Protective role of different extracts of *Bacopa monnieri* on Protein and free amino acid metabolism during Pentylenetetrazole- induced epilepsy

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ABSTRACT:

Epilepsy is a central nervous system disorder in which nerve cell activity in the brain becomes disrupted, with a combination of seizures, uncontrollable muscle jerks or periods of unusual behavior, sensations and sometimes loss of consciousness. Proteins are the essential bio-chemical compounds of the living organisms, which play an important role in cellular metabolism. Since Epilepsy induces tonic-clonic seizures leading to violent muscle contractions, the present study is taken up to study the alterations in the composition of proteins, free aminoacids and transamination reactions in functionally different muscles during PTZ- induced epilepsy and on antiepileptic treatment using different extracts of *Bacopa monnieri* (BM). Rats were divided into eight groups consisted of six rats in each group. (a) control rats treated with saline, (b) PTZ- induced epileptic group (60mg/kg, IP), (c) Epileptic group pretreated with n-hexane extract (nHE), (d) Epileptic group pretreated with chloroform extract (CE), (e) Epileptic group pretreated with Ethylacetate extract (EAE), (f) Epileptic group pretreated with n-Butanol extract (n-BE), (g) Epileptic group pretreated with Aqueous extract (AE) and Epileptic group pretreated with the reference drug, diazepam (DP). BM extract (180mg/kg body weight) was given to the animals for one week prior to the injection of PTZ. The present study reveals that the total protein levels were decreased in different skeletal muscles with subsequent elevation in free aminoacids and transamination reactions during PTZ- induced epilepsy. Pretreatment with different BM extracts to the epileptic rats reversed the alterations suggesting that BM extracts proved to be effective in recovering the pathophysiological abnormalities that were caused due to PTZ-induced epilepsy.

KEY WORDS: *Bacopa monnieri*. Epilepsy, Proteins, Amino acids, Antiepileptic effect, Pentylenetetrazole.

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Contents

1. INTRODUCTION

Epilepsy is one of the most common serious neurological disorders characterized by recurrent seizures that affects 3% of world population (Loscher and Schmidt, 2002) and about 50 million people world- wide (Strine, 2005). Epilepsy can be caused by many different conditions such as stroke, head trauma, complications during child birth, infections (meningitis, encephalitis) and certain genetic disorders. Seizures may also

develop as a consequence of neuropathological abnormality or altered metabolic states. Earlier studies indicated that epilepsy is characterized by recurrent, unprovoked seizures that result from excessive and hypersynchronous electrical dischargers in the brain which manifest in many different ways, such as temporary loss of consciousness, or abnormal motor activity that can range from minor involuntary movements to whole body convulsions (Baxendale et al., 2012). It has been well documented that epilepsy in addition to neuropathological abnormalities is also associated with repetitive rhythmic jerking of limbs due to violent muscle contractions.

Despite the fact that there are formidable array of antileptic drugs available, pharmacotherapy for epilepsy is limited due to high incidence of pharmaicresistance and failure to prevent the development and progression of epilepsy and exhibit substantial side effects. Hence, there is a clinical need for the development of new antileptic therapentics with fewer side effects. Much attention is being paid in recent times towards identifying the bioactive factors from natural medicinal plants for different human ailments including epilepsy. Hence, the present investigation is primarly focused to characterize the significance of *Bacopa monnieri* in the amelioration of pathophysiolological consequences that occurred during PTZ-induced epilepsy, with particular reference to proteins and amino acid metabolism in functionally different skeletal muscles of rat.

2. MATERIALS AND METHODS

2.1 Collection of the plant material

Bacopa Monnieri (BM) plant was collected from Thalakona 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. 428). The leaves were separated from the plant, dried in shade, powdered and powder was used for the extraction of anticonvulsant principles using different solvents.

2.2 Preparation of Plant Extracts

The active principles of the leaves of the plant were extracted in different solvents, such as 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 [Sowmyalakshmi et al., 2005; Vattanajun et al., 2005]. 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 Hahn Rotary Evaporator HS-2005V 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 Hahn Rotary Evaporator. The individual extracts were freeze dried and stored at -20°C until further use.

2.3 Procurement and Maintenance of Experimental Animals

Male adult wistar rats weighing 150±25 grams were used as the experimental animals in polypropylene cages under laboratory conditions of 28±2^{0}C temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity. The rats were fed with standard pellet diet 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/09/a / (i) /CPCSEA /IAEC /07-08/SVU/ZOOL/WR-PS/ dt.30.06.2008.

2.4 Administration of the test substance

Convulsions were induced by an intraperitoneal (i.p.) injection of Pentylenetetrazole (60mg/Kg body weight) dissolved in saline [Ray and Poddar, 1985; Gupta et al., 1999; Santos Junior et al., 2002; Rizwan et al., 2003]. Each fraction of BM extract (180mg/Kg body weight) was dissolved in water and given to the animals for one week prior to the injection of PTZ. A gavage tube was used to deliver the substance by the oral route, which is the clinically expected route of administration of BM. The volume of administration was kept at 1ml to the animal.

2.5 Experimental design for screening of plant extracts for anticonvulsant activity.

The rats were divided into 8 groups, each consisted of 6 rats and used for studying effects of different fractions/extracts of plant, *Bacopa Monnieri*.

Group 1 -Normal saline treated control rats (SC)
Group 2 -Rats treated with PTZ (Epileptic rats)
Group 3 -Epileptic rats pretreated with n-Hexane Extract (nHE+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 (nBE+PTZ)
Group 7 -Epileptic rats pretreated with Aqueous Extract (AE+PTZ)
Group 8- Epileptic rats pretreated with Diazepam (Reference control) (DP+PTZ)

2.6 ISOLATION OF TISSUES

The animals were sacrificed after the treatment by cervical dislocation. Functionally different muscles such as white vastus, red vastus, soleus and gastronemius 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.

2.7 BIOCHEMICAL ANALYSES

The total protein content was estimated by the method of Lowry et al. (1951), total free amino acid content by the method of Moore and Stein (1954). Aspartate aminotransferase (AAT) and Alanine aminotransferase (ALAT) activity levels were assayed by the method of Reitman and Frankel (1957). The activity levels of Branched chain aminotransferases such as Leucine (LAT), Isoleucine (ILAT) and Valine (VAT) aminotransferases were estimated by the method of Taylor and Jenkins (1966).

2.8 STATISTICAL ANALYSES:

Values of the measured parameters were expressed as mean ± SEM. One way – ANOVA (F value) was used to test the significance of difference among more than two arithmetic means, followed by post-hoc test for multiple comparison to test the difference between each two means. The significance was considered at p values < 0.05. All the statistical analyses were performed using Statistical Program of Social Sciences (SPSS) for Windows, version 11.5.

3. RESULTS

3.1 Total Proteins

The total protein content in control rats was found to be highest in Red vastus (RV), followed by Gastrocnemies (GN), Soleus (SOL), and White Vastus (WV). The administration of Pentylenetetrazole(PTZ) caused significant decrease in total proteins of different muscles. Pre-treatment with BM extracts to PTZ- induced epileptic rats caused significant increase in protein content (Table 1).

3.2 Free amino acids

The Free amino acid content in control rats was found to be highest in RV followed by SOL, GN and WV. The administration of Pentylenetetrazole (PTZ) caused significant increase in free amino acid content in different muscles. The free amino acid content was decreased in different muscles during pre-treatment with BM extracts to PTZ-induced epileptic rats (Table 2).

3.3 Transamination reactions:

Aspartate aminotransferase (AAT), Alanine aminotransferase (ALAT), Leucine aminotransferase (LAT), Isoleucine aminotransferase (ILAT) and valine aminotransferase (VAT) activity levels were significantly increased in rat skeletal muscle during PTZ-

induced epilepsy when compared to control rats. However, the activity levels of all aminotransferases were decreased in all the muscles of epileptic rats pretreated with different extracts of BM and diazepam reference control (Table 3, 4, 5, 6, 7).

4. DISCUSSION

The present study reveals that the total protein content was decreased in different skeletal muscles during PTZ-induced epilepsy. The decreased protein content may be correlated to the augmented proteolysis as evidenced by elevated free amino acids. Earlier studies have suggested that oxidative stress plays an important role in the etiology of different types of epilepsy such as Kainic acid (KA) (Dal-Pizzol et al., 2000; Gluck et al., 2000), Iron-Salt induced Seizures (Kabuto et al., 1998), Electro shock induced seizures (Rola et al., 2002) and in the kindling model of complex partial seizures (Frantseva et al., 2000). It is well documented that skeletal muscle adopts unique energy metabolism. In addition to its aerobic energy metabolism, it adopts for short-term anaerobic activity involved in intense muscle activity resulting in profound generation of reactive oxygen species promoting muscle fatigue and tissue damage. Such intense muscle contraction as appeared in seizures, could be a potential factor of oxidative stress. Cooper et al. (2002) have reported that intense or prolonged exercise can lead to an increase in free radicals production exceeding antioxidant defence, causing oxidative stress which has consequences on exercise performance. Earlier studies in our laboratory clearly demonstrated that PTZ inhibits glycolytic and oxidative pathways and also different ATPases causing reduction in ATP/ADP ratio. Hence, the decreased protein content and consequent elevation in free amino acid pool might be implicated to the synergestic oxidative stress caused by PTZ- induced epilepsy coupled with uncontrolled violent muscular activity. Since free amino acids released from protein breakdown have large impact on the regulation of intermediary metabolism and also have significant modulatory effects on nitrogen balance, the possible role of transamination reactions were studied in the present study during induced epilepsy and on pretreatment with different extracts of BM. On par with increased free amino acid pool, the different transamination reactions such as AAT, ALAT, LAT, ILAT and VAT were significantly elevated in different skeletal muscles during PTZ-induced epilepsy indicating increased amino acid turnover and glutamate formation.

Amino acid metabolism comprises a wide array of intricate physiological processes which include maintenance of adenine nucleotide concentration, anaplerotic processes of TCA cycle, neurological functions, acid-base regulations as well as generating precursors for glucconeogenesis and ureagenesis. In addition, the alterations in size and composition of free amino acid pool have significant modulatary effects on protein synthesis and degradation, nitrogen balance as well as intermediary metabolism. Hence, the dynamic free

pool of amino acids are essential to skeletal muscle metabolism and inter-organ relationships especially during increased metabolic demands. In addition, the amino acid pool participates in transamination and trans-deamination reactions within muscle and plays a critical role in regulation of oxidative metabolism. Hence, the elevated free amino acid pool indicate a turnover of proteins or amino acids which normally lead to enhanced transamination and deamination. The reduced TCA cycle activity during PTZ-induced epilepsy as observed in our earlier studies adds credence to the above contention. Although, the amino acids and proteins contribute as substrates for energy sources to a lesser extent, they account for more than 10% total energy expenditure via gluconeogenesis during short supply of carbohydrates and thus amino acids might play an active role in intermediary metabolism in addition to the regulation of energy metabolism during eccentric muscular activity as observed in PTZ-induced epilepsy.

Pretreatment with different extracts of BM to the PTZ-induced epileptic animals caused significant elevation in total protein content and significant depletion in free amino acid pool. Different transaminases such as AAT, ALAT and Branched chain aminotransferases (LAT, VAT and ILAT) were found to be decreased in functionally different skeletal muscles of epileptic animals pretreated with different extracts of BM. It is commonly accepted that excessive free radical production due to PTZ-treatment is harmful to skeletal muscle and its prevention should have beneficial effects. The antioxidants protect cells from oxidative stress, and an increase in intracellular antioxidant levels in muscle might offer greatest protection against oxidative stress. It is well documented that the use of antioxidants, both natural and synthetic in the prevention and cure of various diseases is gaining momentum in the field of ethanopharmacology. Aniya et al. (2005) have reported that *Crassocephalon crepidiodes* showed free radical scavenging activity against chemically induced hepototoxicity. Sekhar Misra et al. (2009) have conclusively demonstrated that the composite methanolic extracts of roots of *Withania sominifera*, leaves of *Ocimum sanctum* and rhizomes of *Zingber officinalis* showed therapeutic protective effect on forced swimming –induced oxidative stress. Further, it has been elucidated that the ingredients such as flavanoids and phenolic compounds present in the plant exhibit antioxidant and anti-stressor activity (Mishra et al., 2000; Prakash and Gupta, 2005). The studies of Lieben Louis et al. (2012) suggest that both skin and flesh of garlic extracts are effective in preventing NE induced oxidative stress in cardiomyocyte hypertrophy and cell death. Several authors have shown that *Bacopa monnieri* was able to prevent lipid peroxidation *in vitro* and *in vivo* (Pawar et al., 2001; Rohini et al., 2004;) and quenches superoxide, hydroxyl and nitric oxide radicals effectively *in vitro* (Pawar et al., 2001; Russo et al., 2003). The general decrease in all the transaminases during pre-treatment with BM indicates reduced turnover of amino acids and possibly direct α-ketoglutarate into the citric acid cycle for further oxidation. The down regulation of transaminase reactions plays a critical role in the regulation of glutamic acid levels in

skeletal muscles during seizure threshold and recovery. Furthermore, it is presumed that the bioactive factors present in the extracts of BM possibly modulate the inter-conversations of amino acids and keto acids through transamination reactions and thus regulate the glutamate metabolism as one of the facets of antiepileptic treatment.

5. Conclusion:

The present study reveals that PTZ-induced epilepsy causes significant reduction in protein content with subsequent elevation in free amino acids and corresponding transamination reactions. These alterations were recovered after pretreatment with different extracts of BM suggesting that the bioactive factors present in the plant extracts might have caused significant protection against the PTZ-induced alterations.

Conflict of interest:

None declared.

6. References

1. W. Loscher, D. Schmidt, New horizons in the development of antiepileptic drugs. Epilepsy Res. 50 (2002) 3-16.
2. T.W. Strine, R. Kobau, D.P. Chapman, D.J. Thurman, P. Price, L.S. Balluz, Psychological distress, comorbidities, and health behaviors among U.S. adults with seizures: results from the 2002 National Health Interview Survey. Epilepsia. 46 (2005) 1133-1139.
3. S. Baxendale, C.J. Holdsworla, P.L. MezaSantoscoy, M.R. Harrieon, J.Fox, C.A. Parkin, P. W. Ingham, V.T. Cunliffe, Identification of compounds with anti-convulsant properties in a zebrafish model of epileptic seizures., Dis Model Mech. 5 (2012) 773-784.
4. S. Sowmyalakshmi, M. Nur-E-Alam, M.A. Akbarsha, S. Thirugnanam, J. Rohr, D. Chendil, Investigation on Semecarpus Lehyam-a Siddha medicine for breast cancer. Planta. 220 (2005) 910-918.
5. A. Vattanajun, H. Wattanabe, M.H. Tantisira, T. Tantisira, Isobolographically additive anticonvulsant activity between *Centella asiatica's* ethyl acetate fraction and some antiepileptic drugs. J. Med. Assoc. Thai. 88 (2005) 131-140.
6. S.K. Ray, M.K. Poddar, Effect of pentylenetetrazol on carbaryl-induced changes in striatal catecholamines. Biochem Pharmacol. 34 (1985) 553-557.
7. M. Gupta, U.K. Mazumder, S.R..Bhawal, CNS activity of *Vitex negundo* Linn. in mice.. Indian J Exp Biol. 37(1999):143-6.
8. J.G. Jr Santos, F.H. Do Monte, M. Russi, P.E. Agustine, V.M. Lanziotti, Proconvulsant effects of high doses of venlafaxine in pentylenetetrazole-convulsive rats. Braz J Med Biol Res. 35 (2002) 469-472.

9. A.N. Rizwan, A.,Ali, Y. Dua, S.N. Pal, K.K Pillai, Effects of gabapentin and antidepressant drug combinations on convulsions and memory in mice.Pol J Pharmacol. 55 (2003) 965–971.
10. O.H. Lowry, N.J. Rosebrough, A.l. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent. J Biol Chem. 119 (1951) 265-275.
11. S. Moore, W.H. Stein, A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J Biol Chem. (1954) 221: 907-13.
12. S. Reitman and S. Frankel, A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvictransaminases. Am J Clin Pathol. 28 (1957) 56-61.
13. R.T. Taylor, W.T. Jenkins, Leucine aminotransferase. I. Colorimetric assays. J. Biol. Chem. 241(1966) 4391-4395.
14. F. Dal-Pizzol, F. Klamt, M.M. Vianna, Lipid peroxidation in hippocampus early and late after status epilepticus induced by pilocarpine or kainic acid in Wistar rats. Neurosci Lett. 291 (2000) 179-182.
15. M..R. Gluck, E. Jayatilleke, A.J. Rowan, CNS oxidative stress associated with the kainic acid rodent model of experimental epilepsy. Epilepsy Res. 39(2000) 63-71.
16. H. Kabuto, I. Yokoi, N.Ogawa, Melatonin inhibits iron-induced epileptic discharges in rats by suppressing peroxidation. Epilepsia. 39 (1998) 237-243.
17. R Rola, M Swiader, SJ.Czuczwar, Electroconvulsions elevate the levels of lipid peroxidation products in mice. *Pol J Pharmacol.* 54 (2002) 521-524.
18. M.V. Frantseva, J.L. Perez Velazquenz, L.R. Mills, Oxidative stress is involved in seizure-induced neurodegeneration in the kindling model of epilepsy. Neuroscience. 97 (2000) 431-435.
19. C.E. Cooper, N.B. Vollaard, T. Choueiri, M.T. Wilson, Exercise, free radicals and oxidative stress. Biochem Soc Trans. 30 (2002) 280-285.
20. Y. Aniya, T. Koyama, C. Miyagi, M. Miyahira, C. Inomata, S. Kinoshita, T. Ichiba, Free radical scavenging and hepatoprotective actions of the medicinal herb, *Crassocephalum crepidioides* from the Okinawa Islands. Biol Pharm Bull. 28 (2005) 19–23.
21. D.S. Misra, R. Maiti, D. Ghosh, Protection of swimming-induced oxidative stress in some vital organs by the treatment of composite extract of *Withania somnifera, Ocimum sanctum* and *Zingiber officinalis* in male rat. Afr J Tradit Complement Altern Med. 6 (2009) 534–543.
22. L.C. Mishra, B.B. Singh, S. Dagenais, Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review. Altern Med Rev. 4 (2000) 334–346.
23. P. Prakash, N.Gupta, Therapeutic uses of *Ocimum sanctum* Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. Indian J Physiol Pharmacol. 49 (2005) 125–131.

24. X.L. Louis, R. Murphy, S.J. Thandapilly, L. Yu, T. Netticadan, Garlic extracts prevent oxidative stress, hypertrophy and apoptosis in cardiomyocytes: a role for nitric oxide and hydrogen sulfide. BMC Complement Altern Med.12 (2012) 140.
25. R. Pawar, C. Gopalakrishnan, K.K. Bhutani, Dammarane triterpene saponin from *Bacopa monniera* as the superoxide inhibitor in polymorphonuclear cells. Planta Med. 67 (2001) 752–754.
26. G. Rohini, K.E. Sabitha, C.S. Devi, *Bacopa monniera* Linn. extract modulates antioxidant and marker enzyme status in fibrosarcoma bearing rats. Indian J Exp Biol. 42 (2004) 776-780.
27. A. Russo, A.A. Izzo, F. Borrelli, M. Renis, A. Vanella, Free radical scavenging capacity and protective effect of *Bacopa monniera* L. on DNA damage. Phytother Res. 17 (2003) 870-875.