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BEHAVIORAL PHARMACOLOGY AND LOCOMOTOR ACTIVITY OF BACTERIAL EXTRACTS FROM CONTAMINATED RICE IN MICE

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ABSTRACT

Rice during its storage contaminate with various microorganisms especially in the tropical countries. Four such bacterial contaminants B1, B2, B3, and B4 were isolated and the extracts were evaluated for behavioral and locomotor activity in mice. Where B2 developed stereotype significantly and B1 extract had with a high significance Though B2 showed a significant change in stereotype behavior but the result of B1 showed a high significant development of stereotype. The B1 extract showed reduction in reactivity and locomotor activity which were of great significance. There was certain inhibition in the CNS activity observed in mice. The future studies of microbial and chemical characterization of the extract isolated in the microorganisms will reflect the mechanism of action.

Keywords: Rice; Microbial Extracts; Blind Screening; Mice.

INTRODUCTION

Rice is one of the principle foods for many countries around the world. India has been considered as one of the most important rice producing country in the world. Though there has been increased demand of production over the last twenty years, but the production and consumption ratio is not achievable due to the post harvest loss of the grain for poor technical storing methods [1].

The temperature and humid condition of India is favourable for the contamination of rice with various microorganisms. Various microorganisms contaminate with rice during growth, harvesting, processing and handling [2]. The contamination of rice with the mycotoxins [3, 4], *Bacillus cereus* [5, 6] and aflatoxin [7] has been studied elaborately. Developing countries face a wide problem of food borne disease after consumption of contaminated food. To protect health of people contaminant free crop has become an important concern worldwide.

Rice at several instances get contaminated and intentionally/ unintentionally may mix with uncontaminated grains. Especially the underprivileged category of the society is at a risk of consuming such mixed up rice. Though rice is consumed after cooking but the risk for the presence of microbial spores after cooking cannot be ruled out completely [8]. The microorganisms generate different types of substances which may produce untoward reactions in animals and human after consumption. Several studies have been carried to emphasize the various microbial contaminants that harm the growth of rice and reflect the toxins obtained from them. Even studies are conducted regarding the better strains of rice that can resist the microbial growth [9]. The present study aimed on the pharmacological screening of the extracts obtained from the microorganisms present in the microbial contaminated rice in the FCI storehouse.

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MATERIALS AND METHODS

Rice sample collection and preparation

The microbial contaminated rice sample was collected from the Food Corporation of India storehouse in a sterile manner. 1 g of contaminated rice was suspended in 10 ml of sterile normal saline from which 10^{-1}, 10^{-2}, $10^{-4}$and 10^{-6} dilutions were made with sterile saline water. 100µL of each dilution was inoculated aseptically on Nutrient agar plates (Peptone, beef extract, agar agar powder obtained from Nice Chemicals P. Ltd., Kochi, Kerela, India) in duplicates and incubated at an ambient temperature of 30°C for 24 h. The plates were observed for colony formation. From these colonies four different colonies were isolated (B1, B2, B3 and B4).

Preparation of extract

Four different strains of bacteria considered as B1, B2, B3 and B4 were obtained from the Nutrient agar medium. The bacterial strains were identified with the Gram Staining procedure [10]. The microorganisms were grown separately on the soyabean casein digest agar medium (HiMedia) plates containing 1 % rice and washed with sterile normal saline to collect the organisms. The different strains of cells were individually suspended in 10 ml of sterile normal saline and were subjected to heating at 10 lbs pressure for 10 mins. After cooling the samples were centrifuged at 10,000 rpm at 4°C for 10 minutes. The supernatant was collected and lyophilized.

Animals

Adult Swiss Webster mice of either sex weighing between 18 and 22 g were obtained from the CPCSEA approved animal house of Dr. B. C. Roy College of Pharmacy and Allied Health Sciences, Durgapur. The animals were housed 5 per cage under a 12-hr/12-hr light/dark cycle (lights on at 06:00, lights off at 18:00) at a constant temperature of 22 ± 1°C. Food and water were available *ad libitum* for 1 week before the experiments. Mice were handled 1 day before the test. This study was performed according to the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and Institutional Animal Ethics Committee (IAEC).

Dosage preparation and administration

The lyophilized extracts were dissolved in sterile normal saline at a concentration of 1 mg/ml. Mice were randomly separated into 4 groups of 5 mice each. Each of the four groups of animals were separately administered with dosing solution of the extract obtained from B1, B2, B3, B4 microorganism orally at a dosing volume of 10ml/kg body weight after taking the control reading for blind screening and photoactometer of individual mice for each group.

Blind Screening

The animals were subjected to Blind Screening by assessing the different parameters after 30 mins of drug administration [11].

- Passivity- The passivity is determined by observing the struggling activity when restrained. The score given accordingly struggles when restrained by neck - 0, when in vertical position - 2, when held in supine position - 4, when held vertically on forepaw - 6, when held by hind paw - 8.
- Reactivity- The reactivity is determined by placing the mice at the closed arena and observing the activity. The normal activity is scored 5. The score is given as less activity - 4, 3, 2, little motion - 1, constant walking - 6, walking with running - 7, agitated spurt - 8.
- Righting Reflex-The animals are individually swung and thrown such that there are two and half turn before falling on a platform for five time. The score in given depending on a different position of fall- standing on four feet, lying on one side, lying on back and regaining normal position slowly, lying on back.
- Pinna and Corneal reflex- The pinna and cornea is touched with a sharp or pointed object and the ear retraction along with the movement of the head and closing of eyelids are noted respectively.
- Grip Strength- The mice are allowed to hang on a wire or bar and the normal score are given as 4. The inactive grip is less than 4 and active grip is more than 4.
- Abdominal Tone- Observed by palpation of abdomen indicated by flaccid, no return of cavity to normal - 0, slight resistance - 1, and extreme resistance - 2.
- Body tone - Compressing both sides of the mouse between thumb and index finger. The scores are flaccid, no return of cavity to normal - 0, slight resistance - 1, extreme resistance - 2.
- Stereotype- The animals are kept in a closed area and scored according to the movements observed. The normal score is 0 i.e., sitting on four paws with normal movements. Repetition of movement - 1, searching movement - 2, Circling - 3, self-biting - 4, walking backwards - 5, licking of lips - 6, tail lashing - 7.
- Catalepsy- The mouse is placed on a bar parallel to the ground elevated 1 inch from the ground. The cut off time is 20sec for the animal; the animal if remain immobile on the bar with its forepaw for more than 20 s it is considered as cataleptic [12].

Photoactometer

The Photoactometer (Make: MVTEX, Ambala, Haryana, India) was used in the study to assess the spontaneous locomotor activity [13]. The equipmemt is a square closed arena (30 x 30 x 30 cm) equipped having six photocells wall. The locomotor activity was recorded by the interruptions of the photocells which has a digital counter. The control reading of each group was recorded by placing individual animal of a particular group in the activity cage for 5 mins. Animals were then treated with the extracts of B1, B2, B3, and B4 and were exposed to the

photoactometer after 30 mins of treatment for 5 mins. The difference in activity of before and after treatment was considered and percentage of decrease in activity was calculated.

Statistical analysis

The data obtained was statistical analysed using ANOVA followed by Student T-Test. The P values < 0.05 were considered significant.

RESULT

Gram staining of the microorganisms

The results of Gram staining showed that both Gram positive and Gram negative strains of bacteria are present in the rotten sample as given in the Table 1.

Effect on the Blind screening

The bacterial extracts administered to the different groups of mice showed different effects. The body tone, abdominal tone, pinna reflex, corneal reflex and righting reflex were similar to the control group of mice (Table 2). The passivity of the extract treated mice was normal similar to the control as all the mice in every group showed grasping when held by the neck (Table 2).

There was no catalepsy observed in any groups both for control and extract treated (Table 3). No rigidity was observed in the limbs of the mice. The normal reactivity score was 5 but it got reduced in the bacterial extract treated groups. The reactivity decreased significantly for B2 as 4.25 ± 0.5 ($p< 0.05$) and B3 as 4.25 ± 0.5 ($p< 0.05$) but a high significant reduction in activity was observed in B1 as 1.25 ± 0.5 ($p<0.001$) as compared to control (Table 3). The grip strength of the control and extract treated mice were normal and no significant change was observed in the treated group (Table 3).

The control animals showed no stereotype activity in the mice. The groups treated with bacterial extract B1 and B2 showed repetition of movement and searching behavior which was significant with a score of 1.8 ± 0.5 ($p<0.001$) and 1.5 ± 0.5 ($p<0.05$), respectively (Table 4).

Effect on the Locomotor Activity

The photoactometer activity after 30 mins of the administration of the bacterial extract of B1, showed a distinguished decrease with 107 ± 17.01 ($p<0.001$) as compared to the control group 363 ± 39.94(Table 5). The administration of B2, B3, and B4 showed the following decrease in locomotor activity 273 ± 27.92 ($p<0.05$), 251 ± 25.76 ($p<0.05$), and 250 ± 16.68 ($p<0.05$) significantly as compared with the controls 346 ± 16.62, 351 ± 23.09 and 331 ± 23.41 respectively.

Table 1. Identification of bacteria by Gram Stain

Name	Gram Stain	Structure
B1	Negative	Coccus
B2	Positive	Coccus
B3	Positive	Coccus
B4	Positive	Bacillus with mucilaginous characteristics

Table 2. Effect of Bacterial Extracts on the Various Parameters of Blind Screening

Groups	Body Tone	Abdominal Tone	Pinna Reflex	Corneal Reflex	Righting Reflex	Passivity
Control	+	+	+	+	5	0
B1	+	+	+	+	5	0
B2	+	+	+	+	5	0
B3	+	+	+	+	5	0
B4	+	+	+	+	5	0

n = 5; + = Present, 5 = Normal activity on 4 paws at all 5 times, 0 = mouse grasped when held by neck.

Table 3. Effect of Bacterial Extracts on the Catalepsy and Reactivity of Mice

Groups	Catalepsy (sec)		Reactivity (score)		Grip Strength (sec)	
	Control	Treated Group	Control	Treated Group	Control	Treated Group
B1	8.00 ± 3.6	11.25 ± 2.62	5 ± 0	1.25± .5**	32.5 ± 1.91	32 ± 2.58
B2	8.75 ± 1.7	8.25 ± 4.64	5 ± 0	4.25 ± 0.5*	32.5 ± 3.41	31.5 ± 3.41
B3	8.00 ± 4.1	8.75 ± 4.85	5 ± 0	4.25 ± 0.5*	30.75 ± 4.79	29.5 ± 1.73
B4	8.25 ± 2.9	9 ± 4.24	5 ± 0	4.25 ± 0.95	33.25± 2.06	29.75 ± 3.86

*All values are Mean ± SD, n = 5, *P<0.05, **P<0.001 when compared with control*

Table 4. Effect of Bacterial Extracts on the Stereotype activity of Mice

Animal Groups	Control	Treated Group
B1	0	1.8 ± 0.5**
B2	0	1.5 ± 0.5*
B3	0	0.5 ± 0.57
B4	0	0 ± 0

*0=Normal activity All values are Mean±SD, n = 5, *P<0.05, **P<0.001 when compared with control*

Table 5. Effect of Bacterial Extracts on the Locomotor Activity using Photoactometer

Groups	Locomotor Activity		% Reduction in Activity
	Before Treatment	After Treatment	
B1	363 ± 39.94	107 ± 17.01**	70.52
B2	346 ± 16.62	273 ± 27.92*	21.09
B3	351 ± 23.09	251 ± 25.76*	28.49
B4	331 ± 23.41	250 ± 16.68*	33.83

*All values are Mean±SD, n = 5, *P<0.05, **P<0.001when compared with control*
Same group of animals were used as control and drug treated.

DISCUSSION

The extracts have been obtained from unknown species of microorganisms which may be peptides or proteins by nature along with lipopolysaccharides. In near future the microbial and chemical characterization of the extracts isolated from the microorganisms will pinpoint the characterization of the compounds. The effect of crude extracts has been noted in behavioral reactivity of mice. The same animals have been considered for control and treated. As the extracts are of biological origin they have the potency to interact with the enzyme or receptor molecules of the body. The effects of the microbial extracts on behavioral pharmacology need to be elaborated. Preliminary study has been done to find the effect on the behavioral activity.

The preliminary pharmacological blind screening study was conducted as the microorganisms and the extracts obtained were unknown. The blind screening was conducted to have knowledge of affected behavior on administration of the extracts. The study of blind screening in all the extracts showed a reduction in the reactivity of the mouse indicating an inhibitory action on the central nervous system or also may be inhibitory action on ganglia and neuromuscular junctions. The reactivity is a psychological characteristic which shows alteration in behavior due to awareness. The inhibitory action may be due to sedative property of the substance as sedation cause suppression of the CNS activity [14]. The unchanged passivity reflects no alteration in motor co-ordination and depressed condition was unachieved by the treatment of the extracts. The unchanged righting reflex, pinna and corneal reflex supported the fact that though inhibitory influence was observed but hypnosis or complete unconsciousness was not developed at the given dose. The grip strength in all the groups was normal concluding that motor coordination was active in the animals. The haloperidol induced catalepsy is due to the inactivation of dopamine receptors by the dopamine antagonist haloperidol [15, 16]. As there was no catalepsy developed after dosing with the extracts so the extracts at the given dose may not have influence on the dopamine receptors or dopamine concentration of the brain. The increased stereotype activity in two groups B1 and B2 indicated the catecholamine levels of the brain may be affected [17, 18].

The reduced reactivity in mice was confirmed by the reduction in locomotor activity study using photoactometer as locomotion is considered to be the index of alertness. The classical animal model for measuring locomotor activity is the use of photoactometer. The extract of B1 showed a significant reduction in the photoactometer value in comparison with the effects of other three extracts. The reduction of such activity indicated that there may be sedation or inhibition of central nervous system functions (depression or anxiolysis) because anxiogenic condition have been reported to cause increased locomotion [19]. The significant reduction in movement was observed for the extract obtained from the microorganism B1 but extracts from other microorganisms also showed decrease in movement. The reduced reactivity in blind screening along with the reduced motor activity could be due to interference of the extract with the GABA (gamma amino butyric acid), the inhibitory neurotransmitter of the brain [20, 21]. The various neurotransmitters of the brain (excitatory and inhibitory) control the body activity either in single or by interacting with one another [22]. Similarly, GABAergic neurons have an important role in the pathogenesis of multiple disorders, though in several instances the GABAergic transmission has been found to interfere with the activity of the various catecholamines of the brain [23, 24] which may sometimes alter homeostasis.

CONCLUSION

In the present study, the effect of microbial extract on behavioral reactivity and motor activity has been evaluated. The result showed that the extracts attenuated the

locomotor activity in the photoactometer. The reduced activity may be either due to the influence of GABA receptors of the brain directly or it may be the cause of interference of GABA with other neurochemicals present in the brain. To establish the involvement of inhibitory neurotransmitter further study is necessary on the pathway through which the extracts show inhibition in the locomotor activity and reduced reactivity in mice.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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