A STUDY ON THE EFFECT OF ALOE VERA EXTRACT ON THE MOTOR AND EXPLORATORY ACTIVITIES IN NORMAL HEALTHY RATS

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ABSTRACT

Aloe vera (AV) has been used in folk medicine for thousands of years, which has led researchers to study its various health benefits. The aim of this study was to evaluate the effect of AV extract on the motor and exploratory activities in normal healthy rats. The open-field test (OFT) was used to provide a quantitative measurement of the animal motor and exploratory activities. The rats were weighed and randomly divided into three groups (six rats in each) identified as: control group (rats received distilled water (DW)), AV 10% group (rats received AV extract 10%), and AV 20% group (rats received AV extract 20%). All rats were administered DW or AV extracts by oral gavage once daily for 2 weeks at constant doses (2 ml/100 g body weight) among groups. The OFT was performed immediately after 2 weeks using LE8811 Actimeter PanLAB device. The results showed that oral consumption 10% and 20% of AV extract slightly reduced rats’ global & locomotor activities, stereotyped movements, distance traveled, and time spent in the fast movements in the OFT. Interestingly, oral consumption 10% and 20% of AV extract completely abolished central rats’ rearing activity (exploration) and slightly increased resting time. Additionally, oral consumption of 10% and 20% of AV extract markedly reduced central permanence time and number of entries to center of the arena. However, only oral consumption of 10% AV extract markedly reduced latency to first entry to center of the arena. Under our experimental conditions, these findings lead to the conclusion that AV extract possesses mild sedative and anxiolytic activities in concentration-dependent manner. Further studies are needed to assess these findings using different doses/concentrations of AV extract and other behavioral tests in rats.

Keywords: Aloe vera; Anxiety; Sedation; Rats

INTRODUCTION

Aloe vera (AV) is a hardy succulent plant belonging to the family Aloeaceae (Pathak and Sharma, 2019). The leaves of the AV plant rise from the base in the rosette pattern (Volkov and Shtesel, 2017). Each AV leaf is composed of three main layers: (i) An inner gel containing 99% water, and the remaining part made of amino acids, glucosamnans, sterols, lipids, and vitamins; (ii) The middle layer is made of latex, which is the bitter yellow sap containing anthraquinones and glycosides; and (iii) The outer thick layer, or rind, has protective function and synthesizes proteins and carbohydrates (Surjushe et al., 2008).

The AV, a magical plant, is widely used in the traditional herbal medicine of Egypt, China, India, Japan, and worldwide (Marshall, 1990; Park and Lee, 2006; Akev et al., 2011; Upton et al., 2012; Kumar et al., 2019). The medicinal benefits associated with AV have been attributed to the polysaccharides found in internal leaf aloe gel (Radha and Laxmi, 2014). Also, there is a synergistic effect with compounds contained in the whole leaf extracts beyond the medicinal properties of the AV plant (Eshun and He, 2004; Yohannes, 2018). Today, AV extract is widely used in medicine worldwide. It has anti-inflammatory, antibacterial, antioxidant, and immune stimulating effects (Prabjone et al., 2006; Sahu et al., 2013).

Anxiety is a frequent negative emotional state characterized by feelings of worry and accompanied by specific behavioral manifestations (Nuss, 2015). Hall (1934) originally described the open-field test (OFT) for the study of emotionality in rats. The procedure consists of subjecting an animal to a novel environment from which escape is prevented by surrounding walls (Doukkali et al., 2016). Oral consumption of AV gel may also have sedative and anxiolytic effects in rodents. In an effort to identify the possible sedative and anxiolytic effects of AV
An extract of Aloe vera has been used to investigate the motor and exploratory activities in normal healthy rats (Belzung, 1999; Prut and Belzung, 2003).

The aim of the current study was to evaluate the effects of AV extract on motor and exploratory activities in the open-field arena, as indicators representing AV-mediated sedation and relaxation in a rat model.

**MATERIALS AND METHODS**

**Ethical Approval**
Animal handling and experimentation were performed in line with approved Institutional Animal Care and Use Committee (IACUC#: 12-214) protocols at the South Valley University (Qena, Egypt) and complied with the ethical standards established by the Egyptian animal welfare laws and policies and followed the national authority (Ministry of Higher Education and Scientific Research, Egypt) guidelines for the detention, use and the ethical treatment of laboratory animals. Also, all animal protocols were approved by the Animal Use Subcommittee and by the Research and Ethical Review Committee of the Faculty of Veterinary Medicine, South Valley University.

**Experimental Animals**
Adult male Wistar albino rats were originally obtained from animal care facility at the Faculty of Medicine, Assiut University (Assiut, Egypt) and subsequently maintained in the animal care facility at the Faculty of Veterinary Medicine, South Valley University. Animals (6 per cage) were kept in the laboratory at 22±2°C with 60% relative humidity under a 12 h light/dark cycle for 2 weeks prior to experimentation. Tap water and food pellets were always freely available.

**Preparation of Aloe vera Extract**
The fresh AV plant leaves was selected to derive crude gel. The gel collected as described in the study of Oyeyemi and Ajani (2015). Briefly, the leaf surfaces were thoroughly washed with tap water and later with distilled water to remove traces of dirt and soil. The fleshy mass of the AV was carefully opened later with distilled water to remove traces of dirt and surfaces were thoroughly washed with tap water and then dried with a dry cloth. The AV gel aqueous extract was freshly prepared every time before use. It contained all the functional ingredients of the crude gel in the same proportion as it appears in the leaf.

**Experimental Design**
Eighteen male Wistar albino rats (12 weeks old and 200-250 g weight) were used in this study. The protocol used in the present study is the same as described previously (Nuriyiani et al., 2014; Moustafa et al., 2015). The rats were randomly divided into three experimental groups (six rats per group) as follows:

**Group I** (Control): rats were gavaged with distilled water (20 ml/kg body weight), once daily for fourteen consecutive days.

**Group II** (AV 10%): rats were gavaged with 10% Aloe vera extract (20 ml/kg body weight), once daily for fourteen consecutive days.

**Group III** (AV 20%): rats were gavaged with 20% Aloe vera extract (20 ml/kg body weight), once daily for fourteen consecutive days.

**Behavioral Testing**
All rats were monitored daily by research staff beginning two weeks prior to the experiment. Before testing, rats were handled once daily for one week to acclimate them to gentle manipulation. Any environmental or physical stress was avoided in order to habituate the rats to manipulation for behavioral testing. The apparatus used in behavioral testing was thoroughly cleaned after each test session with a lightly wet cloth and then dry it with a dry cloth.

**Open-Field Test**
At the end of 14 day's test period, the animals were evaluated regarding motor and exploratory activities in a novel environment using an infrared LE8811 Actimeter system and measured using ActiTrack software (Panlab, Barcelona, Spain) (Mantha et al., 2013). The open-field chamber consisted of a 45 cm (width) x 45 cm (depth) arena of black plexiglass enclosed with four clear acrylic walls (35 cm in height), as well as an infrared frame that produced a 16 × 16 grid of intersecting beams used to track the movement of each rat (Fig. 1A). Infrared beam-break data were used to calculate motor and exploratory-activities. Movement data were analyzed by dividing the arena into an outer, 11.25 cm-wide periphery zone and an interior, 22.5 × 22.5 cm central zone (Fig. 1B). This procedure allows the study of different anxiety-related parameters in rats (Simon et al., 1994).

Behavioral testing was carried out between the hours of 07:30 and 12:30 in order to reduce any diurnal fluctuations. The apparatus was placed in a room homogeneously illuminated at 400 lx. Each rat was placed in the same corner of the arena when beginning the trial, and was allowed to freely explore for 10 minutes. This minimized stress to the animals. Measurements were only recorded during the final 5 min (first 5 min was used only for animal acclimatization). The following parameters were analyzed: (i) global & locomotor activities,
stereotyped movements, mean velocity/speed, total distance travelled, and number of rearing in each user-defined zones; (ii) time spent in fast (>5 cm/s) & slow (<5 cm/s) movements or in rest (0-2 cm/s); (iii) latency time to enter the center zone, number of entries into center zone, and permanence time in each zone. At the end of the testing, the rat was removed and returned to its home cage.

**Statistical Analysis**

The results are presented as mean ± standard errors of the mean (SEM); n represents the number of animals in each group. Data analyses were performed with Origin 6.0 (OriginLab Corp., Northampton, MA, USA) software for Windows. Group means for all measures were compared using unpaired Student’s t test (for two group comparisons) to verify significant differences between groups. Differences were considered statistically significant when a minimum value of P less than 0.05.

**RESULTS**

**Effect of oral consumption of Aloe vera extract on the rats’ global activity**

Global activity is the sum total of locomotor and stereotypic activities. After 2 weeks, oral consumption 10% of Aloe vera extract (2 ml/ 100 g b wt/day) decreased the peripheral and central global activities (232.00±125.08 and 24.50±14.50, respectively), but this did not reach statistical significance when compared with the control animals receiving only distilled water (368.67±95.52 and 50.80±26.66, respectively) in a novel open-field environment (Fig. 2A,B).

Similarly, oral gavage 20% of Aloe vera extract (2 ml/ 100 g b wt/day) for two consecutive weeks was also associated with decreased peripheral and central global activities (249.00±43.67 and 3.33±1.20, respectively), but statistically not significant, compared to the control group (368.67±95.52 and 50.80±26.66, respectively), in the open-field arena (Fig. 2A,B). It seems likely that the concentration-dependent effect of Aloe vera extract on global activity is very sensitive especially in the central area of the open-field arena.

**Fig. 1:** Apparatus used to assess motor and exploratory activities of rats. (A) An open-field chamber with a sample ActiTrack real-time tracking output. Lower frame is used to track motor activity, while the upper frame tracks number of rearing (exploratory activity). (B) Schematic of zones in open-field arena with separation of the peripheral zone (red) and the central zone (blue).

**Fig. 2:** The rats’ global activity in a novel open-field environment. Mean (±SEM) infrared beam breaks of control and treated rats two weeks after oral distilled water/Aloe vera consumption respectively over a 5-minute test period (n = 6 rats per group) in the peripheral (A) and central (B) zones of the open-field arena.
Effect of oral consumption of Aloe vera extract on the rats' locomotor activity

Locomotor movements include movements with displacement. After 2 weeks, oral consumption 10% of Aloe vera extract decreased the peripheral and central locomotor activities (214.50±116.61 and 24.00±14.00, respectively), but this did not reach statistical significance when compared with the control animals (345.33±89.79 and 47.20±24.20, respectively) in a novel open-field environment (Fig. 3A,B).

Likewise, oral gavage 20% of Aloe vera extract for two consecutive weeks was also associated with a decrease in the peripheral and central locomotor activities (232.40±40.58 and 3.00±1.00, respectively), but statistically not significant, compared to the control group (345.33±89.79 and 47.20±24.20, respectively), in the open-field arena (Fig. 3A,B). Aloe vera extract decreased the locomotor activity of rats in concentration-dependent manner, particularly in center zone.

Effect of oral consumption of Aloe vera extract on the rats' stereotyped movements

Stereotyped movements include movements without displacement, i.e. eating and cleaning movements. After 2 weeks, oral consumption 10% of Aloe vera extract decreased the peripheral and central stereotyped movements (17.50±8.63 and 0.50±0.50, respectively), but this did not reach statistical significance when compared with the control animals (23.33±6.04 and 3.60±2.46, respectively) in a novel open-field environment (Fig. 4A,B).

In the same way, oral gavage 20% of Aloe vera extract for two consecutive weeks was associated with a decrease in the peripheral and central stereotyped movements (16.60±3.27 and 0.33±0.33, respectively), but statistically not significant, compared to the control group (23.33±6.04 and 3.60±2.46, respectively), in the open-field arena (Fig. 4A,B). Aloe vera extract decreased the stereotyped movements of rats in concentration-dependent manner, particularly in center zone.

Fig. 3: The rats' locomotor activity in a novel open-field environment. Mean (±SEM) infrared beam breaks of control and treated rats two weeks after oral distilled water/Aloe vera consumption over a 5-minute test period (n = 6 rats per group) in the peripheral (A) and central (B) zones of the open-field arena.

Fig. 4: The rats' stereotyped movements in a novel open-field environment. Mean (±SEM) infrared beam breaks of control and treated rats two weeks after oral distilled water/Aloe vera consumption respectively over a 5-minute test period (n = 6 rats per group) in the peripheral (A) and central (B) zones of the open-field arena.
Effect of oral consumption of Aloe vera extract on the rats’ movements velocity/speed

After 2 weeks, oral consumption 10% and 20% of Aloe vera extract markedly reduced the peripheral mean speed (1.11±0.74 and 1.06±0.22, respectively), but this did not reach statistical significance when compared with the control animals (1.82±0.52) in a novel open-field environment (Fig. 5A).

Fig. 5: The rats’ speed in a novel open-field environment. Mean (±SEM) average speed (cm/s) of control and treated rats two weeks after oral distilled water/Aloe vera consumption respectively over a 5-minute test period (n = 6 rats per group) in the peripheral (A) and central (B) zones of the open-field arena.

Effect of oral consumption of Aloe vera extract on the rats’ distance travelled

After 2 weeks, oral consumption 10% of Aloe vera extract markedly reduced the peripheral and central moved distance (328.13±219.30 and 17.53±15.15, respectively), but this did not reach statistical significance when compared with the control animals (522.33±146.88 and 70.75±36.58, respectively) in a novel open-field environment (Fig. 6A,B).

Fig. 6: Distance travelled by rats in a novel open-field environment. Mean (±SEM) distance travelled (cm) by control and treated rats two weeks after oral distilled water/Aloe vera consumption respectively over a 5-minute test period (n = 6 rats per group) in the peripheral (A) and central (B) zones of the open-field arena.

In contrast, oral gavage 10% and 20% of Aloe vera extract for two consecutive weeks was associated with slight changes in the central mean speed (10.94±9.19 and 12.86±5.12, respectively), but statistically not significant, compared to the control group (11.87±2.89), in the open-field arena (Fig. 5B).

Similarly, oral gavage 20% of Aloe vera extract for two consecutive weeks was associated with a marked decrease in the peripheral and central moved distance (317.96±65.32 and 5.20±2.96, respectively), but statistically not significant, compared to the control group (522.33±146.88 and 70.75±36.58, respectively), in the open-field arena (Fig. 6A,B). Aloe vera extract reduced the moved distance of rats in concentration-dependent manner, particularly in central zone.
**Effect of oral consumption of Aloe vera extract on the rats’ movements and resting**

After 2 weeks, oral consumption 10% and 20% of *Aloe vera* extract markedly reduced the time spent in the fast movements (21.80±17.38 and 21.48±5.83, respectively), but this did not reach statistical significance when compared with the control animals (46.23±15.03) (Fig. 7A). However, oral consumption 10% and 20% of *Aloe vera* extract was associated with slight changes in the slow-movements time (42.33±14.22 and 50.48±11.16, respectively), when compared with the control animals (59.90±11.91) in a novel open-field environment (Fig. 7B).

In contrast, oral gavage 10% and 20% of *Aloe vera* extract for two consecutive weeks slightly increased the rest time (235.87±31.11 and 228.04±14.02, respectively), compared to the control group (193.87±26.58) in the open-field arena but this did not reach statistical significance (Fig. 7C).

In turn, oral consumption 10% and 20% of *Aloe vera* extract apparently reduced the percentage of time that the rats spent in fast movements with slight effect on the percentage of time spent in slow movements, compared with the control animals in a novel open-field environment. However, the percentage of time that the rats spent in resting was apparently increased in rats received 10% or 20% *Aloe vera* extract compared to control group (Fig. 7D).

**Fig. 7:** Time spent in fast and slow movements or in resting in the open-field arena. Mean (±SEM) time spent in fast (A) and slow movements (B) or in inactivity (C) Activity distribution (D), as the percentage of total time (5 minutes), of the control and treated rats two weeks after oral distilled water/*Aloe vera* consumption respectively over a 5-minute test period (n = 6 rats per group) in the open-field arena.

**Effect of oral consumption of Aloe vera extract on the rats’ rearing activity**

Rearing (i.e. standing on rear limbs) is a common measure of exploratory activity used in the open-field test. After 2 weeks, oral consumption 10% of *Aloe vera* extract was associated with a non-significant decrease in number of rearing in the peripheral zone (3.67±2.29), compared to the control group (5.00±1.93), in this behavioral experimental model in rats (Fig. 8A). However, oral consumption 20% of *Aloe vera* extract for two consecutive weeks was not associated with changes in the number of rearing in the peripheral zone (5.60±2.14), compared to the control group (Fig. 8A).

Interestingly, oral gavage 10% and 20% of *Aloe vera* extract for two consecutive weeks completely abolished the rats’ rearing activity in the central zone of the arena compared with the control group (1.17±0.65) in a novel open-field environment (Fig. 8B).
**Effect of oral consumption of Aloe vera extract on the rats’ entries to central zone**

After 2 weeks, oral consumption 10% of Aloe vera extract markedly reduced the latency time to first enter the central zone (19.40±19.40), but this did not reach statistical significance when compared with the control animals (86.88±46.96) in a novel open-field environment (Fig. 9A). While, oral gavage 20% of Aloe vera extract (2 ml/ 100 g b wt/day) for two consecutive weeks was associated with slight changes in the latency time to first enter the central zone (80.80±77.52), compared to the control animals (Fig. 9A).

Additionally, oral gavage 10% and 20% of Aloe vera extract for two consecutive weeks markedly reduced the number of entries into the central zone (1.33±1.33 and 0.80±0.58, respectively), but this did not reach significance when compared with the control group (4.67±2.36) in concentration-dependent manner (Fig. 9B).

**Effect of oral consumption of Aloe vera extract on the rats’ permanence time**

Permanence time is the total time in seconds spent in the specified zones of the arena regardless of the number of entries. Oral consumption 10% and 20% of Aloe vera extract for two consecutive weeks was not associated with changes in the time spent in the peripheral zone (298.03±1.29 and 299.60±0.23, respectively), compared to the control animals (291.77±5.21), in this behavioral experimental model in rats (Fig. 10A).

On the other hand, oral gavage 10% and 20% of Aloe vera extract markedly reduced the time spent in the central zone (1.97±1.23 and 0.40±0.23, respectively), but this did not reach significance when compared with the control group (8.23±5.21) in concentration-dependent manner (Fig. 10B).
DISCUSSION

Aloe vera (AV) is a popular plant in traditional medicine and has multiple therapeutic benefits (Joseph and Raj, 2010; Thombre et al., 2019). Over the past decade, numerous studies carried out in animal models have revealed that AV may possess an important antidepressant and analgesic activities (Sultana and Najam, 2012; Halder et al., 2013). In the current study, we only focus on the precise impacts of AV extract consumption on motor and exploratory activities in normal healthy rats using open-field test (OFT). Measuring the animal's activity in a novel environment may highlight sedative and anxiolytic potentials of the AV extract (Moniruzzaman et al., 2019).

The OFT is an experimental procedure used for evaluation of general activity, locomotor, stereotypic movements, rearings or exploration in rodents (Hrnkova et al., 2007). The animal is placed in the center or close to the walls of the open-field arena and the activity of the animals was recorded via a computer program (Kas et al., 2008). For animal's activity assessment, the open-field arena is divided into two zones: the “central zone” and the “wall or peripheral zone” (Hart et al., 2010). Therefore, the general rats' activities & movements, mean speed, total distance travelled, rearing & entry numbers, rest & permanence time in selected zones, and latency to enter central zone can be easily measured (Lynch et al., 2011).

The present results showed that oral consumption 10% and 20% of AV extract for 2 weeks was associated with a slight decline in peripheral rats' activities and movements. Additionally, oral consumption 10% and 20% of AV extract markedly reduced fast-movements time and slightly increased time of inactivity in concentration-dependent manner. Alteration in motor activity in rats under OFT is considered as an index of sedative effect (Abdollahnejad et al., 2016). Therefore, AV extract may be having a mild sedative effect under our experimental condition.

In addition, the anxiolytic effect of AV extract was indicated by a reduction in the latency to enter the center (Donthula and Dholi, 2018). Indeed, our data also showed that oral consumption of AV extract only at concentration of 10% reduced latency time to enter the center of the arena compared to control and AV 20% groups. Oral consumption 10% and 20% of AV extract completely abolished central rearing or exploration of the rats. Taken together, AV extract may be having an anxiolytic effect in concentration-dependent manner.

It has been reported that AV is effective to treat various central nervous system (CNS) disorders such as multiple sclerosis (Mirshafiey et al., 2010) and convulsion (Rathor et al., 2014). The anticonvulsant and sedative activity of medicinal plants has frequently attributed to its active phytochemical’s compounds such as flavonoids (Cho et al., 2012). Many of the neurotransmitters such as histamine, dopamine, norepinephrine, serotonin, glutamate, orexin and acetylcholine are involved in sleep-wake regulation (Van Erum et al., 2019). It should be mentioned, however, that the implication of any of the mentioned neurotransmitters needs further clarification as our study did not include any contributing examination.

CONCLUSIONS

The present study provides additional evidences which support sedative and anxiolytic effects of AV
extract obtained by folk or traditional medicine. Examining different doses/concentrations of the AV extract and using other behavioral tests should be added. Further experiments are clearly required to determine precisely the specific mechanisms underlying these neurobiological changes in AV-induced sedation in a rat model.

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DISCLOSURES

The authors declare that there are no conflicts of interest regarding publication of this article.

REFERENCES


دراسة عن تأثير مستخلص الصبّر على الأنشطة الحركية في الفئران السليمة الطبيعية

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تستخدم الصبّر في الطب الشعبي منذ آلاف السنين، مما أدّى بالباحثين إلى دراسة فوائده الصحية المختلفة. كان الهدف من هذه الدراسة هو تقييم تأثير مستخلص الصبّر على الأنشطة الحركية في الفئران السليمة الطبيعية. تم استخدام اختبار المجال المفتوح لتوفير قياس كمي للأنشطة الحركية والاستكشافية في الفئران. تم وزن الفئران وتقسيمها بشكل عشوائي إلى ثلاث مجموعات (ستة فئران في كل منها) تم تحديدها على النحو التالي: مجموعة الضبط (ثلاث الفئران الماء المذبوذ)، ومجموعة الصبّر 10% (ثلاث الفئران مستخلص الصبّر 10%)، ومجموعة الصبّر 20% (ثلاث الفئران مستخلص الصبّر 20%). تم تجريب جميع الفئران عن طريق القم لمدة أربعة عشر يوماً متتالية في جرعات ثابتة (2 مل/100 غرام من وزن الجسم) بين المجموعات. تم إجراء اختبار المجال المفتوح مباشرة بعد استخدام جهاز قياس الحركة والاستكشاف (LE8811 Actimeter PanLAB). وأظهرت النتائج أن تناول مستخلص الصبّر بنسبة 10% و20% من طريقة القم يقلل بشكل كبير من الأنشطة العامة والحركية للفئران. والملاحظة المطروحة، والوقت الذي يستغرقه في الحركات السريعة في اختبار المجال المفتوح. ومن المثير للإهتمام أن تناول بنسبة 10% و20% من مستخلص الصبّر عن طريق القم قد أدى تمامًا الأنشطة الاستكشافية للفئران وزاد من وقت الراحة قليلاً. بالإضافة إلى ذلك، أدى تناول بنسبة 10% و20% من مستخلص الصبّر إلى تقليل وقت الدوران المركزي بشكل ملحوظ عند الإدخال إلى وسط الحلبة. ومع ذلك، فإن تناول مستخلص الصبّر عن طريق القم فقط بنسبة 10% يقلل بشكل ملحوظ من زمن الوصول إلى الدخول الأول إلى وسط الحلبة. تؤدي هذه النتائج إلى استنتاج أن مستخلص الصبّر يمتلك أنشطة خفيفة مهددة ومزيلة للقلق يعتمد على التركيز في ظل ظروفنا التجريبية. هناك حاجة إلى مزيد من الدراسات لتقييم هذه النتائج باستخدام جرعات وتركيزات مختلفة من مستخلص الصبّر وغيرها من الاختيارات السلوكيّة في الفئران.