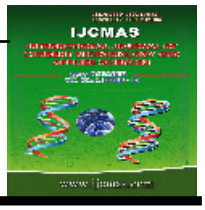
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 10 (2013) pp. 16-25**
http://www.ijcmas.com



Original Research Article

CNS depressant activity of the simple ascidian *Microcosmus exasperatus* Heller, 1878

V.K. Meenakshi*[1], M.I. Delighta Mano Joyce[2], M. Paripooranaselvi[1] and S. Gomathy[1]

[1]Department of Zoology, A.P.C. Mahalaxmi College for Women,
Tuticorin - 628 002, Tamil Nadu, India
[2]Department of Zoology, V.O. Chidambaram College, Tuticorin - 628 008, Tamil Nadu, India
Corresponding author

A B S T R A C T

Among the marine sedentary organisms, ascidians are an interesting but a highly neglected group of animals in India. A variety of biologically active compounds with pharmacological properties have been reported from this group. *Microcosmus exasperatus* is a simple ascidian, belonging to the class Ascidiacea and family Pyuridae. The present study aims at analyzing the CNS depressant activity of the ethanol extract of this species in Swiss albino mice using diazepam as standard drug and carboxy methyl cellulose as control in cocaine induced hyperactivity and phenobarbitone sodium induced sleeping time. Acute toxicity studies, general behavioral profiles, cocaine induced hyperactivity, phenobarbitone sodium induced sleeping time, exploratory behavior and muscle relaxant activity were assessed using standard procedure. Acute oral toxicity results indicated that a dose of 2000 mg/kg body weight brought about irritability, tremor, labored breathing, staggering and convulsions but not mortality. Administration of 150 mg/kg showed significant and very strong depression of alertness, awareness, spontaneous activity, sound and touch response. Strong reduction in pain, righting, pinna reflexes and moderate grip strength was noticed. Hyperactivity due to cocaine was inhibited from 40 through 90 min and a gradual increase in sleeping time was observed in mice treated with the extract and phenobarbitone sodium. A decrease in the exploratory behavior compared to that of control was noted in Y - maze test and head dip test. Muscle relaxant activity using traction and rotarod test showed a significant reduction in motor co-ordination. The active principles present in the ethanolic extract of *Microcosmus exasperatus* can be attributed to the CNS depressant activity.

K e y w o r d s

Microcosmus exasperates; diazepam; cocaine; phenobarbitone sodium; carboxy methyl cellulose; CNS depressant activity.

Introduction

Marine organisms are rich in biologically active metabolites which are of most importance in the pharmaceutical industry. Ascidians are marine, sedentary organisms and they form one of the major components in the biofouling community (Paripoornaselvi *et al.*, 2012, Gomathy *et al.*, 2012). They are capable of creating a toxic condition immediately on their surface as a chemical defensive

mechanism which prevents the attachment of other biofouling organisms (Martin and Bernard, 1991). *Microcosmus exasperatus* is a simple ascidian belonging to the family pyuridae. It is a continuous breeder occurring in the coastal regions of Tuticorin. Various studies such as chemical screening (Meenakshi *et al.*, 2012a,b), anti-bacterial activity (Senthamarai *et al.*, 2012), acute and subchronic oral toxicity (Meenakshi *et al.*, 2012c), anti diabetic activity (Meenakshi *et al.*, 2012d) have been carried out so far. Review of literature reveals that CNS depressant activities have been carried out with many species of plants (Srikanth and Muralidharan 2009, Urmilesh *et al.*, 2011, Deepika *et al.*, 2013,) and two species of colonial ascidians (Rajasekaran *et al.*, 2003, Rajesh and Murugan, 2013). But CNS depressant activity of *Microcosmus exasperatus* has not been attempted at all and hence the present study was designed.

Materials and Methods

Animal material

Microcosmus exasperatus was collected from Tuticorin harbor area with the help of SCUBA diver and identified. A voucher specimen AS 2240 has been deposited in the museum, Department of Zoology, A.P.C. Mahalaxmi College For Women, Tuticorin (Plate 1).

Plate.1 *Microcosmus exasperatus*

Systematic position

Phylum: Chordata, Subphylum: Urochordata, Class: Ascidiacea, Order: Pleurogona, Sub order: Stolidobranchia, Family: Pyuridae, Genus: *Microcosmus*, Species: *exasperates.*

Preparation of powder and extract

The animals were cleaned with sea water, dried at 45^{o} C and homogenized to get a coarse powder which was stored in an air tight container. The extract was prepared from 100 g powder with ethanol as solvent using soxhlet apparatus and cooled to room temperature. It was evaporated in a rotary evaporator to get a residue which was utilized for further investigations.

Experimental animal

Swiss albino mice weighing 20 – 25 g were purchased from the animal house, PSG institute of Medical Science, Coimbatore, India. They were maintained under standard laboratory conditions, with constant 14 hours of darkness, 10 hours of light cycle, and temperature (25±2° C). Clean water and standard pellet diet "ad libitum" (Sai Durga Animal feed, Bangalore, India) were given to them.

Standard drugs

Diazepam - 5 mg/kg (Lupin laboratories Ltd., India), Carboxy methyl cellulose - 5% (SRL Laboratories Ltd., India), Cocaine - 40 mg/kg (M.M. Pharma, New Delhi, India) and Phenobarbitone sodium - 40 mg/kg (Rhone-Poulenc India Ltd., India)

Acute toxicity studies

The acute oral toxicity studies (OECD – 423 guidelines) were carried out by the

acute class method (OECD, 2002). Adult Swiss albino mice of either sex weighing 20-25 g were used. Three animals were selected and 2000 mg/kg bw of the ethanolic extract of *Microcosmus exasperatus* was given orally using intra gastric catheter to overnight fasted mice. They were observed continuously for any gross behavioral changes and toxic manifestations like hypersensitivity, grooming, convulsions, sedation, hypothermia and mortality during the first three hours. If no mortality was observed the experiment was repeated with the same dose of the extract for 7 more days. There after the animals were continuously monitored at regular intervals for fourteen days. Sub- lethal doses of 50,100 and 150 mg/kg bw were used for the following experiments.

General behavioral profiles

The general behavioral profiles were carried out by the standard procedure of (Dixit *et al.*, 1979). The animals were randomly grouped into 5 (n=8). Group I received 5% CMC and acted as control. Group II, III, IV, V were administered with 50, 100, 150 mg/kg of the extract of the *Microcosmus exasperatus* and 5 mg/kg of diazepam respectively using intra gastric catheter. The animals were monitored at an interval of 30 minutes for the first one hour and thereafter hourly for the next 4 hours for the following parameters.

Alertness, awareness, spontaneous activity

The alertness and awareness was recorded visually by placing the animal in different positions and its ability to orient itself without bumps or falls. The normal behavior was scored as (−), little activity (+), moderate flexibility (++), strong response (+++), and abnormal restlessness (++++).

Spontaneous activity was noted by keeping the animal in a bell jar. Moderate activity was scored as (++) and strong activity as (+++). If there is little motion, the score was (+), while if the animal sleeps, it was scored as (−). Excessive or very strong inquisitive behavior like constant walking or running was scored as (++++). The animals were removed from the jar and placed on a table and a similar test was performed with the same scoring (Turner, 1965).

Sound, touch, pain response

Swiss albino mice normally utter no sound. Vocalization on stimulation is an indication of a noxious stimulus. Absence of sound response on treated mice is graded and scored. The mice were touched on various parts of the body like side of the neck, abdomen and groin with a pencil or forceps to record the touch response. A small artery clamp was attached to the base of the tail in order to grade and record the pain response of the mice.

Righting, pinna reflex

Groups of mice were treated with different concentration of the extract of *Microcosmus exasperatus* on the day of experiment. At an interval of 15, 30, and 60 minutes, each mouse was placed gently on its back on an undulated surface made of white iron kept at 30° C. If the animal remained on its back for 30 s, it indicates a loss of righting reflex. By touching the center of pinna with a hair or other fine instrument the reflex was examined.

Grip strength

It is used for assessing the muscular strength or neuromuscular function in rodents. This was noted as the time taken

by the animal to grasp the pencil in the horizontal position and to drop it on the table.

Cocaine induced hyperactivity

The animals were divided into four groups of eight (n=8). Group I was given the vehicle (CMC 5%) and II, III and group IV were administered with 50, 100, 150 mg/kg extract of *Microcosmus exasperatus* for 15 days. After 30 minutes of habituation period, the animals were treated with cocaine (40 mg/kg) and were kept in the activity cages for duration of 90 minutes. Activity was measured as light beam interruptions at a gap of per 10 minutes (Chung *et al.*, 2002).

Phenobarbitone sodium induced sleeping time

The animals were divided into four groups of eight (n=8). Group I acted as control, group II, III and IV received 50, 100 and 150 mg/kg bodyweight of the extract. were given 40 mg/kg phenobarbitone sodium, intraperitonealy 30 minutes after the administration of extract of *Microcosmus exasperatus*. 1 ml of 5% CMC was used as a vehicle control. The sleeping time was recorded, and measured as the time interval between the loss and regaining of the righting reflex (Dandiya and Collumbine, 1956).

Exploratory behavior

This was performed in mice using Y – maze and head dip tests.

Y- maze test

The experimental animals were divided into five groups of 8 albino mice in each. The groups were injected with CMC, extract of *Microcosmus exasperatus* (50, 100, 150 mg/kg) diazepam (5 mg/kg i.p) respectively on the test day. The experiment was carried out at an interval of 30 minutes for 2 hours. The animals were placed in a symmetrical Y – shaped run way (33 cm x 38 cm x 13cm) individually for 3 minutes. The numbers of maze with all 4 feet (an 'entry') were counted (Rushton *et al.*, 1961).

Head dip test

The curiosity of mice can be evaluated using head dip test. Five groups of albino mice (n=8) were placed on top of a wooden box with 16 evenly spaced holes, after the administration of vehicle (1ml of 5% CMC), extract of *Microcosmus exasperatus* (50, 100, 150 mg/kg) and diazepam (5 mg/kg, i.p) respectively. The number of times each animal dipped its head into the holes was counted for the period of 3 minutes (Tomkiewicz *et al.*, 1971).

Muscle relaxant activity

It was studied by using traction and rotarod test.

Traction test

By placing the fore paws of the mice on a small twisted wire, rigidly supported above the bench top, screening was done to perform the traction test. Normally the mice grasp the wire with the fore paws and place at least one hind foot on the wire (within 5 s) when allowed hanging free. Five groups of animals (n=8) were screened previously. On the 15^{th} day, 30 minutes after the administration of vehicle (1 ml of 5% CMC), extract of *Microcosmus exasperatus* (50, 100, 150 mg/kg) and diazepam (5 mg/kg) were

given. Inability to put up at least one hind foot is considered as failure in the traction test (Rudzik *et al.,* 1973).

Rotarod test

It is used to evaluate the activity of drugs interfering with motor coordination. Fresh mice were placed on a horizontal wooden rod (32 mm diameter) rotating at a speed of 16 rpm (Model 7600; Ugo Basile). The animals capable of remaining on the top for 3 minutes or more, in 3 successive trials were selected. They were divided into 5 groups (n=8). After administering the test doses, the animals were placed on the rod at an interval of 30 minutes for 2 ½ hours duration. If the animals failed more than once to remain on the rotarod for 3 minutes they were considered to have passed in the test (Dunham and Miya, 1957).

Statistical analysis

Values are expressed as mean ± SEM. The statistical analysis was done by one- way analysis of variance (ANOVA) followed by Dunnet's test. P values less than 0.05 were considered to be significant.

Results and Discussion

Studies on the CNS depressant activity with the ethanolic extract of *Microcosmus exasperatus* showed the following results.
Acute toxicity studies

Administration of 2000 mg/kg body weight of the ethanolic extract of *Microcosmus exasperatus* did not show any mortality during the 24 hour experimental duration. This single dose induced irritability, tremor, labored breathing, staggering and convulsions but not mortality, indicating its safety. Earlier reports on sub chronic oral toxicity conducted for a period of 14 days showed no adverse effect on liver and renal function (Meenakshi *et al.,* 2012c). Hence it can be concluded that ethanolic extract of *Microcosmus exasperatus*, is nontoxic at the highest limit dose of 2000 mg/kg bw. Further experiments were carried out by selecting sub lethal doses of 50, 100, and 150 mg/kg
.

General behavioural profiles

The results obtained for different parameters of general behavioral profiles are presented in table1. The extract at a dose of 150 mg/kg produced strong response to alertness and awareness. Spontaneous activity, sound and touch responses of the animal showed a significant and very strong depression. Strong reduction in pain, righting, pinna reflex, and moderate response in grip strength was recorded. The general behavioral profile of the mice administered with highest dose of the extract was similar to that of those treated with the standard drug diazepam. Investigations carried out with the extract of different plant species has shown that compounds like saponins and triterpenoids may be responsible for the CNS depressant activity (Srikanth and Muralidharan 2009). Chemical analysis of the extract of *Microcosmus exasperatus* has shown the presence of both these compounds suggesting a similar role in these sedentary ascidians. A block in synaptic transmission of nerve impulse in the afferent pathway can be attributed to the strong reduction in pinna reflex (Ramanathan *et al.,* 2008).

Cocaine induced hyperactivity

The inhibition of hyperactivity in mice

Table.1 Effect of *Microcosmus exasperatus* on general behavioural profiles

Behaviour type	Group I	Group II	Group III	Group IV	Group V
	CMC (1 ml)	50 mg/kg	100 mg/kg	150 mg/kg	Diazepam (5 mg/kg)
Alertness	–	++	++	+++	+++
Awareness	–	++	++	+++	+++
Spontaneous activity	–	+	++	++++	++++
Sound response	–	++	++	++++	++++
Touch response	–	++	+++	++++	++++
Pain response	–	++	++	+++	++++
Righting reflex	–	+	+++	+++	++++
Pinna reflex	–	++	++	+++	++++
Grip strength	–	++	+	++	++++

(–) No effect; (+) slight depression; (++) moderate depression; (+++) strong depression; (++++) very strong depression; n=8.

Table.2 Effect of *Microcosmus exasperatus* on cocaine induced hyperactivity

No. of light Interruptions at an interval of 10 minutes	Group I	Group II	Group III	Group IV
	CMC (1 ml)	50 mg/kg	100 mg/kg	150 mg/kg
10	185 ± 2.48	195 ± 1.27	185 ± 1.53	180 ± 2.25
20	164 ± 1.48	180 ± 1.39*	175 ± 1.17*	135 ± 1.62*
30	145 ± 1.92	160 ± 1.28*	135 ± 1.33*	120 ± 1.07*
40	160 ± 1.44	125 ± 1.66*	110 ± 1.54*	105 ± 1.38*
50	180 ± 1.41	120 ± 1.54*	80 ± 1.30*	100 ± 1.54*
60	140 ± 1.34	110 ± 1.59*	75 ± 1.56*	90 ± 1.21*
70	110 ± 1.56	95 ± 1.18*	65 ± 1.33*	70 ± 0.65*
80	105 ± 1.32	80 ± 1.75*	60 ± 1.27*	50 ± 0.37*
90	95 ± 1.55	75 ± 1.58*	40 ± 1.78*	40 ± 0.94*

Data represented as number of entries in 3 minutes; mean± S.E.M, (n=8). Significance between control and extract treated groups. * $p < 0.05$

Table.3 Effect of *Microcosmus exasperatus* on phenobarbitone sodium induced sleeping time

Treatment	Groups/Dose mg/kg	Sleeping time (min)
CMC	I - 5% 1ml	53 ± 1.88
Extract+ phenobarbitone Sodium	II - 50	94 ± 2.68*
	III - 100	103 ± 3.37*
	IV - 150	118 ± 2.21*

Data represented as mean± S.E.M, (n=8). Significance between control and extract treated groups. * $p < 0.05$.

Table.4 Effect of *Microcosmus exasperatus* on exploratory behavior

Treatment	Groups & Dose mg/kg	Y – maze Test				Head dip Test
		Number of entries after treatment				
		30	60	90	120	
CMC	I - 5% 1ml	9.6 ± 0.11	9.8 ± 0.85	9.9 ± 0.44	10.3±0.29	95 ± 4.20
Extract	II - 50	6.2±0.25	6.5±0.36	6.8±0.36	7.3±0.30	55±2.48
	III - 100	4.5±0.84*	5.2±0.22*	5.5±0.23*	5.1±0.62*	35 ± 1.66*
	IV - 150	3.7±0.45*	3.3±0.75*	3.5±0.94*	3.4±0.28*	30 ± 1.27*
Diazepam	V - 5	3.1±0.44*	3.5±0.51*	3.6±0.28*	3.3 ± 0.18	37 ± 2.56*

Data represented as mean± S.E.M, (n=8). Significance between control and extract treated group. * $p < 0.05$.

Table.5 Effect of *Microcosmus exasperatus* on muscle relaxant activity

Treatment	Groups/Dose (mg/kg)	Traction test	Rotarod test
CMC	**I - 5% 1ml**	**0**	**0**
Extract	**II - 50**	**75***	**50**
	III - 100	**85***	**60***
	IV - 150	**95***	**70***
Diazepam	**V - 5**	**100**	**100**

Data represented as percentage animals showing a negative results mean± S.E.M, (n=8). Significance between control and extract treated groups. * $p < 0.05$.

induced after cocaine injection was evident on treatment with the ethanolic extract of *Microcosmus exasperatus* from 40 through 90 minutes. Normally administration of cocaine releases both dopamine and noradrenalin which stops the normal exploratory, grooming, rearing and gnawing behavior of mice. Dopamine antagonists prevent these effects by destroying the dopamine containing cell bodies in CNS but the noradrenergic system is not inhibited (Srikanth and Muralidharan, 2009; Chung *et al.,* 2002). The hyperactivity in the nigrostriatal dopaminergic system reflects the motor disturbances induced by cocaine. Earlier studies using plant extracts have demonstrated that dopamine antagonists are responsible for preventing hyperactivity (O'Neill and Shaw 1999). In the present study, the hyperactivity induced by cocaine in mice was inhibited by the extract of *Microcosmus exasperatus* showing the presence of dopamine antagonists.

Effect of phenobarbitone sodium induced sleeping time

A graded inclination in the phenobarbitone sodium induced sleeping time in a dose dependent manner was evident on treatment with the extract of *Microcosmus exasperatus* (Table. 3). A significant increase in the hypnotic effect induced by phenobarbitone suggests an elevated sedative activity. This can be related to the interaction of benzodiazepines and related compounds that bind to receptors in the CNS as reported using some plant extracts (Ramanathan *et al.,* 2008). A similar increase in sleeping time has been noticed on treatment with methanolic extract of the colonial ascidians (Rajasekaran *et al.*, 2003, Rajesh and Murugan, 2013).

Exploratory behavior in mice

A decrease in exploratory behavior in y – maze test in a dose related manner was noted in animals treated with the extract of *Microcosmus exasperatus* compared with control. In case of head dip test also there was a marked decrease in response as shown in table 4. Different dose of the extract exhibited a marked fall in exploratory behavior in mice using y – maze and head tip tests similar to that of standard drug. At a low dose, the standard drug diazepam acts as an anxiolytic, anticonvulsant and induce sedation (Onaivi *et al.,* 1992). The same mode of action may be suggested for the reduction in exploratory behavior of the treated groups indicating the presence of CNS depressant agents in the extract. Earlier reports with the extract of other colonial ascidians of Tuticorin water have also revealed a decline in locomotor activity (Rajasekaran *et al.,* 2003, Rajesh and Murugan, 2013).

Muscle relaxant activity

A significant failure was noted in the traction test on administration of the extract. The motor coordination was reduced in the rotarod test in a dose related way (Table. 5). An increase in the number of falls and a decrease in the time on the bar at a higher dose as an indication of muscle relaxant activity were detected in rotarod test (Onaivi *et al.,* 1992). The lack in motor coordination and muscle relaxant activity may be due to the depressant activity of CNS. The extract of *Microcosmus exasperatus* contains alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, quinones, anthraquinones, phenols and aromatic acids. Alkaloids have been indicated to produce CNS depressant action in many reports (Van Wyk and Gericke, 2000, Dewick, 2002, Carlini, 2003, Lewis and Lewis, 2003). The present investigation with the ethanol extract of *Microcosmus exasperatus* showed pharmacological activity identical to that of the standard drugs diazepam, aminobarbitome and an antagonist of cocaine. Though the extract has CNS depressant activity and properties to relieve pain the exact chemical constituent and mechanism of action is yet to be ascertained. Hence further studies on the isolation of the pure compound may lead way to the discovery of drug molecules with potent CNS depressant activity.

Acknowledgement

The authors express their sincere thanks to the UGC, New Delhi- F. No. 39- 588 / 2010 (SR) for financial assistance and to Dr. R. Sampathraj, Director, Samsun Clinical Research Laboratory, Tirupur for animal studies**.**

References

Carlini, E.A., 2003. Plants and the central nervous system. J. Pharmacol. Boichem. Behav. 75: 501-512.

Dandiya, P.C., and Collumbine, H. 1956. Studies on *Acorus calamus* (L.) some pharmacological action of the volatile oil. J. Pharmacol.Exper. Therapeut. 125: 353-59.

Deepika, R., K. Hemamalini., G. Shasipriya and Uma, V. 2013. CNS activity of the methanol extract of *Solanum pubescens* in experimental animal model. J. Pharma. Boisci. 5: 48-51.

Dewick, P.M., 2002. Medicinal natural products: a biosynthetic approach. John Wiley and sons, West Sussex. pp. 1-188.

Dixit, V.K., and Varma, K.C. 1979. Effect of essential oils of rhizomes of *Hedychium spicatum* on central nervous system, Indian. J. Pharmacol. 1: 147-149.

Dunham, N.W., and Miya, T.S. 1957. A note on a simple apparatus for detecting neurological deficits in rat and mice. J. American Pharmacol. Asso.46: 208-209.

Gomathy, S., V.K. Meenakshi., S. Senthamarai., D. Shanmugapriya., M. Paripoornaselvi and Chamundeswari, K.P. 2012. Studies on the distribution of ascidians. Proceedings of 8th all India conference of KAAS. Vol.III Sciences. Zoo. 23-31.

Chung, I.W., A. N. Moore, W.K. Oh, M. F. O'Neill, J.S. Ahn, J.B. Park, U.G. Kang and Y.S. Kim. 2002. Behavioral pharmacology of polygalasaponins indicates potential antipsychotic efficacy. Pharmacol.Biochem. Behav. 71: 191.

Lewis, W.H., and Lewis, P.F. 2003. Medical botany: plants affecting human health. John Wiley and sons,Washington. pp. 1-237.

Martin, W., and Bernard, B. 1991. Marine epibiosis. Possible antifouling defense adaptations in *Polysyncraton lacazei* (Giard). J. Experi. Marine Biol.Ecol. 145: 49-63.

Meenakshi, V.K., S. Gomathy and Chamundeswari, K.P. 2012c. Acute and subchronic oral toxicity of *Microcosmus exasperatus* Heller, 1878. J. Microbiol.Biotechnol.Res. 2: 94-98.

Meenakshi, V.K., S. Gomathy and Chamundeswari, K.P. 2012a. GC-MS analysis of the simple ascidian *Microcosmus exasperatus* Heller, 1878. Inter. J. Chem. Tech. Res. 4: 55-62.

Meenakshi, V.K., S. Gomathy, M. Paripoornaselvi and Chamundeswari, K.P. 2012d. Antidiabetic activity of the ethanol extract of simple ascidian, *Microcosmus exasperatus* Heller, 1878. Inter. J. Chem. Pharma. Sci. 3: 33-39.

Meenakshi, V.K., S. Gomathy, S. Senthamarai, M. Paripoornaselvi and Chamundeswari, K.P. 2012b. GC-MS Determination of the bioactive components of *Microcosmus exasperatus*. Heller, 1878. J.Curr.Chem. Pharm. Sci. 2: 271-273.

OECD (Organization for Economic Cooperation and Development), 2002. OECD Guidelines for the testing chemicals/ selection 4: Health effects test No. 423: Acute Oral Toxicity-Acute Toxic Method. *OECD.* Paris.

Onaivi, E.S., P.A. Maguiri, N.F. Tsai, M.F. Davies and Locu, G.H. 1992. Comparison of behavioral and central BDZ binding profile in three rat lines. J.Pharmacol. Biochem. Behav. 43: 825.

O'Neill, M.F., and Shaw, G. 1999.

Comparison of dopamine receptor antagonists on hyper locomotion induced by cocaine, amphetamine, MK-801 and the dopamine D1 agonist C-APB in mice. Psychopharmacol. 45: 237-250.

Paripoornaselvi, M., V.K. Meenakshi, D. Shanmugapriya, S. Gomathy, S. Senthamarai and Chamundeswari, K.P. 2012. Ascidian biofouling. Proceedings of 8th all India conference of KAAS Vol. III Sciences. Zoo. 6-13.

Rajasekaran, A., P. Thirupathy Kumaresan and Murugesan, S. 2003. CNS depressant activity of the methanolic extract of simple ascidian *Distaplia nathensis.* Inter. J. Chem.Sci. 1: 13-16.

Rajesh, R.P., and Murugan, A. 2013. Central nervous system depressant, anti-inflammatory analgesic and antipyretic activity of the ascidian *Eudistoma virde*. Pharmacol. 65: 69.

Ramanathan S.K., R. Shanmugasundram, P. Sivakumar, R. Nethaji, V. Senthil, N. Venkateswaramurthy and Kanagasabi, R. 2008. CNS activity of the methanol extract of *Careya arborea* in experimental animal model. Bangaladesh. J. Pharmacol. 3: 36- 43.

Rudzik, A.D., J.B. Hester, A.H. Tang, R.N. Staw and Friis, W. 1973. The benzodiazepines Raven Press, New York. pp. 285-297.

Rushton, R., H. Steinberg and Tinson, C. 1961. Effects of an amphetamine-barbiturate mixture by the past experience of rats. Nature. 192: 533-535.

Senthamarai, S., V.K. Meenakshi, S. Gomathy, M. Paripoornaselvi, D. Shanmugapriya and Chamundeswari, K.P. 2012. Antibacterial activity of ascidian *Microcosmus exasperatus* against human pathogens. Proceedings of 8th all India conference of KAAS. Vol. III Sciences. Zoo. 14-22.

Srikanth, J., and Muralidharan, P. 2009. CNS activity of the methanol extract of *Sapindus emarginatus Vahl*. in experimental animal model. J. Sci.Res. 1: 583-593.

Tomkiewicz, M., D. Joyce, R.D. Porsolt and Summerfield, A. 1971. Persistence of dose related behaviour in mice. Nature. 231: 121-123.

Turner, R.A., 1965. Screening methods in pharmacology. Academic Press, New York, 26-35.

Urmilesh Jha, M., P. Chhajed, T. Tushar Shelke, J. Rajesh Oswal and Prafull Adkar, P. 2011. CNS activity of the methanol extract of *Pathenium hysterophorus L.* in experimental animals. Der Phavmacia letter. 3: 335-341.

Van Wyk, B.E., and Gericke, N. 2000. People's plants. A guide to useful plants of South Africa. Briza Publications, Pretoria. pp. 1-84.