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EVALUATION OF PERIPHERAL AND CENTRAL ANALGESIC EFFICACIES OF AYURVEDIC NEW FORMULATION FOR *AMAVATA* DISEASE IN ANIMAL MODEL THROUGH WRITHING TEST AND TAIL -FLICK IMMERSION TEST IN MICEMODEL- AN ANIMAL EXPERIMENTAL STUDY.

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic inflammatory disorder, it is the most common persistent inflammatory arthritis, occurring throughout the world and in all ethnic groups. This is the most marked in those with severe diseases, with a reduction in expected lifespan by 8-15 years. Around 40% of RA patients are registered disable within 3 years, around 80% are moderately to severely disables within 20 years, and 25% will require a large joint replacement. Functional capacity decreases most rapidly at the beginning of disease and the functional status of patients within their 1st year of RA is often predictive of long-term out-come(1).

Aim: In research field RA need more attention for deep research due to, firstly its unbearable symptoms and secondary complication and secondly in modern science there are medicines available for temporary management purpose but with lot of adverse reaction. RA is a non-curable disease, in *Ayurveda*, RA can be corelated with *Amavata* disease (Rheumatoid arthritis).

Methodology: In this present study It has been taken up to see the efficacy of new combined *Ayurvedic* formulation i,e combination of Stem of *Asthishrinkhala* (*Cissus quadrangularis* **L**), bulb of *Sunthi* (*Zingiber offici-*

nale **Roscoe**) and bulb of *Rasona* (*Allium sativum* **L**) with (1:1:1) ration for better management with less side effects. This research firstly done on animal to evaluate central and peripheral activity of research drug, through Writhing Test and Tail -flick immersion test in Mice model.

Keywords: Rheumatoid Arthritis, RA, *Ayurvedic* formulation.

INTRODUCTION

Rheumatoid arthritis is a chronic systemic inflammatory disorder that affects many tissues and organs, including skin, blood vessels, heart, lungs and the muscles. It is mainly effects the joints and specially started with small joints, producing a non-supportive proliferative synovitis that often progress to destruction of the articular cartilage and ankylosis of the affected joints. Now a days RA has taken the foremost place among the chronic joint disorder. In *Ayurveda*, Rheumatoid arthritis can be correlated with *Amavata* disease due to its similarity of sign and symptoms like severe pain, swelling, stiffness etc. *Amavata* as an independent disease at 1st mentioned by *Acharya Madhava kara* in '*Madhava Nidan'* in 19th Century AD[(2)]. *Amavata* is a resultant combined effect of simultaneous aggravation of two pathological entities, *Ama (*Metabolic Toxin*)* and *Vata dosha*. *Ama* is a consequential toxic metabolite of *Agnimandya* and *Vatadosha* is aggravated by its pathological factors. In modern science for RA there are present few medicines with lot of adverse effects,now a days people want relief, medicines from traditional ways. Though it is an incurable disease, its treatment management with proper herbal medicine is very needed. So in this present research a combined *Ayurvedic* new has taken to improve management of *Amavata* disease, this new formulation made of from the stem of *Asthisrinkhala*, buld of *Sunthi* and bulb of *Rasona*. To determine the properly analgesic effectiveness of this new formulation, before clinical trial the animal trial on mice was done by Central analgesic test-Tail-flick immersion test and for peripheral test Writhing test was done.

Material and methods: In this animal research,Tail flick immersion test was done to find out the Central analgesic activity and writhing test for Peripheral analgesic activity of *Ayurvedic* new formulation in Mice model.

Inclusion criteria:

- Animal- Albino mice
- Sex- both male and female
- Weight- (20-30) gm
- Route of administration of drug- oral route
- No. of groups-4
- No. of animals in each groups-6.

Tail-flick immersion Test on Mice: The animals were housed in polypropylene cages and maintained at 24 ± 2^0C under 12h light dark cycle and feed with standard pellet diet and were led to have free access to water. Permission for the study wasobtained from the Institutional Ethical Committee of IPGAE& R at SVSP Kolkata.

Experimental design:

GROUP A	Positive Control,mice were administrated water.
GROUP B	Standard group, mice were administrated orally Ibuprofen 100mg/kg/10ml
GROUP C	Test drug mice were administrated orally research drug (RAS)500mg/kg body wt.
GROUP D	Test drug mice were administrated orally research drug (RAS)700mg/kg body wt.

Fig:1: Different steps of Tail-flick test on mice

Procedure: The tail-flick test is a test of the pain response in animals. It is used in basic pain research and to measure the effectiveness of Central analgesics, by observing the reaction of heat apply. It was first described by D' Amour and Smith in1941.The distal half of each mice tail was placed in a water bath.The tail with-draw latency was recorded with a 10 second time-out. Mice were tested twice for baseline response at 30 minutes prior to drug administration. After baseline, Group C and D mice were administered 500 mg/kg body weight and 700 mg/kg body weight of research drug respectively research and Group B administrated standard drug Ibuprofen 100mg/kg/10 ml in normal saline water and only water in control group A ,30 min before applying the hot water thermal stimulus, which was maintained at 55±1^{0}C. The de-
lay in tail flick reaction by each animal was recorded in seconds as response immediately before (0) and after 30min, 60min, 90 min and 120 min of drug administration. Maximum reaction time of observation was kept at 10 sec throughout to avoid tissue damage. Inhibition of tail flick reaction = $\mathbf{(V_c\text{-}V_t)\ x\ 100/V_c}$= Mean tail flick reaction timing in control group, VT = Mean tail flick reaction timing in the drug treated groups

Writhing Test on mice: The animals were housed in polypropylene cages and maintained at 24 ± 2^{0}C under 12h light dark cycle and feed with standard pellet diet and were led to have free access to water. Permission for the study was obtained from the Institutional Ethical committee of IPGAE & R at SVSP Kolkata.

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GROUP D	Test drug mice were administrated orally research drug (RAS)700mg/kg body wt.

Procedure: The detection of Peripheral analgesic activity of the research drug was evaluated in mice by Acetic acid induce Writhing test method. The control group was orally administered 10 ml/kg saline, Standard group was administered Ibuprofen100 mg/kg/10 ml in normal saline water and research groups were administered 500mg/kg body weight and 700mg/kg body weight of research drug respectively, 30min before injecting intraperitonially 0.1ml, 1%Acetic acid. Writhing reaction by each animal was recorded in counting as response for 30 min period. Inhibition of writhing reaction = $(V_c-V_t) \times 100/V_c$= Mean writhing reaction count in control group, VT = Mean writhing reaction count in the drug treated group.

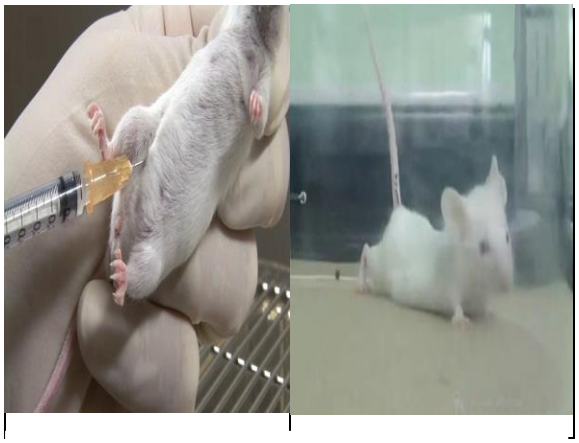

Fig:2: Showing the different steps of Writhing test in mice

Result: It was shown that Inhibition of tail flick reaction time in different treated and control group is as follow-
In Graph presentation and with Table presentation.

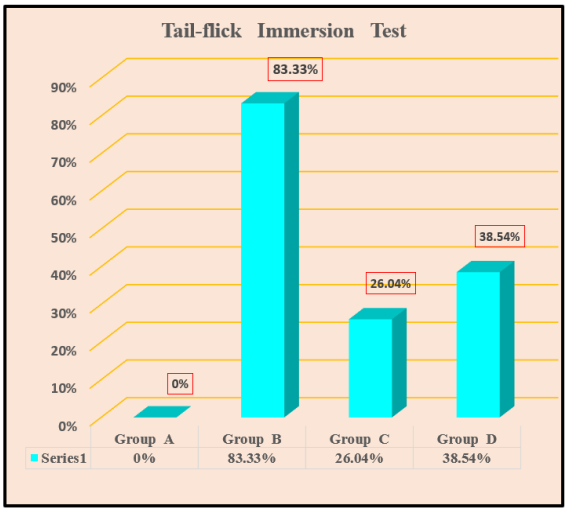

Fig:3: Graph Presentation of Tail-flick immersion test in different groups of mice in compared to control group.

GROUP	0MIN ±SEM	30 MIN ±SEM	60 MIN ±SEM	90 MIN ±SEM	120 MIN±SEM	Percentage of increased reaction time
A	0.93±0.03	0.96±0.02	0.95±0.02	0.95±0.02	0.96±0.03	**0%**
B	0.95±0.03	1.21±0.09	1.48±0.04	1.66±0.04	1.76±0.03	**83.33%**
C	0.91±0.03	0.98±0.01	1.21±0.04	1.31±0.04	1.21±0.05	**26.04%**
D	0.91±0.03	1±0.02	1.2±0.05	1.4±0.03	1.33±0.06	**38.54%**

Table.1.

Showed % of increased reaction time in different minutes in Tail-flick test.

The values are expressed in terms of MEAN ± SE (n=6/group). P<0.01 when compared to control by two way ANOVA

In Writhing test-

Effect of treatment of Aq. Extract of research Drug on PeripheralAnalgesic activity by Writhing-Test on Mice

Table.2.Showed percentage of inhibition of writhing reactions in different animal group in compared with control group where values are expressed in terms of MEAN ± SE (n=6/group). P<0.01 when compared to control one way ANOVA

GROUP	No. of Writhes in 30 min.(Mean ± SEM)	% of Inhibition
Group A	70±0.57	**0%**
Group B	27.16±1.88	**61.2%**
Group C	47.16±0.94	**32.62%**
Group D	38.66±0.80	**44.77%**

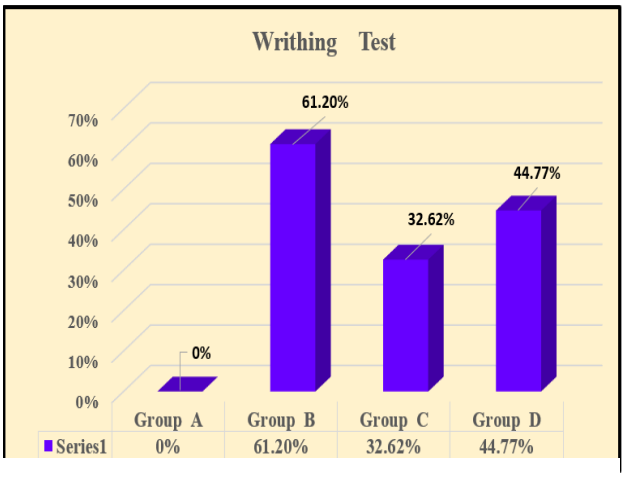

Fig: 4: Graph Presentation of Writhing reactions test in different groups of mice in compared to control group.

DISCUSSION

From the above showed data, it proves that in Tail-Flick test, Standard drug shown max % of increase reaction time in Albino Mice was 83.33%, while research drug at the dose 500mg/kg was 26.04%, and 700 mg/kg was 38.54% respectively. Both groups were compared to the control group. It was a satisfactory result in comparison to control group.

In Writhing test, Group B showed highest effective result i.e., 61.2% then Group C and Group D, that is, research drug group showed 32.62% and 44.77% respectively.

CONCLUSION

After full experiment it was concluded that while comparing between Central and Peripheral Analgesic test in mice it was confirmed that in this research study, the research drug that is combination of *Asthisrinkhala* , *Sunthi* and *Rasona* has Peripheral analgesic effect more than Central analgesic effect. So this research drug can be effective for human kind in future and further study may be continue.

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