ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

CHRONIC ANTICONVULSANT EFFECT OF GHEE PREPARED WITH *HUMBOLDTIA VAHLIANA* (ATTUVANCHI BARK) – AN ETHNOMEDICINAL PRACTICE IN KERALA

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ABSTRACT

Humboldtia vahliana Wight, Ic.tt belongs to the family Caesalpiniaceae is an unarmed erect small tree, commonly called as *Attuvanchi* in Malayalam. Stem bark which is used in the treatment of *apasmara* by tribal healers of Urulanthanni area, Ernakulum district in Kerala, India. *Apasmara* of Ayurveda aptly comes under the epilepsy of modern literature, especially under tonic-clonic/ generalized seizure. Epilepsy (*Apasmara*) is an electrical disturbance resulting from sudden, recurrent, and disorderly discharge of neurotransmitter in brain cells. The prevalence of epilepsy has been estimated at 5-10 persons per1000. In this study ghee prepared with *Humboldtia vahliana* (GHV) was tested for chronic anticonvulsant effect by Maximal electro shock seizure test in female wistar albino rats (150-200mg). The experiment was carried out with 5 groups having 6 albino rats per group. 'Phenytoin' was given to the standard group. Group I, III, IV, V were given distilled water, vehicle (cow's ghee), effective dose of GHV, double dose of GHV respectively. Complete abolition or reduction time (in seconds) of hind limb extension is considered as anticonvulsant activity of drug. At the end of experiment effective dose of GHV and double dose of GHV showed significant chronic anticonvulsant action as compared with vehicle and control. Group IV (effective dose) was able to reduce the Time of Tonic Hind Limb extension (THE) by 44.34%with p value 0.007 (compare with control). Group V (double dose) was able to reduce the Time of Tonic Hind Limb extension by 59.53% with p value 0.004 (compared with control). This change was statistically significant at $p<0.05$.

KEYWORDS: *Apasmara*, MES, *Ghee, Humboldtia vahliana*, Phenytoin, Epilepsy, *Attuavanchi,* GHV,

INTRODUCTION

India has rich floristic and ethnic diversity. A large number of plants are equally used by tribes and folklore traditions in India for treatment purposes. Recording and analysis of these ethanobotanical plants is of great significance in new drug development. The researches in the field of Ayurveda should focus on enriching its pharmacopoeia by focusing on protocols based on concepts of Ayurveda. It will help to bridge the lacunae in the existing literature and thus it offers immense scope for researchers who engaged in validation of traditional drugs. *Humboldtia vahliana* Wight, Ic.tt (*Attuvanchi*) is an ethanobotanical drug include in Caesalpiniaceae family. In Malayalam it is called *Attuvanchi* or *Kurappunnu*. It is an endemic tree in southern Western Ghats especially in the Kerala and Tirunelveli district of Tamilnadu. [1,2]. Its stem bark is used as medicine by tribes in Urulanthanni area, Ernakulum district in Kerala for Apasmara. *Apasmara* of Ayurveda aptly comes under the epilepsy of modern literature, especially under tonic-clonic/ generalized seizure. Epilepsy is the fourth most common neurological disorder and affects people of all ages[3]. Epilepsy accounts for a significant proportion of the world's disease burden, affecting around 50 million people worldwide[4]. The prevalence of epilepsy has been estimated at 5-10 persons per 1000[5]. Epilepsy is a neurological disorder in which a person has two or more recurrent unprovoked seizures caused by abnormal, excessive and hyper synchronous discharge from an aggregate of CNS neurons. The pharmacological actions of the ethanobotanical plants which are not included in any *Samhithas* should be evaluated and extensive clinical trials are needed to confirm the results. To enrich the pharmacopoeia of Ayurveda, inclusion of extra pharmacopoeial drugs in Ayurvedic sciences is need of the hour. These ethanobotanical plants can be included to the Ayurvedic pharmacopoeia of India for its judicious usage as a novel Ayurvedic medicine and has good scope for future researches aiming to develop a safe herbal

drug. It is known that herbal remedies are inherently safer compared with synthetic therapies, even during long-term use.

MATERIALS AND METHODS

The stem barks of genuine *Humboldtia vahliana* were collected from Jawaharlal Nehru tropical botanical garden research and institute, palode and the sample was authenticated by Botanist, JNTBGRI, and Palode. (Figure 1) *Ghee* (cow's ghee) of Agmark standard was used, purchased from Milma out let Thiruvananthapuram. Ghee of *Humboldtia vahliana* was prepared as the classical procedure in *Sarandhara samhitha madhyamakhanda*.[6] Dose was calculated using the table constructed by paget G.E & Barnes T.M considering the human dose of *ghee* as Table no: 1

Serial no	Drug	Quantity
1	*Kalka*(paste) of *Humboldtia vahliana*	83g
2	Ghee(cow's ghee)	500ml
3	*Kashaya* (decotion) prepared with *Humboldtia vahliana* bark	2L

The *Kalka* and the *Kashaya* were mixed together. *Sneha* (*ghee*) was then added, boiled on mild fire and stirred well continuously till *Madyama paka* (devoid of liquid and it is soft like a wick)

Experimental animals

Female wistar albino rats (150-200g) were obtained from SCTIMST (Sree Chitra Tirunal Institute for Medical Sciences & Technology), Thiruvananthapuram. They were grouped and housed in poly-acrylic cages and maintained under standard laboratory conditions. They were fed standard rat feed and water.

Ethical considerations

The experimental protocols were approved by institutional Ethics Committee and a written permission from Institutional Animal Ethical committee, Govt. Ayurveda college, Thiruvananthapuram (order no:2015/GO/Re/S/18/ CPCSEA, dated 09/07/2018)were taken to carry out and complete this study and standard guidelines were followed for the maintenance and use of the experimental animals.

Grouping of animals

The acclimatized animals were weighed and randomly divided into 5 groups having 6 animals in each group. The random selection ascertained unbiased distribution of animal with regard to age, weight etc in each group. The animals were marked for proper identification and kept in separately labeled cages. The dose of each animal was calculated according to the body weight and put in tables for further reference.

48ml. Phenytoin sodium injection (Eptoin), 2ml ampule (50mg/ml) ,of Abbot Health care pvt Ltd. The medicine was procured from a local pharmacy near Govt medical college, Thiruvananthapuram.

Preparation of study drug

Preparation of *Kalka of Humboldtia vahliana*

Sufficient amount of finely powdered bark of *Humboldtia vahliana* taken and added water, grinding in *Khalwayantra (mortar and pestle)* made into 83 g paste.

Preparation of *Kashaya*

1 kg bark of *Humboldtia vahliana* is taken and added to 8 L water and boiled reduced to 2L *Kashaya*. This *Kashaya* was taken for the *ghee* preparation

Group will be named as I, II, III, IV, and V

Group I: Control group -distilled water

Group II: Standard group- phenytoin (2.5mg/ 100gm)

Group III: Effective dose of vehicle (Cow's ghee)

Group IV: Effective dose of ghee prepared with *Humboldtia vahliana* (GHV) (0.864ml for 200gm animal).

Group V: Double dose of ghee prepared with *Humboldtia vahliana* (GHV) (1.728 ml for 200 gm animal).

Drug administration

Group I, III, IV, V were given distilled water, vehicle, effective dose of GHV, double dose of GHV respectively for a period of 15 days in the morning (figure 2).

Procedure of MES test

On the 15th day group I, III, IV, V were given respective drugs, group II was given standard drug (phenytoin) intraperitoneally (figure3). After two hour, convulsions were produced in rats using Electro convulsiometer by delivering current of 150 mA through corneal electrodes for a period of 0.2 seconds. The maximal seizure typically consists of a short period of tonic flexion and prolonged period of tonic extension followed by clonic convulsions and stupor.

Objective parameters

Complete abolition or reduction time of hind limb extension in seconds.

RESULTS

The drug is assumed to have anti convulsion effect if it reduces the duration time of THE (in sec) or abolishes THE. The statistical analysis of time (in seconds) over the tonic hind limb extensor phase of MES convulsions in each group was carried out to establish the effect of the study drug in each group. The arithmetic mean and standard deviation of the values for all groups were calculated and tabulated. (Table no2) The significance of the data across all groups was analyzed by Kruskal-wallis test which is the non-parametric version of ANOVA. The p value corresponding to the Kruskal Wallis test is lower than 0.05, suggesting that one or more treatments are significantly different. Mann- Whitney U test follows.

Figure1: Sample of *Humboldtia vahliana*

Figure 2: Administration of test drug (GHV)

Figure 3: Intraperitoneal injection of phenytoin

Table 2: The arithmetic mean and standard deviation

Group	Mean (the in sec)	SD	Percentage in inhibition of time of the compared with control (%)
Group 1 (control)	13.17	2.48	0
Group 2 (standard)	0.00	0.00	100
Group 3 (cow's ghee)	11.00	0.63	16.48
Group 4 (effective dose)	7.33	2.06	44.34
Group 5 (double dose)	5.33	1.21	59.53

Kruskal- Wallis test P value is 0.02, and then Mann Whitney U test follows

Comparison between the control and other groups and comparison between the experimental drug treated groups are given in table no 3, table no 4

Table 3: Comparison between control and other group

Pairs considered	Mann-Whitney test p-value	Statistical significance
Group 1 vs Group 2	0.002	Significant
Group 1 vs Group 3	0.280	Non-significant
Group 1 vs Group 4	0.007	Significant
Group 1 vs Group 5	0.004	Significant

Table 4: Comparison between the group3, 4and 5

Pairs considers	Mann Whitney test p value	Statistical value
Group 3 vs Group 4	0.005	Significant
Group 3 vs Group 5	0.004	Significant
Group 4 vs Group 5	0.105	Non-significant

Compared with control , Standard group showed 100% reduction in time of Tonic Hind Limb Extension, Group III was able to reduce the Time of Tonic Hind Limb extension by 16.48% with p value 0.280, Group IV was able to reduce the Time of Tonic Hind Limb extension by 44.34%with p value 0.007 and Group V was able to reduce the Time of Tonic Hind Limb extension by 59.53% with p value 0.004.The effective dose and double dose have significant anticonvulsant effect compared with control but there was no significant difference between control and vehicle. There was significant difference in the anti convulsion effect between vehicle (cow's ghee) vs. effective dose of GHV (p value=0.005) and vehicle (cow's ghee) vs. double dose of GHV (p value=0.004) but there was no significant difference between the anticonvulsant effect of effective dose and double dose of *GHV* (p value=0.105). The effective dose and double dose of *GHV* were found to be more effective at end of the study. None of the test drug showed complete abolition of extensor phase of MES convulsions as that of standard drug phenytoin treated group. The study can stated that ghee prepared with *Humboldtia vahliana* have significant anti convulsant effect in effective dose and double dose.

DISCUSSION

Records of indigenous knowledge through ethanobotanical and ethanomedicine studies and its validation through scientific procedure are very important for conservation and utilization of biological sources. The present study is an attempt to validate and evaluate one such ethanobotanical knowledge regarding the drug Attuvanchi, identified as *Humboldtia vahliana* Wight, Ic.tt.

The study was aimed to experimentally evaluate the chronic anticonvulsant activity of ghee prepared with *Humboldtia vahliana*. *Humboldtia vahliana* Wight, Ic.tt a member of family Caesalpiniaceae is an ethno medicinal drug, used for *Apasmara* by tribal healers of Urulanthanni, Ernakulum district, Kerala, India.

By considering the wide range of classical symptoms of *Apasmara* and the modern description of Epilepsy, they are widely equated to one another. The description of *Apasmara* of Ayurveda aptly comes especially under tonic-clonic / generalized seizure of epilepsy. Although various new drugs are available in market their clinical use are limited due to neurotoxicity and side effects. On the other hand herbal medicines like *Humboltia vahliana* are being used due to applicability and efficiency coupled with least side effects.

Lipid soluble drugs are rapidly distributed and absorbed from the gut due to their lipid solubility are known to readily diffuse into the CSF and the brain. Ghee as a lipid medium for preparation is used to facilitate easy digestion and absorption of any drug owing to its *Deepana* (increase gastric activity) and *Yogavahi* (it enhances the properties of other drugs without losing its own act) properties. Moreover ghee by itself is *Medhya, Smrithi vardhana* and *Budhi vardhana* [7]. With this background an attempt has made to prepare ghee of *Humboldtia vahliana* (GHV).

Maximal Electro Shock test model was used for the study, animals treated with effective dose and double dose of ghee prepared with *Humboldtia vahliana* was analyzed along with control. Both the groups had shown significant reduction in Tonic hind limb extension ($p<0.05$ compared with control and vehicle). It is suggestive of anticonvulsant action of the drug.

The presence of flavanoids, phenol, alkaloid, saponin in bark of *Humboldtia vahliana* had been confirmed by phytochemical analysis. It is known that flavanoids, phenol, alkaloid and saponin exert protective action on CNS [8]. Especially flavanoids, alkaloids, saponin have proven anticonvulsant action. Ghee being the *Kalpana* used, it can extract the fat soluble components effectively, and its lipophilic nature allows the drug to cross the blood brain barrier effectively and induce the effect [9].

Hence the anticonvulsant action of medicine (GHV) may be due to the synergistic effect of phytochemicals in the *Humboldia vahliana* and properties of Ghee.

CONCLUSION

The test medicine GHV has chronic anticonvulsant action in both effective dose and double dose compared to control group and vehicle group. *Humboldtia vahliana* is an extra pharmacopieal drug, this study create an acceptance of these ethno botanical drugs among common health care, which otherwise become limited among tribes only. The conservation of ethno botanical medicinal information into knowledge and in validation through scientific procedure is the necessity of the day. Ghee prepared with *Humboldtia vahliana (GHV)* has anticonvulsant effect it is beneficial not only to the diseased community but also help in broadening the horizons of Ayurvedic wisdom.

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Cite this article as:
R.Sreelekshmi, M.S Deepa. Chronic Anticonvulsant Effect of Ghee Prepared with Humboldtia Vahliana (Attuvanchi Bark) - An Ethno medicinal Practice in Kerala. International Journal of Ayurveda and Pharma Research. 2020;8(Suppl 2):61-65.
Source of support: Nil, Conflict of interest: None Declared

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