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Ayurveda

ROLE OF Citrullus colocynthis Schard ON ROTENONE INDUCED BRADYKINESIA IN EXPERIMENTAL RATS

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ABSTRACT The current study aims at investigating the effect of INDRAVARUNI – Citrullus colocynthis Schard on rotenone induced bradykinesia in experimental rats. 42 healthy male Wistar rats were selected for the study and were divided into 7 groups. Before inducing Parkinson's disease, the experimental animals were acclimatized for three days. The Rotenone solution was administered at 2.5mg/kg body weight/day intraperitonealy to all the groups, except for the normal control group which received only the vehicle (DMSO + Triester F 810). The respective treatment groups received their treatment from the day of starting of rotenone injection. Animals developing debilitating phenotype – limiting their mobility, feeding or grooming; were sacrificed and brain samples procured from them. Otherwise, the animals were assessed for their motor scores using Open field Test in three sessions – Day 0 (before induction), Day 2nd & Day 5th.

Ultimately the rats surviving till the 9th day were also sacrificed on 10th day and brain samples procured, which were used for further analysis. One sample from each group was analysed for striatal dopamine levels using HPLC, as a supportive observational study. The BID and TDS dosage groups showed maximum efficacy in preventing bradykinesia.

KEYWORDS : Citrullus colocynthis, Rotenone, Parkinson's disease, Bradykinesia.

INTRODUCTION:

Citrullus colocynthis an herb belonging to *Cucurbitaceae* family is an annual or perennial desert viny plant that grows in sandy arid soils.[1] It is widely used in the Indian system of medicine for different ailments[2] and is specifically prescribed by a classical Ayurvedic text *Sharangadhara Samhita* in the management of *KAMPA VATA* in the form of oil base preparation called *VARUNI TAILA*.[3] The clinical picture of *Kampavata* has a close match with the features of Parkinson's disease.[4,5,6] Parkinson's disease is one of the most common forms of a group of progressive neurodegenerative disorders characterized by *bradykinesia*, rest tremor, muscular rigidity, shuffling gait, and flexed posture[6].

Parkinson's disease as such is characterized by; Loss of ~50–70% of the dopaminergic neurons in the *substantia nigra pars compacta* & a profound loss of dopamine in the striatum[7].

Exposure of rats to the pesticide and complex I inhibitor rotenone reproduces features of Parkinson's disease, including selective *nigrostriatal dopaminergic cell* degeneration[8] and bradykinesia.

Hence, the current study is carried out to determine the effect of *Citrullus colocynthis* on rotenone induced bradykinesia in experimental rats.

MATERIALS AND METHODS:

Animals:

Healthy, adult, male Wistar rats (150-250g) were obtained from the Central animal house facility from J.S.S College of Pharmacy, S.S.Nagar, Mysore. The animals were kept in a well ventilated room and the animals were exposed to 12 hrs day and night cycle with a temperature between 20±3^{0}C. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and rat feed *ad libitum*. All the experiments were performed after obtaining prior approval from CPCSEA. The animals were housed in suitable environmental conditions.

Chemicals:

The chemicals, which were used for the present study, were procured from Sigma Aldrich USA, Indian commercial company PVT.Ltd, Mumbai, Merk chemicals Mumbai.

Collection of plant material and preparation *Varuni Taila:*

Roots of botanically identified *Citrullus colocynythis* were collected from its natural habitat. The roots were cleaned properly and were macerated well to prepare a paste. One part of this paste was added with 4 parts of sesame oil and 16 parts of water. This mixture was cooked on mild fire till the oil part remains, and then was filtered and stored in a glass container.

Study design:

Sample :

42 male Albino Wistar rats of middle aged group were selected for the study and were separated randomly into 7 groups of 6 animals in each (as shown in Table A).

Sr. No	Groups	No. of Rats	Inducing PD	Treatment
1	Negative Control	6	Rotenone + 98%Triester F 810 + 2% DMSO	Not Treated
2	Normal Control	6	Only 98%Triester F 810 + 2% DMSO	Not Treated
3	Vehicle Control	6	Rotenone + 98%Triester F 810 + 2% DMSO	Taila (Plain sesame oil – 1.34ml/Kg)
4	Test group A	6	Rotenone + 98%Triester F 810 + 2% DMSO	Varunitaila (1.34ml/Kg) OD dose
5	Test group B	6	Rotenone + 98%Triester F 810 + 2% DMSO	Varunitaila (1.34ml/Kg) Bid dose
6	Test group C	6	Rotenone + 98%Triester F 810 + 2% DMSO	Varunitaila (1.34ml/Kg) Tds dose
7	Standard Treated	6	Rotenone + 98%Triester F 810 + 2% DMSO	Standard dose (10mg/Kg) of L-Dopa

Table A: Grouping of experimental rats

Preparation of Rotenone solution:

Rotenone solution was prepared as a stock for 3 days in 100% Dimethylsulfoxide (DMSO) and diluted in medium chain triglyceride, Caprylic / Capric Triglyceride (Triester F 810) to obtain a final concentration of 2.5mg/ml rotenone in 98% Triester F 810 & 2% DMSO.

Vortexing the solution creates a stable emulsion of the DMSO containing rotenone &Triester F 810. Fresh stock solution was prepared twice a week and stored in a vial protected from light. Vortexing of the vial several times before each injection was ensured to eliminate the possibility of settling[10].

Schedule of the procedure:

Before inducing PD, the experimental animals were acclimatized for three days. The Rotenone solution thus prepared was administered at 1ml/kg body weight/day intraperitonealy to all the groups, except for the normal control group which received only the vehicle (DMSO +

Triester F 810) [10]. The respective treatment groups received their treatment from the day of starting of rotenone injection[11]. During daily handling, animals were observed closely for the emergence of Parkinson's disease phenotype[10, 11]. Animals developing debilitating phenotype – limiting their mobility, feeding or grooming; were sacrificed and brain samples procured from them [7]. Otherwise, the animals were assessed for their motor scores in three sessions on; ◦ Open field Test – Day 0 (before induction), Day 2nd & Day 5th.Ultimately the rats surviving till the 9th day were also sacrificed on 10th day and brain samples procured, which were used for further analysis.

Open field test[11]

Principle:

The **Open Field Test** (**OFT**) is an experiment used to assay general locomotor activity levels and anxiety in rodents in scientific research. It is a commonly used qualitative and quantitative measure of general locomotor activity and willingness to explore in rodents. The open field is an arena with walls to prevent escape. Commonly, the field is marked with a grid and square crossings. Rearing and time spent moving are used to assess the activity of the rodent. In the modern open field apparatus, infrared beams or video cameras with associated software can be used to automate the assessment process.

In the current study, video camera of VGA resolution was used to monitor the activity of rats using PANLABS, Smart Video Tracking Software 30 day's trial version.

Open field arena was custom prepared using wood ply board measuring 24×24 inch with walls on all the four sides with a height of 12 inches to prevent the escape of rats.

Procedure:

Each animal was placed inside the arena and the following parameters were measured for a period of 5 min: Total distance travelled, Rest time, slow time, Fast time, Rearing duration.
This test was conducted in 3 sessions;

Session 0: Before starting Rotenone Injection and their respective treatments.

Session 1: On 2nd day after starting Rotenone Injection and their respective treatments.

Session 2: On 5th day after starting Rotenone Injection and their respective treatments.

Estimation of Striatal Dopamine levels an observational study[12]:

One Rat brain from each group dissected out of the cranium, were washed in ice cold saline. Then the sub-cortical striatal structures were dissected on ice plate.

Monoamine neurotransmitters DA contents in rat brains were measured by HPLC coupled with electrochemical detector (Alburges et al, 1993). The brain tissue (100mg) was homogenised in an ice-cold solution of 0.4 M percloric acid containing 5 mM sodium bisulfite and 0.04 mM EDTA for avoiding oxidation and then centrifuged at 30,000g for 15 min at 4°C. 10 µl of the resulting supernatant was chromatographed on a C18 RP column using waters 1465 HPLC. The mobile phase consisted of 17.6% methanol (v/v) and 82.4% distilled water containing 0.0876 mM EDTA disodium, 1.512 mM triethylamine, 9 mM DL-10-camphorsulfonic acid, 20 mM $Na_2HPO_4.12H_2O$ and 15 mM citrate at a flow rate of 0.7 ml/min. The measurements were done at electrode potentials of a glassy carbon electrode +650 mV Vs Ag/Agcl reference electrode with waters 1645 electrochemical detector. DA was identified and quantified by comparing their retention times and peak areas to those of standards. The concentration of DA was expressed in ng/g wet tissue.

Statistical Analysis:

All the values were expressed as Mean ± SD. The data was statistically analyzed using One-way ANOVA with Post Hoc Tukey's Multiple comparison test using SPSS software. The values of $p < 0.01$ were considered as significant.

(Note: The Dopamine levels were not subjected to statistical analysis as it was an observational study conducted with only one sample in a group.)

Results:

Open field test Total distance travelled:

There is no significant difference in the mean values of all the groups from session 0 to 1. Whereas, when the values are compared between session 1 & 2, significant decrease occurs in the negative and vehicle control groups with *P value<0.01 (HS).* The Test group A with OD dosage also shows moderately significant decrease with *P value= 0.045 (0.01<P<0.05(MS))*.

When the values are compared between sessions 0 to 2 i.e. pretreatment to final session – the negative control, vehicle control, standard control and OD dosage groups show significant decrease in the total distance travelled.

Rest time:

There is no significant difference in the mean values of all the groups from session 0 to 1. Whereas, when the values are compared between session 1 & 2, moderately significant increase occurs in the negative and vehicle control groups with *0.01<P value<0.05(MS).*

When the values are compared between sessions 0 to 2 i.e. pretreatment to final session – the negative control, vehicle control and OD dosage groups show significant increase in the rest time. The standard control group has also shown moderately significant increase in the rest time from session 0 to 2 with *P value = 0.033* (*0.01<P value<0.05(MS))*

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Slow time:

There is no significant difference in the mean values of all the groups from session 0 to 1. Whereas, when the values are compared between session 1 & 2, moderately significant decrease occurs in the negative and vehicle control groups with *0.01<P value<0.05(MS).*

When the values are compared between sessions 0 to 2 i.e. pretreatment to final session – the negative control, vehicle control and OD dosage groups show significant decrease in the slow time. The standard control group has also shown moderately significant decrease in the slow time from session 0 to 2 with *P value = 0.045* (*0.01<P value<0.05(MS))*.

Fast time:

Statistically there is no significant difference among the sessions with respect to fast time. Although percentage decrease in the fast time lies among the sessions with maximum decrease in the negative control group and minimum decrease in OD, BID and TDS dosage groups.

Rearing duration:

Statistically there is no significant difference among the sessions with respect to rearing duration.

Striatal Dopamine levels:

Striatal dopamine levels estimated with HPLC coupled with electrochemical electrode showed no dopamine peaks at retention time 10 mins in negative and vehicle control groups. Rest of the other groups had dopamine peaks, with highest concentration in TDS dosage group other than the normal and standard control group.

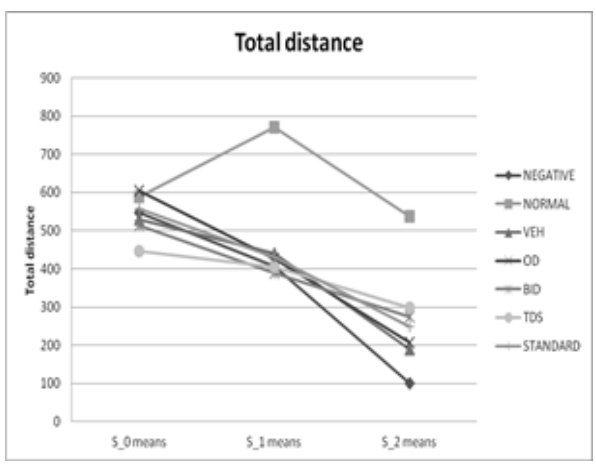


Chart A

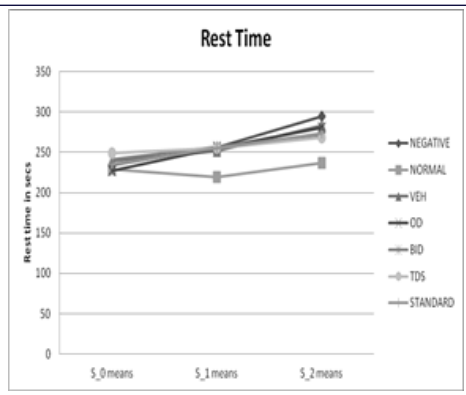
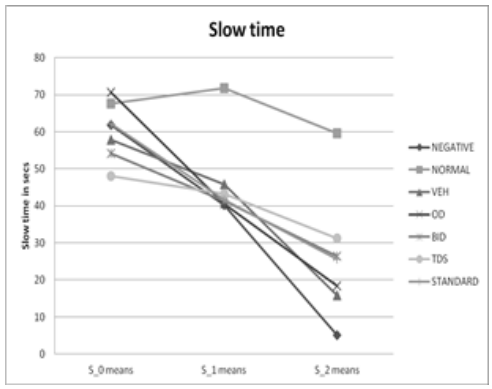


Chart B



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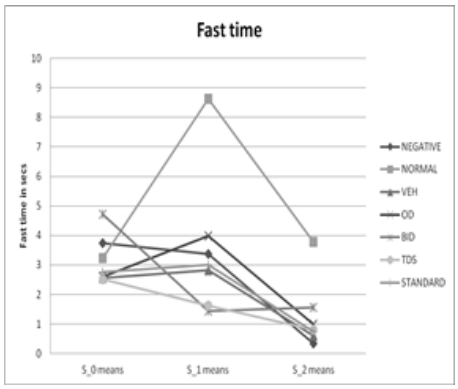

Chart D

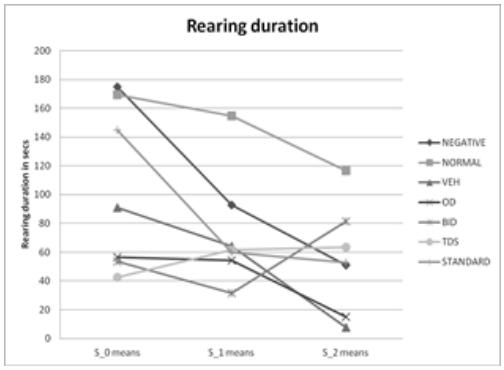

Chart E

Chart (A, B, C, D & E):
Intersession comparison of means of Total distance travelled(A), Rest time(B), Slow time(C), Fast time(D), & Rearing duration(E).

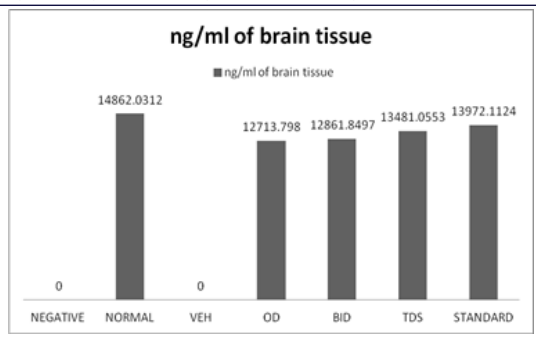

Chart F: Showing striatal dopamine levels in various groups.

DISCUSSION:

Varuni Taila is prescribed by an Ayurvedic text in the management of a clinical condition called *Kampavata* [3], whose features closely match with that of Parkinson's disease [4,5,6]. Parkinson's disease being characterized by bradykinesia as one of the major hallmark, the current study is employed to assess the role of the test drug on Rotenone induced bradykinesia in experimental animals using Open field arena.

The results obtained from the open field test shows that the negative control group has significant decrease in the total distance and slow time; and has significant increase in the rest time. This signifies the induction of the disease with respect to locomotor and behavioral parameters has occurred in the induced rats. The BID and TDS dosage group has shown no significant decrease between the sessions 0 to 2 with respect to total distance travelled and slow time; And no significant increase in the rest time. These results signify that the BID and TDS dosage of Varuni Taila has prevented the drastic fall in the locomotor parameters- total distance and slow time. Mean while it has also prevented increase in the rest time when compared to negative control group. This effect may be due to the Neuro protection caused by the Varuni Taila in the respective dosages, via its pro oxidant - anti oxidant effects.

The TDS dosage group has shown the dopamine concentration of about 13,481.055, which is close to the standard control group. The negative and vehicle control groups have shown no dopamine peak at 10 mins retention time. This loss of dopamine may be associated with dopaminergic cell degeneration in the nigro-striatal tissue due to the rotenone toxicity. The existence of dopamine concentration in other groups may be due to the Neuro- protection caused by Varuni Taila. However a much elaborate study has to be undertaken to infer in this regard.

Conclusion:

The BID and TDS dosage of the test drug showed significant effect in preventing the rotenone induced bradykinesia.

Rotenone toxicity has caused severe depletion of striatal dopamine levels which is prevented by the treatment with test drug VARUNI TAILA and Standard drug L-Dopa.A much elaborate study followed by clinical trial is required to validate the use of this test drug.

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