Behavioral and Memory Boosting Effects of Intellan and Cyanocobalamin in Mice

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Abstract: The aim of this study was to compare the effect of selected drugs, Intellan and cyanocobalamin on some aspects of animal behavior, like effect on memory, depression, gross behavior and exploratory activity. Results of present investigations reveals cyanocobalamin to be a good memory enhancer and intellan also showed memory boosting effects in addition to anti-anxiety activity which affects overall performance.

Keywords: *Centella asiatica*, *Bacopa monniera*, neurotransmitter, acetyl choline, reactive oxidative specie.

INTRODUCTION

Memory is the ability of the brain to store, retain, and subsequently recall information. There are various factors which affects memory like age, change in hormonal or neurotransmitter levels, stress, disease state etc. The central cholinergic system is considered to be the most important neurotransmitter involved in the regulation of cognitive functions. Cholinergic neuronal loss in hippocampal area is the major feature of Alzheimer's disease (AD). Bacopa Monniera (BM) may indirectly, modify Acetylcholine (Ach) concentrations, through its influence on other neurotransmitter systems [1]. *Bacopa Monniera* (BM) is also an antioxidant and it decreases reactive oxidative specie (ROS) levels by enhancing antioxidant enzymes for example Super oxide dismutase, glutathione per oxidase and catalase in frontal cortex, striatum and hippocamopus of rats [1]. This increase in Ach level is by decreasing the AcetylcholineEstrase enzyme activity [2, 3]. So by increasing the Acetyl choline (Ach) level this loss in cognition can be reversed. Another neurotransmitters which may be involved in memory are norepinephrine, epinephrine and dopamine. Recently, it was found that the numeric working memory process involved the function of lateral prefrontal cortex (PFC) [4, 5] whereas the spatial memory was reported to involve hippocampus [6]. Previous studies demonstrated that the numeric working memory could be modulated by dopamine, norepinephrine and other main neurotransmitters [7, 8] and during spatial memory task, both acetylcholine and serotonin (5HT) in hippocampus were simultaneously activated [9]. It has been suggested that the behavioral effects of cholinergic degeneration can be changed by a reduction in noradrenergic function [10]. BM is known to lower nor-epinephrine and increase 5-hydroxy-tryptamine levels in the hippocampus, hypothalamus and cerebral cortex [11]. So both BM and CA which are the important constituents of Intellan syrup, has got the ability to affect these neurotransmitters (NT). Another important ingredient of Intellan syrup is *Centella asiatica* (CA) which is distributed widely in south America and Asia especially in the damp places throughout India and is known as therapeutic agent in folk medicine,capable of improving memory and treating several neurological disorders. It is also used as brain tonic for promoting brain growth and improving memory. It act as an antioxidant and thus exert significant neuro protective effect and proved efficacious in protection of brain against age related oxidative damage [12].

While Vitamin B_{12} also has an important role in cognition and it has been reported by Teruhiko *et al.*, [13] that low levels of vitamin B_{12} are associated with cognitive decline in Japanese adults. Raised homocysteine, low folate and vitamin B_{12} concentration may cause cognitive decline [13]. The deficiency of vitamin B_{12} may lead to different brain damages and depression [14] as well as it may leads to development and progression of dementia [15].

MATERIALS AND METHODS

The study protocol is designed in such a way that the drugs were tested for their effect on CNS For CNS screening 40 mice weighing 20–25 gm bred at animal house of Department of Pharmacology, University of Karachi, were used. The animals were maintained under constant environmental conditions 21 ± 1°C. All animals were given standard diet prepared in the laboratory and water ad libitum for 30 days.

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Selection of Doses

The dosing of intellan and cytacon were done daily in normal doses according to their body weight. Control animals were received water equivalent to the volume of respective doses according to their body weight. Animals were divided in three groups, One served as control , two groups received Intellan and Cyanocobalamin (Cytacon) respectively. All animals received drug orally. Body weight of the animals was measured weekly. Albino mice were divided into 4 groups of 10 animals each (both sexes) and were administered distilled water. Dosing was done for 30 days.

- Group 1 served as Control.
- Group 2 received 0.25 mg of Centella asiatica/ Bacopa Monniera present in 0.05 ml of Intellan (diluted with distilled water).
- Group 3 received 0.3µg of vitamin B_{12} present in 0.04 ml of Cytacon liquid.

After 30 days the mice were sacrificed and their brains were stored at temperature below 4°c in deep freezer.

Behavioral Studies

Following parameters were observed during dosing period:

- Head dip test (learning ability)
- Stationary rod test
- Cage crossing test (movement in an enviornment)
- Open field activity (locomotor activity)
- Swimming induced depression test(anti depressant activity)
- Gross behavior test (mood, alertness, CNS excitation, muscle tone, reflexes, autonomic reflexes).

Open Field Activity

The open field test was used to assess locomotor activity [16]. The apparatus consist of an open topped wooden box (20 x 40 x 40 cm) with the floor arena marked in to 16 squares of 10 cm each. Individual mouse was placed in the lower right hand corner and the squares entered and numbers of rearings and forwardings during 10 minutes of observation were recorded.

Head Dip Test

Head dip test was used with some modification [17], to assess the animal's learning ability. The apparatus is made up of a rectangular wooden box (35 × 45 × 45 cm) with a hole (2.5 cm in diameter) at each corner. The animal was placed in the center of the box and the number of times the animal poked its snout (pokings) in the holes during 10 min of observation was recorded.

Swimming Induced Depression Test

The behavioral despair swim test was described by Porsolt *et al.* [18,19] as a model for testing antidepressant activity. Mice were forced to swim in a restricted space from which they cannot escape, are induced to a characteristic behavior of immobility and assume a floating posture. This behavior reflects a state of despair.

Cage Crossing Test

Transparent perpex cages (26x26x26 cm) with sawdust cover floor were used to monitor the activity in familiar environment. Mice were placed individually in these cages to get them familiarized with the environment, and number cage crossing were counted for 10 minutes.

Stationary Rod Test

In this test mice were allowed to balance on stationary rod after training. Time during which mice maintain balanced and then fall from the stationary rod was noted for each mouse individually.

Inclined Plane Test

The plain consist of two rectangular board connected at one end by a hinge .One board is the base and the other is a movable inclined plane. The mice was allowed to move on the plane and the movement on the plane was observed.

Gross Behavior Test

Mice were daily observed for gross behavioral changes such as awareness, mood, CNS excitation, posture, muscle tone, reflexes and autonomic reflexes.

Statistical Analysis

All values were compared with the controlled and standard drug by taking mean of all of them and the significance of difference between means was

determined by student significance t- test. Values of P<0.05 were considered as significant and P<0.01 as highly significant. All statistical procedures were performed according to the method of Alcaraz and Jimenez [20].

RESULTS

Table **1** shows the effect of drugs on Head dip activity in mice which shows insignificant decrease by Intellan on the 20th day however there was a significant decrease in exploratory activity on the 30th day. Cyanocobalamine significantly increased the head dip activity on day 20th but insignificantly decreased it on 30th day.

Table 1: Effect of Drugs on Head Dip Activity in Mice

Drugs	Days	
	20 Days	30 Days
Control	22.1±3.83	25.9±1.78
Intellan	17±2.3	11.6±2.68**
Cyanocobalamin	32.5±2.43*	23.9±3.29

n=10; Average values ± S.E.M.; *p<0.05 significant as compared to control; ** p<0.001 highly significant as compared to control.

The stationary rod activity is shown in Table **2** shows that there is significant reduced activity time by Intellan on 20th and 30th day, and Cyanocobalamine also shows significant reduction in time on 20th as well as on 30th day.

Table 2: Effect of Drugs on Stationary Rod Activity in Mice

Drugs	Day	
	20 Days	30 Days
Control	106±15.15	103.6 ± 12.6
Intellan	54.7±15.18*	57.9 ± 13.92*
Cyanocobalamin	9.0±2.06**	23.9 ± 3.29**

n=10; Average values ± S.E.M.; *p<0.05 significant as compared to control; ** p<0.001 highly significant as compared to control.

Intellan reduced the cage crossing activity significantly on day 20th and increased it significantly on day 30th as shown by Table **3**. Similarly Cynocobalamin also increased the cage crossing activity significantly on day 20th and on day 30th.

Table **4** shows that Swimming induced depression activity of Intellan treated mice which was reduced significantly on both days. The activity was increased insignificantly by Cyanoacobalamin on day 20th but decreased insignificantly on day 30th.

Table 3: Effect of Drugs on Cage Crossing Activity in Mice

Drugs	Days	
	20 Days	30 Days
Control	42.1 ± 5.15	41.7 ± 4.27
Intellan	18.2 ± 2.41*	63.8 ± 8.76*
Cyanocobalamin	111.6 ± 6.36**	113.5 ± 11.2**

n=10; Average values ± S.E.M.; *p<0.05 significant as compared to control; ** p<0.001 highly significant as compared to control.

Table 4: Effect of Drugs on Swimming Induced Depression in Mice

Drugs	Days	
	20 Days	30 Days
Control	107.8 ± 14.84	107.6 ± 14.2
Intellan	41.4 ± 4.68*	27.2 ± 5.22**
Cyanocobalamin	113 ± 15.3	85 ± 6.04

n=10; Average values ± S.E.M.; *p<0.05 significant as compared to control; ** p<0.001 highly significant as compared to control.

Intellan decreased the open field activity on day 20th and day 30th non-significantly as shown by Table **5** however Cyanoacobalamin insignificantly increased the activity on day 20th and on day 30th .

Table 5: Effect of Drugs on Open Field Activity in Mice

Drugs	Days	
	20 Days	30 Days
Control	160.5±20.15	162.2±15.0
Intellan	127.2±8.6	90.0±9.58
Cyanocobalamin	193.1±12.2	193.4±8.83

n=10; Average values ± S.E.M.

Table **6** shows the effect of drug on body weight of mice which was increased insignificantly by Intellan. Cyanocobalamin treated group showed non significant decreased body weights on 30th day.

Table 6: Effect of Drug on Body Weight of Mice on Day 30

Drugs	30 Days
Control	24.0 ±0.16
Intellan	24.86±1.36
Cyanocobalamin	21.14±0.49

n=10; Average values ± S.E.M.

Table **7** shows that brain weights of mice treated with Intellan and Cyanocobalamin increased insignificantly after day 30th as compared to control.

Table 7: Effect of Drugs on Brain Weight of Mice on Day 30

Drugs	30 Days
Control	0.2538± 0.016
Intellan	0.3075 ±0.016
Cyanocobalamin	0.3175 ± 0.022

n=10; Average values ± S.E.M.

Table **8** shows gross behaviors of both groups taking Intellan and Cyanocobalamin as compared to control.

DISCUSSION

The head dip activity was decreased by Intellan, indicating the decline in exploratory activity which in consistent with the findings of Sulochana *et al.* [21] who also indicated that *Centella asiatica* can reduce head dip activity. The reduction in activity can also be associated to increased learning [22]. The composition of Intellan contain *Centella asiatica* which is contributing to this effect as *Centella asiatica* has shown to posses the central nervous system depressant activity [22], which is contributing to this effect. The other ingredients of intellan include *Coriander sativum*, which has been reported to posses anti anxiety activities and can relieve insomnia. This is one of the use of coriander in Iranian Folk medicine [23].The anti anxiety effect leads to calming action, leading to reduction in head dip activity as observed.

The head dip activity was enhanced by Cyanocobalamin (Cytacon) indicating that it has increased alertness and the animal has shown increased activity. This finding is supported by Servet *et al.*, [24], according to which the geriatric dementia may be related to the decreased levels of vitamin B_{12}, and he also indicated that the cognitive functions are related to vitamin B_{12} availability. This support our observation of overall increased head dip activity by Cyanocobalamine. Vitamin B_{12} is required to synthesize S-adenosyl methionine which is involved in the synthesis of certain neurotransmitters and Catecholamines and is also involved in the brain metabolism. The deficiency of vitamin B_{12} is therefore associated with depression due to less availability of

Table 8: Gross Behavior Chart

Parameters	Control	Drugs			
		Intellan		Cynocobalamin	
		15 Days	30 Days	15 Days	30 Days
Grooming	+++	++	+	+++	+++
Staggering	-	-	-	-	-
Straub's Phen.	-	-	-	-	-
Writhing	-	-	-	-	-
Tremor	-	-	-	-	-
Twitches	-	-	-	-	-
Righting Reflex	+++	+++	+++	+++	+++
Pinna Reflex	+++	+++	+++	+++	+++
Corneal Reflex	+++	+++	+++	+++	+++
Papillary diameter (constriction/Dilatation)	-	-	-	-	-
Eyelid (closure/Exopth alamus)	-	-	-	-	-
Salivation	-	-	-	-	-
Lacrimation	-	-	-	-	-
Defecation	+++	+++	+++	+++	+++
Urination	+++	+++	+++	+++	+++

neurotransmitters involved in regulation of mood. In our observation the mood is elevated and the animal tries to explore and shows enhanced activity.

The effect of intellan on stationary rod indicate that memory and learning was improved after taking Intellan and the animal crosses the rod in half of the time as compared to control. This effect is possibly through activation of Acetyl choline (Ach) as also indicated by Sulochana *et al.*, [21] that *Centella asiatica* can enhance learning and memory probably by influencing cholinergic system and by changing the neuronal morphology.

Bacopa monniera also improves the memory as reported earlier by Nanteetip *et al.* [25] which is also present in Intellan contributing to this effect. On the other hand after the administration of Cyanocobalamin (Cytacon) the animal reached the other side of the rod only in nine seconds which is very significant indicating that vitamin B_{12} can enhance learning and improve memory. This effect is due to increase in the synthesis of neurotransmitters and due to these neurotrans-mitters especially acetylcholine there is marked increase in learning and memory .

The effect on swimming induced depression was also significant. The struggling time was decreased by Intellan possibly due to the presence of ingredients having anxiolytic profile especially *Coriandrum sativum* and *Centella asiatica*. The CNS depressant activity was also reported by Chatterjee *et al*., [22] which supports our observation. Coriander in Iranian folk medicine is reported to treat insomnia, and these effects are contributing to decreased struggling time. Cyanocoba-lamin (Cytacon) after twenty days of treatment increased the struggling time slightly as it enhances the oxygen saturation of the blood and improves the muscular activity leading to increased struggling time. The effect after 30 days is slightly decreased possibly due to the repeated exposure to swimming the animal has become depressed and vitamin B_{12} only tries to improve muscular activity but cannot elevates the mood.

Intellan reduced cage crossings, may be due to the anxiolytic profile of *Centella asiatica* which reduces locomotor activity. But cage crossing was increased on day 30^{th} may be due to increased learning.

The open field and cage crossings both were significantly increased by Cyanocobalamine, because the overall oxygen delivery and mental alertness is increased.

CONCLUSION

We conclude that Cyanocobalamin could prove as a good memory enhancer, but the effect on lipid profile requires periodic assessments. Intellan has shown good memory boosting effects but it also has certain anti-anxiety constituents which can affect the overall performance.

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