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Original Article

Neuro-pharmacological screening of Cyanocobalamine and khamera-e-gao zaban amberi jawahir dar and its Behavioral and Memory enhancing effects in mice

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ABSTRACT

Unani medicines are used since long time ago, among which Khamira's are used for rejuvenation of body homeostasis. The object of the study was to explore the effect of khamira-e gao zaban amberi jawahirdar and the results were compared with the standard drug Cyanocobalamine (vitamin B_{12}). Different CNS parameters had been performed on mice. The drugs were administered to mice and results were observed on day 20 and30. The dose of khamira administered to one group was 0.1 ml containing 0.83 mg dissolved in milk (9 mg dissolved in 10 ml of milk). While 0.04 ml of Cyanocobalamine was given to another group, third group serves as control. The results showed decrease in the time taken to reach the platform in stationary rod by both drugs, while exploratory activities were also found increased in head dip, cage crossing and open field activities. It is concluded from our findings that khamira-e-gao zaban amberi jawahir dar and cyanocobalamine posses marked effect on behavior as well as it also possesses memory enhancing effect.

Key words: Cyanocobalamine (vitaminB_{12}), Khamira-e-Gao Zaban Amberi Jawahir Dar, memory, behavior.

INTRODUCTION

Pakistan has a very rich tradition in the use of medicinal plants for the treatment of various ailments. Global sales of herbal products now exceed a staggering US$40 billion a year [1]. Pakistan has the history of using the unani medicines [2]. This traditional medicine sector has become an important source of health care, especially in urban and ethnic areas of the country [3]. Most of the medicinal plants are found in the temperate climates and subtropical forests of northern Pakistan [4]. The Unani system of medicine is matchless in treating chronic diseases like inflammation of bones, respiratory disorders, brain dysfunctioning, heart diseases, and gastrointestinal disorders, urinary tract infections, and genital diseases. Usually in Unani medicine the 'Khamira', a semi-solid preparation, which is traditionally used for cardiac ailments, such as, arrythmias, cardiac dysfunctioning, and so on. Khamiras are also used as general tonics for other vital organs like the brain, kidney and liver. Due to increase in the incidence of heart diseases, there is a need of a detailed investigations that to be done to evaluate the effects of Khamira is of special significance [5]. The various active ingredients used in these Khamiras have beneficial effect on heart. In most developed countries myocardial infaction (MI) remain the leading cause of death[6]. Vitamin B_{12} also has an important role in cognition and it has been reported by [7] that low levels of vitamin B_{12} are associated with cognitive decline in Japanese adults. Raised homocysteine and low folate and vitamin B_{12} concentration predict cognitive decline in community developing older Japanese adults [7].

MATERIALS AND METHODS

These drugs were selected because of their excessive usage by the population for their reported effects on memory and behavior. Cytacon liquid contains Cyanocobalamin (vitamin B_{12}), while khamera-e-gao zaban contains multiple constituents as mentioned below: Agate green triturated (quartz) , Ambra grasea , Bombax mori , Centaurea behen , Cheirantus cheiri(yellow variety), Coiandrum sativum, Emerald triturated, Lallimantia royleana, Lavandula stoeches Nepata hindustana, Onosma bracteatum, Pearls triturated, Ruby triturated, Saliva haematodes, Santalum

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album (saw dust), Silicates of magnesia and iron, Silver foils (Argentum), Sesame, Excepients

Selection of animals: The study protocol was designed to test the drugs for their CNS effect. For CNS screening 30 mice weighing 20–25 gm bred at animal house of Department of Pharmacology, University of Karachi were used. Constant environmental conditions were maintained at 25 ± 2ºC temperature with 12/12 hours light and dark cycle. Standard animal diet was given to all animals prepared in the laboratory and water ad libitum for 30 days. Two animals were housed per cage at least a week before the start of experiments, for accomadation with the environment. The mice were kept in the experimental room 12 hours before test for familiarization. This research was approved by the ethical committee of the Board of Advanced Studies and Research University of Karachi vide Resol. No. 10 (12) dated: 23-04-2008 & 10-05-2008.

Dosing in mice: The dosing of both drugs i.e. Cyanocobalamin and khamira-e-gaozaban amberi jawahirdar were done daily in normal doses according to the body weight of animals. Equivalent amount of water and as that of respective doses of the drugs were administered orally. Animals were divided in three groups,

Group one= control (0.1 ml water)
Groups two= Cyanocobalamine (Cytacon) (0.04 ml without dilution) and
Group three= khamira-e-gao zaban amberi jawahirdar (0.83 mg dissolved in milk) respectively.

Body weight of the animals was measured weekly. Dosing was done for 30 days. After 30 days the mice were sacrificed and their brain were kept frozen for examination.

Test for CNS screening: For CNS screening different parameters like effect on memory, depression, gross behavior, exploratory activity, etc were determined. Present investigation was intended to examine the Nootropic effect of two selected drugs.

Behavioral studies: Following parameters were observed during dosing period:[8]
Head immersion test
Stationary rod activity
Cage activity (movement in an enviornment)
Open field activity (locomotor activity)
Forced swim test(anti depressant activity)
Gross behavior test (mood, alertness, CNS excitation, muscle tone, reflexes, autonomic reflexes).

Open field activity: The locomotor activity was assessed by open field test [9]. The apparatus consist of an open box having 16 squares of 18 x 18 cm each on the floor. Individual mouse was placed in the corner and the squares entered with all four paws and numbers of rearings were recorded for 10 minutes.

Head immersion test: Head immersion test[10] was used with some modification[11], which is used for the assessment of mice learning ability. The apparatus is made up of a rectangular wooden box with four equal distance holes each side of the wall. The animal was placed in the center of the box and the number of times the animal poked in the holes during 10 min of observation was recorded.

Forced swim test (FST): The forced swim test was described by [12,13] as a model for testing antidepressant activity. Mice were restrained in swim tank from which they cannot escape and forced to swim, till the mice acquired a characteristic behavior of immobility and assume a floating posture. This activity reflected a state of helplessness. The mice struggling time was recorded till the mice became immobile.

Cage activity: The mice were introduced in the cages designed as per protocol [8] used to observe the activity in recognizable surroundings. Mice were placed individually in these cages to get them acclimatized with the environment and cage crossings were counted for 10 minutes.

Stationary rod activity: The mice were trained daily to balance and walk on rod before the test day. The apparatus consist of horizontal wires. For each mouse time was recorded during which mice maintain balanced and then fall from the stationary rod. [14]

Gross Behavior test: For gross behavioral analysis (such as awareness, mood, CNS excitation, posture, muscle tone, reflexes and autonomic reflexes) mice were observed daily.

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 $P<0.001$ as highly significant. All statistical procedures were performed according to the method [15].

RESULTS

Table 1 shows that Two sample t-test shows that the head dip activity was increased non-significantly on day 20 in khamira-e-gao zaban amberi jawahirdar. But this activity was comparatively increased slightly lesser on day30 than day 20 which was also non-significant. Cynocobalamin increased the activity significantly on day 20 ($p<0.05$) but non-significantly decreased on day 30. Table 2 shows that the stationary rod activity was decreased non-significantly on day 20 in khamira-e-gao zaban amberi jawahirdar treated mice. But this activity was comparatively decreased more significantly on day30 ($p<0.001$). Cynocobalamin also reduced the activity time significantly on day 20 ($p<0.001$) and on day 30 ($p<0.001$). Table 3 shows that the cage crossing activity was increased significantly on day 20 in khamira-e-gao zaban amberi jawahirdar treated mice. ($p<0.001$) The cage crossing activity was increased more significantly on day 30 ($p<0.001$) as well. Cynocobalamin increased the activity on day 20($p<0.001$) and day 30 ($p<0.001$). Table 4 shows that Swimming induced depression activity of activity was decreased non-significantly on day 20 and on day 30 in khamira-e-gao zaban amberi jawahirdar treated mice, The activity was increased non-significantly by cynoacobalamin on day 20 but decreased on day 30. Table 5 shows that the open field activity was increased non-significantly on day 20 and on day 30 in khamira-e-gao zaban amberi jawahirdar treated mice as compared to the control. while cynocobalamin increased the activity on day 20 and day30 non-significantly. Table 6 shows that the body weight of mice was found decreased in both groups that is Cyanocobalamin and khamira e gao zaban amberi jawahirdar treated groups nonsignificantly as compared to control on day 30. Table 7 shows that brain weights of mice were increased non-significantly on day 30 in khamira-e-gao zaban amberi jawahirdar treated rabbits as compared to the control and cynocobalamin were increased non significantly after day 30 as compared to control. Table 8 shows gross behaviors of both groups taking khamira and cynocobalamin as compared to control.

DISCUSSION

The head dip activity is increased by khamira –e– gaozaban amberi jawahirdar, as it contains many triturates and it is indicated to be used for increasing the mental alertness. It removes brain weakness and acts as a tonic of brain. This action is supported by our findings that overall activity of the mice was increased indicating that khamera-e-gao zaban amberi jawahirdar increased the alertness and activity. It also contains iron and magnesium, both of which increase the level of O_2 delivery and thereby help in increasing overall activity. It also contains amber grasea which is reported to protect the vital organs from premature degeneration, possibly affecting the cellular integrity and improving it that is depicted here by observing the enhanced activity. The effect on head dip was observed on day 20. After day 30 khamira-e-gao zaban amberi jawahirdar it was increased non significantly but it was slightly decreased as compared to day 20. It shows that khamera-e-gao zaban amberi jawahirdar has prolonged effects.

The head dip activity was enhanced by Cynocobalamin indicating that it has increased alertness and the animal has increased activity significantly at day 20. This finding is supported by [16], where the geriatric dementia may be related to the decreased levels of vitamin B_{12}, and also indicated that the cognitive functions are related to vitamin B_{12} availability. This suggest that the activity in our observation is supported and because of increased levels of vitamin B_{12}, there is overall increased head dip activity. 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 as these neurotransmitters are reduced that are involved in regulation of mood. Here in our observation the mood is elevated and the animal tries to explore and shows enhanced activity. After day 30 the activity decreased non significantly showing that the environment is not novel now for the animal. The reduction in hole poking could be associated to increased learning, as animals learn and do not poke through the holes [17].

The effect of khamira e gao zaban amberi jawahirdar and Cyanocobalamine on stationary rod activity was also observed. The stationary rod activity test is used to assess the effects of drugs on memory and learning. After the administration of Cynocobalamin 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 as was indicated in head dip analysis and due to these neurotransmitters especially acetylcholine, there is a marked increase in learning and memory after cynocobalamin. The effect of Khamira-e-gaozaban amberi jawahirdar on learning and memory was also significant as the animal reached the other side in less time. This effect is due to the quartz and pearls which is present in khamira, and have been reported by [18] to act as a nerve tonic. This

supports our observations that it enhanced the memory. Also, argentums preparations are reported to be tonic and khamira gao zaban also contains argentums, and finally because of amber grasea the cellular integrity is increased and memory is improved.

The effect of drugs on swimming induced depression was very pronounced. Cynocobalamin after twenty days of treatment increased the struggling time slightly as Cynocobalamin 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} tries to improve muscular activity but not elevates the mood and struggling time is decreased. The effect of Khamira-e-gao zaban amberi jawahirdar on swimming induced depression is also significant and it reduced the struggling time. This is due to argentums and other ingredients which may reduce the CNS activity and can act as sedative [18]. Due to tonic ingredients of khamira-e-gao zaban amberi jawahirdar the swimming struggling time is not reduced significantly, and after 30 days it was same as that of cytacon. The ingredients present in khamira -e-gao zaban such as Lallementia Royleana is also reported to be sedative and calming in action, which could further explain decreased struggling time.

The cage crossings were significantly increased by Cynocobalamin, as is evident that overall oxygen delivery and mental alertness is increased and hence the locomotor activity is increased. This observation also supports our previous findings which indicated that Cynocobalamin enhance exploratory and locomotor activities after day20 or even on day 30s and is a nervine tonic. The cage crossing activity was increased by khamira-e-gao zaban amberi jawahirdar after day 20 and even on day 30 with slight decrease. This supports our previous observations that many ingredients of khamira are acting as tonics and due to increased Iron content, possibly the oxygen saturation is complete and the animal enhances the overall performance as well as the locomotor activity [18]. However, on day 30 the activity was slightly reduced possibly due to the sedative effects of some ingredients which become prominent after prolong dosing. The open field activity was also increased by Cyanocobalamin and khamira-e-gao zaban amberi jawahirdar after 20 days as well as after day 30. Cyanocobalamin increased the activity by increasing oxygen delivery and mental alertness is increased and hence the locomotor activity is increased. This observation also supports our previous findings which indicated that Cynocobalamin $_{\text{enhance}}$ exploratory and locomotor activities after day20 or even on day30 as discussed earlier. Khamira e gao zaban amberi jawahir dar after day20 showed increase in open field activity which was decreased slightly on day 30. Reason for this finding is same as discussed earlier. i.e.as the ingredients here act as nervine and tonic reported earlier [18]. So overall motor and locomotor activity was enhanced. However, on day 30 the activity was slightly reduced possibly due to the sedative effects of some ingredients which become prominent after prolonged dosing.

CONCLUSION

In the end of study, it is concluded that cyanocobalamin is still the mainstay for the enhancement of CNS activities and has a memory enhancing activity. The khamira –e-gao zaban amberi jawahirdar also posses benefit for mental activity as well as locomotion also.

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Table – 1: Effect of drugs on Head dip activity in mice

Drugs	Days	
	20 Days	30 Days
Control	22.1±12.1	25.9±5.65
Khamera-e-gao zaban amberi jawahirdar	31.7±10.2(NS)	29.4±15.4(NS)
Cyanocobalamin	32.5±7.69*	23.9±10.4(NS)

Values are mean ± S.D (n=10)

Values are mean ± S.D. (n=8). Significant differences by Student significance Two Sample t-test *p<0.05, **p<0.01 and *** p<0.001 as compared to control mice, following two sample t-test. NS=NON SIGNIFICANT.

Table – 2: Effect of drugs on stationary rod activity in mice

Drugs	Days	
	20 Days	30 Days
Control	106±47.90	103.6 ± 40
Khamera-e-gao zaban amberi jawahirdar	39±10.1(NS)	29.4 ± 15.4***
Cyanocobalamin	9.0±6.51***	23.9 ± 10.4***

Values are mean ± S.D. (n=10). Significant differences by Student significance Two Sample t-test *p<0.05, **p<0.01 and *** p<0.001 as compared to control mice, following two sample t-test.
NS=NON SIGNIFICANT.

Table – 3: Effect of drugs on cage crossing activity in mice

Drugs	Days	
	20 Days	30 Days
Control	42.1 ± 16.3	41.7 ± 13.5
Khamera-e-gao zaban amberi jawahirdar	118 ± 20.9***	104.9 ± 10.7***
Cyanocobalamin	111.6 ± 20.1***	113.5 ± 35.5***

Values are mean ± S.D. (n=10). Significant differences by Student significance Two Sample t-test *p<0.05, **p<0.01 and *** p<0.001 as compared to control mice, following two sample t-test.
NS=NON SIGNIFICANT.

Table – 4: Effect of drugs on Swimming induced depression in mice

Drugs	Days	
	20 Days	30 Days
Control	107.8 ± 46.9	107.6 ± 44.9
Khamera-e-gao zaban amberi jawahirdar	87.6 ± 39.3(NS)	82.4 ± 27.8(NS)
Cyanocobalamin	113 ± 48.5(NS)	85 ± 19.1(NS)

Values are mean ± S.D. (n=10). Significant differences by Student significance Two Sample t-test *p<0.05, **p<0.01 and *** p<0.001 as compared to control mice, following two sample t-test.
NS=NON SIGNIFICANT.

Table – 5: Effect of drugs on open field activity in mice

Drugs	Days	
	20 Days	30 Days
Control	160.5±63.7	162.2±47.5
Khamera-e-gao zaban amberi jawahirdar	191.5±43.6(NS)	141.8±31.4(NS)
Cyanocobalamin	193.1±38.6(NS)	193.4±27.9(NS)

Values are mean ± S.D. (n=10). Significant differences by Student significance Two Sample t-test *p<0.05, **p<0.01 and *** p<0.001 as compared to control mice, following two sample t-test. NS= Non significant.

Table -6: Effect of drug on body weight of mice on day 30

Drugs	30 Days
Control	24±3.06
Khamera-e-gao zaban amberi jawahirdar	22.86±2.79(NS)
Cyanocobalamin	21.14±1.57(NS)

Values are mean ± S.D. (n=10). Significant differences by Student significance Two Sample t-test *p<0.05, **p<0.01 and *** p<0.001 as compared to control mice, following two sample t-test. NS=non significant

Table -7: Effect of drugs on brain weight of mice on day 30

Drugs	30 Days
Control	0.2538± 0.016
Khamera-e-gao zaban amberi jawahirdar	0.3548 ±0.043(NS)
Cyanocobalamin	0.3175 ±0.022(NS)

Values are mean ± S.D. (n=10). Significant differences by Student significance Two Sample t-test *p<0.05, **p<0.01 and *** p<0.001 as compared to control mice, following two sample t-test NS=non significant.

Table-8: Gross behavior chart

Parameters	Control	Drugs			
		Khamera-e-gao zaban amberi jawahir dar		Cynocobalamin	
		20 Days	30 Days	20 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	+++	+++	+++	+++	+++

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