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EVALUATION OF THE ACUTE TOXICITY OF AGNIKUMARA RASA

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ABSTRACT

AgnikumarRasa is a well-known *Ayurvedic* formulation indicated in the treatment of *Jawara* (Pyrexia). It contains *Vatsnabha* (Aconitum) as major and active ingredient and its recommended dose as per *Ayurvedic* literature is one *Ratti* (125 mg). As aconite is a poisonous herb and can produce toxic effect when consumed in improper dose. Acute toxicity study was performed on albino Wistar rats for three different doses (50mg/kg, 300 mg/kg, and 2000 mg/kg). Total 12 Wistar rats were divided in to three groups (Group A, Group B and group C) and were administered the calculated dose related with three doses used for toxicity study. It was observed that there was no mortality in any group, wellness parameters were found normal in group A and group B, only lethargy was found in group C, and hematological parameters were found normal. Histopathology of sacrificed rat from group C showed normal findings in kidney, liver, brain and spleen but inflammation was observed in the connective tissue of the heart. This study reveals the recommended dose (one *Ratti*) of *AgnikumarRasa* is quite safe for humans.

Keywords: *Ayurveda*, *AgnikumarRasa*, Toxicity study

INTRODUCTION

Ayurveda is a science having holistic approach towards life. It is based on the fundamental objective of keeping healthy person healthy and treating the diseased one. *Ayurvedic* classics are enriched with plenty of formulations for treating every kind of disease. Some of these formulations are having herbs only, some of them are having herbs and minerals and some of having heavy metals and poisonous ingredients. It is believed that *Ayurveda* treatment acts slowly but that is not a truth at all, *Ayurvedic* formulations having herbo-mineral and heavy metal ingredient work much faster. *Ayurveda* suggest that by *Bhavna* (Processing) poison can be made as effective as medicine. That time *Ayurvedic* science was so developed that heavy metals and poisonous herbs

and minerals were made highly effective by various purification methods.In *Ayurveda* classics there are so many formulations which contain poisonous herbs or minerals; still these formulations are veryeffective in different diseases. *Agni Kumar Rasa* is such a popular and effective herbal formulation mentioned in the *Ayurvedic* classics. It is chiefly indicated in *Jwara* (Pyrexia)[1]. It contains *Vatsnabha* (Aconitum Chasmanthum) as the chief ingredient (50%) [2], its other ingredients are *Marich* (Piper Nigrum), *Vacha* (Acorus Calamus), *Kooth* (SaussueraLappa), *Mustak* (Cyprus Rotundus) (12.5% each). It is prepared by grinding and mixing all the ingredients processed with *Swarasa*(juice) of *Adraka* (Zingiberofficinale). It is indicated in conditions of *Jwara* in the dose of one *Ratti* (125 mg). It is supposed that its antipyretic property is due to its main ingredient *Vatshnabha* which is a poisonous drug. So, the study was planned to evaluate its Toxicity effect in relation to its dose for the evaluation of its safe dose in humans.

Material and Methods: The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Institute of Biomedical and Industrial Research in accordance with the guideline formulated by CPCSEA, Government of India under CPCSEA Registration No. 1737/PO/Rc/S/14/CPCSEA.

Drug: For the study *AgniKumarRasa* was prepared in the Pharmacy of National Institute of Ayurveda, Jaipur.

Animals for experimentation:

Total 12 Albino Wistar Rats of weight ranging from 55to 220 grams were used in this study.

Mode of Administration: Oral route

Drugs were administered through 2ml disposable syringe fitted with 20 gauze stainless steel needle. Predetermined quantities of drugs were taken in the syringe.

Dose Calculated for Experimental Albino Rats – The dose for experimental study was calculated by extrapolating the human dose to animal's dose based on the body surface area ratio. The normal dose of *Agnikumara rasa* is 125mg/day in *Jwara*. Hence the suitable dose for albino rat was calculated by referring to the table of, Paget and Barnes' [3] i.e.

- Dose of *Agnikumara rasa*= 125mg/day
- Absolute human dose of *Agnikumara rasa* = 125mg/70kg
- Conversion factor (Human to Albino rat) = 0.018

The dose of *Agnikumara rasa* for Albino rat,

= human absolute dose x Conversion factor
= 125 x 0.018
= 2.25mg/ 200gm of albino rat
By converting to mg/kg,

The dose multiplies with suitable factor i.e. 5
= 2.25 x 5
= 11.25mg/ kg albino rat

Housing and feeding conditions

S.No.	Conditions	Requirement
01.	Room Temperature	22ºC (±3ºC)
02.	Humidity	50 – 60%
03.	Light and Dark Period	12/12 Hours
04.	Bedding	Clean Sterilized Husk
05.	Oral Feed	Conventional Laboratory Diets, Like Standard Pellet Chow
06.	Distilled Drinking Water	Unlimited Supply

Preparation of *Agnikumara rasa* in Glycerin for test group

1. For Acute Toxicity Study

Test Group A: 50 mg/ Kg in 10 ml Glycerin
Test Group B: 300 mg/ Kg in 10 ml Glycerin
Test Group C: 2000 mg/ Kg in 20 ml Glycerin

Inclusion criteria: -

Adult healthy albino rats
Rats weighing 55-220gms
Albino rats between 90-120days were included.

Exclusion criteria: -

Unhealthy albino rats
Weight below 55gm and above 220gms
Albino rats of below 90 days and above 120 days were excluded.

Fixation and Marking of identification: -

The albino rat was marked with Picric acid in each group as H, B, T, HB, BT, HT, HBT, Black tail, where:- H,B,T stands for Head, back and tail of albino rats respectively, HB, BT, HT and HBT stands for head and back, Back and tail, head and tail and head, back and tail of albino rats respectively.

Acute toxicity study

Method - According to OECD Guideline 423, Annexure II B (starting dose is 50mg/kg body weight).

S.No.	Group Name	No. of Albino rats	Dose (mg/kg)
2.	Test group A	04	50mg/kg
3.	Test group B	04	300mg/kg
4.	Test group C	04	2000mg/kg

Blood Collection: By Orbital Puncture

Hematological tests: Haemoglobin, WBC, RBC, Neutrophils, Lymphocytes, Eosinophils, Monocytes, Basophiles, Platelets.

Histopathological procedure:

Fixations: The organs were excised out immediately after sacrificing, cleaned of extraneous tissue, cut into pieces of appropriate thickness and transferred to 10% formalin solution. The tissues could remain in it till they are taken up for processing.

Tissue Processing: Tissue processing was done by removal of water by alcohol dehydration, then Infiltration of tissue in benzene as a solvent for paraffin wax and wax impregnation.

Section cutting: The tissue section of thickness of 5 μm was cut with the help of instrument "Spencer type rotating microtone".

Staining: After removing wax slide were treated with Xylene and rehydrated with Ethyl Alcohol in decreasing order concentrations. Finally slide mouth is covered with DPX (Distrene Dibutyl Pthalate Xylene). The slides were then sent for histo-pathological readings which were viewed under a microscope at various magnifications to note down the microscopic features.

Acute Toxicity Study: As per OECD Guidelines 423 [4], Wellness parameters of animals were observed continuously during the first 30 min after dosing and observed periodically (with special attention given during the first 4 hours) for the next 24 hours and then daily thereafter, for 14 days. All observations were systematically recorded with individual records being maintained for each animal. Observations included changes in skin and fur, eyes and mucous membranes and behavioral pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality.

1.Wellness Parameters: - Changes in wellness parameters i.e. Skin and fur, eyes, mucous membrane, salivation, lethargy, sleep, coma, convulsion, tremors, diarrhea, morbidity and mortality were compared with that of control animals with

the time duration of 30 minutes, 4 hours, 24 hours, 48 hours, one week and on 2 weeks.

2. Weight: -Body weights of animals were recorded before the administration of test drug on 1st day of the study and thereafter on the 7th and 14th day of the experiment. Changes in the weight of individual animals were calculated and compared with that of the control animals.

3. haematology

3. Histopathology: histopathology was performed for the sacrificed rat for the histopathological changes.

Observation and Results:

Wellness parameters: It was observed that all wellness parameter was normal in test Group A and test group B but in test group C lethargy was observed at the duration of 30 minutes, 4 hours and 24 hours and 48 hours, after 48 hours lethargy disappeared.

Weight: The following table provides data pertaining to change in initial and final body weight of albino rats of all groups. An apparent weight gain was observed all the treated groups.

Table 1: Effect of Test drug *Agnikumara rasa* on body weight of Albino rats at treatment of 50mg/kg, 300mg/kg and 2000mg/kg

GROUP	**TREATMENT**	**BODY WEIGHT**		**% of change**
		Before Treatment M1±SD1	**After Treatment M2±SD2**	
Test group A	50mg of test drug	103.00± 17.35	115.67± 16.29	12.3
Test group B	300mg of test drug	103.00± 20.75	122.33± 23.57	18.7
Test group C	2000mg of test drug	85.00± 29.46	89.00± 26.87	4.7

All the haemetological parameters were found normal in group B and group C.

Table 2: Results on haematological parameters.

S.No.	Haematological Parameters	Test group B (300mg/kg)	Test group C (2000mg/kg)	Normal Range
1.	Haemoglobin	12.5	12.9	11.5-16.1gm%
2.	WBC	7.8	8.4	6.6-12.6x10^3/mm^3
3.	RBC	8.2	7.1	6.76-9.75x$10^{6/}$$mm^3$
4.	Neutrophils	2.51	2.15	1.77-3.38x10^3/mm^3
5.	Lymphocytes	7.95	6.42	4.78-9.12x10^3/mm^3
6.	Eosinophils	0.04	0.06	0.03-0.08x10^3/mm^3
7.	Monocytes	0.02	0.01	0.01-0.04x10^3/mm^3
8.	Basophils	0.0	0.0	0.00-0.03x10^3/mm^3
9.	Platelets	344	287	150-460x10^3/mL

Histopathological studies:

No mortality was seen in any group. There were not any changes in wellness parameters of any group except of test group C, where all rats were found lethargic for the duration of 30 minutes to 48hrs. Therefore, one rat of Test group C (2000mg/kg) was sacrificed to observe the histopathological changes in the organs. There was no pathological change in any organ except of heart in which mild change was seen. In heart the nuclei appeared normal, but inflammation in the connective tissue was observed, the nuclei and cardiac muscle fibers were found well arranged.

Brain: The section of the cerebellum showed the normal histology of the cerebellum and its layers-the outer cerebella cortex and inner medulla. The

cerebella cortex presented three layers; the outer molecular, intermediate Purkinje and inner granular, layers. In the molecular layer were sparsely distributed cells. The Purkinje cell layer, which is one-cell thick, showed large pyriform shaped Purkinje cells with deeply stained basophilic nucleus. The granular layer presented numerous, thickly populated cell.

Kidney: It showed normal architecture of renal glomeruli with intact Bowman's capsule. There was minor aggregation in brush bordered cuboidal epithelium lining the proximal convoluted tubules. Simple cuboidal epithelium lining the distal convoluted tubules and Macula densa cells were found very prominent.

Spleen: Showed Normal Morphology of Lymphocyte, Red Blood Cells, Neutrophils, Macrophages and Platelets.

Heart: The nuclei appeared normal, but inflammation in the connective tissue was observed, the nuclei and cardiac muscle fibers were well arranged.

Liver: It was observed that the sections conformed to normal histological features. The sinusoids in the sections of the treated rats were devoid of occlusions and were not distorted.

DISCUSSION

Agnikumara Rasa is an effective formulation for the treatment of *Jwara (*pyrexia*)* mentioned in the *Ayurvedic* classics and its antipyretic effect in animal model has been revealed by a study [5]. It contains Aconite as its chief active ingredient therefore it is very necessary to evaluate its safe dose which is one *Ratti* (125 mg) as per recommended in *Ayurvedic* literature. In present study acute toxicity study was performed on wistar rats on three different doses 50 mg/kg, 300mg/kg and 2000 mg/kg. All groups were kept under observation for 14 days, where in Test Group A & B all the rats were normal. Wellness parameters were found normal except of group C (2000mg/kg) where lethargy was found. Mortality was not observed after 24hr, even at the highest dose level studied that was 2000 mg/kg in wistar albino rats. This clearly showed that the actual LD50 is much above 2000 mg/kg. All the haematological parameters like haemoglobin, red blood cells (RBC), white blood cells (WBC), neutrophils, lymphocytes, eosinophils, monocytes, platelets were found to be normal in *Agnikumara Rasa* treated group. Histopathology of one rat (HB) belonging to group C had done to find out any histopathological abnormal change. On the observation it was found that in the histopath slide of heart section showed that the nuclei appeared normal, but mild inflammation in the connective tissue was observed, the nuclei and cardiac muscle fibers were well arranged. That may due to the given dose which is far higher than the therapeutic dose of *Vatsnabha*. As we observed that in both the test group A & B, there were not found any type of toxic symptom except Test group C. From this, it can be inferred that the test sample is not likely to produce any marked toxicity at the dose level used in the therapeutic settings. *Ayurveda* believes that the major difference between drug and poison is of the 'dose'. If a drug is used in over quantity it becomes poison and if a poison is used in proper dose it becomes medicine and properties of any *Dravya* can also be changed by introducing them with various combinations by *Yukti* and by different purification methods (*Sanskara*) [6]. The dose of *AgnikumarRasa* recommended by *Acharya* reflects their high knowledge regarding the safety and efficacy aspect of any drug.

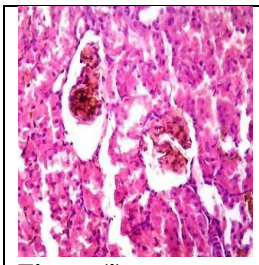
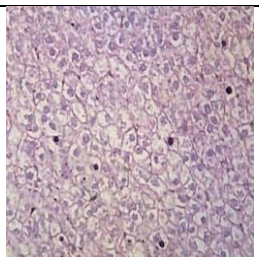


Figure (i)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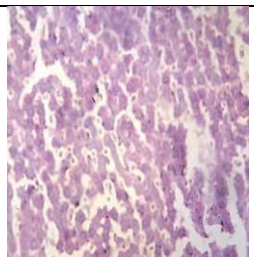
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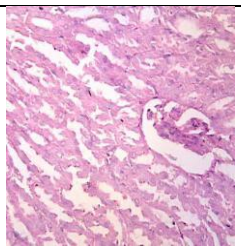
Figure (iv)

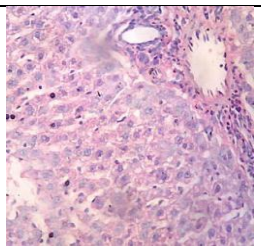
Figure (v)

Microscopic picture of different organs (i) Kidney, (ii)Spleen, (iii) liver, (iv)brain & (v)Heart of sacrificed albino rat from group C (2000mg/kg)

CONCLUSION

Based on over all findings from the acute toxicity study, it can be concluded that the drug *Agni Kumar Rasa* is a safe herbal remedy at the mentioned dose in literature.

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