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# Behavioral and Memory enhancing effects of Memory Enhancing Milk Toffee Developed by Incorporation of O. bracteatum Extract

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## Abstract

*Product development, is the life-blood of companies and societies. The present study was designed to explore the behavioral and memory enhancing effects of the first ever nutraceutical (memory enhancing) milk toffee produced by incorporating of Eastern Medicine O. bracteatum. The results were compared with the efficacy of Eastern khamira-e-gaozaban amberi. Experimental animal mice administered with milk toffee and ware gone through various CNS parameters to check the effects. The effects of both were recorded on day 20 and 30. To the first group dose of milk toffee was 0.05gm without dilution and 0.83 mg dissolved in milk was administered to second group, and the third one was control. A distinct decrease in time in the stationary rod test was observed by both newly prepared milk toffee as well as khamira, while exploratory activities such as cage crossing, open field and head dip activities were found to be increased in the test animal. The newly produced milk toffee possesses memory enhancing effect and improved behavior effects*

**Keywords:** memory, milk toffee, behavior, *O. bracteatum*

## Introduction

Eastern herbal (Unani) medicines are a traditional way and considered as an important source of treatment of various diseases in Pakistan (Hussain, Saeed *et al.* 2006, Organization 2001). These herbal medicines have successfully been used to treat respiratory diseases, liver dysfunction, gastrointestinal and urinary tract infections, genital diseases, cardiovascular diseases and even for brain dysfunction (Ahmad, Rehman *et al.* 2010). *Onsomabracteatum (gaozaban)*, belongs to the family *Boraginaceae. O. bracteatum* is a key ingredient in many Eastern medicines and Ayurvedic forms. It contains lipids, carbohydrates, alkaloids, phenolic compound, aliphatic ketones, naphthazarines and naphtaquinones. The dried leaves and flowers of the wall of *Obracteatum* forms a medicine, which is used as a tonic, rejuvenating, laxative, diuretic and provide a soothing effect in the internal part of organs of the digestive tract. It helps as a spasmolytic (Kumar, Kumar *et al.* 2013). *O. bracteatum* has been proven to produce positive impact on enhancing memory, mental alertness and behavior (Anser, Najam *et al.* 2014)]. *O. bracteatum* contains pharmacologically active substances with antidiarrhoeal and antimicrobial properties (Gautam, Bithel et al. 2017). *O. bracteatum* appeared to have magnificent antioxidant properties and is reported to have anti-anxiety and depression reducing impact (Asif, Hayee et al. 2019). *O. bracteatum* is found useful in asthma, bronchitis, heart diseases, diuretic, alterative, syphilis as well as leprosy (Kirtikar and Basu 1975). Due to its memory enhancing effects, afirst ever memory enhancing milk toffee was produced by incorporating of Eastern Medicine *O. bracteatum*in the Department of Food Science and Technology, Jinnah University for Women (Raza 2021).

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The present study was designed to explorethe behavioral and memory enhancing effects of first ever produced memory enhancing milk toffee by incorporation of *O. Bracteatum* extract and the results were compared with the khamira-e gaozaban amberi. Different CNS parameters were performed on experimental animal mice.

**Materials and Methods**

The khamira-e gaozaban amberi was purchased from an herbal store located at Nazimabad, Karachi.

**Selection of animals and environment**

CNS screening of milk toffee was carried out on 30 mice weighing 20–25 gm were used. 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 accommodation with the environment. The mice were kept in the experimental room 12 hours before test for familiarization.

**Dosing in mice**

The daily dose both milk toffee and khamira was based on body weight of mice. Equivalent amount of water and as that of respective doses were administered orally. The study design was to devide the mice in three groups,1) control fed with 0.1 ml water 2)fed with milk toffee (0.05gm without dilution) and3)fed with khamira-e-gaozaban amberi (0.80 mg dissolved in milk) respectively for 30 days. Measurement of the body weight of the experimental animal was carried out every week.

**Test for CNS screening**

For CNS screening in present investigation the Nootropic effect of two selected products was examined.

**Behavioral studies:**

The locomotor activity was assessed by open field test (Saillenfait and Vannier 1988). Head immersion test with some modifications (Devi 2001) was used for the assessment of mice learning ability. The antidepressant activity was analyzed by the forced swim test (Porsolt, Le Pichon *et al.* 1977, Porsolt, Anton *et al.* 1978). The mice were introduced in the cages designed as per protocol (Najam and Anser 2011) used to observe the activity in recognizable surroundings. The mice were trained daily to balance and walk on rod before the test day and time of stationary rod test was recorded for each mouse (Najam 2003). Gross behavioral analysis of mice was carried out daily (Najam and Anser 2011).

**Statistical analysis**

All behavioral tests were repeated 10 times and standard deviation was estimated. Statistical software SPSS version 16.0 was used for the statistical analysis (Alcaraz, Jimenez *et al.* 1989).

**Results and discussion**

Table 1 shows that two sample t-test shows that the head dip activity was not increased significantly on day 20 in khamira-e-gaozaban amberi. But this activity was comparatively increased slightly lesser on day 30 than day 20 which was also non-significant. In milk toffee increased the activity

significantly on day 20 but non-significantly decreased on day 30. The head dip activity is increased by milk toffee indicating memory enhancing milk toffee 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 memory enhancing toffee has increased the alertness and activity and also help in increasing overall activity. The effect on head dip was observed on day 20. After day 30 it was increased non significantly but it was slightly decreased as compared to day 20. It shows that the memory enhancing toffee has prolonged effects. These findingsare supported by the results reported earlier (Alcaraz, Jimenez *et al.* 1989). This suggest that the activity in the observation of current study is supported as there is overall increased head dip activity was observed.

**Table 1:** Head dip activity Examination

| Group | 20 Days | 30 Days |
|---|---|---|
| | **No ± SD** | **No ± SD** |
| `Control Group | 23.2±12.1 | 26.3±5.65 |
| Khamera-e-gaozaban amberi | 31.7±10.2(NS) | 29.4±15.4(NS) |
| Memory enhancing milk toffee | 32.5±7.69 | 23.9±10.4(NS) |

NS=Non Significant.

**Table 2:** Stationary rod activity Examination

| Group | 20 Days | 30 Days |
|---|---|---|
| | **No ± SD** | **No ± SD** |
| ControlGroup | 105.9±47.90 | 104 ± 40 |
| Khamera-e-gaozaban amberi | 39±10.1(NS) | 29.4 ± 15.4 |
| Memory enhancing milk toffee | 9.0±6.51 | 23.9 ± 10.4 |

NS=Non Significant.

Table 2 shows that the stationary rod activity was decreased non-significantly on day 20 in khamira-e-gaozaban amberi treated mice. But this activity was comparatively decreased more significantly on day30. The memory enhancing milk toffee reduced the activity time significantly on day 20 and on day 30. The stationary rod activity test is used to assess the effects of drugs on memory and learning. Mice fed with milk toffee took nine second to reach the other side of the rod indicates that the newly prepared milk toffee has produce positive effects on improving memory and enhancing learning of the mice. This effect, there is a marked increase in learning and enhancing memory after milk toffee administration was observed.

**Table 3:** Cage Crossing Activity Examination

| Group | 20 Days | 30 Days |
|---|---|---|
| | **No ± SD** | **No ± SD** |
| Control Group | 41.9 ± 16.3 | 40.9 ± 13.5 |
| Khamera-e-gaozaban amberi | 108 ± 20.9 | 109.9 ± 10.7 |
| Memory enhancing milk toffee | 111.6 ± 20.1 | 113.5 ± 35.5 |

Table 3 mice fed with khamira-e-gaozaban amberi has shown increase in the cage crossing activity on day 20. The cage crossing activity was increased more significantly on day 30 as well. The memory enhancing toffee increased the activity on day 20 and day 30. The cage crossingwas significantly increased by

newly prepared milk toffee, which shows increase in locomotor activity and mental alertness. The cage crossing activity was increased by khamira-e-gaozaban amberi after day 20 and even on day 30 with slight decrease. Many of the 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 (Aslam 2006). However, on day 30 the activity was slightly reduced possibly due to the sedative effects of some ingredients which become prominent after prolong dosing.

**Table 4:** Effect of drugs on open field activity in mice

| Group | 20 Days | 30 Days |
|---|---|---|
| | **No ± SD** | **No ± SD** |
| Control Group | 159.9±63.7 | 162.5±47.5 |
| Khamera-e-gaozaban amberi | 191.5±43.6(NS) | 141.8±31.4(NS) |
| Memory enhancing milk toffee | 193.1±38.6(NS) | 193.4±27.9(NS) |

NS=Non Significant.

Table 4 shows that the open field activity was increased non-significantly on day 20 and on day 30 in khamira-egaozaban amberi treated mice as compared to the control. while milk toffee increased the activity on day 20 and day30 non-significantly. The open field activity was also increased by newly prepared milk toffee and khamira-e-gaozaban amberi after 20 days as well as after day 30. These results indicate that overall motor and locomotor activity was enhanced by the newly produced memory enhancing milk toffee.

**Table 5:** Gross behavior chart

| Parameters | Control | Drugs | | | |
|---|---|---|---|---|---|
| | | **Khamera-e-gaozaban amberi** | | **Memory enhancing milk toffee** | |
| | | **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/Exopthalamus) | - | - | - | - | - |
| Salivation | - | - | - | - | - |
| Lacrimation | - | - | - | - | - |
| Defecation | +++ | +++ | +++ | +++ | +++ |
| Urination | +++ | +++ | +++ | +++ | +++ |

**+++: appreciable improvement, ++: moderate improvement, +: trace improvement, - :not observed**

Table 5 shows gross behaviors of both groups taking khamira and milk toffee as compared to control.

**Table 6:** Swimming Induced Depression Examination

| Group | 20 Days | 30 Days |
|---|---|---|
| | **No ± SD** | **No ± SD** |
| Control Group | 107.8 ± 46.9 | 107.6 ± 44.9 |
| Khamera-e-gaozaban amberi | 87.6 ± 39.3(NS) | 82.4 ± 27.8(NS) |
| Memory enhancing milk toffee | 113 ± 48.5(NS) | 85 ± 19.1(NS) |

NS=Non Significant.

Results of swimming induced depression are summarized in Table-6. The mice fed with khamira-e-gaozaban amberi have shown a non-significant decreasing pattern for the swimming induced depression activity from day 20 to day 30. The activity was decreased non-significantly by memory enhancing milk toffee from day 20 to day 30. The effect of milk toffee on swimming induced depression was very pronounced, shows improvement in the muscular activity.

## Conclusion

In the end of study, it is concluded that newly prepared memory enhancing toffee has enhancement of CNS activities; has a memory enhancing activity but also possess benefits for mental activity as well as locomotion also.

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