The assessment of effectiveness of a novel antidepressant, Agomelatine on anxiety and depression induced by fluoride intoxication by means of Open-Field and Hot-Plate tests in mouse model *(BalB-C)*

Başaran KARADEMİR

The Faculty of Applied Sciences, Iğdır University, Iğdır, Türkiye

ORCID: 0000-0002-6604-9021

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Corresponding author
basaran_k@hotmail.com

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ABSTRACT

It is well known that fluoride (F) poisoning causes anxiety and depression, and Agomelatine, an analogue of melatonin, has been reported to be effective on anxiety and depression. Therefore, the aim of this study is to investigate the short-term efficacy of Agomelatine application on anxiety and depression caused by F intoxication via Open-Field and Hot-Plate tests. Forty male *BalB-C* mice, aged 5-6 months, constituted the research material for this study. Subjects were randomly divided into 4 groups (Healthy-Control, Fluorosis-Control, 25 mg/kg Agomelatine, 50 mg/kg Agomelatine). Healthy-Control group (HC) received tap water, containing 0.3 ± 0.05 mgF/L. Fluorosis-Control group (F) received drinking water containing 40 mgF/L. Other two experimental groups (25 and 50) received drinking water containing 40 mgF/L and a single dose of Agomelatine (25 and 50 mg/kg respectively). The effect of Agomelatine on anxiety and depression induced by high dose F was evaluated using Open-Field and Hot-Plate tests compared to control groups. Fluorosis caused to decrease in Rearing, Grooming and Square numbers of Open-Field test and to increase Defecation counts ($P<0.05$). Agolematine applications enabled to normalize the Open-Field Test data. Similarly, according to the Hot-Plate findings, low reaction time caused by fluorosis increased in Agomelatine groups ($P<0.05$). According to those results, psychological improvement was observed in patients with fluorosis compared to the control group after Agomelatine applications. Consequently, according to Open-Field and Hot-Plate tests findings, it could be concluded that Agomelatine has a curative effect on anxiety and depression induced by F toxicity.

Introduction

It is well known that fluoride (F) is highly electronegative halogen (11, 19). Areas contaminated with F are reported to exist widely in both natural and industrial environments (7, 16, 31). Therefore, human and animal populations are under the risk of F toxicity and subsequent fluorosis. It is also well known that F poisoning damages metabolism, hormones (15, 23), hard (19-21) and soft tissues (10, 14) in animals and humans. Moreover, the brain and all other nervous system are also affected by F intoxication (11, 24, 28). In this case, it is an inevitable fact that brain functions and locomotor activities are also affected by F intoxication (7, 24, 30, 33, 37). Neurological and psychiatric disorders have also been reported in cases of F toxicity, such as; mental retardation, memory impairment, learning disruption, lethargy, memory, and concentration impairment, thinking difficulties etc. (7).

Agomelatine is a novel synthetic analogue of the melatonin hormone that has been used in the treatment of psychiatric disorders such as depression and anxiety (5, 29, 38). Its therapeutic effect is via its agonist effects on MT1 / MT2 receptors (1, 9, 26, 36) and antagonist effects on 5-hydroxytryptamine-2C (5-HT_{2C}) receptor (5, 6, 29).

It is reported that the Open-Field test is used as an indicator of the emotional state (depression, anxiety, etc.) in animal models with the conditions described above. The

Open-Field test is also used to investigate the effectiveness of antidepressant drugs on animal models (24, 28, 30, 33, 37). However, psychological problems such as depression and anxiety are known to reduce tolerance to distressing situations (2). The Hot-Plate test is used to evaluate the tolerance level to heat stress on animal models (3, 13, 22). In terms of its effect on 5-HT_{2C} receptors, Agomelatine is likely to have an effect on pain-heat tolerance (5, 6, 29, 36).

Depression and anxiety is an important problem in animals as well as humans and is often overlooked. The prevalence of fluoride toxicity with the increase of industrialization causes these two situations to interact with each other. There is rather limited information in the literature about Agomelatine, which has been recently used as a novel antidepressant in psychological state disorders after fluorine intoxication. This research will reveal the short-term effectiveness of Agomelatine in the treatment of these mental disorders. The results of this study will also guide further research examining the long-term use of Agomelatine in similar situations.

For the aforementioned reasons, the aim of this study was to investigate the short-term effects of Agomelatine on mice exposed to F intoxication and suffering from depression and anxiety diagnosed by Open-Field and Hot-Plate tests.

Materials and Methods

Experimental animals and design: This study was conducted in 40 male mice (*BalB-C*) 5-6 months old and weighing 25.0 ± 1.8 g. The subjects were divided into 4 equal groups randomly and kept under stable temperature (20 ± 0.5 C) and artificial lighting condition with tungsten lamp (12 hours dark and 12 hours light). Subject groups were designed as Healthy-Control group (Group HC), Fluorinated group (only exposed to 40 mgF/L, Group F), First experimental group received fluorinated drinking water and 25 mg/kg/bw Agomelatine (Group 25) and Second experimental group received fluorinated drinking water and 50 mg/kg/bw Agomelatine (Group 50). The amount of Agomelatine applied was determined as normal and maximum dose, considering the previous studies (1, 5, 6, 17, 26, 29, 38). Agomelatine were given the experimental groups in 0.3 ml 1% Hydroxyethyl-cellulose solution via intra peritoneal (1, 36). Intra peritoneal 0.3 ml 1% Hydroxyethyl-cellulose solution also applied the other control groups. Agomelatine application time accepted as first application start time for Open-Field and Hot-Plate tests (min. 0^{th}). All experimental groups except Group HC received drinking water containing 40 mgF/L for 3 mounts, control group received only tap water containing 0.3 ± 0.05 mgF/L. These drinking waters and commercial food were given *ad libitum* during experiment. Ingredients of Commercial food, purchased from Bayramoğlu Yem ve Un San. Tic. A.Ş. (ISO 9001:2000, ISO 22000:2005), were presented in Table 1.

Table 1. Nutritional content of commercial feed received by experimental animals, reported by commercial firm (Bayramoğlu A.Ş.).

Diet Composition	Amounts and Units	Diet Composition	Amounts and Units
Dry matter	88%	Phosphorus	0.75%
Crude protein	17%	NaCl	0.6%
Crude cellulose	12%	Vitamin A	5000 IU/kg
Crude ash	10%	Vitamin D_3	600 IU/kg
Acid insoluble ash	1%	Vitamin E	25 mg/kg
Calcium	1.5%	Metabolic energy	2600kcal/kg

Raw materials for this composition were barley, corn, corn chaff, corn gluten, wheat, rye chaff, cotton seed meal, sunflower meal, dicalcium phosphate, vitamins and minerals.

Timing design of Agomelatine application for Open-Field and Hot-Plate tests: It is reported that the plasma peak value of Agomelatine is between 45 and 90 minutes after a single dose administration and its half-life is approximately at 2 hours (1, 6, 17, 36, 38). Therefore, Open-Field test was performed at three times (0^{th}, 60^{th} and 90^{th} minutes) and "Hot-Plate" test was also carried out one-shot at 90^{th} minute to observe reactions of subjects after Agomelatine application. The Open-Field test does not hurt the subjects, but not Hot-Plate test. Therefore, three different effective time points for Open-Field test procedures following single dose of Agomeletine application were preferred. But, a single time point in the middle of the effective time interval was chosen for the Hot-Plate test application.

Open-Field Test and Procedures: The Open-Field test is used to assess behavioral changes such as anxiety and depression for animal models. It is also used to demonstrate the efficiency of drugs in such cases (24, 33, 37, 30). For Open-Field test application, a closed area of 80x80 cm with a transparent barrier was used, divided into 64 equal squares with permanent lines (18). The subjects were left in the middle of the test area and their behaviors were recorded with a video camera for 5 minutes. Each behavior (Rearing, Grooming, Crossed Squares and Defecation) was counted from this video.

Hot-Plate Test and Procedures: Hot-Plate test is an experimental method used to determine the pain threshold in an animal model (3, 13, 18, 22). For this purpose, a

heater plate adjusted to 50 ºC was used. The perimeter of the heating plate was surrounded by transparent material. "Hind Paw Licking" or "Jumping off" movements were identified as expected reaction. If any of these actions were observed, the test was terminated and the time recorded as "Reaction Time". In the event of no reaction, the test was scheduled to be terminated at 45 seconds and the Reaction Time considered as 45th second. The Hot-Plate test was performed in the 90th minute following the Agomelatine application.

Special Apparatus, chemicals and their preparations: Fluoridation of drinking water: Tap water already has included F in the level of 0.3 ± 0.05 mgF/L. The required reinforcement for 40 mgF/L level was provided by the addition of Sodium Fluoride (NaF, Merck 106449). The F content of the drinking water was confirmed by means of ion meter equipped with F ion selective electrode (Orion 4-Star portable ion meter and F ion-selective electrode - Orion 9609BNWP) (16, 19, 20, 21).

Agomelatine (N-[2-(7-Methoxy-1-naphthalenyl) ethyl]-acetamide, Sigma-Aldrich A1362) were used in 1% Hydroxyethyl-cellulose (Sigma-Aldrich 54290) intraperitoneally (IP) (1).

Statistical Analysis: Firstly, normality test (Kolmogorov-Smirnov) was performed for all test data (Hot-Plate and Open-Field) according to the groups.

One-Way ANOVA test was applied to check the significance of the difference between the Hot-Plate test data groups, which show normal distribution according to the groups (P>0.05). Homogeneity test result in One-Way ANOVA was insignificant (P>0.05). Tukey HSD was performed as Multiple Comparisons.

Normality test results for Open-Field test data were insignificant for Rearing and crossed Squares count data (P>0.05, Parametric data) and significant for Grooming and Defecation count data (P<0.05, Nonparametric data).

Repeat Measures (RM) ANOVA and Freadman tests were used to compare groups with each other over time (RM ANOVA for parametric, Friedman for nonparametric groups). Within RM ANOVA, Mauchly's test of Sphericity was significant (P<0.05) for Grooming and insignificant for crossed Squares groups. For this reason, Greenhouse-Geisser test was taken into consideration in the evaluation of Grooming data and Tamhane's T2 multiple comparisons test. But sphericity assumed for evaluation of crossed Squares data and Tukey HSD test for multiple comparisons was performed.

Grooming and Defecation data were illustrated in Figures 2 and 4 apparently; the course of the groups over time (HC, F, 25 and 50) was compared using the Friedman test and Wilcoxon test was used for multiple comparisons. The Kruskal-Wallis test was used to check for differences between the groups' data at the same time (0th, 60th and 90th minutes). Mann-Whitney U test was used for multiple comparisons of these time data. Bonferroni correction was performed manually for both Wilcoxon and Mann-Whitney U tests.

Results

All test data including Open-Field and Hot-Plate were analyzed for being normal distribution. Kolmogorov-Smirnov test results as the smallest values were as follows; for Open-Field test groups are as follows; Rearing; P>0.05, Grooming; P<0.05, Squares; P>0.05 and Defecation: P<0.05 and for the Hot-Plate data groups: P>0.05

The relationship among the Open-Field test parameters was examined with Spearman's Correlation test and the results are presented in Table 2.

Table 2. Spearman's Correlation test results (r) for Open-Field test parameters including Rearing, Grooming, Squars and Defecation.

	Grooming	Squars	Defecation
Rearing	0.322**	0.221*	-0.204*
Grooming		0.508**	-0.457**
Squars			-0.347**

*: P<0.05, **: P<0.01

For Rearing data, the RM ANOVA test was performed to reveal the relationship of groups with each other over time. Since the Sphericity value was found to be significant (P<0.01), the Greenhouse-Geisser test was taken into account for the interaction of time and groups (P<0.001). Tamhame's T2 Post-Hoc test was used for multiple comparisons of the groups. All of these test results are illustrated in Figure 1.

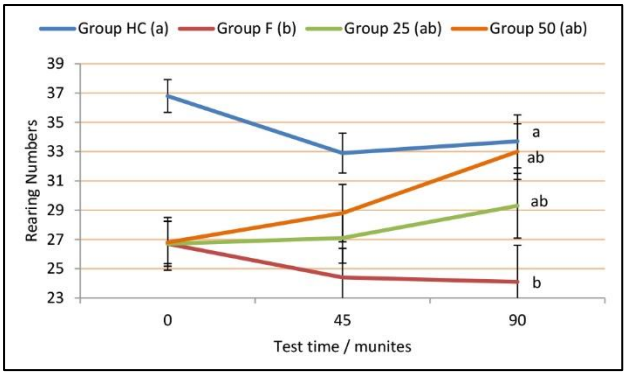

Figure 1. Course of Rearing groups over time and statistical comparisons with RM ANOVA test.
a,b: The difference between groups having different superscripts is statistically significant (P<0.01).

One-Way ANOVA test result of Rearing were as follows for minutes 0th; P<0.001 (Post Hoc; Group HC have statistical differences with all other groups) and minutes 90th; P<0.05 (Post Hoc; Group HC have no statistical differences with Groups 25 and 50, but not Group F).

Grooming and Defecation data, which are the two parameters of Open-Field test, did not show normal distribution (Kolmogrov Simirnov; P<0.05). Therefore, RM ANOVA, a parametric test, could not be used to analyze the relationship of the groups over time. Although the course of the groups can be seen clearly on the graphs, the situation should be clarified with statistical tests. For this purpose, nonparametric tests were used. Firstly, the course of each group (HC, F, 25 and 50) over time was evaluated separately with the Friedman test and results indicated with superscripts on the right side of the graphs (Figure 2 and 4). Then, Wilcoxon test was used to reveal Multiple Comparisons. Secondly, the differences of the groups (HC, F, 25 and 50) for each time point (0th, 45th and 90th) were demonstrated by the Kruskal-Wallis test and the results are shown in superscripts at the top of the graphs (Figure 2 and 4). Multiple Comparisons for these groups were performed by the Mann-Whitney U test (Table 3 and 4).

Wilcoxon test results (for Friedman test important groups) of Grooming data for both 25 and 50 mg Agomelatine groups are as follows: The difference between 0th -45th and 0th-90th minutes was found to be significant (P<0.01), but the difference between 45th- 90th minutes was not significant (P>0.05).

In case of Figure 2 and Table 3 are evaluated together, it was determined that the Grooming numbers were higher in the Healthy Control (HC) group than in the other Fluoride applied groups, and significant differences detected between them at the 0th minute (P<0.001). However, over time, a dose-dependent increase was observed in the treatment groups (Group 25 and 50). While the difference between the treatment groups and the F group gain a significancy in the 90th minute data (P<0.01), the differences between the treatment groups and the HC group decreased depending on the dose (P<0.05), and even the difference between the HC group and Group 50 was found to be statistically insignificant (P> 0.05).

For crossed Squares data, the RM ANOVA test was performed to reveal the relationship of groups with each other over time. Since the Sphericity value was found to be significant (P>0.05), "Sphericity assumed" option has been considered for the interaction of time and groups (P<0.001). Tukey HSD Post-Hoc test was employed for multiple comparisons of the groups. All test results are presented in Figure 3.

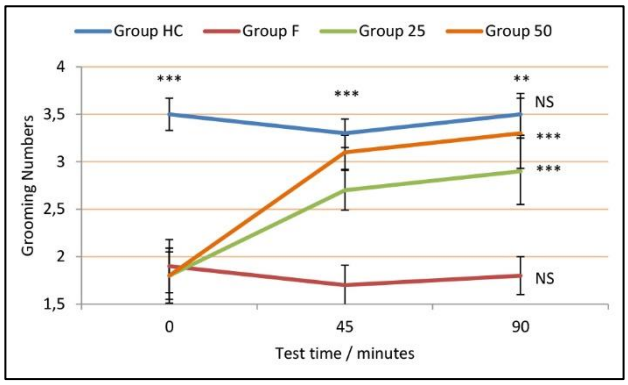

Figure 2. The course of the groups (HC, F, 25 and 50) within time (0th, 45 and 90th minutes) according to the number of Grooming data in the Open-Field test The asterisks at the top of the graph are used for statistical comparisons of independent variables (Kruskal-Wallis test) according to time points, while the asterisks on the right side of the graph are used to indicate the statistical differences of time dependent variables (Friedman test) of individual groups in their course over time.
NS: P>0.05, *: P<0.05, **: P<0.01, ***: P<0.001.

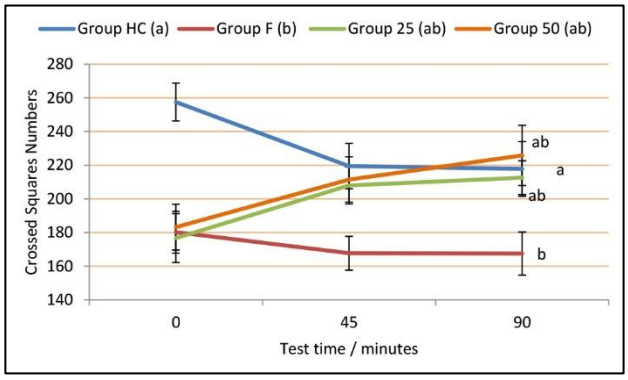

Figure 3. Course of Crossed Squares groups over time and statistical comparisons with RM ANOVA test.
a,b: The difference between groups having different superscripts is statistically significant (P<0.01).

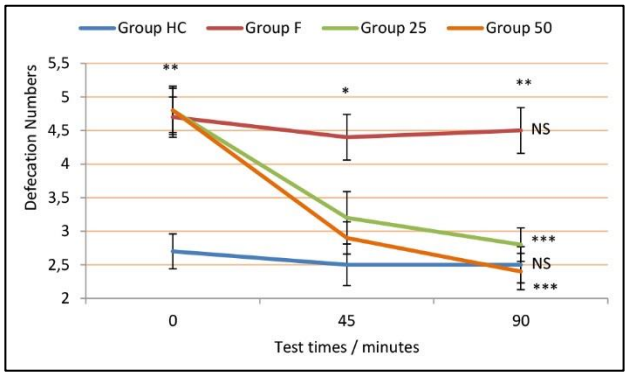

Figure 4. The course of the groups (HC, F, 25 and 50) within time (0th, 45 and 90th minutes) according to the number of Defecations data in the Open-Field test. The asterisks at the top of the graph are used for statistical comparisons of independent variables (Kruskal-Wallis test) according to time points, while the asterisks on the right side of the graph are used to indicate the statistical differences of time dependent variables (Friedman test) of individual groups in their course over time.
NS: P>0.05, *: P<0.05, **: P<0.01, ***: P<0.001.

Table 3. Mann-Withney U test results (for Kruskal-Wallis test important groups) for Grooming data.

	0th Minutes			45th Minutes			90th Minutes		
Groups	F	25	50	F	25	50	F	25	50
HC	***	***	***	***	*	NS	***	*	NS
F		NS	NS		**	***		**	**
25			NS			NS			NS

NS: Not Significant, *: P<0.05, **: P<0.01, ***: P<0.001.
Statistical comparisons were among groups HC, F, 25 and 50 within each time group (0th, 60th and 90th minutes).

Table 4. Mann-Withney U test results (for Kruskal-Wallis test important groups) for Defecation data.

	0th Minutes			45th Minutes			90th Minutes		
Groups	F	25	50	F	25	50	F	25	50
HC	***	***	***	**	*	NS	***	NS	NS
F		NS	NS		**	**		***	***
25			NS			NS			NS

NS: Not Significant, *: P<0.05, **: P<0.01, ***: P<0.001.
Statistical comparisons were among groups HC, F, 25 and 50 within each time group (0th, 60th and 90th minutes).

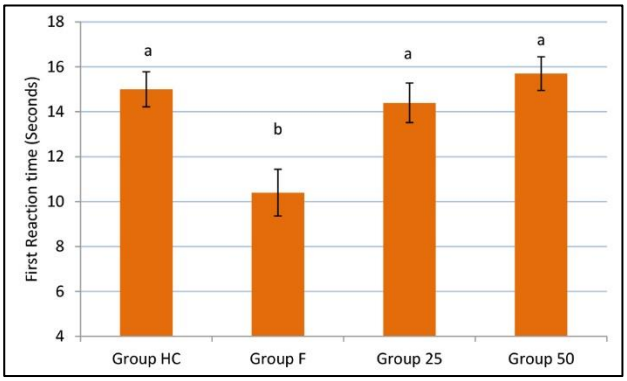

Figure 5. The first reaction time data of the groups in the hot-plate test and their statistical comparisons.
a,b: The difference between groups having different superscripts is statistically significant (P<0.01).

One-Way ANOVA test result of crossed Squares were as follows for minutes 0th; P<0.001 (Post Hoc; Group HC have statistical differences with all other groups) and minutes 90th; P<0.05 (Post Hoc; Group HC have no statistical differences with other groups).

Wilcoxon test results (for Friedman test important groups) of Defecation data for both 25 and 50 mg Agomelatine groups are as follows: The difference between 0th-45th and 0th-90th minutes was found to be significant (P<0.01), however, the difference between 45th- 90th minutes was significant with the P value of P<0.05 and P<0.01 for 25 mg/kg and 50 mg/kg Agomelatine groups respectively).

In the event of Figure 4 and Table 4 were consider together, it was observed that the defecation numbers of the Healthy Control (HC) group at the 0th minute were significantly lower than the other Fluoride applied groups (P<0.001). However, a dose-related decrease was observed in the treatment groups (Group 25 and 50) over time. While a significant difference was found between the treatment groups and the F group in the 90th minute data (P<0.001), it was observed that the difference between the treatment groups and the HC group became statistically insignificant depending on the dose (P>0.05).

Analysis of differences between the groups of Hot-Plate test data, which is parametric data, was performed employing the One-Way ANOVA test and Tukey HSD was performed as Post Hoc Multiple Comparisons. Analysis of Hot-Plate test results having homogeneous subsets is presented in Figure 5.

Discussion and Conclusion

First of all, in the research, it was determined that oral fluoride toxicity in mice caused some changes on the Open-Field and Hot-Plate tests findings. It is reported in the literature that similar findings are obtained in depression and anxiety states (3, 13, 22, 24, 28, 30, 33, 37). The second important outcome of this study is the determination that Agomelatine, a new antidepressant (8, 26, 38), brings the above-mentioned findings closer to normal levels in a short time depending on the dose.

Agomelatine (N-[2-(7-metoksinaftalen-1-yl)etil] acetamid) is a novel antidepressant agent that has been investigated for the treatment of depression and anxiety in recent years. It is reported that Agomelatine is a synthetic analogue of melatonin and has similar agonist effect on MT1 and MT2 receptors (8, 26, 38). It is also reported that Melatonin given exogenously in mental disorder work as an antidepressant and has a healing effect (9). At the same time, Agomelatine has an antagonist effect on 5-HT_{2C} receptors of serotonin, this effect strengthens its anti-depressant activity (5, 6, 29).

Open-Field test is one of the most used tests to determine the emotional state of the experimental animal before any procedure and the changes that may occur after the procedure (22, 12, 34). However, it is an animal model method used to assess loco-motor functions and hypo-locomotion has been reported in depressed and anxious (stressed) mice (18, 28, 33, 37). It has been reported that fluorosis affects the behavior of the subjects, thus changing the Open-Field test results (11, 28, 33).

In this context, there are some scientific studies on the Open-Field test results on different species of subjects who were exposed to toxic amounts of F intoxication with drinking water. In the shortest form, the results of some of these studies are as follows; The Open-Field test results of Oyagbemi et al. (30) showed an increase in motility with giving of 300 mgF/L in drinking water to Wistar rats. The test results of Lu et al. (25) showed a stability in motility with giving of 50 mgF/L in drinking water to mice. The results of Lopes et al. (24) were stable motility for 10-50 mgF/L in drinking water to mice. Mullenix et al. (28) reported a decrease in motility for Open-Field test results with given 75, 100, 125 mgF/L to Sprague-Dawley rat Weanlings. For the ICR mice given 100 mgF/L, Wang et al. (37) reported that a decrease in Open-Field Test results. As a motility data in Open-Field Test, Pereire et al. (33) reported a decrease for male rats given 100 mgF/L. Decreased data were reported by Kıvrak (18) for Open-Field test parameters including Rearing, Grooming and Crossed Square number except defecation count for the Swiss mice receiving 40 mgF/L. At the same time, Kıvrak stated that the number of defecation increased in mentioned study. As can be seen from the reported results, this situation is not clear, conflicting and causes confusion.

If one evaluates the effect of fluorosis alone on Open-Field test results in this study, for the first data (minute 0^{th}) of all parameters (Rearing Grooming, Square and Defecation), it will be clearly seen that there is a significant difference between the HC group and the F toxicity groups (F, 25 and 50) ($P<0.05$) (Figures 1-4). Open-Field test findings of this presented study were similar with some studies previously conducted by Mullenix et al., Wang et al. Pereira et al.ve Kıvrak (18, 28, 30, 33), nevertheless, contrasts with the findings reported by Oyagbemi et al., Lu et al. and Lopes et al. (24, 25, 30).

The different results in these studies above may be originated from different factors including the dose of F applied, the species and breed of subjects, environmental factors and personnel errors. However, the decline of some parameters (Rearing, Grooming and Crossed Square numbers) of this study presented may have been caused by developmental and neuro-developmental toxicity caused by F toxicity (7, 23, 28). On the other side, it has been reported that lethargy is an important symptom of neurological and psychiatric disorders (7, 18). Therefore, as a more logical reason, depression and anxiety induced by F intoxication may have also caused lethargy in subjects of the present study. As can be seen from the charts (Figure 1-4), it was determined that after the application of Agomelatine depending on the dose, the effects of F intoxication decreased and even situation reached in some parameters better than group of HC.

On the other hand, the defecation count findings of the Open-Field test of this study exhibited a different situation compared to other parameters (Figure 1-4). This parameter, unlike other parameters, showed an opposite course with bullish direction. Bowel movements are controlled by a complex mechanisms including sympathetic and parasympathetic nervous systems. Psychological conditions affect bowel movements through the sympathetic and parasympathetic nervous system. It has been reported that bowel movements increase with the increase of Vagal tone in cases of depression and anxiety (4, 32, 35). In this study presented similar to the above information, an increase in defecation was found in subjects exposed to F intoxication. In the advanced stages of the study, it was observed that Agomelatine applications neutralized the increase in defecation after F intoxication and returned it to the HC group data.

The relationship between Open-Field test parameters was examined using Spearsman's correlation test. Significant positive correlation was detected among the parameters Rearing Grooming and Square ($P<0.05$). However, there was significant negative correlation between the Defecation parameter and the others above ($P<0.05$) (Table 2). This correlation situation also confirms the opposite direction of motion mentioned above. The above-mentioned conditions of Open-Field test parameters of this presented study were also in line with the findings of previous studies (18, 28, 33).

A striking finding in the Open-Field test data of both control groups (Rearing, Grooming, Crossed Squares Number and Defecation) is that although a small decrease was observed from the data of minutes 0^{th} to 45^{th}, this decrease did not occur in the later time of the work (90^{th} minute) (Figures 1-4). Zador (39) uses the expression "*a long-lasting change in behavior that is the result of experience*" for the learning term in animal psychology. The reason for this initial fall in question may be because the subjects see an environment they do not know for the first time, and in later times they react more stable than they are used to the environment (12, 22, 27, 34). In fact, this first decline may have been in Agomelatine groups, but since the subjects were under the influence of the administered Agomelatine, these decreases may not have been noticed (Figures 1-4).

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On the other hand, the Hot-Plate test is a method that is generally used to investigate the effect of the analgesic agents on nociceptive system in animal models, based on the determination of the tolerance level against heat (3, 13, 22). However, there is very limited information about the use of the Hot-Plate test in psychiatric disorders such as depressive and anxious cases related to fluorosis. In the study presented, Hot-Plate test results also showed a significant difference according to reaction time between HC and other fluoridised groups ($P<0.01$). This also proves that in the case of depression and anxiety caused by F toxicity, mice have a lower tolerance to heat and react earlier.

Chenaf et al. (6) testified that Agomelatine administration reduces neuropathic pain in animal models through melatonergic, spinal 5-HT_{2C}, and Alpha-2 receptors. In this study also, in cases of depression and anxiety caused by fluorosis, it was observed that Hot-Plate test reaction time returned to normal in a short time depending on the dose after the administration of Agomelatine (Figure 5). These results reveal an interesting and novel effect of Agomeletine on the treatment of psychological disorders caused by F toxicity and prove applications neutralize the low tolerance to heat caused by fluorosis.

In the evaluation of both test results together, it was determined that F toxicity changed the results of Open-Field and Hot-Plate test, and Agomelatine applications neutralized the effects of F intoxication in the short term depending on the dose and even made them better than the healthy control group.

Agomelatine is reported to have acute and chronic treatment options (17). This presented study was conducted on the short-term effects of Agomelatine, namely its acute effects. Since psychiatric diseases such as depression are generally emotional diseases with a chronic course (7), experimental studies on the long-term use of Agomelatine are also needed.

As a result, it was observed that Agomelatine, a novel antidepressant, has a dose-dependent therapeutic effect on depression and anxiety caused by F intoxication in animal model (mouse). According to these results, it has been thought that new studies should be carried out in this direction that they may have positive effects on depression and anxiety caused by fluorosis in both humans and animals.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study was approved by the Animal Experiments Local Ethics Committee with the number KAÜ-HADYEK-014-047.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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