International Journal of Current Research in Medical Sciences
ISSN: 2454-5716
www.ijcrims.com
Volume 2, Issue 10 -2016

Original Research Article

DOI: http://dx.doi.org/10.22192/ijcrms.2016.02.10.009

Analytical Standardization of Brahmi Nei and Effect of Siddha Methodologies on Spasticity in Cerebral Palsy

*Arul Mozhi P[1]., Pattarayan R[2]., Deivanayagi.S[3]., Banumathy.V[4]
[1]Lecturer, National Institute of Siddha, Chennai, Tamil Nadu
[2] Professor (Rtd), National Institute of Siddha, Chennai, Tamil Nadu
[3]Asst.Professor, Sri Sairam Institute of Technology, Chennai, Tamil Nadu
[4]Director National Institute of Siddha, Chennai, Tamil Nadu
*Corresponding author: *drarulmozhi@yahoo.co.in*

Abstract

The objective of the study is to analyze the standardization and to determine the effect of Brahmi Nei with massage and Varmam on spasticity of children with spastic cerebral palsy. In pre clinical standardization, organoleptic, physicochemical, microbial load, specific pathogen, pesticide, aflatoxins and HPTLC study were done. In clinical study, among the 250 children 210 Spastic CP satisfied the inclusion criteria and were divided in to three groups (N=70 Nos). Group –I treated as an active control, Group –II received Brahmi Nei, Group - III received Brahmi Nei along with external therapies such as massage with Vasavu ennai and Varmam twice a day. Experimental period was 90 days and spasticity was recorded 0^{th} day and followed by every 30^{th} day. Group III had decreased spasticity in different group of muscles which are compared with other two groups. Finally, it can be concluded that Brahmi Nei along with Vasavu ennai massage and Varmam has a clinical efficacy on spasticity in cerebral palsy children.

Keywords: Cerebral palsy, Brahmi Nei, Vasavu ennai, Spasticity.

1. Introduction

In India, Siddha system of medicine owes its origin to medicinal ideas and practices of a class of Tamil sages. It has classic sasthric formulations such as herbs, minerals, metals and salts all have been used for pediatric population. The purpose of this research work is to develop recommendations to the evaluate Siddha methodologies and Medicines. Brahmi Nei is a polyherbal formulation of Siddha medicine. In the present study, the selected Brahmi Nei is an important formulation mentioned in sasthric Siddha literature for the treatment of neurological disorders. The standardization any medicine is important for the reproducibility of the therapeutic effect. In this study, organoleptic, physicochemical, microbial load, specific pathogen, pesticide, aflatoxins and HPTLC study were done for Brahmi Nei. Cerebral palsy (CP) is described as “a group of disorders of the movement and posture that are attributed to non-progressive disturbances are often accompanied by disturbances of spasticity and rigidity.” The prevalence of cerebral palsy is estimated to be 1.5- 3 per 1000 live births, with variations possibly differences in ascertainment and classification (Andersen, G. L et.al 2007, Blair, E.

2010). During the last years, focus of care for children with cerebral palsy has shifted from a main emphasis on motor function, towards participation and minimizing limitations of activity. A large number of treatment options have been available for spasticity children with cerebral palsy such as oral Baclofen, Tizanidine, Dantrolene, Diazepam and Gabapentin. Not all children with spasticity benefit from this treatment (Orsnes GB, 2000) the incidence of adverse drug effects (drowsiness, sedation and muscle weakness) were high. Children with localized or multifocal spasticity injections have benefit of Botulinum toxins (Lim EC-H, 2008) formation of antibodies against has been demonstrated (Muller K et.al 2009). Neurosurgery such as rhizotomy and orthopaedic surgery (tendon lengthening and soft tissue releases) may be the options (Ward AB, 2008). In view of all of the above, an immediate and urgent need exists to look for an alternative form of therapy such as natural products. Different treatment modalities can improve the quality of life to the disabled children and these can include Siddha bio pharma products included Brahmi Nei (Tamil Nadu Siddha Medical Board 1995, 116) as internal medicine, Vasuvu Ennai (Siddha Hospital Pharmacopeia Part-I) for thokkanam (Massage) and Varama therapy (Kannan Rajaram 2008, R.Thiyagarajan 1985) as external, all of which have been used in the Siddha system of medicine for many centuries either singly or in various combination. In order to limit this issue, efforts were undertaken to study the result of the use of a combination of these therapy.

2. Materials and Methods

2.1 Preparation of Experimental Formulations

Brahmi Nei as internal medicine and Vasavu Ennai for thokkanam (Massage) were identified for this study. Raw drugs to prepare the products were purchased from the market and fresh plants were collected from wild sources. The raw materials have got authentication from Department of Medicinal Botany, National Institute of Siddha, and Chennai.

2.1.1.Birahmi Nei

Birahmi Nei was prepared as described as in the sasthric Siddha literature. Briefly, it was prepared by adding paste of *Zingifer officinale* Linn., (Dried Rhizome) *Piper longum* Linn. (Dry fruit), *Alpenia officinarum* Linn. (Dried Rhizome), *Feronia elephantum* Linn.(seed), Induppu, *Caryyota urens* Linn.(pal jaggery), *Gurkuma aromatic* Linn.(Rhizome) each 14gms, in freshly prepared *Bacopa monniera* Linn.(5.44kg), *Acorus calamus* Linn.(1.36 kg), *Alpenia galanga* Linn.(1.36kg), and in vessel having Cow's milk (5.44kg), Cow's Ghee,(2.72 kg). Above mixture was heated and filtered after acquiring completion test. In this way, Birami Nei was prepared.

2.1.2.Vasavu Ennai

Vasavu Ennai also was prepared as described as in the sasthric Siddha literature. Briefly, it was prepared by adding paste of *Hemidesmus indicus* Linn.(100gms), in freshly prepared juice of *Citrus aurantifolia* Linn., *Aloe barbadensis* Linn., in vessel having *Cocos nucifera* Linn oil and Ricinus communis Linn., oil each one litre. Above mixture was heated and filtered after acquiring completion test and boiled in medium flame with continuous stirring and monitoring of paakam. The boiling was stopped and the oil was filtered using a washed and dried white filter cloth when chikku patham was attained. In this way, Vasavu Ennai was prepared.

2.3 Organoleptic and Physicochemical studies

Organoleptic, physicochemical, Microbial load, Specific pathogen, Pesticide, Aflatoxins and HPTLC study was done in Regional research institute of Unani Medicine, Chennai. The physicochemical testing employed for Brahmi Nei profiling which is depend on the specific characteristics of formulation as per method given in PLIM guidelines and Siddha Pharmacopoeia of India (SPI) contains various parameters for testing such as 1. Description– Colour, Odour, 2. Weight/ml, 3.Refractive index at 25 ºC, 4. Iodine value, 5.Saponification value, 6. Acid value,

7.Test for heavy metals – Lead, Cadmium, Mercury, Arsenic, 8.Microbial contamination - Total bacterial count, Total fungal count, 9.Test for specific Pathogen - *E. coli*, *Salmonella* spp., *S.aureus*, *Pseudomonas aeruginosa*, 10. Pesticide residue - Organochlorine pesticides, Organophosphorus pesticides, Pyrethroids.

2.4 HPTC

The procedure recommended for the analysis of TLC and HPTLC analysis as per Wagner H and Bladt S, 1996. The sample was applied for the thin layer chromatography and High performance Thin layer chromatography study with suitable solvent system. The sample (petroleum ether extract - 2µl (A) & 4µl(B)) were applied in TLC aluminium sheet silica gel 60 F 254 (E.MERK) and plate was developed using the solvent system Toluene:Ethyl acetate: Glacial acetic acid (6:0.4:0.4). After development the plate was allowed to dry inair and examined under UV-254nm, 366nm and visible light (Vanillin-Sulphuric acid).

Instrument : CAMAG (CAMAG – Automatic TLC sampler, scanned and visualiser)
Spray gas : N2
Lamp used : Deuterium and Tungston Lamp

2.5 Clinical Study

The present study was a prospective, open label, non-randomized, outpatient and inpatient based, single centered drug trial conducted in the department of Kuzhanthai Maruthuvam (Pediatric), National institute of Siddha, Chennai. It was conducted during 2011 to December 2016 after obtaining approval from the Institute Ethics Committee (NIS/IEC/2011/3/48). Single batch of Brahmi Nei and Vasavu ennai were prepared for the entire study. The first 250 children with spastic cerebral palsy were screened during the study period. Children of either sex between the age group of 3 to 12yrs, who were diagnosed with spastic cerebral palsy, were identified to include the study. Other type of cerebral palsies and along with seizure disorder, Spinal deformities, impaired vision Autistic Spectrum Disorders, ADD/ADHD (Hyper activity), Mental Retardation, Visual Impairments and Blindness, Hearing Loss and Deafness, Down Syndrome, Spina Bifida, Traumatic Brain Injury were excluded from the study. 210 children satisfied the inclusion criteria and were willing to participate in the study, signed the informed consent. The parents of children who were enrolled was informed about the study, trial drug, possible outcomes and the objectives of the study in the language and terms understandable for them.

Children were divided in to three groups (N=70 Nos). Group –I treated as an active control received regular OPD medicines, Group –II received internal medicine Brahmi Nei (3 yrs to 5 yrs - 8 ml, 6 yrs to 9 yrs - 10 ml, 10 yrs to 12 yrs - 12 ml) twice a day. Group - III received internal medicine Brahmi Nei along with external therapies such as massage with Vasavu ennai and Varmam (Kondai kolli, Natchathira kaalam, Thilartha varmam, Pidari kaalam) twice a day. Experimental period was 90 days and spasticity was recorded 0^{th} day and followed by every 30^{th} day. Experimental formulations were assigned to each subject and regular study drug reconciliation was performed to document the drug assigned, consumed, and remaining are logged on the drug reconciliation form with sign & date.

Assessment of Spasticity - Ashworth scale

The most commonly used definition of spasticity is described by Lance (1980) i.e “Spasticity is a motor disorder characterized by a velocity-dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks, resulting from hyperexcitability of the stretch reflex, as one component of the upper motor neuron syndrome” (Scholtes VA et.al 2006). Several methods have been developed and used to assess spasticity. The most commonly used test in clinical practice is the Asworth scale (Ashworth B, 1964). The test is based on the assessment of resistance to passive strech of muscle group at one non specified velocity in Gastrocnemous and Soleus, Hip adductors, Thigh flexors and extensors, Triceps and biceps, Shoulder girdle and trunk muscles, Forearm flexors and extensors.

- 0 - No increase in muscle tone
- Slight increase in tone with a catch and release or minimal resistance at end of range
- As 2 but with minimal resistance through range following catch
- More marked increase tone through ROM
- Considerable increase in tone, passive movement difficult.
- 5 - Affected part rigid

2.6 Statistical Analysis

All of the analyses were performed using the SPSS statistical software, version 20.0. The results are expressed as mean values ± SD. Statistical significance was tested by means of analysis of variance (ANOVA), paired students t-test for within-group comparison and the independent student t-test was used for comparisons between the two therapy groups and the group means were compared by Duncan's Multiple Range Test (DMRT). Values were considered statistically significant when at $p < 0.05$ (Duncan BD, 1957).

3. Results and Discussion

Table.1 Observations of organoleptic properties of Brahmi Nei Analytical Parameters

S.No	Tests	Observations
1.	Colour	Yellowish green
	Odour	Fragrant
	Taste	Characteristic
2.	Weight/ml	100 ml

The Brahmi Nei was yellowish green in colour and had characteristic ghee smell, fragrant odder of Brahmi and characteristic taste.

Table -2 Physicochemical parameter's findings of Brahmi Nei

S.No	Tests	Observations
1.	Refractive index at 25 ºC,	1.4552 – 1.4582
2.	Acid value	5.21 to 10.91
3.	Iodine value	32.35 – 34.75
4.	Saponification value	159.02 - 227.2
5.	**Test for heavy metals**	
	Lead, Cadmium, Mercury, Arsenic	NAD

Table. 3 Analysis of Microbial load, Specific pathogen, Pesticide and Aflatoxins

1.	**Microbial contamination**	
	Total bacterial count	Less than 10 cfu/ml
	Total fungal count	Less than 10 cfu/ml
2.	**Test for specific Pathogen**	
	E. coli, Salmonella spp., *S.aureus Pseudomonas aeruginosa*	Absent
3.	**Pesticide residue**	
	Organochlorine and Organophosphorus pesticides, Pyrethroids,	Absent
4.	Test for Aflatoxins (B1,B2,G1,G2)	Absent

Table.4 Rf Value petroleum ether extract of unsaponifiable matter of Brahmi Nei

Solvent System	Rf values		
	UV-254 nm	UV-366 nm	Visible light (Vanillin – Sulphuric acid)
Toluene: Ethyl acetate: Glacial acetic acid (6:0.4:0.4)	0.57 Green	0.52 Blue	0.61 Grey
	0.47 Green	0.48 Blue	0.50 Grey
		0.42 Red	0.42 Grey
		0.37 Blue	0.39 Dark Grey
			0.19 Grey
			0.12 Grey

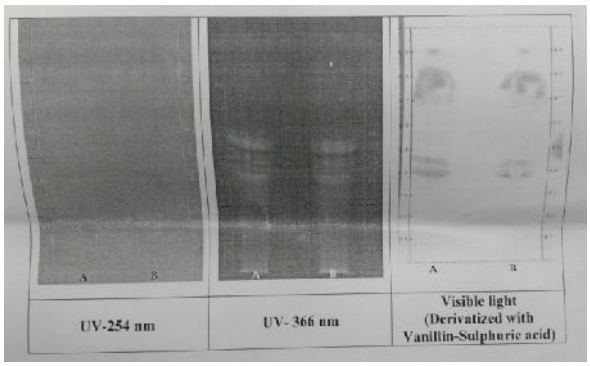

Fig.1 TLC photodocumentation of petroleum ether extract of unsaponifiable matter of Brahmi Nei

HPTLC study was done just to obtain the fingerprints of preparation and it was also carried out to get the standard markers for this study. TLC photo documentation was done for the samples of Brahmi Nei as showed in the Fig.1. HPTLC densitometry scans of petroleum ether extract of unsaponifiable matter of Brahmi Nei at 366 nm which showed 6 peaks which covered the area of corresponding Rf values as showed in Fig. 2.

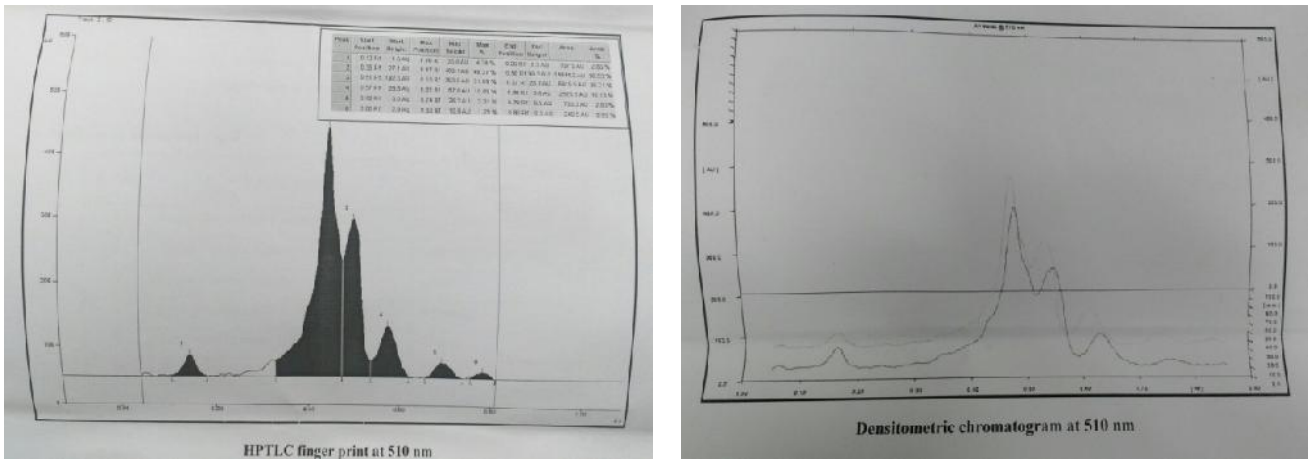

Fig.2 HPTLC Dencitometric scan of petroleum ether extract of unsaponifiable matter of Brahmi Nei

Table.5 Spasticity changes muscles as per the Asworth scale

Days	Groups					
	Group I		Group II		Group III	
	Mean	SD	Mean	SD	Mean	SD
Day 0	3.59	1.03	3.69	1.08	3.60	1.03
Day 30	3.51	0.88	3.50	0.94	3.21	0.95
Day 60	2.96	1.06	3.50	1.02	2.91	1.07
Day 90	3.00	1.09	3.39	1.13	2.46	1.22

Spasticity changes in the different clinical groups at 90 days of treatment. The number of children in each group is given within parentheses. Values are given as mean ± S.D for 70 children in each group.

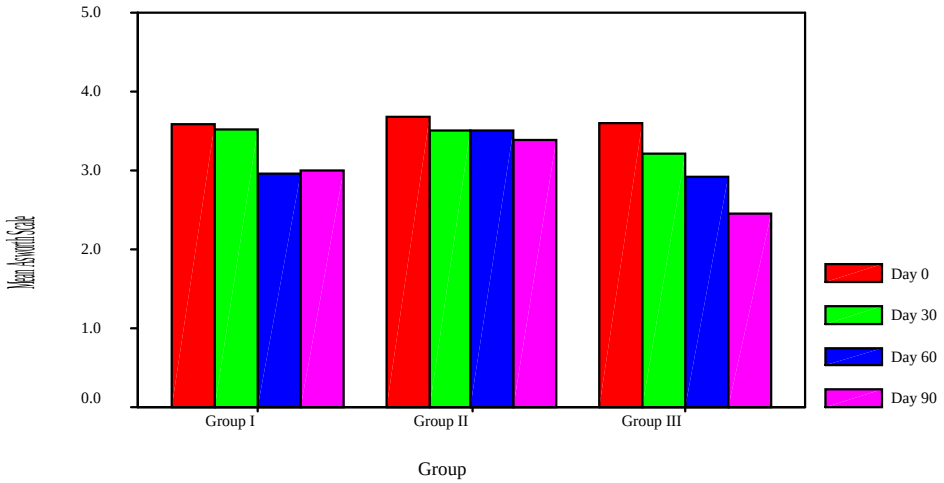

Fig. 3 Spasticity Changes

Brahmi Nei and Vasavu ennai were prepared as per the standard operative procedure mentioned in the sasthric siddha literature. Brahmi Nei was subjected to physico-chemical analysis and HPTLC. The acid value indicates the presence of free fatty acids in the brahmi Nei. The free fatty acid is the responsible of rancidity compund, falvour and stability. Lesser free fatty acid make them less rancidity. It suggests that Brahmi Nei contains contains less free fatty Acids and chances of rancidity are less (Yadav KD et.al 2013). Refractive index is used in determining the identity and purity and the results showed the given sample was having more purity. The saponification value indicates the average molecular weight or chain length of all fatty acids present. It improves the absorption rate to the intestine there by increase nutritional value and therapeutic values. In the present study higher saponification value in Brahmi Nei shows that it contains shorter chain fatty acids so that absorption rate will be more (Yadav KD et.al 2013). The iodine value indicates the degree of unsaturation of fat, which in turn denotes the less rancidity of fats and also having health benefits. In this study, Brahmi Nei contains more Iodine value which suggests the presence of higher unsaturated fatty acid bonds and the chance of rancidity will be less. Unsaponifiable matter indicates the non - fatty matter which contains active components. In Brahmi Nei increased value of unsaponifiable matter was 159.02 - 227.2 suggests that it contains more non fatty active volatile components. It has shown that all the levels of Lead, Cadmium, Mercury, Arsenic toxic heavy metals analyzed were not detectable in the Brahmi Nei. The implication of the present findings may be taken into consideration of the experimental formulation may be safe for the children. For the evaluation of microbial contamination, total bacterial and fungal content were less than 10 cfu/ml. In specific pathogen analysis *E. coli*, *Salmonella* spp., *S.aureus*

Pseudomonas aeruginosa were totally absent. All the pesticide residue and aflatoxins showed absent (Table -3). In a nutshell, Brahmi Nei reaches children with zero contamination and safe.

HPTLC study was done to obtain the fingerprints of the Brahmi Nei and it was also done to get standard markers. In the present study, HPTLC densitometric scan of petroleum ether extract of unsaponifiable matter of Brahmi Nei at 366 nm showed 6 peaks which covered the area of corresponding Rf values. Maximum spots were observed in our sample which indicates more active constituents in it. These are the standard markers of the components which can be used as referral standards.

In this clinical study we tested the influence of adding varmam and massage to Brahmi Nei on the level of spasticity of children with spastic cerebral palsy. Based on the results of this study, while compared to the Group II of children showed no significant reduce in spasticity, children of Group I and Group III showed significant reduction in spasticity all group of muscles. This study indicating that the combined therapy of internal medicine and external medicine had superior action as far as reduction in spasticity is concerned. We observed that the single internal medicine with Brahmi nei had no significant action as far as reduction in spasticity is concerned. There were significant decrease in Group I and Group III. In Siddha literature CP is under the Vadha disease therefore, the therapeutic management is considered to be internal medicine, (Thokkanam) massaging and varmam. Since Brahmi primary use is to enhance cognitive function, research has been focused on the mechanism behind these properties. The triterpenoid, saponins and their bacosides are responsible for increase the muscle tone through enhance nerve impulse transmission. The bacosides aid in repair of damaged neurons by enhancing kinase activity, neuronal synthesis, and restoration of synaptic activity, and ultimately nerve impulse transmission and boosting the synthesis of new protein in the brain (Singh HK, Dhawan BN 1997). Massage with Vasavu ennai which soothe the sensory nerve endings, they produce a hyperemic effect causing the arterioles dilate in musculature, and reduce stiffness (Shailaja U et.al 2013). Massage is considered to enhance muscle relaxation, (Nordschow, 1962) reduce muscle tension (Dubrosky V,1962) and soreness (Tiidus P and Shoemaker J,1995) and post- sequently, improve performance (Rinder A and Sutherland C, 1995). Massage is also thought to provide a soothing, sedative, invigorating feeling and can give the comfort (Tiidus P, 1997). Varma therapy is one of the method of treatment is prevalent in southern parts of Tamilnadu and Kerala. The stimulation of particular points in human body in appropriate pressure gives relief from the spasm.

4. Conclusion

In general, based on the results of this study it was found that Saponification value and Iodine value were higher which indicates higher active constituents were present in Brahmi Nei and it can reduce the chance of rancidity thereby increase the quality. The implication of the present findings such as microbial contamination, specific pathogen, aflatoxins, pesticide residues and heavy metals may be taken into consideration of the experimental formulation may be safe for the children. HPTLC showed that maximum number of spots in UV 366 nm. The marker found in HPTLC may be identified and used as referral standards. Hence the work can be used for the quality assessment and standardization of Brahmi Nei. In this clinical study, it can be concluded that Brahmi Nei along with Vasavu ennai massage and Varmam has a definitive action as well as clinical efficacy on spasticity in cerebral palsy children in contrast to that seen in regular OPD treatment and Brahmi nei. The effects of internal and external therapies may be due individual drugs' multipronged action. Further study is required for scientific validation to prove its clinical efficacy in multicentre clinical study.

References

Andersen, G. L., Irgens, L. M., Haagaas, I., Skranes, J. S., Meberg, A. E., & Vik, T. (2008). Cerebral palsy in Norway: prevalence, subtypes and severity. Eur J Paediatr Neurol, 12(1), 4-13. doi: 10.1016/j.ejpn.2007.05.001

Ashworth B. Preliminary trial of carisoprodol in multiple sclerosis. Practitioner 1964;192:540-542.

Blair, E. (2010). Epidemiology of the cerebral palsies. Orthopedic Clinics of North America, 41(4), 441-455. doi: 10.1016/j.ocl.2010.06.004

Dubrosky V. Changes in muscle and venous blood flow after massage. Soviet Sports Rev 1982; 4: 56-7

Duncan BD: Multiple range test for correlated and heteroscedastic means. *Biometrics* 1957, 13:359-364.

Kannan Rajaram,Varma maruthuva adippadaigal, A.T.SV.S. siddha maruthuva kallori, pudukkadai (po)., 2008; 1-78

Lim EC-H, Seet RCS. Botulinum toxin: description of injection techniques and examination of controversies surrounding toxin diffusion. Acta Neurol Scand 2008;117:73–84

Muller K, Mix E, Saberi FA, Dressler D, Benecke R. Prevalence of neutralizing antibodies in patients treated with botulinum toxin type A for spasticity. J Neural Transm 2009;116:579–85.

Nordschow M, Bierman W. The influence of manual massage on muscle relaxation: effect on trunk flexion. J Am Phys Ther 1962; 42 (10): 653-7

Orsnes GB, Sørensen PS, Larsen TK, Ravnborg M. Effect of baclofen on gait in spastic MS patients. Acta Neurol Scand 2000;101:244–8.

R.Thiyagarajan, Siddha maruthuvam-sirappu: varmam, 1ed., Commissionerate of Indian medicine and Homeopathy, Chennai,1985; 129-158

Rinder A, Sutherland C. An investigation of the effects of massage on quadriceps performance after exercise fatigue. Complement Ther Nurs Midwifery 1995; 1: 99-102

Shailaja U, Rao PN, Arun Raj GR. Clinical study on the efficacy of Samvardhana Ghrita orally and by Matrabasti in motor disabilities of Cerebral Palsy in children. Int J Res Ayurveda Pharm 2013; 4:373-7.

Siddha Hospital Pharmacopeia Part-I

Singh HK, Dhawan BN (1997) Neuropsychopharmacological effects of the Ayurvedic nootropic *Bacopa monniera* Linn (Brahmi). Indian J. Pharmachol. 29:S359–S365.

Tamil Nadu Siddha Medical Board 1995, 116

Tiidus P, Shoemaker J. Effleurage massage, muscle blood flow and long term post-exercise recovery. Int J Sports Med 1995; 16 (7): 478-83

Tiidus P. Manual massage and recovery of muscle function 79. A literature review. J Orthop Sports Phys Ther 1997; 25: 107-12

Ward AB. Spasticity treatment with botulinum toxins. J Neural Transm 2008;115:607–16.

Yadav KD, Reddy KR, Agarwal A. Preliminary physico – chemical profile of Brahmi Ghrita. AYU 2013; 34: 294 - 6.http://dx.doi.org 10.4103/0974 8520.123130 and http://en.wikipedia.org/wiki/Saponification_value 2013.

Access this Article in Online	
	Website: www.ijcrims.com
	Subject: Siddha Medicine
Quick Response Code	

How to cite this article:
Arul Mozhi P., Pattarayan R., Deivanayagi.S., Banumathy.V. (2016). Analytical Standardization of Brahmi Nei and Effect of Siddha Methodologies on Spasticity in Cerebral Palsy. Int. J. Curr. Res. Med. Sci. 2(10): 82-89.
DOI: http://dx.doi.org/10.22192/ijcrms.2016.02.10.009