OPEN ACCESS

Kashf Journal of Multidisciplinary Research
Vol: 02 - Issue 09 (2025)
P-ISSN: 3007-1992 E-ISSN: 3007-200X
https://kjmr.com.pk

EFFECT OF *VITIS VINIFERA* ON MARBLE BURYING BEHAVIOUR IN MICE

Muhammad Aslam[1], Nuzhat Sultana[2], Hammad Ahmed[3]

[1]Department of Pharmacology, Faculty of Pharmacy, University of Sindh, Jamshoro 76080, Pakistan.
[2]Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Karachi 75270, Pakistan.
[3]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Ziauddin University, Karachi 75600, Pakistan.

Corresponding Author: (maslam.solangi@usindh.edu.pk, Pharmacologist1@yahoo.com)
DOI:(*https://doi.org/10.71146/kjmr616*)

Article Info

Abstract

Background: Approximately 2% of people worldwide suffer from obsessive-compulsive disorder, a common mental health issue. Although obsessive-compulsive disorder was thought to be among the rarest mental disorders in previous decades, research conducted in recent years has shown that it is really one of the most prevalent mental disorders. The use of grapes in traditional medicine has been supported by several scientific investigations. In this study, we assessed the effect of *Vitis vinifera* on marble burying behaviour in mice

Material and method: Every mouse was placed in a cage with usual dimensions of (22 x 32 x 13.5 cm) containing a 5 cm layer of sawdust and 24 glass marbles (1.5 cm in diameter). The test compound, the vehicle and fluoxetine, the standard drug, were administered and the animals were kept in the cages for next 30 min. The number of marbles buried in the sawdust, during 30 min, was recorded for every single mouse.

Result: The results of the analysis reveal that the number of marbles buried by the mice were significantly decreased in the mice receiving *Vitis vinifera* juice as compared to saline treated group. The results are comparable to the standard drug, fluoxetine.

Conclusion: *Vitis vinifera* juice has shown a significant effect on the marble burying behaviour in mice. In previous studies, *Vitis vinifera* has shown modulating effect on the amine neurotransmitters, especially, serotonin. This effect of the herb might be responsible for it effect on the marble burying behaviour of the mice.

Keywords: *Marble burying behaviour, Vitis vinifera, Obsessive-compulsive disorder, Fluoxetine.*

Introduction:

Approximately 2% of people worldwide suffer from obsessive-compulsive disorder (OCD), a common mental health issue (Murphy, 2010 & Voderholzer, 2022). Although obsessive-compulsive disorder was thought to be among the rarest mental disorders in previous decades, research conducted in recent years has shown that it is really one of the most prevalent mental disorders (Tavakolizadeh, 2016). The most prevalent of the "obsessive compulsive and related disorders," which are now grouped together in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, and the International Classification of Diseases, Eleventh Revision, is obsessive-compulsive disorder (OCD), which is often underdiagnosed and undertreated. Furthermore, much study on psychobiology, phenomenology, medication, and psychotherapy has helped to better identify, assess, and treat OCD, a neuropsychiatric disorder.(M S, Prabakar, 2022).

Repetitive obsessions, compulsions, or a mix of the two are characteristics of this illness. Compulsions are repetitive behaviors that are frequently carried out to counteract obsessions and are therefore seen as comforting. Persistent and recurring impulses, ideas, or pictures that are invasive and typically viewed as unpleasant and unsettling are known as obsessions (Abramowitz, 2020). Because OCD has a wide range of symptoms, these compulsions and/or obsessions can take many different forms, such as silent word repetition, ordering, or a dread of acting inappropriately (Heyman, 2006). OCD frequently develops gradually, and if appropriate treatment is not received, the illness usually progresses over time (Abramowitz, 2009 & Fontenelle, 2020).

The burying behavior assay was first used as a novel model to evaluate compulsive-like behaviors by Njung'e K. and Handley SL. Since then, it has been a useful tool in behavioral research (Njung’e, 1991). They stated that glass marbles are suitable for evaluating compulsive-like behavior because they are not considered an instinctual fear stimulus and do not cause an adverse reaction. Rather of being a reaction to aversion or fear, marble burying in mice is thought to be an unintentional behavior that displays obsessive tendencies. Rats and mice instinctively exploit readily available bedding to conceal unpleasant causes of discomfort in their wild and lab environments (Aboul-Fotouh, 2022). In order to avoid and protect against the confined hazard, burrowing behavior involves utilizing the snout and forepaws to forward-shove the diggable material over the source of aversion (de Boer, 2003). This distinctive behavior is thought to be a self-protective reaction that reproduces the animal's anxiety state. It is usually directed at various aversive or hazardous stimuli, such as small predators like scorpions, food with a disagreeable taste, dead conspecifics, or electric prods (Wilkie, 1979; Deacon, 2006 & De Brouwer, 2019).

Due to its use in the production of wine, Vitis vinifera is a natural product that is widely grown worldwide (Ali, Maltese, Choi & Verpoorte, 2009). The use of grapes in traditional medicine has been supported by several scientific investigations. However, in order to determine the significance of grapes in neurological disorders, scientific research on their pharmacological actions is required. In this study, the effect of the herb on the marble burying behaviour of the mice is assessed.

Materials and Methods:
The collection of plant and preparation of juice:

The White Kishmish variety of *Vitis vinifera* fruits were purchased from local markets in Karachi, Pakistan. Following verification, the fruits were squeezed through a cotton cloth to extract fresh juice. The juice was extracted from fresh fruit each day. About 80–100 mL/100 g was the yield.

Animals' selection:

For this investigation, male Swiss albino mice weighing between 20 and 25 grams were chosen. The mice were kept in polypropylene cages, with five mice per cage. Standard mouse pellets and unlimited water were given to the animals. Six hours before to the medication delivery and during the trial, the animals were denied nourishment.

Division of mice into different groups:

Group I: Normal control, given normal saline 8 ml/kg, *p.o.*
Group II: Treatment group, given VVJ 4 mL/kg, *p.o;*
Group III: Treatment group, given VVJ 8 mL/kg, *p.o;*
Group IV: Positive control, given Fluoxetine 10 mg/kg, *p.o;*

Dose:

Vitis vinifera juice and normal saline were administered through oral route. The dosing was carried out once daily at 10 a.m. for 60 days.

Marble burying test: Every mouse was placed in a cage with usual dimensions of (22 x 32 x 13.5 cm) containing a 5 cm layer of sawdust and 24 glass marbles (1.5 cm in diameter). The test compound, the vehicle and fluoxetine, the standard drug, were administered and the animals were kept in the cages for next 30 min. The number of marbles buried in the sawdust, during 30 min, was recorded for every single mouse (Saadat, 2006; Dixit et al., 2014). The test was conducted on 7^{th}, 15^{th}, 30^{th} and 60^{th} day of the study, that is, the animals were examined 4 times during the 60 days of the study.

Statistical analysis: One-way ANOVA and Newman-Keuls *post hoc* test were used to calculate the statistical significance. The data is expressed as mean ± SEM. Statistically significant difference was accepted at $P < 0.05$. GraphPad Prism version 5.00 was used as statistical software.

Results:
Marble burying test:

A one-way ANOVA was used to test the difference in the number of marbles buried by four groups of mice. The number of marbles buried by mice showed significant difference between the four groups of animals, ($F_{3, 12} = 689$, $P < 0.001$) The means of treated groups (VVJ 4 mL/kg and VVJ 8 mL/kg) were compared with the means of the normal control (saline-treated) group using Newman-Keuls *Post hoc* test. The results of the analysis reveal that the number of marbles buried ($P < 0.001$) was significantly decreased in treated groups when compared with the saline-treated group (Table 1,2,3 and 4)... Moreover, administration of Fluoxetine (10 mg/kg, *p.o.*) significantly decreased the number of marbles buried ($P < 0.001$) in treated mice.

The test was conducted on 7th, 15th, 30th and 60th day of the study, that is, the animals were examined 4 times during the 60 days of the study

Table 1: Effect of *Vitis vinifera* juice on the marble burying behaviour of mice on the 7th day of the study

Treatment	Marbles buried
Control (Saline 8 mL/kg)	19.10 ± 0.37
V. vinifera juice 4 mL/kg	15.70 ± 0.49*
V. vinifera juice 8 m L/kg	15.70 ± 0.33*
Fluoxetine 10 mg/kg	18.50 ± 0.40

Number of animals per group (n) = 6.

The observations are given as mean ± S.E.M.
***P<0.001, **P<0.01, *P<0.05; ANOVA followed by Newman-Keuls test.

Table 2: Effect of *Vitis vinifera* juice on the marble burying behaviour of mice on the 15th day of the study

Treatment	Marbles buried
Control (Saline 8 mL/kg)	19.30 ± 0.33
V. vinifera juice 4 mL/kg	14.50 ± 0.34***
V. vinifera juice 8 m L/kg	10.20 ± 0.32***
Fluoxetine 10 mg/kg	7.70 ± 0.42***

Number of animals per group (n) = 6.

The observations are given as mean ± S.E.M.
***P<0.001, **P<0.01, *P<0.05; ANOVA followed by Newman-Keuls test.

Table 3: Effect of *Vitis vinifera* juice on the marble burying behaviour of mice on the 30th day of the study

Treatment	Marbles buried
Control (Saline 8 mL/kg)	19.20 ± 0.24
V. vinifera juice 4 mL/kg	13.90 ± 0.60***
V. vinifera juice 8 m L/kg	9.80 ± 0.29***
Fluoxetine 10 mg/kg	7.70 ± 0.36***

Number of animals per group (n) = 6.

The observations are given as mean ± S.E.M.
***P<0.001, **P<0.01, *P<0.05; ANOVA followed by Newman-Keuls test.

Table 4: Effect of *Vitis vinifera* juice on the marble burying behaviour of mice on the 60th day of the study

Treatment	Marbles buried
Control (Saline 8 mL/kg)	19.40 ± 0.54
V. vinifera juice 4 mL/kg	13.50 ± 0.42***
V. vinifera juice 8 m L/kg	9.50 ± 0.26***
Fluoxetine 10 mg/kg	7.70 ± 0.39***

Number of animals per group (n) = 6.

The observations are given as mean ± S.E.M.
***P<0.001, **P<0.01, *P<0.05; ANOVA followed by Newman-Keuls test.

Discussion:

This is the first study to show that compulsive behavior can be influenced by *Vitis vinifera.* Marble-burying activity is a sign of compulsive behavior, which is typically linked to OCD (Njung'e, 1991; Deacon, 2006 & Dixit, 2020). OCD is a neuropsychiatric disorder characterized by persistent, intrusive thoughts and/or repetitive activities that cause severe distress and interfere with day-to-day functioning (Gaikwad, 2014). Serotonin deficiency is linked to OCD, an anxiety-related condition (Stein, 2007). Despite being classified as an anxiety condition, OCD symptoms are usually not alleviated by routinely used antianxiety medications like benzodiazepines. SSRI's and clomipramine are more successful in lessening the intensity of OCD symptoms, according to clinical research (Wilson, 2021).

SSRIs' anti-obsessional effects increase synaptic 5HT release in the orbitofrontal cortex by gradually desensitizing the presynaptic auto receptors on 5HT neurons and their nerve terminals(Stein, 2007). An important component of evaluating anxiety is behavioral testing (Millan, 2001). Because the frequency of marble-burying is constant during repeated trials, showing obsessive behavior, the marble-burying behavior test is seen to be a helpful model for assessing anti-OCD therapy. The applicability of the test in OCD research is further supported by the fact that antidepressants like tricyclics and SSRIs have been demonstrated to lessen marble-burying behavior (Greist, 1995). In rodents, marble-burying behavior is an unconditioned, species-specific protective response that is unrelated to any immediate physical threat and does not decrease with repeated trials (Njung'e, 1991).

Male mice's marble-burying behavior is considerably reduced when SSRIs and tricyclic antidepressants are administered acutely.28,34 These results imply that male mice's marble-burying behavior is more indicative of obsessive behavior than anxiety (Greist, 1995). To evaluate compulsive behavior, this model was employed in the present study. According to the current investigation, *Vitis vinifera* decreased marble-burying behavior which is comparable to that of fluoxetine.

Serotonin neurotransmitter abnormalities may be a contributing factor or a result of OCD, according to the pathophysiology of the condition. Anxiety modulation is thought to be influenced by serotonin. Serotonin has to attach to receptor sites on nearby nerve cells in order to transmit chemical impulses between neurons. Serotonin receptors are thought to be comparatively underactive in OCD sufferers. This theory is consistent with the observation that many OCD sufferers benefit from SSRIs, a class of antidepressants that make serotonin more accessible to nearby nerve cells. *Vitis vinifera* has shown modulating effect on the amine neurotransmitters, especially, serotonin (Aslam, 2015). This effect of the herb might be responsible for it effect on the marble burying behaviour of the mice.

Conclusion:

Vitis vinifera juice has shown a significant effect on the marble burying behaviour in mice. In previous studies, *Vitis vinifera* has shown modulating effect on the amine neurotransmitters, especially, serotonin. This effect of the herb might be responsible for it effect on the marble burying behaviour of the mice.

References:

Murphy DL, Timpano KR, Wheaton MG, Greenberg BD, Miguel EC. Obsessive-compulsive disorder and its related disorders: a reappraisal of obsessive-compulsive spectrum concepts. Dialogues Clin Neurosci. 2010; 12(2): 131-48.

Voderholzer U, Favreau M, Rubart A, Staniloiu A, Wahl-Kordon A, Zurowski B, et al. Therapie der Zwangsstörungen: Empfehlungen der revidierten S3-Leitlinie Zwangsstörungen. Nervenarzt. 2022; 93(7): 678-87.

Tavakolizadeh J, Safarzade S. The prevalence of obsessive-compulsive disorder in the population of 18–66-year-old city of Gonabad-Iran in 2016. Res J Pharm Technol. 2018; 11(10): 4222.

M S, Prabakar S, Rao UM. Cognitive behavioral therapy in children and adolescents. Res J Pharm Technol. 2022; 15(3): 1330-6.

Abramowitz JS, Reuman L. Obsessive compulsive disorder. In: Zeigler-Hill V, Shackelford TK, editors. Encyclopedia of Personality and Individual Differences. Cham: Springer; 2020. p. 3304-6.

Heyman I, Mataix-Cols D, Fineberg NA. Obsessive-compulsive disorder. BMJ. 2006; 333(7565): 424-9.

Abramowitz JS, Taylor S, McKay D. Obsessive-compulsive disorder. Lancet. 2009; 374(9688): 491-9.

Fontenelle LF, Hasler G. Understanding the course of obsessive–compulsive disorder: a meta-analysis and qualitative review. J Obsessive Compuls Relat Disord. 2020; 27: 100587.

Njung'e K, Handley SL. Evaluation of marble-burying behavior as a model of anxiety. Pharmacol Biochem Behav. 1991; 38(1): 63-7.

Aboul-Fotouh S, Eid A, El-Khayat Z. Marble-burying behavior in rodents: uncovering the neurobiology, relevance, and reliability. J Pers Med. 2022; 12(11): 1921. doi:10.3390/jpm12111921.

de Boer SF, Koolhaas JM. Defensive burying in rodents: ethology, neurobiology and psychopharmacology. Eur J Pharmacol. 2003; 463(1-3): 145-61. doi:10.1016/S0014-2999(03)01278-0.

Wilkie DM, MacLennan AJ, Pinel JPJ. Rat defensive behavior: burying noxious food. J Exp Anal Behav. 1979; 31(3): 299-306.

Deacon RMJ. Digging and marble burying in mice: simple methods for in vivo identification of biological impacts. Nat Protoc. 2006; 1(1): 122-4.

De Brouwer G, Fick A, Harvey BH, Wolmarans W. A critical inquiry into marble burying as a preclinical screening paradigm of relevance for anxiety and obsessive-compulsive disorder: mapping the way forward. Cogn Affect Behav Neurosci. 2019; 19(1): 1-39. doi:10.3758/s13415-018-00653-4.

Ali, K., Maltese, F., Choi, Y., & Verpoorte, R. (2009). Metabolic constituents of grapevine and grape-derived products. *Phytochem Rev*, *9*(3), 357-378.

Saadat, K. (2006). The acute and long-term neurotoxic effects of MDMA on marble burying behaviour in mice. *Journal of Psychopharmacology*, *20*(2), 264-271.

Dixit, M., Thakre, P., Pannase, A., Aglawe, M., Taksande, B., & Kotagale, N. (2014). Imidazoline binding sites mediates ant compulsive-like effect of agmatine in marble-burying behavior in mice. *European Journal of Pharmacology*, *732*, 26-31.

Dixit PV, Sahu R, Mishra DK. Marble burying behavior test as a murine model of compulsive-like behavior. J Pharmacol Toxicol Methods. 2020; 102: 106676.

Gaikwad U. Pathophysiology of obsessive-compulsive disorder: affected brain regions and challenge towards discovery of novel drug treatment. In: Obsessive-Compulsive Disorder - The Old and the New Problems. London: Intech Open; 2014. doi:10.5772/57193.

Stein DJ, Ipser JC, Baldwin DS, Bandelow B. Treatment of obsessive-compulsive disorder. CNS Specter. 2007; 12(S3): 28-35.

Wilson SA, Nagaraj A, Vaidyanathan L. Calocybe indicia extract modifies GABA and serotonin levels to alleviate anxiety in experimental adult zebrafish models. Res J Pharm Technol. 2021; 14(9): 4789-94.

Millan MJ, Dekeyne A, Papp M, Rochelle CDL, MacSweeny C, Peglion JL, et al. S33005, a novel ligand at both serotonin and norepinephrine transporters: II. behavioral profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine. J Pharmacol Exp Ther. 2001; 298(2): 581-91.

Greist JH, Bandelow B, Hollander E, Marazziti D, Montgomery SA, Nutt DJ, et al. WCA recommendations for the long-term treatment of obsessive-compulsive disorder in adults. CNS Spectr. 2003; 8(S1): 7-16.

Muhammad Aslam, and Nuzhat Sultana. *Vitis Vinifera* juice ameliorates depression-like behavior in mice by modulating biogenic amine neurotransmitters. Bangladesh Journal of Pharmacology, 2015, 10 (4); 753-758.