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Research Article

EVALUATION OF *BACILLUS CEREUS* AND *BACILLUS PUMILUS* METABOLITES FOR CNS DEPRESSANT AND ANTICONVULSANT ACTIVITIES

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ABSTRACT

Objective: The present work was focused on testing the metabolites of the two bacteria *Bacillus cereus* and *Bacillus pumilus* for central nervous system (CNS) depression and anticonvulsant activity.

Methods: CNS depressant activity was performed using photoactometer, skeletal muscle relaxant activity by rotarod method and anticonvulsant activity by Maximal electric-shock (MES) method.

Results: The methanolic extract of both bacteria exhibited significant CNS depressant activity in mice with 58.10% and 54.54% change in locomotor activity in comparison with control. The methanolic extracts of both bacteria also exhibited skeletal muscle relaxation properties with a 52.90% and 53.64% in comparison with control. However, none of the sample extracts showed any significant anticonvulsant activity.

Conclusion: The obtained result promises that both the bacteria can be exploited for bioactive molecules as CNS depressants with therapeutic potential.

Keywords: *Bacillus cereus, Bacillus pumilus,* CNS depressant, Anticonvulsant.

INTRODUCTION

The central nervous system (CNS) mainly comprises of the brain and spinal cord. The CNS processes the information with the help of chemical messengers which can act as neurotransmitters, neuromodulators, neuroregulators, neuromediators and neurotropic factors [1].

The modern day life is highly competitive and more demanding of mental skills. The working conditions are becoming increasingly stressful. As a result, is the surge in incidence of variety of psychiatric disorders. According to world health report (WHO 2001) approximately 450 million people suffer from a mental or behavioral disorder, but only a small minority of them receives even the most basic treatment. This amounts to 12.3% of global burden of disease and expected to rise to 15% by 2020 [2].

The recent advances in science and technology have contributed to an enormous improvement in the quality of mankind. The CNS acting drugs are invaluable therapeutically because they can produce specific physiological and psychological effects. The path breaking research in psychopharmacology has lead to flow of drugs for specification. For example, Benzodiazepines (Diazepam, Alprazolam, larazepam, nitrazipam etc) are the most frequently prescribed synthetic drugs for variety of disorders particularly against anxiety, depression, epilepsy and insomnia [3]. But the chronic use of Benzodiazepines can cause deterioration of cognitive function, physical dependence and tolerance [4]. For the past 30 years the barbiturates have been replaced by the benzodiazapines that are less addictive and have less abuse potential.

Epilepsy is a common neurological disorder. It is a collective term given to a group of syndromes that involves spontaneous, intermittent, abnormal electrical activity in the brain [5]. An ideal epileptic drug should suppress all seizures without causing any unwanted side effects. Unfortunately, it is observed that the presently available antiepileptic drugs are unable to control seizures effectively in as many as 25% of the patients. The conventional antiepileptic agents like phenytoin, carbamazeipine and sodium valporate have reported several side effects, mainly neurotoxicity. Since majority of antiepileptic drugs are to be consumed life long, the administration of other drugs predisposes to the risk of drug interaction [6, 7]. Thus it is necessary to investigate for antiepileptic agents that are safe, efficacious and free from toxicity. The main intention of treating an epileptic is to not only to eliminate the occurrence of seizures but also to help him to have a self sustained life.

In this context, there is resurgence of interest in medicines from natural sources. It may be from plants, animals or microbial origin. The drugs obtained from the natural source will always have significantly lesser side effects than that observed with synthetic drugs and comparably with near equal efficacy. Variety of drugs from plant sources have been tested and are in use for psychopharmacological effects and are found to be effective in the treatment of psychiatric disorders [8, 9]. The drugs obtained from animal life, both from terrestrial and oceanic origin have showed a wide variety of chemical compounds like terpenes, polyketides, actogenins, peptides etc with structural diversity and biomedical importance [10, 11, 12]. One of the major problems of these natural products coming into clinical trials is supply issue. The concentration of active compounds in these organisms are often is in minute quantities [13]. Hence, scientists have to look for alternative natural source without extinction of the respective species.

Besides plants and animals, the other alternative natural source that we can look for is microorganisms. There are many microorganisms associated with plants and animals and their metabolites have striking structural similarity of natural products suggesting that microorganisms are the real producers of these metabolites [13].In the present study, the metabolic extracts of two bacteria *Bacillus cereus* and *Bacillus pumilus* were tested for locomotor activity in mice which is an index of wakefulness (alertness) of mental activity using photoactometer and muscle relaxant property by Rota-rod apparatus. The metabolites were also tested for antiepileptic property by maximal electric-shock method.

MATERIALS AND METHODS

Solvent extraction and preparation of samples

The two bacteria *B. cereus* and *B. pumilus* were grown separately in large quantity in nutrient broth medium and incubated for three days at 35°C. The broth was centrifuged to separate the cells at 10,000 rpm for 20 minutes. The clear supernatant containing the metabolites was collected. The metabolites of both organisms were subjected to successive solvent extraction with petroleum ether, ethyl acetate and methanol (1:1) in a separating funnel. All the three solvent extracts were dried in separate plates. The *B. cereus* (BC) petroleum ether extract was labeled as sample BC-1, ethyl acetate

extract as BC-2 and methanol extract as BC-3. Similarly the *B. pumilus* (BP) extracts were labeled as BP-1, BP-2 and BP-3 respectively.

Selection and preparation of experimental animals

Healthy young adult Swiss albino mice weighing between 25-30 grams were used. Each animal, at the commencement of its dosing were between 8 to12 weeks old. The temperature in the experimental animal room was maintained around 25^{0}C (±3^{0}C). The relative humidity was maintained between 50-60 %. They were kept in normal lighting for 12 hours and 12 hours dark. For feeding, conventional laboratory diet with an unlimited supply of drinking water was provided. The animals were marked to permit individual identification and kept in their cages for at least 5days prior to dosing to allow for acclimatization to the laboratory conditions. The test substance was administered in a single dose by intraperitoneal route. The experimental protocols were approved by institutional animal ethical committee (Ref.No.NCP/IAEC/CL/02/12/2010-11) prior to the experiments. Procurement of animals and all the experiments were carried in the department of pharmacology, national college of pharmacy, shimoga, Karnataka.

Toxicity studies

In toxicity studies the LD_{50} was determined using mice. The LD_{50} studies for all the test samples were carried out as per OECD guidelines 423.

Evaluation of CNS depressant activity using photoactometer

The locomotor activities of the animals were measured using a photoactometer. The instrument consists of a transparent cage which is 25×48×18 cm^3 in size. It has a wire mesh at the bottom. Six lights and six photo cells are placed in the outer periphery of the bottom in such a way that a single mouse can block only one beam. Technically its principle is that a photo cell is activated when the rays of light falling on photo cells are cut off by animals crossing the beam of light. The photo cells are connected to an electronic automatic counting device which counts the number of "cut offs".

Healthy Young Swiss albino mice weighing between 25-30 grams were used. The selected animals were divided into eight groups of six animals each. Before the administration of the test samples, standard and control, the basal activity score for all the animals were recorded using photoactometer. The groups 1, 2, and 3 received the sample extracts of *B. cereus* BC-1, BC-2 and BC-3 respectively. Similarly, groups 4, 5 and 6 received the sample extracts of *B. pumilus* BP-1, BP-2 and BP-3 respectively. All the samples were prepared in sterile water at a dose of 50mg/kg body weight. The group 7 received the standard drug Diazepam at 5 mg/kg body weight. The control group received plain water of 1ml each (group eight). After 1 hour of administration of the standard drug and the test samples the locomotor activity was observed. The scores were recorded for all the animals and percentage change in locomotor activity was calculated by the following formula:

Change in motor activity = (A-B)/A ×100

Where; A: Basal score, B: Score after treatment [14]

Evaluation of muscle relaxant activity

This activity was studied by placing the animals on rotating rods. The difference in the fall off time from the rotating rod between the control and diazepam-treated animal (standard) was taken as an index of muscle relaxation. Similarly, the samples are also compared with control and standard.

Healthy Young Swiss albino mice weighing between 25-30 grams were used. The mice were placed on a horizontal wooden rod (diameter 3cms) that was 50 cms above the bench in order to discourage the animals from jumping off the roller. The rod was set for a rotation of 20 revolutions/minute. After the preliminary runoff naïve animals, those that did not remain on the rod for 5 consecutive minutes were discarded. The selected animals were divided into eight groups of six animals each. The groups 1, 2, and 3 received the sample extracts of *Bacillus cereus* BC-1, BC-2 and BC-3 respectively. Similarly, groups 4, 5 and 6 received the sample extracts of *Bacillus pumilus* BP-1, BP-2 and BP-3 respectively. All the samples were prepared in sterile water at a dose of 50mg/kg body weight. The group 7 received the standard drug Diazepam at 5 mg/kg body weight. The control group received plain water of 1ml each (group eight). After 1 hour of administration of the standard drug and the test samples the mice were placed on the rotarod. The time taken for each mouse to fall off the rotarod was recorded as the endurance time. The percentage decrease in time spent on rotarod was calculated [15].

Evaluation of anticonvulsant activity by maximal electro-shock (MES) Induced convulsions

Different types of epilepsies can be induced in laboratory animals. The convulsions in mice can be induced by giving high voltage current near the brain or by using suitable CNS stimulating chemicals (Chemo-convulsions e.g. Pentylenetetrazole). In MES convulsions, electric shock is applied through the corneal electrodes (or ear electrodes) and through optic stimulation cortical excitation is produced. The MES-convulsions are divided into five phase such as tonic flexion, tonic extensor, clonic convulsions, stupor, recovery or death. A substance is known to possess anticonvulsant property if it reduces or abolishes the extensor phase of MES-convulsions [16].

Healthy Young Swiss albino mice weighing between 25-30 grams were used. Before the administration of the test samples, standard and control, the mice were first tested by giving current of 80 mA for 0.2 seconds using elecro-convulsiometer. Those animals which showed characteristic course of convulsions were selected for experiment. The selected animals were divided into eight groups of six animals each. The groups 1, 2, and 3 received the sample extracts of *B.cereus* BC-1, BC-2 and BC-3 respectively. Similarly, groups 4, 5 and 6 received the sample extracts of *B.pumilus* BP-1, BP-2 and BP-3 respectively. All the samples were prepared in sterile water at a dose of 50mg/kg body weight. The group 7 received the standard drug Phenytoin at 25 mg/kg body weight. The control group received plain water of 1ml each (group eight). After 1 hour of administration of the standard drug and the test samples the electric shock was induced. The different phases of convulsions i.e. tonic flexion, tonic extensor, clonic convulsion, stupor and recovery time or death were observed. The time (seconds) spent by the animals in each phase was recorded. The % protection provided by the standard and test samples was calculated. [5]

RESULTS

Toxicity studies

All the test samples (BC-1, BC-2, BC-3, BP-1, BP-2 and BP-3) exhibited LD_{50} at 500mg/kg body weight. Hence, the dose level was fixed at 50mg/kg body weight (The $^1/_{10^{th}}$ of the LD_{50} dose is equal to therapeutic dose).

Loco motor activity

Bacillus cereus

Among the *B.cereus* samples, the sample BC-3 inoculated mice showed reduced locomotor activity with a score of 251±12.23 in 10 minutes. The score recorded before treatment was 600±2.25. This value amounts to 58.1% change in locomotor activity in comparison with control.

The sample BC-1 with a score of 300±4.78 (575±1.52 before treatment) and BC-2 with a score of 350±7.25 (590±4.87 before treatment) exhibited 47.82% and 40.67% change in locomotor activity respectively. The dose inoculated, the locomotor scores of mice before and after treatment and percentage change in activity are shown in Table 1.

Bacillus pumilus

Among the *B.pumilus* samples, the BP-3 inoculated mice showed reduced locomotor activity with a score of 250±8.89 (550±2.23 before treatment) in 10 minutes. This shows 54.54% change in locomotor activity in comparison with control. The samples BP-2 with a score of 310±7.23 (575±2.32 before treatment) and BP-1

with a score of 300±5.35 (550±3.25 before treatment) showed 46.08% and 45.45% change in activity respectively (Table 1).The activity of all the test samples was comparatively less with that of standard which exhibited 87.05% change in loco motor activity.

Table 1: CNS depressant activity of *B.cereus* and *B.pumilus* metabolites in mice using Photoactometer

Treatment groups	Dose mg/kg body weight	Mean of locomotor activity scores in 10 min		% change in activity
		Before treatment	After treatment	
Control (distilled water)	-	555±0.96	560±0.36	0
Diazepam (standard)	5mg	610±1.36	75±12.69**	87.05
BC-1	50mg	575±1.52	300±4.78**	47.82
BC-2	50 mg	590±4.87	350±7.25**	40.67
BC-3	50 mg	600±2.25	251±12.23**	58.1
BP-1	50 mg	550±3.25	300±5.35**	45.45
BP-2	50 mg	575±2.32	310±7.23**	46.08
BP-3	50 mg	550±2.23	250±8.89**	54.54

Values are mean ±SEM, n=6, ** p<0.001 significant (compared to control)

BC – *Bacillus cereus*; BC-1 Petroleum ether extract; BC-2 Ethyl acetate extract; BC-3 Methanol extract

BP – *Bacillus pumilus*; BP-1 Petroleum ether extract; BP-2 Ethyl acetate extract; BP-3 Methanol extract

Skeletal muscle relaxant activity

Bacillus cereus

Among the *B.cereus* samples, the sample BC-3 showed more skeletal muscle relaxant activity in mice with a fall off time of 365±16.23 seconds when compared to 775±20.22 seconds fall off time before treatment. This shows 52.90% decrease in time spent by the mice on revolving rod when compared with control. The sample BC-1 and BC-2 with a fall off time of 420±18.20 seconds (800±7.59 before treatment) and 452±15.24 seconds (770±18.2 before treatment) exhibited 47.5% and 41.29% decrease in time when compared to control. The dose inoculated, the fall off time of mice before and after treatment, and percentage decrease in time spent on the rotating rod for standard and test samples are shown in Table 2.

Bacillus pumilus

The BP-3 sample showed better skeletal muscle relaxation in mice with a fall off time of 350±2.36 seconds when compared to 755±16.25 seconds recorded before treatment. This shows 53.64% decrease in time when compared with control. The sample BP-2 and BP-1with a fall off time of 410±22.22 seconds (775±5.65 before treatment) and 385±19.93 seconds (720±8.2 before treatment) exhibited 47.09% and 46.52% decrease in time spent on rotarod when compared to control (Table 2)

Table 2: Skeletal Muscle relaxation activity of *B.cereus* and *B.pumilus* metabolites in mice using Rotarod

Treatment groups	Dose mg/kg body weight	Fall off time (sec)		% decrease in time
		Before treatment	After treatment	
Control (distilled water)	-	750±8.73	730±7.7*	0
Diazepam (standard)	5mg	725±2.73	125±7.68**	82.75
BC-1	50mg	800±7.59	420±18.2**	47.5
BC-2	50mg	770±18.2	452±15.24**	41.29
BC-3	50mg	775±20.22	365±16.23**	52.90
BP-1	50mg	720±8.2	385±19.93**	46.52
BP-2	50mg	775±5.65	410±22.22**	47.09
BP-3	50mg	755±16.25	350±2.36**	53.64

Values are mean ±SEM, n=6, ** p<0.001 significant (compared to control)

All the test samples showed less skeletal muscle relaxation in comparison to standard which exhibited 82.75% activity. But test samples showed significant muscle relaxant activity along with standard (p<0.001).

Anticonvulsant activity

Bacillus cereus

All the test samples of *B.cereus* (BC-1 to BC-3) failed to show any significant anticonvulsant activity. A maximum of 19.92% protection was shown by BC-3 sample in comparison with control. This percentage of protection is far less when compared to 90.90% protection exhibited by the standard. The test samples BC-1 and BC-2 exhibited 13.55% and 10.82% protection respectively.

Bacillus pumilus

The test samples of *B.pumilus* also failed to show any significant anticonvulsant activity with a maximum of 18.10% protection shown by BP-3 sample in comparison with control. The samples BP-2 and BP-1 exhibited 10.82% and 4.45% protection respectively. The duration spent at different phases of convulsions and percentage protection obtained for all the samples and standard are shown in Table 3.

DISCUSSION

The aim of the studies reported here was to detect the possible CNS depressant action of *B,cereus* and *B.pumilus* metabolites. The motive behind to carryout depressant activity was the signs and symptoms of depression shown by the animals during determination of LD_{50} studies which is mandatory and also essential for fixation of the test dose before carrying any pharmacological studies on animal models. In the present studies the successive solvent extracts of metabolites obtained from *B. cereus* and *B. pumilus* were tested for CNS depressant activity by locomotor scores using photoactometer, skeletal muscle relaxant activity by rotarod method and

anticonvulsant activity by maximal electro-shock method. The study revealed that *B.cereus* metabolites is having little better CNS depressant activity with 58.10% depression when compared to *B.pumilus* metabolites which showed 54.54% of CNS depressant activity. Regarding skeletal muscle relaxation, the *B.pumilus* metabolites have shown more skeletal muscle relaxant activity with 53.64% decrease in time on rotarod when compared to 52.90% exhibited by *B.cereus.* However, none of the test samples exhibited any significant anticonvulsant activity. Literatures are available on plant and animal originated metabolites with CNS depressant activities and are in use since centuries [15, 17]. The reports on pharmacologically active metabolites of microbial origin are comparatively less. Most of the studies reported are on antimicrobial, anticancer, immunomodulatory, anti-inflammatory, antioxidant, enzyme inhibitors and antiparasitic activities [18, 19, 20]. But very less information is available regarding evaluation of microbial metabolites acting on CNS. Moreover, microbial metabolites with anticonvulsant activity are seldom reported.

Table 3: Anticonvulsant activity of *B.cereus* and *B.pumilus* metabolites by MES method

Treatment groups	Dose mg/kg body weight	Duration in various phases (time in seconds)				Recovery or death	% protection
		Flexion	Extensor	Clonic	Stupor		
Control (water)	---	2.29 ± 0.17	10.99±0.21	5.50±0.15	9.30±0.50	Recovery	0%
Phenytoin (standard)	25 mg	1.28±0.95	1.0±0.50	1.50±0.90	1.18±0.14	Recovery	90.90%
BC-1	50 mg	3.35±0.45	9.50±0.55	3.0±0.45	10.1±0. 60	Recovery	13.55%
BC-2	50 mg	2.50±0.34	9.95±0.35	3.5±0.91	8.5±0.80	Recovery	10.82%
BC-3	50 mg	2.20±0.12	8.80±0.75	3.60±0.75	6.8±0.75	Recovery	19.92%
BP-1	50 mg	3.44±0.34	10.50±0.86	4.40±0.88	10.0±0.85	Recovery	4.45%
BP-2	50 mg	4.50±0.48	9.80±0.45	4.20±0.50	7.0±0.88	Recovery	10.82%
BP-3	50 mg	2.50±0.86	9.00±0.20	2.55±0.65	10.8±0.88	Recovery	18.1 0%

There are some reports on neuroactive compounds obtained from microorganisms. *Antarticum vesiculatum* and *Psychroserpens burtonensis*, the two bacteria obtained from antartic region are known to produce neuroactive compounds [21] and Komadoquinone A, a neuritogenic compound has been isolated from *streptomyces* species [22]. "Lactacystin", a low molecular weight metabolite with neurotrophic factor like activity which would be useful to treat patients suffering from neurological diseases has been reported by Barde [23].

A mycotoxin obtained from *Penicillium nigricans* with CNS depressant activity has been reported by Chaudhuri *et al* [24]. Among bacterial metabolites the ethyl acetate extracts of four bacterial strains obtained from marine source have exhibited CNS depressant activity [25]. Kamat *et al* [26] have reported bacterial metabolites with antianxiety and ant dementia activity. Jebasingh *et al* [27] have reported CNS depressant activity for chloroform extracts of *Bacillus megaterium* associated with cone snail and *pseudomonas aeroginosa* from tubeworm, both obtained from marine source.

The antibiotics like Dicloxacillin, Aminobenzylpenicillin, Chloramphenicol, Spiramycin have reported to have smooth muscle relaxation activity [28]. The metabolites of the two bacteria tested in the present study were originally isolated from soil samples during screening for antibacterial activity and proved to be good antibiotic producers [29]. The antibiotic fraction in the metabolite may be responsible for smooth muscle relation and depressant activity. An aromatic aminoacid obtained from *Pseudoalteromonas rubra* is known to have myorelaxant properties [21]. The crude extracts obtained from *streptomyces* species has been reported to have dose dependant CNS depression [30].

Compounds having sedative action may also exhibit anticonvulsant activities [31]. The sedative activity exhibited by *B.cereus* and *B.pumilus* metabolites prompted us to carryout anticonvulsant activity. Many literatures are available on bioactive compounds obtained from plant and animal sources with anticonvulsant activity [6, 32, 33]. Isatin derivatives of natural origin (both plant and animal) have been reported to exhibit considerable pharmacological actions such as anticonvulsant, antianxiety and psychoactive activity. They are found in plants of the genus *Isatis, Calanthe discolor*, etc, which are also found as a component of the secretion from the paratid gland of *Bufo* frogs [34, 35, 36]. Similar kind of isatin derivative 6-(3'-methlbuten-2'-yl) has been reported from bacteria *Streptomyces albus* [37]. "pimprinine" an extracellular alkaloid produced by *streptomyces* species has been reported to have anticonvulsant activity by MES test in mice. It is also reported to inhibit effectively tremorine-induced tremors and analgesia in mice [38].

In the present studies the metabolites of *B.cereus* and *B.pumilus* tested for CNS depressant activity and skeletal muscle relaxant activity have shown promising results whereas the metabolites of both bacteria exhibited poor anticonvulsant activity. The CNS depressant, skeletal muscle relaxant and anticonvulsant activities for metabolites of these two bacteria have not been reported in the past. Hence, as per the available literature and to the best of our knowledge, this may be the first report on these activities for these two bacteria.

CONCLUSION

The present study indicated the potential CNS depressant as well as skeletal muscle relaxant activity for both *B.cereus* and *B.pumilus* metabolites. The metabolites can be further tested for other activities like antianxiety, induction of relaxation and sleep. Further purification and identification of the active compounds responsible for sedative activity and exploration of the chemical structures of the same can lead to potentially useful compounds of biomedical importance. However, none of the test samples exhibited any significant anticonvulsant activity.

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