Subacute Oral Toxicity Study of Arsenic Compound Sameerpannag Rasa in Albino Rats

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Abstract

Background: Despite the long tradition of thousands of years and acceptance in common public, Ayurvedic medicines of mercurial, metallic and mineral origin are seen with fear and doubt because in toxicology mercury, metals etc. are known as toxic to the human body. The main reason for dispute lays in the method of analysis of the Ayurvedic metallic and mercurial medicines. The usual method of analysis of medicines for heavy metals is usually atomic absorption spectro-photometry etc. where elements can be detected but the molecular form is not detected. Arsenic in Sameerpannag Ras is more than a thousand times higher than the permissible limit of Arsenic in medicines, still successful use of this medicine is Ayurveda practice brings the hypothesis that it is non-toxic in therapeutic dose.

Method: Sameerpannag Ras is a medicinal compound made with three Arsenic ores and Sulphur. Proper animal ethics committee approval was taken, 150 Albino rats were divided into five groups viz control, vehicle control, therapeutic dose, 2X dose and 5X dose. Subacute oral toxicity study was conducted as per OECD guidelines, general behaviours, blood analysis and histopathology study of vital organs were done to access toxicity.

Results: Histopathology study reveals that the consumption of Sameerpannag Rasa in therapeutic dose as well as in five times the therapeutic dose for 28 days did not cause any significant changes in tissues of liver, kidney, lungs, heart or brain. The behaviour of animals was unchanged during the study, also there was no mortality during the study period.

Conclusion: Sameerpannag Ras prepared as per classical Ayurvedic pharmaceutical procedure consumed in therapeutic dose is non-toxic in acute toxicity study. Irrespective of the Arsenic as its major constituent, the pharmaceutical method makes it not-toxic, hence re-establishing its safety and relevance in Ayurveda system of medicine.

Keywords: *Sameerpannag Ras, Ayurveda, Arsenic, Toxicity, heavy metal.*

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Introduction

Ayurveda believes that even a fatal poison when used with skill and knowledge proves to be a good medicine and a medicine used ignorantly acts as a poison. Accumulated toxicity data on the hazardous effects of heavy metals have made health scientists afraid of heavy metals. As a result, renewed interest in the beneficial effects of metals and minerals is often viewed

with skepticism. Hence it is high time that instead of blindly following the text and prescribing the medicine, one should test it thoroughly about its side effects, dose duration and toxicity in the target organ of body. These tests will allow us to form those guidelines regarding contra-indications of our drug and also unravel the myths and ambiguities about mercurial and metallo-mineral medicines[1]. The contents of this drug include mercury and minerals containing Arsenic and sulfur. The dose and vehicle of the drug is mentioned but the duration of dose is not found anywhere and whether it can be used for repeated dose or not is also not mentioned. So the topic of present study was testing of sub-acute oral toxicity of Sameerpannag Rasa in albino rats. This study helps us to know the safe dose and dose duration of Sammerpannag Rasa .Its effect on general behavior, food consumption, body weight, hematology and blood-biochemistry and histology of albino rats[2,3].

Aim and objectives:

- To study the effect of Sammerpannag Rasa on hematological parameter of Wistar albino rats.
- To study the effect of Sammerpannag Rasa on blood biochemical picture of Wistar albino rats.
- To study behavioural changes induced if any, in Wistar albino rats due to feeding of Sammerpannag Rasa for a period of 28 days.
- To study the effect of Sammerpannag Rasa on histo-structural changes if any, in Wistar albino rats.
- To determine the sub-acute oral toxicity of Sameerpannag Rasa.

Materials and Method

1. **Testing of sub acute oral toxicity in albino mice:** The objective of the study was to determine the sub-acute oral toxicity of Sameerpannag rasa in albino rats, following the OECD guidelines, 407 adopted on 27th July 1995. In the assessment and evaluation of the toxic characteristics of a chemical or a drug, the determination of oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained by acute testing, which has been already carried out, as mentioned earlier(prior to commencement of sub-acute oral toxicity studies). The test material i.e. Sameerpannag Rasa is orally administered by gavage daily 7 days per week in graduated doses to three experimental groups of Wistar albino rats. One dose level, per group, for a period of 28 days. As per, Aushadhigunadharma shastra betel leaf is mentioned as a vehicle so fresh Juice of betel leaf prepared was selected as suitable vehicle for Sameerpannag Rasa and the same was administered in the animals from control group as vehicle control group to test whether it has got any toxicity on its individual use. Also plain control group without any dose was kept to compare the observations with experimental group. During the period of drug administration the animals from all 5 groups were observed closely, each day for signs of toxicity, body weight, food consumption per day, were recorded weekly and the dose volume was changed accordingly. Blood samples were collected at the end of sub-acute toxicity study for Hematological and clinical Biochemical analysis. All animals were autopsied and their liver and kidneys were tested for Histopathology.

Observations and Statistical analysis:

Table 1: Data showing comparison of body weight of test groups to control group from 0 to 4 weeks in male & female albino rats

Group	Mean diff	SD-1	SD-2	SE	T	P
V-I male	41.5	15.67	4.481	9.4	4.41	<0.05
V-II male	30.16	19.32	4.481	11.45	2.633	>0.05
V-III male	25.5	14.54	4.481	8.78	2.902	<0.05
V-IV male	9.667	12.20	4.481	7.154	1.286	>0.05
V-I female	1.167	4.726	6.062	4.421	0.262	>0.05
V-II female	26.833	14.28	6.062	8.951	2.995	<0.05
V-III female	23.167	9.07	6.062	6.288	3.677	<0.05
V-IV female	6.167	4.041	6.062	4.188	1.466	>0.05

Table 2: Hematological data (unpaired t test) in males

Parameter	Mean diff	SD-1	SD-2	SE	T	P
V-I						
WBC	5.533	4.09	0.624	2.38	2.3	>0.05
Lymph	3.5	3.65	0.611	2.13	1.6	>0.05
Moncyt	0.133	0.152	0.173	0.13	1.0	>0.05
Grancyt	1.8	0.49	0.057	0.86	6.2	<0.05
RBC	0.12	0.135	0.545	0.32	0.3	>0.05
Hb	0.4	0.43	0.866	0.56	0.7	>0.05
Group-V-II						
WBC	4.9	2.835	0.624	1.67	2.9	<0.05
Lymph	1.833	1.81	0.611	1.11	1.6	>0.05
Moncyt	0.266	0.15	0.173	0.13	2.0	>0.05
Grancyt	2.7	0.98	0.057	0.56	4.7	<0.05
RBC	1.5	1.16	0.545	0.98	1.5	>0.05
GroupV-III						
WBC	3.4	3.29	0.624	1.93	1.7	>0.05
Lymph	2.1	2.34	0.611	1.39	1.5	>0.05
Moncyt	0.0	0.1	0.173	0.11	0.0	>0.05
Grancyt	1.2	0.86	0.057	0.49	2.4	>0.05
RBC	0.6	1.13	0.545	0.72	0.8	>0.05
Group V-IV						
WBC	4.63	8.81	0.624	5.1	0.9	>0.05
Lymph	3.86	7.26	0.611	4.2	0.9	>0.05
Moncyt	0.03	0.32	0.173	0.1	0.1	>0.05
Grancyt	0.6	1.2	0.057	0.71	0.8	>0.05
RBC	2.29	2.26	0.545	1.34	1.7	>0.05

Table 3: Hematological data (unpaired t test) in female

Parameter	Mean diff	SD-1	SD-2	SE	t	P
Group V-I						
WBC	0.86	1.19	3.19	1.96	0.4	>0.05
Lymph	1.26	0.83	2.49	1.51	0.8	>0.05
Moncyt	0.00	0.11	0.11	0.09	0.0	>0.05
Grancyt	0.4	0.28	0.75	0.46	0.8	>0.05
RBC	0.65	2.03	3.04	2.1	0.3	>0.05
Hb	1.63	3.71	6.09	4.1	0.3	>0.05
Group V-II						
WBC	1.9	1.55	3.19	2.04	0.9	>0.05
Lymph	1.4	0.85	2.49	1.15	0.9	>0.05
Moncyt	0.0	0.05	0.11	0.11	0.0	>0.05

Parameter	Mean diff	SD-1	SD-2	SE	t	P
Grancyt	0.46	0.66	0.75	0.46	0.8	>0.05
RBC	1.56	2.0	3.04	2.11	0.3	>0.05
Group V-III						
WBC	0.26	4.6	3.19	0.32	0.8	0.05
Lymph	0.43	3.05	2.49	2.27	0.1	0.05
Moncyt	0.06	0.2	0.11	0.13	0.4	0.05
Grancyt	0.86	0.4	0.75	0.49	0.7	0.05
RBC	0.78	1.61	3.04	1.99	0.3	0.05
Group V-IV						
WBC	1.36	2.08	3.19	2.20	0.6	>0.05
Lymph	0.6	2.56	2.49	2.06	1.5	>0.05
Moncyt	0.0	0.05	0.11	0.07	0.0	>0.05
Grancyt	0.86	0.40	0.75	0.49	1.7	>0.05
RBC	0.29	2.3	3.04	2.2	0.1	>0.05

Table 4: Biochemistry data in males

Parameter(Sr)	Mean diff	SD-1	SD-2	SE	t	P
Group V-I						
BUN	5.0	8.86	6.8	6.49	0.7	>0.05
T.Protein	0.86	0.88	0.513	0.59	1.4	>0.05
Al.phos.	66.33	36.5	54.19	37.8	1.7	>0.05
SGPT	13.00	13.3	7.76	8.89	1.4	>0.05
Group V-II						
BUN	0.36	8.8	6.8	6.05	0.5	>0.05
T.Protein	0.3	1.7	0.513	1.07	0.2	>0.05
Al.phos.	43	31.0	54.19	36.0	1.1	>0.05
SGPT	50	17.1	7.76	10.8	4.5	<0.05
Group V-III						
BUN	11	1.52	6.8	4.0	2.6	>0.05
T.Protein	1.6	0.15	0.513	0.3	5.1	<0.05
Al.phos.	93.0	28.3	54.19	35.9	2.6	>0.05
SGPT	14.0	13.6	7.76	9.06	1.5	>0.05
Group V-IV						
BUN	0.758	10.2	6.8	7.0	0.3	>0.05
T.Protein	0.76	0.2	0.513	0.3	2.4	>0.05
Al.phos.	48.6	95.9	54.19	35.9	0.7	>0.05
SGPT	35.6	21.19	7.76	13.0	2.6	>0.05

Table 5: Biochemistry data in females

Parameter(Sr)	Mean diff	SD-1	SD-2	SE	T	P
Group V-I						
BUN	8.0	12.66	7.5	8.49	0.9	>0.05
T.Protein	0.1	0.305	0.404	0.29	0.3	>0.05
Al.phos.	35	44.2	14.9	26.9	1.2	>0.05
SGPT	6.3	8.18	18.23	11.5	0.5	>0.05
Group V-II						
BUN	1	3.05	7.5	4.6	0.2	>0.05
T.Protein	1.6	0.5	0.404	0.47	2.1	>0.05
Al.phos.	90.3	52.7	14.9	31.6	2.8	<0.05
SGPT	9.33	0.57	18.23	10.5	0.8	>0.05
Group V-III						
BUN	9.66	3.05	7.5	4.37	2.2	>0.05
T.Protein	0.33	0.5	0.404	0.27	1.2	>0.05
Al.phos.	5.33	52.73	14.9	30.8	0.1	>0.05
SGPT	15.66	0.577	18.23	12.1	1.3	>0.05
Group V-IV						
BUN	7.3	8.5	7.5	6.54	1.1	>0.05
T.Protein	0.4	1.0	0.404	0.67	0.6	>0.05
Al.phos.	144.6	81.9	14.9	48.1	3.0	<0.05
SGPT	17	8.32	18.23	11.5	0.2	>0.05

Discussion and Results

As per principles of medicine, the histological, biochemical, haematological and clinical changes have to be significantly relevant to reach up to certain diagnosis.[1-4]

- The test group animals H, 2H and 5H showed normal and healthy weight gain and food intake throughout the study. The mean percent body weight gain of animals of control male group was 19.4%. While of vehicle control group, H, 2H and 5H was 29.4%, 44.5%, 37.66%and 33.86% after the completion of 4 weeks. In females the mean percent body weight gain of control group was 6.1%. In vehicle control group H, 2H and 5H it was 3.12%, 6.56%, 21%and 21%.
- All survived animals were observed healthy and showed no abnormal behavioral changes. This particularly indicates the safety of Sammerpannag Rasa.
- The RBC count is normal in all the groups. This shows that there is no toxic effect of Sameerpannag rasa on the test groups. The Hemoglobin levels are within normal limits in all animals The test group 5H female rats showed leukopenia but it do not has any relevance with its biochemical, clinical and histological changes. But possibility of initiation of an allergic drug reaction cannot be denied clearly[4].
- In the present study SGPT of all animal lies within normal level, except in Group2H animal no 1, 2 and 3 which show significant rise in SGPT levels. but histological picture shows no evidence of cell necrosis of liver. Similarly the SGPT levels of 5H group are normal with normal picture of liver histology. There were no associated clinical symptoms like dark stool, diarrhea, tiredness etc. seen in the animals. Regardless of this they show increased food consumption and body weight gain. Hence the rise of SGPT level is not significant from toxicity point of view[5].

- The Sr. Alkaline phosphatase values lie with in normal limits in all the groups.
- In present study the levels of total protein are raised in 5H group which may be due to higher dose of Sammerpannag Rasa . The histology of liver is normal in 5H group, but it shows evidences of primary cellular changes to the kidney. Hence it can be said that High doses of Sammerpannag Rasa minutely though, has affected the renal function .No mortality occurred in any group. No clinical signs like dark stool, diarrhea, or fatigue etc. The biochemical parameters of H and 2H groups were within normal limits as compared to control and vehicle control groups.
- Histology of liver in 5H group shows NAD with mild lobular and periportal mononuclear cells, which is of no toxicological significance.
- However in renal pathology the animals in 5H group shows focal tubular necrosis, which indicates damage to the renal cells, this change is not seen in control or vehicle control group. These changes may be due to Sameerpannag rasa in high dose. The Sr. protein levels are also raised in males of this group, but females show normal values. Because cells are constantly adjusting structure and function to accommodate changing demands and extracellular stresses. Cells tend to preserve their immediate environment and intracellular milleu with a relatively narrow range of physiological parameters, they maintain normal homeostasis. The cells encounter pathologic stimuli, they can undergo adaptation, achieving a new steady state and preserving viability. The principle adaptive responses are atrophy and hypertrophy. If the adaptive capability is exceeded, cell injury develops. Within certain limit, injury is reversible and cells resume to a stable baseline. However, in severe or persistent stress results in irreversible injury. Indeed, the observed changes in these animals are subjected to persistent increased load on glomerular filtration, ultimately the interstitial tubular cells, which were undergone adoption by hypertrophy (an increase in the size of individual cells) to generate required filtration rate[6]. Thus, due to this adaptation of cell the pathological stimuli are observed without hampering clinical, haematological and biochemical values. Hence, BUN values were not affected during this course. Particularly, tubular necrosis and cloudy changes observed in these animals are reversible.

The study shows pathological changes in kidney in animals given 5 times higher dose and all normal values in H group, hence it should be concluded that Sammerpannag Rasa is safe to be given for repeated dose at dose level of 60-120mg.

Conclusions

- Prepared Sameerpannag Rasa has not showed sub-acute toxicity by oral route in albino rats on the basis of food consumption data.
- Prepared Sameerpannag Rasa has not showed sub-acute toxicity by oral route in albino rats on the basis of Body weight gain data.
- Prepared Sameerpannag Rasa has not showed sub-acute toxicity by oral route in albino rats on the basis of Behavioural changes.
- Prepared Sameerpannag Rasa has not showed sub-acute toxicity by oral route in albino rats on the basis of Clinical signs observed.
- Prepared Sameerpannag Rasa has not showed sub-acute toxicity by oral route in albino rats on the basis of Haematological parameters considered.
- Prepared Sameerpannag Rasa has not showed sub-acute toxicity by oral route in albino rats on the basis of Biochemical parameters.
- Prepared Sameerpannag Rasa was found safe at therapeutic dose levels when administered for a limited duration of time.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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