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ALTERATIONS IN THE MUSCLE CARBOHYDRATE METABOLISM DURING PENTYLENETETRAZOLE-INDUCED EPILEPSY: PROTECTIVE ROLE OF CENTELLA ASIATICA

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ABSTRACT

The aim of this study is to investigate the anticonvulsant effect of different extracts of *Centella asiatica* (CA) in functionally different muscles with reference to carbohydrate metabolism during pentylenetetrazole (PTZ) induced epilepsy and also during pre-treatment with different CA extracts. The rats were randomly divided into 7 groups having 6 in each group: (1) Control group received saline, (2) PTZ-induced epileptic group (60 mg/Kg, i.p.), (3) Epileptic group pretreated with n-hexane extract (n-HE), (4) Epileptic group pretreated with chloroform extract (CE), (5) Epileptic group pretreated with ethyl acetate extract (EAE), (6) Epileptic group pretreated with n-butanol extract (n-BE) and (7) Epileptic group pretreated with aqueous extract (AE). PTZ-induced epilepsy increased the glycogen, glucose and lactate contents and decreased the levels of total carbohydrates (TC) and pyruvate (PYR), Lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) activities in all the muscles (White vastus, Red vastus, Soleus and Gastrocnemius). Pre-treatment with different CA extracts showed a conspicuous recovery in the levels of glycogen, glucose and lactate contents and LDH, ICDH, SDH and MDH activity levels. From the results, it is presumed that the bioactive factors present in different extracts of CA offered protection against PTZ- induced alterations occurred in different muscles.

Key words: Epilepsy, Anticonvulsant, *Centella asiatica*, Pentylenetetrazole, Carbohydrates.

INTRODUCTION

Epilepsy is the most frequent neurodegenerative disorder affecting more than 50 million people world wide[1]. It is well established that impaired GABAergic activity and exaggerated glutamatergic activity are thought to contribute to the various types of epilepsy[2]. The epileptic seizures occur via alterations in the behavior of neural networks in the brain that induce synchronized bursting interspersed by periods of normal electrical activity[3]. The abnormal activity of the brain is transmitted to the rest of the body as incorrect signals, resulting in abnormal muscular activity or convulsions (Convulsions-ecure-com.html). On the basis of multiple neurophysiological mechanisms exhibited during epilepsy, a few antiepileptic drugs have been emerged with different target specifities. However, many antiepileptic drugs (AEDs) show very narrow therapeutic window and showed either limited efficacy or severe adverse effects. From the survey of literature, it is obvious that screening of phytochemicals with particular reference to anticonvulsant/

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antiepileptic activity was performed by number of workers for the past few years from other countries and much is awaited from our country, which is endowed with rich heritage of flora and fauna. *Centella asiatica* (CA), one of the multipurpose miracle herbs of oriental medicines, has been used in ayurvedic preparations in the treatment of mental fatigue and anxiety[4]. The extracts of CA also showed antidepressant activity[5], improving learning deficits[6] and protection against convulsions induced by pentylenetetrazole and Strychnine[7]. Even though much work has been done on the anticonvulsant effects of this medicinal plant as reported in the foregoing account, no systematic investigation was carried out on the neurobiological role of *Centella asiatica*, with particular reference to anticonvulsant and neuroprotective activity. In addition to the neuropathological abnormalities associated with the epilepsy, it has been well documented that seizure are characterized by repetitive, rhythmic jerking of limbs resulting from involuntary muscle twitching and loss of muscle tone (Convulsions-ecure-com.html). The main overt symptoms of epileptic patient include stiffening of muscles for 30 seconds to one minute (Tonic phase) followed by the phase of muscle jerking convulsions (Clonic phase). Although many reports are available on the neurochemical and neurophysiological abnormalities during epilepsy, not much is known on the influence of epilepsy on muscle metabolism. Hence, the present study is undertaken to examine the anticonvulsant effect of different fractions of *Centella asiatica* on selected biochemical parameters in functionally different types of rat skeletal muscle with particular reference to carbohydrate metabolism.

EXPERIMENTAL

Materials and methods

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^{o}C 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*. The rats were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 438/01/a/cpcsea/dt 17.07.2001 in its resolution No: 9/IAEC/SVU/2007/dt 04.03.2007.

Selection of drug

Pentylenetetrazole (PTZ), a convulsing 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 identified 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). The leaves were separated from the plant, dried in shade, powdered and powder was used for the extraction of anticonvulsant principle/s using different solvents.

Preparation of plant extracts

The active principles of the leaves of plant were extracted into different solvents, methanol, water, n-hexane, chloroform, ethyl acetate and n-butanol, since these solvents were predominantly used by several investigators for extracting anticonvulsant principle(s) from various plants[8,9]. 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 various organic solvents of increasing polarity (starting with the lipophilic solvent n-hexane, ending with the more hydrophilic n-butanol). The solvent from each extract was distilled and concentrated under reduced pressure in the Buchi rotavapour. Finally the extracts were freeze dried and were used for further studies.

Induction of epilepsy

Convulsions were induced by an intraperitoneal (i.p.) injection of pentylenetetrazole (60 mg/Kg body weight)[10-14].

Administration of the tested substance

Each fraction of CA extract (200 mg/Kg body weight) was dissolved in saline and given to the animals for one week prior to the injection of PTZ[13]. A gavage tube was used to deliver the substance by the oral route, which is the clinically 5 expected route of administration of CA[9]. The volume of administration was kept at 1 mL/Kg b.w. to the animal. The rats were divided into 7 groups, each consisted of 6 rats and used for studying the effects of different fractions/ext racts of plant, *Centella asiatica*.

Group 1 - Normal saline treated control rats (SC)

Group 2 - Rats treated with PTZ (PTZ)

Group 3 - Epileptic rats pretreated with n-hexane extract (n-He + PTZ)

Group 4 - Epileptic rats pretreated with chloroform extract (CE + PTZ)

Group 5 - Epileptic rats pretreated with ethyl acetate extract (EAE + PTZ)

Group 6 - Epileptic rats pretreated with n-butanol extract (n-Be + PTZ)

Group 7 - Epileptic rats pretreated with aqueous extract (AE + PTZ)

Isolation of tissues

The animals were sacrificed after the treatment by cervical dislocation. Functionally different muscles such as white vastus, red vastus, soleus and gastrocnemius muscles were separated and frozen in liquid nitrogen (-180^{o}C) and stored at -40^{o}C until further use. At the time of analyses, the tissues were thawed and selected parameters were estimated by employing standard methods.

Procurement of chemicals

All chemicals used in the present study were Analar grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

Biochemical analyses

The total carbohydrate content was estimated by the method of Carroll *et al.*[15]. Glycogen was estimated by the method of Kemp and Van Hejnigen[16]. Glucose was estimated by the method of Mendal *et al.*[17]. Lactic acid in the muscle was estimated by the method of Barker and Summerson[18] as modified by Huckabee[19]. Pyruvate content of the muscle was estimated by the method of Friedmann and Hangen[20]. The activity levels of lactate (LDH), succinate (SDH), and malate (MDH) dehydrogenases were estimated by the method of Nachlas *et al.*[21] with slight modifications as described by Prameelamma and Swami[22].

Isocitrate dehydrogenase activity was assayed by the method of Korenberg and Pricer[23] as modified by Mastanaiah *et al*.[24].

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 for different parameters. Difference between control and experimental assays was considered as significant at $P < 0.05$.

RESULTS AND DISCUSSION

Different parameters of glycolytic and oxidative pathways were estimated in different muscles of rat during PTZ-induced epilepsy and during pre-treatment with different CA extracts. Total carbohydrates were decreased in all the muscles during PTZ-induced epilepsy which were elevated in the muscles of epileptic animals pre-treated with different CA extracts. Glycogen and glucose levels were elevated in all the muscles during epilepsy which were recovered to normalcy in the epileptic rats pre-treated with different extracts of CA. Increased lactate and decreased pyruvate levels were recorded in all the muscles during PTZ-induced epilepsy. Where as lactate levels were decreased and pyruvate levels were increased in some muscles during treatment with different CA extracts. Although 7 significant changes were not observed in the lactate and pyruvate levels in some muscles, the levels were recovered to normalcy during pre-treatment with different extracts of CA. Decreased lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) activities were recorded in some muscles during epilepsy, which were elevated or reached to normalcy in the epileptic animals pre-treated with different extracts of CA (Tables 1-4).

Table 1: Alterations in the carbohydrate metabolism in white vastus of wistar male albino rat during PTZ-induced epilepsy and on pre-treatment with different extracts of centella asiatica

(The values of total carbohydrates, glycogen, glucose and lactate were expressed as mg/g wet wt; pyruvate levels expressed as µ moles/g wet wt. and LDH, ICDH, SDH, and MDH activities were represented as µ moles of formazan formed/mg protein/ hour).

WV	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
	5.092	4.440*	5.661*	6.864*	6.252*	5.921*	5.575*
TC							
	± 0.010	± 0.007	± 0.018	± 0.013	± 0.022	± 0.021	± 0.009
		(-12.81)	(11.18)	(34.8)	(22.79)	(16.29)	(9.48)
	0.945	1.243*	0.894*	0.887*	0.866*	0.888*	0.914*
GLY							
	± 0.024	± 0.012	± 0.008	± 0.012	± 0.011	± 0.009	± 0.011
		(31.56)	(-5.37)	(-6.11)	(-8.39)	(-6.03)	(-3.29)
	0.911	1.218*	0.889	0.888	0.882	0.900	0.891
GLU							
	± 0.054	± 0.049	± 0.052	± 0.057	± 0.055	± 0.062	± 0.048
		(33.71)	(-2.41)	(-2.486)	(-3.23)	(-1.22)	(-2.17)

Cont…

WV	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
	1.322	1.768*	1.216*	1.035*	1.293	1.164*	1.266
LAC							
	± 0.059	± 0.042	± 0.067	± 0.012	± 0.068	± 0.026	± 0.048
		(33.77)	(-8.04)	(-21.73)	(-2.192)	(-11.97)	(-4.22)
	86.4	48.159*	89.761	104.233*	93.511	114.333*	92.733
PYR							
	± 3.069	± 3.664	± 7.070	± 10.023	± 5.956	± 4.780	± 4.79
		(-44.26)	(3.89)	(20.64)	(8.23)	(32.33)	(7.33)
	2.307	1.365*	3.273*	2.906*	2.832*	2.689*	3.074*
LDH							
	± 0.272	± 0.059	± 0.128	± 0.115	± 0.006	± 0.073	± 0.137
		(-40.83)	(41.85)	(25.96)	(22.74)	(16.57)	(33.23)
	1.043	0.853*	1.087	1.207*	1.069	1.183*	1.112
ICDH							
	± 0.021	± 0.039	± 0.037	± 0.041	± 0.047	± 0.041	± 0.026
		(-18.17)	(4.24)	(15.68)	(2.49)	(13.45)	(6.64)
	2.048	1.311*	3.014*	2.647*	2.573*	2.430*	2.682*
SDH							
	± 0.272	± 0.059	± 0.128	± 0.115	± 0.006	± 0.073	± 0.137
		(-35.99)	(47.18)	(29.25)	(25.62)	(18.66)	(30.96)
	1.219	1.110*	1.869*	1.803*	1.816*	1.704*	1.569*
MDH							
	± 0.043	± 0.029	± 0.017	± 0.026	± 0.021	± 0.023	± 0.020
		(-8.96)	(53.36)	(47.92)	(49.01)	(39.8)	(28.75)

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.

Table 2: Alterations in the carbohydrate metabolism in red vastus of wistar male albino rat during PTZ-induced epilepsy and on pre-treatment with different extracts of centella asiatica

(The values of total carbohydrates, glycogen, glucose and lactate were expressed as mg/g wet wt; pyruvate levels expressed as µ moles/g wet wt. and LDH, ICDH, SDH, and MDH activities were represented as µ moles of formazan formed/mg protein/ hour).

RV	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
	8.681	7.067*	8.960	10.131*	9.645*	9.086	9.568*
TC							
	± 0.017	± 0.016	± 0.013	± 0.031	± 0.028	± 0.015	± 0.023
		(-18.59)	(3.21)	(16.7)	(11.11)	(4.66)	(10.22)

Cont…

RV	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
GLY	0.957	1.072*	0.934	0.933	0.934	0.955	0.935
	± 0.007	± 0.010	± 0.007	± 0.007	± 0.003	± 0.012	± 0.008
		(12)	(-2.42)	(-2.5)	(-2.36)	(-0.17)	(-2.263)
GLU	0.766	0.842*	0.743	0.742	0.724	0.764	0.744
	± 0.007	± 0.010	± 0.007	± 0.007	± 0.003	± 0.012	± 0.008
		(9.98)	(-3.02)	(-3.13)	(-5.44)	(-0.21)	(-2.82)
LAC	1.430	1.558	1.287	1.335	1.284*	1.413	1.498
	± 0.038	± 0.077	± 0.029	± 0.005	± 0.073	± 0.031	± 0.003
		(8.92)	(-9.99)	(-6.65)	(-10.18)	(-1.16)	(4.74)
PYR	92.940	64.389*	97.894	107.401*	110.180*	118.777*	101.658*
	± 7.268	± 5.559	± 2.126	± 4.931	± 3.069	± 6.91	± 4.14
		(-30.72)	(5.33)	(15.56)	(18.55)	(27.8)	(9.38)
LDH	1.519*	1.213	1.542	1.543	1.637	1.563	1.541
	± 0.007	± 0.010	± 0.007	± 0.007	± 0.003	± 0.012	± 0.008
		(-20.16)	(1.524)	(1.579)	(7.789)	(2.9)	(1.42)
ICDH	0.754	0.604*	0.864*	0.947	0.912*	0.807	0.787
	± 0.029	± 0.016	± 0.026	± 0.037	± 0.021	± 0.071	± 0.026
		(-19.88)	(14.53)	(25.58)	(21)	(6.98)	(4.37)
SDH	1.260	0.954*	1.283	1.284	1.378*	1.262	1.282
	± 0.007	± 0.010	± 0.007	± 0.007	± 0.003	± 0.012	± 0.008
		(-24.3)	(1.838)	(1.904)	(9.39)	(0.132)	(1.715)
MDH	2.612	1.884*	2.808	3.435*	2.866*	2.836*	2.829*
	± 0.040	± 0.036	± 0.031	± 0.025	± 0.047	± 0.021	± 0.032
		(-27.86)	(7.52)	(31.51)	(9.70)	(8.57)	(8.309)

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

Table 3: Alterations in the carbohydrate metabolism in Soleus muscle of wistar male albino rat during PTZ-induced epilepsy and on pre-treatment with different extracts of *Centella asiatica*

(The values of total carbohydrates, glycogen, glucose and lactate were expressed as mg/g wet wt; pyruvate levels expressed as µ moles/g wet wt. and LDH, ICDH, SDH, and MDH activities were represented as µ moles of formazan formed/mg protein/ hour).

SOL	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
	6.269	5.206*	7.282*	9.158*	8.147*	8.222*	6.720
TC							
	± 0.020	± 0.016	± 0.017	± 0.016	± 0.011	± 0.020	± 0.016
		(-16.96)	(16.16)	(46.08)	(29.95)	(31.15)	(7.2)
	0.927	1.232*	0.922	0.784	0.807	0.793	0.920
GLY							
	± 0.008	± 0.003	± 0.009	± 0.008	± 0.005	± 0.007	± 0.006
		(32.95)	(-0.593)	(-15.4)	(-12.92)	(-14.47)	(-0.755)
	0.736	1.041*	0.731	0.593*	0.616*	0.602*	0.729
GLU							
	± 0.008	± 0.003	± 0.009	± 0.008	± 0.005	± 0.007	± 0.006
		(41.5)	(-0.747)	(-19.4)	(-16.28)	(-18.22)	(-0.951)
	1.269	1.703*	1.252	0.977*	1.074*	0.897	1.249
LAC							
	±0.029	± 0.011	± 0.020	± 0.026	± 0.02	± 0.005	± 0.024
		(34.17)	(-1.36)	(-23.02)	(-15.39)	(-29.31)	(-1.6)
	82.041	55.493*	86.988	89.564*	100.467*	103.363*	84.018
PYR							
	±8.606	± 3.069	± 2.427	± 2.455	± 2.577	± 1.904	± 5.50
		(-32.36)	(6.03)	(9.17)	(22.46)	(25.99)	(2.41)
	1.489	1.184*	1.494	1.632*	1.609*	1.623*	1.496
LDH							
	±0.008	± 0.003	± 0.009	± 0.008	± 0.005	± 0.007	± 0.006
		(-20.51)	(0.36)	(9.592)	(8.047)	(9.01)	(0.47)
	1.06	0.973*	1.243*	1.229*	1.197*	1.390*	1.060
ICDH							
	±0.030	± 0.016	± 0.012	± 0.040	± 0.029	± 0.034	± 0.026
		(-8.23)	(17.25)	(15.96)	(12.91)	(31.14)	(0.031)
	1.23	0.925*	1.235	1.373*	1.350*	1.364*	1.237
SDH							
	±0.008	± 0.003	± 0.009	± 0.008	± 0.005	± 0.007	± 0.006
		(-24.83)	(0.447)	(11.61)	(9.74)	(10.9)	(0.56)

Cont…

SOL	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
	3.243	3.134	3.894*	4.074*	4.450*	4.460*	3.594*
MDH							
	±0.043	± 0.029	± 0.017	± 0.026	± 0.021	± 0.023	± 0.020
		(-3.37)	(20.06)	(25.61)	(37.22)	(37.52)	(10.81)

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.

Table 4: Alterations in the carbohydrate metabolism in Gastrocnemius muscle of wistar male albino rat during PTZ induced epilepsy and on pre-treatment with different extracts of *Centella asiatica*

(The values of total carbohydrates, glycogen, glucose and lactate were expressed as mg/g wet wt; pyruvate levels expressed as µ moles/g wet wt. and LDH, ICDH, SDH, and MDH activities were represented as µ moles of formazan formed/mg protein/ hour).

GN	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
	7.033	5.704*	7.200	8.720*	7.942*	7.869*	7.374
TC							
	± 0.031	± 0.018	± 0.016	± 0.014	± 0.021	± 0.010	± 0.024
		(-18.89)	(2.38)	(23.99)	(12.93)	(11.88)	(4.85)
	0.885	1.210*	0.879	0.698*	0.752*	0.751*	0.881
GLY							
	± 0.010	± 0.008	± 0.006	± 0.004	± 0.011	± 0.008	± 0.008
		(36.74)	(-0.64)	(-21.16)	(-15.04)	(-15.14)	(-0.433)
	0.694	1.019*	0.688	0.507*	0.561*	0.560*	0.690
GLU							
	± 0.010	± 0.008	± 0.006	± 0.004	± 0.011	± 0.008	± 0.008
		(46.85)	(-0.816)	(-26.99)	(-19.18)	(-19.3)	(-0.552)
	2.62	3.018*	2.615	2.236*	2.393	2.459	2.570
LAC							
	± 0.011	± 0.068	± 0.007	± 0.105	± 0.295	± 0.019	± 0.019
		(15.2)	(-0.203)	(-14.66)	(-8.66)	(-6.14)	(-1.908)
	75.303	49.941*	75.501	79.659	101.26*	98.09*	78.812
PYR							
	± 2.455	± 4.448	± 2.093	± 6.505	± 3.138	± 4.599	± 5.38
		(-33.68)	(0.263)	(5.785)	(34.47)	(30.26)	(4.66)
	1.447	1.122*	1.453	1.634*	1.580*	1.581*	1.451
LDH							
	±0.010	± 0.008	± 0.006	± 0.004	± 0.011	± 0.008	± 0.008
		(-22.47)	(0.39)	(12.94)	(9.2)	(9.26)	(0.264)

Cont…

GN	Saline	PTZ	PTZ+n-HE	PTZ+CE	PTZ+EAE	PTZ+n-BE	PTZ+AE
	1.011	0.874*	1.013	1.054	1.240*	1.217*	1.028
ICDH							
	±0.043	± 0.024	± 0.051	± 0.015	± 0.038	± 0.031	± 0.026
		(-13.51)	(0.197)	(4.22)	(22.68)	(20.4)	(1.648)
	1.188	0.863*	1.194	1.375*	1.321*	1.322*	1.192
SDH							
	±0.010	± 0.008	± 0.006	± 0.004	± 0.011	± 0.008	± 0.008
		(-27.37)	(0.476)	(15.76)	(11.2)	(11.27)	(0.32)
	1.839	1.578*	1.886	2.306*	2.255*	2.152*	2.032*
MDH							
	±0.028	± 0.023	± 0.017	± 0.027	± 0.031	± 0.017	± 0.017
		(-14.18)	(2.55)	(25.37)	(22.64)	(17.01)	(10.52)

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.

Carbohydrates play not only a structural role in the cell but may serve as a reservoir of chemical energy. Carbohydrates are the major sources of energy fuels for metabolic process readily assimilable, though fats yield more energy[25]. Muscle utilizes carbohydrates as the major source of energy for mechanical activity and kinesiological efficiency of the animal. The immediate source of energy for muscular contraction is ATP and these biological currencies are replenished ultimately by carbohydrates or fats or proteins. Selected parameters of glycolytic and oxidative pathways of carbohydrate metabolism were studied in different muscles of rat during PTZ-induced epilepsy and on pre-treatment with different extracts of *Centella asiatica*. 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. On treatment with CA extracts the total carbohydrate levels were increased which might be due to the synthesis of carbohydrates through glycogenesis and gluconeogenesis replenishing the loss of carbohydrates that occur during epileptic seizures. The glycogen levels were increased in different muscles of PTZ treated animals, which indicate possible 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 8 with the glycogen, glucose levels were also increased in all the muscles during PTZ-induced epilepsy, which might be implicated to the increased conversion of glycogen to glucose for the onward glycolytic pathway. On contrary to this, glycogen and glucose levels were non-significantly decreased and/or recovered to normalcy during pre-treatment with CA extracts suggesting lesser utilization of these components through anaerobic glycolysis. Lactate is the end product of glycolysis under anaerobic conditions and the rate of lactate production is considered as an index of physiological stress in the biological systems[27-28]. The lactic acid production and accumulation suggest the tissue capacity to withstand anaerobiosis. The levels of lactic acid also indicate the prevalence of anaerobiosis in the tissues and the tissue specific resistance or susceptibility to anaerobic conditions. In the present study, lactate levels were increased during PTZ induced epileptic condition. Although, the extent of changes in lactate and pyruvate were not uniform in all tissues, lactate levels were decreased and pyruvate levels were increased during pre-treatment with CA extracts. Increased lactate content during PTZ treatment suggests induction of lacticacedemia in different muscles and CA extracts reduce such metabolic acidosis and protect muscles

from any architectural damage caused due to PTZ- induced epileptic seizures. The formation of pyruvate, an important end product of glycolysis was found to be low during PTZ-induced epilepsy indicating greater mobilization of pyruvate to lactate through reverse pathway of NADH2 dependent lactate dehydrogenase. The decreased levels of pyruvate and elevated levels of lactate during induced epilepsy indicate prevalence oxygen deficiency in the intracellular milieu with advancement of treatment. NAD-Lactate dehydrogenase (LDH) is a key enzyme of glycolysis and catalyses the reversible oxidation of lactate to pyruvate in the terminal step of glycolysis. The reaction catalyzed by LDH interlinks anaerobic and aerobic oxidation of glucose. The activity of LDH 9 was significantly decreased in all the muscles during PTZ- induced epileptic condition when compared to their respective controls indicating down regulation of oxidative metabolism due to lesser feeding of pyruvate into the TCA cycle. Pre-treatment with different CA extracts significantly increased the NAD-LDH activity in all the muscles which implies that the bioactive factors of CA extracts favor greater conversion of lactate to pyruvate and subsequent feeding of pyruvate into Kreb's cycle for further oxidation. The reduced levels of oxidative enzymes of TCA cycle i.e. ICDH, SDH and MDH during induced epilepsy indicate depressed oxidative metabolism in mitochondria and reduced turnover of carbohydrates and energy output[29]. The decreased activities of mitochondrial enzymes could also be attributed to the low feeding and /or availability of substrates, loss of structural integrity of mitochondria and prevalence of hypoxic condition ultimately leading to energy crisis during epilepsy. However, pre-treatment with different CA extracts to the epileptic animals caused marked elevation in the activities of all the oxidative enzymes thus promoting flux of reduced equivalents into oxidative phosphorylation and compensates the energy crisis that might have occurred during epilepsy. The present findings demonstrate that the CA extracts and the bioactive factors present in the CA extracts offer protection against induced epilepsy by restoring oxidative metabolism and reduce the risk of metabolic dysfunction that occurred during epilepsy.

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