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RESEARCH ARTICLE
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ANTIANXIETY POTENTIAL AND ACUTE TOXICITY ASSESSMENT OF FRAGARIA ANANASSA FRUIT AND LEAVES EXTRACT

Riffat Farooqui[1], Syeda Afroz[2*], Hafiza Touseef Sayyar[3], Syeda Hafiza Afsheen Zafar[4], Fatima Rizvi[5], Waqas Ahmed Farooqui[6].

[1] Department of Pharmacology Dr. Ishrtul Ibad Institute of Oral Health Sciences. Dow University of Health Sciences. Karachi.
[2*]Department of Pharmacology. Faculty of Pharmacy and Pharmaceutical Sciences. University of Karachi.
[3]Department of Pharmacology. Bahria University Health Sciences. Campus Karachi.
[4]Department of Pharmacology. Bahria University Health Sciences. Campus Karachi.
[5]Department of Pharmacology DIMC. Dow University of Health Sciences. Karachi.
[6]School of Public Health. Dow University of Health Sciences. Karachi.

***Corresponding author:** Dr. Syeda Afroz
* Department of Pharmacology. Faculty of Pharmacy and Pharmaceutical Sciences. UOK
safroz@uok.edu.pk

ABSTRACT

Background: Anxiety is life threatening and uncontrollable phobia with a wide range of symptoms and marked number of causes. A variety of pharmacologic agents are used to treat anxiety and depression but the potential side effects lead to the search from newer agents with fewer side effects. Pharmacologist add natural plant-based components to treatments as supplements. The Fragaria Ananassa plant has therapeutic significance and is being employed in various ways.

Objective: The purpose of this study was to determine whether three different doses of fruit and leaves extract of Fragaria Ananassa at different time intervals had any antianxiety effects and to also assess its toxicity index.

Methods: This study was carried out in forty-eight Swiss albino mice (20–30 g of 6–8 weeks). Anxiolytic effect was determined by open field test at three different doses of ethanolic extract of Fragaria Ananassa fruit extract (FE) and leaves extract (LE) (i.e. 100mg/kg, 300mg/kg and 500mg/kg). The specific parameters measured in open field test (OFT) which indicate the spontaneous behavior of the animal. Acute toxicity test was done described by lorke. A p-value of <0.05 was considered statistically significant.

Objectives: This study intended to assess the anxiolytic properties and safety of Fragaria ananassa fruit and leaves extracts in Swiss albino mice through the Open Field Test and acute toxicity evaluation.

Results: Different doses of Fragaria Ananassa fruit extract and leaves extract have shown statistically insignificant antianxiety effect. However clinically significant improvement on different anxiety parameters in mice is observed with different doses of fruit and leaves extracts. There was no toxicity or mortality observed during the acute toxicity study.

Conclusion: Using OFT, clinically significant improvement in anxiety is observed with strawberry fruit and leaves extracts and its LD50 is greater than 5000mg/kg.

1.Introduction

Mental illness account for approximately 14% of the worldwide burden of disorder, depression and anxiety serving as the leading causes of disability globally[1, 2].

In 2017, the World Health Organization (WHO)reported over 260 million people experienced anxiety disorder[3]

Drug therapy for it involves the use of antidepressants with anxiolytic action and benzodiazepine medications[4].However, these prescription drugs have serious adverse effects that include drowsines s, pharmacological tolerance, and drug dependency[5, 6]. The strawberry is widely regarded as a tasty and nutrient-dense fruit with significant nutritional value and as an ideal human source of vitamins, carbs, lipids, and proteins. In Pakistan, strawberries are used all year long to make processed jelly, jams, and squashes in addition to being eaten fresh[7, 8]. According to research, strawberry polyphenols can prevent illnesses linked to oxidative stress, have both direct and indirect antibacterial, anti-allergy, and antihypertensive effects, and can block the actions of various biological enzymes as well as receptors[9]. The Open Field test examines anxiety-like behavior by having the animal move around a wide field for a predetermined period of time. The outcomes include latency to entrance and time spent in the central area[10, 11, 12]. With the use of OFT, the present study examines the effects of varying Fragaria Ananassa fruit and leaf extracts dosages on several anxiety parameters in mice at different time intervals. The acute toxicity testing is done on experimental animals in order to identify any possible risks that a test substance may produce and to characterize how it works.[13]. The short-term evaluation and assessment of Fragaria Ananassa fruit and leaf extracts possible toxicity were also examined at various dosages.

2.Materials and Methods

Experimental Animals

The study was carried out after approval from ASRB/No/06254/Pharm. November 18, 2021, as per ASRB Resol. No. 10 (P) 05 dated 30-08-2021. Animals used for this study were male albino Swiss mice (6 –8 weeks, 20–30 g). These animals were procured from the institutional animal house. Throughout the experiment, they were kept in conventional settings with a 12-hour light-dark cycle and a temperature of 25 ± 2 ◦C after spending a week getting used to the animal house amenities.

Preparation of Ethanolic Extract of Fragaria ananassa Fruit and Leaf Extracts

Fresh Fragaria Ananassa with leaves were collected from the local market of Karachi, Pakistan, in the months of January 2023. The plant material was authenticated by the department of pharmacognosy, College of Pharmacy, University of Karachi, as Fragaria Ananassa FAF-14-20 and FAL-13-20. The Fragaria Ananassa fruits and leaves were washed with distilled water to remove impurities then dried at room temperature (25 °C). The fruits and leaves were blended to a mesh size of 1 mm. The fruit (2 kg) and leaves (800 g) were macerated in five litters of 98% ethanol for three days at room temperature, shaking them occasionally, in preparation for extraction. A rotating evaporator set at 40 degrees Celsius was used to filter and eliminate the extracted solvent. After being freeze-dried, the extract was kept for future research at 20^{o}C.

Experimental Design

Forty-eight males outbreed of 6-8 weeks old mice strain Swiss albino with average body weight of 20-30 grams were divided into eight groups of six mice each, as follows:

1) Control Group (Received 1ml distilled water)
2) Standard Group (Clonazepam 0.5 mg/kg an antianxiety) as a comparison group.
3) Fragaria Ananassa fruit extract (with dose of 100 mg/kg)
4) Fragaria Ananassa fruit extract (with dose of 300 mg/kg)
5) Fragaria Ananassa fruit extract (with dose of 500 mg/kg)
6) Fragaria Ananassa leaves extract (with dose of 100 mg/kg)
7) Fragaria Ananassa leaves extract (with dose of 300 mg/kg)
8) Fragaria Ananassa leaves extract (with dose of 500 mg/kg)

Antianxiety Evaluation

Open Field Test

Following an hour of medication, each animal was positioned in the middle of the OFT apparatus, which is a basic wooden cubic box measuring 68 cm by 68 cm by 45 cm. The floor is divided into 16 squares, which represent the center and outer squares[14]. For five minutes, the number of box crossings, entries into the central zone, time spent in central zone, rearing, and grooming were all recorded. The entire session was captured on camera, which was positioned 1.5 meters above the box.

Acute Toxicity Test

To find the toxic dose that Lorke described in 1982, an acute toxicity test was performed[15].

Phase 1

The experimental albino mice were divided into 6 groups each containing 3 animals having average weight of 25–30 g. Group 1, II and 111 of animals were administered different doses i.e. 10 mg/kg, 100mg/kg and 1000 mg/kg of Fragaria ananassa fruit extract and group IV, V and VI of animals received Fragaria ananassa leaves extract respectively. The animals were placed under observation for 24 hours to monitor their behavior as well as mortality if occur.

Phase 2

This phase involves the use of six animals, which are distributed into six groups of one animal each. The animals were administered higher doses (1600mg/kg, 2900mg/kg and 5000 mg/kg) of s Fragaria ananassa fruit and leaves extracts and then observed for 24 hours for behavior as well as mortality.

Statistical Analysis

The data obtained from the experiment was evaluated by SPSS windows version 27 using mean difference from control (p-value using RM two-way ANOVA and post-hoc (LSD with Bonferroni correction at $\alpha = 0.05/16 = 0.003125$)

3.RESULTS

Antianxiety characteristics using Open Field Test

In this study, the outcome measurements showed no significant group-time interaction, for box crossing (BC) ($F_{14,80}$=0.814, MSE=856.2, p=0.652, $\eta_p^2 = 0.125$), for entry in central zone (ECZ) ($F_{14,80}$=0.607, MSE=2.704, p=0.852, $\eta_p^2 = 0.096$), Time spend in central zone (TSCZ) ($F_{14,80}$=1.100, MSE=29.726, p=0.371, $\eta_p^2 = 0.161$), for Rearing ($F_{14,80}$=0.161, MSE=15.431, p=1.000, $\eta_p^2 = 0.027$), and for Grooming ($F_{14,80}$=1.302, MSE=3.779, p=0.225, $\eta_p^2 = 0.186$) (Table 01).Table 01 showed that box crossing (BC) is insignificantly reduced with all animal groups at 1st, 2nd and 3rd week in comparison to the control group. Maximum insignificant reduction in box crossing BC is observed with CLO (Clonazepam) (50± 19.4) at 1st week. Maximum insignificant increased in entry in central zone (ECZ) is observed with CLO (7.8 ± 2.04) at first week and among test groups FE100 (4.1±1.72) and FE500 (4.1 ±2.40) at first week. Maximum time spent in central zone (TSCZ) is seen with CLO (18 ± 7.0), FE500 (18 ± 12.07) at first week in comparison to the control group (7.3 ± 2.0). Maximum increased in rearing is seen with CLO (13 ± 4.0) at 2nd week, CLO (11 ± 4.17) at 3rd week and FE100 (11 ± 6.48) at 2nd week. Maximum grooming is seen with CLO (7.17 ± 2.78) at 2nd week.

Intra-group comparison

Mean comparison from 1st week of antianxiety characteristics (TSCZ, Rearing, and Grooming) using Open Field Test represents clinically significant improvement on TSCZ with FE500mg/kg group (≤0.001) at 3rd week and clinically significant improvement on grooming with LE100 mg/kg group (0.001) at 2nd week. (Table 02b)

Inter-group comparison

Box Crossing (BC)

At 1st, 2nd and 3rd week of drug administration CLO group showed clinically significant improvement ($p<0.001$) in BC when compared to the control animal group. At 2nd week BC with FE100 mg/kg group showed clinically significant improvement. At 2nd and 3rd week BC with test groups i.e. FE 300 mg/kg, FE 500 mg/kg, LE 100 mg/kg, LE 300 mg/kg and LE 500 mg/kg showed clinically significant improvement ($p<0.001$) in comparison to the control group. (Table 03)

Entry in Central Zone (ECZ)

At 1st, 2nd and 3rd week only CLO group showed clinically significant improvement ($p<0.001$) on ECZ in comparison to the control group. (Table 03)

Grooming

At 1st, 2nd, 3rd week, CLO group showed clinically significant (<0.001) improvement on grooming in comparison to the control group. At 1st week FE100 mg/kg group, at 3rd week FE500 mg/kg group and at 1st and 3rd week LE100 mg/kg showed clinically significant (<0.001) improvement on grooming in comparison to the control group. (Table 03) However, mean improvements in BC at 1st week is better with CLO group (79.3) as compared to BC at 2nd and 3rd week with CLO groups (72.2 and 74.0 respectively). Mean improvements in BC at 2nd week is better with FE100 mg/kg group (58.8) as compared to BC in 3rd week with FE100 mg/kg group (54.7).

Mean improvements in BC at 3rd week is better with FE300 mg/kg group (65.2) as compared to BC in 2nd week of FE300 mg/kg group (64.8). mg/kg Mean improvements in BC at 3rd week is better with LE100 mg/kg (64.2), LE300 mg/kg (64.8) and LE500 (72.8) groups as compared to BC in 2nd week with LE100 mg/kg (59.5), LE300 mg/kg (58.2) and LE500 mg/kg (70.8) groups. (Table 03) Mean improvements on ECZ at 3rd week is better with CLO group (-4.8) as compared to ECZ at 1st and 2nd week of CLO groups (-4.7 and -4.5 respectively). Mean improvement in TSCZ is better with CLO (11.2) and FE500 mg/kg (11.2) group at 1st week. Mean improvement on grooming at 3rd week with CLO group (-6.5) is better as compared to the grooming at 1st and 2nd week of CLO groups (-5.7 and -5.2 respectively). Mean improvement on grooming is better with FE100 mg/kg (-48) at first week FE500 mg/kg at 3rd week (-4.2) and LE100 mg/kg at 3rd week (-4.5). (Table 03)

Acute Toxicity Result:

In our study Fragaria ananassa fruit and leaves extracts were found safe as no changes in behavior and mortality is observed in all animal groups received different doses of FA fruit and leaves extracts during phase 1 (10 mg/kg to 1000mg/kg) and phase 2 (1600mg/kg to 5000mg/kg).

TABLES

Table 01: Mean ± SD of antianxiety characteristics using Open Field Test

Antianxiety Characteristics	Control N = 6 (%)	CLO N = 6 (%)	FE100 N = 6 (%)	FE300 N = 6 (%)	FE500 N = 6 (%)	LE100 N = 6 (%)	LE300 N = 6 (%)	LE500 N = 6 (%)
BC (count)								
1st Week	129 ± 48.5	50 ± 19.4	93 ± 36.8	69 ± 31.6	93 ± 27.3	113 ± 56.0	92 ± 36.9	62 ± 7.60
2nd Week	129± 30.4	57 ± 26.3	70 ± 48.4	64 ± 23.7	58 ± 25.1	69 ± 12.8	71 ± 8.93	58 ± 12.5
3rd Week	131 ± 37.5	58 ± 20.3	77 ± 21.3	67 ± 18.2	59 ± 31.6	68 ± 30.6	67 ± 43.4	59 ± 32.1
ECZ (count)								
1st Week	3.1 ± 1.72	7.8 ± 2.04	4.1 ± 1.72	3.6 ± 1.96	4.1 ± 2.40	3.8 ± 1.16	3.6 ± 1.75	3.5 ± 2.25
2nd Week	2.8 ± 0.75	7.3 ± 2.80	3.3 ± 1.75	3.0 ± 1.54	3.1 ± 1.83	3.1 ± 0.96	2.5 ± 1.37	2.0 ± 1.09
3rd Week	2.6 ± 1.21	7.5 ± 2.88	2.0 ± 1.26	1.5 ± 0.54	1.3 ± 0.51	1.1 ± 0.40	1.3 ± 0.51	1.3 ± 0.51
TSCZ (seconds)								
1st Week	7.3 ± 2.0	18 ± 7.0	13 ± 7.0	8.8 ± 5.81	18 ± 12.07	12 ± 5.85	13 ± 9.0	8.8 ± 5.49
2nd Week	8.8 ± 2.22	17 ± 4.07	10 ± 6.08	9.6 ± 3.20	14 ± 7.55	11 ± 4.53	10 ± 5.57	13 ± 9.48
3rd Week	5.8 ± 2.31	11 ± 6.26	6.5 ± 1.22	5.3 ± 2.0	4.8 ± 2.85	6.3 ± 2.58	9.3 ± 6.0	9.3 ± 4.88
Rearing (count)								
1st Week	7.3 ± 3.07	9 ± 3.18	8.1 ± 3.12	8.5 ± 2.07	8.0 ± 3.84	8.1 ± 2.31	7.6 ± 2.16	7.1 ± 1.60
2nd Week	9 ± 4.35	13 ± 4.0	11 ± 6.48	10.5 ± 4.23	10 ± 5.0	8.5 ± 4.0	9.1 ± 3.43	10 ± 1.72
3rd Week	10 ± 1.94	11 ± 4.17	10 ± 3.16	9.00 ± 3.89	8.3 ± 4.45	9.0 ± 4.19	9.5 ± 5.43	8.3 ± 4.41
Grooming (count)								
1st Week	1.8 ± 1.16	7.5 ± 3.01	6.6 ± 3.38	4.00 ± 0.89	4.5 ± 1.37	6.1 ± 1.72	4.3 ± 1.96	3.8 ± 0.75
2nd Week	2 ± 0.89	7.17 ± 2.78	5.3 ± 3.35	3.5 ± 1.87	3.3 ± 1.03	2.6 ± 1.21	2.6 ± 1.86	3.3 ±1.03
3rd Week	1.3 ± 0.81	7.8 ± 2.71	3.8 ± 2.40	4.1 ± 2.13	5.5 ± 2.25	5.8 ± 2.31	4.5 ± 2.25	3.5 ± 1.04

BC: Box crossing; ECZ: Entry central zone, TSCZ: Time spend in central zone, CLO: Clonazepam, FE: Fruit extract, LE: Leaf extract

Table 02a: Mean comparison from 1st week of antianxiety characteristics (BC, ECZ) using Open Field Test

Antianxiety Characteristics	Control N = 6 (%)	CLO N = 6 (%)	FE100 N = 6 (%)	FE300 N = 6 (%)	FE500 N = 6 (%)	LE100 N = 6 (%)	LE300 N = 6 (%)	LE500 N = 6 (%)
BC (count)								
2nd Week, Δ	0.2	-7.0	22.8	5.7	35.7	44.0	21.3	4.7
Improvement (%)	-16.4	-15.1	23.2	3.7	35.6	30.7	10.6	4.1
P-value[£]	0.992	0.667	0.165	0.751	0.036	0.010	0.194	0.798
3rd Week, Δ	-2.7	-8.0	15.8	2.7	34.0	45.8	25.7	3.3
Improvement (%)	-8.1	-35.0	-15.0	-6.4	36.6	32.0	21.1	1.4

P-value[£]	0.882	0.657	0.381	0.882	0.064	0.014	0.167	0.853
ECZ (count)								
2nd Week, Δ	-0.33	-0.50	-0.83	-0.66	-1.00	-0.66	-1.16	-1.50
Improvement (%)	11.1	0.1	26.1	2.2	-16.7	-5.0	26.1	-13.5
P-value[£]	0.736	0.613	0.400	0.500	0.314	0.500	0.241	0.134
3rd Week, Δ	-0.500	-0.33	-2.17	-2.17	-2.83	-2.67	-2.33	-2.17
Improvement (%)	2.8	10.4	-44.7	-47.8	-58.3	-66.9	-39.7	-40.1
P-value[£]	0.603	0.729	0.029	0.029	0.005	0.008	0.019	0.029

BC: Box crossing; ECZ: Entry central zone, CLO: Clonazepam, FE: Fruit extract, LE: Leaf extract, Δ: mean difference from 1st week, [£]RM two-way ANOVA and post-hoc (LSD with Bonferroni correction at $\alpha = 0.05/16 = 0.00312$

Table 02b: Mean comparison from 1st week of antianxiety characteristics (TSCZ, Rearing, Grooming) using Open Field Test

Antianxiety Characteristics	**Control N = 6 (%)**	**CLO N = 6 (%)**	**FE100 N = 6 (%)**	**FE300 N = 6 (%)**	**FE500 N = 6 (%)**	**LE100 N = 6 (%)**	**LE300 N = 6 (%)**	**LE500 N = 6 (%)**
TSCZ (seconds)								
2nd Week, Δ	1.50	-1.7	-3.0	0.83	-4.83	-1.17	-2.7	4.7
Improvement (%)	33.4	1.7	7.8	56.8	56.3	3.4	13.5	60.1
P-value	0.669	0.635	0.394	0.812	0.173	0.740	0.449	0.188
3rd Week, Δ	-1.50	-7.50	-6.7	-3.50	**-13.7**	-6.0	-3.8	0.50
Improvement (%)	-18.5	-22.2	-38.5	-18.8	-45.7	-38.9	11.7	17.1
P-value	0.652	0.029	0.050	0.295	**≤0.001**	0.077	0.252	0.880
Rearing (count)								
2nd Week, Δ	3.50	2.83	2.83	2.0	2.16	0.33	1.50	3.0
Improvement (%)	58.8	45.7	55.5	29.7	49.2	24.3	32.2	45.7
P-value	0.128	0.215	0.215	0.379	0.341	0.883	0.509	0.190
3rd Week, Δ	2.50	1.0	1.83	0.50	0.33	0.83	1.83	1.16
Improvement (%)	57.3	9.7	40.2	15.6	32.6	21.1	34.2	28.0
P-value	0.285	0.667	0.431	0.829	0.886	0.720	0.431	0.616
Grooming (count)								
2nd Week, Δ	0.17	-0.33	-1.33	-0.50	-1.17	**-3.50**	-1.66	0.50
Improvement (%)	-13.9	19.0	-18.4	-9.7	-18.1	-55.2	-25.0	-8.1
P-value	0.871	0.745	0.197	0.626	0.258	**0.001**	0.109	0.626
3rd Week, Δ	-0.50	0.33	-2.83	0.16	1.0	-0.33	0.16	0.33

Improvement (%)	-19.4	40.2	-32.1	4.4	31.4	-25.0	27.9	-2.5
P-value	0.694	0.793	0.030	0.896	0.433	0.793	0.896	0.793

TSCZ: Time central zone, CLO: Clonazepam, FE: Fruit extract, LE: Leaf extract, Δ: mean difference from 1st week, [£]RM two-way ANOVA and post-hoc (LSD with Bonferroni correction at α = 0.05/16 = 0.003125)

Table 03: Mean comparison from control of antianxiety characteristics (BC, ECZ, TSCZ, Rearing and Grooming) using Open Field Test

Antianxiety Characteristics	**CLO N = 6 (%)**	**FE100 N = 6 (%)**	**FE300 N = 6 (%)**	**FE500 N = 6 (%)**	**LE100 N = 6 (%)**	**LE300 N = 6 (%)**	**LE500 N = 6 (%)**
BC (count)							
1st Week	**79.3 (<0.001)**	36.2 (0.090)	59.8 (0.006)	36.0 (0.092)	15.7 (0.456)	37.0 (0.083)	66.8 (0.003)
2nd Week	**72.2 (<0.001)**	**58.8 (<0.001)**	**64.8 (<0.001)**	**71.0 (<0.001)**	**59.5 (<0.001)**	**58.2 (<0.001)**	**70.8 (<0.001)**
3rd Week	**74.0 (<0.001)**	54.7 (0.004)	**65.2 (<0.001)**	**72.7 (<0.001)**	**64.2 (<0.001)**	**64.8 (<0.001)**	**72.8 (<0.001)**
ECZ (count)							
1st Week	**-4.7 (<0.001)**	-1.0 (0.371)	-0.5 (0.653)	-1.0 (0.371)	-0.7 (0.549)	-0.5 (0.653)	-0.3 (0.764)
2nd Week	**-4.5 (<0.001)**	-0.5 (0.599)	-0.2 (0.861)	-0.3 (0.726)	-0.3 (0.726)	-0.3 (0.726)	0.8 (0.382)
3rd Week	**-4.8 (<0.001)**	0.7 (0.364)	1.2 (0.116)	1.3 (0.074)	1.5 (0.045)	1.3 (0.074)	1.3 (0.074)
TSCZ (seconds)							
1st Week	-11.2 (0.011)	-5.8 (0.173)	-1.5 (0.723)	-11.2 (0.011)	-5.0 (0.241)	-5.8 (0.173)	-1.5 (0.723)
2nd Week	-8.0 (0.021)	-1.3 (0.692)	-0.8 (0.804)	-4.8 (0.155)	-2.3 (0.489)	-1.2 (0.620)	-4.2 (0.170)
3rd Week	-5.2 (0.029)	-0.7 (0.771)	0.5 (0.827)	1.0 (0.663)	-0.5 (0.827)	-3.5 (0.132)	-3.5 (0.132)
Rearing (count)							
1st Week	-2.5 (0.125)	-0.8 (0.604)	-1.2 (0.469)	0.7 (0.678)	-0.8 (0.604)	-0.3 (0.836)	0.2 (0.917)
2nd Week	-1.8 (0.468)	-0.2 (0.947)	0.3 (0.895)	0.7 (0.791)	2.3 (0.357)	1.2 (0.509)	0.7 (0.791)
3rd Week	-1.0 (0.702)	-0.2 (0.949)	0.8 (0.750)	1.5 (0.567)	0.8 (0.750)	0.3 (0.899)	1.5 (0.567)
Grooming (count)							
1st Week	**-5.7 (<0.001)**	**-4.8 (<0.001)**	-2.2 (0.068)	-2.7 (0.026)	**-4.3 (< 0.001)**	-2.5 (0.036)	-2.0 (0.091)
2nd Week	**-5.2 (<0.001)**	-3.3 (0.006)	-1.5 (0.200)	-1.3 (0.254)	-0.7 (0.566)	-0.7 (0.566)	-1.3 (0.254)
3rd Week	**-6.5 (<0.001)**	-2.5 (0.045)	-2.8 (0.024)	**-4.2 (0.001)**	**-4.5 (<0.001)**	-3.2 (0.012)	-2.2 (0.080)

BC: Box crossing; ECZ: Entry central zone; TSCZ: Time spend in central zone; CLO: Clonazepam, FE: Fruit extract, LE: Leaf extract, Δ: mean difference from control (p-value using RM two-way ANOVA and post-hoc (LSD with Bonferroni correction at α = 0.05/21 = 0.0024)

1. DISCUSSION

Anxiety is an uncomfortable mental state characterized by nervousness, apprehension and worry or anxiety over an unknown or well-defined prospective danger[16]. The frequency of the disease stays unchanged despite a continuous growth in the creation of antianxiety medications; this may be due to the imprecise neurobiological knowledge of pathogenesis or the uneven effectiveness of present therapies[17]. As berries contain a considerable quantity of polyphenols and anthocyanins, which are linked to anxiolytic action at the preclinical level, research on the possible effects of berries on anxiety has garnered significant attention[18, 19]. In Pakistan, some areas of Punjab and KPK provinces produce strawberries as a yearly crop[20].

Research was conducted to evaluate the phenolic profiles of Fragaria x ananassa cv. Festival fruit and leaves from crops produced in the tropical highlands of Costa Rica. The results indicated that the leaves of the plant had a 13-fold greater antioxidant activity (ORAC) and a 122-fold greater total polyphenol amount than the fruits[21]. With the goal to find novel, effective natural remedies for the symptoms of anxiety, Open Field Test OFT evaluated the anxiolytic properties of Strawberry fruit and leaf extracts in the present research. The specific parameters measured in open field experiment include in our study are number of box crossings (BC), number of entry in central zone (ECZ), time spend in central zone (TSCZ), rearing and grooming which indicate the spontaneous behavior of the animal. Regarding the parameter of BC, ECZ, TSCZ, rearing and grooming there were no significant differences observed. However, among test groups FE500 group showed insignificant anxiolytic behavior supported by maximum insignificant reduction in BC, maximum insignificant increased on ECZ, maximum insignificant increase on TSCZ. FE100 group showed insignificant anxiolytic behavior supported by maximum insignificant increased on ECZ, rearing and grooming. LE500 group showed insignificant anxiolytic behavior supported by maximum insignificant reduction on BC count (Table 01). Interestingly mean comparison from 1st week of antianxiety characteristics (TSCZ, Rearing, Grooming) using Open Field Test represents significant improvement in anxiolytic behavior with FE500 mg/kg group (≤0.001) supported by significant clinical improvement on TSCZ and with LE100 mg/kg group (0.001) supported by significant clinical improvement on grooming (Table 02b). The parameters evaluated in the behavioral assessment test e.g. OFT a most prevalent sign of anxiety, such as greater entries and time spent in the center as opposed to the periphery[22]. In our study no significant changes on rearing behavior is observed. However better clinical improvement in anxiety behavior on different anxiety parameters are seen at 2nd and 3rd week with CLO group and animals on Strawberry fruit and leaves extracts i.e. at 2nd and 3rd week maximum better clinical improvement in BC is observed with FE500 group 36% and 37% respectively (Table 02a). On ECZ at 2nd week maximum better clinical improvement is observed with FE10 mg/kg 0 group (26%) and LE300 mg/kg group (26%) and at 3rd week LE100 mg/kg group (67%) (Table 02a). On TSCZ at 2nd week maximum better clinical improvement is observed with LE500 mg/kg group (60%) and at 3rd week with FE500 mg/kg group (46%). On grooming at 2nd week maximum better clinical improvement is observed with LE100 mg/kg group (55%) and at 3rd week CLO group (40%) (Table 02b). Furthermore mean intergroup comparison from control group of anxiety characteristics (BC, ECZ, TSCZ, rearing and grooming) using Open Field Test showed statistically significant clinical improvement in anxiety behavior i.e. with CLO, FE100 mg/kg, FE300mg/kg, FE500mg/kg, LE100mg/kg, LE300mg/kg and LE500 mg/kg groups supported by statistically significant (≤0.001) improvement in BC, ECZ and grooming with CLO group and statistically significant improvement (≤0.001) in BC and grooming with FE100 mg/kg, statistically significant clinical improvement (≤0.001) in BC count with FE300mg/kg group, statistically significant improvement (≤0.001) in BC and grooming with FE500 mg/kg group, statistically significant improvement (≤0.001) in BC and grooming with LE100 mg/kg groups and statistically significant improvement (≤0.001) in BC count with LE300 mg/kg and LE500 mg/kg mg/kg. (Table 03). The observations supported the anxiolytic effects of the FA fruit and leaves extracts treatment.

Overall results with CLO group and all test groups showed antianxiety characteristics but these antianxiety characteristics are not dose dependent nor duration of treatment dependent. The potential of strawberry fruit to lessen anxiety is not as well-established as that of various foods or substances.

Still, a few of the ingredients in Fragaria ananassa may help lower anxiety. It has been found that strawberry fruit contain several anthocyanidin glycosides as well as phenolic substances such as ellagic acid, ellagic acid-glycoside, and coumaryl glycoside. Antioxidant, cancer prevention, anti-inflammatory, and anti-neurodegenerative qualities are also attributed to the Fragaria ananassa fruits[23, 24]. Certain flavonoids and anthocyanins have been demonstrated to have anxiolytic properties based on their interaction for GABA-A receptors[25]. Acute toxicity results of Fragaria ananassa fruits and leaves extracts showed no mortality at doses between 10mg/kg to 5000 mg/kg. Our findings are corroborated by Ibrahim DS[26], who also noted that animals did not die at any of the extract dosages up to 800 mg/kg.

2. CONCLUSION

In conclusion, these results imply that adding Fragaria ananassa fruit and leaves extracts in pharmacotherapy of anxiety disorders may potentially play a significant role in easing their symptoms. Given the high concentration of antioxidants and polyphenols found in strawberry fruits and leaves, nutritional strategies could be a means of reducing some of the detrimental effects of long-term exposure to oxidative stress. The findings of acute toxicity of Fragaria ananassa fruit and leaves revealed that these extracts are non-toxic with oral dose of 5000 mg/kg/day.

Novelty Statement

Given the promising anxiolytic effects of Fragaria ananassa fruit and leaves extracts in animal model, it is surprising that more robust effects were not observed. Limitations of this study include a small sample size, the antioxidant capacity and its effects as functional food on animal health have not assessed. However, some further studies such as long-term dose effects and some mechanistic studies are needed to fully evaluate the possible mechanisms, underlying the anxiolytic-like effects of fruit and leaves extracts.

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