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Pre-clinical Study

Title of Article:
Efficacy of *Malla Sindoora* prepared by adopting new Technology: Pre-Clinical approach

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ABSTRACT

Rasoushadhis got popular day by day due to its unique assimilatory organometallic constitution. If Good Rasashastra practice is blended with scientific physico chemical and instrumental gadgets for up-to-date analysis, the drug designing in pharmaceutical field can excel to the top. MallaSindoorais one of the unique Rasa Yoga, with Parada, Gandhaka&Malla prepared by Kupipaka method having indications in Vataroga, Amavata, Visuchika etc. In present study the evaluation of its Analgesic activity on albino rats is carried out. Humans share majority of their genes with mice and rats. The physiologies of humans and these animals are very similar. They are susceptible to many of the same health problems as men. Also, the short life span allows animals to be studied throughout their entire life.Thus research on animals is important in understanding diseases and developing ways to prevent them. Also, the pharmaceutical research requires the experimental use of animals for drug control regulatory approvals, as conducting experimental study prior to human trial is mandatory. Even though MallaSindoora is time tested, we need to give a base to understand its analgesic action through in vivo study. Therefore to prepare MallaSindoora by Classical and VMF and systematically observe the pharmaceutical process as well as to accord technological evidences and evaluate analgesic activity in vivo, the present study entitled "Efficacy of MallaSindoora prepared by adopting new technology . A Pre-Clinical approach " has been undertaken.

Objective :To carry out the Analgesic activity of MallaSindoora 1 &2 on Albino rats by Using Analgesiometer.

Keywords : MallaSindoora, MallaSindoora prepared by classical method (MS-1), Mall Sindoora Prepared by using Electrical Vertical Muffle Furna (MS-2), Albinorats, Analgesic Activity, Experimental Study.

INTRODUCTION

Rasa Shastra or 'Vedic Chemistry' is an offshoot of Ayurveda that was developed around the period, when Buddha existed, i.e. more than 2500 years ago. Although it developed relatively late in the history of Ayurveda, it is today considered a very important aspect of Ayurvedic medicine by those who are experienced in preparing and using these medicines. Indian alchemy developed a wide variety of chemical processes for the transmutation of metals and preparation of elixir of life, in which mercury occupied a prime position. The literature on Rasashastra is perceptibly voluminous and methodical in the presentation of a variety of processes whose number is exhaustless. Of these processes, KupipakvaRasayana deserves special mention because of its minimal dosage, augmenting effect and long lasting potency.

MallaSindoora is one of the important classical KupipakvaRasayana containing, HingulothaParada, ShuddhaGandhaka and ShuddhaMalla in 1:1:1/2 proportions. It is Sagandha, Saagni, Bahirdhuma, KantasthaKupipakvaRasayana potentiated with Agni samskara for 36-48 hours. The process converts the metal in to a chemical compound with necessary medicinal benefits like treatment of Vataroga, Amavata, and Visuchika etc. All together 7 varieties of MallaSindoora has been mentioned in classics. All are different in the form of ingredients, their quantity, time duration of processing and their therapeutic indication.

The similarities being all are kupipakwa rasa: Parada, Gandhaka, & Malla are the common ingredients in all types of MallaSindoora. In the present study below mentioned preparation was undertaken which is prepared by Classical & VMF method and their comparative Analgesic activity on Albino rats has been done.

Pharmaceutical preparation :

MallaSindoora[2]

Ingredients:

SuddhaMalla[4]	: 5 tola
Suddha Parada	: 10 tola
Suddha gandhaka[3]	: 10 tola
Kumari swarasa[5]	: Q.S.

Procedure:

Take the ingradients in above said quantity, prepare the kajjali[6]. And after kajjali siddhi laxanas add fine powder of shodhitaMalla and give the bhavana with Kumari swarasa. And allowed it to dry. Fill it in kachakupi[7]keep it in valuka yantra[8] give kramagni[9]. This method of preparation is adopted in the present study.

Duration of Agni	: 36 - 48 hrs.
Dose	: ¼ - ½ Ratti
Anupana	: Grita,Madhu, Adrakaswarasa.
Rogaghnata	: Swasa,Kasa, Sannipata, Unmada, Apatantraka, Hysteria, Amavata, Visuchika, Vataroga, Prameha

Experimental Study:

Any new drug which is developed for any purpose has to undergo mandatory experimental study on animals for various reasons like setting the dosage, studying its pharmacology, toxicology and safety. In the same way evaluating Comparative Analgesic activity of MallaSindoora prepared by Classical and VMF method has been undertaken.

Aims and objectives of the study:

1) To screen the Analgesic activity of MallaSindoora - Classical (M.S -1) and MallaSindoora – VMF (M.S- 2) on Albino Rats.

Material and Methods:

Animals : Wister strain Albino-rats of either sex between 150-200gms.

Drugs : Gum acacia[10] as control Diclofinac Sodium[11] as standard drug. M.S -1 & M.S - 2 as Test drug.

Equipment : Tuberculin syringe (1ml), Rat feeding tube, Weighing balance, Hand Gloves, Camera

Glass wares: Glass Beakers, Test tubes, Stirrer, Measuring jar.

Analgesiometer:

This device was first introduced by D Amour F.E and Smith D.L in the year of 1941. This was used in present study for screening the analgesic action. The apparatus employed here is supplied by Techno Analgesiometer Lucknow, U.P. which consists of nichrome wire, which can be electrically heated and hence provides point source of application of radiant heat to the rat tail. Nichrome wire is surrounded by a water jacket with an inlet and outlet for water to flow continuously to keep the surroundings cool when the nichrome wire is heated.

Methods: The methods employed for the study of analgesic activity was – Tail flick method using Analgesiometer12.

Tail flick method: For our study, albino rats of either sex (weighing 150-200 gms) were used. The trial animals were shaved. The animals were arranged into four groups

of six each. One served as control and received 2% suspension by mouth, where as the second group, as standard, which received Diclofenac sodium, and third group, served as trial group-1 which received drug MallaSindoora – 1 and Group four served as trial group-2 which received drug MallaSindoora – 2 under evaluation. The drugs were administered with the help of gavage needle supported with tuberculin syringe prior to the application of stimulus. Stimulus was applied every 30minutes, 0, 30, 60, 90, 120 minutes cut off time of 30 sec to avoid any thermal injury to the tail and each time the reaction time is noted in all groups.

Albino rats of either sex weighing 150-200gms were held in suitable restrainer with tail protruding out. Radiant heat is applied over the tail on a single spot with the help of an Analgesiometer. The animals are selected by preliminary screening. Those showing variations of more than one second between 2 reaction times at 15 minutes interval, or more than 3 seconds from the group means are discarded. All the reaction times or results are expressed as mean increase over basal ±SE. The results are analyzed using one - way ANOVA followed by Dunnet Multiple gacacia
comparison test by using Graph Pad In stat, (Inc 5755 Oberlin Drive) Statistical Software.

Rocedure :

Animal study was conducted in Vijaynagar Institute Of Medical Science , Bellary with
Ref no : VVIMS/STD.II/PG-AYU/IAEC/01/2011-12.
Fixation and Preparation of Rat Dose:
The classically advised, normal human adult dose of MallaSindoora is 1/4th to 1/2 ratti, which is equal to 31.25mg-62.5mg. This was converted into animal dose based on Paget and Burner's surface area ratio which works out to be 5.62 mg /kg body weight.
i.e. Rat dose / kg body wt. = 0.018 x Human dose x 5
= 0.018 x 1/2 ratti x 5
= 0.018 x 62.5 x 5
Therefore Rat dose of MallaSindoora = 5.62 mg / kg body weight. Similarly, total daily dose of Diclofenac sodium4 is 10mg/kg body weight. The drugs were prepared as a suspension by triturating with water and gum acacia and solution of Diclofenac sodium was prepared in distil water. The test and standard drugs were prepared as a suspension by triturating with 2% gum acasia. Control drug was dissolved in distilled water.

Table No 1 : Shows drugs according to Groups

Group	Purpose	Number of Rats	Drug
Group I	To serve as Control	6	2% Gum acacia
Group II	To serve as Standard	6	Diclofenac sodium
Group III	To serve as Trial	6	MallaSindoora - 1
Group !V	To serve as Trial	6	MallaSindoora -2

Control Group:

In the group I of control – 2% gum acacia was administered which was prepared by adding 100ml of distilled water to 2 gm of gum acacia. Then this diluted 2% gum acacia was calculated for rat dose of 5ml of kg body wt and used (i.e. 1ml / 200g rat).

Standard Group:

Drug: Diclofinac Sodium. Rat dose of Diclofinac Sodium: 10 mg/kg wt
For 200g rat : 2 mg/200g wt

Dissolution

Standard dose for 200g rat is 1 ml,2mg drug should be in 1 ml of suspension of Diclofinac Sodium dissolved in 2% Gum acasia. Hence 20 mg Diclofinac Sodium is dissolved in 10ml.
Hence 20 mg Diclofinac Sodium is dissolved in 10ml.

Trial Group:

Drug : M.S-1 & M.S-2
Rat dose of : 5.62 mg/kg wt
For 200g rat : 1.12mg/200g wt

Dissolution - 20 mg of M.S-1 & 20 mg of M.S-2 are dissolved in 10ml of Gum acasia respectively.

Administration of Drugs:

The assigned drugs were administered orally using the rat feeding tube.

- Each rat of Group I received 2% Gum acasiasolution.
- Each rat of Group II received 10mg/kg body
- weight of Diclofinac Sodium suspended in 2% Gum acasia.
- Each rat of Group III received 5.62 mg/kg body wt ofM.S-1 suspended in 2% Gum acasiasolution.
- Each rat of Group IV received 5.62 mg/kg body wt ofM.S-2 suspended in 2% Gum acasiasolution.

Images of Animal Experimental Study :

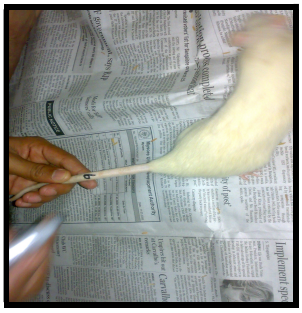
Fig.No: 01

Analgesiometer

Fig.No: 02

Weighing Rats

Fig.No: 03



Numbering rats

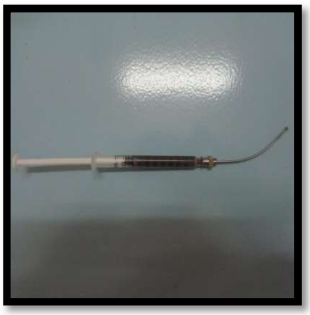
Fig.No: 04

Grouping of Rats

Fig.No: 05

Suspension of drugs

Fig.No: 06



Tuberculin syringe with Rat feeding tube

Fig.No:07

Fig.No: 08

Oral feeding of the drug **Tail Flick of Rat**

Table No 2 : Shows Dose of Control group, Standard group, Trial group.

Control group Rat No.	Weight of Rat ingms	Dose of Rat in ml	Standard group Rat No.	Weight of rat in gms	Dose of Rat in ml	Trial group - 1(M.S-1) Rat No.	Weight of rat in gms	Dose of Rat in ml	Trial group-2 (M.S-2) Rat No.	Weight of rat in gms	Dose of Rat in ml
C1	200	1	S1	190	0.95	T1	150	0.42	T1	180	0.5
C2	200	1	S2	200	1	T2	180	0.5	T2	160	0.44
C3	200	1	S3	190	0.95	T3	165	0.46	T3	165	0.46
C4	200	1	S4	200	1	T4	160	0.45	T4	190	0.53
C5	200	1	S5	180	0.9	T5	150	0.42	T5	200	0.56
C6	150	0.75	S6	150	0.75	T6	170	0.47	T6	180	0.5

RESULTS

1) Tail Flick Method

Table No 3: Shows the results of experimental study.

Groups	0min	30 min	60 min	90 min	120 min	180 min
Control	3.11 ± 0.27	3.68 ± 0.16	3.06 ± 0.37	2.33 ± 0.42	2.16 ± 0.30	2.48 ± 0.19
Standard	3.75 ± 0.35	4.38 ± 0.18	4.35 ± 0.10	4.36 ± 0.22	6.5 ± 1.1	2.25 ± 0.17
Trial-1	3.25 ± 0.30	4.96 ± 0.35	4.73 ± 0.37	4.25 ± 0.11	5.16 ± 1.45	2.9 ± 0.31
Trial-2	3.5 ± 0.44	4.43 ± 0.30	5.06 ± 0.66	4.53 ± 0.24	3.91 ± 0.35	3.0 ± 0.38
F	0.62	3.912	4.187	14.169	3.65	1.568
df	3,20	3,20	3,20	3,20	3,20	3,20
P value	**0.604^{ns}**	**0.0238***	**0.018***	**0.0001****	**0.03***	**0.228^{ns}**

All the values are expressed in Mean ± S.E.M seconds.
n s – not significant , * - P < 0.05 , ** - P < 0.01
Statistics Applied: One – Way Anova followed by DUNNET'S MULTIPLE Comparison Test by using graph pad In stat software. From the above table it can be observed that the test drug 1 & 2 was showing good analgesic activity after 30, 60, 90 &120 min of administration. This was statistically highly significant at P < 0.0001.

After 30 min: The P value is 0.0238, considered significant, variation among the column mean is significantly greater than expected by chance. Dunnet's Multiple Comparison test: if the q value is greater than 2.540 the p value is less than 0.05. After 60 min: The P value is 0.018, considered significant, variation among the column mean is significantly greater than expected by chance. Dunnet's Multiple Comparison test: if the q value is greater than 2.540 the p value is less than 0.05. After 90 min: The P value is 0.0001, considered extremely significant, variation among the column mean is significantly greater than expected by chance. Dunnet's

Multiple Comparison test: if the q value is greater than 2.540 the p value is less than 0.05.

After 120 min: The P value is 0.03, considered significant, variation among the column mean is significantly greater than expected by chance. Dunnet's Multiple Comparison test: if the q value is greater than 2.540 the p value is less than 0.05. After 180 min: The P value is 0.228, considered not significant, variation among the column mean is significantly greater than expected by chance.

DISCUSSION

In the present study an attempt has been made to evaluate the comparative analgesic activity of MallaSindoora 1 & 2 by Tail Flick Method by using Analgesiometer. All the parameters were noted with their time duration in seconds using stopwatch. Tail-flick latency was assessed by the analgesiometer. All drugs were given orally to the respective group rats as a suspension in gum acacia. The strength of the current passing through the naked nichrome wire was kept constant at 4 ampere. The application site of the heat on the tail was maintained within 2 cm, measured from the root of the tail. Cut-off reaction time was +10 s to avoid any tissue injury during the process. Randomly selected 24 rats weighing 150 – 200 gm were equally divided into four groups of 6 each. The group I of control was administered 2% gum acacia suspension in rat dose of 5 ml / kg body weight, group II of standard was treated with Diclofenac sodium 10mg/ kg body weight of rats. Group III & IV of trail was treated with M.S-1 & M.S-2 respectively, with 5.62 mg /kg body weight. All were given orally.

The Experimental data are analyzed according to One wayAnnova test followed by Dunnett's multiple comparison. The statistical analysis showed that the both test drugs M.S-1 & 2, has significant Analgesic action after 30, 60, 90 &120min, at level of significance $p < 0.01$. When experimental data are analysed by t-test, it shows both Mall Sindoora 1& 2 are having same efficacy after 30, 60, 90 and 120 min.

With this one can say that both the test drugs are playing a role in management of pain (Analgesic action) in rats when compared with control group, though was not up to the mark when compared with standard drug (Diclofenac sodium). Various factors may be playing role here such as dosage, pharmaco dynamic and kinetic properties of the test drugs.

CONCLUSION

- The Analgesic activity of both Malla Sindoora-1 & 2 are screened by Tail-Flick Method by using Analgesiometer experimented on albino rats showed statistically significant Analgesic effect of both M.S-1 & 2 are seen after 30, 60, 90 and 120min intervals respectively ($P<0.01$) in Tail Flick Method.
- By observing un-paired t-test it can be said that both MallaSindoora 1 & 2 are having same efficacy.
- So laborious Classical Kupi Method can effectively replaced by VMF in a view of Pre-clinical studies. Further clinical trial has to be carry out to conclude the efficacy of the drug.

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