Grape Seed Extract Attenuates Anxiety-Like Behaviors in Experimental Model of Dietary-Induced Hypercholesterolemia in Rats

ZIENAB ALREFAIE, M.D.
The Department of Physiology, Faculty of Medicine, Cairo University

Abstract

Objective: Grape seed oligomeric proanthocyanidins (OPCs) have proved many beneficial and protective effects. The present work aimed to assess the possible anxiolytic effect of grape seed extract containing OPCs in dietary-induced hypercholesterolemia which has not previously been investigated.

Methods: 30 adult male Wistar rats were included into 3 groups (N=10); control group (normal rat chow-fed group), high cholesterol group (fed with chow with 4% cholesterol+1% cholic acid) and high cholesterol+grape seed extract group (fed with the high cholesterol chow+grape seed extract 100mg/kg/day by oral gavage). The experiment lasted for 4 months, then animals were subjected to behavioral testing using open field and elevated plus maze tests.

Results: The high cholesterol diet resulted in hypercholesterolemia which was greatly reduced by grape seed extract administration. Hypercholesterolemic rats showed anxiety-like behaviors in the open field and elevated plus maze testing when compared to normal chow-fed rats. Administration of grape seed extract together with the high cholesterol diet resulted in significant anxiolytic effect in hypercholesterolemic rats.

Conclusion: The present results showed that grape seed extract reduced serum cholesterol level and anxiety-like behaviors associated with high-cholesterol diet.

Key Words: Grape seed — Extract — Anxiety — Hypercholesterolemia — Rats.

Introduction

CHANGES in serum cholesterol levels may have a direct impact on mental performance and behavior as cholesterol plays an integral role in the structure and function of the cell membrane and may also affect neurotransmission in the central nervous system [1].

Serum cholesterol levels have been found to be related to the pathophysiology of mood disorders. Steegmans et al., [2] and Rafter [3] have observed significant correlation between lower cholesterol levels and severity of depressive symptoms. On the contrary, many studies have reported high cholesterol levels in patients with anxiety disorders [4,5].

Grape seeds have high content of oligomeric proanthocyanidins (OPCs) which are end products of flavonoid biosynthetic pathway [6]. OPCs are increasingly recognized as having beneficial effects on human health [7]. OPCs also exerted a spectrum of biological, pharmacological, therapeutic and chemoprotective properties. In addition, OPCs showed better scavenging ability than vitamin C and E against oxidative stress [8]. OPCs were also found to display a wide variety of biological functions related to modulation of carcinogenesis both in vitro and in vivo studies [9,10].

No previous research has assessed the effect of grape seed extract containing OPCs on anxiety-like behavior in hypercholesterolemic rats.

Aim of the study:

The present study aimed to assess the possible effect of grape seed extract on anxiety-like behavior that could associate dietary-induced hypercholesterolemia. The hypothesis is that high plasma cholesterol could result in anxiety-like behavior in rats which could be alleviated by grape seed extract ingestion.

Material and Methods

Animals:

30 Adult male Wistar rats weighing 200-250g were obtained from the animal house, Research Center, Faculty of Medicine, King Abdulaziz University. Rats were housed in the rodent facility with controlled temperature and humidity under 12h/12h light-dark cycles.
All experimental procedures were carried out in accordance with the guidelines of animal care and approved by the college research ethics committee.

Animals were divided randomly into 3 groups; control group (fed with normal rat chow), high cholesterol fed group (fed with rat chow supplemented with 4% cholesterol and 1% cholic acid) and high cholesterol supplemented with grape seed extract group (fed with the high cholesterol diet and received grape seed extract 100mg/kg/day by oral gavage) [12].

At the end of the experiment which lasted for four months, animals were tested for anxiety-like behavior using open-field and elevated plus-maze tests with 2 recovery days between the tests.

Grape seed extract preparation:
Grape seed proanthocyanidins were prepared according to Karthikeyan et al., 2007 [13]. Grapes were collected at optimal maturity and seeds were manually separated. Seeds were dried at 70°C for 72 hours, then ground to a fine powder. The powder was extracted in a Soxhlet extractor with petroleum ether (60°C for 6h) to remove the fatty material. The defatted powder was re-extracted in a Soxhlet apparatus for 8 hours with 200m1 acetone/water/acetic acid (90:9.5:0.5) at 60°C. The extract was concentrated by rotator evaporation under vacuum at 70°C to get crude extract. The crude extract was purified using flash column chromatography. The product was stored in vacuum desiccators, and the weighed dose was dissolved in saline prior giving to the rats.

Open field test:
The open field test is a common behavioral test used to assess the anxiety responses of rodents. The open field consists of a Plexiglas box (45x45x22cm). Each rat was placed in the center of the apparatus to initiate a 10min test session. The total distance traveled (cm) and periods of immobility (s) were recorded and quantified using an automated tracking system (Mot2; TSE Systems). Also the number of entries into the center of the field and the time latency (s) to enter the center were measured [14].

Elevated plus maze test:
The elevated plus maze test has been used to assess internal conflict between voluntary approaches and withdrawal tendencies as a rodent model for human anxiety [15].
The elevated plus maze apparatus consists of a plus-shaped two open and two closed arms, each with an open roof. The apparatus is elevated 50cm from the floor. All arms are 10cm in width and 50cm in length and joined at the center to form a 10cm² central platform. The two closed arms opposite each other are surrounded by 40cm high walls.

At the start of testing, the rat was placed on the central platform, and was allowed to move freely for 5min. The cumulative time spent on each arm and the number of entries into the open or closed arms were recorded.

The behavior in the maze was recorded using a video camera mounted on the ceiling above the center of the maze and was relayed to the S-MART program (Etho vision, Noldus, Wageningen, The Netherlands).

The standard measure of anxiety was indicated by the percentage of the time spent in the open arms of the maze and the percentage of open arm entries. A smaller percentage indicates less open arm exploration or more anxiety. In addition, the total distance moved (cm) and the velocity of movement (cm/s) were also measured.

Statistical analysis:

The results were expressed as the Mean±Standard deviation (SD). The data were calculated and analyzed by Onaway analysis of variance (ANOVA) using SPSS (Version 20; SPSS Inc., Chicago, IL, USA). The statistical significance of the differences among groups was further analyzed using post-hoc test.

In all analyses, p<0.05 was considered significant.

Results

Results of the present study showed that dietary ingestion of high cholesterol chaw with cholic acid did significantly increase serum cholesterol level (p<0.001).

Ingestion of grape seed extract containing OPCs with the high cholesterol diet significantly attenuated the marked increase in serum cholesterol produced by high cholesterol diet alone although it was still higher compared to normal-chaw fed rats (Fig. 1).

It is well documented that if the animals are having anxiety, their ambulation in the open field will be decreased while the period of immobility will be correspondingly increased. The present results showed that high cholesterol diet resulted in significant decrease in the total distance travelled in the open field and significant increase in immobility period (p<0.001 for both). In addition, there was significant increase in the time latency to enter the center of the field (p<0.001), and fewer entries into the center (p<0.01) (Fig. 2).

Co-ingestion of grape seed extract with the high cholesterol diet reversed the findings obtained in the open field in high cholesterol fed group; it increased total distance moved and number of entries into the center, although values did not reach significance. Grape seed extract also decreased immobility period (p<0.01) and time latency to enter the center of the field (p<0.001) (Fig. 2).

The elevated plus maze test depends on the fact that the anxiety-prone animals will prefer to stay in the dark closed arms of the apparatus than in the open arms.

It is quite evident from the results of the elevated plus maze test that the hypercholesterolemic animals spent significantly less time in open arms and more time in closed arms as indicated by the % time spent in open arm to total time and % entries into open arm to total entries (p<0.001 for both). These changes were significantly reversed by grape seed extract administration (Fig. 3).

Other measures assessed in the elevated plus maze test included total distance moved and velocity of movement. Both parameters showed significant decrease in high cholesterol fed rats when compared to control rats (p<0.001) and significant increase in response to grape seed extract supplementation compared to hypercholesterolemic rats although values were still lower than control rats (Fig. 3).
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Fig. (2): Anxiety-like behavior in the open field in all groups. Total distance moved (cm) (A), immobility time (s) (B), number of entries into center (C) and latency to enter the center (s) (D). P1: Significance when compared to control group. p2: Significance when compared to high cholesterol fed group.

Fig. (3): Anxiety-like behavior in the elevated plus maze in all groups. Total distance moved (cm) (A), velocity (cm/s) (B), % time spent in open arms (C) and % entries into open arms (D). P1: Significance when compared to control group. p2: Significance when compared to high cholesterol fed group.
Discussion

The present results showed that the high cholesterol diet significantly elevated serum cholesterol, while the ingestion of grape seed extract with the high cholesterol diet prevented the marked increase in serum cholesterol induced by this diet alone.

The present results also demonstrated that dietary-induced hypercholesterolemia resulted in anxiety-like behaviors in rats, while the co-administration of grape seed extract reduced these behaviors.

Thus, the results of the present study support the possibility that grape seed extract could have hypocholesterolemic and anxiolytic effects in dietary-induced hypercholesterolemic model.

It is generally believed that cholesterol itself does not easily cross the blood brain barrier and that brain and peripheral cholesterol levels are independently regulated. Peripheral cholesterol can be taken up by the brain via its conversion into 27-hydroxycholesterol [16]. High levels of serum cholesterol may cause an increased flux of cholesterol from the circulation into the brain through the conversion between cholesterol and oxysterol, leading to increased levels of brain cholesterol.

One suggested mechanism for the deleterious effects of hypercholesterolemia on the brain is the amyloid deposition and diminished amyloid \(\beta\) degradation leading to intraneuronal accumulation of amyloid \(\beta\) oligomers and subsequent synapse loss [17,18]. Measures used to reduce serum cholesterol were found to decrease neuronal formation of \(\beta\) amyloid [19].

Elevated cholesterol levels may also alter the fluidity of the neuronal membrane, and thereby the conformation of 5HT receptors or 5HT transporters. This could be another mechanism by which hypercholesterolemia could be related to anxious behavior. Hypercholesterolemia might also operate through inhibition of dendritic outgrowth, in addition to comorbid cerebrovascular disease leading to cerebral hypoperfusion and neurodegeneration [20].

Reduced neurotrophic factors could also be among the deleterious effects of hypercholesterolemia on human brain [21].

Sooksawate and Simmonds [22], have observed that elevated cholesterol may directly contribute to anxiety by altering the sensitivity of the GABA receptors.

In accord with previous investigators [20,22], the present results demonstrated that dietary induced hypercholesterolemia was associated with anxiety-like behaviors in both open field and elevated plus maze.

In agreement with the present observations, studies in humans reported high cholesterol levels in patients with anxiety disorders [4]. The mean anxiety score was also higher in patients with hypercholesterolemia in the study of Vural et al., [23]. In addition, elevated premenstrual serum cholesterol was observed in females suffering from anxiety disorders [24].

Because the exploration in the elevated plus maze is based on a natural fear of open and elevated spaces, the number of entries into the open arms and the time spent in open arms are negatively correlated with the anxiety level of the subject.

In the present study, results showed overt effects of grape seed extract ingestion on anxiety like behavior in hypercholesterolemic rats. Grape seed extract produced anxiolytic-like effects on the elevated plus maze as indicated by increased percentage of open arm entries and percentage of time spent in open arm. Grape seed extract administration also decreased immobility time and increased distance travelled in the open field.

Similar to the present finding, proanthocyanidins significantly reduced the anxiety/agitation that occurred during the initial phase of levo thyroxin treatment in hypothyroid patients [25].

Although the underlying cause of anxiety was different, but the finding of Cornelli et al., [25], provides an evidence for the possible anxiolytic effect of proanthocyanidins in human anxiety patients.

In rats, Moreira et al., [26], showed that acute intraperitoneal administration of proanthocyanidin-rich fraction increased the frequency of open arm entries in the elevated plus-maze test consistent with the existence of anxiolytic effects.

Postulated mechanisms for the possible neuroprotective effect of grape seed proanthocyanidins have been reported. They provided near complete protection in terms of DNA damage, as well as abolished apoptotic and necrotic cell death in induced neurotoxicity in mice. They also were shown to modulate cell cycle/apoptosis regulatory genes and to significantly decrease tumor necrosis factor alpha-induced adherence of T-cells [27].
Besides to the previously suggested mechanisms, grape seed extract showed anti-oxidant, anti-lipid peroxidative and anti-apoptotic effects in high cholesterol fed rats in the study of Thiruchenduran et al., [12].

Conclusion:
The present results demonstrated that grape seed extract containing OPCs reduced serum cholesterol level and anxiety-like behaviors associated with high-cholesterol diet.

These results suggest that grape seed extract might be an effective nutritional additive to lower cholesterol level and alleviate anxiety associating hypercholesterolemia.

References
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