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ORIGINAL ARTICLE

"Effect of Withania Somnifera and it's Active Principle in Protein and Antioxidant Vitamins on Experimentally Lesioned Basal Ganglia"

[1]A.Mary Antony Praba*, [2]Venkatramaniah, [3]Chidambaram

ABSTRACT

Huntington's disease is a fatal neurodegenerative disorder characterized by a continuous, choreiform movements or dance like movements of the limbs and face. Being a life threatening disorder it is necessary to produce a model of chorea in animals for research proceeding. For this study we produced a model of Huntington's chorea by injecting 0.5µl of kainic acid into the basal ganglia bilaterally and analysed the effect of Withania somnifera on it. The Sprague Dawly rats were divided into 8 groups CO, LC, WS100, WS125, WS150, WD5, WD50 and WD100, with 6 animals each. All rats except CO and LC were pre-treated with either ethanolic extract of Withania somnifera (WS) or the active principle (WD), 10 days prior to the lesion surgery. Lesion surgery was performed in all the rats except CO group rats, the brain tissues were collected within 24 hrs and the protein level and antioxidant Vitamins Vit C and Vit E were analysed. The protein and antioxidant vitamin analysis with 24 hours shown a decrease in the LC rats when compared with the CO rats and with the drug treated group rats the proteins level was increased and the vitamin levels were highly decreased.

KEY WORDS: Huntington's disease, Withania somnifera, Antioxidant, Vitamins.

Introduction

Huntington's disease is a fatal neurodegenerative genetic disorder named after George Summer Huntington who first described the disorder in 1872 [3], that causes defects in behavior, cognition, and uncontrolled rapid, jerky movements. Chorea, was derived from the Greek word for "dance"[2]. Huntington's disease mainly associated with basal ganglia degeneration [9] which is composed of the caudate nucleus and putamen leads to lack of coordination and an unsteady gait. There is no cure for HD, and full-time care is required in the later stages of the disease [10].

Herbal medicine have been always a part of mankind's healing armamentarium and supports the wellness by enhancing the body's inherent healing potential. The World Health Organization (WHO) estimates that 80% of the world's population presently uses herbal medicine for some aspect of primary health care [4].

[1]Assistant Professor of Anatomy,
Tagore Medical College, Chennai,
[2]Assistant Professor of Anatomy,
[3]Professor of Radiology,
Sri Lakshmi Narayana Institute of Medical Sciences,
Ossudu, Pondicherry

***Corresponding Author**
A. Mary Antony Praba,
Assistant Professor of Anatomy,
Tagore Medical College, Chennai.
Email id –fio7rio@yahoo.co.in
Telephone number –09941178552.

WITHANIA SOMNIFERA

Ashwagandha, also called as "Queen of Ayurveda" is a important plant in Ayurveda, the Indian traditional medicine (fig-1). This herb was used 4000 years plus in India. The roots of the plant are categorized as rasayana (fig-2) was the part mostly used in herbal medicine.

At present, 12 alkaloids, 35 Withanolides have been isolated from the roots of the plant. Withanolide A is an important secondary metabolite that posses high medicinal value and anti- antioxidant properties. Withanolide A is one of the important phytochemical currently being explored for it's activity in nerve cell regeneration. That property of this herb made us to employ the herb in this study.

Material and Methods

Materials

Steriotaxic frame (INCO), micromotor hand piece, kainic acid (Sigma Aldrich US.), Hamilton syringe, Withanolide A (Sigma Aldrich, US), Ethanolic extract of Withania somnifera (by the method of saxhelation), spectrophotometer, homogenizer, pH meter, centrifuge, micropipettes, and chemicals.

Methodology

Animals

Adult male Sprague Dawly rats (200–240 gm) were selected 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.

Drug

Oral dosage was avoided to reduce the wastage of drug and to minimize the stress condition of rats.

Ethanolic Extract of Withania Somnifera

Three I.P. dosage of WS was selected they are WS100, WS125 and WS150. The drugs were dissolved in normal saline [6], the volume was adjusted to 1ml.

Withanolide A

Three dosages of WD was prepared they are, WD5, WD10 and WD100, the volume was adjusted to 1ml for each animal. The administration of drug was started 10 days prior to make the lesion and also after the lesion so as to access the protective role by scavenging the free radicals in striatum and was given around 10 O' clock every day.

Lesion Surgery

The animals were maintained in empty stomach 10hr before the procedure and were anaesthetized using Pentathol sodium 40mg/kg IP [11]. With the help of the stereotaxic frame the bregma was found and the striatum was marked 2.2mm anteriorly and 3mm bilaterally. Kainic acid was dissolved in 0.9% NaCl [11]. 0.5micrograms in 0.5µl of kainic acid was taken in a Hamilton syringe and was into the striatum which was present in 5mm depth. The injection was at the rate of 1 µl per 1 minute [11]. The syringe was withdrawn and the scalp was sutured with proper care.

Biochemical Study

All the animals were sacrificed within 24hr by cervical dislocation, the brain tissues were collected from that striatum was quickly removed and placed in ice-cold saline (fig-3,4). Using ice cold Tris-Hcl buffer (0.1M, pH 7.4) 10% homogenate was prepared by motor driven Teflon - glass tissue homogenizer. The homogenate was centrifuged at 2000 rpm, 4°C for 15 minutes [7] and the supernatant was used for the following analysis.

a. Protein [5]

b. Vitamin C [8]

c. Vitamin E [1]

The results were tabulated, analysed and the discussion follows. The statistical analysis was done by one way anova where 0.05<P.

Result and Discussion

Analysis of Protein (chart-1):

The protein level of animals belongs to LC group were significantly low when compared with CO group of animals. The WS100, WS125 and WS150 drug group of animals were showing significantly increased levels of proteins, with significantly high values of protein in WS125 (df=3,20. F=80) group of animals. The animals groups WD5, WD50 and WD100 also were showing significant increase in the protein levels when compared with the LC, with the highest values with WD100 (df=3,20. F=89) group of animals.

Analysis of Vitamin C (chart-2):

The LC animals shown a slight decrease in the Vit C level when compared with the CO group of animals. The drug group animals WS100, WS125 and WS150 were showing a decrease in the Vit C level. In that the animal group WS125 shown maximum decrease in Vit C when compared with the LC animals. The drug group WD5, WD50 and WD100 were also showing a decrease in the Vit C level when compared with LC and CO group with the lowest value in the WD100 group of animals. This says the drug groups WS125 (df=3,20. F=996) and WD100 (df=3,20. F=884) were very much effective in stimulating the utilisation of Vit C against free radicals within 24 hours.

Analysis of Vitamin E (chart-3):

The analysis of Vit E level within 24 hours of lesion shown a slight decrease but was not significant with the CO group. The animal groups WS100, WS125 and WS150 shown a significant decrease when compared with the CO and also with the LC group of animals. The animal groups WD5, WD50 and WD100 were also showing significant decrease in the Vit E level in comparison with the LC and CO group of animals. In the animal group WD100 the Vit E level was very much decreased than the other group of animals. By this study we can conclude the drug dosages WS125 (df=3,20. F=3034) and WD100 (df=3,20. F=3461) were good in neuroprotection by inducing the utilisation of Vit E in free radicals scavenging.

Charts

Chart-1 Chart Showing The Level Of Protein In Basal Ganglia Within 24 Hours Of Lesion

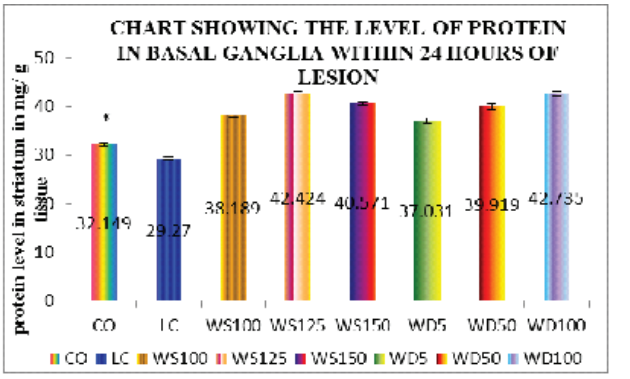


Chart-2 Chart Showing The Level Of Vit C In Basal Ganglia Within 24 Hours Of Lesion

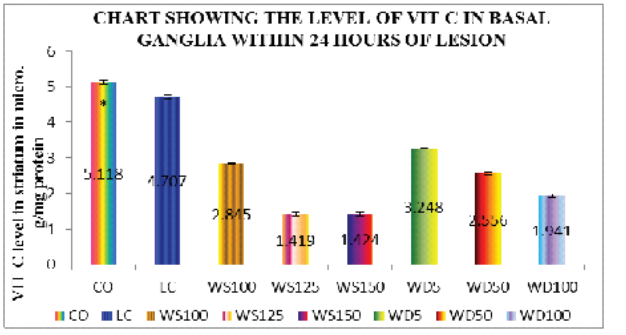
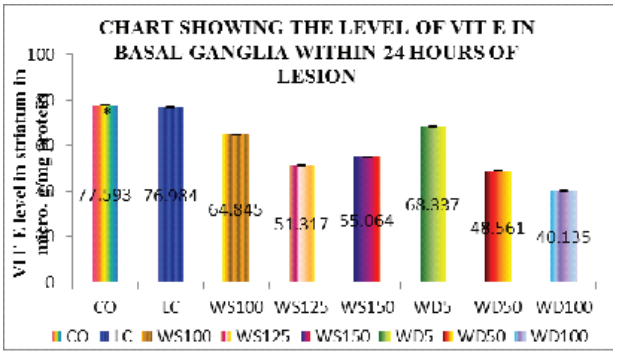


Chart-3 Chart Showing The Level Of Vit E In Basal Ganglia Within 24 Hours Of Lesion



Tables

Table -1 shows the mean value of protein, Vit C and Vit E in different animal groups.

S.no	Animal groups	Mean value of protein (mg/ g tissue)	Mean value of Vit C (micro. g/mg protein)	Mean value of Vit E (micro. g/ mg protein)
1	CO	32.14966	5.118336	77.59313
2	LC	29.2702	4.707889	76.9841
3	WS100	38.18993	2.8456	64.84586
4	WS125	42.42487	1.419007	51.31759
5	WS150	40.57158	1.424574	55.06409
6	WD5	37.03125	3.248256	68.33788
7	WD50	39.91931	2.556394	48.56137
8	WD100	42.73591	1.941249	40.13559

Conclusion

(table-1)

Huntington's chorea is a neurodegenerative disorder mainly because of nerve cell degeneration in striatum. There is no cure for the disorder and and physical injuries will reduce the life expectancy and eventually causes death within 10 to 20 years. Free radicals and other reactive species are considered to be an important causative factor in the development of neurodegenerative diseases. And so antioxidant properties of medicinal plants are attracting more researchers in medicine, to counteract ROS and to minimize the neurodegeneration.

By all the above said biochemical assays it was confirmed that in the LC animals the antioxidant system was not stimulated immediately after the lesion and so the protein level was not increased and the vitamins were not utilised properly to reduce the deleterious effects of the free radicals produced due to the lesion surgery. But in the drug group of animals the protein level was immediately stimulated to produce the enzymes in excess to reduce the adverse effects of the free radicals. The drugs were also induced the vitamins to be utilised more to reduce the effects of the free radicals. These effects were best studied by the drug groups WS125 and WD100. With these group of drugs the protein was stimulated maximum and the vitamin utility was also maximum. So these group of drugs can protect the nervous system at the highest.

So by this study we can conclude that the plant withania somnifera and it's active principle withanolide A in the dosage of WS125 and WD100 were considered as good neuroprotectors by scavenging the free radicals in high significance with the other groups and so they can be further developed into drugs in the treatment of Huntington's chorea and neurodegenerative disorders.

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Figures

Fig-1 The plant withania somnifera

Fig-2 The root of the plant withania somnifera

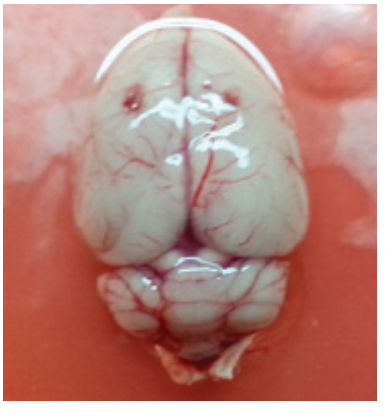

Fig-3 showing the lesioned brain kept in ice cold saline

Fig-4. Figure showing the striatum of CO animals after removing the cortex

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