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The Effect of Different Doses of Beetroot Juice on Exercise Performance: An Experimental Study

Farklı Dozlardaki Pancar Suyunun Egzersiz Performansına Etkisi: Deneysel Bir Çalışma

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This study was prepared based on the findings of Melike Nur Eroğlu's thesis study titled "The effect of beetroot juice taken at different doses on submaximal running performance" (Bursa: Bursa Uludağ University; 2020).

ABSTRACT Objective: This study aimed to investigate the effects of different doses of beetroot juice (BJ) on submaximal exercise performance in elite male short-distance runners. **Material and Methods:** Twelve elite male short-distance runners (mean age: 19.0±1.54 years, mean body weight: 66.0±4.7 kg, mean height: 176.5±5.3 cm, mean body fat: 7.68±2.78%) voluntarily participated in this single-blind, placebo-controlled, crossover design trial. The study design involved three separate occasions with one-week intervals between sessions. Participants consumed BJ containing different nitrate (NO_3^-) doses (0.04 mmol as placebo, 6 mmol, or 10 mmol) three hours before each exercise trial. Participants performed treadmill exercise tests until exhaustion at 85% HR reserve, during which time to task failure (TTF), HR, ratings of perceived exertion (RPE), and rating of perceived hunger (RPH) were recorded. Blood pressure (BP) was measured before BJ ingestion, pre and post exercise. All trials were conducted between 8 and 12 AM. **Results:** When blood pressures were examined, a statistically significant difference was found the both BJ trials (6 mmol and 10 mmol NO_3^-) compared to placebo trial ($p<0.05$). Significant differences in TTF and RPE were observed only in the 10 mmol NO_3^- trial ($p<0.05$). There were no statistically significant differences in RPH and HR results between the trials ($p>0.05$). **Conclusion:** In male elite short-distance runners, 10 mmol NO_3^- BJ dose may enhance performance, while the 6 mmol NO_3^- BJ dose did not show such an effect.

Keywords: Performance; beetroot juice; ergogenic aids; blood pressure; fatigue

ÖZET Amaç: Bu çalışma, elit erkek kısa mesafe koşucularında farklı dozlardaki pancar suyunun submaksimal koşu performansı üzerindeki etkilerini incelemeyi amaçlamıştır. **Gereç ve Yöntemler:** Tek kör, plasebo kontrollü, çapraz tasarımlı deneme modeli ile yapılan bu çalışmaya on iki elit düzeyde kısa mesafe koşucusu (yaş: 19,0±1,54 yıl, vücut ağırlığı: 66,0±4,7 kg, boy: 176,5±5,3 cm, vücut yağı yüzdesi: 7,68±2,78) gönüllü olarak katılmıştır. Çalışma tasarımı, denemeler arasında bir haftalık ara verilecek şekilde üç ayrı test sürecini içermiştir. Katılımcılar, test sürecinden üç saat önce farklı nitrat (NO_3^-) dozlarını içeren pancar suyu tüketmişlerdir (plasebo olarak 0,04 mmol, 6 mmol veya 10 mmol). Katılımcılar, kalp atım hızı (KAH) rezervinin %85'inde tükeninceye kadar koşu bandında koşmuştur. Bu süre zarfında KAH, hissedilen yorgunluk ve açlık dereceleri kaydedilmiştir. Pancar suyu tüketiminden önce, test öncesi ve sonrası kan basıncı ölçülmüştür. Tüm denemeler sabah saat 8 ile 12 arasında gerçekleştirilmiştir. **Bulgular:** Kan basınçları incelendiğinde; 6 mmol ve 10 mmol NO_3^- pancar suyu denemeleriyle plasebo denemesi arasında istatistiksel olarak anlamlı bir fark bulunmuştur ($p<0,05$). Testi bitirme süreleri ve hissedilen yorgunluk derecelerinde sadece 10 mmol NO_3^- denemesinde anlamlı bir fark görülmüştür ($p<0,05$). Hissedilen açlık dereceleri ve KAH sonuçlarında, denemeler arası istatistiksel olarak anlamlı bir fark bulunmamıştır ($p>0,05$). **Sonuç:** Erkek elit kısa mesafe koşucularında, 10 mmol NO_3^- pancar suyu performansı artırabilirken, 6 mmol NO_3^- pancar suyu dozu böyle bir etki göstermemiştir.

Anahtar Kelimeler: Performans; pancar suyu; ergojenik yardımcılar; kan basıncı; yorgunluk

In a highly competitive setting, maintaining overall health, providing fuel for training, and facilitating recovery afterwards necessitates the consumption of an adequate and well-balanced diet. In addition, athletes use nutritional supplements to increase their performance during the season.[1,2]

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Ergogenic aids are called supports that help a person to exercise, increase exercise efficiency, increase recovery after exercise and/or prevent injury during intense training.[3] One of these supplements is nitrate (NO_3^-). There are many studies that showing that supplements containing NO_3^- have a vasodilating effect, making oxygen use more economical in athletes and prolonging the endurance time of the athlete.[4,5] NO_3^- is converted to nitrite and subsequently to nitric oxide (NO) by various physiological mechanisms in the body. NO plays a significant role in numerous physiological responses, including but not limited to blood flow and pressure regulation, energy metabolism, skeletal muscle perfusion, contractile function, enhancement of muscle mitochondrial efficiency, and promotion of glucose uptake. These physiological changes have a positive effect on the oxidative energy metabolism, which is active during high-intensity endurance sports.[6]

NO functions as a vasodilator in the human body, thereby inducing a reduction in blood pressure (BP). Therefore, unlike other supplements that are valuable for athletes, nitrate supplementation may provide cardiovascular health benefits for both sedentary individuals and athletes by reducing resting BP.[7]

Numerous studies have been conducted to assess both aerobic and anaerobic performance by giving beetroot juice (BJ) containing certain doses of nitrate to elite and non-elite team players and individual athletes doing sports in different branches. In these studies, a number of tests (treadmill tests and bicycle ergometer for aerobic performance and blood lactate level for anaerobic performance, etc.) were used to evaluate aerobic and anaerobic performance.[5,8]

In studies on sports performance, no adverse effect was observed when BJ containing nitrate at various doses ranging from 4.2 mmol to 16.8 mmol/day were given.[9] When the literature were reviewed, few studies comparing different doses were found. For example, in a study examining the ergogenic impact of various doses of BJ ingestion in elite kayakers, it was determined that the performance-enhancing effect of 9.6 mmol NO_3^- consumption resulted in a 1.7% increase in a 500 m test, whereas the 4.8 mmol dosage did not yield a significant improvement in the outcomes of the 1,000 m test.[10]

Although most studies show ergogenic effects of BJ at a supplemental dose of 6-8 mmol NO_3^-, it is emphasized that higher doses should be consumed in elite athletes.[6] The aim of this study was to examine the impact of various doses of BJ on submaximal exercise performance in short-distance runner male athletes.

MATERIAL AND METHODS

PARTICIPANTS

The research group consists of 12 elite short distance runner male athletes who participated voluntarily in this study. Participants were 19.0±1.54 age, 66.0±4.7 kg of body weighted, 176.5±5.3 cm of height, 7.68±2.78% body fat and 60.98±5.16 kg body lean muscle mass. Written informed consent was obtained from each individual after explanation of the experimental procedures and their associated risks. The study was approved by the Bursa Uludağ University Faculty of Medicine Clinical Research Ethics Committee (date: October 6, 2019 no: 2019-17/10) and was conducted in compliance with the guidelines outlined in the 2008 Helsinki Declaration.

FAMILIARISATION

Participants first attended the laboratory for familiarisation, determination of treadmill speed at target heart rate (HR) and anthropometric measurements. During this session, height (m) [stadiometer (Seca, Germany)], body mass (kg), body lean muscle mass (kg) and body fat (%) (Tanita BC 418, Tokyo-Japan) were determined.

A treadmill test was conducted to determine the appropriate treadmill speed that would correspond to 85% intensity of the HR, as calculated using the Karvonen method. Participants were equipped with a smartwatch (Polar V800, Kempele, Finland) and a heart rate monitor band, before commencing running on a treadmill (Profitness 3600, Taiwan) set at 0% inclination and a speed of 8 km/h. The treadmill speed was increased to 10 km/h after 3 minutes, followed by an incremental increase of 2 km/h every 3 minutes until the athlete reached exhaustion. Throughout the test, the participants' HR were monitored, and the corresponding treadmill speed for the target HR was determined.

EXPERIMENTAL DESIGN

The study design was randomized cross-over, placebo-controlled and single-blind. Three separate occasions (one week between session) as they arrived at the laboratory, participants were provided with BJ containing either different N03 doses. Before intaking BJ and pre-exercise, resting BP was measured. Three hours after intaking the BJ, all participants performed treadmill exercise test until exhaustion at 85% HR reserve. During the exercise, time to task failure (TTF), HR, BP, ratings of perceived exertion (RPE), rating of perceived hunger (RPH) were measured. BP measurement was repeated after the exercise. All trials were performed between 8 and 12 AM.

HR DATA COLLECTION

During the exercise, a smart watch and a HR band (Polar V800, Kempele, Finland) were used to record HR with application [Elite HRV (Heart Rate Variability), NC, USA].[11]

PHYSICAL EXERCISE PROTOCOL

Participants walked on a treadmill at 5.5 km/h at a 1% incline for 5 minutes, then ran at 85% of their HR reserve until exhaustion occurred at their predetermined running speeds. Participants were encouraged to continue the trial as long as possible. The exhaustion point was set as the end time of the trial.

BP MEASUREMENT

Participants rested for 10 min both before intake BJ and when they arrived for the trial, and resting BP were measured with an electronic sphygmomanometer (Omron M2, Kyoto, Japan).[12] The measurement was repeated 5 minutes after the exercise.

RPH

The visual analogue scale (VAS) was employed to RPH.[13] During exercise, participants' hunger ratings were assessed every 5 minutes using the VAS (10 cm) and the average scores were recorded.

RPE

For the measurement RPE, the Borg Scale with a score between 6-20 points was used.[14] During exercise (every 5 minutes), the participants were asked about the RPE and the average scores were recorded.

DIETARY AND EXERCISE-TRAINING STANDARDIZATION

Training during the 24-hour period before each performance trial was standardized for each individual by scheduling each testing day for an identical time of the training week. Dietary intake was standardized during the 24-hour period before each trial using a combination of replication of usual diet and standardized diet techniques. Specifically, subjects were instructed to follow their normal training diet and fluid intake for 24 hours before each trial but were asked to refrain from all caffeine and alcohol during this period. Participants were informed about the foods containing high amounts of NO_3^- (spinach, beetroot, carrot, radish, lettuce, etc.) that they should avoid 48 hours before the day they of the tests. Subjects were also instructed to avoid using chewing gum and mouthwash (to preserve oral bacteria that may facilitate NO_3^- reduction) and refrain from all other ergogenic supplements before trial. During the 3-hour period before each trial, participants received no food or liquid other than water to ensure hydration status. Instructions on how to keep a food diary were provided.[6] A copy of the food diary kept before the first trial was provided to subjects with the instructions to replicate this eating pattern as closely as possible before subsequent trials, with a further record being kept to assess compliance. On arrival at the laboratory on a trial day, subjects' diaries were qualitatively assessed for compliance with these dietary instructions. Semiquantitative analysis of these dietary records revealed that all subjects complied with the study requirements, avoiding the specified dietary constituents.

BEVERAGE INTAKE

The nitrate content in BJ varies depending on the soil and the season in which it is cultivated. In the study, red beet roots grown in the fields of Beypazarı were used. Before the study, the BJ was analysed by TUBITAK Bursa Test and Analysis Laboratory Directorate with the decision dated 04.02.2019 and numbered GT20190028 and the NO_3^- in 1 litre of BJ was reportes in mg and mmol value. According to the report, placebo containing 0.4 mmol (24.5 mg) was calculated 10 mL, BJ containing 6 mmol (367 mg) NO_3^- was calculated 146 mL and BJ containing 10 mmol (611 mg) NO_3^- was calculated 244 mL.

Participants were informed that they would consume three different BJ mixtures throughout the study, which were presented in dark-colored bottles. According to the results of the analysis, BJ corresponding to 6 and 10 mmol nitrate are completed with water to 250 mL. The placebo beverage was prepared by adding water, mineral water, and enough BJ (10 mL) for taste. The participants were not given any information about the BJ mixture, and there was no discussion about the content and benefits of the drinks. This approach aimed to prevent participants from making assumptions about potential benefits. Participants were aware that they were consuming BJ mixtures each time, but they did not know the source of the taste difference and the exact content of the beverages.[15]

STATISTICAL ANALYSIS

Descriptive statistics such as arithmetic means and standard deviations were calculated for the demographic information and parameters obtained from the data collection tools. In the data analysis, a two-way analysis of variance (ANOVA) was employed for repeated measures. When sphericity assumption could not be met in the between-group comparisons, analysis was conducted using Greenhouse-Geisser corrections. Post-hoc testing using the LSD (Least Significant Difference) test was performed when significant differences were detected between groups. All statistical analyses were conducted using the SPSS 22 (IBM, USA) software package, and significance levels were set at 0.05.

RESULTS

Of the 12 participants, only 2 were able to correctly guess content of BJ, indicating that the blinding of the BJ had been successful. No major adverse reactions were reported by any participant.

The results of each BJ trial are presented in Table 1. According to two-way repeated measures placebo and BJ trials demonstrated a significant effect on TTF, RPE, (p=0.034, p=0.028, respectively). When comparing the effects between doses, it was observed that the 10 mmol dose significantly extended the TTF and reduced fatigue compared to the placebo condition (p=0.013). However, no significant difference in effects was found between the 6 mmol dose and the placebo trial (p=0.085). Furthermore, there were no statistically significant effects observed on HR and RPH (p=0.455, p=0.61, respectively).

Two-way repeated measures analysis did not demonstrate a significant time x trial interactive effect on systolic blood pressure (SBP) (p=0.051). However, a significant difference was observed in the effects of all trials and times on SBP (p=0.00, p=0.02, respectively) (Figure 1).

Two-way repeated measures analysis did not demonstrate a significant time x trial interactive and time effect on diastolic blood pressure (DBP) (p=0.494, p=0.142, respectively). However, significant variation was observed when examining the overall effects of the trials on DBP (p=0.00) (Figure 2).

DISCUSSION

The results of our study suggest that 10 mmol BJ increased TTF during treadmill exercise, reduced RPE, and decreased both SBP and DBP. However, our

TABLE 1: Effects of nitrate supplementation on treadmill exercise measures.

Variable	Placebo	6 mmol	10 mmol	f	p value
TTF (min)	14.03±2.33	15.13±2.25	18.56±3.17[a,b]	5.696	0.034*
HR	176.25±6.31	175.92±4.27	176.83±5.68	0.742	0.455
RPE_{mean}	11.08±3.37	10.42±2.27	9.17±2.36[a,b]	8.874	0.028*
RPH_{mean}	3.22±1.907	3.25±2.179	3.19±2.125	0.203	0.61

Values are means±standard deviation; [a]p<0.05 compared to placebo; [b]p<0.05 compared to 6 mmol; HR: Heart rate; TTF: Time to task failure; RPE: The rating of perceived exertion; RPH: The rating of perceived hunger.

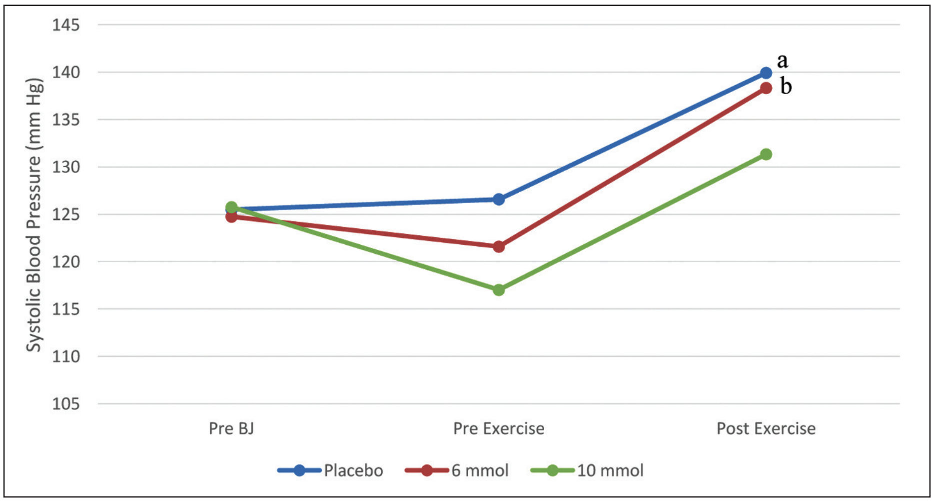

FIGURE 1: Effects of BJ dose trial on systolic blood pressure on pre BJ, pre and post exercis;. BJ: Beetroot juice; [a]One-way ANOVA: Significant trial effect between placebo and 10 mmol NO3 (p=0.00); [b]One-way ANOVA: Significant trial effect between placebo and 6 mmol NO3 (p=0.011).

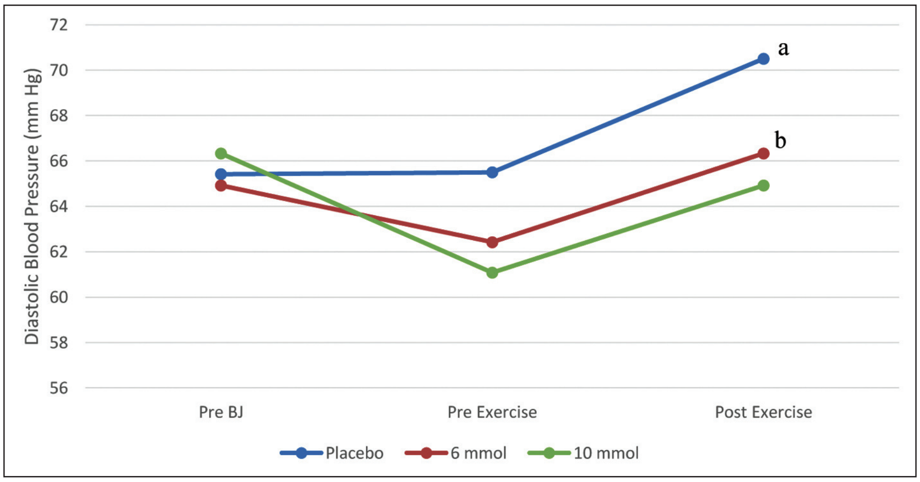

FIGURE 2: Effects of beetroot juice dose trial on diastolic blood pressure on pre BJ, pre and post exercise; BJ: beetroot juice; [a]One-way ANOVA: Significant trial effect between placebo and 10 mmol NO3 (p=0.00); [b]One-way ANOVA: Significant trial effect between placebo and 6 mmol NO3 (p=0.00).

findings did not demonstrate any significant effects of 10 mmol BJ on HR and RPH. Except for TTF and RPE, the effects of the 6 mmol and 10 mmol trials were similar.

Based on the available references, it appears that dietary nitrate supplementation may have a positive effect on submaximal running time. Several studies have reported that dietary nitrate supplementation can improve exercise performance, including submaximal running time. For example, a study found that acute supplementation of L-arginine and nitrate improved submaximal running economy at 10 km/h and 14 km/h, as well as 5-km time-trial running performances in elite athletes.[16] Similarly, another study reported that dietary nitrate supplementation with BJ reduced the O_2 cost of submaximal walking and running exercise tolerance in young healthy males and improved submaximal exercise endurance in club level cyclists.[17] Other studies have also reported that dietary nitrate supplementation can reduce the oxygen cost of submaximal exercise and increase exhaustion time at submaximal workloads.[1,18] Finding of the current our study is that a 10 mmol of NO_3^- BJ may improve (by ~5 min) submaximal running performance in elite short distance runners; however, a 6 mmol dose is unlikely to elicit an ergogenic effect. However, some studies have found no significant effects of dietary nitrate supplementation on exercise performance.[19] The different results observed in the investigations could be attributed to variances in the participants' training status and the timing of the supplementation. Overall, more research is needed to determine the effects of dietary nitrate on submaximal running time.

BJ is known to provide cardiovascular benefits through its effects on blood circulation and oxygen cost due to its high NO_3^- content.[6] In our study, BJ did not affect the mean HR during exercise. The results regarding HR seem to be consistent with previous studies.[4,12,20,21] A study by Muggeridge et al. found that acute ingestion of BJ did not significantly alter heart rate during submaximal exercise in professional cyclists.[22] However, a study by Lansley et al. found that chronic ingestion of BJ for 6 days significantly reduced resting HR in professional soccer players.[5] In a study by Cermak et al., acute ingestion of BJ was found to significantly reduce HR during submaximal exercise in well-trained cyclists.[4] Overall, the effects of BJ on HR in professional athletes appear to be mixed and may depend on various factors such as the duration and dose of BJ ingestion, the type of exercise performed, and the specific population studied.

Several studies have reported that dietary nitrate supplementation can improve exercise tolerance and reduce muscle fatigue, as well as lower the perception of effort and leg muscle pain during exercise.[9,23,24] As in this study, Kent et al. in their study looking at the repeated sprint performance of 12 male team athletes under hypoxic conditions, found an improvement in RPE between groups consuming placebo and BJ containing 13 mmol NO_3^-.[21] We also found that 10 mmol nitrate was effective for elite short distance runners (by ~-2 points). However, in a study with a 5 km run, the RPE in the last 1.8 km was found to be lower with BJ.[25] Another study (n=8; m=5, f=3) reported no significant difference in RPE between the BJ (13 mmol NO_3^-) and placebo group.[26] The contrasting results can be attributed to the timing of BJ consumption and differences in the sample population.

A well-established effect of dietary nitrate supplementation is a reduction in SBP either with or without a concomitant reduction in DBP.[7,8,27-29] The reduction in BP has been suggested to result from the reduction of nitrite to NO, which is known to promote endothelial relaxation via its role in cyclic guanosine monophosphate synthesis.[30] Webb et al. reported that peak reductions in BP occurred 2.5 to 3-h post ingestion, with changes in SBP persisting for longer than changes in DBP.[7] Consistently, we observed a significant reduction in systolic SBP with a dosage of 10 mmol (~6 mmHg) and a dosage of 6 mmol (~2 mmHg). In terms of DBP, we also observed a decrease of 5 mmHg in both the 6 mmol and 10 mmol dosage groups compared to the placebo trial.

According to the available references, there is no direct evidence on the effect of BJ on RPH. In a study, BJ has been found to inhibit key carbohydrate-metabolizing enzymes associated with type II diabetes.[31] In a separate study, taking into account the beneficial effects of BJ through its impact on NO, it has been suggested as a potential component of therapeutic interventions for patients with insulin resistance.[6,32] Considering these observed effects, we aimed to investigate its potential impact on athletes. But, our findings did not reveal any significant effect of BJ on RPH.

Our study has several limitations. BJ is a supplement rich in NO_3^- known to elevate levels of circulating nitrite and NO.[33] However, in our study, we did not measure these levels prior to the participants' intake of either placebo or BJ. Furthermore, the sample size of our study, consisting of 12 subjects, although appropriate for this type of investigation, may have limited our ability to detect subtle changes that could arise from BJ administration.

CONCLUSION

The results of our study suggest that 10 mmol BJ increased TTF during treadmill exercise, reduced RPE, and decreased both SBP and DBP. However, our findings did not demonstrate any significant effects of 10 mmol BJ on HR and RPH. Except for TTF and RPE, the effects of the 6 mmol and 10 mmol trials were similar. These findings suggest that BJ can be a dependable ergogenic supplement that can enhance both cardiovascular health and sports performance. However, further studies involving elite athletes are required to validate the results of this investigation.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

***Idea/Concept:** Şerife Vatansever, Melike Nur Eroğlu; **Design:** Şerife Vatansever, Melike Nur Eroğlu; **Control/Supervision:** Şerife Vatansever, Melike Nur Eroğlu; **Data Collection and/or Processing:** Şerife Vatansever, Melike Nur Eroğlu; **Analysis and/or Interpretation:** Şerife Vatansever, Melike Nur Eroğlu; **Literature Review:** Şerife Vatansever, Melike Nur Eroğlu; **Writing the Article:** Şerife Vatansever, Melike Nur Eroğlu; **Critical Review:** Şerife Vatansever; **References and Fundings:** Şerife Vatansever, Melike Nur Eroğlu.*

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