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Research Article

Comparative Study of Different Samples of *Chandamarutha sindoora* for Neuro-Regenerative Activity – An Experimental Study

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ABSTRACT

Abstract: Chandamarutha Sindoora is a classical Siddha and Ayurvedic Pakshaghata/stroke/Hemiplegia, Vata Vyadhi, Kushta etc. diseases. However scientific validation and evidences are lacking and yet to be elucidated. Aim of the study: To prepare three different samples of Chandamarutha Sindoora, analytically assess them and to establish the scientific evidences and to explore the most efficacious sample for neuro-regenerative mechanism among three different Chandamarutha Sindoora samples, evaluated experimentally through rat model. Materials and methods: Preparation of CMS- Three samples of Chandamarutha Sindoora according to the reference Rasayoga Sagara, Agastya Vaidya Chandrika and Ayurveda Vijnana Kosham with the same ingredient i.e; Rasa Karpooora, Rasa Pushpa, Rasa Sindoora, Shuddha Hingula and Shuddha Gandhaka, in different ratios, have been referred, to prepare and to study their Neuroregenerative activity on neurodegeneration induced rat model. The pharmaceutical preparation of Chandamarutha Sindoora was done according to Arappu Chendooram method of Siddha system, after subjecting the ingredients for proper Shodhana and by giving Bhavana with egg white. Chemical identification of Chandamarutha Sindoora (CMS) was performed using analytical parameters like pH, moisture content, ash value etc and instrumental analysis like XRD, heavy metal content (ICPMS). Neuroregenerative activity was evaluated in AlCl₃ induced neurodegenerative rat model. During the study behavioural assessment, cook's pole climbing test were performed and at the end of experiment, the brain samples were collected and sent for histopathological study. Results: Chemical standardisation were showing supportive results and also, heavy metal limits were found to be less or not detectable and XRD analysis evidences the proper preparation of Chandamarutha Sindoora by matching the peaks of standard Chandamarutha Sindoora. Among three samples, CMS - 3 sample shows best Neuroregenerative activity than CMS - 2 than CMS - 1. Although all three CMS samples exhibited Neuroregenerative activity, CMS 3 showed better performance in the open field test, behavioural assessment, and Cook's

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pole climbing test than CMS - 2 and CMS - 1. Histopathological analysis also showed minimal or no neuronal degeneration in the hippocampus and cerebrum also evidence the Neuroregenerative effect exhibited by the sample CMS - 3. Conclusion: Disease control group showed decreased activity in motor function, behavioural activity and open field behaviour and histopathological result shows degenerated and pyknotic neurons in cerebrum, CA - 1 and CA - 3 regions of hippocampus. Treatment with all CMS samples showed effectiveness in Neuro-regeneration and comparatively CMS - 3 sample given better results than CMS - 2 and CMS - 1.

INTRODUCTION

Ayurveda has a long tradition of utilizing Herbo-mineral formulations for the management of chronic and difficult-to-treat disorders. These formulations (*Rasaushadhis*) occupy a significant place in Ayurvedic therapeutics due to their rapid action, smaller dose, enhanced bioavailability, and long shelf life. The components of these formulations undergo specific *Shodhana* (purification), *Marana* (Incineration) etc pharmaceutical processing, which are believed to enhance their therapeutic efficacy while minimizing their toxicities.

Among these formulations, *Chandamarutha Sindoor* is a well-known herbo-mineral preparation mentioned in both Ayurvedic and Siddha system, containing purified mercurial compounds and Sulphur as major ingredients. However, variations in ingredient proportions and manufacturing methods may influence its physicochemical characteristics, safety, and therapeutic efficacy.

The *Chandamarutha Sindoor* (CMS) is described in several classical texts including Rasayoga Sagara, Agastya Vaidya Chandrika and Ayurveda Vijnana Kosham where it is primarily indicated for Neurological conditions such as *Pakshaghata*. In the present study three different samples of *Chandamarutha Sindoor* were prepared based on

the formulations described in these texts. The *Chandamarutha Sindoor* containing ingredients such as *Rasa Sindoor*, *Rasa Karpoora*, *Rasa Pushpa*, *Hingula* and *Gandhaka*, combined in varying proportions, according to the respective textual references and compared to identify the most efficacious formulation with superior Neuro-regenerative and neuroprotective activity.

Among Siddha formulations, *Chandamarutha Chendooram* has been traditionally indicated for conditions such as paralysis, facial palsy, hemiplegia, neuromuscular disorders, and other degenerative diseases.

Pakshaghata, described under *Vata Vyadhi* in Ayurveda, is characterized by loss of motor function, weakness, pain, and impaired movements affecting one half of the body. Clinically, it closely resembles Hemiplegia, which commonly occurs following cerebrovascular accidents (stroke).¹ Stroke is one of the leading causes of long-term disability worldwide, resulting in significant physical, psychological, social, and economic burden.² Although the available stroke management by different medical systems mainly focuses on thrombolysis, rehabilitation, and symptomatic care.³ Complete neurological recovery remains challenging, necessitating the exploration of effective complementary therapeutic approaches.

Neurodegeneration involves oxidative stress, neuroinflammation, excitotoxicity, and progressive neuronal loss. Hence, there is growing interest in scientifically validating traditional formulations that may possess neuroprotective properties.⁴

Recent experimental studies have demonstrated that *Chandamarutha Chendooram* exhibits significant neuroprotective activity by reducing oxidative stress, neuroinflammation, and neuronal



damage in experimental models. These findings support the need for further pharmaceutical processing and experimental validation of the formulation.⁵

Ayurvedic classics advocate the use of *Vatahara*, *Balya*, *Rasayana*, and *Medhya* drugs in the management of *Pakshaghata*. *Balya*, and neuroprotective properties may help to improve nerve conduction, reduce neuronal damage, promote tissue regeneration, and enhance functional recovery.

Therefore, the present study is undertaken to prepare *Chandamarutha Sindoor* according to classical methods, evaluate its pharmaceutical and analytical characteristics, and experimentally assess its neuroprotective efficacy, in Aluminium Chloride induced Neuro-degeneration model in Wistar Albino rats. The study aimed to generate scientific evidence supporting safety, quality and therapeutic potential of this classical formulation.

MATERIALS AND METHODS

Pharmaceutical study:

1. Identification, collection and purification of raw drugs:

The ingredients required for the preparation of *Chandamarutha Sindoor* are collected from local market and are analysed for genuinity. The *Chandamarutha Sindoor* contains intermediate products like *Rasa Karpoora* (pooram), *Rasa Pushpa* (veeram), *Hingula* and *Gandhaka*, which are subjected to purification process. The *Rasa Sindoor* purchased from the market was added during the procedure without purification. *Rasa Karpoora* and *Rasa Pushpa Shodhana* was done according to Siddha source of literature. *Hingula* and *Gandhaka Shodhana* was done according to

Rasa Tarangini and *Ayurveda Prakasha* text books.

2. Preparation of Chandamarutha Sindoor:

All the ingredients were taken, weighed according to the different ratios as mentioned in the texts such as *Rasayoga Sagara*, *Agastya Vaidya Chandrika* and *Ayurveda Vijnana Kosham* and were triturated with egg white until it became red coloured *Sindoor* form. This methodology was explained under the category of *Chendooram* preparation in Siddha medicine.

1. Veeram Shodhana or Rasa Pushpa shodhana:

It was performed according to Siddha Formulary of India.

Ingredients	Quantity
<i>Sauveeram / Rasa Pushpa</i>	100gms
<i>Karpoora / Camphor</i>	20gms
Tender coconut water	800 ml

- 20g of Camphor was taken, grinded to powder and mixed with 800ml of Tender Coconut water. It was taken in a mud pot.
- *Pottali* of *Veeram* or *Rasa Pushpa* was made with a clean Kora cloth and was hung from a wooden stick, which was kept over the mud pot to form a *Dola Yantra*.
- *Pottali* height was adjusted so as to stay it above the liquid throughout the procedure.
- Steaming of the *Pottali* was done for 1 hour after which the purified *Veeram* or *Rasa Pushpa* was collected dried under the Sun and stored in a glass container.

2. Pooram Shodhana (Rasa Karpoora) Shodhana:



It was performed according to Siddha Formulary of India.

- *Nagavalli Patra* was washed with water to remove impurities and ground with *Maricha* to form a *Kalka* in a clean and dry *Khalwa Yantra*.
- The above prepared *Kalka* was added to 1350 ml of water in a mud pot. *Pottali* of *Rasa Karpoora* was made with a clean Cora cloth and tied to a wooden stick.
- The *Pottali* was suspended in the above liquid till it gets completely immersed and the pot was kept over the gas stove for heating.
- Continued the heating till the liquid was reduced to 1/4th portion.
- When cooled the *Pottali* was removed, *Rasa Karpoora* was collected and washed with warm water. Then it was dried and stored in a glass Jar.

3. Gandhaka Shodhana⁶

It was performed according to A.P. 2/21-24

- *Gandhaka* was grinded to powder form.
- In an Iron pan equal amount of Ghee was taken and melted over *Mandagni*.
- To this powdered *Ashuddha Gandhaka* was added. when all the *Gandhaka* got melted, it was filtered through the cloth into a vessel containing milk.
- Milk was then discarded & *Gandhaka* was washed thoroughly with hot water and then kept for drying under shade.

- After drying, it was powdered again and the same procedure was repeated for 2 more times.
- After completion of the process, *Shuddha Gandhaka* was dried, powdered and stored in a glass jar.

4. Hingula Shodhana⁷

It was performed according to Rasa Tarangini 9/12

- *Ashuddha Hingula* was taken and powdered in a *Khalwa Yantra*.
- *Ardraka Swarasa* was added to it and *Bhavana* was carried out till the *Hingula* got completely dried.
- This was repeated for 7 times by adding fresh *Ardraka Swarasa* during each *Bhavana*.
- After 7th *Bhavana* the *Shodhita Hingula* was collected and dried under shade.

5. CHANDAMARUTHA SINDOORA (CMS) - 1

Reference: *AYURVEDA VIJNANA KOSHAM*⁸

SH. RASA KARPOORA / POORAM	4 Parts	26.67 gm
SH. RASA PUSHPA / VEERAM	1 Part	6.67 gm
SH. GANDHAKA	1 Part	6.67 gm
SH. HINGULA	8 Parts	53.33 gm
RASA SINDOORA	1 Part	6.67 gm
EGG WHITE	—	24 ml

Procedure

- Each ingredient was taken according to the ratio mentioned in the text and triturated separately to made into fine powder.



- Then it was added one by one in to a *Khalwa Yantra* and triturated in clockwise direction with a stroke of 25/min.
- 24ml of Egg white was added and *Bhavana* was done until the whole Egg white was dried and dry powder of *Sindoora* coloured preparation was obtained.

6. CHANDAMARUTHA SINDOORA (CMS) - 2

Reference: *RASAYOGA SAGARA*⁹

Ingredients and Quantity:

SH. RASA KARPOORA / POORAM	8 Parts	25 gm
SH. RASA PUSHPA / VEERAM	2 Parts	6.25 gm
SH. GANDHAKA	2 Parts	6.25 gm
SH. HINGULA	4 Parts	12.50 gm
RASA SINDOORA	16 Parts	50 gm
EGG WHITE	—	25 ml

Procedure

- Each ingredient was triturated separately and made into fine powder. Then the powder was added one by one in to a *Khalwa Yantra* and trituration was continued at the rate of 25strokes/min to made into a homogenous mixture.
- Egg white was added to the mixture after filtering and *Bhavana* was done until the whole Egg white was dried and *Sindoora* like preparation was obtained. It took around 6hrs for completing the procedure.

7. CHANDAMARUTHA SINDOORA (CMS) - 3

Reference: *AGASTYA VAIDYA CHANDRIKA*¹⁰

Ingredient and Quantity:

SH. RASA KARPOORA / POORAM	14 Parts	9.72 gm
SH. RASA PUSHPA / VEERAM	14 Parts	9.72 gm
SH. GANDHAKA	1 Part	694.4 mg
SH. HINGULA	114 Parts	79.17 gm
RASA SINDOORA	1 Part	694.4 mg
EGG WHITE	—	24 ml

Procedure:

- Each ingredient was triturated and made into fine powder separately.
- Then the ingredients were added to a *Khalwa yantra* and sufficient quantity of Egg white was added and *Bhavana* was done until the whole Egg white was dried and *Sindoora* like preparation was obtained.
- It took around 6hrs for completing the procedure.

Experimental study:

Wistar albino rats were taken randomly from the well- established animal house attached to S.D.M. Centre for Research in Ayurveda and Allied Sciences.

An experimental model for the study of Neuroregenerative activity is selected in such way that it would satisfy the following condition:

- To evaluate the Neuroregenerative action of *Chandamarutha Sindoora* on Aluminium chloride-induced Neurotoxicity.
- To estimate Neuroregenerative activity of 3 samples of *Chandamarutha Sindoora* and to determine the most efficacious sample.



Table no. 2.2 showing grouping of rats

GROUPS	DRUG USED	NO. OF ANIMALS
1	Normal control	6
2	Disease control	6
3	Trial drug CMS 1	6
4	Trial drug CMS 2	6
5	Trial drug CMS 3	6

Group 1: The rats were given normal tap water & Standard laboratory diet.

Group 2: The rats were injected with Aluminium Chloride for 45 days.

Group 3: The rats were injected with Aluminium Chloride ($AlCl_3$) followed by trial drug *Chandamarutha Sindoor* -1, administered orally through feeding tube after 1 hour of injection for 45 days.

Group 4: The rats were injected with Aluminium Chloride ($AlCl_3$) followed by trial drug *Chandamarutha Sindoor* - 2 administered orally through feeding tube after 1 hour of injection for 45 days.

Group 5: The rats were injected with Aluminium Chloride ($AlCl_3$) followed by trial drug *Chandamarutha Sindoor* - 3 administered orally through feeding tube after 1 hour of injection for 45 days.

Body weight, open field behaviour test and behavioural despair test was conducted on 7th day from the day of administration of dosing. On 45th day the same tests were conducted and the parameters such as number of outer squares, middle squares, inner squares crossed, rearing, grooming, faecal pellets and freezing time was assessed in every rat of each group and values were noted.

From 15th day onwards training for Cook's Pole Climbing apparatus test was started for 7 days and

on 7th day assessment of retention of Conditioned Avoidance Response was conducted in each rat in each group and the values were noted.

On 45th day all rats from each group were Anaesthetised and blood collected from the Orbital Plexus for Blood Acetyl-cholinesterase test and hours later 2 rats from each group were sacrificed through deep ether Anaesthesia and Brain was removed by dissection and weighed and sent for Histopathology studies by storing in 10% Formalin.

RESULTS:

Results of pharmaceutical study

Table no.3.1 showing results of Shodhana of ingredients.

Sr. No	Ingredients	Weight before Shodhana	Weight after Shodhana
1	<i>Hingula</i>	160gm	165gms
2	<i>Gandhaka</i>	250gm	200gms
3	<i>Pooram</i>	100gm	92gms
4	<i>Veeram</i>	100gm	94gms

Table no 3.2 showing final result of Chandamarutha Sindoor

Sr. No	Ingredients	CMS -1	CMS-2	CMS-3
1	<i>Rasa Pushpa</i>	6.67gm	6.25gm	9.72gm
2	<i>Rasa Karpoora</i>	26.67gm	25gm	9.72gm
3	<i>Rasa Sindoor</i>	6.67gm	50gm	694.4mg
4	<i>Sh. Gandhaka</i>	6.67gm	6.25gm	694.4mg
5	<i>Sh. Hingula</i>	53.33gm	12.50gm	79.17gm
Total quantity obtained after Bhavana with egg white		102gm	103gm	102gm
Colour of final the product		Bright red	Bright red	Dark red

Results of analytical study



Organoleptic characters were analysed. Sample 1 dark red in colour with faint characteristic odour.
and 2 were bright red in colour and sample 3 was All the samples were tasteless and lustreless.

Table no. 3.4 showing analytical parameters of *Chandamarutha Sindoor* 1,2& 3

Parameters	Results n=3 %w/w (Avg±SD)		
	CMS 1	CMS 2	CMS 3
Samples			
Ph	5.75	5.62	5.82
Loss on drying (Moisture content)	5.01±0.00	3.94±0.06	1.97±0.02
Total ash	24.12±4.14	19.65±0.26	8.26±0.00
Acid insoluble ash	4.20±0.00	2.67±0.03	1.06±0.01
Water soluble ash	6.41±0.02	2.97±0.02	2.22±0.02
Alcohol soluble extractive value	5.56±0.03	4.92±0.02	9.9±0.01
Water soluble extractive value	10.28±0.00	7.82±0.01	4.91±0.02
Particle size (µm)	100	100	100

Experimental study results:

EFFECT OF *CHANDAMARUTHA SINDOORA* FOR NEURODEGENERATIVE ACTIVITY

Experimental evaluation in the $AlCl_3$ - induced Neurodegenerative model of Rats demonstrated

improvement in Open field test Behavioural parameters, Cholinesterase activity, Brain weight and Histopathological changes in Brain tissue. Among the three formulations of *Chendamarutha Sindoor* samples, CMS - 3 exhibited comparatively better neuroprotective and Neuroregenerative activity followed by CMS 2 and CMS 1.

Table 3.6 showing effect of test drug on open field behaviour test:

GROUPS	NO. OF SQUARES CROSSED		
	OUTER SQUARE	MIDDLE SQUARE	INNER SQUARE
NORMAL CONTROL	98.67±20.54	4.50±1.23	1.33±0.33
DISEASE CONTROL	5.80±3.49**	3.00±2.00	1.00±0.63
CMS 1	8.33±3.14	2.00±0.93	1.33±0.80
CMS 2	13.83±7.22	2.88±2.83	1.50±1.50
CMS 3	3.83±2.10	1.33±0.84	0.33±0.33

Table no 3.7 showing effect of test drug on open field behaviour test

GROUPS	REARING	GROOMING	FECAL PELLETS	FREEZING TIME
NORMAL CONTROL	26.17±6.88	15.17±4.85	1.33±0.42	11.00±9.81
DISEASE CONTROL	0.80±0.37**	1.60±1.16	1.80±0.58	168.0±34.99*
CMS 1	4.667±2.07	11.67±6.66	2.33±0.33	140.0±45.61
CMS 2	2.16±2.16	20.83±15.09	4.00±1.18	163.3±34.03
CMS 3	5.16±2.16	5.66±3.66	2.66±0.61	78.67±35.34

Table no 3.8 showing effect of test drug on behaviour despair test

GROUPS	IMMOBILITY FREQUENCY	IMMOBILITY TIME
NORMAL CONTROL	16.83±1.27	46.50±12.16
DISEASE CONTROL	4.80±2.03**	9.40±5.04**
CMS 1	2.00±1.36	7.66±6.54
CMS 2	2.16±0.87	4.83±2.38



CMS 3	0.83±0.83	2.00±2.00
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Table no 3.9 showing effect of test drug on cook's pole climbing test

GROUPS	LATENCY TO JUMP	TIME SPENT ON POLE
NORMAL CONTROL	30.83±8.79	22.50±6.67
DISEASE CONTROL	23.60±8.25	13.80±5.08
CMS 1	24.50±6.06	10.67±0.66
CMS 2	31.00±9.13	7.10±1.83
CMS 3	13.67±6.00	33.83±8.05

Table no 3.10 showing Blood Cholinesterase results

GROUPS	CHOLINESTERASE
NORMAL CONTROL	766.0±45.92
DISEASE CONTROL	578.2±57.30*
CMS 1	498.0±22.09
CMS 2	580.2±27.80
CMS 3	559.2±54.64

Table no 3.11 showing Brain weight of rats

GROUPS	BRAIN WEIGHT
NORMAL CONTROL	0.88±0.024
DISEASE CONTROL	1.54±0.03**
CMS 1	1.45±0.04
CMS 2	1.38±0.12
CMS 3	1.65±0.03

Discussion:**Discussion on Pharmaceutical study**

As the drug CMS is an important formulation in both Siddha and Ayurveda systems of medicine, the *Shodhana* of *Gandhaka* and *Hingula* was done according to the reference from Ayurvedic literature while *Shodhana* of *Pooram* (*Rasa Karpooora*) and *Veeram* (*Rasa Pushpa*) was done according to the reference from Formulary of Siddha Medicine.

Rasa Karpooora and *Rasa Pushpa*: As there was difference in properties of *Pooram* (HgCl_2) and *Veeram* (Hg_2Cl_2) in modern literature, so as in the procedures of *Shodhana*. In *Pooram Shodhana* the *Pottali* was immersed completely in the boiling liquid of *Dola Yantra* where as in the *Veeram*

Shodhana the *Pottali* was tied above the boiling liquid.

From the above changes in both procedures, we can infer that immersion of *Rasa Pushpa Pottali* in *Shodhana Dravya* may dissolve the content and can reduce the quantity or there may be chances of vaporization of chemical constituents due to heat. Hence steaming by hanging above the liquid was done. Whereas *Rasa Karpooora* was hung, by immersing in the tender coconut water, during *Dhola Yantra Swedana* might helped to reduce the toxicity of *Rasa Karpooora*. It was also noted that as per the modern chemistry the rise in temperature will increases the solidity and reduces the solubility of *Rasa Karpooora*, which may be the actual reason to immerse the *Pottali* in Tender coconut water, as mentioned in the Siddha classic.



Even though the HgCl_2 (*Rasa Karpoora*) is said to be soluble in water unlike the Hg_2Cl_2 (*Rasa Pushpa*) there was loss of only 8gm *Shodhita Rasa Karpoora* after *Swedana* procedure which may be due to the erosion of superficial surface of *Rasa Karpoora* by the liquid media.

Gandhaka Shodhana: Milk and Ghee used during the procedure might helped to remove the toxicity and fat-soluble impurities. During purification of *Gandhaka* profuse fumes of Sulphur dioxide was evolved and the *Ugra Gandha* of *Gandhaka* was much reduced after *Shodhana*.

Hingula Shodhana: The quantity of *Bhavana Dravya* decreased from 1st to 7th *Bhavana*, may be because of the remnant moisture content of earlier *Bhavana* present with the *Hingula*. No shining particles were seen after the 7 days of trituration with *Ardraka Swarasa* which indicates proper trituration.

Market Sample of *Rasa Sindoor* was taken and utilized as one of the ingredients in the *Chandamarutha Sindoor* preparations according to the ratios mentioned the Classics and the colour and appearance of *Rasa Sindoor* was bright red and powdery without any shining.

In the present study the preparation of CMS was done as per the method of *Arappu Chendooram* (trituration) of Siddha medicine instead of *Kupipakva* method or *Damaru Yantra* method. In *Kupipakva Rasayana* preparations the colour of the final product may vary depending on the ingredient (*Svarna vanga*- golden yellow colour, *Rasa karpoora* – white colour etc) but most of them were named as *Sindoor* because of the red colour (*Rasa Sindoor*, *Shila Sindoor* etc). whereas in Siddha all the *Sindoor* / *Chendooram* preparations resemble various shades of red colour, hence the name *Chendooram* was

attributed and they are prepared with different techniques.

Discussion on analytical study

Determination of pH around 5.75-5.82, helps in identification of acidic nature of the samples. LOD was 5.01 in the CMS1 sample which is significantly high compared to 1.97 in the CMS 3 sample. Which indicates the moisture content of the sample in percentage. Value of total ash determines the presence of inorganic material in the sample which was 24.12% in CMS 1 and very less in CMS 3 i.e; 8.26 percentage. Similarly, water soluble ash and acid insoluble ash determines the percentage amount of ash that is soluble in water and insoluble in acid respectively which was more in sample 1 compared to 2 and 3. Alcohol soluble extractive value was found to be highest in Sample 3 and water-soluble extractives was found to be more in sample 1, and hence proper conclusion cannot be drawn with these values. Finally, determination of particle size of the *Chandamarutha Sindoor* through microscopy was done which shown same particle size i.e; 100 μm in all the 3 samples.

XRD analysis revealed the samples are standard, as the peaks matches the standard *Chandamarutha Sindoor* and it showed the presence of Mercuric Chloride, Mercurous Chloride, Mercuric Sulphide, Sulphur and traces of hydrated Calcium Sulphate. The presence of Calcium may be because of egg white added during preparation of the final compound. Most of the peaks in all the samples were resembling to each other and CMS 2 and CMS 3 almost nearer to each other. The highest peaks were produced because of the compound HgS and then Hg_2Cl_2 and HgCl_2 . The Market sample of *Rasa Sindoor* added in the preparation had the analytical report demonstrated that all tested parameters were within the standard limit.



(Hg-85.78%w/w, Sulphur- 13.05% w/w, XRD profile-confirmed characteristic peaks of HgS)

To analyse heavy metal content in the drug, ICPMS (Inductively Coupled Plasma Mass Spectrometry) was used and it revealed the presence of mercurial compounds as major constituent may be because the drug was primarily constituting Mercury based compounds and other heavy metals were not detected or may be detected in negligible amount.

Hence the analytical study given best supportive results nearest to the standard, confirming all the three samples were pure and safe to administer.

DISCUSSION ON EXPERIMENTAL STUDY:

Despite numerous research efforts made in treating *Pakshaghata* / Hemiplegia, there are still no satisfactory therapies for neuronal damage after stroke. Most of the current treatments are directed towards alleviating the symptoms of the disease and not on the treatment of the root cause of neurodegeneration.

In stroke conditions, current strategy only removes the clot and restores the cerebral blood flow, but fails to address the existing events such as excitotoxicity, inflammation and oxidative stress. These events may cause rigorous degeneration of neurons in the vital regions of the brain which determines the intensity of physical disability in stroke patients.

As $AlCl_3$ causes neurodegeneration or neuronal damage in rat model, hence this model was selected to study the Neuroregenerative activity or repair of neurons by administering the *Chandamarutha Sindoor* through oral route and the outcomes were compared to identify the most efficacious sample.

Aluminium (Al) has been associated with the modification of many biomolecules that are associated with neurotoxicity and the occurrence of various neurodegenerative condition inducing oxidative stress, inflammation, and neuronal damage.

Mechanisms such as oxidative stress and inflammation play a significant role in mediating neurodegenerative processes. Aluminium chloride ($AlCl_3$) is a well-documented neurotoxicant, which induces oxidative stress and inflammation, which makes it relevant to neurodegenerative activity study in animal models.

DISCUSSION ON RESULTS OF EXPERIMENTAL STUDY

The comparative study of the three samples was assessed using behavioural parameters such as the Open Field Test, Behavioural Despair Test, and Cook's Pole Climbing Test, along with neurochemical and histopathological evaluations. The findings revealed varying degrees of neuroprotective activity among the three formulations.

CMS-3 demonstrated better restoration of behavioural and neurochemical parameters, including locomotor activity, cognitive performance, suggesting its effectiveness in improving functional deficits associated with neurodegeneration. CMS-2 exhibited moderate neuroprotective activity and showed improvement in open field test, behavioural despair test, blood acetylcholinesterase and histopathological parameters. CMS 1 showed comparatively low parameters among CMS 2 and CMS 3. *Chandamarutha Sindoor* sample 3 produced the most pronounced histopathological protection, with a marked reduction in pyknotic and degenerated neurons in the cerebrum and



hippocampal regions, indicating its ability to preserve neuronal architecture.

Overall, all three formulations exhibited neuroprotective potential against $AlCl_3$ -induced neuronal damage. CMS-3 showed superior

efficacy in behavioural and neurochemical assessments and demonstrated greater protection at the tissue level. Therefore, the efficacy of the formulations was assessed by correlating behavioural, neurochemical, and histopathological findings to identify the most effective formulation.



Fig 1 Raw Rasa karpura



Fig 2 Rasa karpura shodhana



Fig 3 Raw Rasa Pushpa



Fig 4 Rasa Pushpa Shodhana



Fig 5 Melted Gandhaka



Fig 6 Melted Gandhaka in Ksheera



Fig 7 Shodhita hingula



Fig 8 Rasa Sindoor



Fig 9 Preparing CMS



Fig 10 Chandamarutha sindoor



Fig 11 Injecting AlCl₃



Fig 12 Behavioural despair test



Fig 13 Open field test



Fig 14 Cook's pole climbing test

CONCLUSION:

- According to the conceptual study, the drug CMS possesses *Prabhava* due to its unique

combination of ingredients during pharmaceutical processing's. *Prabhava* remains the highest in the hierarchy of pharmacological actions of a drug especially



the *Rasoushadhi*'s. As *Rasa Dravyas* are useful in *Asadhya Rogas*, *Chandamarutha Sindoor* has the potential to treat many severe diseases including *Pakshaghata*, *Vatavyadhi*, *Arbuda*, *Granthi*, and *Mahodara* etc.

- Pharmaceutically, *Chandamarutha Sindoor* can be prepared with different proportions of *Rasa Karpoora*, *Rasa Pushpa*, *Gandhaka*, *Hingula*, and *Rasa Sindoor*. The classical method of processing is uncommon and should be carried out with utmost care. The formulation can be prepared by following the procedure adopted in the present study. To make the pharmaceutical process easier, *Rasa Karpura* and *Rasa Pushpa* were purchased from the market and relevant *Shodhana* techniques were carried out as mentioned in the *Siddha* practices. *Rasa Sindoor* was bought from the local market, *Hingula* and *Gandhaka* were purified according to *Ayurvedic* classics before mixing them in different ratios and *Bhavana* was given with Egg white.
- Experimental evaluation in the $AlCl_3$ - induced Neurodegenerative model of Rats demonstrated improvement in Open field test Behavioural parameters, Cholinesterase activity, Brain weight and Histopathological changes in Brain tissue. Among the three formulations of *Chendamarutha Sindoor* samples, CMS 3 exhibited comparatively better neuroprotective and Neuroregenerative activity followed by CMS 2 and CMS 1.
- Based on the findings of the present study, it can be concluded that *Chandamarutha Sindoor* prepared according to the classical references possesses significant potential in managing Neurodegenerative conditions associated with neuronal damage.

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