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Original article

Investigating the Avocado (Persea americana) fruit's anti-anxiety potentials in rat models

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ABSTRACT

Background and aim: Anxiety has an effect on the common regular living of a human as it causes fatigue and restlessness. In the current study, an effort was undertaken to investigate the anti-anxiety behavior of male albino by the treatment of Avocado Powder and Juice in vivo.
Methods: Avocado Powder 10 % (AP1) and 15 % (AP2) substituted from the diet and Avocado Juice 100 mL/kg (AJ1) and150ml/kg body weight (AJ2) rat over control rats.
Results: The oral intake of Avocado powder and Juice caused a significant decrease in the body weight gain, daily feed intake, and feed efficiency ratio (FER) in all experimental groups tested as compared to control. Also, the activity of the antioxidant enzymes like SOD, GST, GPX, and Catalase is not much influenced by the intake of avocado fruit. This significant result has confirmed the effectiveness of this fruit for the treatment of anxiety. The anti-anxiety effect of the avocado fruit was tested by exploring the behavioral changes tests in experimental rats. All the experiments conducted showed that the intake of dose AP2 and AJ2 has significantly decreased the number of head dips and cage crossing and increased the time spent in light side in light–dark transition box test, and increased time spent in open arm in elevated plus maze test.
Conclusions: This result proved that the avocado fruit as powder then as juice have an anxiolytic effects and will be a better alternative for people with an anxiety disorder.

1. Introduction

In life, as a part of everyday life, a person experiences anxiety disorder, one of the most common mental disorders that occurs due to a fear situation and worries. It is an entirely natural human feeling, and in case these feelings occur for a longer time period, it causes distraction in both physical and mental health (Cha et al., 2005).This anxiety disturbs the regular everyday living of a human as it is an adjunct to fatigue and restlessness, thereby creating difficulty in focus. The occurrence of anxiety might be due to multiple factors such as stress, alcohol intake (Quitkin et al., 1972), caffeine intake and may be due to withdrawal of particular drugs. Also, this might occur with other mental disorders (Healy, 2008) like major depressive disorder or bipolar disorder etc.

Usually, anxiety could be treated by using minor tranquilizers. In the main, Phenobarbital and other long-acting barbiturates are widely used as anti-anxiety medicine prior to the introduction of benzodiazepines in 1960 s. Furthermore, the barbiturates were replaced by benzodiazepines for the reason that in overdose, they have a high margin of safety. Conversely, benzodiazepines are associated with ill-treatment, addiction, and withdrawal symptoms (Stein and Stein 2008). But, Buspirone, a non-benzodiazepine, is used as an alternative choice for the treatment of anxiety; distinctly, they are not associated with dependence and withdrawal symptoms like benzodiazepines.

Various anti depressants are also proved successful in the treatment of a range of anxiety disorders such as panic disorder, Generalized Anxiety Disorder (GAD), Post-Traumatic Stress Disorder (PTSD), and Obsessive-compulsive disorder (OCD). Currently, a small number of synthetic anti-anxiety medicines are available and also the currently approved anti-anxiety drugs are associated

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with the varied complexities for anxiety patients (Cloos and Ferreira 2009). Consequently, there is an increasing requirement for medicines that are herbal base as they have extensive and therapeutic applications since they produce only slight side effects. In these circumstances, many superfluous side effects caused by benzodiazepines have provoked researchers to investigate novel compounds for anti-anxiety medicine with fewer side effects (Grundmann et al., 2007).

Although, compared to synthetic medicines, most herbal derivatives were declared free from side effects and less toxic in Ayurveda (Pari and Maheswari 1999). According to the intensity of anxiety, the treatment can be varied. In general, dietary intake and lifestyle modification will be useful for the initial treatment of anxiety and also psychological treatments like Psychotherapy (Lader, 1994) and Cognitive behavior therapy (CBT) (Reinecke et al., 1998). Most commonly, fruits are rich source of nutrients and are associated with better mental health and lessened symptoms of depression.

In this view, Avocado fruits have high nutritional quality and are rich in vitamins, minerals, proteins, and fibers, as well as high concentrations of unsaturated fatty acids that provide more benefit to human health (Yahia et al., 2017). Furthermore, it is also reported that the various bioactive phytocompounds such as phenolic acids, hydroxybenzoic acids, hydroxycinnamic acids, condensed tannins, flavonoids, procyanidins and flavonols produced by the avocado peel and seed had shown enormous biological activities like antioxidant and anti-inflammatory properties (Hurtado-Fernandez et al., 2011; Figueroa et al., 2018A; Figueroa et al., 2018B). With all these observations, the current study were proposed to examine the anti-anxiety effect of the fruit *Persea Americana* (avocado) based on the exploratory behavior using a rat model.

2. Materials and Methods

2.1. Experimental Animals:

Forty male albino rats weighing 100gm ± 10gm were used for the study. Rats have resided in a cage that was clean and clear. The cage was maintained at 23 °C with free access to food and water. The rats were become accustomed to laboratory conditions before being exposed to experiment.

2.2. Preparation of Avocado juice and powder

To feed the rats, avocado fruits were purchased from a local market. The avocado juice was prepared by removing the peel and seed of the fruit. The fruit pulp was crushed and initially filtered through muslin cloth and finally through sterilized Whatmann No.1 filter paper. The prepared juice was kept at 0–8 °C until use. The avocado powder was prepared by the dehydration and drying of avocado pulp by applying a low temperature of 45 °C for 48 h and blended into a powder.

2.3. Details of dose administration and experimentation:

Male albino rats were equally divided into 5 groups each group containing 8 rats. Group 1 was taken as normal control and given regular basal diet. Group 2–5 were taken as treated and given avocado powder juice. The experimental design was as follows:

Group 1: Normal Control (rats fed with basal diet).
Group 2: Avocado Powder (AP) 10 % substituted from the diet (AP1).
Group 3: Avocado Powder (AP) 15 % substituted from the diet (AP2).
Group 4: Avocado Juice (AJ) 100 mL/kg body weight rat (AJ1).
Group 5: Avocado Juice (AJ) 150 mL/kg body weight rat (AJ2).

2.4. Biological Effects:

The biological values of different treatments were assessed by the determination of the Body Weight Gain and Feed Intake (FI), which was evaluated at the end of the experimental period, and Feed Efficiency Ratio (FER) that was calculated twice a week. The mean of the body weight gain, feed intake and feed efficiency ratio were also taken and recorded (Chapman et al., 1959).

3. Biochemical characterization of anti-oxidant enzymes:

3.1. Superoxide dismutase (SOD) assay

The SOD activity level in blood serum was determined as described in a previously published protocol (Kakkar et al., 1984).The SOD activity was examined by using sodium pyruvate phosphate and phenazine methosulphate. 150 μL of the serum was mixed with 600 μL of 0.052 mM of sodium pyrophosphate buffer (pH 7.0) containing 50 μL of 186 mM phenazine methosulphate as a substrate. The reaction was initiated by adding 100 μL of 780 μM of NADH and 500 μL of acetic acid at 1 min. interval. The color change was determined by recording the results at 560 nm in a spectrophotometer and expressed in mmol/l protein.

3.2. Glutathione-S-transferase (GST) assay

The level of GST in blood serum was determined as described in the previously reported protocol (Habig et al., 1974). The reaction comprised of 150 μL of the serum to 720 μL of sodium phosphate (0.1 mM), 150 μLof GSH (1 mM) and 14.5 μLof chloro-2,4-dinitrobenzene (CDNB). The optical density was taken at340 nm in a spectrophotometer to determine the formation of the CDNB conjugate. The GST level was estimated and expressed in mmol/l.

3.3. Glutathione peroxidase (GPx) assay

The GPxlevel in serum was estimated using the protocol (Mohandas et al., 1984). The reaction was formed by the simultaneous addition of 1.59 mL of 0.2 M of potassium phosphate buffer (pH 7.4), 0.5 mL of glutathione reductase (1 1 U/mL), 0.1 mL of 1 mM of sodium azide, 0.01 mL of 0.25 mM of H_2O_2 (as a substrate)and 0.1 mL of 0.2 mM of NADPH. The GPx level in serum was estimated and expressed in mmol/l.

3.4. Catalase (CAT) assay

The CAT level was estimated by the protocol as described in (Chance and Maehly 1955).The changes in the Catalase activity were taken by recording the absorbance of the serum sample at 240 nm after every 30 s. The Catalase activity was estimated and expressed in μ /l.

3.5. Determination of anti-anxiety activity

The treated and control rats were examined using Head dip test Cage crossing activity Light and dark test Elevated plus maze test for the exploration of the anti-anxiety effect of the fruit avocado. All the experiments were conducted and evaluated at day intervals 0, 10 and 21 days.

3.6. Head dip test

The apparatus for the head dip test consists of a wooden rectangular box enfolding an area of 35x45 cm^2 with a hole board on the floor, elevated from the ground in a way that the rats would be able to peek through the holes. The hole board contains 12 holes each of 2.5 cm in diameter in about four walls. Before the test started all rats were habituated with the environment and allowed to move freely. The rats were placed in the center of the hole board individually and the number of head dips through the holes was recorded (Clark et al., 1971) for five minutes at day intervals 0, 10, and 21 days of treatment. The apparatus was cleaned thoroughly with 70 % alcohol after each reading (Hall and Ballachey 1932).

3.7. Cage crossing activity

The apparatus used for the analysis of Cage crossing activity consists of a transparent, Plexiglas cage of 26 × 26 × 26 cm^3.The rat was introduced in the Plexiglas cage at the center by gently placing its tail. The activity of each rat was observed for 5 min, and the number of cage crossings was counted and recorded. The apparatus was cleaned thoroughly with 70 % alcohol after each reading was taken (Hall and Ballachey 1932).

3.8. Light and dark test

The rodents favor dark places generally and so the locomotion of Light and dark areas was tested by the method described by Crawley and Goodwin (1980) for the prediction of anxiolytic or anxiogenic activity of rat. The locomotion apparatus of 20 × 40 × 40 cm^3 box area was sectioned into two equal compartments like light and dark compartment was taken. The dark one ispainted black with a black lid and light side compartment has transparent sides with a transparent lid accompanied by an illumination lamp with an opening in the center.

The rat models were recognizable with the place where the apparatus was placed. The rat was introduced individually at center of light illuminated compartment. Then, the rats were allowed to move freely in the light and dark compartments for about 5 min. The time spent by the rats in each compartment was noted and recorded (Sarfaraz et al., 2015). The apparatus was cleaned thoroughly with 70 % alcohol after each reading (Hall and Ballachey 1932).

3.9. Elevated ***plus maze test***

The elevated plus maze was used to confirm the anxiolytic or anxiogenic activity of the rats. The plus shaped apparatus was designed for this test. The maze has two arms that are enclosed with two open and two closed arms. The height of the apparatus was elevated 40–70 cm above the floor (Carobrez and Bertoglio 2005). The rats were introduced carefully and gently at the center of the plus shaped apparatus. The rodents were allowed to move for 5 min freely in the apparatus and the total number of transitions from enclosed arm to open arm was observed. The apparatus was cleaned thoroughly with 70 % alcohol after each reading was taken (Hall and Ballachey 1932).

3.10. ***Statistical*** *analysis*

All the result by experimentation was **s**tatistically analyzed using SPSS 19 (Statistical Package for Social Sciences 19). The mean value of all the treatments was compared with control and with each other. The data were expressed as mean ± S.E.M from 8 rats [n = 8]. The two way ANOVA (analysis of variance) followed by post hoc Tukey's test was performed for the calculation of the significance difference between means. The values of $p < 0.05$ is considered significant, $p < 0.01$ is moderately significant and $p < 0.001$ is highly significant.

4. Results

4.1. Biological effect of Avocado powder and Juice on the experimental rats

The biological values of different treatments were assessed at the end of the experiment and recorded the treatment of Avocado powder and Juice over different parameters such as the body weight gain, feed intake and feed efficiency ratio (FER) of experimental rats (Table 1). At a maximum, the intake of AP1& AJ1 has a non-significant decrease in the body weight gain(66.3 g)&(68.4 g), Daily feed intake(18.4 g/w) &(17.9 g/w) and FER (0.054) & (0.056) respectively when compared to all other treatments and control.

4.2. Biochemical characterization of anti-oxidant enzymes:

The blood Serum analyzed to examine the effect of Avocado powder and Juice on antioxidant enzymes such as SOD, GST, GPX and CAT at the end of the experiment showed that the treatment of Avocado powder and Juice has not influenced the activity of the enzymes SOD, GST, GPX and CAT that are responsible for oxidation (Fig. 1).

4.3. Determination of anti-anxiety activity:

The anti-anxiety effect of Avocado powder and Juice on head dip activity compared to control was shown in Fig. 2. At first, the rat tried to escape from the head dip apparatus due to its neophobic nature. Then, the neophobic responses reduced over time when exposed to the apparatus, continuously lessening the number of head dips result leading to the reduction of anxiety. Generally, all the treatments reduced the number of head dips of all rats significantly when compared to control after 21 days. The treatment of AP2 and AJ2 reduced the number of head dips by 11.1 and 12.6, respectively, whereas in control, the average number of head dips was 26.1.

The anti-anxiety effects of Avocado powder and Juice on the cross-caging activity are presented in Fig. 3. It showed that the activity of crossing the cage was reduced, which showed the anti-anxiety effect. In this experiment, the treatment of AP2 and AJ2 has expansively reduced the mobility of rats to 3.8 and 3.1 in comparison with control.

In the Light and Dark Box Test, the administration of Avocado powder and Juice in rats increased the time spent in alight area showing anxiolytic activity of the avocado (Fig. 4). As rodents usually prefer a dark area, the rats fed with AJ1 and AJ2 has spent more time of 142 and 149 s in the light area compared to normal control which was recorded at123 seconds.

The Anxiolytic activity of Avocado powder and Juice was examined by calculating the time spent in the open arm in the Elevated Plus Maze Test of the rats (Fig. 5). In general, rodents use to hide and do not prefer the open arm for a long time. However, the rats that were given the feed of AP2 and AJ2 spent more time, 101 and 104 s, in the open arm rather than preferring the closed arm as compared to the control.

5. Discussion

The use of natural products derived from plants for the treatment of anxiety is desirable due to its advantages that include low cost,

Table 1
Biological Effect of Avocado powder and Juice on weight gain, feed intake and feed efficiency ratio (FER) of experimental rats.

Parameters	Groups				
	Normal Control	AP 10 % Diet	AP 15 % Diet	AJ (100 mL/kg BW)	AJ (150 mL/kg BW)
Body Weight gain (g)	88.25 ± 4.61	66.32 ± 3.21**	71.51 ± 6.11*	68.41 ± 7.13**	74.02 ± 8.11
Feed intake (g/w)	19.84 ± 2.26	18.44 ± 1.32	18.90 ± 1.30	17.97 ± 1.22	18.86 ± 1.19
FER	0.076 ± 0.003	0.054 ± 0.003*	0.060 ± 0.001	0.056 ± 0.002*	0.068 ± 0.004

Values are the means ± S.D n = 8 rats/group. The data were analyzed statistically by two way ANOVA. P values were represented as * P < 0.05; * * P < 0.01; *** P < 0.001: Significant with normal control group.

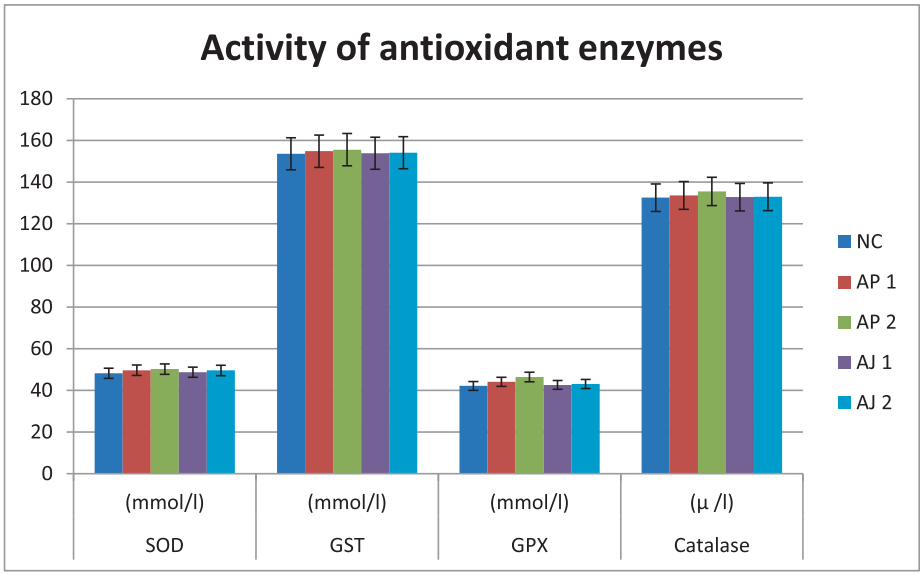

Fig. 1. Effect of Avocado powder and Juice on various serum antioxidant enzymes (SOD, GST, GPX and CAT). Values are the means ± S.D n = 8 rats/group. The data were analyzed by the two way ANOVA for statistical analysis and post hoc Tukey's test.

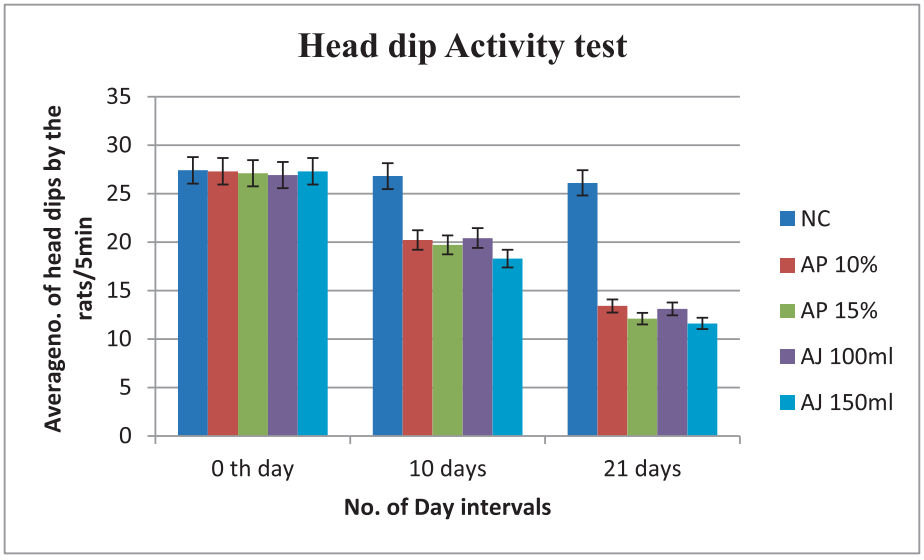

Fig. 2. Number of head dips by the rats treated with Avocado powder and Juice in Head dip Activity test. Values are the means ± S.D n = 8 rats/group. The data were analyzed by the two way ANOVA for statistical analysis and post hoc Tukey's test.

safety, and effective use. Avocado (*Persea Americana*) has achieved substantial popularity in recent times and is frequently marketed as a "super food". It is due to its unique nutritional composition, antioxidant content and rich biochemical profile (Bhuyan et al., 2019). So, in our current study, the anti-Anxiety Effect of Avocado powder (AP) and Juice (AJ) on experimental rats were examined and analyzed in different formulations like AP1,AP2, AJ1 and AJ2, and all these treatments showed that they have anti-anxiety activity.

The results of decrease in body weight, Feed intake, and FER for most treated groups compared to control due to consumption of

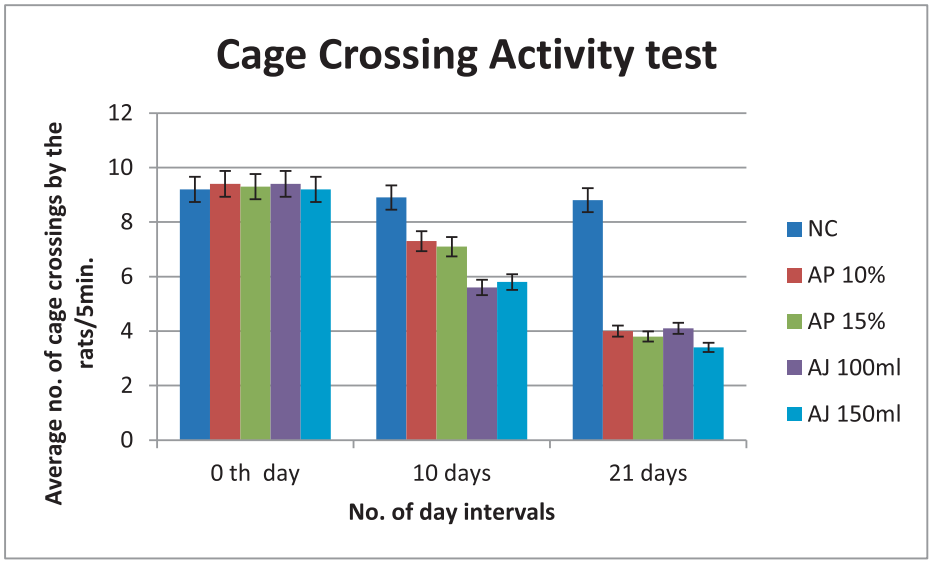

Fig. 3. Number of cage crossings by rats treated with Avocado powder and Juice inCage Crossing Activity test. Values are the means ± S.D n = 8 rats/group. The data were analyzed by the two way ANOVA for statistical analysis and post hoc Tukey's test.

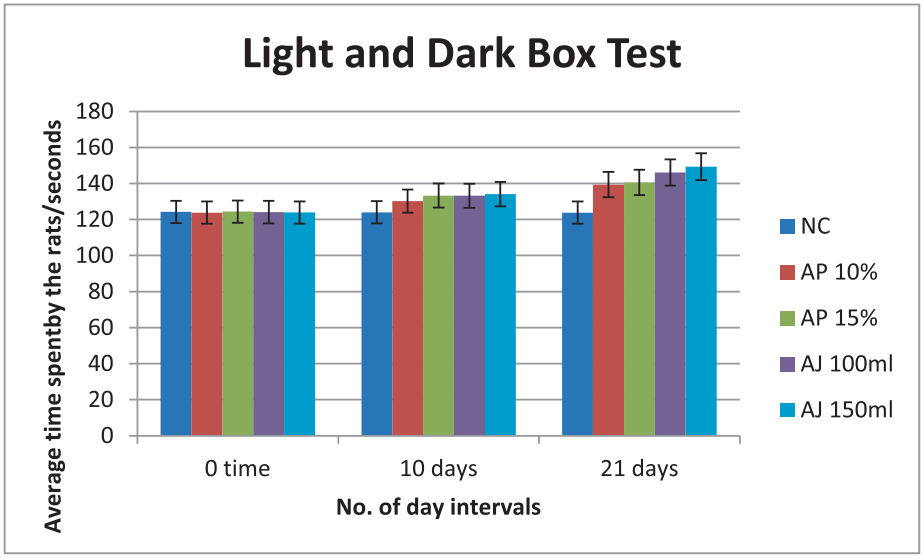

Fig. 4. Time spent on light area box by the rats treated with Avocado powder and Juice in the Light and Dark Box Test. Values are the means ± S.D n = 8 rats/group. The data were analyzed by the two way ANOVA for statistical analysis and post hoc Tukey's test.

both AV and AJ have confirmed the application of avocado juice as an anti-anxiety agent. The data obtained is in consistent with the results of Canetti et al. (Canetti et al., 2002), who studied food in relation to anxiety.

It is also observed that there is only a slight change in the level of antioxidant enzymes such as SOD, GST, GPX, and CAT due to the ingestion of Avocado powder and Juice compared to normal control. This result proved that the intake of avocado juice or powder supported non-significantly the increase of the normal activity of antioxidant enzymes, thereby removing the free radicals, scavenging superoxide compounds, or by activating the detoxification/defensive processes within the body. Also, it indicated that the ingestion of avocado never affects the cellular oxidation level (Kovacheva-Ivanova et al., 1994).

The anti-anxiety effect of Avocado powder and Juice on the head dip activity test showed that the treatment of AP2 and AJ2 has extensively reduced the mobility of rats due to anxiety compared to control. It also shown that the activity of crossing the cage was drastically reduced, which showed the anti-anxiety effect. In this test, the treatment of AP2 and AJ2 has expansively reduced the mobility of rats due to stress caused by the new place. These observations on the head dip and cage crossing activity test were in accordance with the results of Zubair et al. (Zubair et al., 2017), who evaluated the effect of coconut milk on anxiety using albino mice.

Further, the Anxiolytic activity of Avocado powder and Juice has shown highly significant that the rats fed with AJ1 and AJ2 spent more time in the light arena compared to normal rats, which indicated that the result was highly significant. These results agreed with the results that transition of the rodents from the dark to the light side is an indication of an anti-anxiety effect (Bourin and Hascoët 2003). The anxiolytic activity of Avocado powder and Juice explored was proved the anti-anxiety activity of avocado powder and juice by increasing the survival of rats in the open arm

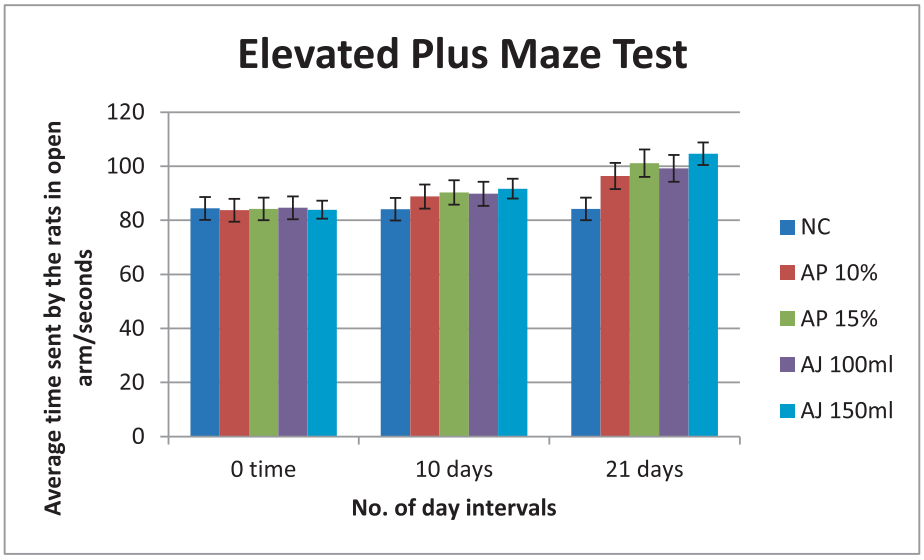

Fig. 5. Time spent in open arm by the rats treated with Avocado powder and Juice in Elevated Plus Maze Test. Values are the means ± S.D n = 8 rats/group. The data were analyzed by the two way ANOVA for statistical analysis and post hoc Tukey's test.

for a long time. Concurrently, the result obtained was consistent with the results of Kulkarni et al. (2016) who reported a substantial anxiolytic activity of *Punica granatum* (*Linn.*) fruit juice in mice in the Elevated plus Maze model.

All the results obtained in the study confirmed the presence of anxiolytic activity in avocado fruit, mainly if it is taken as a dosage of AP2 and AJ2 formulation. This may be due to the fact that avocado fruit has high nutritional value and is highly rich in protein, Vitamins A, B,D and E, and the pulp of the fruit were enriched with different oils (Ayaz et al., 2017). Ultimately, the consumption of avocado fruit by people might reduce all types of anxiety disorders.

6. Conclusion

Although avocado fruit was taken as powder and juice formulations, which reduces the anxiety level particularly the powder treatments, thus due to its bioactive flavonoids and polyphenolic compounds that are responsible for the anxiolytic activity and therefore may affecting positively mental hormones. Also, a complete analysis of the natural bioactive compounds would be proved to be useful for the development of novel drugs and deciphering the mechanism of the anxiolytic activity that must be useful for humans in the future.

Compliance with ethical standards

The Author(s) declare(s) that the work is in compliance with ethical standards.

Availability of data and materials

All the data is contained in the manuscript.

Author contributions

All authors have equal contributions in software collections, MF: Experimental design, collecting data and writing-original draft preparation, MI: Revising the experimental design, writing-review and editing. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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