"A RESEARCH PROTOCOL FOR ASSESSMENT OF RELATION BETWEEN PITTADHARA KALAA AND MAJJADHARA KALAA WITH INTERVENTION OF CHITRAKADI VATI IN ROTENONE INDUCED PARKINSON DISEASE IN WISTAR RATS."

AUTHORS:

*DR. PANKAJ S. MUSALE[1],DR. PRITI DESAI[2], AKHILESH AGRAWAL[3], DR. AMOLI BELSARE[4]

Designation and Affiliation of Authors:

1] Asso. Prof. & H.O.D. Rachana SharirDepartement, Shree RMD Ayurved College & Hospital, Waghaldara, Dist: Valsad, Gujrat, India. Mob No. 9422757676, Email Id: drpankajmusale83@gmail.com

2] Professor and Head, Dept. of Rachana Sharir, Mahatma Gandhi Ayurved College, Hospital & Research Centre, Datta Meghe Institute of Medical Sciences (Deemed to be University), Wardha, Maharashtra, India. Mob. No- 9923020425, Email Id:- drprdesai@gmail.com,

3] Research Consultant, Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Wardha.

4] Professor, Electronics & Tele. Engineering, Yeshwantrao Chavan College of Engineering, Nagpur

Corresponding Author:

Dr. Pankaj S Musale

Article Type: Study Protocol

Conflict of Interest: None

Funding: Self

IAEC/DRC Approval:

IAEC Approval: DMIMS (DU)/IAEC/2018-19-08 dated 13th Nov 2018.

DRC Approval: DMIMS/PhD. Regn/2019/96 dated 22nd Feb 2019.

ABSTRACT

The Kalaa is that the structure intervening between dhatus and their ashayas which are seven in number.As per Commentry by Dalhanacharya the kalaa which is pittadhara also the majjadhara kalaawhich is described in visha-vegantara in kalpasthana. This relationship has also clinical importance in Ayurvedic treatment factor. This relation has not yet being studied. As kalaa is that the structure intervening dhatu and it's ashaya, the majja dhatu is concerened with Majjadharakalaa while the Grahani is that the site of PittadharaKalaa.. In present study chitrakadivati is taken into account because the treatment of pittadharakalaa and Parkinson disease is assumed to be the disease of Majjadharakalaa and which is that the disorder of central systema nervosum as per modern science.During this experimental study the drug chitrakadivatiis given to rotenone induced Parkinson disease model of wistar rats. Behavioural assessments like open field test, ladder climbing test and wire hanging test are going to be done before the beginning of the treatment, and so on 6^{th} day and at last after 1 hour of last dose. The biochemical estimation i.e. Aspartate and alanine transaminases (AST and ALT), Serum urea, Serum creatinine are assayed after the last dose.We anticipate that there is also an improvement in Parkinson disease after giving Chitrakadivati. Hence here is an effort to determine protocol for the assessment of relationship between pittadhara and majjadharakalaa.

KEYWORDS:

Kalaa, PittadharaKalaa, Majjadharakalaa, Parkinson disease, Chitrakadivati

BACKGROUND:

Kalaais thebody structure describe in classical ayurvedic text which is one in all the foremost difficult topics to grasp scientifically. So there's a necessity of scientific understanding of concepts like exact form, structure, function and importance of kalaashareer. The kalaa is that the structure intervening between dhatus and their ashayas which are seven in number. Kalaa must be exceedingly minute and invisible to the unaided eye [1].

The concept of kalaahad been explained by Acharya Sushrutaduring which there's mentioned the instancelike, on cutting the wood, its pith is observed, and likewise dhatu is found dissecting the musculature. Kalaa are the structures which are covered by ligaments spread as membranous structure like amniotic membrane and streaked with slesma (mucus)[2]. Seven varieties of Kalaawith significant definition are explained by Sushrutaamong which sixth is Pittadharakalaa which holds four forms of annapana (food and drink)[3]and is placed in between amashaya and pakvashaya. Pittadhara kalaa is that the site of internal agni which holds food, digests it, absorbs useful part and liberates waste part. If it gets affected with vitiation of dosha, it liberates undigested food material[4]. The body remains healthy because of normal functioning of grahani supported by agni.

According to Dalhanacharya the kalaa which is pittadhara also the majjadhara kalaa which is precise in keeping withShareer [5]. Though the abovementioned relation is explained invisha-vegantara in kalpasthana, it is havinggreat significance in clinical practice.

As kalaa is that the structure intervening dhatu and it's ashaya, the majja dhatu is concerened with Majjadharakalaa.In accordance with Induteeka, it's Medodhatu, which turns into Mastulunga and it'sMedodhatu again which gets became Majjadhatu. Mastulunga could be asadyahpranaharamarma. Mastulunga is present inside the skull. Majja is the term which can be correlated with marrow, as in bone-marrow (asthi-majja). So majja is supposed to be concurrent with the systema nervosum and which is enclosedinside bone resembling bone marrow[6].Therefore the cranium is that the shell of the brain while the vertebrae are the shell of the spinal cord. The spinal cord and brain are the components of central nervous system. Therefore in Ayurveda, systema nervosum and the bone marrow are assumed as homologous anatomical structure. During this study Parkinson disease is assumed to be the disease of Majjadharakalaa and which is the disorder of central nervous system as per modern science [7].

Pittadharakalaa is situated in between Aamashaya and Pakvashaya. As described in Ayurvedic context, partially digested food in Aamashaya is hold by the action of Pitta so propelled to the subsequent part of Grahani foradditional function like Shoshan and Mumchan. Therefore theGrahaniis the site of PittadharaKalaa where Pachakagni works. In present study chitrakadivati is taken into account because the treatment of pittadharakalaa which is that the drug employed ingrahanichikitsa [8]. Each ingredient of this chitrakadivati has no direct relevance for improving the diseases of Majjadharakalaa. During this experimental study the drug chitrakadivatiis given to rotenone induced Parkinson disease model of wistar rats, the improvement in Parkinson diseasewill establish relation of Pittadharakalaa with Majjadharakalaa.

AIM &OBJECTIVES:

Aim: To assess relationship between pittadharakalaa and majjadharakalaa with intervention of chitrakadivati in parkinson disease in wistar rats.

Objectives:

- To Study the MajjadharaKalaa in perspective of Shareer.
- To evaluatechanges in behavioural and biochemical parameters in Group I with intervention of 0.9ml Nacl as a control group.
- To evaluatebehavioural and biochemical parameters in Group II with intervention of Rotenone.
- To evaluate behavioural and biochemical parameters in Group III with intervention of Syndopa and Rotenone.
- To evaluate behavioural and biochemical parameters in Group IV with intervention of ASCV (aqueous solution of chitrakadivati) and rotenone.
- To evaluate behavioural and biochemical parameters in Group V with intervention of Syndopa, ASCV and rotenone.
- To comparebehavioural and biochemical parameters among all groups.

MATERIAL METHODS:

Selection of Animals:

The male adult healthy albino wistar rats having weight in between 150 to 220gm aregoing to be collected from the registered animal house of DMIMS (DU) Wardha, Maharashtra, India. The procedure of this experiment has been sanctioned by IAES (Institutional Animal Ethics Committee)of DMIMS.All rats are going to be accommodated in cages of polypropylene, under maintenance of standardfarming conditions. The rats are going to be supplied with diet of standard pellet and adequate water throughout duration of the study.

Procurement of drugs

Chitrakadi Vati is going to be procured from GMP certified Pharmaceuticals. The vati will be powdered to form homogenous aqueous solution. Rotenone (Catalogue No: R8875-1G) will be purchased from Sigma Alderich.

Test for acute oral toxicity of ASCV: [9]

The test of acute oral toxicity will be conducted to figure outLD99 value of solution of chitrakadivati. Experiments are prearranged using young healthy albinowistar rats weighing 150-220 g.

Assignment of animals

The animals are going to be separated into five groupsafter randomization. Eachgroup consists of six rats. They'll be recognized by employing a yellow stain marks. In every group, excluding one rat (control), the others are going to be marked on different parts as Group I - Head, Group II- Body, Group III- Tail, Group IV- Head and Body to simplifystudy.

Diet and accommodationof animals:

Albinowistar rats are going to be housed in polypropylene cages measuring 55cm x 32.7cm x 19 cm with standard pellet diet and the temperature is going to be maintained at 23 ± 2^{0}C 2ºC. Illumination issynchronized to produce twelve hrs of sunshine &twelve hrs of dim for every day of 24 hrs.Everygroup of rats in cagesare going to be acknowledged by laminated card showing the information such as serial no. of cage, quantity of wistar rats in cageand weight of the each rat it contained, name of drug, dose and route of administration. The rats are going to be feed with diet of standard pillets and water ad lib.

Route of administration:

The study of acute oral toxicity of Chitrakadivati is going to be performed as per guideline of OECD. Total 6 albino wistar rats will be randomly selected in each group of experiment of acute oral toxicity. The rats are going to be fasted before administration of drug for duration of 12 hrs. There will be free access during fast.After the 12 hrs of fast, the rats are going to be weighed and test extract of Chitrakadivati will be given by oral route as Group I: 1000

mg/kg, Group II: 2000 mg/kg, Group III: 3000 mg/kg and Group IV: 4000 mg/kg. Following the dosing of ASCV, feeding of ratsis going to be withheld for the period of two hrs.

Assessment of symptoms:

The clinical assessment such as mortality of rats and signs which includes changes in skin, eyes, fur and mucous membrane are going to be noted. The assessment will be done initially after the duration of four hours and subsequently after 72 hrs and 7 days of infusion of drug i.e. Chitrakadivati. The behavioral assessment such as locomotion, rearing, position of body, tremors and gait are going to be observed for complete seven days. The other parameters such as pain response, righting reflex and grip strength are also going to be observed during experiment.

Methodology

30 Male wistar rats having weight 150 to 220 gm arealienated into five groups. Each group consist of 6 albino wistar rats.

Table 1: (GroupWise drug, dose &duration)

GROUP	DRUG	DOSE	ROUTE	DURATION
I	0.9% Nacl	2 ml	Per Oral	12 Days
II	Rotenone	12 mg/kg per day	Per Oral	12 Days
III	Rotenone +	12 mg/Kg per day	Per Oral	12 Days
	Syndopa	10 mg/Kg per day		
IV	Rotenone+	12 mg/Kg per day	Per Oral	12 Days
	ASCV	45 mg/Kg per day		
V	Rotenone+	12 mg/Kg per day	Per Oral	12 Days
	ASCV +	45 mg/Kg per day		
	Syndopa	10 mg/Kg per day		

Behavioural Assessment

Behavioural assessments are going to be carried out before the begining of the treatment;soon 6th day and final behavioural quantification are done after 1 hour of last dose.

Test in Open field apparatus: [10]

This test is going to be done for assessment of locomotion, exploration and anxiety with the help of open field apparatus. This apparatus is the wooden box measuring 72cm x 72cm x 36cm and the floor of the box is divided into 16 squares by marker pen. Each rat is going to bepositionedinside the open field apparatus in centre. The three parameters such as ambulation, rearing and grooming are going to be assessed individually for five minutes.

(i) Ambulation: The crossing of number of lines by rat with four paws.
(ii) Rearing: Frequency of standing of rats on hind paws.

(iii) Grooming: Observation of duration of licking and self scratching of rat while stationary.

Open field apparatus used for experiment is going to be cleaned before each assessment. The solution of ethanol 5% will be used for cleaning. The purpose of cleaning is to get rid of bias because of odour of previous animal.

Test of Ladder climbing [11]

For the assessment of this test, the instrument of grip walk is going to be used. This apparatus is kept 2 cm apart with inclination at 45^0 angles. The experimental rat is going to be positionedon the surface of ladder and prepared to climb on the equipment. Then movement of experimental rat will be noted with score which indicates the activity of muscles.

Test of Hanging wire [12]

The test of Hanging wire is going to be conducted for the assessment of strength of forelimbs. The equipment made up of wire of stainless steel having 90cm length with diameter of 3mm. The wire is presetin horizontal direction between 2 vertical stands at 60cm height. The soft cushioned surface is prepared below the instrument. This test is going to be performed on the experimental rat and might be forced to gripthe central location of the steel wire with its forepaws. The latency(s) to fall from the steel wire to the soft cushioned surface is going to be noted. After reaching lag time over 120 seconds the experimental rat will be released from the steel wire and lag duration is going to be noted as 120 secs. The experiment will be executed for three times and the value of longest duration will be considered as the final value for evaluation. In between consecutive trials there will be resting pause of 3 min.

Biochemical estimation [13]

For the estimation of biochemical parameters the experimental rats are going to be anesthetized. After proper anaesthesia the blood sample will be collected from retino orbital site. After collection, blood sample will be allowed to clot for the duration of thirty minutes by keeping uninterrupted at room temperature. The serum from each sample will be separated by centrifugation. After separation of serum the biochemical test such as serum urea, serum creatinine, aspartate and alanine transaminase (AST and ALT)are going to be done with the help of analyser at Central Research Laboratory of DMIMS. A number of related studies were reported in GBD study [14-16].

RESULTS:

We anticipate that the Chitrakadivatii.e drug for pittadharakalaa may have better results in the treatment of Parkinsons disease i.e. disease of Majjadharakalaa.

CONCLUSION:

There will be relationship between pittadharakalaa and majjadharakalaa with intervention of chitrakadivati in parkinson disease in wistar rats.

REFERENCES:

1. Sharma P.V, Sushrut Samhita of Acharya Sushrut, with Nibandhasangraha Commentary, Sharirsthana, Chapter 4/5,1st Edition, Varanasi: Choukhanmba Orientalia Reprint 2007, 355p.
2. Sharma P.V, Sushrut Samhita of Acharya Sushrut, with Nibandhasangraha Commentary, Sharirsthana, Chapter 4/6,7, 1st Edition, Varanasi: Choukhanmba Orientalia Reprint 2007, 355p.
3. Sharma P.V, Sushrut Samhita of Acharya Sushrut, with Nibandhasangraha Commentary, Sharirsthana, Chapter 4/18, 1st Edition, Varanasi: Choukhanmba Orientalia Reprint 2007, 356p.
4. Sharma P.V, Sushrut Samhita of Acharya Sushrut, with Nibandhasangraha Commentary, Sharirsthana, Chapter 4/18, 1st Edition, Varanasi: Choukhanmba Orientalia Reprint 2007, 356p.
5. Sharma P.V, Sushrut Samhita of Acharya Sushrut, with Nibandhasangraha Commentary, Kalpasthana, Chapter 4/40, 1st Edition, Varanasi: Choukhanmba Orientalia Reprint 2007, 574p.
6. DesaiPriti., National Conference Proceeding Book "Kalanveshan-2019", A Modern Perspective Towards Interpretation Of 'Pittadharasaevamajjadharaiti'.,
7. Musale Pankaj S, Desai Priti, Critical review of verse "EvamaYevaPittadharaSaevaMajjadharaiti", International Journal of Ayurvedic Medicine, vol 11(1), 1-5
8. Tripathi Ravidatta , Charauk Samhita of Charakacharya,, Drudhabal, , Chikitsasthana, Chapter 15/96,97, 1st Edition, Delhi: Chaukhambha Sanskrit Pratisthan, 374p.
9. Dharmalingam Subha1, Natesan Geetha, Journal of Scientific and Innovative Research 2017; 6(3): 113-115.
10. Walsh RN, Cummins RA. The open-field test: a critical review. Psychol Bull 1976;83:482-504.
11. Cummings BJ, Engesser-cesar C, Cadena G, Anderson AJ. Adaptation of a ladder bear walking task to assess locomotor recovery in mice following spinal cord injury. Behav Brain Res 2007;177:232-41.
12. Tillerson JL, Miller GW. Grid performance test to measure behavioral impairment in the MPTP treated a mouse model of Parkinsonism. J Neurosci Meth 2003;123:189-200.
13. Vijayalakshmi *et al.*Int J Pharm Pharm Sci, Vol 9, Issue 11, 159-164.
14. James, Spencer L, Chris D Castle, Zachary V Dingels, Jack T Fox, Erin B Hamilton, Zichen Liu, Nicholas L S Roberts, et al. "Estimating Global Injuries Morbidity and Mortality: Methods and Data Used in the Global Burden of Disease 2017 Study." *Injury Prevention* 26, no. Supp 1 (October 2020): i125–53. https://doi.org/10.1136/injuryprev-2019-043531.
15. James, Spencer L, Chris D Castle, Zachary V Dingels, Jack T Fox, Erin B Hamilton, Zichen Liu, Nicholas L S Roberts, et al. "Global Injury Morbidity and Mortality from 1990 to 2017: Results from the Global Burden of Disease Study 2017." *Injury Prevention* 26, no. Supp 1 (October 2020): i96–114. https://doi.org/10.1136/injuryprev-2019-043494.
16. Murray, Christopher J L, Cristiana Abbafati, Kaja M Abbas, Mohammad Abbasi, Mohsen Abbasi-Kangevari, Foad Abd-Allah, Mohammad Abdollahi, et al. "Five Insights from the Global Burden of Disease Study 2019." *The Lancet* 396, no. 10258 (October 2020): 1135–59. https://doi.org/10.1016/S0140-6736(20)31404-5.