

EVALUATION OF SEDATIVE EFFECTS OF DAILY CONSUMPTION OF YOGHURT IN RATS

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ABSTRACT

Yoghurt is one of the most popular fermented dairy foods worldwide and possesses several health-beneficial properties. Yoghurt provides numerous nutrients and bioactive components which may modulate many physiological and psychological processes. This study aimed to assess the possible sedative effects of daily consumption of yoghurt in normal, healthy rats. The plain yoghurt was made directly using fresh cow's milk and starter culture. Rats were weighed, randomly divided into two groups (12 rats in each group), and orally gavaged once a day for seven consecutive days as follows: Control group (distilled water, 2 ml/100 g b.wt) and yoghurt group (plain yoghurt, 2 ml/100 g b.wt). Motor activity and exploratory behavior of the animals were measured actimetry, using the infrared LE8811 Actimeter Panlab system, in order to count the number of global, locomotor, and stereotype activities, number of rearing, total distance travelled, mean velocity/speed, and resting time. Daily consumption of yoghurt was associated with a significant reduction of rat global, locomotor, and stereotyped activities compared to control group. The total distance traveled, mean speed, and number of rearing also decreased but not reaching significance. Interestingly, daily consumption of yoghurt accentuated resting state in rats. Together, these data demonstrate for the first time that daily consumption of yoghurt for seven consecutive days could decrease rat motivity and promote calmness resulting in a sedative effect, which may be effective for the treatment of anxiety and depression.

Keywords: Yoghurt; Sedation; Psychomotor Activity; Rats

INTRODUCTION

Consumption of fermented dairy foods has increased in nearly all world regions, but at different speeds (Deeth and Tamime, 1981). Yoghurt is the major dairy food made from the milk by lactic acid fermentation through the action of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* culture (Tamime and Robinson, 2007). In some countries, *Lactobacillus helveticus* and *Lactobacillus lactis*, are mixed with the previous starter culture (McKinley, 2005). When a large amount of lactic acid produced, the milk coagulated and called yoghurt (Tamime and Robinson, 1999).

Yoghurt can be classified as plain/natural, fruit or flavored yoghurt based on flavor and set, stirred, drinking or frozen yoghurt based on the manufacturing methods (Shah *et al.*, 2000). Yoghurt

can be also classified into two different groups namely, standard culture yoghurt and bio- or probiotic yoghurt (Chandan and Kilara, 2013).

Yoghurt is considered as a functional highly nutritious food that contains essential nutrients such as carbohydrates, proteins, lipids, vitamins, and minerals (Tamime and Robinson, 2007). Apart from the nutritive constituents, consumers can intake the active bacterial culture and bioactive components formed during fermentation from yoghurt (EFSA, 2010; Zare *et al.*, 2011; Murette *et al.*, 2017). Yoghurt consumption has many health benefits in terms of enhancing immunity, controlling inflammation, modulating gut microbiota, and improving cholesterol metabolism (Frias *et al.*, 2016; Murette *et al.*, 2017).

Yoghurt was regarded as a potential vehicle for probiotic (Tamime and Robinson, 2007; CDC, 2009; Frias *et al.*, 2016; Murette *et al.*, 2017). Probiotic bacteria are living organisms that can inhabit the gut and contribute to the health of the host (Gareau *et al.*, 2010). Accumulating clinical evidence suggests that probiotics can modulate the stress response and

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improve mood and anxiety symptoms in patients with chronic fatigue and irritable bowel syndrome (Silk *et al.*, 2009). Yoghurt also contains several nutrients with potential sleep-promoting properties (Milind and Jyoti, 2014).

In an attempt to understand the impact of daily yoghurt consumption in the modulation of physiological and psychological processes in healthy rats, we undertook an open-field test to evaluate the locomotion and exploration of a novel environment as an index for the fear and anxiety levels. Additionally, to determine some possible sedative effects of daily consumption of yoghurt in rats we evaluated its influence on the three types of information regarding the animal spontaneous motor activity (global, locomotor, and stereotyped activities) and number of rearing.

MATERIALS AND METHODS

Ethical Approval

Animal handling and experimentation were performed in line with approved Institutional Animal Care and Use Committee (IACUC#: 12-214) protocols at the South Valley University (Qena, Egypt) and complied with the ethical standards established by the Egyptian animal welfare laws and policies and followed the national authority (Ministry of Higher Education and Scientific Research, Egypt) guidelines for the detention, use and the ethical treatment of laboratory animals. Also, all animal protocols were approved by the Animal Use Subcommittee and by the Research and Ethical Review Committee of the Faculty of Veterinary Medicine, South Valley University.

Experimental Animals

Adult male albino rats were purchased from the animal house of the Faculty of Medicine, Assiut University, Assiut, Egypt and shipped to our facility in environmentally controlled ground vehicles. The animals were housed in cages (55 x 40 x 20-cm), under standard laboratory conditions (22 ± 2°C temperature, 60 ± 5% humidity, in a 12-h light-dark cycle), with food and water *ad libitum*. Environmental factors (such as cage type and size, colony size, bedding, and environmental enrichment) were standardized between cages. They were kept for two weeks under this condition to adapt the laboratory conditions before the start of the experiment.

Yoghurt's Starter Culture

Commercial yoghurt culture (YC-X11, Thermophilic yoghurt culture - YoFlex[®]), containing *Streptococcus Thermophilus* and *Lactobacillus Bulgaricus*, was obtained from CHR-Hansen, Horsholm, Denmark.

Plain Cow's milk Yoghurt preparation

Fresh cow's milk was pasteurized at 90°C for 20 min and cooled to 45°C. After cooling, pasteurized cow's milk was inoculated with 3% (v/v) Yoghurt's starter

culture (YC-X11) and incubated at 45°C for 4 h until complete coagulation (Aswal *et al.*, 2012). Yoghurt samples were cooled to refrigerator temperature (~4±1°C) and stored for 7 days.

Experimental Design

The experiment was carried out on twenty-four adult male albino rats (200-250 g) distributed in two groups of 12 animals each treated as follows:

Group I (Control): Rats were gavaged orally with distilled water (2 ml/100 g body weight/day) for one week and fed unrestricted amounts of a standard chow diet.

Group II (Yoghurt): Rats were gavaged orally with plain yoghurt (2 ml/100 g body weight/day) for one week and fed unrestricted amounts of a standard chow diet. The dose of yoghurt was 2 ml/100 g body weight/day, whose consumption is equivalent to the children consumption of 100 ml yoghurt/day (WHO, 2006).

Behavioral studies

The open field activity measurement was performed as previously described (Prut and Belzung, 2003; Araki *et al.*, 2015). Briefly, rat's motor activity and exploration of a novel environment were assessed using an infrared LE8811 Actimeter system (Panlab, Barcelona, Spain). The test apparatus consisted of a 45 cm (width) x 45 cm (depth) arena of black plexiglass enclosed with four clear acrylic walls (35 cm in height) (Fig. 1). A square frame mounted outside of the arena created a 16 x 16 grid of intersecting infrared beams used to track the motor activity of each rat in real-time. A second frame placed above the lower frame was used to track the number rearing of each rat, set to detect hind-leg rearing.

Locomotor measures were recorded using ActiTrack software (Panlab), which uses infrared beam data to calculate the following parameters: (i) number of spontaneous global activity (total number of beam breaks); locomotion with displacement, and stereotyped movements without displacement; (ii) number of rearing; (iii) total distance travelled in the actimeter (cm); (iv) mean velocity/speed (cm/s); and (v) time spent in resting (in sec or as %). The recorded data are easily exported in a format compatible with the Excel program for Windows, through SeDaCom32 computer software.

In the 7th day, testing was conducted during the light phase between 07:30 h and 12:30 h for all rats. Rats were brought into the testing room in their home cage a half hour prior to testing for habituation. Each rat was then released near the wall of the arena and left undisturbed to explore freely the new environment for a period of 10 min. This minimized stress to the animals. Measurements were only recorded during the final 5 min (first 5 min was used only for animal acclimatization).

During testing, the lighting of the experimental room was about 400 lx so as not to inhibit normal exploratory behavior. At the end of testing, the rat

was removed and returned to its home cage and the surface and walls of the arena were wiped clean with 30% isopropanol.

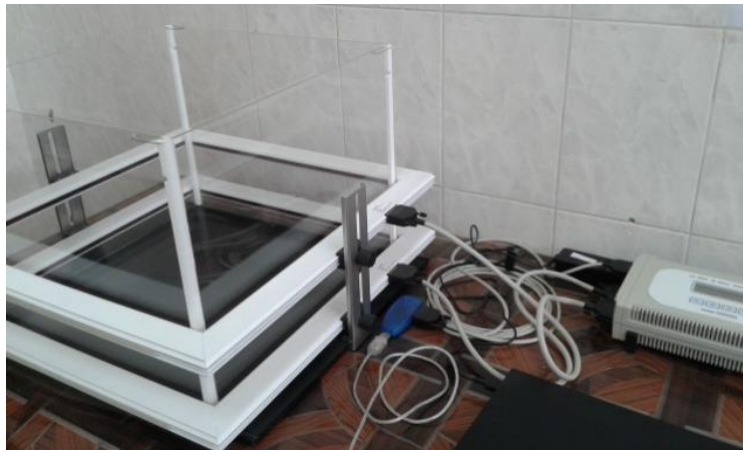


Fig. 1: Infrared LE8811 Actimeter (Panlab) system for motor activity tracking in rodents. A Novel open-field apparatus connects with a sample ActiTrack real-time tracking output. Lower frame is used to track motor activity, while the upper frame tracks number of rearing.

Data Analysis and Statistics

All data were expressed as the mean \pm standard error of the mean (SEM). Plots were constructed with SigmaPlot 2001 for Windows, Version 7.0 software (SPSS Inc., Chicago, IL, USA). Statistical analysis of the behavioral data was done with Microcal™ Origin 6.0 software (Microcal Software Inc., Northampton, MA, USA) by student's *t* test. Statistical significance was established when $P < 0.05$.

RESULTS

To study the effects of daily consumption of yoghurt on modulation of the physiological and emotional states in normal, healthy rats, the plain cow's milk yoghurt has been administered orally via gavage for

seven consecutive days. Sedative effect corresponded to a decrease in the percentage of motor activity compared with the control group (Girzu *et al.*, 1997).

Effect of daily yoghurt consumption on the spontaneous global activity

Spontaneous global activity consisted of the sum of locomotion and stereotyped movements. The oral gavage of yoghurt (2 ml/ 100 g b wt/day) for 7 days was associated with a significant ($p < 0.05$) decrease of rat spontaneous global activity (243.64 ± 41.38), compared to control group (421.25 ± 68.66) in this experimental behavioral model using normal, healthy rats (Fig. 2).

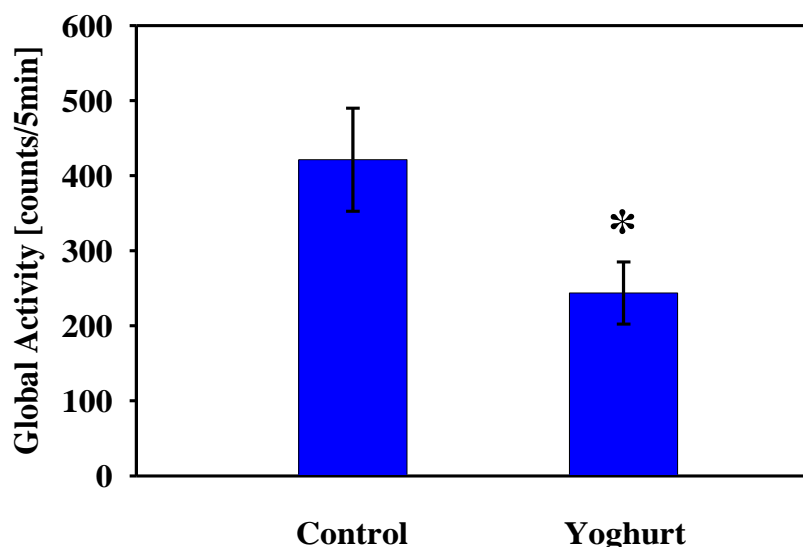


Fig. 2: Global activity in the open-field test. Data shown represent the mean (\pm SEM) infrared beam breaks by adult male albino rats 7 days after oral administration of distilled water (in control group, $n = 11$ rats) or plain yoghurt (in yoghurt group, $n = 12$ rats). * $p < 0.05$ vs control.

Effect of daily yoghurt consumption on the locomotor activity (locomotion)

Ambulatory locomotor activity (locomotion) refers to the movement made by the rat during the analyzed interval, i.e., distance walked in each sample where the position of the rats is different from the position of the previous sample and different from the position of the second sample back in time (Carino *et al.*,

2017).

Daily oral administration of yoghurt significantly ($p < 0.05$) decreased the locomotor activity of rats (226.00 ± 37.78), compared to control group (388.92 ± 63.81) in this experimental behavioral model (Fig. 3).

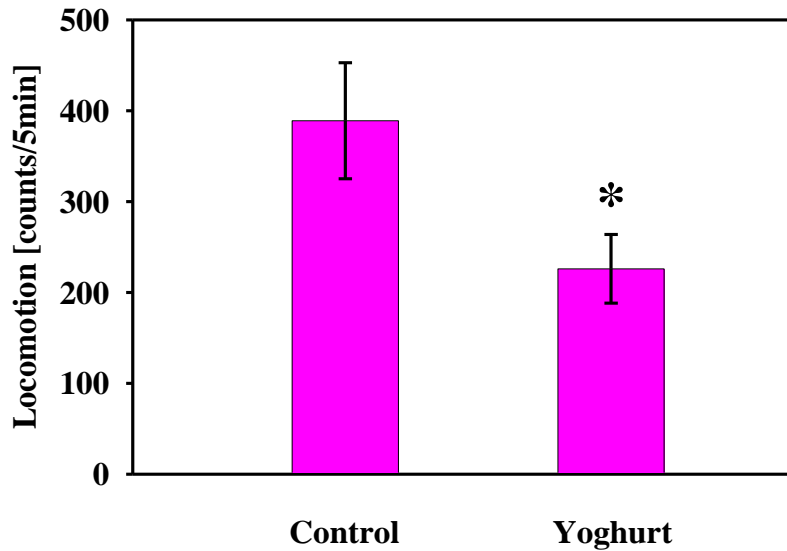


Fig. 3: Locomotion in the open-field test. Data shown represent the mean (\pm SEM) infrared beam breaks by adult male albino rats 7 days after oral administration of distilled water (in control group, n =11 rats) or plain yoghurt (in yoghurt group, n = 12 rats). * $p < 0.05$ vs control.

Effect of daily yoghurt consumption on the stereotyped movements

Stereotyped movements during the analyzed interval, indicate the number of samples where the position of the rats is different from the position of the previous sample and equal to the position of the second sample

back in time (Carino *et al.*, 2019).

The treatment with yoghurt in rats (yoghurt group) was associated with a statistically significant ($p < 0.05$) decrease of the rat stereotyped movements (17.64 ± 4.07), comparing with the control group (32.33 ± 5.25), during the session of experimentation (Fig. 4).

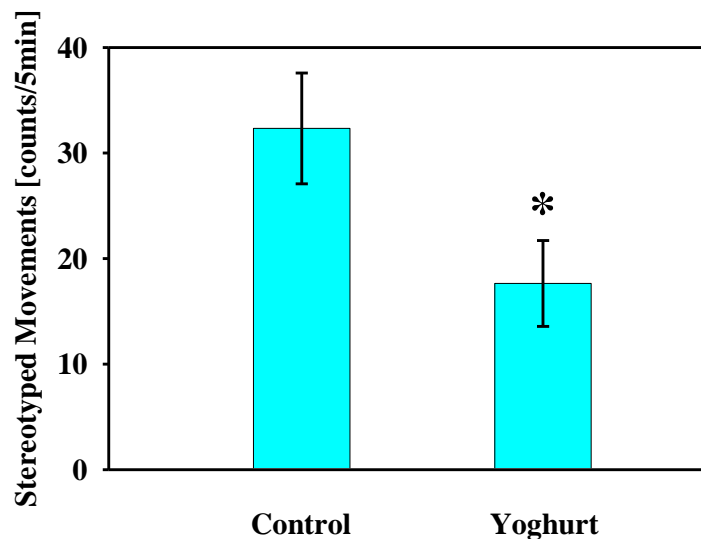


Fig. 4: Stereotyped movements in the open-field test. Data shown represent the mean (\pm SEM) infrared beam breaks by adult male albino rats 7 days after oral administration of distilled water (in control group, n =11 rats) or plain yoghurt (in yoghurt group, n = 12 rats). * $p < 0.05$ vs control.

Effect of daily yoghurt consumption on the number of rearing

The animals administered with yoghurt showed a marked decrease of the number of rearing (3.27 ± 0.76), but not reaching significance compared to

control group (8.58 ± 3.12), in this behavioral test (Fig. 5A). While, there was nearly no effect of yoghurt consumption on mean duration of rearing compared to control group (Fig. 5B).

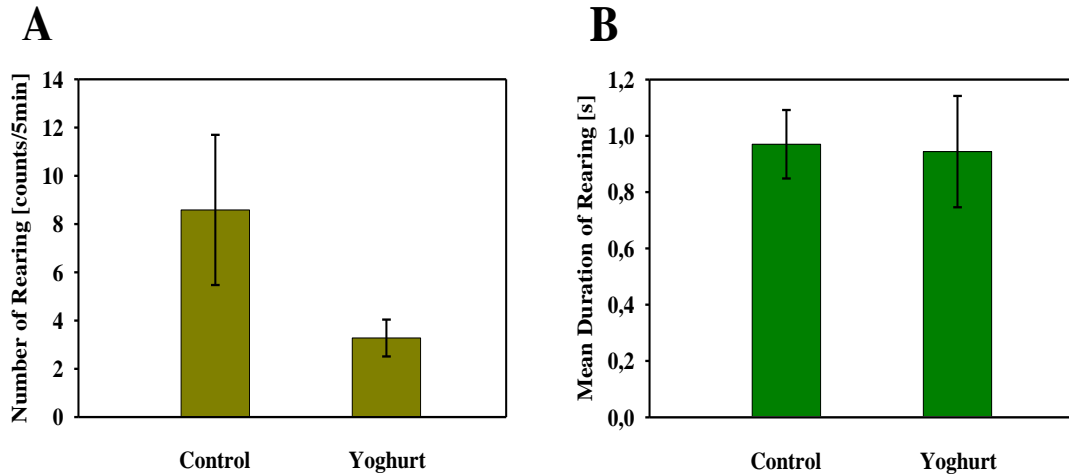


Fig. 5: Number and mean duration of Rearing in the open-field test. Data shown represent the mean (\pm SEM) number of rearing (A) and rearing duration in seconds (B) of adult male albino rats 7 days after oral administration of distilled water (in control group, n =11 rats) or plain yoghurt (in yoghurt group, n = 12 rats).

Effect of daily yoghurt consumption on the total distance travelled

The animals administered yoghurt showed a marked decrease of the total distance travelled ($324.35 \pm$

65.31), but not reaching significance compared to control group (610.88 ± 121.91), in this behavioral test (Fig. 6).

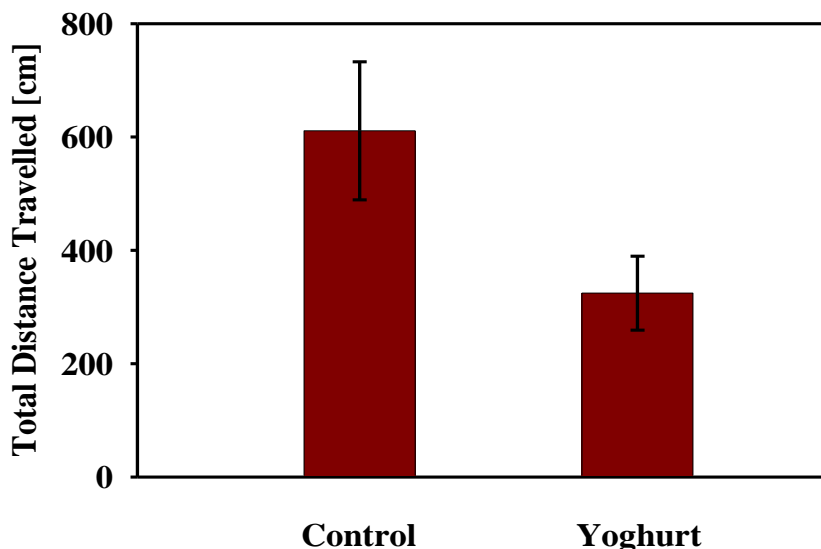


Fig. 6: Total distance travelled (cm) in the open-field test. Data shown represent the mean (\pm SEM) total distance travelled (cm) by adult male albino rats 7 days after oral administration of distilled water (in control group, n =11 rats) or plain yoghurt (in yoghurt group, n = 12 rats).

Effect of daily yoghurt consumption on the mean velocity/speed

The animals which were administered with yoghurt showed a great decrease of mean velocity/speed

(1.08 ± 0.22), but not reaching significance, compared to control group (2.03 ± 0.41), in this behavioral test (Fig. 7).

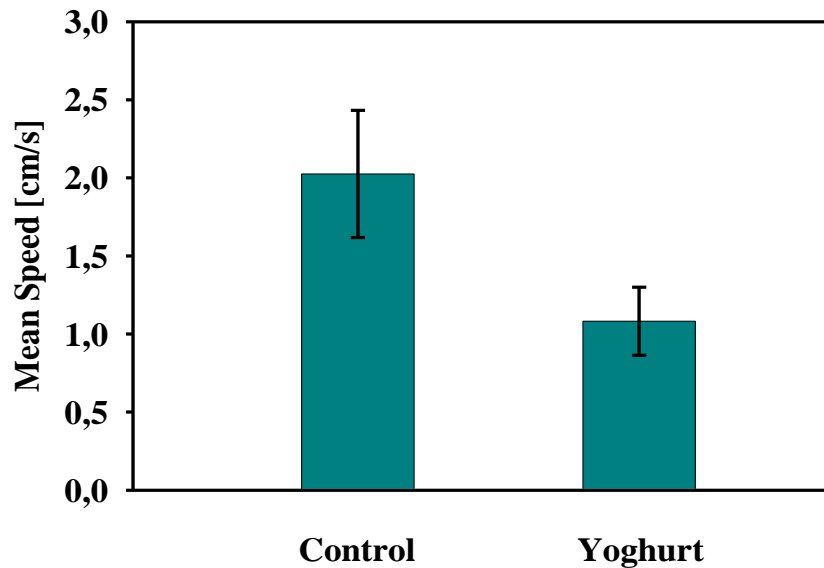


Fig. 7: Average speed in the open-field test. Data shown represent the mean (\pm SEM) average speed (cm/s) of adult male albino rats 7 days after oral administration of distilled water (in control group, n =11 rats) or plain yoghurt (in yoghurt group, n = 12 rats).

Effect of daily yoghurt consumption on the resting time

Resting time consists of sleeping, cleaning and eating time. The animals which were administered with yoghurt (yoghurt group) showed a significant

($p < 0.05$) increase of mean resting time (232.11 ± 10.47), compared to control group (183.62 ± 16.59), in this behavioral test (Fig. 8A). The percentage of resting time of yoghurt fed animals was increased by 16% compared to control group (Fig. 8B).

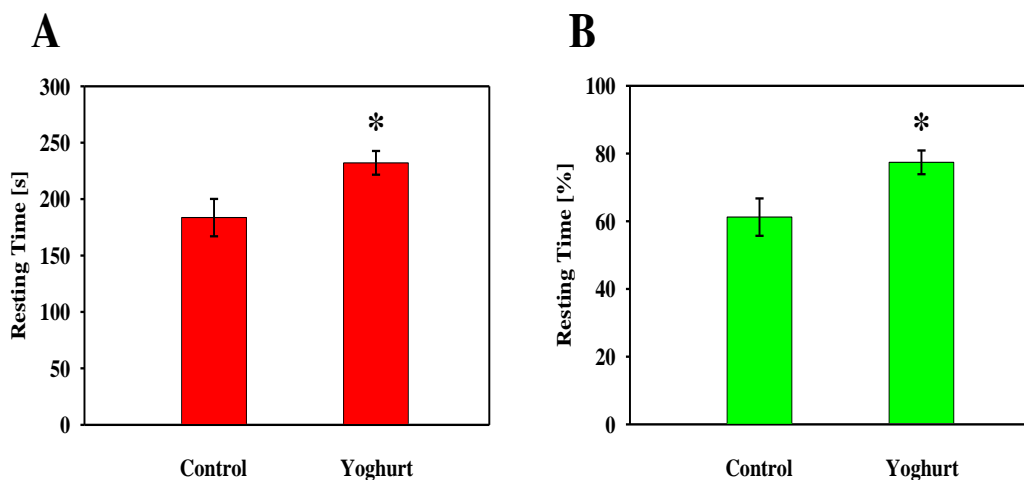


Fig. 8: Resting time in the open-field test. Data shown represent the mean (\pm SEM) rest time in seconds (A) or as the percentage of total time, 5 minutes (B), of adult male albino rats 7 days after oral administration of distilled water (in control group, n =11 rats) or plain yoghurt (in yoghurt group, n = 12 rats). * $p < 0.05$ vs control.

DISCUSSION

The open-field test (actimetry) is a widely used model for the evaluation of emotional responses of the animals, especially the rodents ((Boissier and Simon, 1965). We use a fully-automated infrared actimeter system to record the spontaneous psychomotor

abilities and cognitive functions in normal, healthy rats. We obtained the number of locomotor activity, stereotyped movements, and rearing, in a definite session of time. The locomotor activity and number of rearing indirectly signify the animal state of fear and anxiety of the new environment (Kas *et al.*, 2008; Hart *et al.*, 2010). On the other hand, the stereotype

movements suggest the self-maintenance of animal personal hygiene (Lynch *et al.*, 2011).

Locomotion is dependent on proper control of muscle activity. Muscle relaxation is commonly associated with sedation (Bonetti *et al.*, 1982), which may be indicated by less locomotion in the actimeter. Myorelaxation may also impair the exploratory activity of the rats and thus affect their performance in the actimeter. The obtained results show that in the experimental behavioral model using normal, healthy rats, yoghurt consumption for seven consecutive days diminished both motor activity and the number of rearing. This could correspond somehow to sedation in humans.

The previous study by Jaatinen *et al.* (2014) showed that daily consumption of yoghurt enriched with bioactive components may aid in stress coping. Yoghurt rich in *Lactobacillus*, a probiotic bacterium, may help alleviate symptoms of depression and anxiety, a finding that could lead to new strategies for treating psychiatric conditions (Marin *et al.*, 2017) and insomnia (Yamamura *et al.*, 2009). Moreover, tryptophan is present in small amounts in most protein foods and in higher amounts in yoghurt, milk, oats, bananas, dates, poultry, eggs and peanuts (Kitano *et al.*, 2014). After consumption, tryptophan is metabolically transformed to bioactive metabolites, including serotonin and melatonin (Friedman, 2018). Both of these are brain chemicals that induce relaxation and sleepiness (Peuhkuri *et al.*, 2012).

In this study, daily yoghurt consumption produces a significant decrease in motivity compared with rats treated with distilled water alone. Furthermore, daily consumption of yoghurt accentuates resting time in normal, healthy rats. This sedative and hypnotic effect of the yoghurt could contribute to impacts of yogurt's components (e.g., probiotics, tryptophan, and vitamins) that induce relaxation and calmness.

CONCLUSIONS

The present study demonstrates for the first time that daily consumption of plain cow's milk yoghurt possesses significant sedative and hypnotic-like features that may have a potential therapeutic value for treatment of anxiety and depression symptoms in patients with sustained and systemic convulsions. However, further precise studies are required to verify its activity in other experimental models and its precise mechanism of action should be determined.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding publication of this paper.

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تقييم التأثيرات المهدنة للاستهلاك اليومي للزبادي في الفئران

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الزبادي هو واحد من منتجات الألبان المخمرة الأكثر شعبية في جميع أنحاء العالم وله العديد من الفوائد الصحية. يوفر العديد من العناصر الغذائية والمكونات النشطة بيولوجيا والتي قد تؤثر على العديد من العمليات الفسيولوجية والنفسية. تهدف هذه الدراسة إلى تقييم التأثيرات المهدنة للاستهلاك اليومي للزبادي في الفئران. تم تحضير الزبادي باستخدام حليب البقر الطازج والبادئ. تم وزن الفئران، قسمت بشكل عشوائي إلى مجموعتين (١٢ فأر في كل مجموعة) ، وتُعطى عن طريق الفم مرة واحدة يوميًا لمدة سبعة أيام متتالية على النحو التالي: مجموعة التحكم (الماء المقطر، ٢ مل / ١٠٠ جم) ومجموعة الزبادي (زبادي، ٢ مل / كجم). تم تقييم النشاط الحركي والسلوك الاستكشافي للحيوانات باستخدام جهاز مقياس النشاط، من أجل قياس عدد الأنشطة الكلية والحركية والنمطية، عدد مرات الوقوف عالياً، المسافة الإجمالية المقطوعة، متوسط السرعة، ووقت الراحة. ارتبط الاستهلاك اليومي للفئران من الزبادي مع انخفاض كبير في النشاط الكلي والحركي والنمطي مقارنة بمجموعة التحكم. المسافة الإجمالية المقطوعة وعدد مرات الوقوف عالياً انخفضت أيضا ولكن لم تصل إلى الفرق المعنوي. ومن المثير للاهتمام، تناول الزبادي يوميا يبرز حالة الراحة في الفئران. في الختام، يمكن للاستهلاك اليومي للزبادي أن يقلل من الأنشطة الحركية النفسية ويعزز الهدوء الذي ينتج عنه تأثير مهدئ، والذي قد يكون مفيداً في الطب في علاج بعض المشاكل النفسية مثل القلق والإكتئاب.