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ANXIOLYTIC-LIKE EFFECTS OF SYRINGIC ACID: A BEHAVIORAL STUDY

SİRİNJİK ASİDİN ANKSİYOLİTİK BENZERİ ETKİLERİ: DAVRANIŞ ÇALIŞMASI

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ABSTRACT

Objective: The current study investigated the possible anxiolytic-like effects of syringic acid (SA) administration against deltamethrin (DTM) exposure in rats subjected to behavioral tests.

Materials and Methods: Wistar albino male rats weighing 250-270 g were randomly divided into four groups as control (0.5 mg/kg corn oil), DTM (1.28 mg/kg), SA (25 mg/kg) and DTM+SA groups (1.28 mg/kg DTM, 25 mg/kg SA). Anxiety-like behaviors were evaluated by an open field test and a marble-burying test (Noldus Ethovision System).

Results: The SA treatment revealed a significant effect on time spent in the inner zone (100.36±0.04 sec, $F_{(3.36)}$=71.13, η2=0.877, p=0.0001, p<0.05), number of crossings in the center (21.42±1.23 (n), $F_{(3.36)}$=13.13, η2=0.522, p=0.0056, p<0.05) and fecal scores (2.9±0.21(n), $F_{(3.36)}$=51.51, η2=0.811, p<0.05) via the open field. SA treatment exhibited a significant difference in the marble test compared with the other groups ($F_{(3.36)}$=77.64, η2=0.962, p<0.0001).

Conclusions: The findings of this study represent the first step toward understanding the anxiolytic effects of SA, and our results suggest that SA treatment may be beneficial for anxiety-related disorders. Therefore, SA should be evaluated in a new drug design to increase the understanding of underlying anxiety disorders, and DTM may be a good resource for modeling anxiety behavior in Wistar rats.

Keywords: Anxiety, behavior, marble test, open field test, syringic acid

ÖZET

Amaç: Bu çalışma, davranış testlerine tabi tutulan sıçanlarda deltametrin (DTM) maruziyetine karşı sirinjik asit (SA) uygulamasının olası anksiyolitik benzeri etkilerini araştırmak için yapılmıştır.

Gereç ve Yöntem: Ağırlıkları 250-270 gr olan Wistar albino erkek sıçanlar randomize olarak kontrol (0.5 mg/kg mısır yağı), DTM (1,28 mg/kg), SA (25 mg/kg) ve DTM+SA (1,28 mg/kg DTM, 25 mg/kg SA) olmak üzere dört gruba ayrıldı. Anksiyete benzeri davranışlar açık alan testi ve misket gömme testi (Noldus Ethovision System) ile değerlendirildi.

Bulgular: Sirinjik asit tedavisi ile açık alan testinde, iç kısımda geçirilen süre (100,36±0,04 sn, $F_{(3.36)}$=71,13, η2=0,877, p=0,0001; p<0,05), merkezdeki geçiş sayısı (21,42±1,23 (n), $F_{(3.36)}$=13,13, η2=0,522, p=0,0056; p<0,05) ve dışkı skorlarında (2,9±0.21 (n), $F_{(3.36)}$=51,51, η2=0,811; p<0,05) anlamlı farklılık ortaya çıktı. SA tedavisi misket gömme testinde diğer gruplara göre anlamlı bir farklılık gösterdi ($F_{(3.36)}$=77,64, η2=0,962; p<0,0001).

Sonuçlar: Bu çalışmanın bulguları SA'nın anksiyolitik etkileri üzerine ilk adımı temsil ediyor ve sonuçlarımız SA tedavisinin anksiyete ile ilişkili bozukluklar için faydalı olabileceğini gösteriyor. Bu nedenle SA, altta yatan anksiyete bozukluklarının anlaşılmasını artırmak için yeni bir ilaç tasarımında değerlendirilmelidir ve DTM, Wistar sıçanlarında anksiyete davranışını modellemek için iyi bir kaynak olabilir.

Anahtar Kelimeler: Açık alan testi, anksiyete, davranış, misket gömme testi, sirinjik asit

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INTRODUCTION

Anxiety is a mental, behavioral and physiological state aroused in animals by an actual or potential threat (1). It is outlined by intensified arousal, anticipation, neuroendocrine, autonomic activation and particular response patterns frequently with a behavioral shift from continuing responses to an escape or other protective actions (2). The purpose of these escapes is to improve coping with unexpected situations. Although the adaptive form of anxiety is not sufficient, the anxiety can display neuroticism, which might later change the way an animal copes with stressful conditions in regular life (2). In rats, anxiety-like behaviors are assessed by a series of tests by recording their response to a new and intimidating situation (3-5). The marble burying test is the most frequent test used to assess anxiety. The open-field test (OFT) is used to evaluate the animal's motor activity and their response to an unusual environment in case of anxiety or curiosity (5-8). The anxiety behavior probably concerns some form of defensive burying behavior in rats and is mainly evaluated by the marble burying test (9). Moreover, fecal scores, time spent in the center, and training time likely measure emotional aspects in OFT, including other patterns of anxiety (10). The advantages of both tests include the highest validity, convenience of use, spontaneous behaviors, accuracy, and evaluation of abnormal behaviors. The use of familiar housing cages reduces the habituation period. The marble test also demonstrates the inclination of rats to dig in natural surroundings. However, these procedures are confined to general animal behavior such as time spent in specific zones and short times, implying that they only evaluate reactions to novelty. However, it has been stated that the OFT and marble burying tests may have a predictive effect in monitoring new antidepressants, anxiolytics, and antipsychotics (11, 12).

There is a necessity to raise awareness of the use of safe doses, and this forces researchers to evaluate the neuroprotective efficacy of phytochemicals (13, 14). Syringic acid (SA) is one of the phenolic acid patterns in several dry fruits, pumpkin, dates, olives, açaí palm, grapes, red wine, honey, and other plants (13, 14). Current developments in anxiety research have led to a refreshed interest in SA treatment, and it might prevent any adverse effects that are considered a barrier to standard drugs (14, 15). Therefore, novel treatment methods should be urgently explored.

Deltamethrin (DTM) is a Type II pyrethroid insecticide used to control a range of insects in agricultural and home settings (15). Deltamethrin causes several autonomic and neuroendocrine reactions that suggest high-stress levels, likely due to neurotoxicity (15). It has been shown that DTM increased anxiogenic responses and that stress had an additive effect on DTM exposure (16). Therefore, the present study investigates whether SA treatment may decrease anxiety against DTM exposure and the possible anxiogenic effects of DTM in rats via behavioral tests.

MATERIALS AND METHODS

Syringic acid ($C_9H_{10}O_5$; Cat-ID: 530-57-4; SA≥95%) was purchased from Santa Cruz (California, USA), dissolved to 5.78 mg/ml at 25°C and administrated at a dose of 25 mg/kg. Deltamethrin (DTM; $C_{22}H_{19}Br_2NO_3$) was purchased from Decis®; Bayer AG (Leverkusen, Germany) and administered at 1.28 mg/ml. DTM and SA were dissolved in corn oil (0.5 ml), and effective doses were selected as described by previous studies (15, 17, 18).

Animals

The present study was conducted on Wistar Albino (*Rattus Norvegicus*) male rats (2 months of age, weighing 250-270 gm) obtained from the Experimental Animals Breeding and Research Center of the Akdeniz University. The animals were placed in propylene cages (27x48x20 cm) under controlled temperature and humidity (21±2°C; 50±5 %) on a 12-hours light/dark schedule, with free access to food and water. The analyses were conducted according to the national regulations and rules of the Institutional Animal Ethical Committee (IAEC) of the Akdeniz University (Date: 18.10.2021, No: 1370). The rats were acclimatized for one week before treatment, and all applications were made to reduce the number of animals.

Experimental design

The animals were randomly assigned to the four groups of ten males: Control, DTM, SA and DTM+SA. The Control (0.5 ml corn oil), DTM (0.5 ml corn oil+1.28 mg/kg), SA (0.5 ml corn oil+25 mg/kg) and DTM+SA (0.5 ml corn oil+1.28 mg/kg DTM; 25 mg/kg SA) were administered to the adult Wistar albino rats for two months by oral gavage daily.

Behavioral procedures

The rats were kept in the test room for one hour before the behavioral tests to avoid setting influences and maintain their basal anxiety levels. Behavioral tests were recorded by a Noldus Ethovision XT video software system (Noldus Information Technology, Wageningen, The Netherlands).

Open-field test (OFT)

The OFT evaluated the movements and anxiety-like behaviors of the rats by measuring center activity to assess response to the new environment (19, 20). The OFT was conducted as defined by Denenberg et al (21). Evaluations were carried out in an arena (80×80×40 cm) split into 16 squares (20x20 cm) as reported by Denenberg et al. (21) (Figure 1). The equipment was wiped with a 70% alcohol and water solution before settling the animals to defeat the potential bias produced by the odors of the

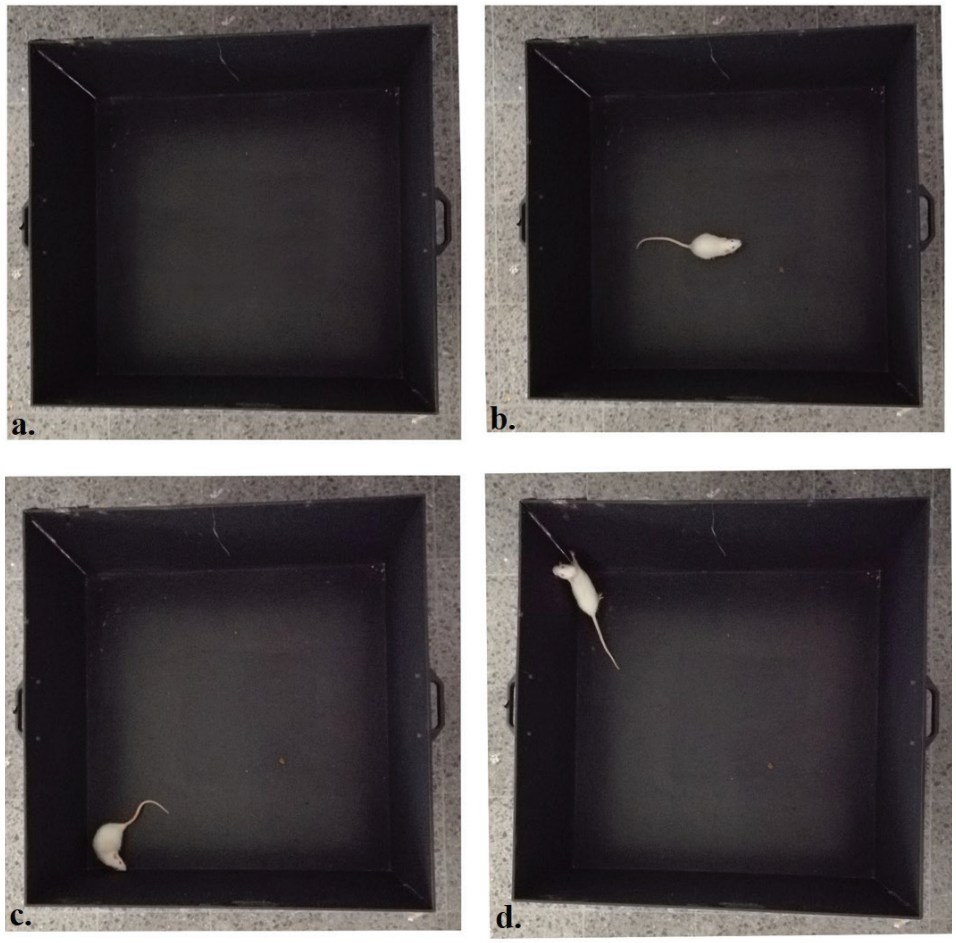

Figure 1: Open field test (OFT) was used to assess the anxiety. a: The test was conducted on a square, black matte-based assembly with a wall height of 40 cm and a base of 80x80 cm. The field was divided into 16 circles equal to 20 cm2 with the Noldus Ethovision System and the activity was recorded. b: Rats were placed at the center of the field. c: Their movements were recorded for 5 minutes. d: The Noldus software system allows us to designate this area and multiple other regions of the test to track exploratory activity.

previous rats. Each rat was placed in the core of the OFT, respectively (Figure 1). The number of crossings in the center and time spent in the inner/outer zone were noted for 5 minutes. The total time spent in the inner/outer zone, inner/total distance ratio, number of crossings in the center, and total fecal scores were recorded by the Noldus Ethovision XT software (Wageningen, The Netherlands).

Marble burying test

The marble burying test is used to evaluate habitual action with high-grade efficacy, defensive anxiety, repetitive behavior, and compulsivity. It is a tool for assessing anxiety-like behavior with sensitivity to anxiolytics (22). The test was carried out as described by Sahgal and Sprowles et al. (23, 24). This test recorded the number of marbles buried by a rat in a new environment. The test was conducted in a propylene cage (45×21×20 cm) with its ground coated with a 5-10 cm uniform layer of sawdust. Twenty-four standard glass marbles (1.5 cm) were distributed equally on the sawdust. The rats were settled in the test cage, and movements were observed for 20 minutes by the Noldus Ethovision software. At the end of the test, animals were removed from the cages, and then the number of buried marbles was recorded. The marbles were cleaned before each new trial.

Data analysis

SPSS 25.0 (IBM SPSS software, USA) and GraphPad Prism 8.2 (GraphPad Software, San Diego, CA) were used for all statistical analyses. Descriptive statistics of continuous variables were given with the mean, standard deviation,

standard error of the mean, and coefficient variation of groups for categorical variables. The Shapiro Wilk test was used as a test of normality. The parameters of the marble burying test and OFT were analyzed by one-way ANOVA. The Post-Hoc Tukey test was used in multiple comparisons. For all statistical comparisons, $p<0.05$ was taken to indicate statistical significance.

RESULTS

Time spent in inner/outer zone (sec.)

Time spent in the outer zone, identified in Figure 2a, including thigmotaxis or wall-hugging behavior, shows anxiety-related behavior. The rats subjected to DTM presented higher anxiety degrees than DTM+SA and SA treated rats on thigmotaxis. As shown in Figure 2b, one-way ANOVA revealed significant effects of SA treatment in the time spent in the inner zone (100.36±0.04, p=0.0001, p<0.01) compared with control (69±0.04, p=0.000, p<0.01) (Table 1). Moreover, a significant decrease in total time spent in the inner zone was noted in the DTM group (25.71±0.03, p=0.000, p<0.01) (Figure 2b). The DTM-treated rats spent a significantly greater amount of time exploring the periphery of the arena than the center (274.28±14.37, p=0.000, p<0.01). SA administration caused a significant increase in time spent in the inner zone ($F_{(3.36)}$=51.62, R^2=0.8114, p=0.0001,

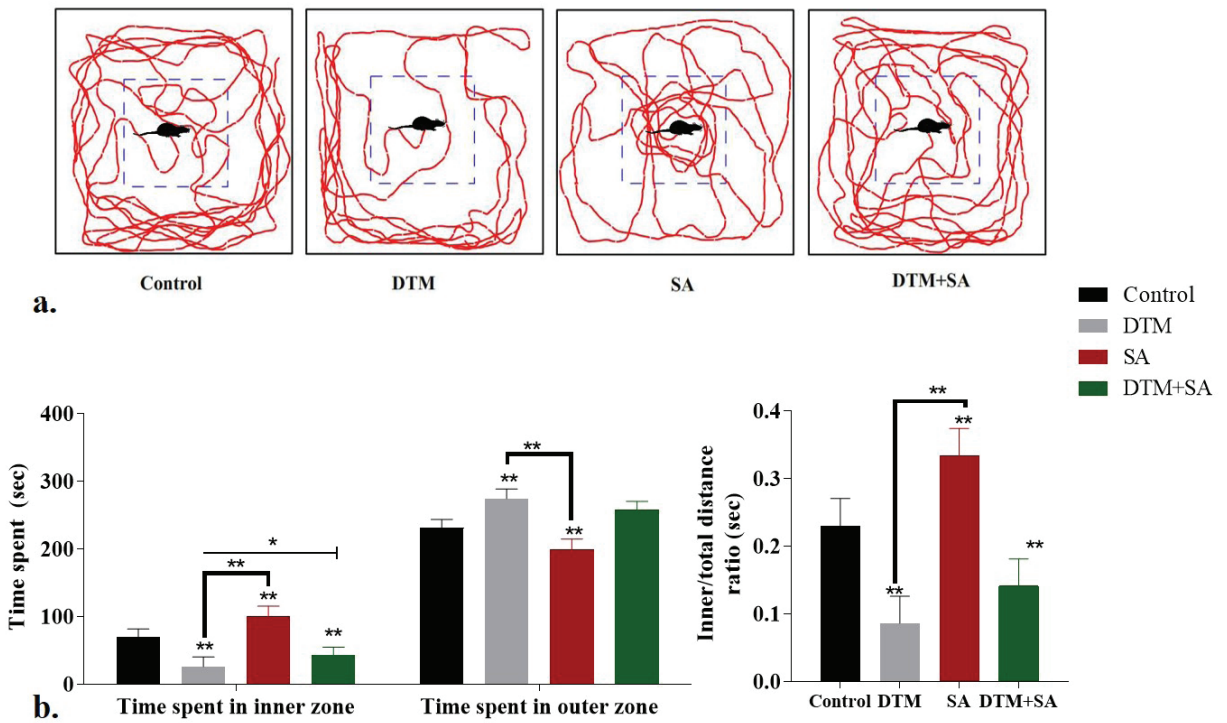

Figure 2: The results of time spent (inner/outer zone) in the OFT and the inner/total distance ratio. **a.** The effect of SA on OFT in rats. (Control, n=10; DTM, n=10; SA, n=10; DTM+SA, n=10) are shown. Each track represents the total distance travelled by the rats during the 5 min period. The SA-treated rats cross into the center of the maze at regular intervals while the DTM group remained closely in proximity to the walls indicating increased thigmotaxis and anxiety-related behavior. Greater time spent in the outer zone is recorded as increased thigmotaxis and anxiety-related behavior *p<0.05 **b.** Time spent in the inner zone (sec.), time spent in the outer zone (sec.), and the inner/total distance ratio (sec.) were evaluated as parameters of anxiety. DTM significantly decreased the total time spent in the center (sec) (25.71±0.03, p<0.01). SA significantly increased the time spent in the center (sec) (100.36±0.04, p<0.01) compared with the control (69±0.04, p<0.05) and DTM+SA (42.27±0.02, p<0.01) ($F_{(3.36)}$=71.13, η2=0.877; p=0.0001, p<0.01). DTM significantly increased the time spent in the outer zone (sec) (274.28±14.37, p<0.01). SA significantly reduced the time spent in the outer zone (sec) (199.63±14.96, p<0.01) compared with the control (231±12.54, p<0.05) and DTM+SA (257.72±12.37, p<0.01) ($F_{(3.36)}$=51.23, η2=0.991, p=0.0001, p<0.01). DTM significantly decreased the inner/ total distance ratio (sec) (0.08±0.01, p<0.01). SA significantly increased the inner/ total distance ratio (sec) (0.33±0.01, p<0.01) compared with the control (0.23±0.01, p<0.05) and DTM+SA (0.14±0.01, p<0.01) ($F_{(3.36)}$=51.62, η2=0.8114; p=0.0001, p<0.01). Asterisks (*) signify a statistically significant effect; *p<0.05. **p<0.01, ***p<0.001 according to the Tukey post-hoc test for multiple comparisons

p<0.01) and it was considerably greater than in the DTM group (p<0.01). Similarly, a considerable increase in the time spent within the OFT was recorded in the DTM+-SA group in comparison with the control (42.272±0.02, p=0.0008, p<0.01) (Figure 2b). The SA-treated rats spent significantly more time exploring the center of the arena than the outer zone (199.63±14.96, p=0.000, p<0.01), demonstrating anxiolytic-like behavior (Table 1). The administration of SA and DTM were investigated in correlation coefficient statistical tests to detect anxiety-like behavior in OFT. DTM exposure was significantly correlated with anxiogenic behavior. The SA treatment was positively correlated to the time spent in the inner zone (r=0.78, p<0.001) (Figure 4a). As seen in Figure 4a, the coefficient of variations was statistically significant between the groups ($F_{(3.9)}$=6.679, p=0.0115).

The number of crossings in the center (n)

As shown in Figure 3a, we observed that the number of crossings in the center was considerably raised in the SA group ($F_{(3.36)}$=13.13, R^2=0.5225) (21.42±1.23, p=0.0056, p<0.05) when compared to the control (16.13±0.9, p<0.05)

Table 1: Means, standard deviations, and One-Way analyses of variance in OFT and Marble Burying Test (DTM, SA)

Groups	Control		DTM		SA		DTM+SA		*F*(3. 36)	*η2*
Parameters	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**		
Time spent in inner zone (sec.)	69	0.04	25.71	0.03	100.36	0.04	42.272	0.02	71.13***	0.877
Time spent in outer zone (sec.)	231	12.54	274.28	14.37	199.63	14.96	257.72	12.37	51.23***	0.991
Inner/total distance ratio (sec.)	0.23	0.013	0.08	0.018	0.33	0.015	0.14	0.012	51.62***	0.811
The number of crossings in the center (n)	16.13	0.901	12.14	0.594	21.42	1.231	17.08	1.321	13.13***	0.522
Fecal (defecation) scores (n)	3.70	0.260	6.71	0.285	2.90	0.211	5.77	0.222	51.51***	0.811
The number of buried marbles (n)	11.75	0.675	20.22	0.741	4.20	0.587	7.81	1.042	77.64***	0.962

***: p<0.001, OFT: Open Field Test, DTM: Deltamethrin, SA: Syringic Acid

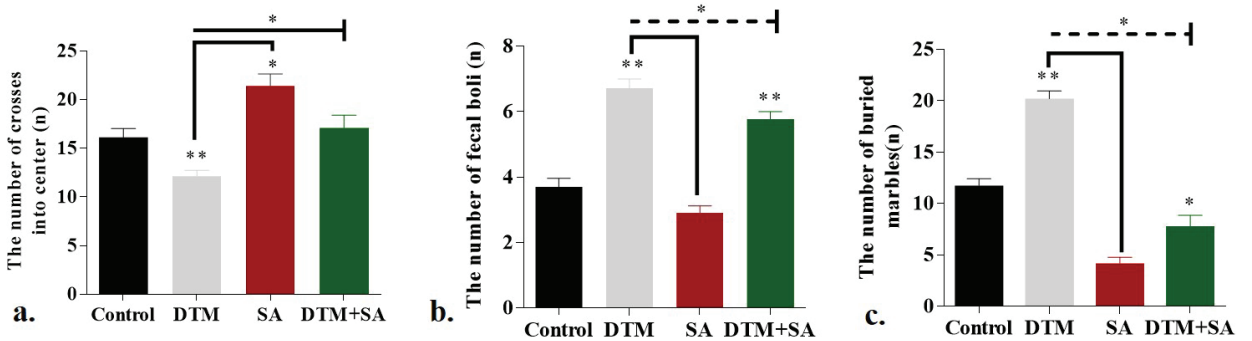

Figure 3: The results of the number of crosses in the center and the fecal scores in OFT and the number of buried marbles in the marble test. **a.** DTM significantly reduced the number of crossings of the center (p<0.01). SA (21.42±1.23, p<0.05) significantly increased the number of crosses in the center compared with DTM (12.14±0.59, p<0.01) and DTM+SA (17.08±1.32, p>0.05) ($F_{(3.36)}$=13.13, R^2=0.5225) **b.** A significant reduction was detected in DTM+SA (5.77±0.22, p<0.05) and SA (2.9±0.21, p<0.01) for the fecal scores (n) compared with the DTM (6.71±0.28, p<0.01) ($F_{(3.36)}$=51.51, R^2=0.8111, p<0.0001). **c.** The DTM group (20.22±0.74, p<0.01) significantly buried more marbles (n) than the control (11.75±0.67, p<0.05), DTM+SA group (7.81±1.04, p<0.05), and SA group (4.2±0.58, p<0.01) ($F_{(3.36)}$=77.64, R^2=0.8661, p<0.0001). Asterisks (*) signify a statistically significant effect; *p<0.05, **p<0.01, ***p<0.001 according to the Tukey post-hoc test for multiple comparisons (DTM: Deltamethrin, SA: Syringic Acid)

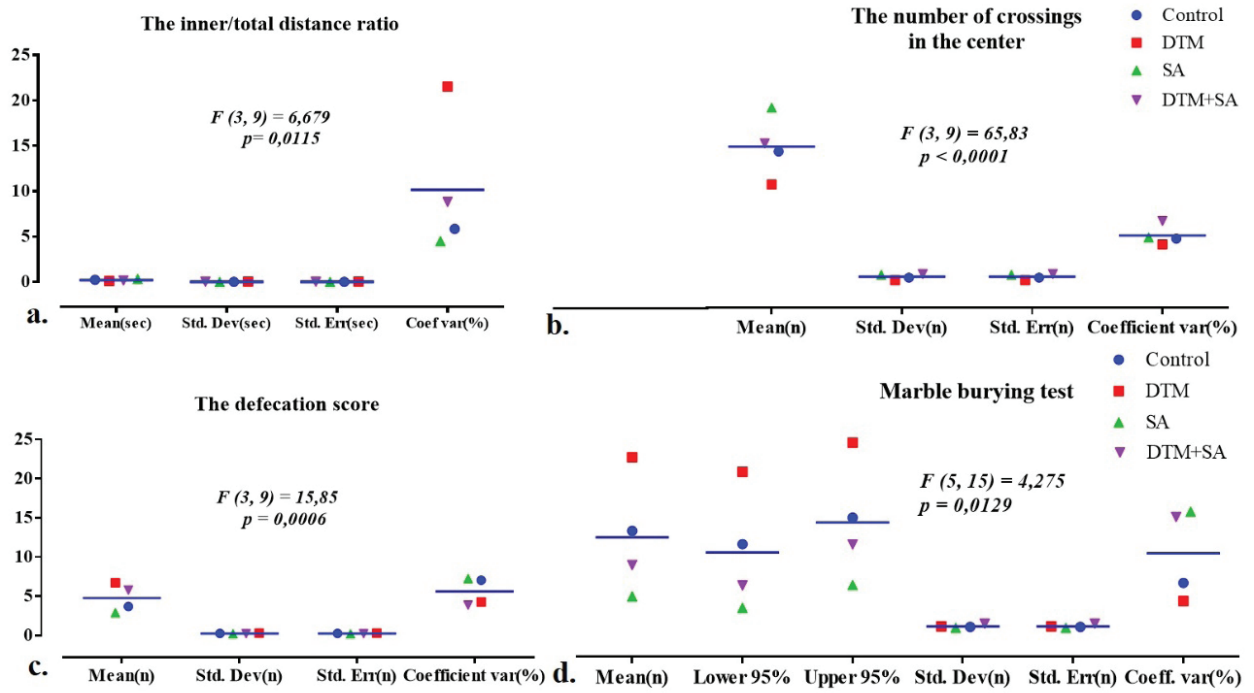

Figure 4: The mean, std. deviation, std. error of mean and coefficient variation of groups **a.** The coefficient variations of the inner/total distance ratios were 5.84% (Control), 21.51% (DTM), 4.50% (SA), and 8.79% (DTM+SA), respectively; $F_{(3,9)}$=6.679, p=0.0115. **b.** The coefficient variations of the number of crossings in the center were 5.58% (Control), 4.9% (DTM), 5.75% (SA), and 7.73% (DTM+SA), respectively; $F_{(3,9)}$=65.83, p<0.0001. **c.** The coefficient variations of defecation score were 7.04% (Control), 4.26% (DTM), 7.26% (SA), 3.85% (DTM+SA) respectively; $F_{(3,9)}$=15.85, p=0.0006. **d.** The lower 95% CI of mean of the marble burying test were 10.2217 (%) (Control), 18.5455 (%) (DTM), 2.87174 (%) (SA), 5.45902 (%) (DTM+SA), respectively; $F_{(5,15)}$=4.275, p=0.0129. The upper 95% CI of mean were 13.2783 (%) (Control), 21.8989 (%) (DTM), 5.52826 (%) (SA), 10.1774 (%) (DTM+SA), respectively. The coefficient of variation were 5.75% (Control), 3.67% (DTM), 13.98% (SA), and 13.34% (DTM+SA), respectively. (DTM: Deltamethrin, OFT: Open Field Test, SA: Syringic Acid, SD: Standard Deviation, SEM: Standard Error of Mean)

and a remarkable reduction in crossing numbers was observed in the DTM group (12.14±0.59, p=0.0415, p<0.05) compared to the control (Table 1). The DTM+-SA group (17.08±1.32, p=0.0106, p<0.05) exhibited a notable rise in the number of crossings in the center when compared to the DTM group (p=0.0515, p>0.05). SA was positively correlated with anxiolytic behavior (r=0.91, p<0.001), whereas DTM was negatively correlated with anxiolytic behavior. As seen in Figure 4b, the coefficient of variations was statistically significant between the groups ($F_{(3,9)}$=65.83, p<0.0001).

The fecal scores (n)

As seen in Figure 3b, fecal scores increased in the DTM group (6.71±0.28, p=0.000, p<0.05) when contrasted with the control (17.08±1.32, p=0.0001, p<0.01). DTM+SA rats (5.77±0.22, p=0.0001, p<0.01) and SA rats (2.9±0.21, p=0.041, p<0.05) ($F_{(3,36)}$=51.51, R^2=0.8111, p<0.0001) results are also provided (Table 1). SA-treated rats decreased the fecal scores and increased the exploration. The coefficient of variations was statistically significant between the groups ($F_{(3,9)}$=15.85, p=0.0006) (Figure 4c).

Marble burying test (n)

As shown in Figure 3c, the DTM rats (20.22±0.74, p=0.000, p<0.01) buried more marbles than the control group (11.75±0.67, p=0.0119, p<0.05). The DTM+SA group (7.81±1.04, p=0.0056, p<0.05) and the SA group (4.2±0.58, p=0.000, p<0.01) ($F_{(3,36)}$=77.64, R^2=0.8661, p<0.0001) results are also provided (Table 1). There was a considerable increase in the number of buried marbles in the DTM group (p=0.000002, p<0.001) when contrasted with the control group and a decrease in the SA and DT-M+SA rats (p=0.000001, p<0.05). The behavioral tracking software revealed a notable rise in the buried marbles in the DTM group in comparison to the DTM+SA group (n=10, p<0.001 (One-way ANOVA)). As seen in Figure 4d, the coefficient of variations was statistically significant between the groups ($F_{(5,15)}$=4.275, p=0.0129).

DISCUSSION

We suggest that the anxiolytic effects of SA should be evaluated in a new drug design to increase the understanding of underlying anxiety disorders, and DTM can be a good resource for modeling the anxiety behavior in Wistar rats. The current study confirmed that a 25 mg/kg dose of SA could be more effective in treating DTM-induced anxiety over time spent inner/outer zones, number of crossings, fecal scores, and buried marbles. It appears to demonstrate significant translational validity as a model of SA on anxiety and it may be beneficial in promoting more high-throughput time/cost-efficient drug discovery. Phenolic acid compounds have been shown to exert anxiolytic-like effects through various mechanisms other than those used in daily practice (25). It has been indicated that phenolic acid can reduce anxiety symptoms by increasing the electrical activity of pyramidal neurons and neuroprotective activity (25, 26). Dalmagro et al. investigated the antidepressant-like effects of the SA treatment; however, the anxiolytic effects of SA have not been elucidated (26). Dalmagro et al. reported that acute or subchronic SA administration had an antidepressant-like effect in the behavioral tests in mice due to the neuroprotective activity of SA (26). Several studies have shown the effectiveness of SA in reducing the signs and symptoms of neurological and neuropsychiatric disorders such as Alzheimer's disease, Parkinson's disease, depression, hepatic encephalopathy, short and long-term learning and memory deficits, cognitive impairments, and behavioral and cerebral dysfunctions (13-15, 17, 18, 26-29). It has been reported that the administration of SA significantly reduced neural, biochemical, and behavioral abnormalities in Alzheimer's rats, indicating that SA has both therapeutic and neuroprotective effects against neurodegenerative disorders (29). Most recently, it has been reported that SA has been revealed to have a significant role in excitatory neurotransmitters and reducing behavioral dysfunctions due to its antioxidant and anti-inflammatory effects (14). Chowdhury et al. stated that the higher OFT and marble burying test scores of SA were more effective than the standard drugs against several neurological disorders (27). The current data suggest that SA significantly reduces anxiety via different activation patterns that may contribute to the differences in coping with stress. Moreover, a docking study of SA showed that it was a promising candidate against several neurological impairments compared to the standard drugs. In this way, SA could be considered for further *in vitro* and *in vivo* analysis of therapeutic potentials (27). SA treatment against DTM can increase the therapeutic effects of SA by enhancing the synthesis and release of neuroprotective factors (14, 15, 18). Recent studies have found that the administration of SA decreased the behavioral disturbances in neurodegenerative disorders (14, 15, 17, 18, 28, 29). Ferah Okkay et al. reported a decrease in locomotor and exploratory behaviors in the thioacetamide group, whereas SA treatment improved behavioral impairments in rats (28). Furthermore, they confirmed that SA could improve locomotor activity and decrease anxiety-like behaviors caused by thioacetamide (28). The findings of the current study were consistent with previous studies. However, it is hard to draw a definite conclusion about the anxiolytic role of SA treatment because we detected behavioral differences only in the marble burying test and OFT from the wide range of anxiety-related behavioral tests such as the elevated plus-maze. The applied behavioral tests in the present study are mainly on analyses that can assess anxiety-like behaviors, as they design an essential methodological device in neurobehavioral toxicology and pre-clinical research.

The development of anxiety can be strongly influenced by toxicological factors or various activated neurotransmitter mechanisms of DTM. Experimental studies have shown that increased neurotransmitter release at multiple levels in cortical regions improves the efficacy of interconnected networks in anxiety (30, 31). A further possible finding is that DTM is responsible for anxiety-related behavioral changes in the marble burying test and OFT; therefore, it can be used to design various experimental models in translational neuroscience. Bhattacharya et al. reported that decreased activity after DTM exposure might result from enhanced anxiety because they observed a decreased number of crossings, a decline in time spent in the inner zone, and increased defecation scores in the DTM group (30). Ricci et al. reported that behavioral and neurochemical methods induced the anxiogenic effects of DTM in rats, and DTM had a similar effect to anxiogenic drugs via serotonin neurotransmission (31). Similarly, in our study, DTM decreased locomotion, prolonged immobility, rearing repetition, and decreased the time spent in the inner zone.

Sprowles et al. asserted that selective serotonin reuptake inhibitors treated rats exhibiting anxiety-like behavior and compulsivity in the marble test (24). Likewise, in the current study, the enhanced anxiety-like performance and shorter duration in the inner zone were noted in the DTM group when contrasted with the SA group. The long-term SA-treated rats exhibited a notable reduction in the number of buried marbles compared to the DTM and control groups ($F_{(3.36)}$=77.64, R^2=0.8661, $p<0.0001$). Rats treated with SA alone and DTM+ SA revealed a significant decrease in burying behavior as compared with the DTM group ($p=0.000001$, $p<0.05$) (Figure 3c). This outcome depends on the consequences of the anxiolytic effects of SA, and increased time spent in the center indicates the anxiogenic effects of DTM. Wistar rats are sensitive to a wide range of anxiogenic compounds of DTM and thus further confirming their translational value

in affective research for high-throughput anxiolytic drug discovery. Moreover, SA is the best candidate for drug design based on the positive actions on anxiety.

Although multidisciplinary fields are essential agents for interpreting the underlying effects of new drug treatments, the behavior of rats represents the final output of the central nervous system. It should be the base for pre-clinical evaluations of novel drugs (32). SA has the potential to be a viable curative agent for the treatment of anxiety in the future due to the possible anxiolytic effects and improved behavioral dysfunctions for innovative drug design. Therefore, the strategic development in this field that is considerably noteworthy is the implementation of new translational models over anxiety.

Limitations

In further studies, the effect of SA on anxiety should also be evaluated by an elevated plus-maze. Moreover, an experimental animal study might not be sufficient to decide the anxiolytic manner of SA. It can explain only a part of the anxiety in humans; therefore, the anxiolytic-like effects of SA must be strengthened by further clinical observations on humans.

CONCLUSION

The findings of the current study represent the first step in the anxiolytic effects of SA, and our results suggest that a SA treatment can be beneficial for anxiety-related disorders. Therefore, it should be evaluated in a new drug design to increase the understanding of underlying anxiety disorders, and DTM can be a good resource for modeling the anxiety behavior in Wistar rats.

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