

ANTICONVULSANT EFFECT OF DIFFERENT EXTRACTS OF *BACOPA MONNIERI* ON CHOLINERGIC METABOLISM DURING PENTYLENETETRAZOLE-INDUCED EPILEPSY**Paturu Suseela, Gunduluru Swathi and Wudayagiri Rajendra****Division of molecular biology, Dept. of Zoology, Sri Venkateswara University, Tirupati-517502, Andhra Pradesh, India**Corresponding Author Email: rajendraw2k@yahoo.co.in**ABSTRACT**

The study was carried out to investigate the anticonvulsant effects of different extracts of *Bacopa monnieri* (BM) against Pentylenetetrazole induced-epilepsy, with special reference to cholinergic metabolism of functionally different skeletal muscles. 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 diazepam (DP). BM extract (180mg/kg body weight) was given to the animals for one week prior to the injection of PTZ. Increased Acetylcholine content and decreased acetylcholinesterase activities were observed in skeletal muscles during PTZ induced seizures. On par with the reference drug, diazepam, the cholinergic activity was reversed during pretreatment with different extracts of BM. These findings suggest that the bioactive factors present in specific fractions of BM ameliorate the cholinergic dysfunctions that occur during PTZ-induced epilepsy.

KEY WORDS*Bacopa monnieri, Epilepsy, Acetylcholine, Acetylcholinesterase, Antiepileptic effect, Pentylenetetrazole.***INTRODUCTION**

Epilepsy is one of the most common serious neurological disorders characterized by recurrent seizures that affect 3% of world population [1] and about 50 million people world- wide [2]. 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 discharges 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 [3]. Studies on human epileptic tissue described a spontaneous synchronous activity present intriguing evidence for a defect in glutamatergic and GABAergic signaling leading glutamate excitotoxicity [4].

Although the neurophysiological mechanisms are well defined in different types of epileptic seizures, the impact of epilepsy on the muscle metabolism is not yet fully understood. It is reported that tonic-clonic epileptic seizures cause sustained muscle contraction lasting a few seconds to minutes [5]. Baxendale have reported that exposure of 2-day-old zebrafish embryos to the convulsant PTZ rapidly induce intense neuromuscular activity and increased locomotor

behavior the characteristic features of seizures in mammals.

Despite the fact that there are formidable array of antiepileptic drugs are available, pharmacotherapy for epilepsy is limited due to high incidence of pharmacoresistance and failure to prevent the development and progression of epilepsy and exhibit substantial side effects. Hence there is a clinical need for new antiepileptic therapeutics 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 primarily focused to characterize the significance of *Bacopa monnieri* in the amelioration of neuropathological consequences occurred during PTZ-induced epilepsy, with particular reference to cholinergic activity in different skeletal muscles.

MATERIALS AND METHODS

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.

Preparation of Plant Extracts

The active principles of the leaves of 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 [6,7]. 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 vapour 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 vapour Rotary Evaporator. The individual extracts were freeze dried and stored at -20°C until further use.

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 $28\pm 2^{\circ}\text{C}$ 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.

Convulsions were induced by an intraperitoneal (i.p.) injection of Pentylentetrazole (60mg/Kg body weight) dissolved in saline [8, 9, 10, 11].

Administration of the test substance

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.

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)

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°C) and stored at -40°C until further use. At the time of analyses the tissues were thawed and used. Selected parameters were estimated by employing standard methods.

BIOCHEMICAL ANALYSES

The level of ACh content and activity of AChE were estimated by the method of Hestrin [12] as given by Augustinson [13] and with slight modification of the Ellman [14] respectively in different brain regions of control and experimental animals.

STATISTICAL ANALYSES

Values of the measured parameters were expressed as mean \pm 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 multiple comparison to test the difference between each two means. The significance was considered at p values < 0.05 . All the statistical analyses were processed using Statistical Program of Social Sciences (SPSS) for Windows, version 11.5.

RESULTS

The Acetylcholine (ACh) content was estimated in different skeletal muscles of the rat during Pentylentetrazole (PTZ) induced epilepsy and on pre-treatment with anticonvulsant extract of *Bacopa monnieri* (BM).

Animals with PTZ induced seizures had significantly elevated levels of ACh in skeletal muscles when compared with controls. The highest increase was noticed in GN (Table1). Animals pre-treated with n-HE, EAE, n-BE of BM extracts and DP caused significant reduction in Ach in different muscles. The decrease was highest and significant with DP. The effect of n-BE is greater than the effect of EAE and n-HE. The ACh content showed no-significant change in CE and AE.

The activity levels of Acetylcholinesterase were decreased in different muscles of epileptic animals and the highest decrease was recorded in WV followed by RV, SOL, and GN (Table2). The AChE activity levels were increased in different muscles of rats pre-treated with n-HE, EAE, n-BE of BM and DP. The levels of AChE were more significant in nHE extract when compared to EAE and n-BE extracts in all the muscles.

DISCUSSION

Acetylcholine (ACh) is an excitatory neurotransmitter at the neuromuscular junction (NMJ), but also functions as an inhibitory neurotransmitter in many target organs of the parasympathetic nervous system (PSNS). AChE is an enzyme that terminates the signal transmission at the cholinergic synopsis by rapid hydrolysis of the neurotransmitter ACh. The enzyme AChE converts Acetylcholine to in inactive metabolites choline and acetate. This enzyme is abundant in the synaptic cleft, which has a main role in rapidly clearing free acetylcholine from the synapse that is essential for proper muscle function. Acetylcholine exerts its effect by binding to the receptors at the neuronal postsynaptic membrane causing excitation contraction coupling leading the muscle contraction. Therefore the pathophysiology of cholinergic neurotransmitter system at the

neuromuscular junction plays a prominent role in understanding the kinesiological efficiency of different skeletal muscles during induced epilepsy and also during antiepileptic treatment.

In the present study an increased Acetylcholine levels and decreased AChE activity levels were observed in functionally different skeletal muscles during PTZ-induced epilepsy. It is well established that excessive levels of ACh in tissue can produce epileptiform activity. [15,16]. Increased ACh content due to AChE inhibition provide compelling evidence that activation of nAChRs exacerbate epileptiform activity in different skeletal muscles as observed in CNS [17]. It has been reported that several cholinergically mediated behaviours would be disrupted if AChE activity is decreased. It is also clear from the studies that accumulation of Ach due to inhibition of AchE cause specific neurobehavioral symptoms such as uncoordinated contraction leading to convulsions and tumours, a characteristic feature of epilepsy. Earlier studies in our laboratory have reported similar increase in Ach and decrease in AchE activity in different regions of rat brain during PTZ-induced epilepsy. Although there are no specific reports on the sequelae of pathophysiology of epilepsy in skeletal muscles, it is presumed that neurobehavioural symptoms such as uncoordinated contractions in the skeletal muscle might be correlated to the Ach accumulation during PTZ-induced epilepsy.

The cholinergic activity in different muscles showed differential changes during pre-treatment with different extracts of BM. On par with diazepam the Ach content was decreased and AchE activity was elevated in PTZ-induced epileptic animals during pre-treatment with different extracts of BM except CE and AE. Decreased cholinergic excitation with some extracts indicate the contracting effects of these extracts on cholinergic hyper excitability.

Although not related to the skeletal muscles, it has been reported that the sepolamine, the antiepileptic drug, caused significant increase in AChE activity levels [18]. Similar increase in AChE activity was also reported with Gabapentin and Alprazolam [18],

phenytoin and Brahmi Rasayana [19]. It is obvious from the results that selected extracts of BM exerted a conspicuous effect on the recovery of PTZ-induced seizures. The medicinal importance of Brahmi with particular reference to antiepileptic and insomnia [20], has been well documented in Indian traditional literature such as Athar-ved, Charak Samhita, Susrutha Samhita [21].

Several lines of evidence indicate that epilepsy is known to produce reactive oxygen species leading to oxidative stress [22]. Which may cause catalytic inefficiency of several enzymatic proteins including AChE. Pre-treatment with selected extracts of BM caused significant recovery in AChE activity thus protecting the deleterious effects of Acetylcholine that is accumulated during PTZ-induced epilepsy. Earlier investigations reported similar effect of BM on brain mitochondrial AChE activity of morphine treated rats [23]. In consonance with this [24] reported that oral administration of BM extract markedly reduce the memory deficits as well as acetylcholine concentrations, choline acetylase activity and muscarinic receptor binding in the hippocampus and frontal cortex. It is also reported that the BM extracts, which have high amount of saponins, exhibit antidiabetic [25] and antioxidant [26] activities. Animal studies have shown that Bacosides present in BM extracts exert antioxidant activity in different regions of brain [24]. Chowdary have demonstrated that Brahmi extract modulated the expression of important enzymes involved in generation and scavenging of reactive oxygen species in the brain. In vitro research has demonstrated that Brahmi exerts a protective effect against DNA damage in astrocytes [27] and human fibroblasts [27]. Vohara have reported that the cognitive effects of BM may be the results of the herbs antioxidant properties and for its cholinergic effect. From the results coupled with earlier reports it is obvious that the bioactive factors such as saponins and bacosides that are present in BM extracts might have offered a protective role from the oxidative stress caused during PTZ-induced epilepsy. It is also well established that BM possess antioxidant activity and hence BM extract possibly ameliorate neurodegenerative disorders associated with the over

whelming oxidative stress. Although the exact mechanism of action of BM is not known, there is evidence that the neuroprotective action could be attributed to a combination of cholinergic modulation and antioxidant effects.

The increase in ACh levels coupled with the decrease in AChE activity in different skeletal muscle seems to aid in the convulsing effect of PTZ. In contrast, the increased AChE and decreased ACh with selected plant extracts in skeletal muscle implicate anticonvulsant effect of *Bacopa monnieri* extracts. From these result it is obvious that selected extract of *Bacopa monnieri* exhibited conspicuous effects on the recovery of PTZ-induced seizures. The present data suggest that the *Bacopa monnieri* extracts have anticonvulsant effect and reduce the seizure manifestations and accompanying biochemical changes and highlights the possible role of antiepileptic therapy for better seizure control.

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TABLES

Table 1: Changes in the Acetylcholine content in different muscles of rat during PTZ-induced epilepsy and pre-treatment with different extracts of *Bacopa monnieri*.

	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	PTZ+ DP
Mean± SD	6.957±0.135	9.706**±0.155	5.296**±0.158	9.988*±0.140	6.727±0.156	6.729±0.157	9.970±0.127	4.158**±0.023
Mean±SD	4.754±0.111	6.99**±0.135	4.563±0.098	7.343**±0.114	4.240**±0.127	3.942**±0.110	7.956**±0.031	4.322**±0.184
Mean± SD	4.453±0.153	6.872**±0.046	4.303±0.449	7.233**±0.161	4.070±0.113	4.424±0.145	8.314**±0.135	3.363**±0.226
Mean± SD	4.370±0.134	8.383**±0.128	4.155*±0.050	8.492**±0.108	4.355±0.154	3.951**±0.160	8.751**±0.097	3.252**±0.034

The values are expressed as Mean ± SEM of six individual observations
*Significant at p>0.05 compared with saline control;**Significant at p>0.01 compared with saline control

Table 2: Changes in the Acetylcholinesterase content in different muscles of rat during PTZ-induced epilepsy and pre-treatment with different extracts of *Bacopa monnieri*

	SC	PTZ	PTZ+N-HE	PTZ+CE	PTZ+EAE	PTZ+N-BE	PTZ+AE	PTZ+ DP
Mean± SD	6.701±0.142	3.322**±0.159	6.927±0.125	2.385**±0.115	7.257**±0.196	6.823±0.165	2.238**±0.134	9.158**±0.062
Mean±SD	5.525±0.113	3.850**±0.110	6.243**±0.135	2.981**±0.150	6.831**±0.114	5.733±0.177	2.742**±0.176	7.321**±0.086
Mean± SD	5.264±0.149	3.835**±0.165	6.030**±0.095	2.914**±0.444	5.458±0.303	5.715*±0.142	2.377**±0.180	7.063**±0.033
Mean± SD	5.026±0.094	4.072**±0.084	9.924**±0.201	3.873**±0.075	7.442**±0.014	5.210±0.078	2.331**±0.137	7.832**±0.026

The values are expressed as Mean ± SEM of six individual observations
*Significant at p>0.05 compared with saline control;**Significant at p>0.01 compared with saline control



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