Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231– 6345 (Online)
An Open Access, Online International Journal Available at www.cibtech.org/sp.ed/jls/2014/04/jls.htm
2014 Vol. 4 (S4), pp. 2215-2221/Behzad et al.
Research Article

INVESTIGATION OF THE EFFECT OF GUM OLIBANUM ON LEARNING AND SHORT-TERM MEMORY

[*]Adele Matini Behzad[1], Homa Hajisadeghi[2] and Ali Mirarab[3]
[1]*Department of Animal Science, Saveh Branch, Islamic Azad University, Saveh, Iran*
[2]*Department of Physical Education & Sport Sciences, Islamic Azad University, Taft Branch, Iran*
[3] *Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran*
**Author for Correspondence*

ABSTRACT
Learning and memory are considered as highest functional levels of the central nervous system. Thus, the problems associated with these processes had been noteworthy for the researchers and to date, widely studies has been designed in this field. The aim of this study is to Investigation of the effect of Gum olibanum extract on learning and memory of rats. The study was a Lab Trial type which conducted on 20 adult male Wistar rats weighing 250 g. The study was conducted using boxing Shuttle device. The rats which randomly divided into 4 groups (including one control group and three experimental groups) were analyzed. Data were analyzed in SPSS ver.19.0 by using ANOVA and Scheffe tests. Within three days, the mean score of the learning was increased from 67.9±7.7 in control group to 80.7±8.8 in group with dosage of 400 mg/kg (p=0.022). Also the mean score of the memory was increased from 50.2±11.5 in control group to 84.5±10.6 in group with dosage of 400 mg/kg (p=0.022). The results revealed that, Gum olibanum extract effectively increases the short-term memory and learning. It seems that further studies with larger sample sizes and including parameters such as long-term memory, could be effective in determining the impact of Gum olibanum extract on learning and memory.

Keywords: *Central Nervous System, Learning, Memory, Gum Olibanum*

INTRODUCTION
Learning is a neural process whereby organisms through practice, change their behavior while the memory is attributed to the process of knowledge storage (Berne and Levy, 1993). So learning and memory are considered as one of the highest functional levels of the central nervous system.
Nowadays the importance of learning and memory disorders which involved the humanity in problems such as Alzheimer, is not hidden to anyone;
As the National Health Research Center in the US in 2000, expressed concern about the prevalence of this phenomenon, in his reports and has predicted that by 2030, only in the US, 5.8 million people will be affected by this disease and its complications (National Institute of Aging, National Institutes of Health (USA), 2003).
Humankind has long recognized the value of medicinal plants and was used the plants to treatment of any disease and pain. One of these medicinal plants is Gum olibanum which was used as medicine from ancient times.
Therefore, various studies relying on herbal medicine has increased considerably in this area. Investigation of the effect of caffeine (Joshi and Parle, 2005), piperin (Dhingra *et al.*, 2004), glycyrrhiza glabra (Abe and Saito, 2000), Crocus sativus (Parle *et al.*, 2004), Myristica fragrans (Al-Zuhair *et al.*, 1996), Cardamol (Rao *et al.*, 2005), Centella asiatica (Thakur and Mengi, 2005), Eclipta alba (Nickavar *et al.*, 2003), Nigella sativa (Joshi and Parle, 2006), Ocimum sanctum (Nwosu, 1999), and finally Gum olibanum, on learning and memory were designed in experimental models to achieve this end. Gum olibanum is a resin gum which obtained from some species of Gum olibanum. The plants of this genus are shrubs form and included in *Burseraceae* family.
This family includes 24 spices and the most common species which are the sources of Gum olibanum, are *B.carteri*, *B. Frereana*, *B. Serrata* and *B. Papyrifera*.

Gum olibanum in the Iranian market, is obtained from two spices; the first one is *B. carteri* that is native of the Red Sea beaches especially in the northeast of Africa, and Somalia and Ethiopia are the main producers of that. The second one is *B. Serrata* which grows in various parts of India. Humankinds have been recognized this resin from ancient times and used that as incense. It has been used in ancient Iran for incense and disinfection. Also in Avesta (Mahajan *et al.,* 1995), it is mentioned as an effective drug for the treatment of various diseases including cancer, nausea, diarrhea and memory enhancing drug. Studies on the Gum olibanum represents significant pharmacological effects of this resin on a wide range of diseases associated with brain disorders such as amnesia, insomnia, insanity and rheumatic fever (Duke, 2001).

Chemical compounds and elements of the Gum olibanum are included, insoluble in alcohol Sorin and Arabin (Alaei *et al.,* 1999) and free Olibaneurzon and α and β essence of penin, Hudolin, Borneol, cariophilen, simen and karon (Nwosu, 1999).

Researches has been done in the way of learning by conditioned stimuli, revealed that; Gum olibanum extracts enhances learning speed and memory in rats and also its use in pregnancy and lactation period, enhances learning and memory in the next generation rats (Krohn *et al.,* 2001). It seems that this process is because of increasing the number of dendritic spines, synaptic branches and neuronal synapses (Shailesh *et al*., 2007).

MATERIALS AND METHODS

Current study is a Lab Trial type research which conducted on 20 adult male wistar rats with 250 g weight were divided into four groups and each group consisted of 5 rats. 15 min before experiment, the control group has been injected by distilled water and solvent and three other groups has been injected intra peritoneal by doses of 100, 200, 300, and 400 mg of Gum olibanum extracts per kg of body weight. The experiments were conducted on four groups of animals in order to assessing the short-term memory and learning.

Extraction

For preparing aqueous-alcoholic extract, 100g of Gum olibanum has been exposed to 330 ml of ethanol (300 ml, 70% ethanol and 30 ml, 80% ethanol). Then, after two days, the solution was passed through a filter paper and the filtrate evaporated at 27 °C and purified extract of Gum olibanum was obtained. To preparing different doses of this dried extract, we need to solvent; because the dried powder (which obtained from extract), is not soluble in the water. For this purpose, the organic solvent DMSO (dimethyl sulfoxide) was choice as a solvent with least side effects on dried extracts of Gum olibanum.

Animals kept at room temperature of about 21±1° C and humidity of 55±10 % and also the rats placed in the same conditions of light and food and exposed to the period of 12 hour light (07:00 to 19:00 o'clock). For all experimental groups 15 minutes before the start of the experiment, the solution was injected intraperitoneally. A day before the exam, in the stage called primary education, the animals become familiar with the methods and tools and is without injection and grouping. The main experiment is consisted of short-term memory and learning process. The shuttle box is used to understanding and evaluating learning and memory in animals such as mice or rats. In which light is used as a conditioning stimulus. Also electrical stimulation plays the role of unconditioned stimulus in this box. In current study, the rats were totally relaxed and quietly entered the shuttle box. Then the rats were given 15 minutes to reach full compliance with conditions and away from the stress have been readied to start the experiment. Then the animal was placed in the light chamber of the shuttle box and after closing the entrance door of the shuttle box, the light was turned on by switch. The guillotine door in the wall went up and after turning on the light, the animals were allowed to leave the light chamber in 10 minutes and went to the dark compartment. After 10 seconds, if the animal did not do it, electric shock (unconditional stimulus) was performed and the animal was forced to leave the light chamber. The duration of electrical shock was 1500 microseconds and intensity of the 200-volt. Upon entrance to the dark compartment guillotine door was closed and the resting phase between 5 and 40 seconds was started. Animals were driven back to light chamber and the process was repeated again. Conditioned response was defined as avoidance of the

shock for 10 seconds in the presence of light (conditioned stimulus). Response time was considered since the beginning of the conditioned stimulus (light) to escape the animal toward the dark chamber. If the response time is less than 10 seconds, it was considered as Conditioned response and this meant that the animals are responded to conditioned stimulus (light). If the response time is much than 10 seconds, it's considered as unconditioned response and this meant that the animals is not responded to conditioned stimulus (light) and only applying an unconditioned stimulus (electric shock) forced it to responded. Finally, after completion of the test, rats were monitored for a week to observation any possible patience behavior. Data were analyzed in SPSS (19 ver.) by using ANOVA and Scheffe tests.

RESULTS AND DISCUSSION

Results

The least score of learning in control group was 54.8 and maximum of it was 70.2 and mean score of control group was 67.9±7.7.

- In the first experimental group (dose of 100 mg/kg), the maximum score of learning was 76.6 and the minimum of it was 65.5 and the mean score of this group was 73±12.4.
- In the second experimental group (dose of 200 mg/kg), the maximum score of learning was 82.8 and the minimum of it was 70.4 and the mean score of this group was 76.1±15.6.
- In the third experimental group (dose of 400 mg/kg), the maximum score of learning was 87.2 and the minimum of it was 73.1 and the mean score of this group was 80.7±8.8 (table 1).

Scheffe method was used to binary comparison and it was observed that there's a significant difference between groups receiving different doses of Gum olibanum compared to the control group ($p>0.05$).

Table 1: Average score of Learn (0-100 points) within three days *

Group	Number of samples	maximum	minimum	mean±SD
Control	5	70.2	54.8	67.9±7.7
Dose (100 mg/kg)	5	76.6	65.5	73±12.4
Dose (200 mg/kg)	5	82.8	70.4	76.1±15.6
Dose (400 mg/kg)	5	87.2	73.1	80.7±8.8
total	20	86.6	34.4	80.7±8.8

**p=0.022*

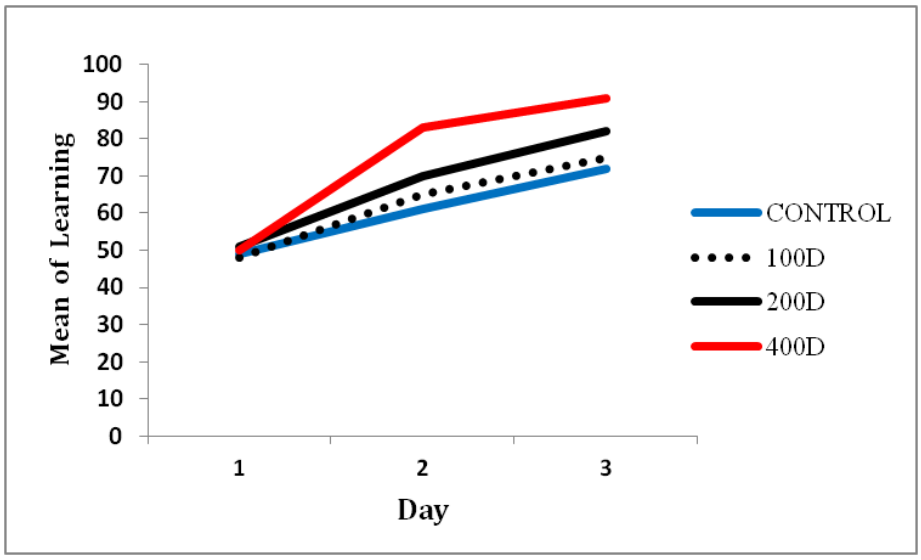

Figure 1: Comparison of different dosages of Gum olibanum on learning

The results test showed that level of learning had a significant increase in rats that received Gum olibanum rather than the control group and also it has been increased by dose increment.

Furthermore, the findings were also observed for short-term memory, revealed that:
The least score of short-term memory in control group was 26.6 and maximum of it was 73.2 and mean score of control group was 50.2±11.5.

- In the first experimental group (dose of 100 mg/kg), the maximum score of short-term memory was 86.5 and the minimum of it was 43.2 and the mean score of this group was 67.2±15.8.
- In the second experimental group (dose of 200 mg/kg), the maximum score of short-term memory was 90.5 and the minimum of it was 49.9 and the mean score of this group was 72.5±14.7.
- In the third experimental group (dose of 400 mg/kg), the maximum score of short-term memory was 99.9 and the minimum of it was 73.2 and the mean score of this group was 84.5±10.6 (table 2).

These differences were analyzed by analysis of variance (ANOVA) and significant differences were observed (p=0.002). It means that the different doses of Gum olibanum have different effects on short-term memory of rats. Scheffe method was used to binary comparison and it was observed that there's a significant difference between groups receiving different doses of Gum olibanum compared to the control group (p>0.05).
The results revealed that Gum olibanum caused an increase in short-term memory and also increasing concentrations of Gum olibanum, leads to increment of short-term memory function.

Table 2: Average score of short-term memory (0-100 points) within three days ❁

Group	Number of samples	maximum	minimum	mean±SD
Control	5	73.2	26.6	50.2±11.5
Dose (100 mg/kg)	5	86.5	43.2	67.2±15.8
Dose (200 mg/kg)	5	90.5	49.9	72.5±14.7
Dose (400 mg/kg)	5	99.9	73.2	84.5±10.6
Total	20	99.9	26.6	68.4±19.7

❁P-Value =0/024

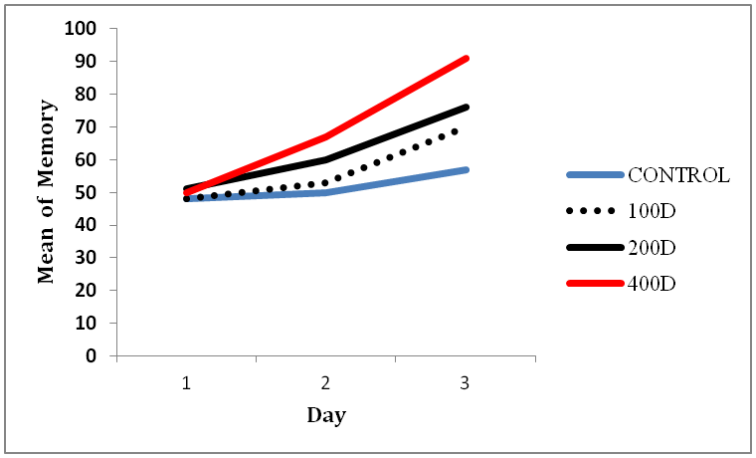

Figure 2: Comparison of different dosages of Gum olibanum on memory

Discussion and Conclusion

Gum olibanum have about 25% insoluble in alcohol gum, a resin, and a little essence (Poeckel and Werz, 2006). The insoluble in alcohol part is consist of arabin and bassorin and soluble in alcohol part is consist of a resin called Olibanoresene, a free acid called bosolic acid, bitter substance and essence (Safayhi *et al.*, 1992). In Gum olibanum essence, in addition of pinene, dipanthe, and Phellandrene, there's a type of alcohol called olibanol. Bosolic acid is 4 groups of penthacylic tritrepnoids and is the main constituent of Gum olibanum resin, which is free or combined with other materials (Vahidi and Dashti, 2002). Bosolic acid are the main effective materials of resin gum and also responsible for its therapeutic effects. Bosolic

acid are from specific and non-competitive inhibitor of 5-lipoxygenase enzyme which is key enzyme in biosynthesis of leukotrienes (Behnamrasuli *et al.*, 2001).

The current study, has been investigated the impact of Gum olibanum resin on learning and short-term memory by using an experimental assessing method. According to the results of current research, using Gum olibanum resin extract, leads to improvement of learning and memory function in adult male wistar rats. By increasing the Gum olibanum extract an ascending trend has been observed in the scores of learning and memory. totally, according to the findings, which is done with the animal model, it has been showed that, in terms of the mean scores of short-term memory, Gum olibanum has a significant effect in improving memory and learning, which is consistent with other studies. As short-term memory in rats, have been improved considerably, following the receipt of the plant species used to increase intelligence and memory. As an example; Vahidi *et al.,* (2002), have been reported the acceptable effects of Gum olibanum on short-term memory improvement (Vahidi and Dashti, 2002). Also Behnamrasooli (2001) has been showed that 500 mg/kg dose of Gum olibanum in critical periods of growth and development of nervous system of fetal and infant growth, leading to a significant increase in learning ability, especially to enhancement of the memory (Behnamrasuli *et al.*, 2001; Ugiura *et al.*, 1994; Sugiura *et al.*, 1995). Also studies of Sugiura during years of (1994-2000), has been showed beneficial effects of hydroalcholic extract of Saffron on short-term memory of rats (Duke, 2001). Studies have been shown that the cholinergic system, particularly acetylcholine plays an important role in learning and memory (Sugiura *et al.*, 2000). α-pinen is a compound which is in Gum olibanum (Buccafusco and Terry, 2000), has an inhibitory effect on AchE (Savelev *et al.*, 2003) that reinforced the cholinergic system in the brain. Probably Gum olibanum through this mechanism can increase the power of learning and memory. The studies have been showed that bosolic acid and partly COX-I of the Gum olibanum is considered as inhibitor (Siemoneit *et al.*, 2008). Cyclophosphamide oxygenize involved in the pathology of Alzheimer's disease, but the effect of these inhibitors in the treatment of this disease has little or no effect. Another hypothesis about the ways which Gum olibanum improve memory and learning is its impact on hepatic gluconeogenesis and lipid peroxidation. Gum olibanum extract has been protected liver against injury and toxicity induced by carbon tetrachloride paracetamol and thioacetamide in laboratory animals. This protective effect is most likely due to the inhibition of the activity of 5-lipoxygenase enzyme. Clinical studies on Gum olibanum indicated that it's extract reduces cholesterol and increase HDL levels It seems that the reduction of cholesterol, leading to increased membrane fluidity of brain cells in the hippocampus area and thus lead to improved memory performance (Bourre *et al.*, 1997). In addition, the ant diabetic properties of Gum olibanum have been proven. This effect is due to the effect on hepatic gluconeogenesis (Al-Awadi *et al.*, 1991; Kavitha *et al.*, 2007; Sharma *et al.*, 2007). According to other studies it's believed that; Insulin receptors in the brain that play an important role in cognitive functions such as memory and teach (Ozkan *et al.*, 2005), are impacted on this way. Using the resin extract in current study, showed favorable effects on memory. But judging about the role of this plant in increasing the performance of learning and memory required performing additional studies to be more reviewed.

ACKNOWLEDGMENT

The work reported here is funded by Alvand Institute and the authors would like to thank Dr. Alireza Asgaripour the head of Alvand Institute and Dr. Hamed Matini Behzad for useful comments in data analyzing.

REFERENCES

Abe K and Saito H (2000). Effects of saffron and its constituent crocin on learning behaviour and long-term potentiation. *Phytotherapy Research* **14** 149-52.

Alaei H, Moatar F, Toori L Alaei H, Moatar F and Toori L (1999). Effects of the abstract of Oliban on learning and memory. *Journal of Qazvin University of Medical Sciences* **11** 21 – 28.

Al-Awadi F, Fatania H and Shamte U (1991). The effect of a plants mixture extract on liver gluconeogenesis in streptozocin induced diabetic rats **18** 163 - 8.

Al-Zuhair H, el-Sayeh B, Ameen HA and al-Shoora H (1996). Pharmacological studies of cardamom oil in animals. *Pharmacological Research* **34** 79-82.

Behnamrasuli M, Hoseinzadeh H and Ghafarimoghadam G (2001). Empowering effect of Frankincense extract on memory. *Tarbiat Moalem University Journal of Science* 1 – 14.

Berne RM and Levy MN (1993). *Physiology*, 3rd edition (Mosby-Year Book) New York 260-78.

Bourre JM, Dumont OL, Clement ME and Durand GA (1997). Endogenous synthesis cannot compenstate for absence of dietary oleic acid hn rats. *Nutrition* 127488-493.

Buccafusco JJ and Terry AV Jr (2000). Multiple central nervous system targets for eliciting beneficial effects on memory and cognition. *Journal of Pharmacology and Experimental Therapeutics* **295** 438 - 46.

Dhingra D, Parle M and Kulkarni SK (2004). Memory enhancing activity of Glycyrrhiza glabra in mice. *Journal of Ethnopharmacology* **91** 361-65.

Duke JA (2001). *Handbook of Phytochemical Constituent of GRAS Herbs and other Economic Plants*, 1st edition (CRC Press) Washington 285 - 6.

Joshi H and Parle M (2005). Effect of pipeline on memory and behavior mediated via monoamine neurotransmitters. *Journal of Traditional and Complementary Medicine* **23** 39-43.

Joshi H and Parle M (2006). Evaluation of nootropic potential of Ocimum sanctum Linn, in mice. *Indian Journal of Experimental Biology* **44** 133-36.

Kavitha JV, Rosario JF, Chandran J, Anbu P and Bakkiyanathan (2007). Hypoglycemic and other related effects of Boswellia glabra in alloxaninduced diabetic rats. *Indian Journal of Physiology and Pharmacology* **51** 29 - 39.

Krohn K, Rao MS, Raman NV and Khalilullah M (2001). HPLC analysis of anti inflammatory triterpenoids from Boswellia serrata. *Phytochemical Analysis* **12** 374-6.

Mahajan B, Taneja SC, Sethi VK and Dhar KL (1995). Two triterpenoids from Boswellia serrata gum resin. *Journal of Phytochemistry* **39** 453 - 5.

National Institute of Aging, National Institutes of Health (USA) (2003). Progress report on Alzheimer's disease: taking the next steps. Washington, DC: National Institutes of Health 1154-56.

Nickavar B, Faraz M, Javidnia K and Roodgar Amoli M (2003). Chemical Composition of the Fixed and Volatile Oils of Nigella sativa L, from iran. *Zeitschrift für Naturforschung* **5**(8) 629-631.

Nwosu MO (1999). Herbs for mental disorders. *Fitoterapia* **70** 28 - 63.

Ozkan Y, Yilmaz O and Oztuk AI (2005). Effect of trioxidanT combinatian (Vitnami E,Vitamin C and alfa –lipoic acid)with isulin on lipid and cholstrol Level and fatty acid composition of brain tissue in experimental diabetic and non diabetic – rat. *Cell Biology International* **29** 754-60.

Parle M, Dhingra D and Kulkarni SK (2004). Improvement of mouse memory by Myristica fragrans seeds. *Journal of Medicinal Food* **7** 157-61.

Poeckel D and Werz O (2006). Boswellic acids: biological actions and molecular targets. *Current Medicinal Chemistry* **13** 3359-69.

Rao SB, Chetana M and Uma Devi P (2005). Centella asiatica treatment during postnatal period enhances learning andmemory in mice. *Physiology & Behavior* **86** 449-57.

Safayhi H, Mack T, Sabieraj J, Anazodo M, Subramanian LR and Ammon HPT (1992). Boswelli acids: novel, specific, nonredox inhibitors of 5- lipoxygenase. *Journal of Pharmacology and Experimental Therapeutics* **261** 1143-6.

Savelev S, Okello E, Perry NS, Wilkins RM and Perry EK (2003). Synergistic and antagonistic interactions of anticholinesterase terpenoids in Salvia lavandulaefolia essential oil. *Pharmacology Biochemistry and Behavior* **75** 661 - 8.

Shailesh AS, Ishwarsinh SR, Bhanubhai NS, Dharmesh AP, Vijay KP and Bharat KS *et al.*, (2007). Estimation of boswellic acids from market formulations of Boswellia serrata extract and 11-keto -

boswellic acid in human plasma by high performance thin-layer chromatography. *Journal of Chromatography B* **848** 232-8.

Sharma A, Mann AS, Gajbhiye V and Kharya MD (2007). *Phytochemical Profile of Boswellia Serrata: an Overview* **1** 1.

Siemoneit U, Hofmann B, Kather N, Lamkemeyer T, Madlung J, Franke L, Schneider G, Jauch J, Poeckel D and Werz O (2008). Identification and functional analysis of cyclooxygenase-1 as a molecular target of boswellic acids. *Biochemical Pharmacology* **15** 503 – 13.

Sugiura M, Shoyama Y, Satio H and Abe K (1995). Ethanol extract of Crocus sativus antagonizes the inhibitory action of ethanol on hippocompal long-term potation in vivo **9** 100-104.

Sugiura M, Shoyama Y, Satio H and Abe K (2000). Effects of saffron extract and its constituent crocin on learning behaviour and long-termpotentiation **14** 149-52.

Thakur VD and Mengi SA (2005). Neuropharmacological profile of Eclipta alba (Linn.) Hassk. *Journal of Ethnopharmacology* **102** 23-31.

Ugiura M, Shoyama Y, Saito H and Abe K (1994). Crocin (crocetin di-gentiobiose ester) prevents the inhibitory effect ofethanol on long-term potentiation in the dentate gyrus in vivo. *Journal of Pharmacology and Experimental Therapeutics* **271** 703-707.

Vahidi AR and Dashti MH (2002). Effect of caffeine on memory and learning of rat: a survey project. Yazd: Shahid Sadooghi University of Medical Sciences and Health Services.