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# Effect of *Vitis vinifera* on memory and behaviour mediated by monoamines

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**Abstract**

Objective: To investigate the effect of methanolic extract of *Vitis vinifera* (VVE) and methanolic fraction (MF) of methanolic extract on memory and behaviour mediated by monoamines. Methods: The effect of VVE and MF on memory was studied using Passive shock avoidance paradigm, Elevated plus maze (EPM) paradigm and Object recognition test. The effect of extract on clonidine induced hypothermia, lithium induced head twitches, haloperidol induced catalepsy and sodium nitrite induced hypoxia was observed to study the behaviour mediated by noradrenaline, serotonin, dopamine and Acetylcholine respectively. Scopolamine (0.3 mg/kg) was used to induce cognitive dysfunction. Piracetam (100 mg/kg) served as a standard nootropic drug. Results: Methanolic fraction (MF) of extract significantly ($P<0.05$) reversed scopolamine-induced amnesia in mice. The VVE and MF exhibited significant ($P<0.05$) nootropic activity in all three models of memory. VVE and MF decreased lithium induced head twitches, modified haloperidol induced catalepsy and decreased percentage mortality induced by sodium nitrite. However, clonidine induced hypothermia was not modified. Conclusion: Thus, the raisins of *Vitis vinifera* contain bio-active principle(s) which possess prominent nootropic activity. It also modified 5-HT, DA and Ach mediated behaviour.

**Key Words:** *Vitis vinifera*, nootropic, noradrenaline, serotonin, dopamine, acetylcholine

## 1. Introduction

Cognitive deficits have long been recognized as severe and consistent neurological disorders associated with numerous psychiatric and neurodegenerative states.

Learning is defined as the acquisition of information and skills, while subsequent retention of information is called memory. Nootropics belongs to the class of psychotropic agents with selective facilitatory effect on the cognitive function of CNS such as intellectual performance, learning and memory [1]. A large number of medicinal plants that have been reported as intelligence promoters such as *Celastrus paniculatus* [2], *Lawsonia inermis* [3], *Bacopa monnieri* [4], *Centella asiatica* [5], *Albizzia lebbeck* [6], *Panax ginseng* [7], *Butea monosperma* [8], and *Moringa oleifera* [9] possess nootropic activity. *Vitis vinifera* Linn.

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(Vitaceae) known as common grape-vine or raisins is widely cultivated throughout tropics of India. It has varied uses in Ayurvedic and Unani system of medicine. Raisins possess laxative, cooling, expectorant, antioxidant, diuretic, aphrodisiac and stomachic properties [10].

Several mediators like acetylcholine, noradrenaline, dopamine, serotonin, gamma-aminobutyric acid, glutamate, nitric oxide, and peptides influence cognitive behaviour of the animal [11]. Biogenic amines are reported to be involved in learning and memory processes [12]. However, experimental evidence to support memory-enhancing property of *Vitis vinifera* is negligible.

The present study was therefore undertaken to evaluate the nootropic activity of *Vitis vinifera* by using acceptable behavioural paradigms. Studies on behaviour influenced by noradrenaline (NA), dopamine (DA), serotonin (5-HT) and acetylcholine (Ach) were also carried out to explain the observed actions.

## 2. Materials and methods

### *2.1 Preparation of extract*

*Vitis vinifera* raisins (1kg), was purchased locally, authenticated (by Dr.S.C.Pal, Pharmacognosist, NDMVP Samaj's College of Pharmacy) and cut into pieces. It was defatted with petroleum ether (60 - 80°C) using Soxhlet's extractor. The marc was dried and successively extracted with methanol. The extract was concentrated under vacuum.

The methanol extract (VVE) (63.83% w/w yield) was subjected to column chromatographic fractionation using solvents of varying polarity to obtain ethyl acetate fraction (10.82% w/w yield) and methanol fraction (MF) (53% w/w yield). Alumina (neutral) was used as a stationary phase. VVE and MF were used for further investigation. The selection of doses were based on Irwin (1959) schedule. Preliminary phytochemical screening confirmed the presence of saponins, tannins, phenolic compounds and flavonoids [13].

### *2.2 Animals*

Albino mice (20-25 g) and Albino rats (125-150 g) of both sexes were obtained from Serum Institute, Pune. Animals were housed into groups of five under standard laboratory conditions of temperature 25 ± 1°C with free access to food (Hindustan Lever, India) and water. Food but not water was deprived 4 h before the experiment. The experiments were performed during the light portion (09-14 h). The Institutional Animal Ethical Committee approved the protocol of this study.

### *2.3 Drugs and Chemicals*

Piracetam (Uni-UCB, India), Scopolamine and Clonidine (German Remedies, India), Haloperidol (RPG Life sciences, India) and Lithium sulphate (Glenmark, India) were used for the study. Pet ether, methanol and ethyl acetate were obtained from Modern Scientific, Nashik. All drug solutions were prepared in distilled water and administered intraperitoneally. The VVE and MF were suspended in saline and administered orally 60 min before testing. The extracts and fraction did not exhibit any toxicity signs upto1000mg/kg.

### *2.4 Passive shock avoidance paradigm*

The method used was essentially the same as described earlier [14]. Mice were placed individually on the electric grid and allowed to explore the maze for 1 minute. The stimulus (20 V) with AC current of 5 mA was then applied and latency to reach the shock free zone (SFZ) was recorded three consecutive times as basal reading. Animals that reached the SFZ in 2 minutes in the first trial were selected for the

study. After 1 hour of the first trial, each animal was put on the grid again and latency to reach SFZ and the number of mistakes (descents) the animal made in 15 minutes were recorded as parameters for acquisition and retention respectively. Mice in groups of five each received VVE (30,100 mg/kg, p.o.), MF (30,100 mg/kg, p.o.) or Piracetam (100 mg/kg, i.p.) either alone or 30 min before Scopolamine (0.3 mg/kg, i.p.) administration.

*2.5 Elevated plus maze (EPM)*

EPM consisted of two open arms (35 x 6 cm) and two enclosed arms (35 x 6 x 15 cm). The arm was connected together with a central square of 5 x 5 cm. The maze was elevated to the height of 25 cm. The maze was placed inside a light and sound attenuated room. Mice were placed individually at the end of an open arm of the elevated plus maze (EPM) facing away from the central platform and the time it took to move from the end of open arm to either of the closed arms (transfer latency, TL) was recorded [15].

On the first day, the mice were allowed to explore the maze for 5 minutes after the measurement of TL. On the 2nd day and 9th day, mice were placed again on the EPM as before and TL was noted again. The TL was expressed as retention scores on 2nd day and 9th day for each animal by calculating the "inflexion ratio (IR)" the formula described earlier [16].

$$IR = (L_1-L_0)/L_0$$

Where $L_0$ = initial TL and $L_1$ = TL on the 2nd day and 9th day. TL measured on the first and second day served as parameters for acquisition and retrieval respectively. Mice in groups of five each received VVE (30,100 mg/kg, p.o.), MF (30,100 mg/kg, p.o.) or Piracetam (100 mg/kg, i.p.) either alone or 30 min before Scopolamine (0.3 mg/kg, i.p.) administration.

*2.6 Object recognition test*

The apparatus [17] was formed by a white colored wooden box (70 x 60 x 30 cm) with a grid floor that could be easily cleaned with hydrogen peroxide after each trial. The objects to be discriminated were placed at diagonally opposite corners of the box and were in two different shapes: pyramids of 8 cm side and cylinders of 8 cm height. On the day before test, mice were allowed to explore the box without any object for 2 min. On the day of test in the first trial ($T_1$), two identical objects were presented in two opposite corners of the box, and the amount of time taken by each mouse to complete 20 seconds exploration was measured. Exploration meant directing the nose at a distance less than 2 cm to the object and / or touching with the nose. During the second trial ($T_2$, 90 minutes after $T_1$) a new object replaced one of the objects presented in $T_1$ and mice were left in the box for 5 minutes. The time spent for exploring new (N) and familiar (F) objects were recorded separately. Care was taken to avoid place preference and olfactory stimuli by randomly changing the role (familiar or new object) and the position of the two objects during $T_2$, and cleaning them carefully. Mice in groups of five each received VVE (30,100 mg/kg p.o.), MF (30,100 mg/kg, p.o.) or Piracetam (100 mg/kg, i.p.) either alone or 30 min before Scopolamine (0.3 mg/kg, i.p.) administration.

*2.7 Behavioral Studies*

*2.7.1 Clonidine induced hypothermia (NA mediated behaviour)*

Rats were divided into three groups of five animals each. Rats were treated with vehicle, VVE (100 mg/kg p. o.) and MF (100 mg/kg p. o.) one h before administration of clonidine (100 µg/kg i.p). Rectal temperature was recorded every 30 min after clonidine (100 µg/kg) treatment till 180 min [18].

*2.7.2 Haloperidol induced catalepsy (DA mediated behaviour)*

The mice in groups of five each were treated with vehicle, VVE (100 mg/kg p.o.) and MF (100 mg/kg p.o.) one h before administration of haloperidol (1mg/kg, i. p.). Mice were gently removed from their home cages and their forepaws placed over a glass horizontal bar, 0.5 cm in diameter and 30 cm long, which was fixed at a height of 4 cm above the working surface. Duration of catalepsy was recorded from the time all animals were placed over the bar till the time they removed both forepaws from the bar or climbed over the bar [19].

*2.7.3 Lithium induced head twitches (5-HT mediated behaviour)*

Rats were divided into four groups of five animals each. Rats received lithium sulphate (200 mg/kg i.p.) one h after vehicle or VVE (100 mg/kg, p.o.) and MF (100 mg/kg, p.o.) treatment. The number of head twitches was recorded for one h after Lithium sulphate administration [20].

*2.7.4 Sodium nitrite induce hypoxia (Ach mediated behaviour)*

Chemical hypoxia was induced by subcutaneous injection of sodium nitrite (250 mg/kg) 60 min after the administration of vehicle, VVE (100 mg/kg, p.o.) and MF (100 mg/kg, p.o.) treatment (doses that exhibited nootropic activity). The percentage mortality due to respiratory arrest was noted [21]. Each group consisted of 5 animals.

*2.8 Neurotoxicity test*

In this test a knurled rod (2.5 cm in diameter) was rotated at a speed of 15 rpm. All animals were trained to remain on the rotating rod for 5 min. A normal mouse could maintain its equilibrium for long periods. In a drug treated mouse, the neurological deficit was indicated by the inability of the mouse to maintain equilibrium for 3 min in each of the 3 trials as described earlier [22]. VVE and MF was administered at doses of 100 mg/kg doses and the animals were tested for neurological deficit 30 min after the drug treatment. The control group received diazepam at a dose of 1 mg/kg.

**Table 1.** Effect of VVE, MF of *Vitis vinifera* on passive shock avoidance paradigm.

| Treatment (mg/kg) | Latency to reach SFZ in sec | Number of mistakes in 15 min |
|---|---|---|
| Control | 21.11 ± 2.23 | 16.8 ± 3.07 |
| Piracetam (100) | 7.80 ± 3.44* | 7 ± 0.31* |
| VVE (30) | 18.03 ± 2.06 | 10.2 ± 0.48 |
| VVE (100) | 12.1 ± 1.07* | 7 ± 0.31* |
| MF (30) | 9.812 ± 1.36* | 7.4 ± 1.03* |
| MF (100) | 11.54 ± 0.94* | 6.80 ± 1.24* |
| Scopolamine (0.3) | 34.89 ± 4.13* | 25.8 ± 1.68* |
| MF (100) + Scopolamine (0.3) | 9.42 ± 0.97* # | 7.20 ± 1.39* # |

n=5, *P<0.05 as compared with vehicle treated group (ANOVA followed by Dunnett's test).

#P<0.05 as compared with scopolamine treated group

Values are mean ± SEM. SFZ = Shock free zone. VVE = Methanolic extract of *Vitis vinifera*. MF = Methanolic fraction of methanolic extract of *Vitis vinifera*

**Table 2.** Effect of VVE, MF of *Vitis vinifera* on haloperidol (1 mg/kg) induced catalepsy in mice and lithium sulphate (200 mg/kg) induced head twitches in rats

| Treatment | Duration of catalepsy time at (sec) | | | | | Head Twitches |
|---|---|---|---|---|---|---|
| (mg/kg) | 15 min | 30 min | 60 min | 90 min | 120min | |
| Control | 27.4±1.16 | 59±6.8 | 120±3.5 | 160.2±8.6 | 133± 9.4 | 41.25±1.88 |
| VVE(100) | 22.8±1.77 | 53.8±2.01 | 100±3.5* | 130±9.4* | 121±4.0* | 22.25±1.49* |
| MF (100) | 27.6±2.76 | 57.8±2.54 | 111.4±8.42 | 123±9.73* | 100.2±5.93* | 15.25±2.21* |

n=5.The observations are mean ± SEM. *P<0.05, as compared to vehicle control (ANOVA followed by Dunnett's test)

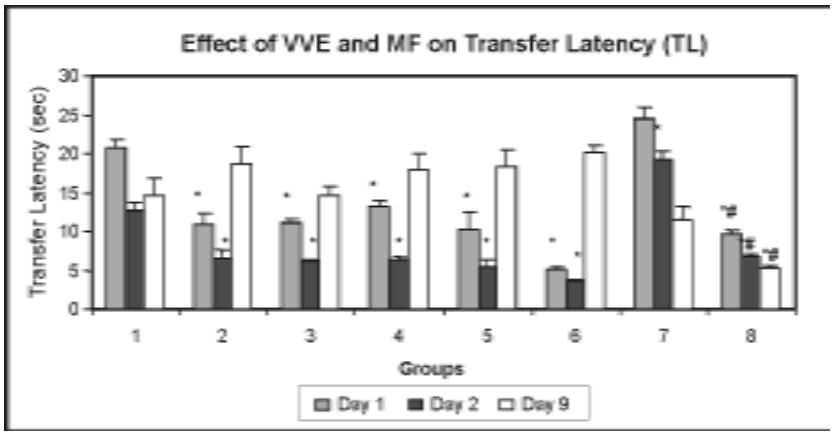


**Fig 1a:** Effect of VVE and MF of *Vitis vinifera* on Transfer Latency

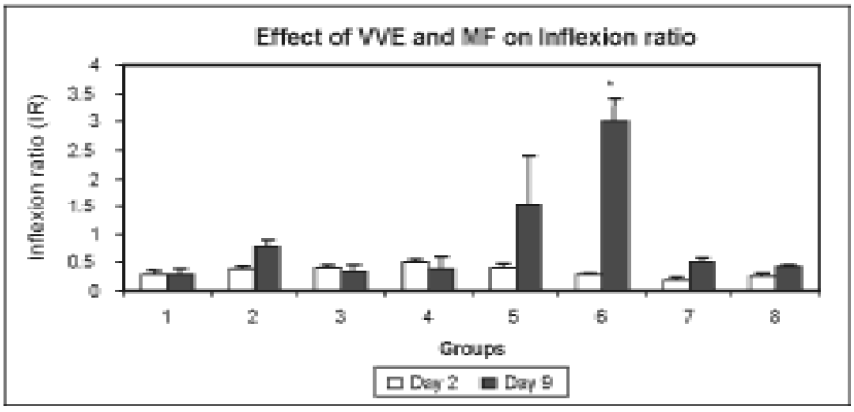


**Fig 1b:** Effect of VVE and MF of *Vitis vinifera* on Inflexion ratio

**Fig-1:** Effect of VVE, MF of Vitis vinifera on elevated plus maze apparatus

n=5, *P<0.05 as compared with vehicle treated group #P<0.05 as compared with scopolamine treated group (ANOVA followed by Dunnett's test). Values are mean ± SEM. VVE = Methanolic extract of Vitis vinifera. MF = Methanolic fraction of methanolic extract of Vitis vinifera. Group 1= Control, Group 2= Piracetam (100), Group 3 = VVE (30), Group 4 = VVE (100), Group 5 = MF (30), Group 6 = MF (100), Group 7 = Scopolamine (0.3), Group 8 = MF (100) + Scopolamine (0.3)

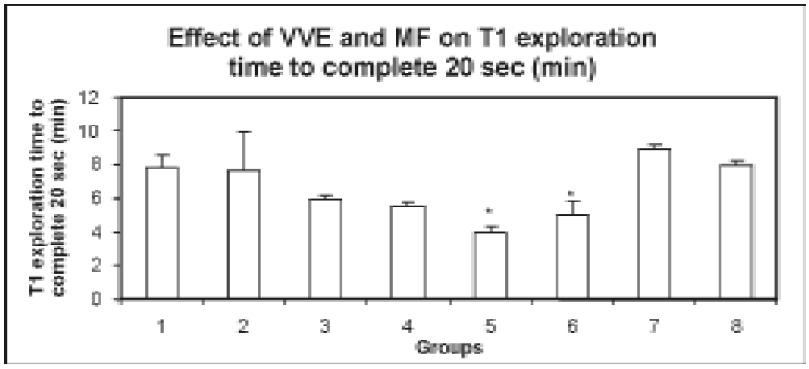


**Fig 2a:** Efect of VVE and MF of Vitis vinifera on T1 exploration time

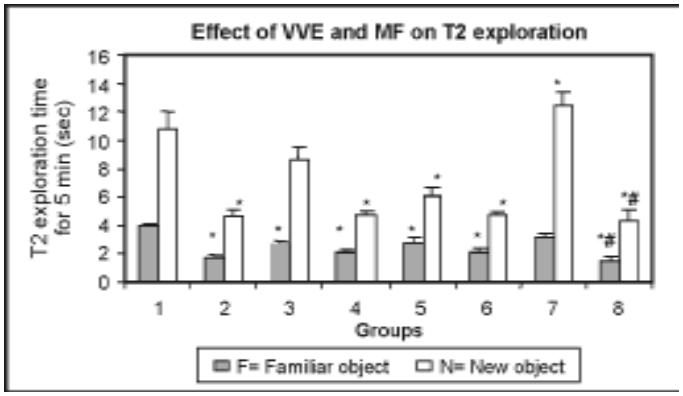


**Fig 2b:** Efect of VVE and MF of Vitis vinifera on T2 exploration time

**Fig 2:** Effect of VVE, MF of *Vitis vinifera* in Object recognition apparatus

n=5, *P<0.05 as compared with vehicle treated group (ANOVA followed by Dunnett's test). #P<0.05 as compared with scopolamine treated group Values are mean ± SEM. VVE = Methanolic extract of Vitis vinifera. MF = Methanolic fraction of methanolic extract of Vitis vinifera. Group 1= Control, Group 2= Piracetam (100), Group 3 = VVE (30), Group 4 = VVE (100), Group 5 = MF (30), Group 6 = MF (100), Group 7 = Scopolamine (0.3), Group 8 = MF (100) + Scopolamine (0.3)

*2.9 Statistical analysis*

All data are shown as mean ± SEM. Statistical analysis was performed with one-way ANOVA followed by Dunnett's test. Differences of p<0.05 was considered statistically significant.

## 3. Results

*3.1. Passive shock avoidance paradigm*

VVE (100 mg/kg) and MF (30 and 100 mg/kg) significantly (P<0.05) reduced the latency to reach SFZ and the number of mistakes. Scopolamine (0.3 mg/kg) significantly increased (P<0.05) the latency to reach SFZ and the number of mistakes. Piracetam (100 mg/kg) significantly (P<0.05) reduced the latency to reach SFZ and the number of mistakes. Thus, piracetam also antagonized the cognitive dysfunction induced by scopolamine. The MF antagonized the effect of scopolamine. The observations are given in Table 1.

*3.2 Elevated plus maze (EPM)*

The transfer latency on the elevated plus maze was expressed as inflexion ratio (IR). The MF (100 mg/kg) significantly (P<0.05) increased the IR on day 9. Piracetam (100 mg/kg) significantly (P<0.05) shortened the TL on the first and the second day. Scopolamine (0.3 mg/kg) significantly (P<0.05) increased the TL on the first day. VVE (30 and 100 mg/kg) and MF (30 and 100 mg/kg) significantly (P<0.05) reduced the TL on the first and the second day. MF (100 mg/kg) antagonized the effects of scopolamine. The observations are given in Fig1.

*3 .3 Object recognition test*

VVE (100 mg/kg) and MF (30 and 100 mg/kg) restored object discrimination and decrease in $T_1$ exploration time. MF (100 mg/kg) significantly (P<0.05) decreased duration of $T_1$ exploration time and was able to discriminate between familiar and novel objects with an intertrial time of 90 min and also antagonized the effect of scopolamine. The observations are given in Fig 2.

*3. 4 Behavioral Studies*

*3.4.1 Clonidine induced hypothermia (NA mediated behaviour)*

In vehicle treated rats, clonidine produced a fall in rectal temperature and the peak effect was observed 150 minutes after its administration. However, VVE (100 mg/kg) and MF (100 mg/kg) did not modify clonidine induced hypothermia.

*3.4.2 Haloperidol induced catalepsy (DA mediated behaviour)*

In vehicle treated group, haloperidol produced peak catalepsy at 90 minutes. The VVE (100 mg/kg) and MF (100 mg/kg) significantly (P<0.05) decreased the duration of catalepsy at 90 min. The observations are given in Table 2.

*3.4.3 Lithium induced head twitches (5 HT mediated behaviour)*

In vehicle treated rats, lithium sulphate produced 41.25 ±1.88 head twitches. VVE (100 mg/kg), MF (100mg/kg) significantly (P<0.05) reduced the number of head twitches to 22.25±1.49, 15.25±2.21 respectively. The observations are given in Table 2.

*3.4.4 Sodium nitrite induce hypoxia (Ach mediated behaviour)*

The animals receiving the vehicle showed 100% mortality after sodium nitrite injection while animals treated with VVE (100 mg/kg) and MF (100 mg/kg) showed only 40% and 20% mortality respectively.

*3.5 Neurotoxicity test*

Mice treated with VVE (100 mg/kg) and MF (100 mg/kg) were able to maintain equilibrium on the rotating rod for more than 3 min, whereas the animal treated with diazepam exhibited incordination and fall off time was significantly (p<0.05) reduced to 43.62 ± 0.10 sec.

## 4. Discussion

The present study suggests that *Vitis vinifera* possess nootropic activity in view of its facilitatory effect on retention of learned task. Passive avoidance behaviour is based on negative reinforcement and is used to examine short-term memory. Both VVE and MF meet the major criteria for nootropic activity. The elevated plus maze is a widely accepted model to study nootropic activity. The shortening of transfer latency and increase in IR by VVE as well as MF indicated improvement of memory in absence of cognitive deficit. Shortening of the transfer latency by VVE and MF, as shown by increased inflexion ratio is in accordance with the hypothesis of Itoh *et al* [15]. This indicates that VVE and MF improved memory in absence of cognitive deficit [23]. Object recognition is a form

of nonspatial memory, based on the spontaneous exploratory behaviour of mice towards objects that have no special meaning for them [17]. Under these conditions object recognition may be considered a form of episodic memory that lasts for at least 90 min. When Piracetam (100 mg/kg) was administered a decrease in the exploration time of the familiar object and a significant difference in the time spent in exploring the familiar and novel objects were found, indicating that discrimination between the two objects was restored but did not affect $T_1$ exploration time. The administration of VVE and MF restored object recognition by reducing the time spent in exploring the familiar object. Although involvement of central cholinergic system is well established, the role of other neurotransmitter systems cannot be ignored. Sodium nitrite converts haemoglobin into methemoglobin and the oxygen carrying capacity is reduced to such extent that the animal can not breath and dies due to respiratory arrest and agents improving cholinergic transmission decrease the mortality rate [21]. The diminished effect of sodium nitrite in presence of VVE, MF indicates that they increase cholinergic transmission and this could be the reason for their nootropic activity. The present study has attempted to correlate the effect on monoamine-mediated behaviour with the nootropic activity. Several studies have indicated that an increase in serotonergic transmission can interfere with learning acquisition and memory consolidation [24]. In our study, we have observed that both VVE and MF diminished serotonergic transmission as observed from decrease in the lithium induced head twitches, a response which reflects on the serotonergic function.

Controversial reports are available on the involvement of DA activity in learning and memory. Piracetam, in one study, exhibited increase in dopaminergic activity [25]. But VVE and MF improved the DA transmission for 60-120 min. The amnesic effect of electroconvulsive shock, which is attenuated by piracetam is known to produce a marked increase in the turnover of rat brain NA [26]. Both VVE and MF did not modify clonidine-induced hypothermia. The results of the rotarod test prove that *Vitis vinifera* extract does not have neurotoxic effect.

Both VVE and MF contain saponins which like saponins of fenugreek [27] or ginseng may improve memory. Thus the present study draws conclusion that the dry grapes is raisins could serve as an important nootropic agent.

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