes in autonomic or behavior responses. After 14 days representative rats from each group were sacrificed for observing pathological changes. From general observations and histopathological examination of kidney, heart and liver it was concluded that Amrutkalanidhi rasa found to be non-toxic and safer to use.

KEYWORDS: Acute toxicity study, Wistar albino rats, herbo mineral, CCRAS guidelines

INTRODUCTION

Fever in Ayurveda is a disease as well as a symptom present in most of the diseases. Fever is present at the time of birth as well as at the time of death. It is also a major symptom present in COVID 19 pandemic. Amrutkalanidhi rasa[1] is a medicine mentioned in Yogratnakara under *jwara chikitsa adhyaya*. Contents of this medicine include purified *Vatsanabha churna* (*Aconitum ferox/ balfourii*), *Maricha churna* (*Piper nigrum*), *Kapardika bhasma* (*cowry shells*). Out of which *Vatasanabha* is a toxic herb which is used after purification process as

mentioned in ayurvedic texts. *Kapardika Bhasma* is a mineral compound. Safety and toxicity studies are important part of research in pre-clinical studies. Till date no safety profile of this formulation is available, without which drug is not accepted worldwide. Hence Acute toxicity study of Amrutkalanidhi rasa was conducted according to the CCRAS[2] (The Central Council for Research in Ayurvedic Sciences) guidelines.

MATERIAL AND METHOD

1. Method of preparation of Amrutkalanidhi rasa

Authentication of all raw materials were carried out from certified labs. Purification of *Vatsanabha* (*Aconitum balfourii*) was done in 2 steps according to the method mentioned in Yogratnakara.[3]

Step 1: By immersing cut pieces of *Vatsnabha* roots in *gomutra* (cow urine) containing mud vessel in sunlight for 3 days.

Step 2: By boiling in vessel containing cow milk for 3 hours.

Vatsanabha then collected, washed and dried.

Purification of *Kapardika* i.e cowry shells were done by boiling in vessel containing *kanji* (acidic medium) for 3 hours. *Kapardika bhasma* was prepared by method mentioned in Rasa Tarangini[4] by giving 2 *gajaputas.*

Fine powders of all raw materials were made separately by passing through sieve no. 85. Purified *Vatsanabha churna, Kapardika bhasma* and *Maricha churna* were mixed in proportion of 2:5:9 respectively and triturated with water in order to form pills of 60 mg (*mudga pramana vati*).

2. Acute toxicity study

Duration of trial**:** 14 days

Animal species: Wistar albino Rat

Weight: 150-250g.

Gender: Male

Study center: The acute toxicity study was conducted in Department of pharmacology, Bombay veterinary college, Parel, Mumbai.

Selection of animal species: Adult male Wistar albino rats weighing about 150 to 250 g were used for the experiment.

Housing condition and feeding schedule

The rats were kept in properly numbered large polypropylene cages with stainless steel top grill having facilities for pelleted feed. The animals were maintained in 12 hours light and dark cycle at 28 °C ± 2°C in a well-ventilated animal house under natural conditions in large polypropylene cages. All animal experiments were performed according to ethical guidelines suggested by the institutional animal ethics committee (IAEC). The protocol was approved in IAEC.

Experimental design

The animals were acclimatized to the laboratory conditions 1 week prior to the test. Before the test, animals were randomized and assigned to the treatment groups.

All the rats were randomly distributed in 3 groups, containing 6 animals per group (n=6) The treated group received maximum orally administered volume i.e. the compound (Amrutkalanidhi rasa) dissolved in gum acacia to make syringeable consistency.

According to GENERAL GUIDELINES FOR SAFETY/TOXICITY EVALUATION OF AYURVEDIC FORMULATIONS by CCRAS - Dose levels and route of administration: The limit of 2gm/kg or at least 10 times of the intended clinical therapeutic dose whichever is less, using the same route as recommended for human.

As therapeutic dose in rat is 5.40mg/kg its 10 times dose approx. 50 mg will be taken as limit dose.

Table 1: Group distribution for acute toxicity in Wistar rats.

GROUP	Group ID	Route of administration	No. of Animals
Group I (Gum acacia)	Vehicle Control	Oral	6
Group II 5mg/kg (Test drug)	Treatment – 1 (Therapeutic equivalent dose)	Oral	6
Group III 50 mg/kg (Test drug)	Treatment-2 10 times therapeutic equivalent dose	Oral	6

Group I: Control group - Animals were treated with gum acacia which was used as vehicle. (10% 2ml /200gm).

Group II: and Group III were treated with Amrutkalanidhi rasa (Test drug) in dose of 5mg/kg and 50 mg/kg i.e the therapeutic equivalent dose of humans in rats and ten times therapeutic

equivalent dose of human in rats (according to CCRAS guidelines of toxicity study) respectively.

Animals were dosed individually and observed continuously for 24 hrs for 14 days to detect any changes in autonomic or behavioural responses viz spontaneous activity, irritability, urination, salivation.

Mortality, general signs, and physical observation of the animals for symptoms such as diarrhea, lethargy, equilibrium, convulsions, shuffling, skin and fur problems, salivation, tremors, tears, breathing difficulty, equilibrium sensation, excitement, dermatitis were observed.

After 14 days representative rats from each group were sacrificed for observing pathological changes.

Histopathology of kidney, heart and liver was performed.

OBSERVATION

Table 2: General observation of Acute toxicity study.

Parameters	Group I	Group II	Group III
Eyes and mucous membranes	NAD	NAD	NAD
Respiratory Rate	NAD	NAD	NAD
Salivation	NAD	NAD	NAD
Excitation	NAD	NAD	NAD
Motor activity	NAD	NAD	NAD
Mortality	NIL	NIL	NIL
Skin and fur	NAD	NAD	2 animals showed slightly ruffled hairs
Lethargy	NAD	NAD	NAD
Diarrhoea	NAD	NAD	NAD
Behavioural patterns	No abnormal changes noted	Equivalent to Normal	Equivalent to Normal

NAD : No Abnormality detected.

Histopathology observations

NAD: No Abnormality Detected. **MNC:** Mononuclear Cell Infiltration.

Grades of Severity of Lesions

+: **Minimal**: Very small amount of change < 10%

++: **Mild:** Lesion is easily identified but of limited severity 11-25%

+++: **Moderate**: Lesion is prominent 26 to 75%.

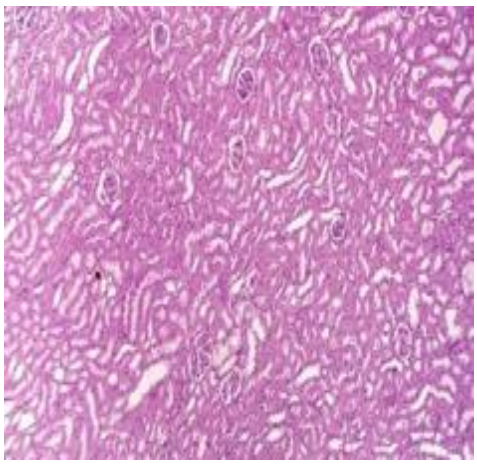
++++: **Severe:** the degree of changes is either as complete 76 – 100% as possible or great enough in intensity or extent to expect significant tissue or organ dysfunction.

Grades of extent of lesions: Focal, Multifocal, Diffuse// Whole of the section.

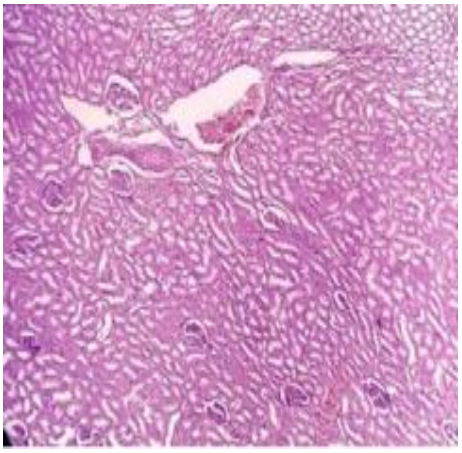
Table 3: Microscopic histopathological observation for changes in Liver, Kidney and Heart tissues.

Sr.No.	Slide No.	Study Group	Microscopic Observations (Histopathology)		
			Kidney	**Liver**	**Heart**
1	A-1	Group- I	NAD	NAD	NAD
2	A-2	Group- I	NAD	NAD	NAD
3	A-3	Group- I	Multifocal congestion (+); Multifocal haemorrhages (+); Multifocal tubular degenerations (++)	Multifocal congestion (++); Multifocal haemorrhages (+); Focal hepatic degeneration (+)	NAD
4	B-1	Group- II	NAD	NAD	NAD
5	B-2	Group- II	NAD	NAD	NAD
6	B-3	Group- II	Multifocal congestion (+); Multifocal haemorrhages (+); Multifocal tubular degenerations (+)	NAD	NAD
7	C-1	Group- III	NAD	NAD	NAD
8	C-2	Group- III	NAD	NAD	NAD
9	C-3	Group- III	NAD	NAD	NAD

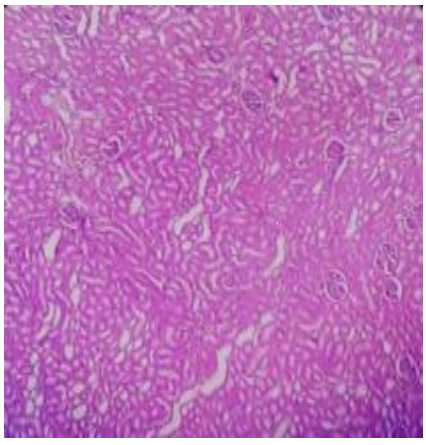
Slides of Histopathological examination of Kidney.



Microphotograph of kidney from group I showing mild nature of focal congestion (H&E, 100X).



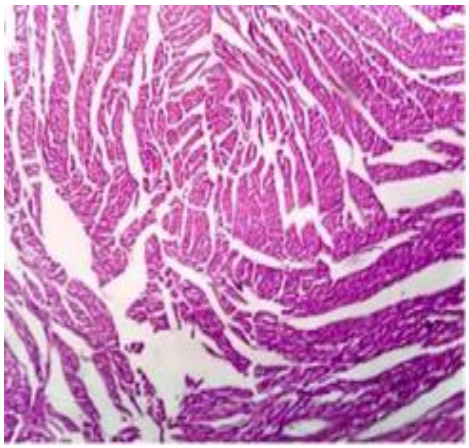
Microphotograph of kidney from group II showing normal Histoarchitectural details (H&E, 100X).



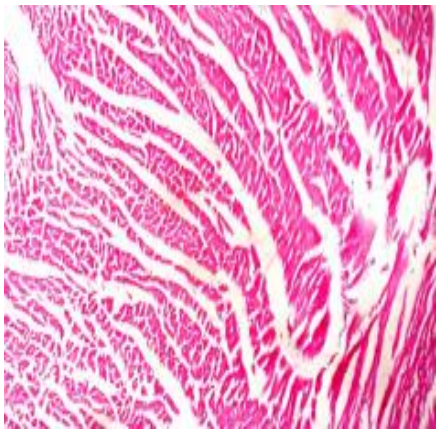
Microphotograph of kidney from group III showing normal Histoarchitectural details (H&E, 100X).

Fig 1: Microscopic examination of Kidney Tissues.

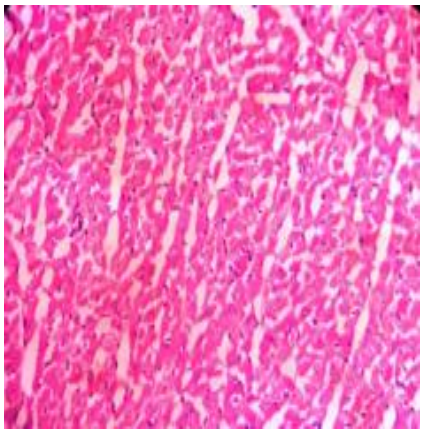
Slides of Histopathological examination of heart



Microphotograph of heart from group I showing normal Histoarchitectural details (H&E, 100X).



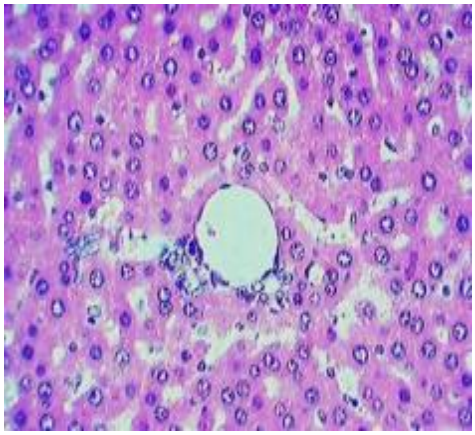
Microphotograph of heart from group II showing normal Histoarchitectural details (H&E, 100X)



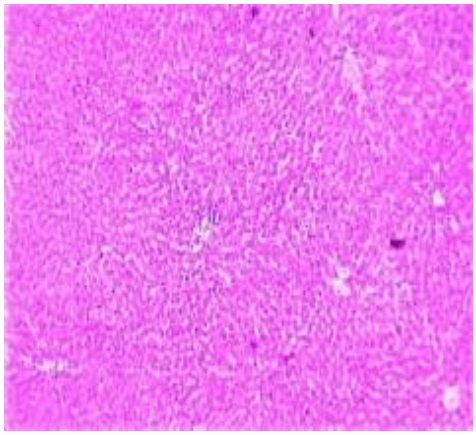
Microphotograph of heart from group III showing normal Histoarchitectural details (H&E, 400X).

Fig.2: Microscopic examination of Heart tissues.

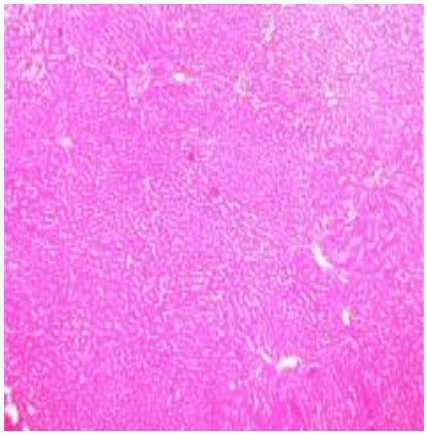
Slides of Histopathological examination of liver



Microphotograph of liver from group I showing mild grade of hepatic degeneration (H&E, 400X).



Microphotograph of liver from group II showing normal Histoarchitectural details (H&E, 100X).



Microphotograph of liver from group III showing normal Histoarchitectural details (H&E, 100X).

Fig 3: Microscopic examination of Liver tissues.

DISCUSSION

Amrutkalanidhi rasa is mentioned in *Yogaratnakara* in *jwara chikitsa adhyaya*. Purified *Vatsanabha churna* is one of the contents of this *Vati*. *Vatsanabha* is highly toxic in its original raw form. *Kapardika bhasma* is also present in *Amrutkalanidhi rasa*. Hence, an acute toxicity study was carried out in order to validate its safety. In the experimental study, Wistar albino rats are selected to carry out acute toxicity study as there is a similarity between anatomical and physiological systems of humans and Wistar albino rats as both are mammals.

Dose calculation

Rat dose = human dose × 0.018 (referring to table of Paget and Barnes, 1964)

Per kg dose in rats = human dose × 0.018 × 5.

In acute toxicity study

According to GENERAL GUIDELINES FOR SAFETY/TOXICITY EVALUATION OF AYURVEDIC FORMULATIONS by CCRAS- Dose levels and route of administration: The limit of 2gm/kg or at least 10 times of the intended clinical therapeutic dose whichever is less, using the same route as recommended for human.

As therapeutic dose in rat is 5.40mg/kg its 10 times dose approx. 50 mg will be taken as limit dose.

CONCLUSION

There were no abnormal changes observed in rats in various parameters such as salivation, respiratory rate, eyes and mucous membrane, behaviour pattern etc. in the 14 day-interval of acute toxicity study.

The study results of "acute oral toxicity" indicates that under experimental set up and laboratory environment, the *Amrutkalanidhi rasa* was found non-toxic up to the dose level of 50 mg/kg dose rate by oral route.

Histopathology study of sacrificed rats also shows no major abnormality in reports. This shows that *Amrutkalanidhi rasa* is non-toxic and safe to use.

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