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Rose Petal Tea as an Alternative Anxiolytic for Stress Management: An *In vivo* study Based on Elevated Plus Maze and Open Field Test

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Abstract
Aim: Medically stress is managed with anxiolytics with a plethora of adverse effects. Stress can be holistically managed by using herbal tea such as Rose petal tea. The aim of this study was, for the 1st time, to establish the efficacy of Rose petal tea as an anxiolytic. **Method:** Male Swiss albino mice were used for Open Field test (OFT) and Elevated Plus Maze (EPM) anxiety model without causing any psychological or physical trauma induced stress. Diazepam (at the doses of 1mg/kg body weight and 0.25mg/kg body weight) was used as standard and garden variety of *Rosa chinesis* (at doses 2g/kg body weight and 1g/kg body weight) was the sample. The control group had no intervention. Parameters such as line crossing, time spent in center, time spent in thigmotaxis were measured for OFT and number of entries in open and closed hands, time spent in the open and closed hands were measured for EPM. Additionally rearing, grooming, urination, defecation and weight changes were also monitored for both tests. **Results:** For the 7-day trial the Rose petal tea showed very close results in compared to the standard drug Diazepam. There was marked weight loss by the use of the sample which was not seen in the other groups. **Conclusion:** Rose petal tea can be implemented for holistically managing stress and can act as a fantastic anxiolytic instead of common anxiolytics and can avert the side effects while combating weight gain due to stress by being rich is flavonoids and antioxidants.

Keywords
Stress management, Rose petal tea, CAM, Alternative anxiolytic, Open Field Test, Elevated Plus Maize

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INTRODUCTION
Stress is a psychosomatic reaction of the mind and body to situations or triggers that an individual finds hard to deal with and it causes anxiety at the least. Stress over time leads to many physiological and psychological dysfunctions in the body [1]. Stress leads to a plethora of such effects as cardiovascular disease, respiratory issues, diabetes, allergy,

miscarriage, anxiety and panic disorders, depression, low immune response etc. and is also a major cause of suicide in many backgrounds of people [2-5]. Suicide among Japanese workers is at an all-time high. It's high time the world takes note of this notorious daily life mate and tries to learn and develop ways to cope with this before it takes over our lives and reduces the quality of life.

Current anxiolytics such as the Benzodiazepine varieties have had major breakthrough since their inception in 1957 and are still used world-wide. Although they are widely used, still benzodiazepines come with their set of side effects such as sedation, muscle relaxation, alcohol incompatibilities, amnesia and addiction [6]. Diazepam is a common anxiolytic used worldwide for the lower side effects. Anxiolytics sometimes are not even the drug of choice since they might not be effective for certain patients or that the adverse effects of dependence and tolerance becomes a nuisance than a boon. Stress can also be effectively managed by holistic and alternative and complementary medicinal systems such as Yoga, meditation, mindfulness, healthy eating, exercise, acupressure, acupuncture, building relaxation rituals like massaging or drinking herbal teas such as rose, bamboo, chamomile, peppermint etc. [7,8].

Rose, sometimes termed as king of flowers, is revered the world over for its aesthetics and beneficial properties as well as the use of it for various skincare regiments and rituals. In a comprehensive review on rose especially rose hip (fruit of wild rose variety) the authors found rose hip to have anticancer effect, anti-inflammatory activities, containing loads of antioxidants to fight free radicals (which contribute to its use in skincare), hepatoprotective action, weight reduction, antidiabetic effect amongst others [9]. *Rosa damascene* has been shown to have antibacterial and antifungal effect [10]. Rose water is used in skincare, cooking and as a perfuming and purifying agent at various cultural and religious gathering [11]. Rose oil is an expensive and very much sought out for the therapeutic and healing properties. Rose petal tea is traditionally Drank in the Middle Eastern, Chinese and Japanese cultures and recently has gained western attention [12]. Rose has calming attribute which lead us to the current study on the beneficial and stress busting effect of rose petal tea. To the best of our knowledge this study is a first reporting of the therapeutic effect of such. The current study is the first sample we studied from our "Stress management using Alternative Medicinal approaches" project. The broad aim of this project was to scientifically establish anxiolytic and calming implicates used in various culture across the world. Rose has been as integral part of Middle Eastern culture and we wanted to prove the prospects of Rose as an alternative anxiolytic which has become a norm for stress and anxiety management.

Open field Tests (OFT) is one of the well-known psychological analytical tools to ascertain stress and its responses in laboratory animals such as mice, rats, hamsters and due to its easy workability and no requirement of training and the test has been also used in other animal species as well [13]. Elevated Plus maze (EPM) is another highly effective and versatile psychological testing available to the psychiatry research realm. It is used in a plethora of situations such as testing new treatment of anxiety-related pharmacological agents, testing anxiolytic and anxiogenic agents, drugs of abuse, hormonal product testing etc. [14]. The tests were chosen to be Implicated in this research based on their simplicity and efficacy. The detailed protocol is discussed in section 2.3 and 2.4.

2. MATERIALS AND METHODS

2.1 Sample preparation

Garden variety of *Rosa chinesis* was collected from the Herbal Garden Of Department of Pharmacy, Primeasia University, Dhaka, Bangladesh by the authors. They were collected at their maturity during the blooming season from February through May 2018. The flowers were washed and dried under shade at a temperature-controlled room (25^{0}C). The petals were removed before drying. After drying the dried petals was then stored in airtight jars in a temperature-controlled room until future use. About 350 gm of dried rose petals were harvested. For the dosage 1gm and 2 gm of the loose tea would be steeped in purified water for 10 mins with the temperature kept at 60-70^{0}C so as to preserve as much of the bioactive as possible allowing much extraction to have taken place.

2.2 Animal

Male Swiss Albino mice (27gm-31gm) were procured from Jahangirnagar University, Bangladesh for the experimental procedure. They were housed at a separate facility in temperature and light controlled condition. 6 mice were put in each cage for each group of Control (no change marked C1-6), Standard Low dose (Diazepam, Sigma Aldrich, 0.25mg/kg body mass marked SL1-6) and Standard High dose (Diazepam, Sigma Aldrich, 1mg/kg body weight marked SH 1-6), Rose petal tea low doses (1g/kg body weight marked as RA 1-6) Rose petal tea high dose (2 gm/kg body weight marked RB 1-6). The mice were fed AdLibitum and kept in very hygienic and calm

condition. Extraneous stressors were removed as much possible so to reduce their exposure to stressful environment, hence ensuring an acceptable experimental model. The tests were chosen very carefully to avoid any means of cruelty and mishandling. Force-feeding of sample tea was done in a soothing manner. Overall it was taken into grave consideration to avoid stressing and not testing them in any harmful, painful or cruel manner. All standard ethical norms were maintained during the entire duration of the study. The study was conducted after gaining ethical board permission from Biomedical Research Center, Dhaka University, Bangladesh with the reference number BMRC/EC/2017-2018/237. The total duration for trial was 4 weeks with each test undertaken for 7 days with a week habituation at the beginning and another at the end to see residual effects if any. Weights were measure the first time before the start of the Trial at Day 1 and the last time was measured at the last day of trial to explore if there was any effect of the samples on the weight.

2.3 Open Field Test maze

A wooden box of dimensions 50cm x 50cm x 35cm was built with no roof so the maze replicates an open field. The floor of the maze is marked into squares with a center space left in middle. The centers along the edge of the walls are marked with a different colour then the center. The floor and walls are smooth so there is no risk of splinters. The maze was placed away from the mice and in a well-lighted area. Each mouse was allowed to roam freely as their wish inside the field during testing period. The mice were removed from their home cage one by one and very gently left in the center of the maze. The stopwatch was switched on and calculations were started in parameters such as Thigmotaxis (wall hugging and staying in the squares near the walls), Rearing (standing up on hind legs, without the support of wall on their own), grooming (toughing the face and other motions with their paws), number of times the lines of the squares crossed and the total amount of time spent both in the center and around the walls were all measured carefully. The frequency of urination and defecations was taken into consideration. Since an anxious mouse will urinate and defecate more than normal during a stressful situation. A calmer mouse will explore the center and do rearing and grooming more which will be a mean for testing the efficacy of the sample. The procedure was carried out alternatively for 7 days. The experimental design for this procedure has been followed from the protocol of Seibenhener and Wooten [13]. The results were tabulated in worksheets such as Table 2.1.

Table 2.1: An example of a worksheet for Open field test.

Parameters	M1	M2	M3	M4	M5	M6
Line crossing						
Thigmotaxis time						
Time spent in the center						
Rearing						
Grooming						
Urination						
Defecation						

M= mice number

2.4 Elevated T-Maze

The second instrument for the anxiety model was an elevated T-Maze build out of plywood. The four runways (each side 2-inch-wide and 11.75 inches long) were open in two ends and closed with walls (of height 6 inches) in the other two. The runway was secured on mounted stands, attached to the runway base and height from the floor to the base of runway was a foot each. The closed ends mimic their natural habitat of closed space while the open end poses an element of risk and causes anxiety in the animals. The parameters measured in this test were total time spend in open ends and closed ends along with the number of entries in the open and closed places. Along with these parameters such as rearing, grooming, urination and defecations were also taken into consideration. A calm mouse will try and explore the open ends which will be a mean of finding the efficacy of the drug or treatment given. The procedures were carried for 7 days in alternative order. The experimental protocol has been heavily scripted from the work of Walf and Frye [14]. A sample worksheet for the Elevated-Plus maze is given in table 2.2.

Table 2.2: Worksheet for Elevated Plus maze

Identity of mice	Time spent		No. of Entry		Urination	Defecation	Rearing	Grooming
	Open	Closed	Open	Closed				
M1								
M2								
M3								
M4								
M5								
M6								

3. RESULTS AND DISCUSSION

The thought of implementing the selected sample Rose petals came from the widespread use of rose petal tea in the Middle Eastern belt along with Arabic peninsula. Rose is a venerated flower and its aesthetics lead to a variety of uses from skin beautification to calming the nerves. Rose is pretty available and affordable which is another reason to choose this for its scientific implications in stress management. Pill popping is not the number one choice of the health-conscious generation we have now and pharmacologically the anxiolytics are very much connected to various side effects such as dependency, drowsiness, sedation, confusion, stomach upsets, depression, sexual dysfunction and even suicidal thoughts [15,6]. Stress is an unavoidable human experience and an integral part of life. The new age medicine depicts that drugs and medicines are not very favourable to our emotional and psychological wellbeing. In 2015 a group of researchers embarked on a the ambitious ESCAPE programme (The Effects of Stress on Cognitive Aging, Physiology and Emotion) which scientifically proved the effect of stress (the daily life style related types) on the cognitive setup of human along with its effect on aging, physiological and psychological aspects [5]. The study concluded that stress has both short term and long-term adverse effect on the quality of life. The complementary and alternative realm depicts that stress requires to be handled with understanding, patience and natural therapeutic means with a change in lifestyle [16]. Herbal infusions have been connected to a wide variety of benefits one of which could be stress relief or temporary relief.

The present study embarked on the journey to scientifically establish the efficacy of Rose petal tea in transcending calmness in the experimental subjects. Both the Elevated Plus Maze (EPM) and Open Field Test (OFT) has been utilized to include as many variables into the analysis and improving the soundness of the experimental design. OFT tested the following parameters: Time spent in Center (depicted as High-risk zone to rodents), Thigmotaxis (hugging the wall to avoid the open, the number of lines crossed (used for calculating Ambulation), urination, defecation and grooming. Two more parameters were calculated using the Center duration, Thigmotaxic duration and Line crossed which are % CT or percentage of center versus thigmotaxic duration and % CL or percentage of center duration versus total lines crossed. The %CT and % CL are two very important analytical parameters which helped us to ascertain the activity, exploration and anxiety. Urination, defecation, rearing and grooming were determined as supporting parameters as in some experiments they were seen to show higher effects than others. For the other analysis of Elevated Plus Maze (EPM), the parameters of number of Entry in the Open area (High risk zone) versus Total number of Entries were depicted as OE/TE, the parameter of Time in Open versus Total Time as OT/TT along with other parameters of urination, defecation, rearing and grooming were also calculated. Each trial lasted for 7 days per test, per group in alternative days to reduce experimental bias of getting used to. Below Table 3.1-3.4 are depiction of the 7-day trial summary of the various parameters tested in the course of this research for both the OFT and EPM procedures.

The comparative depiction of the %CT (percentage presence in center cumulating to having lower stress level), % CL (comparative percentage of center vs. line crossing cumulating to having lower stress level) of Open Field Test (OFT) is done in Tables 3.1 and 3.2 .The depiction of OE/TE (entry to open area vs. total number of entry cumulating to having lower stress level), OT/TT (time spent in open area vs. total time spent cumulating to having lower stress level) for the Elevated Plus Maze (EPM) in Tables 3.3 and 3.4. Both the high and low doses were analysed and shown as compared to the control and Standard drug groups. The parameters of urination and defecation was found to be very negligent amount in both OFT and EPM tests and was therefore concluded to not having any effect on the testing parameters of either doses or drugs used. There have been some studies which concluded that defecation and urination didn't show

effect and was gleaned as unrelated to stress which supported the theory that emotionality in rodents are multidimensional in nature [17]. Our study can conclude that urination and defecation didn't show any significantly acceptable range of effect on the specimens tested be that the drug or the experimental treatment for their stress levels.

For the current study the parameter of rearing was found to be insignificant. Rearing is standing on their hind legs unattached to any support and usually in the center spaces meaning their level of stress being low. In the current study the rodents were seen to show very low frequency (0-2 times/session) of rearing for all the groups which can only be summarized that rearing was not a significant parameter for this study. Grooming on the other hand was seen to be a significant parameter with wide range in results. The general trend for grooming was seen to be high in the initial few days and near the end of the trial going down again. Grooming is usually displayed more in novel situations and is usually claimed to be a type of displacement response which could be the reason why initially the animals showed higher frequency and as the novelty wore off the frequency decreased [18].

Table 3.1: Comparative depiction of the %CT and %CL of OFT testing for Rose High dose, Standard drug high dose and control group.

Days	% CT Rose High dose	% CL Rose High dose	%CT Std High dose	%CL Std High dose	%CT Control	%CL Control
Day 1	4.0457	11.81263	4.531259	12.06506	1.856634	7.32172
Day 2	3.000541	10.85859	3.774792	11.48406	0.970903	5.45631
Day 3	2.316038	6.19137	2.701975	6.987126	0.301729	4.026786
Day 4	1.709891	6.17284	2.467253	6.492019	0.651839	2.222812
Day 5	1.576583	8.58209	1.980119	7.771289	1.121766	1.422993
Day 6	2.191603	9.815951	2.338761	10.20456	1.954121	2.275229
Day 7	2.84589	11.32466	3.346891	12.54232	2.827763	3.586207

*** %CT comparison was found to be significant *p<0.05*; ** %CL was found to be very significant *p<0.001*.**

Table 3.2: Comparative depiction of the %CT and %CL of OFT testing for Rose Low dose, Standard drug Low dose and control group.

Days	%CT Rose Low dose*	%CL Rose Low dose**	%CT Std Low dose*	%CL Std Low dose**	%CT Control	%CL Control
Day 1	2.456378	8.07621	2.715838	8.30243	1.856634	7.32172
Day 2	1.920402	5.098039	2.33716	5.803119	0.970903	5.45631
Day 3	0.678421	4.70984	1.01845	5.021345	0.301729	4.026786
Day 4	0.886928	3.09803	1.29699	3.688553	0.651839	2.222812
Day 5	1.354605	2.02291	1.493117	3.096517	1.121766	1.422993
Day 6	2.10901	3.54671	2.443671	4.332154	1.954121	2.275229
Day 7	3.30821	5.21093	3.801567	6.030208	2.827763	3.586207

*** %CT comparison was found to be significant *p<0.05* ; ** %CL was found to be very significant *p<0.001*.**

3.3: Comparative depiction of the OE/TE and OT/TT of EPM testing for Rose High dose, Standard drug high dose and control group.

Days	OE/TE (%) Rose High dose*	OT/TT (%) Rose High dose*	OE/TE (%) Std High dose*	OT/TT (%) Std High dose*	OE/TE (%) Control	OT/TT (%) Control
Day 1	45.67217	3.53517	46.35765	3.81335	20.1385	2.81649
Day 2	50.11231	6.913756	51.5	8.620039	22.8766	2.93243
Day 3	56.45161	14.3211	63.08333	15.82979	40.8289	3.95187
Day 4	67.44186	9.970674	71.75	11.35765	50.579	5.4991
Day 5	60.21212	6.111677	61.30961	7.23906	42.7826	4.00971
Day 6	44.44444	3.716508	58.12821	5.00311	27.0833	1.88235
Day 7	29.00035	2.46109	29.84721	3.43129	17.037	0.77299

***%OE/TE & OT/TT are significant (p<0.05)**

3.4: Comparative depiction of the OE/TE and OT/TT of EPM testing for Rose Low dose, Standard drug Low dose and control group.

Days	OE/TE (%) Rose Low Dose	OT/TT (%) Rose Low dose	OE/TE (%) Std Low dose	OT/TT (%) Std Low dose	OE/TE (%) Control	OT/TT (%) Control
Day 1	25	1.8721	26.25	4.89683	20.1385	2.81649
Day 2	36.11111	3.950104	37.41667	4.56777	22.8766	2.93243
Day 3	45.30119	4.748603	47.64286	7.79736	40.8289	3.95187
Day 4	64.70588	7.816712	55	8.21177	50.579	5.4991
Day 5	54.03002	4.329609	55.89313	7.43198	42.7826	4.00971
Day 6	40.05716	2.223159	41.56213	5.5621	27.0833	1.88235
Day 7	26.09121	1.763908	27	5.27889	17.037	0.77299

***%OE/TE & OT/TT are significant (*p<0.05*)**

Table 3.5: Depiction of the pre- and post-trial weights of the experimental animals.

Groups	Mean Pre-Trial Weight (gm)	Mean Post-Trial Weight (gm)	% Change	Inference
A/Control	28.33333	32.16667	+ 13.5	Wight gained
B/Std High	28.33333	31	+ 9.4	Weight gained
C/Std Low	28.5	30.16667	+5.8	Weight gained
D/Rose High	29.33333	24	-18.2	Weight lost
E/Rose Low	29	28.16667	- 2.9	Weight lost

The main hypothesis of these experiments was that the rose petal tea will bring calmness in experimental animals which will be seen as an increase in their risk-taking approaches as well as increase in exploratory behaviors. The calmer a rodent is the more time it will spend in open spaces and will also be more active in the center spaces be it for OFT or EPM. The OFT evaluates by taking the presence of the rodents in the center quadrants, the thigmotactic period, how many quadrants they cross (line crossing) etc. For the Rose High dose group, the results can be seen to be very close to the Standard high dose if not surpassing the later.

The rose petal tea is seen to have quite close and similar degree of efficacy to reduce anxiety and produce calming effect which is translated to the rodents showing higher risk assessment and venturing in the center more. The more calm and less stressed a rodent is the more it will try to experiment with open spaces and may have lesser tendency to hide. As the biological response of rodents are to stay in dark enclosed spaces so the more, they feel the urge to cross lines all around the OFT test ground, the more it is ascertained that the anxiolytic is showing positive action. The general trend of action of the anxiolytic across the 7-day trial is seen to follow a similar trend for both the diazepam and rose. The %CT values are seen to be lower compared to the %CL values. The %CT and %CL from the OFT procedure for high dose of Standard Diazep am (Std) for day 1 were 4.531 and 12.065 whereas for Rose high dose the values were 4.045 and 11.812 respectively (Table 3.1). The values for the low doses for %CT and %CL of Standard Diazepam (Std) for day 1 was 2.715 and 8.302 and for Rose they were 2.456 and 8.076 respectively (Table 3.2). The values can be seen to be very close with the standard drug Diazepam (Std) slightly above than the Rose tea. Figure 3.1 (a-b) depicts %CT and %CL of Rose High as compared to Standard high dose while Figure 3.1 (c-d) is showing the %CT and %CL of Rose Low dose as compared to Standard Low dose. The trend can be seen to start declining from day 2 till day 5 after which the results climb up until day 7. This pattern of declining and again increasing can be attributed to the rodents having higher degree of stress for the test procedures as they prefer to stay in close dark habitat rather than a lighted room. Repeated exposure to OFT apparatus has been shown to have a time dependent pattern [19]. Also another study has concluded that in some strain repeated exposure decreases their activity due to habituation which could be the reason of the decline and then the increase in activity after the novelty wore off and they became habituated and become more comfortable to explore the fields of OFT and EPM [20].

It is interesting to see that although the results had a decreasing tend after the novelty has worn off from day 2 but at the end of the trial the levels again got back to near the initial states as can be seen from both the tables and figures. The results for the last day (day 7) of the trial for %CT and %CL from the OFT procedure for high dose of Standard Diazepam (Std)

was 3.346 and 12.542 whereas for Rose high dose the values were 2.845 and 11.324 respectively (Table 3.1). The values for the low doses for %CT and %CL of Standard Diazepam (Std) for day 7 was 3.801 and 6.030 and for Rose they were 3.308 and 5.210 respectively (Table 3.2). The last day results ended at slight lower levels but somewhat close to the day 1 results. This can therefore be concluded that once the rodents acclimatized with the test their general anxious trait was reduced and with repeated use of Rose petal tea the stress level of the rodents were decreased.

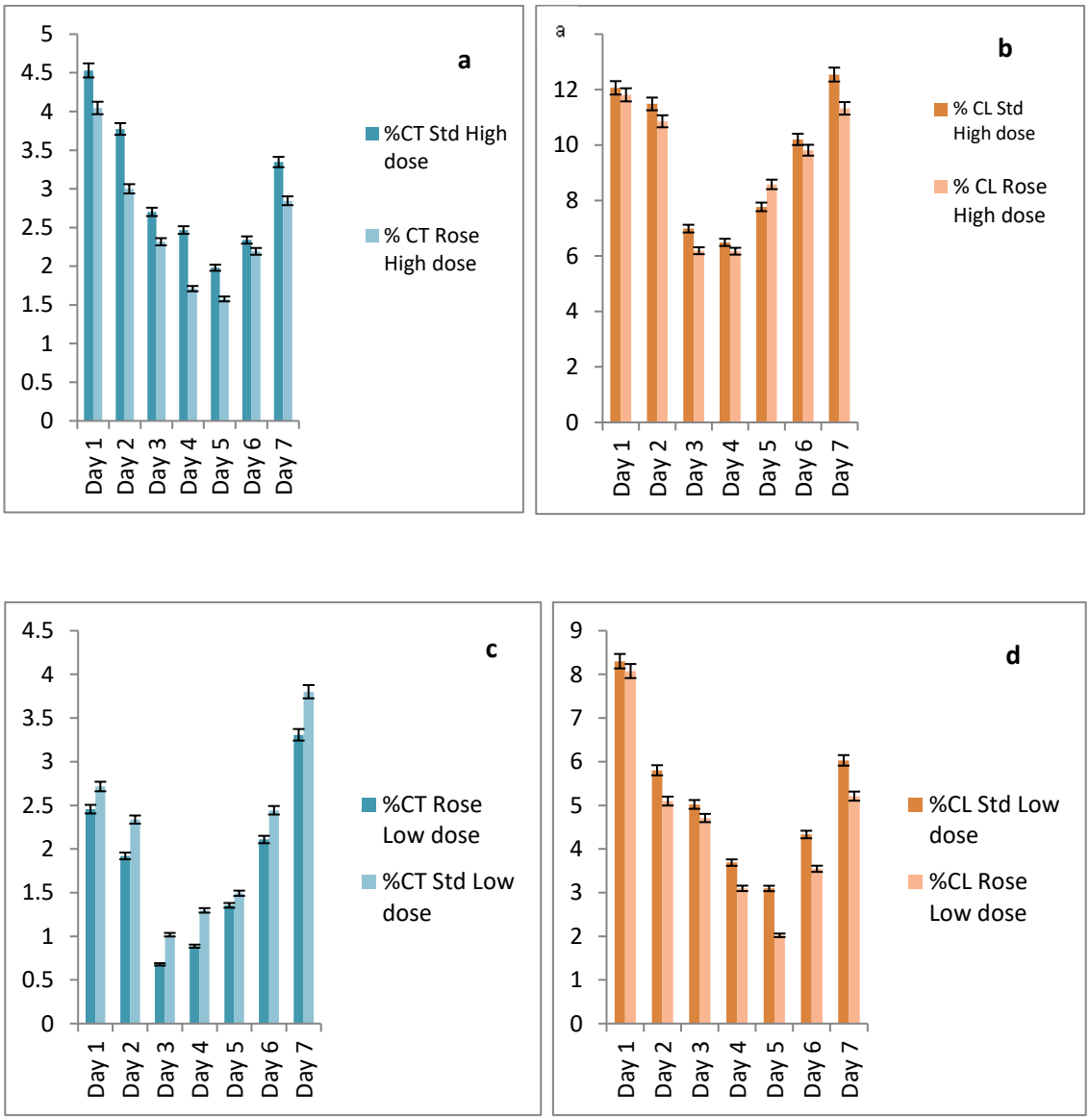

Figure 3.1: Results for Open Field Test (OFT) (a) %CT Standard drug High dose vs Rose High dose;(b) %CL Standard drug high dose vs. Rose High dose; (c) %CT Standard Low dose vs. Rose Low dose; (d) %CL Standard Low dose vs. Rose Low dose. %CT comparison was found to be significant *p<0.05* while %CL was found to be very significant *p<0.001*.

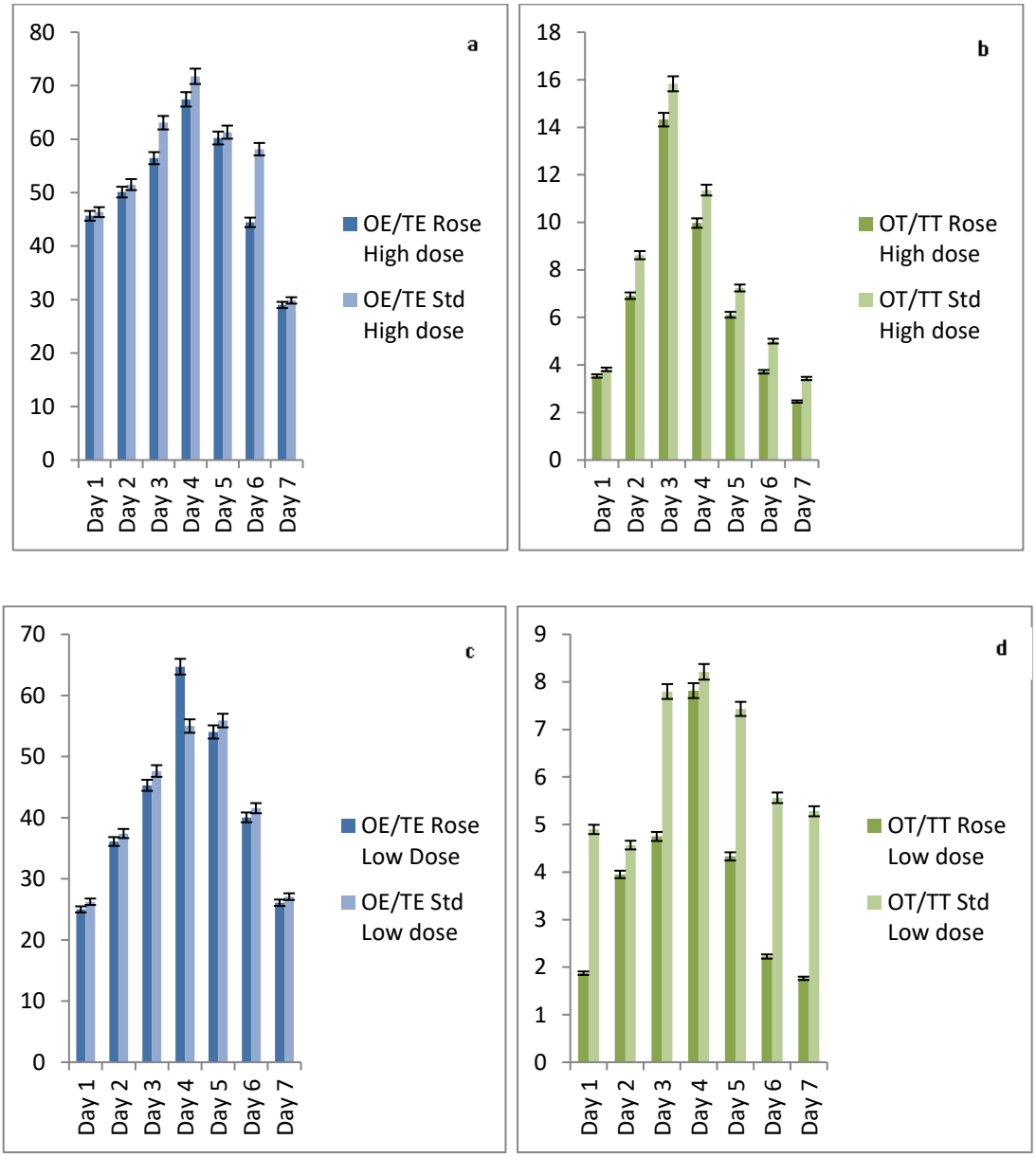

Figure 3.2: Results for Elevated Plus Maze (EPM) (a) OE/TE Standard drug High dose vs Rose High dose; (b) OT/TT Standard drug high dose vs. Rose High dose; (c) OE/TE Standard Low dose vs. Rose Low dose; (d) OT/TT Standard Low dose vs. Rose Low dose. OE/TE and OT/TT comparison were found to be significant *p<0.05*.

Results from the Elevated Plus maze are depicted in Tables 3.3 and 3.4 as well as in Figures 3.2 a-d. The %OE/TT and OT/TT of high dose is shown in Table 3.3 and Figure 3.2 a and b whilst the OE/TT and OT/TT of low doses are depicted in Table 3.4 and Figure 3.2 c and d respectively. The Elevated Plus Maze (EPM) test procedure is said to have Face validity which means the test procedure has the ability to measure what it what purported to observe. Rodents have a high aversion to open, brightly lit and high places so a more agitated rodent will hide in enclosed spaces while a calmer one will show some degree of exploring open arms. The EPM also has predictive validity along with Face validity [14]. Some studies design EPM to be carried on right after OFT tests which is said to increase open arm exploration. But in this study, we did the OFT and EPM on separate trial periods to test the independent results of each procedure. In some comparative study showed that on second exposure there were drops in baseline activity although it is possible to keep the baseline to

stay stable if the test is done for a 3 weeks period [21].

Elevated Plus Maze (EPM) test is another preferentially used technique to ascertain the activity of anxiolytics and was the second test we utilized to verify the efficacy of Rose petal tea as a mode of stress reduction and calming alternative to anxiolytic such as Diazepam. The results of EPM can be seen (Figure 3.2 a-d) to follow a different trend than OFT. In OFT the result started on a high note followed by a declining trend until 4th day and after that increasing back to the initial high state whilst for EPM the trend was inverse. There was a low level of activity to start with, which was followed by a gradual increase in activity until the highest activity was seen to be on day 4 followed by a declining rate. This inverse result can be a resultant reason of not being pre-exposed to EPM which is based on the element of height. Pre-exposure has been seen to improve activity so the sudden elemental fear of height could have been the reason for the low starting level of activity [14]. As the days went by the rodents were more accustomed and the fear decreased followed by a decline which can also be there continued exposure giving them additional stress. Bolivar et al. [20] stated in their study that repeated exposure can cause some strains to increase their activities while some others will show habituation and their activity levels may decrease and there are some strains that show no change in behavior due to repeated exposure.

There is a similar activity report for both OFT and EPM, that being the Rose petal tea showing slight less but commendable and closer to standard Diazepam activity. For OE/TE the number of entries in the open ends versus the total number of entries were calculated. While the high rose dose was seen to be very closely contending with standard for three of the criteria except rose low dose for EPM testing. The OT/TT of rose tea has been slightly further in activity than the other parameters tested so far. Table 3.2 and 3.3 shows the results for high and low doses respectively. The Day 1 OE/TE and OT/TT of Rose High dose were 45.67217 (%) and 3.53517 (%) while for Standard Diazepam the results were 46.35765 (%) and 3.81335 (%). The results for Day1 OE/TE and OT/TT of Rose low dose were 25 (%) and 1.8721(%) whilst for Diazepam they were 26.25 (%) and 4.89683 (%) respectively. The highest activity was seen on the 4th day with the activity to be as high as 67.44% of OE/TE and 9.97% ($p<0.05$) of OT/TT activity of Rose high dose and 71.75% and 11.35% ($p<0.05$) for Diazepam high dose respectively.

The last parameter left to discuss was the weight change if any on the treatments of the various implicates. Table 3.5 is depicting the pre- and post-trial weight of the experimental animals in respect to their treatments. The animals stayed a period of 1 month in the lab and they were given standard pellet diet at regular intervals and they being in their early stage of lives would be required to put on weight in normal circumstances. The table will show that the control group with no intervention gained weight by 13.5% and the Standard high and low dose group gained 9.4% and 5.4% respectively. Both the rose groups showed an interesting case as they underwent weight reduction in the course of the trial. Rose high and low dose reduced weight by 11.7% and 4.5%. This can hence be implied that Rose petal tea has the ability to reduce weight. It is prudent to note that stress is invariably connected to weight gain due to the hormone Cortisol. It is preparing our body for flight or flight by reducing metabolism and storing fat hence the predominant weight gain during stressful times [22]. The study can show that Rose petal tea is having dual property of not only reducing anxiety but also stress related weight gain can be reduced also whereas Diazepam didn't have any effect on weight reduction.

Natural anxiolytics as well as sedative hypnotics include cammomile, valeria root, St. John's wort etc. Sedation-hypnosis or anxiolytics usually works by being agonist / antagonist of various neurotransmitters such as cholecystokinin, excitatory amino acids like butyric acid, serotonin (5-hydroxy tryptamine) and nor-adrenaline. These complex resorts of neurotransmitters are maintained by the gamma amino butyric acid or $GABA_A$ receptors which are the chloride ion channels. The function of these channels is to hyperpolarize and generally reducing the excitatory capability of the CNS. Benzodiazepines such as Diazepam (the standard drug used in this study) bind to GABA receptor allosterically as an agonist thus creating a calming sensation. On the other hand, the mechanism by which rose petal tea is bringing on this similar sensation does not fall under the criteria of the study, but it can be implied based on literature review that rose being a plethora of flavonoids could be a reason for inducing calmness. A study done some time ago by Medina et al [23] found some flavonoids that bind to benzodiazepine receptors and giving similar action. A later study [24] found chrysin (5, 7-dihydroxyflavone), apigenin (5, 7, 40-trihydroxyflavone) and 6, 30-dinitroflavone, derived from natural source to be more potent than diazepam. Zhao et al. [25] isolated the following

compounds from *Rosa chinesis* and they were succinic acid (1), methyl succinate (2), ethyl gallate (3), protoatechuic acid (4), vanilllic acid (5), shikimic acid (6), methyl 3-O-(β-D-glucopyranosyl) gallate (7), benzyl 6'-O-galloyl-β-D-glucopyranoside (8), phenylethyl 6'-O-galloyl-β-D-glucopyranoside (9), catechol (10), hypericin (11), kaempferol-3-O-α-L-arabinopyranoside (12), kaempferol-3-O-β-D-glucopyranoside (13), and pinocembrin-7-O-β-D-glucopyranoside. The sample was a rich source of flavonoids and phenolic acid which could have worked in the similar mechanism as the flavoinoids.

Both the Open Field Test (OFT) and Elevated Plus Maze (EPM) have shown significant positive effect on the experimental animals in reducing their anxiety and thus lowering the stress level. The more stressed a rodent is the more it will show aversion towards open space and stay away in closed spaces. Then in stress they show a variety of stress-induced behaviours such as thigmotaxis, stretch-attend pose, urination, defecation etc. all of which indicates that the animal is hesitant to move from the current position to new territories. So, when they cross lines and stay in center considerably more than it can be safely assumed that the rodents are hence more in exploratory mode which means they are feeling calmer. The results suggest that both the Rose petal tea and standard drug Diazepam showed positive effect in reducing anxiety.

The tea can therefore be proposed as part of the lifestyle and can be helped to keep the stress level down at a significant rate. The repeated use of anxiolytic causes many side effects of dependency and other uncomfortable and unhealthy effects whereas the repeated use of Rose petal tea being a natural therapy is seen in this study to show similar degree of efficacy. Therefore, rose tea could be proposed instead of anxiolytic as a part of therapeutic routine for anxiety disorders.

4. CONCLUSION

It can be concluded from this study that Rose petal tea can effectively reduce stress, can act as anxiolytic significantly contending diazepam which is the current world favorite. Stress is a part of modern life and being complacent about it or managing it by taking anxiolytics routinely has been seen to not be the best holistic and healthy way of coping with anxiety and stress. Instead rose petal tea could be introduced as a beneficial and effecting stress reliever and could be a part of lifestyle as a preventive and therapeutic alternative to anxiolytics.

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DECLARATION OF CONFLICT

None to declare.

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