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Acute Toxicity & Anti-pyretic Activity of Classical Ayurvedic Drug Jwaraghani Gutika with or without Parad in Yeast Induced Pyrexia

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Abstract—
Objective- *The objective of the present work was to study the acute toxicity and anti-pyretic activity of Jwaraghani guitka with Parad and Jwaraghani gutika without Parad (modified). Jwaraghani gutika is a herbo-mineral preparation containing Parad (mercury) and prescribed as an anti-pyretic drug in Ayurvedic classics. But the hazardous effects of mercury on human health and environment impose ban on mercury use and the phase out date by year 2020. So the present work has been taken into consideration and Jwaraghani gutika was prepared with or without Parad.*
Materials & Methods- *Jwaraghani gutika as per reference of A.F.I. and Jwaraghani gutika modified were prepared. Further acute toxicity study at dose of 300mg/kg and 2000mg/kg and anti-pyretic activity at a dose of 90mg/kg in brewer's yeast induced pyrexia were done in albino wistar rats. Result- Jwaraghani gutika with or without Parad found to be safe at a dose of 2000mg/kg body weight in experimental animals. Jwaraghani gutika significantly reduces temperature in first, second, third and fourth hour after drug administration but its therapeutic efficacy was not found equivalent to standard (PCM) group where as Jwaraghani gutika without Parad significantly reduces temperature in first, second and third hour and have equal therapeutic effect to standard (PCM) group. Conclusion- Both the samples of Jwaraghani gutika exhibited statistically significant anti-pyretic activity in different ways. So, it can be recommended for further studies.*

Keywords*: Jwaraghani gutika with or without Parad, acute toxicity, anti-pyretic activity*

1. INTRODUCTION

In the current kinetic era, rasaushadhis (herbo-mineral & metallic preparation) have given Ayurveda a complete novel health care look. The innate qualities like quick action, less dose, tastelessness, prolonged shelf life, better palatability of Rasaushadhis have helped them to conquer the compliance of the patients. Parad (mercury) is the main ingredient of most of the Rasaushadhis and one among these is Jwaraghani gutika (tablet) described in our classical texts. [1-4] Jwaraghani gutika is a herbo-mineral preparation indicated in all types of Jwara.[1] However the use of metallic preparations has raised concerns and debate in scientific community in the recent years.[5] However, a global assessment on mercury and its compounds by United Nations Environmental Programme found sufficient evidence of adverse impacts of mercury on human health and the environment.[6] After that, in January 2013, the intergovernmental negotiating committee concluded its fifth session by agreeing on the text of the Minamata Convention on Mercury. The Minamata Convention on Mercury is a global treaty to protect human health and the environment from the adverse effects of mercury.[7] The major highlights of Minamata Convention on Mercury include ban on new mercury mines and trade of mercury and phase out of existing ones by year 2020.[8] This will creates big issues to the reputation of Ayurveda as India chiefly depends on other countries for mercury. So, in the present study a step has been taken to test acute toxicity profile and antipyretic effect of Jwaraghani gutika and Jwaraghani gutika modified (without Parad).

2. MATERIAL & METHODS-

2.1. Pharmaceutical Study

2.1.1. Jwaraghani Gutika

Jwaraghani gutika was prepared as per reference of A.F.I.[9] Firstly Kajjali was prepared by triturating sudha Parad and sudha Gandhaka in equal amount [10] for 120hrs. Then powder of all ingredients were taken in khalva yantra and triturated with indravaruni moola swarasa till the contents get dried. Trituration was done 8 hrs daily. The same process was repeated for two more times. Gum acacia in 30 % concentration of the triturated mixture was taken and a homogenous solution was prepared. This solution was added to triturated mixture and mixed thoroughly in a stainless steel

vessel and pass through sieve no. 22. These granules are dried in a hot air oven for 2hr at 50°C temp. Then the granules were mixed with sodium starch glycolate (10%) and compressed into tablets with the help of tablet punching machine.

2.1.2. Jwaraghani Gutika Modified (without Parad)

Shodhana of asudha Gandhaka was done. Then powder of all ingredients were taken in khalva yantra and triturated with indravaruni moola swarasa till the contents get dried. Trituration was done 8 hrs daily. The same process was repeated for two more times. Gum acacia in 30 % concentration of the triturated mixture was taken and a homogenous solution was prepared. This solution was added to triturated mixture and mixed thoroughly in a stainless steel vessel and pass through sieve no. 22. These granules are dried in a hot air oven for 2hr at 50oC temp. Then the granules were mixed with sodium starch glycolate (10%) and compressed into tablets with the help of tablet punching machine.

Table 1: Preparation of Jwaraghani gutika

Ingredients	Quantity
Sudha Parad	50gm
Sudha Gandhaka	50gm
Elva	50gm
Akarkara	50gm
Haritaki	50gm
Pippali	50gm
Indravaruni fruit	200gm
Indravaruni moola swarasa ist bhavana	700ml
Indravaruni moola swarasa 2nd bhavana	350ml
Indravaruni moola swarasa 3rd bhavana	215ml
Weight after bhavana	580gm
Gum acacia	174gm (30%)
Sodium starch glycolate	58gm (10%)

Table 2: Preparation of Jwaraghani gutika modified

Ingredients	Quantity
Sudha Gandhaka	50gm
Elva	50gm
Akarkara	50gm
Haritaki	50gm
Pippali	50gm
Indravaruni fruit	200gm
Indravaruni moola swarasa 1st bhavana	750ml
Indravaruni moola swarasa 2nd bhavana	300ml
Indravaruni moola swarasa 3rd bhavana	225ml
Weight after bhavana	595gm
Guma cacia	178.5gm
Sodium starch glycolate	59.5gm

***Note*:** Parad was not used in Jwaraghani gutika modified. Gum acacia was used as a binder and sodium starch glycolate as disintegrating agent.

2.2. Experimental study

2.2.1. Acute toxicity study

Acute toxicity was done according to OECD guideline 423 ANNEX 2c

Oral Acute Toxicity study According to OECD Guideline 423[10]

The experimental study was carried out at **Institute of Biomedical and Industrial Research, Jaipur** after obtaining permission from Institutional Animal Ethics Committee with **Approval number- IBIR/IAEC/ 2015/II/2**.

Acute toxicity study was conducted on mice to determine the minimum lethal dose of the drug. Wistar albino mice of either sex weighing between 90-190gm fasted overnight was used for the study. Three animals are used for each group. The dose level to be used as the starting dose is selected from one of two fixed levels 300 and 2000 mg/kg body weight. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.
All test animals (including those that die during the test or are removed from the study for animal welfare reasons) should be subjected to gross necropsy. Microscopic examination of organs showing evidence of gross pathology in animals surviving after 14 days had been considered because it may yield useful information.

2.2.2. Antipyretic Study

Preparation of animals

The animals are randomly selected, marked with Picric acid H (Mark on head), B (Mark on Back), T (Mark on Tail), HT (Mark on head and Tail), HB (mark on head and Back), BT (Mark on Tail and Back) for individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Number of animals and dose levels
Thirty animals are used in total having 6 rats in each group.
Inducing Pyrexia
Pyrexia was induced by subcutaneous injection of 20 % w/v of brewer's yeast (10ml/kg) in distilled water. Basal rectal temperature was measured before the injection of yeast, by inserting digital thermometer to a depth of 2 cm into the rectum. The rise in rectal temperature was recorded 18 h after yeast injection.

Administration of doses
Calculated dose had been administered orally with help of oral feeding needle after inducing pyrexia.

Formula for dose calculation is

200 gm rat dose= 0.018 X Adult dose
1 kg rat dose= 0.018 X Adult dose X 5

Observations
Rectal temperature with tele thermometer.

Table 3: Groups for Anti-pyretic study

Group No.	Group name
Group 1	Normal control(without inducing pyrexia)
Group 2	Pyrexia Induced control(received distilled water 5 ml/kg oral)
Group 3	Pyrexia induced test sample I(Jwarghani gutika 90mg/kg)
Group 4	Pyrexia induced test sample II(Jwarghani gutika Modified 90mg/kg)
Group 5	Pyrexia induced Standard drug(Paracetamol 50mg/kg)

Fig. 1: Grouping of Rats for Anti-Pyretic Activity

Fig. 2. Anti-pyretic study

3. RESULTS

3.1. Statistical Analysis

According to routine behavioral observations, heamatological analysis and histopathological analysis it was found that the test sample at dose 300 mg/kg and 2000mg/kg is safe and found no morbidity and no mortality. No any changes found in heamatological and histopathological observations.

The results of antipyretic study were expressed as Mean ± SEM. Comparison between each hr. and each group were performed by analysis of variance (ANOVA). In all tests the criterion for statistical significance was $P < 0.05$.

4. DISCUSSION

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive.[11] Normally the infected or damaged tissue initiates the enhanced formation of proinflammatory mediator's (cytokines like interleukin 1beta, alpha, beta and TNF- alpha), which increase the synthesis of prostaglandin E2 (PGE-2) near peptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature.[12] In acute toxicity of samples of Jwaraghani gutika, the routine behavioral observations, hematological analysis and histopathological analysis shows that the test samples at dose 300 mg/kg and 2000mg/kg were safe with no morbidity and mortality. In test group 1st (Jwaraghani gutika), the temperature recorded before drug administration was 38.47 ^{0}C which got reduced to 37.48 ^{0}C after one hr, 37.62^{0}C after 2nd hr. 36.63^{0}C after 3rd hr. and 37.53^{0}C 4th hr. In test group second(Jwaraghani gutika without Parad), mean temperature observed before treatment was 37.92 ^{0}C which reduced to 35.77^{0}C after 1st hr., 36.35^{0}C after 2nd hr., 36.50^{0}C after 3rd hr and 37.93^{0}C after 4th hr of drug administration. In Standard group before treatment mean temperature was observed 37.97^{0}C and 1st hr. after treatment it was 35.83^{0}C, at 2nd hr. was 36.50^{0}C, at 3rd hr. was 36.53^{0}C and at 4th hr. was 36.68^{0}C as shown in table no. 4. The data of table no.5 reveals that, in 1st hr after drug administration test sample II have significant effect($P< 0.0001$) and brings the body temperature in normal range as compared to the other groups whereas the effect of test sample 2nd last for 3hrs($P<0.0001$) after drug administration. Also in test group 1st, temperature decreases after 1hr which was again increases after 2 hrs and then again reduced after 3hrs and remains even after 4thhr($P<0.05$) of drug administration. These variations in body temperature may be because the level of drug in blood is not maintained due to inappropriate drug absorption. The significant anti-pyretic activity of Jawarghani gutika is due to its ingredients as Piperine, the major constituent of *Piper longum* have anti-pyretic activity produced by significant reduction in rectal temperature that may be due to inhibitory effect in

prostaglandin secretion.[13] Flavanoids have been known for their anti-inflammatory, anti-bacterial and anti-viral activities by inhibiting cylo-oxygenase enzymes (mediate the synthesis of PGE2) activities and there by prevent the synthesis of PGE2 (ultimate mediator of febrile response[14] that possibly responsible for anti-pyretic activity of Indravaruni fruit and Haritaki. Beside this, ethanolic extract of Indravaruni root has appreciably reduces the expression levels of pro-inflammatory cytokines viz. TNF-α, PGE2 & COX-2.[15] As pyrexia is a part of inflammatory response so this mechanism of action of Indravaruni root extract also leads to anti-pyretic effect. Along with this, the Ethanolic extract of Akarkara root has significant anti-pyretic activity.[16]

So, study further continues with chronic toxicity study and pharmacokinetic study (ADME) of the drug.

5. CONCLUSION

From the analysis of the data generated, test sample I shows significant temperature changes but not show equal therapeutic effect to standard (PCM) group where as test sample II show significant temperature changes and have equal therapeutic effect to standard (PCM) group.

So with the study it can be concluded that if we prepare Jwaraghani gutika as Jwaraghani gutika modified, it will produce significant therapeutic effect and the issues related to toxicity of mercury get also excluded.

Table 4: Temperature Observation

Test Group	Rectal Temperature (^{o} C)											
	B.T.	S.E.M	18 hr after yeast admin.	S.E.M	1 hr	S.E.M	2 hr	S.E.M	3 hr	S.E.M	4 hr	S.E.M
Normal Control Group	36.02	0.098	36.53	0.217	36.75	0.112	36.07	0.228	36.58	0.154	36.50	0.203
Induced Control Group	36.15	0.250	38.83	0.042	38.67	0.136	38.48	0.158	38.12	0.221	38.32	0.178
Test Sample I Group	36.03	0.123	38.47	0.333	37.48	0.307	37.62	0.352	36.63	0.330	37.53	0.141
Test Sample II Group	36.05	0.112	37.92	0.214	35.77	0.163	36.35	0.141	36.50	0.161	37.93	0.167
Standard Group	36.00	0.151	37.97	0.254	35.83	0.167	36.50	0.169	36.53	0.193	36.68	0.275

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	26.58	16	1.661	F (16, 125) = 6.072	P < 0.0001
Row Factor	78.52	4	19.63	F (4, 125) = 71.75	P < 0.0001
Column Factor	24.72	4	6.179	F (4, 125) = 22.58	P < 0.0001
Residual	34.20	125	0.2736		

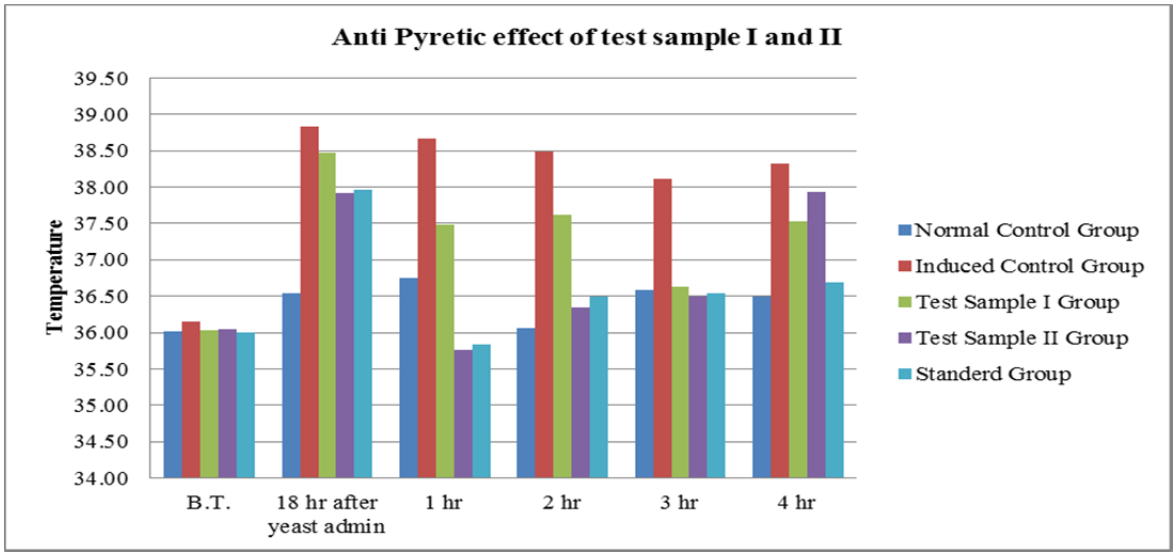
Table 5: Two way- ANOVA with Dunnett's Multiple Comparisons Test

1 Hr				
Dunnett's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Induced Control Group vs. Normal Control Group	1.920	1.173 to 2.667	Yes	****
Induced Control Group vs. Test Sample I Group	1.190	0.4430 to 1.937	Yes	***
Induced Control Group vs. Test Sample II Group	2.900	2.153 to 3.647	Yes	****
Induced Control Group vs. Standard Group	2.840	2.093 to 3.587	Yes	****

2 Hr				
Dunnett's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Induced Control Group vs. Normal Control Group	2.410	1.663 to 3.157	Yes	****
Induced Control Group vs. Test Sample I Group	0.8600	0.1130 to 1.607	Yes	*
Induced Control Group vs. Test Sample II Group	2.130	1.383 to 2.877	Yes	****
Induced Control Group vs. Standard Group	1.980	1.233 to 2.727	Yes	****

3 Hr				
Dunnett's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Induced Control Group vs. Normal Control Group	1.540	0.7930 to 2.287	Yes	****
Induced Control Group vs. Test Sample I Group	1.490	0.7430 to 2.237	Yes	****
Induced Control Group vs. Test Sample II Group	1.620	0.8730 to 2.367	Yes	****
Induced Control Group vs. Standard Group	1.590	0.8430 to 2.337	Yes	****

4 Hr				
Dunnett's multiple comparisons test	**Mean Diff.**	**95% CI of diff.**	**Significant?**	**Summary**
Induced Control Group vs. Normal Control Group	1.820	1.073 to 2.567	Yes	****
Induced Control Group vs. Test Sample I Group	0.7900	0.04300 to 1.537	Yes	*
Induced Control Group vs. Test Sample II Group	0.3900	-0.3570 to 1.137	No	Ns
Induced Control Group vs. Standard Group	1.640	0.8930 to 2.387	Yes	****



Graph 1: Anti Pyretic effect of test sample I and II

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