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Antiepileptic Ayurvedic Medicinal Herb: *Centella Asiatica*

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ABSTRACT
Centella asiatica (gotukola) has been used as a medicine in the Ayurvedic tradition of India for thousands of years. It is listed in the historic Susruta samhita, an ancient Indian medicinal text. In China, gotukola is one of the reported "Miracle elixirs of life". In 19th century, Gotukola and its extracts were incorporated into the Indian pharmacopeia and considered as the Food for the brain. This study evaluated the anticonvulsant effect of chloroform extract of *Centella asiatica* (CA) with particular reference to carbohydrate metabolism in different types of rat muscles. The rats were randomly divided into 4 groups having 6 in each group: i.e. Control group received Saline, PTZ-induced epileptic group (60 mg/kg b.w op/ 1 day), Epileptic group pretreated with chloroform extract (CE), and Epileptic group pretreated with Diazepam (DP; Reference control) (2 mg/kg b.w/ip/ day). The CA extract is administered at the dose of 200 mg/kg body weight orally for one week. The experimental results were observed that the decreased content of Total carbohydrates in the muscles i.e. White Vastus (WV), Red Vastus (RV), Soleus (Sol) and Gastrocnemius (GN); increased the glycogen and glucose levels during PTZ-induced epilepsy in all the muscles. The reversal changes were observed on pre- treatment with the chloroform extract and diazepam. Hence, it is evident that the different bioactive factors of *Centella* offered protection against PTZ-induced epilepsy.

Keywords: Epilepsy, Anticonvulsant, *Centella asiatica*, Pentylenetetrazole, carbohydrate, rat muscle.

INTRODUCTION
Centella asiatica (CA) is a small herbaceous annual plant of the family Apiaceae, native to Asia, also known as Gotu Kola. The active constituents of *Centella* include triterpenoid glycosides i.e. asiatic acid, asiaticoside, madecassic acid, madecassoside, oxyasiaticoside, and centelloside[1,2] ; saponin glycosides (1.4-3.4%) (brahmiside, brahminoside); flavonol glycosides (quercetin-3-glycoside and Kampferol-3-glycoside); flavonoids viz., naringin, quercitin, rutin, cathecin, kampeferol and apigenin; phytosterols such as ß-sitosterol, stigmasterol and camposterol and a volatile oil consisting of vallerin, camphor, cineole and terpene acetate that comprises 35% of the total oil content (Gotu kola, centella asiatica, the Goddes of the Supreme Wisdom). Gotukola also contains naturally occurring vitamins A, B, C, G, K, tannins (24.5%); essential oils (0.8-1%); monoterpenes, sesquiterpenes, several aminoacids (lysine, alanine, phenylalanine, serine, aspartic acid, glutamic acid); fatty acids (palmitic, oleic and linoleic acids); resin (8.9%); an alkaloid named hydrocotyline and elements Calcium, Magnesium and Sodium[3].

Extracts of *Centella asiatica* have also successfully treated in surgical wounds, skin grafts, gangrene, and traumatic injuries[4]; chronic skin lesions and leprosy wounds[5]. *Centella asiatica* showed

wound healing activity[6] ; anti-anxiety activity[7] ; anti-hepatoma activity[8] ; cognition-enhancement in rats[9]. *Centella asiatica* was effective in improving microcirculation in venous hypertension and diabetic microangiopathy[10] . It was also used in the treatment of tuberculosis, syphilis, amoebic dysentery and common cold, also known as anti-aging plant[11] . It also showed protection against radiation induced damage in liver[12], lead poisoning in CNS[13] , age related oxidative damage and colon tumorigenesis. It was also used in the treatment of anaemia, blood disorders, bronchitis, urinary disorders and splenomegali. It is also an active constituent in ayurvedic formulations like Mentat, Memorin, Mentalin, Mental Alertness, Abana (Heart care), Geriforte (Gericare), Anxocare etc.

Hence, the present study selected biochemical parameters in carbohydrate metabolism in different muscles of rat during Pentylenetetrazole induced epilepsy and antiepileptic effect of chloroform extract of medicinal plant, *Centella asiatica*.

MATERIALS AND METHODS

Procurement and Maintenance of Experimental Animals

Male adult Wistar rats weighing 150±25 grams were used as the experimental animals in the present investigation. The rats were purchased from the Indian Institute of Science (IISc), Bangalore, maintained in the animal house of the department in polypropylene cages under laboratory conditions of 28 ± 2^0C temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai) and water ad libitum.

Ethical guidelines

The rats were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 1538/01/A/CPCSEA/ dt:14.08.2015 in its resolution No:09/(i)/a/ CPCSCA/ IAEC/ SVU/ WR/KSP/Dt. 04.03.2016.

Selection of Drug

Pentylenetetrazole (PTZ), an anticonvulsant drug, was selected for the present study. It was obtained as commercial grade chemical from Sigma chemicals, USA.

Collection of the plant material

Centella asiatica (CA) plant was collected from Tirumala hills and indentified by a botanist, Department of Botany, S.V.University, Tirupati. A voucher specimen was deposited in the herbarium of the Department of Botany, S.V.University, Tirupati (Voucher no. 1688).

Preparation of Plant Extract

The active principles of the leaves of plant were extracted into chloroform, since this solvent was predominantly used by several investigators for extracting anticonvulsant principle(s) from various plants [14,15]. Powdered plant material was soaked in methanol for 2 days at room temperature and the solvent was filtered. This was repeated 3-4 times until the extract gave no coloration. The extract was distilled and concentrated under reduced pressure in the Buchi rotovapour R-114 yielding a gum-like residue, which was then suspended in water and extracted with chloroform.

Induction of Epilepsy

Convulsions were induced by an intraperitoneal (i.p.) injection of Pentylenetetrazole (60mg/Kg body weight) in saline [16,17].

Administration of Test substance

Ethnolic extract of CA (200mg/Kg body weight) was dissolved in saline and given to the animals for one week prior to the injection of PTZ [18]. A gavage tube was used to deliver the substance by the oral route, which is the clinically expected route of administration of CA [19]. The volume of administration was kept at 1ml/kg/ animal. Diazepam, an anticonvulsant drug, was dissolved in normal saline and given intraperitoneally (2mg/Kg bw i.p.) for one week to the experimental animals (Reference control).

Drugs ,Chemicals and apparatus

All chemicals used in the present study were Analar grade (AR) and obtained from the following scientific companies: Sigma, Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India). Pentylenetetrazole and diazepam were obtained from Sigma Aldrich (St. Louis, MO, USA). In the present investigation Barnstead Thermoline water purification plant for nanopure water, Kubota KR centrifuge and Hitachi U-2000 Spectrophotometer and other standard equipments were used for biochemical analyses.

Isolation of Tissues

The animals were sacrificed after the treatment by cervical dislocation. The muscle was isolated immediately and placed on a chilled glass plate. Functionally different muscles such as white vastus (WV), red vastus (RV), soleus (SOL) and gastrocnemius (GM) muscles were separated and frozen in liquid nitrogen (-180^{0}C) and stored at -40^{0}C until further use. At the time of analyses the tissues were thawed and used. Selected parameters were estimated by employing standard methods.

Experimental design for screening of plant extracts for anticonvulsant activity

The rats were randomly divided into 4 groups having 6 in each group: i.e. Control group received Saline, PTZ-induced epileptic group (60 mg/kg b.w./ i.p/ 1 day), Epileptic group pretreated with chloroform extract (CE) and Epileptic group pretreated with Diazepam (DP; Reference control) (2 mg/kg b.w/i.p). The chloroform extract was administered at the dose of 200 mg/kg body weight orally for one week.

Biochemical analysis

The total carbohydrate content was estimated by the method of Carroll *et al*[20]. The total carbohydrate content was expressed as mg of glucose/gm wet weight of the tissue. Glycogen was estimated by the method of Kemp and Van Hejnigen[21] . The glycogen content was expressed as mg glucose equivalents/gram wet weight of the tissue. Glucose was estimated by the method of Mendal et al[22]. The glucose content was expressed as mg glucose/gram wet weight of the tissue.

Statistical treatment of data

All assays were carried out with six separate replicates from each group. The mean, standard error (SE) and Analysis of Variance (ANOVA) were done using SPSS statistical software (11.5 ver.) for different parameters. Difference between control and experimental assays were considered as significant at $P<0.05$.

RESULTS & DISCUSSION

Total Carbohydrates

The total carbohydrates were estimated in different muscles of the rat with reference to the administration of convulsant drug pentylenetetrazole (PTZ) and the treatment with anticonvulsant chloroform extraction of *Centella asiatica* (CA). The decrease in total carbohydrate levels in the white vastus, red vastus, soleus and gastrocnemius muscles of PTZ treated rats indicates utilization of carbohydrates to meet energy demands during PTZ induced epileptic conditions[23]. The results are presented in table (1).

On treatment with chloroform extract the total carbohydrate levels were increased which might be due to the synthesis of carbohydrates through glycogenesis and gluconeogenesis. Although not to the same extent, the total carbohydrates were increased in combination treatment (PTZ+CE extract) suggesting that extract help to replenish the loss of carbohydrates that occur during epileptic seizures[24]. Table(1).

GLYCOGEN & GLUCOSE

Glycogen is a major storage form of carbohydrate in animals for biological function and the maintenance of the glycogen reserves is an important feature of the normal metabolism[25] . The amount of glycogen present in tissues varied widely with diet and physiological status[26] . Many cells store glycogen for the purpose of

having glucose available for further use. Muscle glycogen is present to serve as a fuel reserve for the synthesis of ATP within that tissue for increased muscular activity.

The glycogen and glucose levels were increased in all the muscles during PTZ-induced epilepsy. Whereas, in plant extract treated rats the glycogen content was decreased. Similarly, with the combination treatment (CE +PTZ) the glycogen content was increased, to lesser extent than the PTZ treatment. The results are presented in table (2, 3).

The glycogen levels were increased in PTZ treated animals due to mobilization of stored reserves and mobilization of glycogen from liver to the skeletal muscle in order to meet the energy demands during epileptic condition. On par with the glycogen, glucose levels were also increased in all muscles during PTZ-induced epilepsy which might be due to increased conversion of glycogen to glucose for the onward glycolytic pathway.

On Contrary to this, glucose levels were decreased during treatment with CA extract and lesser increment in combination treatment which suggest that lesser utilization of glucose through anaerobic glycolysis. However, the elevated levels of TCA cycle activity during treatment with chloroform extract and in combination treatment suggest maintenance of normal glycolytic activity that contribute to the formation of pyruvate and subsequent TCA cycle activity; in addition to the mobilization of glycogen from liver to all the metabolic tissues including muscle. Since the anticonvulsant drugs suppress the normal physical activity, it is possible that the energy yielding components such as carbohydrates, lipids etc might be utilized to a lesser extent despite the maintenance of normal metabolic activity.

CONCLUSION

The present findings demonstrate that there is significant diminution in the glycolytic and oxidative potential of all functionally different muscles of rat during PTZ-induced epilepsy. Chloroform extracts of Centella asiatica considerably reduce the risk of metabolic dysfunction that occurred during epilepsy. Thus, these extract and more particularly the bioactive factors present in the antiepileptic treatment. However, further in depth studies are required to understand the physiological mechanism of different bioactive compounds present in the CA extracts and to suggest that the therapeutic approaches of these compounds with particular reference of anticonvulsant and neuroprotective activity.

REFERENCES

1. Inamdar PK , Yeole RD, Srivastava de Souza, NJ. Stability study of the active constituents in the Centella asiatica extract formulations. Drug Dev. Industr. Pharm 1996; 22 (5): 211 –216.
2. Maquart FX, Chastang F, Simeon A, Birembaut P, Gillery P and Wegrowski Y. Triterpenes from Centella asiatica stimulate extracellular matrix accumulation in rat experimental wounds. Eur. J. Dermatol.1999; 9: 289-296.
3. Sivaprasad K. Neuroprotective upshot of Centella asiatica against pentylenetetrazole induced epilepsy in rats with reference to protein metabolism. Int. Journl of Recent Scientific Research 2017; 8 (12) : 22555-22559.
4. Kartnig T. Clinical applications of Centella asiatica (L.) Urb. J. Herbs, Spices and Medicinal plants 1988; 3: 146-173.
5. Lawrence JC. The morphological and pharmacological effects of asiaticoside upon skin in vitro and in vivo. Eur. J. Pharmacol. 1967; 1: 414-424.
6. Coldren CD, Hashim P and Ali JM. Gene expression changes in the human fibroblast induced by Centella asiatica treterpenoids. Planta Med 2003; 69: 725-732.

7. Bradwejn J, Zhou Y, Koszyeki D and Shlik J. A double-blind, placebo-controlled study on the effects of Gotu Kola (Centella asiatica) on acoustic startle response in healthy subjects. J. Clin. Psychopharmacol 2000; 20: 680-684.
8. Lin, LT, Liu, LT, Chiang, LC and Lin CC. In vitro anti-hepatoma activity of fifteen natural medicines from Canada. Phytother. Res 2002; 16: 440-444.
9. Gupta YK, Veerendra Kumar MH. Effect of Centella asiatica on pentylenetetrazole induced kindling, cognition and oxidative stress in rats. Ind. J. Pharmacol 2003; 35: 128-136.
10. Incandela L, Cesarone MR, Cacchio M, De Sanctis MT, Santavenere, C and D'Auro MG. Total triterpenic fraction of Centella asiatica in chronic venous insufficiency and in high-perfusion microangiopathy. Angiology 2001; 52 (Suppl. 2): S9-S13.
11. Mehmood I, Mohammed Ahmad, ZF. Screening of some Indian Medicinal plants for their antimicrobial properties. J Ethnopharmacol 1998; 62:183-93.
12. Sharma R, and Sharma J. Modification of gamma ray induced changes in the mouse hepatocytes by Centella asiatica extract: in vivo studies. Phytother. Res 2005; 19: 605-611.
13. Saxena G and Flora SJ. Changes in brain biogenic amines and haem biosynthesis and their response to combined administration of succimers and Centella asiatica in lead poisoned rats. J. Pharm. Pharmacol 2006; 58: 547-559.
14. Sowmyalakshmi S, Nur-e-Alam M, Akbarsha M A, Thirugnanam S, Jurgen Rohr & Chendil D. Investigation on semecarpus lehyam-a siddha medicine for breast cancer. Planta 2015; 220: 910-18.
15. Visweswari G, Siva Prasad K, Chetan P S, Lokanatha V, Rajendra W. Evaluation of anticonvulsant effect of Centella asiatica (Gotu kola) in pentylenetetrazol-induced seizures with respect to cholinergic neurotransmission. Epilepsy & Behavior 2010; 17: 332-35.
16. Santos Junior JG, Do Monte FHM, Russi M, Agustine PE, Lanziotti. Proconvulsant effects of high doses of venlafaxine in pentylenetetrazole-convulsive rats. Brazilian Journal of Medical and Biological Research 2012; 35: 469-472.
17. Rizwan AN, Ali A, Dua Y, Pal SN, Pillai KK. Effects of gabapentin and antidepressant drug combinations on convulsions and memory in mice. Pol. J. Pharmacol. 2013; 55: 965–971.
18. Vattanajun A, Wattanabe H, Tantisira M H & Tantisira T. Isobolographically additive anticonvulsant activity between Centella asiatica's Ethyl Acetate fraction and some antiepileptic drugs. J. Med. Assoc. Thai 2005; 88: S131-40.
19. Ghosh K, Indra N, Jagadeesan G. The ameliorating effect of Centella asiatica chloroform extract on albino rats treated with isoniazid. J Basic Clin Physiol Pharmacol 2017; 28(1): 66-77.
20. Carroll NV, Longley RW, Roe JH. The determination of glycogen in liver and muscle by use of anthrone reagent. J Biol Chem 1956; 220: 583-93.
21. Kemp A, Van Heijningen AJ. A colorimetric micro-method for the determination of glycogen in tissues. Biochem J 1954; 56(4): 646-8.
22. Mendel, B Kemp, A, Myers, DK. A calorimetric micro - methods for determination of glucose; Biochem. J 1954; 56: 639 - 646.
23. Kanchi Siva Prasad, G Sudharani, M. Anil Kumar. Alterations in the muscle carbohydratemetabolism and protective role of Centella asiatica; Acta Chemi phram India 2011; 1 (1), 20-31.
24. Kanchi Siva Prasad. Neuroprotective upshot of Centella asiatica against pentylenetetrazole; International Journal of Recent Scientific Research 2017; 8 (12): 22555-22559.

25. Turner LV, Manchester KL. Effects of denervation on the glycogen content and on the activities of enzymes of glucose and glycogen metabolism in rat diaphragm muscle. Biochemical Journal 1972; 128 (4) 789-801; DOI: 10.1042/bj1280789.
26. Brooks and Fahey. Human Bioenergetics and Its Applications Exercise Physiology. John Wiley and Sons, New York 1984; pp. A-5–A-14.

Table1: Changes in the **Total carbohydrates** content in different muscles of rat during PTZ-induced epilepsy and treatment with chloroform extract of Centella asiatica.

Muscle	Saline control	Chloroform Extract (CE)	Pentylenetetrazole (PTZ)	PTZ+Diazepam
White Vastus	5.092	8.401*	4.440*	6.865*
	±0.010	±0.011	±0.007	±0.013
		(64.96)	(-12.81)	(34.80)
Red Vastus	8.681	11.339*	7.067*	10.132*
	±0.017	±0.023	±0.016	±0.031
		(30.60)	(-18.59)	(16.70)
Soleus	6.269	10.399*	5.205*	9.158*
	±0.020	±0.022	±0.016	±0.016
		(65.87)	(-16.96)	(46.08)
Gastrocnemius	7.033	10.007*	5.704*	8.721*
	±0.031	±0.015	±0.018	±0.014
		(42.28)	(-18.89)	(23.99)

All the values are mean, ±SE of six individual observations.

Values in '( ) 'parentheses are % change over saline control .

*Values are significant at $P < 0.05$ in Scheffe test.

(Values are expressed in mg/g wet wt of the tissue)

Table 2: Changes in the **Glucose** content in different muscles of rat during PTZ-induced epilepsy and treatment with chloroform extract of Centella asiatica.

Muscle	Saline control	Chloroform Extract (CE)	Pentylenetetrazole (PTZ)	PTZ+Diazepam
White Vastus	0.911	0.889*	1.219*	0.891
	±0.054	±0.057	±0.049	±0.048
		(-2.486)	(33.71)	(-2.17)
Red Vastus	0.766	0.742*	1.072*	0.744*
	±0.007	±0.007	±0.010	±0.008
		(-3.132)	(39.98)	(-2.827)
Soleus	0.736	0.878*	1.041*	0.743
	±0.008	±0.008	±0.003	±0.006
		(19.40)	(41.50)	(0.951)
Gastrocnemius	0.694	0.881*	1.019*	0.697
	±0.010	±0.004	±0.008	±0.008
		(26.99)	(46.85)	(0.552)

All the values are mean, ±SE of six individual observations.

Values in ’( ) ‘parentheses are % change over saline control .

*Values are significant at P < 0.05 in Scheffe test.

(Values are expressed in mg/g wet wt of the tissue)

Table3: Changes in the **Glycogen** content in different muscles of rat during PTZ-induced epilepsy and treatment with chloroform extract of Centella asiatica.

Muscle	Saline control	Chloroform Extract (CE)	Pentylenetetrazole (PTZ)	PTZ+Diazepam
White Vastus	0.945	0.887*	1.243*	0.914
	±0.024	±0.012	±0.012	±0.011
		(-6.11)	(31.56)	(-3.29)
Red Vastus	0.957	0.933*	1.263*	0.935
	±0.007	±0.007	±0.010	±0.008
		(-2.50)	(32.00)	(-2.263)
Soleus	0.927	1.069*	1.232*	0.934
	±0.008	±0.008	±0.003	±0.006
		(15.40)	(32.95)	(0.755)
Gastrocnemius	0.885	1.072*	1.210*	0.883
	±0.010	±0.004	±0.008	±0.008
		(21.16)	(36.74)	(0.433)

All the values are mean, ±SE of six individual observations.

Values in ’( ) ‘parentheses are % change over saline control .

*Values are significant at P < 0.05 in Scheffe test.

(Values are expressed in mg/g wet wt of the tissue)