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Original Research Article

A STUDY ON THE BEHAVIOUR OF HUNTINGTON'S CHOREA RAT MODELS ON ROTAROD: TREATED WITH WITHANOLIDE A AND THE ETHANOLIC EXTRACT OF WITHANIA SOMNIFERA

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ABSTRACT

Background: Huntington's disease (HD) is a fatal neurodegenerative disease named after George Summer Huntington who first described the disorder in 1872. Huntington's disease is associated with basal ganglia degeneration which is called as the controlling center of extra pyramidal motor system that exerts an inhibitory effect on cerebral motor cortex. This will filters the unwanted motor movements and so refines the motor movements. Degeneration of neurons of basal ganglia reduces the inhibitory output and so leads to Huntington's disease. At present there is no cure for this disease and trials are going on to treat symptoms, slow the progress of the disease and repairing the damages caused by disease. So there is a necessity to produce an animal model of HD by using a neurotoxin kainic acid for research purpose. By this study we produced a simple and effective rat model of HD which is more mimicking the human model of HD. We also analyzed the role of the extract of a herbal plant Withania somnifera and its active principle withanolide A in preventing the nervous system of HD rat models.

Results: The activity of the herbal drug was analyzed by using rotarod apparatus. Both the drug group animals behaved normally in the rotarod against the lesion control animals and proved the efficacy of the drug employed.

Conclusion: Present days treatments are mostly given to reduce the progress of HD and to treat the symptoms. Complete curation of HD is not up to the mark. But by taking these herbal drugs by daily basis we can prevent the occurrence of HD as these drugs are very good in neuroprotection.

KEY WORDS: Huntington's disease, Extrapyramidal motor system, Cerebral motor cortex, Rotarod, Withania somnifera, Withanolide A.

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BACKGROUND

Huntington's disease (HD) is a neurodegenerative genetic disorder that causes defects in behavior, cognition, and uncontrolled rapid, jerky movements. HD is associated with basal ganglia degeneration [1] mainly by the degeneration of the indirect pathway cells of the striatum. This degeneration of striatal neurons projecting to external globus pallidus (GPe), leads to disinhibition of the indirect pathway, increased inhibition of substantia nigra, and therefore, reduced output of the basal ganglia. Huntington's disease is also known as Huntington's chorea [2] because it is characterized by a continuous, choreiform movements of the body (especially the limbs and face). Eventhough transgenic rat models are available for Huntington's model they are not fully mimicking the human model as they don't show striatal neural damage [3] and produce late onset of the

disease (15-24 months age). So by this study a simple and effective stereotaxic model of HD was produced using kainic acid as the lesion inducer which is more mimicking the human model.

Withania somnifera or Indian ginseng [4]. was considered as the queen of Indian medicine also said to have roles in nervous system by Ayurvedic legends. Recent studies proved that neuronal regeneration was possible in certain regions like hippocampus and sub ventricular zone and withanolide A-one of the active phytochemical of withania somnifera also was found to be inducing it [5]. But studies were not done proving its regenerative ability in stiratum. So we selected this herbal plant as our drug of choice to pretreat the animals and to analyze the neuroprotective role of the drug in striatum.

MATERIALS AND METHODS

Animals: We used adult male Sprague Dawly rats weighing (200–240 gm) for this study and maximum effort was taken to minimize the unwanted stress to the animals and to reduce the number of animal to be used for this study. Animals were divided into 5 groups with 6 animals in each group. They are control group (CO), lesion control (LC), sham control (SC), withania somnifera ethanolic extract 25mg/kg body weight (WS 125) and withanolide A 100mg/kg body weight (WD 100). The drugs were dissolved in normal saline [6] and the volume was adjusted to 1ml for each animal. The administration of drug was started 10 days prior to lesion surgery so as to access the protective role of the drug in striatal neurons. The dosage was given IP. around 10 O' clock every day.

Rotarod test for balance and motor coordination [7]: Striatum as a component of basal ganglia is mainly involving in the coordination of movements, so analyzing the motor coordination of the animals will clearly say the protective level of the drug given.

The rotarod test is used to assess the motor coordination and balance of the rodents so as to determine the brain function. The rats will be trained for 10 days prior to lesion surgery to keep their balance on a rotating rod and after lesion they will be checked for balance and coordination using a rotarod rotating at a speed of 10 rotations per minute.

Training the animals in rotarod apparatus: The apparatus contains an accelerating, motor driven apparatus with a grooved rotating cylinder that facilitates the animals grip. The cylinder will be divided into 5 units with wall for 5 animals to be tested at a time. The unit has a standard fall height of 40cm, a foot plate and a digital monitor to notify the latency time. At time when the rat fell on the foot plate the time on monitor stops. The rod can spin in both clockwise and counterclockwise directions with the speed 0-100 RPM acceleration Rate.

Trials:

- It consists of three trials separated by 15 min inter-trial intervals for 10 consecutive days
- Rats should be kept in their home cages and acclimate to the testing room for at least 15 min
- Turn on the Rotarod apparatus and set the accelerating mode 10rpm in 180sec [8].
- Try to have the rats on the rod to keep their balance and allow positioning of all the rats in their respective lanes.
- Once all the rats are "ready" push the start button and the rod will be accelerating for 10 rpm to 180sec.
- Train the rats to balance in the rod for the given time.
- Leave a 15 mininter trial interval between consecutive trials of the same batch of rats
- Clean the area with 70% alcohol at the end of each trial.

Lesion Surgery [9] (Fig. 1): The animals were maintained in empty stomach 10hr before the procedure and were anaesthetized using pentothal sodium. A small incision was made in the scalp region and the connective tissue was removed to find the bregma. With the help of stereotaxic frame the striatum was marked 2.2mm anteriorly and 3mm bilaterally. Kainic acid was dissolved in 0.9% NaCl. 0.5micrograms in 0.5µl of kainic acid was taken in a micro syringe and was injected into the striatum bilaterally to a depth of 5mm (Fig. 2). The syringe was withdrawn and the scalp was sutured with

proper care. Proper antibiotic care was given for 3 days.

Fig. 1: Showing the process of lesion surgery.

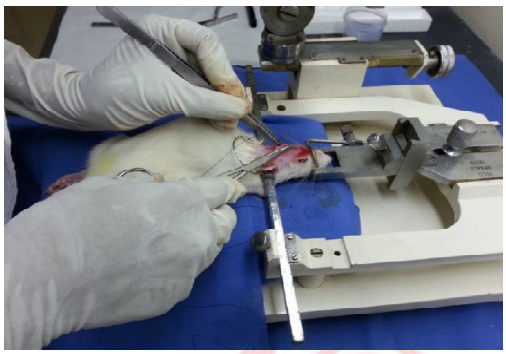
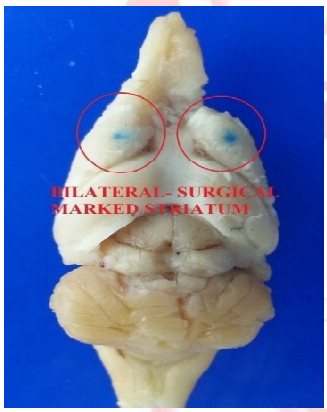


Fig. 2: Showing the bilateral surgically marked area.



Testing rats in rotarod apparatus

- Acclimate the rats to the testing room for at least 15 min
- Turn on the Rotarod apparatus and set the accelerating mode 10rpm for 180sec.
- Keep the rats on the rod and allow positioning of all the rats in their respective lanes.
- Once all the rats are "ready" push the start button and the rod will be accelerating for 10 rpm to 180sec.
- If the rats fall on the foot plate note the latency period and remove them from the apparatus.
- At the end of test, weigh each rat and take note of the body weight on the data sheet.

Parameters: Latency time – time the rat fall on the foot plate.

Scores:

- Low latency time – poor balance and poor motor coordination
- No latency or 180 seconds – good motor coordination

RESULTS AND DISCUSSION

Following lesion surgery after 30 minutes the LC animals exhibited choreiform movements both in the head region and limbs. The choreiform movements were continuous and continued for around 1 hour. During that period the face and the limbs of the LC animals were shivering continuously and were fading after 1 hour. Choreiform movements were found more on the fore limb than the hind limb (Fig. 3). Mild choreiform movements were also found in the body of the LC animals and the posture also was abnormal. But the animals belong to WS125 and WD100 group did not show any abnormal movement or posture as the pretreatment of the drugs preventing the striatum from lesion surgery (Fig. 4).

Fig. 3: Showing the LC animals with Huntington's chorea.

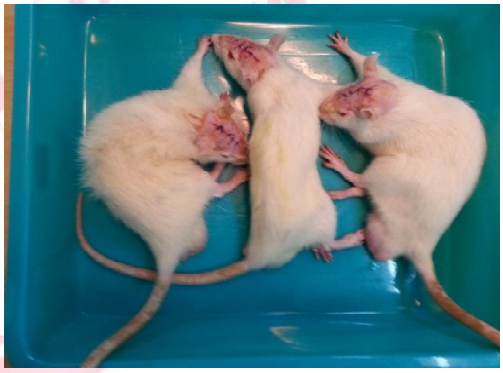


Fig.4: Showing the animals without choreiform movements.

Fig. 5: Showing the control animals in rotarod apparatus.

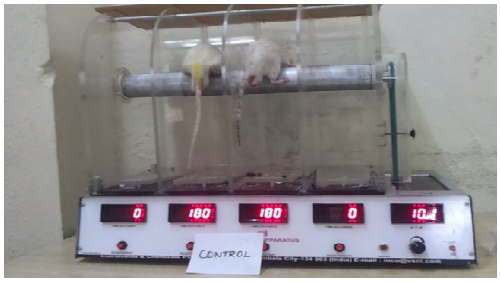
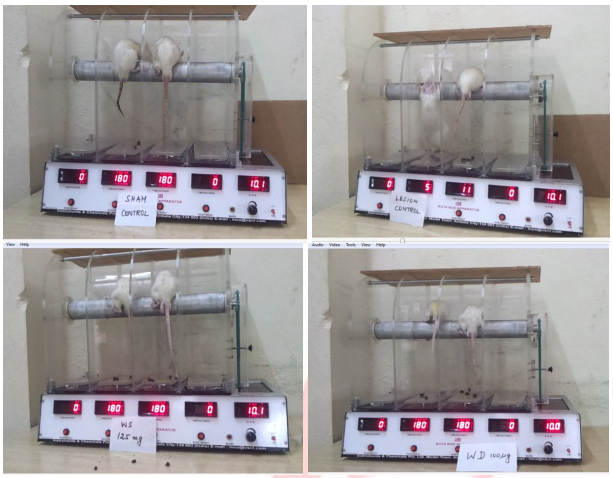
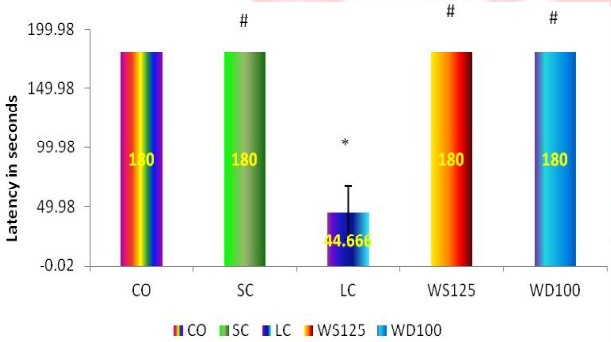


Fig. 6: Showing the latency time of other group of animals in rotarod apparatus.



Graph 1: Showing the latency of the animals in Rotarod test.



As the LC animals had lesion surgery without any protective drugs so they produced Huntington's chorea. Due to this disorder they lost their motor co-ordination and balance and were not able to stay on the rotarod till the end of the test time the 180 seconds (Fig. 5). They fell very often from the rod and their latency time was significantly less in comparison with the CO animals. The SC animals finished the task successfully without any latency time and were equivalent with the CO group. The drug group animals WS125 and WD100 also were finished the task without any latency (Fig. 6) (Graph-1). This says the drug employed in this study was good in protecting the striatum and so the animals behaved normally.

CONCLUSION

Chorea, which derived from the Greek word for "dance [1]," is characterized by continuous, writhing movements of the entire body. HD affects the whole brain, but certain areas are more vulnerable than others. According to Rosenblot and Leroi, [1] Huntington's disease is associated with basal ganglia degeneration. The earliest symptoms are problems with mood or cognition. A general lack of coordination and an unsteady gait often follows. There is no cure for HD, and full-time care is required in the later stages of the disease [10]. The neuronal degeneration and other complications such as pneumonia, heart disease, and physical injury from falls reduce the life and eventually cause death within 10 to 20 years.

In this study we pretreated the animals with the formulated drugs and performed lesion surgery to produce HD which is equivalent with human HD. The animals were also trained in rotarod during the time of pretreatment. Five days after the surgery animals were tested for their motor co-ordination in rotarod. The LC group animals were not performing well in this parameters and falling into the foot plate often. The WS125 and WD100 animal groups finished the task of 180 minutes balancing on the rotarod without falling into the foot plate. This is because both the drugs were effective in protecting the striatum and so the animals maintained their co-ordination and balance on the rotarod even after the lesion surgery.

Herbal medicines have always been a part of mankind's healing armamentarium and supports the wellness by enhancing the body's inherent healing potential [11]. As prevention is always better than cure and these herbal drugs are more preventive without side effects we can very much employ this herbal drugs in our daily food supplements like coffee, tea or other drinks so as to boost and protect our nervous system from such neurodegenerative disorders.

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Conflicts of Interests: None

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