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Research Article

Effects of essential oils aroma therapy on stress-ladened solitary carnivores: Changes in anxiety-related behavior and cortisol concentration

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Abstract

Aromatherapy is a popular enrichment that reduces stress. We hypothesize that essential oils positively affect captivated carnivores to cope with stress. The solitary carnivores, namely, Asiatic Wolf, Jungle Cat, Asiatic Lion, Common Leopard, and Stripped Hyena, were studied for this purpose during the pre-enrichment, enrichment, and post-enrichment phases. The focal monitoring method was used to observe animal behaviors for 384 hours over the 2 months. The carnivores were provided sensory enrichment with essential oils: Lavandula *angustifolia*, *Eucalyptus globulus* Labill, *Citrus limon Linn*, and *Mentha piperita Linn*. The FGCs evaluated the cortisol levels by ELISA. The Asiatic wolf is found to be the most stressed carnivore among all studied animals. The efficacy order of the oils in reducing stress was found to be Lavender > Eucalyptus > Peppermint > Lemon. The frequency of stereotypic behavior and cortisol levels in these carnivores were significantly reduced with the effectiveness of essential oil. The aromatherapy reduced cortisol level values ranged from 82.6±0.927 to 46±0.707, 155±0.707 to 93.4±0.927, 385±0.710 to 289.2±1.067, 285.4±1.029 to 190±0.709 and 205.4±0.930 to 176±0.708 in Asiatic Wolf, Jungle Cat, Common Leopard, Asiatic Lion and Hyena respectively. It is concluded that essential oils and aromatherapy help alleviate stress and anxiety and improve animal behavior. Aromatherapy may be applied as short-term sensory enrichment for captive wildlife.

Keywords: Stress, Animal behavior, Aromatherapy, Essential oils, Sensory enrichment, Wildlife, ELISA

Introduction

The current rate of extinctions caused by human activity is unprecedented and extremely challenging to reverse. There have been severe decreases in the populations of mammalian carnivores, especially the larger terrestrial species. Overall, carnivores face a greater risk than mammal species do (26.9% of carnivore species are endangered, compared to 22.7% of mammal species (Ellis & Wells, 2010). Carnivores (order Carnivora) play a crucial role in ecosystems, therefore protecting them helps protect other species and entire ecosystems. The loss or degradation of habitat is a major factor in the plight of carnivores. The assistance of in situ conservation is probably the most significant thing a zoo can offer in this situation.Instead of leading a carefree existence, captive animals may be subjected to hardships for which they were not originally adapted. Large-range carnivores are less tolerant of captivity than their more restricted wild counterparts. Animals' typical behavior patterns can be disrupted, leading to anxiety and displeasure. Animals subjected to a captive lifestyle are at increased risk of developing psychiatric problems that are strikingly comparable to those seen in humans (Singh et al., 2015) . Animals' emotional and physical wellbeing suffers greatly in captivity. Animal behavior is the most reliable measure of health, both mental and physical. Most carnivores that are kept in captivity show signs of stress and/or psychological disorder; this is concerning considering the challenges of conserving species with large home ranges. A person experiences stress when their body reacts physiologically to a situation it perceives as dangerous. As a dynamic process, enrichment here modifies an animal's environment, broadening its range of natural and species-specific behaviors and skills. Many people who work with captive carnivores believe that the animals will become used to their new environments. The difference between natural and captive habitats has far-reaching consequences for the zoopopulations' exhibited behavior repertoire, making management difficult (Carroll et al., 2014). Furthermore, if the stress response is continuously triggered, confinement may have lasting effects on physiology.To address this challenge, zoos have implemented enrichment strategies(Ali et al., 2015; Nemat et al., 2013; Quirke & O'Riordan, 2011) to boost environmental complexity (Singh et al., 2015) and reduce negative, aberrant, and stereotypical behaviors that emerge during captive production.Captive populations in modern zoos are managed with the help of environmental enrichments to ensure their continued health. Enrichment is an ongoing procedure that modifies an animal's surroundings to broaden its range of natural and species-specific behaviors and skill (Ayaz et al., 2017). The display for the public and the reproductive success of the animals housed there can both benefit from enrichment's ability to increase stress

resilience. Animals benefit from enrichment at zoos, and it helps when they're released back into the wild after being kept there (Damasceno et al., 2017). Providing animals with interesting and novel environments is crucial for their well-being. Essential oils are used in aromatherapy, a form of Complementary and Alternative Medicine (CAM) (List, 2004) that is becoming increasingly popular for the treatment and prevention of a wide range of medical disorders.Aromatherapy is closely linked to the alleviation of stress. The emotional and physical well-being of all living organisms is being negatively impacted by the worldwide epidemic of stress. Essential oils are a highly concentrated form of volatile chemical compounds found in plants (Ali et al., 2015). Essential oils have been shown to have beneficial effects on relaxation, pain relief, blood pressure, and overall bodily function. Clinical aromatherapy has the potential to be a holistic alternative treatment that works on a person's "whole" being. Aromatherapy can be traced back to the ancient Indian practice of Ayurveda. The stress hormone cortisol can be used as a biomarker because of its role in a number of immunologic and metabolic processes.The collection of blood samples necessitates invasive methods, such as the rough handling of animals, which might cause a spike in cortisol and skew the results. Alternatively, collecting a sample of feces or urine is preferred because it does not involve any invasive procedures and yields objective data (Amaya et al., 2020). Carnivorous animals in zoos sometimes suffer from psychological issues like isolation, depression, and behavioral abnormalities. While olfactory enrichment's primary purpose is to enhance the lives of captive animals, it has the potential to have other good effects as well. Olfactory enrichment, for instance, has the potential to have positive effects on reproductive success (Ogata et al., 2020) because fragrances may evoke both behavioral and physiological responses. Carnivores often use their sense of smell to help them find food. Structure of odor plumes and patterns of odor dispersion greatly influence the most effective olfactory search and localization procedures. With a dazzling array of odoriferous organs at their disposal, carnivores make full use of the olfactory opportunities afforded by their urine and excrement (Fay & Miller, 2015). Our primary objective is to improve the quality of life for at-risk zoo carnivores by creating and evaluating novel, short-term sensory enrichments using essential oils. In this context, we carriedout a preliminary study that aimed to assess the effects of a series of essentialoils (namely, lavender oil, eucalyptus oil, lemon oil, and peppermint oil)on behavioral and physiological indicators of stress in five captive solitary carnivore species: Asiatic Lion (*Panthera leo leo*), Common Leopard (*Panthera pardus*), Stripped Hyena (*Hyaena hyaena*), Jungle cat (*Felis chaus*), and Asiatic or Indian Wolf (*Canis lupus pallipe*). Research like these can help with the design of better systems for managing caged animals. Aromatherapy with

essential oils is commonly used to alleviate stress.Essential oils like lavender, cedarwood, vetiver, eucalyptus, and peppermint have been shown to have a calming impact in previous research. Anxiety and depression can be alleviated with the help of these essential oils (Ogata et al., 2020).There is a dearth of research that evaluates the efficacy of aromatherapy with essential oils in reducing anxiety-related behavior and cortisol concentration in carnivores. Effectiveness in domestic animals and humans, as well as in sheltered cats and dogs, and in zoohoused exotic animals, informed the selection of essential oils for (Gök et al., 2015; Laidlaw, 2000). The deforestation, logging, and hunting that threaten the habitat and survival of these species across their ranges (Han & Parker, 2017) have led to the current classifications of the carnivore species studied here as Endangered (Asian lion), Vulnerable (Common leopard), Near Threatened (Stripped hyena) Asiatic or Indian Wolf (*Canis lupus pallipe*), and Least Concern (Jungle cat). Therefore, it is especially important to develop and implement plans to enhance the welfare and conservation success of these species while they are kept in captive. Our hypothesis for this study is that essential oils can help reduce stress in captive carnivores, as evidenced by a variety of changes in both behavioral (increased normal active and passive behavior and decreased stress-related behavior) and physiological (decreased fecalglucocorticoid concentrations or FGCs) indicators of well-being.

Material and methods

Study site

The chosen study site, the Small Cat Section of the Lahore Zoo 31.556006°North and 74.325959° East at Mall Road, Lahore, Pakistan, is one of the oldest zoos in the world and it covers an area of 10 hectares (25 acres). The zoo was established in 1872 and under the supervision of the Punjab Wildlife and Parks Department (Carroll et al., 2014). All experimental procedures done in this study were carried out after acquiring permission dated 26-07-2023 from the Bio-ethical Committee of the University of Punjab.

Animals under study

The current research work was carried out on five carnivore animals, as given below, that were housed solitary in small enclosures. The main objective of this sensory enrichment was to improve the physical and physiological conditions of these stressed animals. A detailed description regarding age, gender, IUCN status, and carnivores under study is given in Table-1.

1. Asiatic Lion (*Panthera leo leo*)
2. Common Leopard (*Panthera pardus*)
3. Stripped Hyena (*Hyaena hyaena*)

4. Jungle cat (*Felis chaus*)
5. Asiatic or Indian Wolf *(Canis lupus pallipe*)

Table-1: Detailed Description of Animals Under Study

Animals / Description	Asiatic Wolf	Jungle Cat	Common Leopard	Asiatic Lion	Stripped Hyena
Age	7.5 years	7 years	9 years	5 years	8 years
Gender	Female	Male	Male	Female	Male
Health status	Healthy	Healthy	Healthy	Had epilepsy at an early age, have distorted vision	Healthy
History	Zoo born	Zoo born	Wild confiscated in 2015	Zoo born	Brought from circus in 2020
Conditions of enclosure	Sand ground with concrete, wooden logs, water tanks, sun exposure, no vegetation, no pool, high accessibility to visitors.	Sand ground with concrete, wooden logs, water tanks, sun exposure, no vegetation, no pool, high accessibility to visitors.	Sand ground with concrete, wooden logs, water tanks, sun exposure, no vegetation, no pool, and high accessibility to visitors.	Sand ground with concrete and marble, wooden logs, water tanks, sun exposure, no vegetation, no pool, andhigh accessibility to visitors.	Sand ground with concrete and marble, wooden logs, water tanks, sun exposure, no vegetation, no pool, and high accessibility to visitors.
Outdoor cage area	486 sq ft, Front 27', Side 18'	51.75 sq ft, Front 8', Sides 5', 9"	742 sq ft, Front 23', 9", Side 31', 3"	625.62 sq ft, Front 35', 9", Side 17', 6"	625.62 sq ft, Front 35', 9", Side 17', 6"
Feedind Scale	04 kg meat (fresh beef) in summers and 05 kg meat in winters for 6 days of week	01 kg meat (fresh beef) in summers and 1.5 kg meat in winters for 6 days of week	05 kg meat (fresh beef) in summers and 06 kg meat in winters for 6 days of week	07 kg meat (fresh beef) in summers and 09 kg meat in winters for 6 days of week	04 kg meat (fresh beef) in summers and 05 kg meat in winters for 6 days of week
IUCN status	Endangered	least concerned	Vulnerable	Endangered	Near threatened
Distribution	In deserts of Cholistan and Thar, lower hills of the	Riverine tracts of the Indus and the cultivated and irrigated lands	In Punjab Baluchistan, Khyber Pakhtunkhwa, Sindh, Azad	Gir Forest National Park in Gujarat	Hill ranges of Baluchistan, Sind Kohistan and Quetta city in Baluchistan

	Baluchistan pleatue.	of Punjab and Sindh	Jammu and Kashmir		

Focal method of behaviour monitoring

The behavior of study animals was observed through the focal method of monitoring animal behavior (Altmann, 1974). Table 2 indicates an ethogram of observed behaviors along with the description and their categories as follows:-

1- Normal behaviors

(a) Normal active behaviors

(b) Normal passive behavior

2-Stereotypic behaviors

Table-2. Ethogram of Recorded Behaviors in Carnivores

Behavior category	**Sr. #.**	**Behavior**	**Description**
Stereotypic behavior	1	Pacing	Walking around a specific route in a cage without a purpose or destination.
	2	Unusual sounds	Making sounds other than normal roaring. These may be growling sounds or shrieking sounds showing pain or frustration of the animal.
	3	Aggressiveness	Unusual anger display and roaring.
	4	Head tossing	Shaking head aberrantly and repeatedly due to anger and boredom.
	5	Over grooming	Licking body fur beyond the level of cleaning or grooming resulting in removal of fur from parts of body.
	6	Auto-mutilation	Self-injury or self-harm by smashing or rubbing body with the bars of the cage or with the walls showing frustration.
Normal passive behavior	1	Laying,	Relaxation position of the animal showing a passive, less energy phase.
	2	Sleeping	Natural rest state of unconsciousness of animal.
Normal active behavior	1	Playing	Chasing, wrestling, pawing, stalking and rushing are common play behaviors. Cubs jump on females, play with tails, wrestle each other and roll on their backs Solitary plying, acrobatic move, playing with branches/wood logs, running
	2	Stretching	Act of physically reaching out of limbs showing fatigue from being in one position and also comfort in the space.
	3	Social activity	Lions of parental groups called “prides” contain heritably associated females and cubs and they communicate using sound, touch and pheromones.

	4	Grooming	Brushing or cleaning the coat of the animal by licking or using paws to remove dirt etc. from the body.
	5	Climbing or digging	Rising up to a higher position in a playful manner.
	6	Territory marking	Leave urine and scat, and scrape the ground to mark territory using their scent Urine marking mostly performed by males however females occasionally spray.
	7	Communication	Body touching and sitting together, food offering, foraging together, lip smacking, chasing/moving together

Behavioral data collection

The behaviors of five captive carnivores were studied in three phases from 1st March 2022 to 3rd May 2022 (Table-3) as given below:-

Phase-1: Pre-Enrichment Phase (1st March-10th March,2022)

During this phase, carnivores were observed for Normal and Stereotypic behaviors without essential oils sensory therapy.

Phase-2: Enrichment Phase (11th March -23rd April, 2022)

During the Enrichment phase, carnivores were introduced to aromatherapy with four different kinds of essential oils.

Phase-3: Post-Enrichment (24th April – 3rd May,2022)

During the post-enrichment Phase, the carnivores under study were again observed for Normal and Stereotypic behaviors without essential oils sensory therapy.

Behaviors observation times and duration

During all phases of research, the behaviors were monitored for 06 hours/day from 10:00 am to 4:00 pm with 5 mins intervals for two months. We recorded a total of 384 hours of observations over the study period.

Table 3. Environmental Conditions and Observation Times During Behavior Monitoring

Phase	Time interval	Overall weather	Temperature	Humidity	Observation time
Phase-1	1st March-10th March,2022	sometimes sunny, mostly cloudy	28-29◦ C	14% ±0.02	11:00 am to 4:00 pm
Phase-2	11th March to 23rd April,20222	Mostly sunny, Sometimes rainy and cloudy	29 to 35◦ C	30 to 54 %±0.06	11:00 am to 4:00 pm

Phase-3	24th April to 3rd May,2022	Mostly Sunny, sometimes scattered clouds, hot	35 to 38◦ C	37%±0.12	11:00 am to 4:00 pm

The frequency and duration of different types of carnivores' behaviors observed through the focal method of animal behaviors were recorded using the following formulas:-

Frequency = Number of times a behavior is recorded for an animal / Total number of observations for that animal (Kritsidima et al., 2010)

Duration = Minutes performing the behavior / Total hours of observation (List, 2004)

Sensory enrichment protocol

Essential oils

The short-term sensory enrichment was carried out with the administration of 04 different types of Essential oils, namely, Lavender oil (*Lavandula angustifolia*), Eucalyptus oil *(Eucalyptus globulus Labill)*, Lemon oil *(Citrus limon Linn)*, Peppermint oil *(Mentha piperita Linn)*. These oils, in packing of 30 ml each, were purchased from Saeed Ghani Oils, Lahore- Pakistan.

Short-term Sensory Aromatherapy

During the Enrichment Phase, all essential oils like Lavender oil, Eucalyptus oil, Lemon oil, and Peppermint oil were administrated to all carnivores under study. Each essential oil was diluted to a ratio of 1:6. For a single cage, 2 ml was diluted in 12 ml distilled water. A total of 14ml essential oil was administered on the wooden logs present in the cages following the concentrated scent provision mechanism (Saad et al., 2015). The oils were sprayed on wooden logs at 9:00 a.m., just before the release of carnivores from night rooms. Phase 2 was further divided into four sub-phases (Table-4) consisting of 11 days each (7 days of Pre-aromatherapy and 4 days Post-aromatherapy). After completion of each sub-phase the carnivores were given a bath, and all the wooden logs and cages were properly washed to remove the effect of administrated oil.

Table 4. Details of Aromatherapy Trials

Enrichment Phase	**Days of Aromatherpy Trials**	**Esssential Oil Therapy**	**Location and Objects**
Sub-Phase-1	7 days 11thMarch-17th March,2022	Pre-aromatherapy	Wooded logs at Exhibit Cage

	4 days 18thMarch-21st March,2022	Post- aromatherpy	
Sub-Phase-2	7 days 22nd March to 28th March,2022	Pre-aromatherapy	Wooded logs at Exhibit Cage
	4 days 29thMarch-1st April,2022	Post- aromatherpy	
Sub-Phase-3	7 days 2nd April to 8th April,2022	Pre-aromatherapy	Wooded logs at Exhibit Cage
	4 days 9thMarch-12th April,2022	Post- aromatherpy	
Sub-Phase-3	7 days 13th April to 19th April,2022	Pre-aromatherapy	Wooded logs at Exhibit Cage
	4 days 20thMarch-23rd April,2022	Post- aromatherpy	

*Aromatherapy was given to all carnivores under study (Asiatic Lion, Common Leopard, Stripped Hyena, Jungle cat)

Fecal collection, preservation, and ELISA processing

To determine fGCM concentrations in the study animals, fecal samples were collected through a non-invasive sampling technique without any harm or discomfort to subjects (Ben-Ari, 2001; Khan et al., 2018) at the end of all study phases in triplicate order before behavioral observations. A total of 30 fecal samples were collected from every study animal (03 samples after the Pre-enrichment Phase, 24 samples after the Enrichment phase, and 03 after the Post-enrichment Phase). In this way, 150 fecal samples were collected during the study period after wearing gloves and lab coats under the supervision of veterinary staff. A sample was considered fresh when the consistency was soft, the color was a shiny dark brown, the sample was still warm, and the smell was persistent.

Samples were preserved and processed for ELISA as follows:-

1. The 150 samples were properly labeled with time, date, and location.
2. The samples were transferred to the conservation biology lab, University of Punjab, Lahore, through storage boxes containing ice packs and saved at -20^{o}C temperature in the lab till completion of research work.
3. Samples were thawed at room temperature and crushed after dehumidification at 60^{o}C.
4. Then 0.5g of every crushed sample was dissolved in 15 ml of 80% methanol and vortexed for 10 minutes.

5. After the vortex, the mixture was centrifuged for 15 minutes at 2000 rpm to pellet the sample.
6. The supernatant was stored in an air-dried Eppendorf and a refrigerator for further analysis, while residual fecal pellets were transferred into a waste bag for discarding.

Estimation of fecal cortisol level

The above-prepared samples were used to estimate fecal cortisol levels through Enzyme-Linked Immunosorbent Assay (ELISA) by following (Bashaw et al., 2016; Yarnell et al., 2016). The fecal cortisol concentration was estimated with the help ELISA Kit CALBIOTECH (A Life science Company) Calbiotech, Inc. 1953Cordell Ct., El Cajon, CA 92020 U.S.A at Conservation Biology Lab, Institute of Zoology University of Punjab. The following standard curve was obtained through which the cortisol concentration of samples was calculated in ng/ml at 450nm absorbance (figure-1).

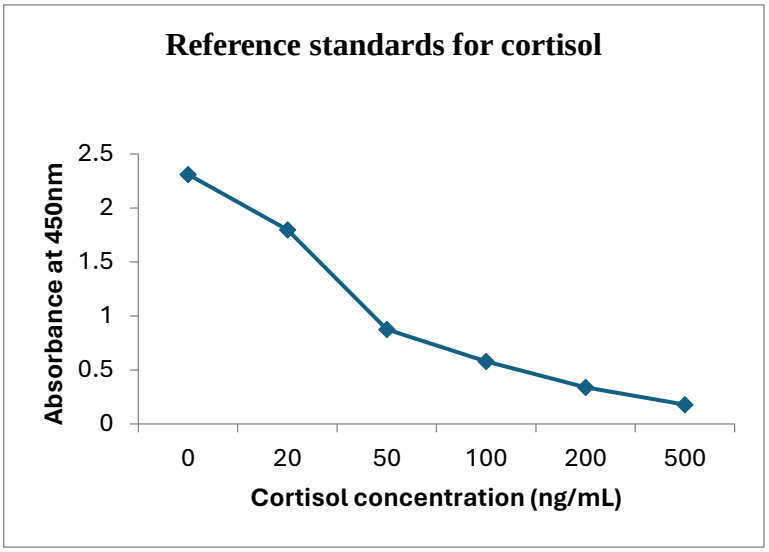

Figure 1. Standard curve for cortisol concentration

Statistical analysis of results

All statistics were done using Origin2022. The confidence level was set as $\alpha = 0.05$. One-way analysis of variance (ANOVA) with post-HOC TUKEY'S test was conducted on the data to statistically analyze significant differences between the behaviors of animals. The Pearson correlation was applied on the effect of cortisol concentrations and essential oils on different animals.

Results

Behavior analysis

Asiatic wolf (*Canis lupus pallipe*)

The Figure 2 shows the frequency of different behaviors exhibited by an Asiatic wolf before and after applying different essential oils (Lavender oil, Eucalyptus oil, Lemon oil, and Peppermint oil). Of all four oils, Lavender oil had the most positive effects on wolf behavior. The frequency of stereotypic behavior like pacing, head tossing, and auto-mutilation decreased significantly after the aromatherapy with essential oils, as the behavior was observed for three hours before and after the aromatherapy. However, there was no significant induction of normal active behavior in wolves. The wolf sniffed the oils after the aroma therapy, but the other behaviors like grooming, climbing, stretching, and licking, were not observed. Eucalyptus oil had some reducing effects on stereotypic, while the remaining two oils had minor reduction.

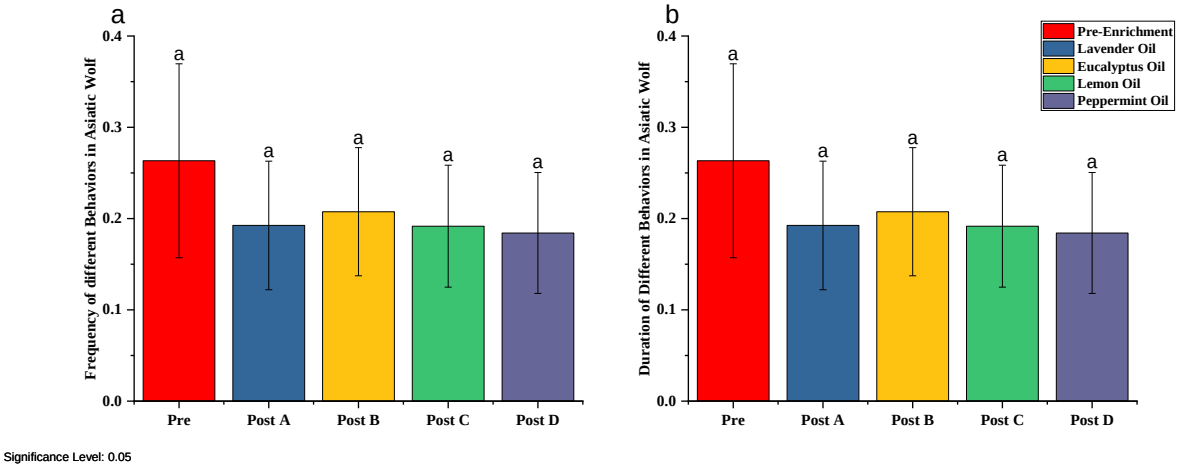

Figure 2: (a) Change in frequency of different behaviors studied in different phases (pre-enrichment (before aroma therapy) and Post-enrichment(after aroma therapy) in Wolf. (b) Change in behavior during the observational period of three hours. Significant changes between means within each period indicate different letters (P 0.05, post-HOC, TUKEY'SANOVAs). For every therapy, the bars depict the means and standard error.

Jungle cat (*Felis chaus*)

Jungle Cat showed the most favorable results with the lavender oil (figure 3). It correctly sniffed and licked it, showing the sign of liking. There was an obvious decline in the frequency of stereotypic behaviors and an increase in passive behavior due to lavender oil. However, it showed avoidance behavior to the odour of the other three oils, i.e. eucalyptus,

lemon, and peppermint. Nevertheless, eucalyptus was quite helpful in relaxing the jungle cat as the frequency of pacing and aggressiveness was reduced slightly.

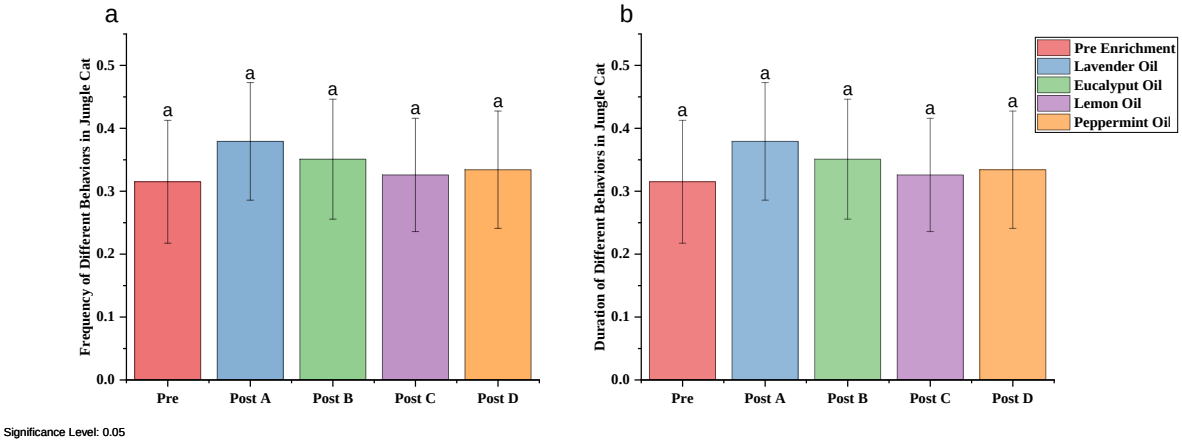

Figure 3. (a) Change in frequency of different behaviors studied in different phases (pre-enrichment (before aroma therapy) and Post-enrichment(after aroma therapy) in Jungle cat. (b) Change in behavior during the observational period of three hours. Significant changes between means within each period indicate different letters (P 0.05, post-HOC, TUKEY'SANOVAs). For every therapy, the bars depict the means and standard error.

Leopard *(Panthera pardus)*

The leopard was found to be less stressed as compared to the wolf and jungle cat. Leopard sniffed the lavender oil, showing some kind of liking, resulting in a reduction in its stereotypic behavior and an increase in normal behavior (figure 4). It sniffed and licked the eucalyptus oil. It had a relaxing effect on Leopard, enhancing its passive behavior more than the lavender oil, but had not as such a positive effect on active behavior. However, lemon oil and peppermint oil had little positive effect on its behavior.

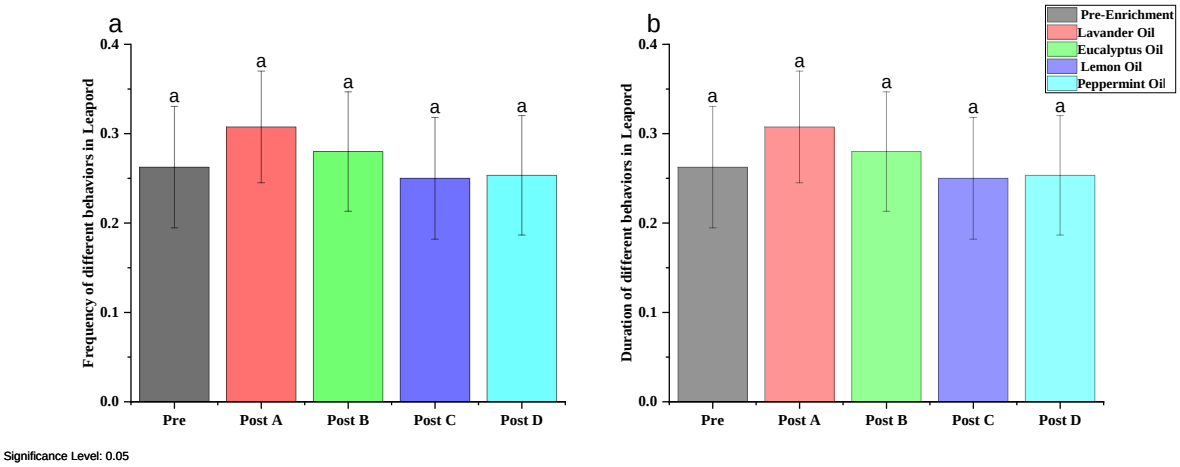

Figure 4. (a) Change in frequency of different behaviors studied in different phases (pre-enrichment (before aroma therapy) and Post-enrichment(after aroma therapy) in Leopard. (b) Change in behavior during the observational period of three hours. Significant changes between means within each period indicate different letters (P 0.05, post-HOC, TUKEY'SANOVAs). For every therapy, the bars depict the means and standard error.

Lion *(Panther leo leo)*

Lavender had an overall positive effect on lion. It reduced the stereotypic behavior and increased the normal behavior, both active and passive. Eucalyptus oil had a much positive effect on passive behavior. Both lemon and peppermint oil have a very minor reduction in stereotypic behavior and a slight to almost zero effect on normal behaviors (figure 5).

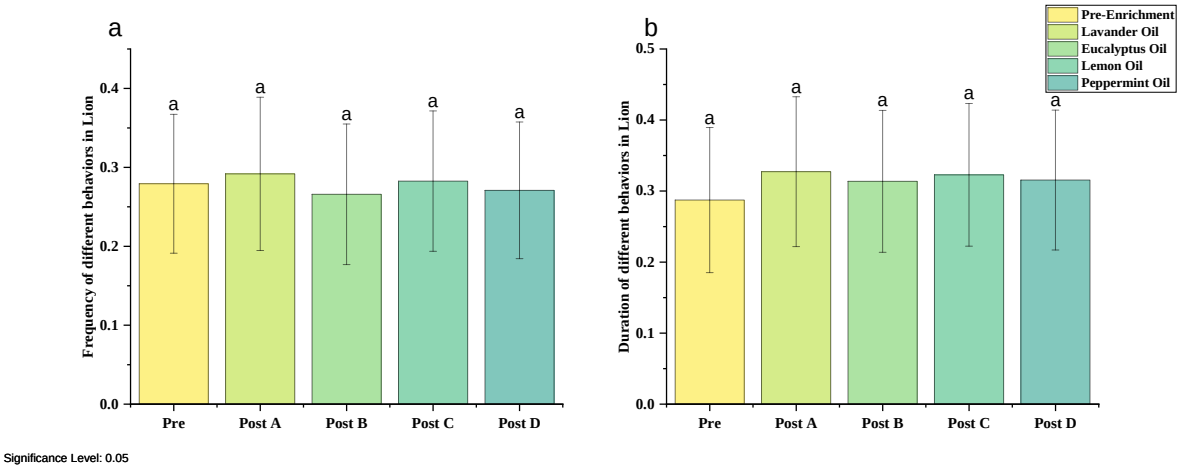

Figure 5. (a) Change in frequency of different behaviors studied in different phases (pre-enrichment (before aroma therapy) and Post-enrichment(after aroma therapy) in Lion. (b) Change in behavior during the observational period of three hours. Significant changes between means within each period indicate different letters (P 0.05, post-HOC, TUKEY'SANOVAs). For every therapy, the bars depict the means and standard error**.**

Hyena *(Hyaena hyaena)*

Hyena was found to be a very passive animal. Before enrichment, it was found doing pacing sometimes. But the most prevalent behaviors it showed were normal passive behaviors like sleeping and lying. Lavender oil showed the maximum decrease in stress levels and a mild effect on the overall performance of Hyena. It didn't induce any active behavior. The remaining three oils had very slight to almost no effect on stereotypic behavior. It sniffed the oils to a small extent and showed no liking response (figure 6).

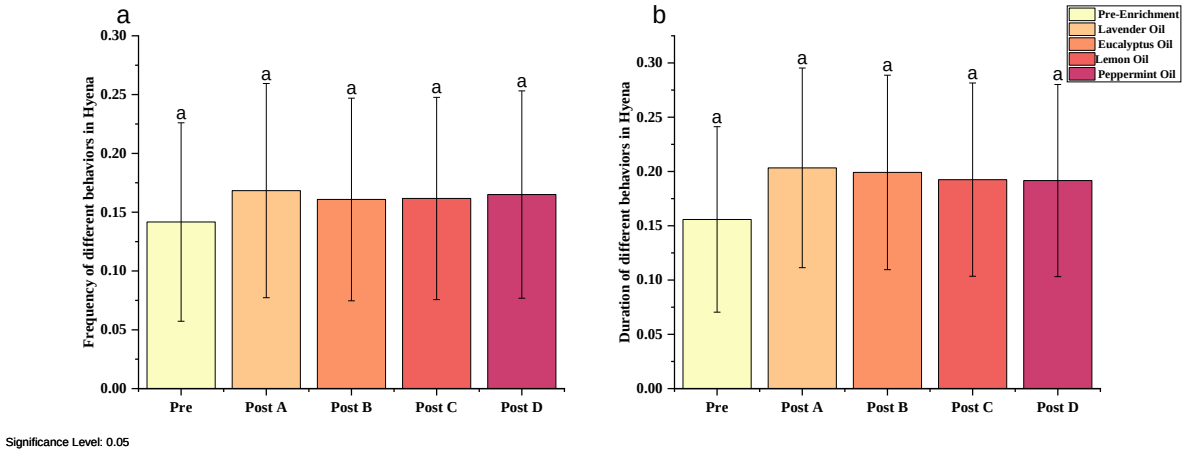

Figure 6. (a) Change in frequency of different behaviors studied in different phases (pre-enrichment (before aroma therapy) and Post-enrichment(after aroma therapy) in Hyena. (b) Change in behavior during the observational period of three hours. Significant changes between means within each period indicate different letters (P 0.05, post-HOC, TUKEY'SANOVAs). For every therapy, the bars depict the means and standard error.

Estimated cortisol concentration after short-term aromatherapy

The overall effect of aromatherapy on cortisol concentration is given in Table 5. It is obvious in figure7 that the cortisol level was measured to be decreased from 82.6±0.927 to 46±0.707 by Lavender oil. Eucalyptus oil also causes a good reduction in cortisol levels. However, the remaining two oils had little reducing effect. The cortisol level was measured again in ten days (Post-enrichment) after the Enrichment Phase, and it was again raised to almost initial values. It means cortisol levels seemed to increase again after aromatherapy,

supported by the statistical analysis [figure 7(I)]. The ELISA cortisol results in Jungle Cat also supported the focal monitoring because there was a reduction in cortisol levels due to lavender oil. The pre-cortisol level was found to be 155±0.707, which lessened to 93.4±0.927. Lavender oil aromatherapy showed positive results that must be considered [figure 7(II)]. The post-enrichment reading was again high, which indicated that cortisol levels again increased in the absence of aromatherapy. Statistical analysis showed that the cortisol was again levelled up to 379±0.701 after the enrichment phase in the leopard [figure 7(III)]. In the case of Lion, the maximum reduction was due to lavender oil from 285.4±1.029 to 190±0.709. However, after the enrichment phase, cortisol level was again increased up to the initial value [figure 7(IV)]. ELISA results in Hyena favour Lavender oil as it decreased the cortisol level from 205.4±0.930 to 176±0.708. The post-enrichment reading was as high as before, indicating the short-term enrichment nature of aromatherapy [figure 7(IV)].

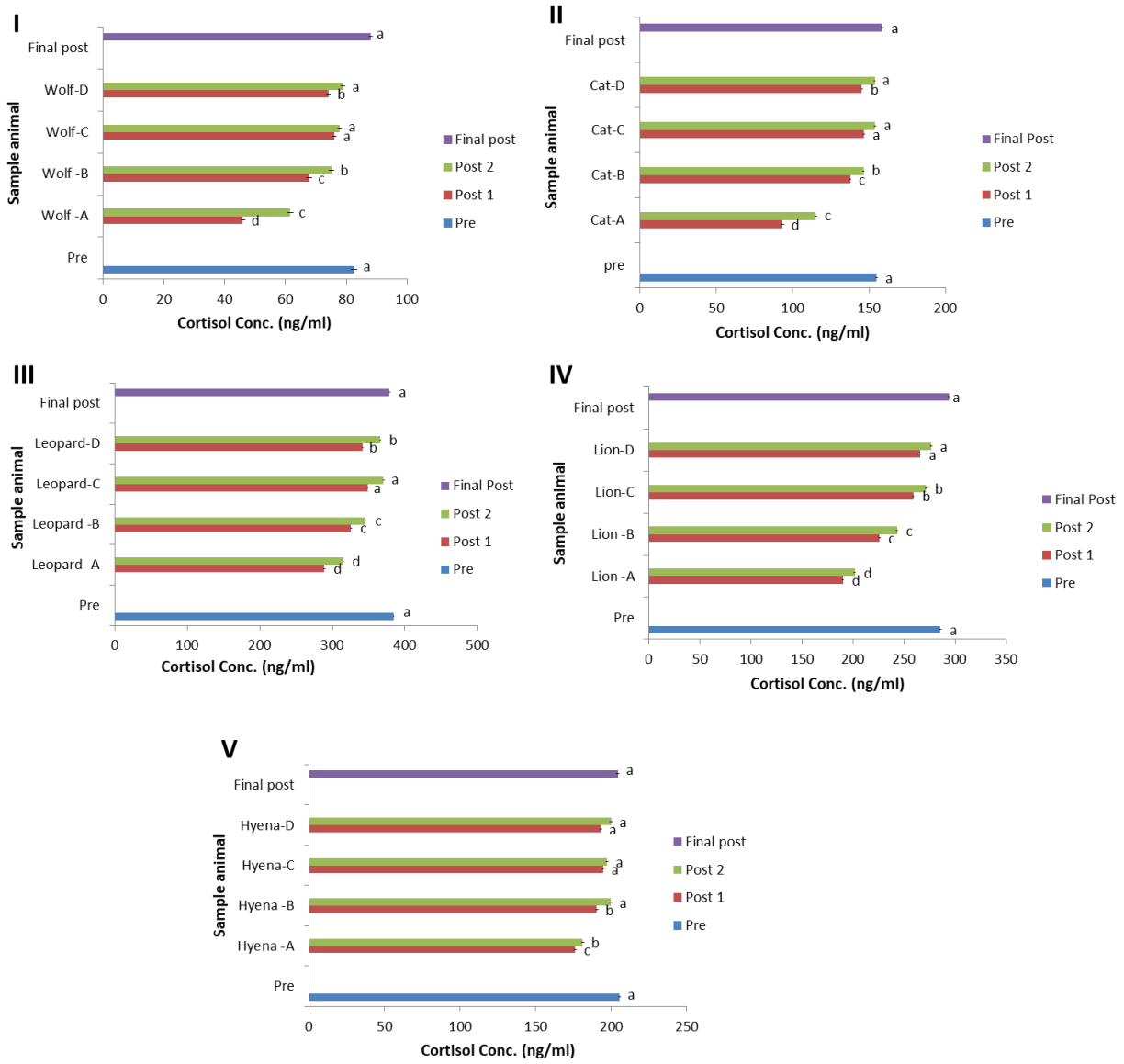

Figure 7 (I, II, III, IV, V, VI). Cortisol Variations in sample animals throughout the study period. Significant changes between means within each period indicate different letters (P 0.05, ANOVAs). For each therapy, the bars show the means and SE. Labels: I (Asiatic Wolf), II (Jungle Cat), III (Leopard), IV (Lion), V (Hyena)

Table 5. Estimated Cortisol Concentration in ng/ml in Study Animals After Sensory Enrichment

Study Animals	Pre- Enrichment Phsae	Enrichment Phase								Post-Enrichment Phase
		Lavender oil		Eucalyptus Oil		Lemon Oil		Peppermint Oil		
		Pre-Enrichmen	Post-Enrichmen	Pre-Enrichmen	Post-Enrichmen	Pre-Enrichmen	Post-Enrichmen	Pre-Enrichmen	Post-Enrichmen	
Asiatic Wolf	82.6±0.927	46±0.707	61.6±0.927	67.8±0.860	75.2±0.860	76±0.707	77.8±0.583	74.2±0.583	79±0.707	88±0.607
Jungle Cat	155±0.707	93.4±0.927	115.2±0.583	137.6±0.5099	146.4±0.5099	146.6±0.583	153.8±0.800	145.2±0.583	153.6±0.517	158.8±0.683
Common Leopard	385±0.710	289.2±1.067	315±0.707	325.8±0.860	346±0.707	349±0.707	370.8±0.663	342±0.707	366±0.699	379±0.701
Asiatic Lion	285.4±1.029	190±0.709	201.8±0.860	225.6±1.031	243±0.707	259±0-705	271.6±1.077	265.4±1.208	276.6±0.927	298.8±0.860
Hyena	205.4±0.930	176±0.708	181±1.00	190.4±0.927	199.6±1.288	194.6±0.5099	197.2±0.860	193±0.800	199.8±1.067	204.2±1.061

Correlation between different phases of treatment and animals

Table 6 shows the correlation coefficients between different phases and treatments involving various essential oils, including Lavender, Eucalyptus, Lemon, and Peppermint Oils. This suggests that the animals consistently exhibited normal passive behavior after aromatherapy with these essential oils. The findings also support the effectiveness of these essential oils in reducing cortisol levels in animals, which is a positive result since reduced cortisol levels are often associated with reduced stress and anxiety.

Table 6. The Pearson Correlation Coefficient (r) Between the Cortisol Concentration and the Short Term Sensory Aroma Therapy Effectiveness

Oils	**Pre-Enrichment Phase**	**Enrichment Phase**								**Post-Enrichment Phase**
		Lavender Oil		**Phase**		**Lemon Oil**		**Peppermint Oil**		
		Pre-Aroma therapy	**Pre-Enrichment**	**Pre-Aroma therapy**	**Post-Aroma therapy**	**Pre-Aroma therapy**	**Post-Aroma therapy**	**Pre-Aroma therapy**	**Post-Aroma-therapy**	
	Pre	1								
Lavender Oil	Pre-Aroma therapy	0.9793	1							
	Post-Aroma therapy	0.9853	0.9964	1						
Eucalyptus Oil	Pre-Aroma therapy	0.9928	0.9923	0.9965	1					
	Post-Aroma therapy	0.9953	0.9909	0.9956	0.9997	1				
Lemon Oil	Pre-Aroma therapy	0.9994	0.9832	0.988	0.9957	0.9974	1			
	Post-Aroma therapy	0.9996	0.9788	0.9859	0.9941	0.9963	0.99952	1		
Peppermint Oil	Pre-Aroma therapy	0.9986	0.9783	0.9812	0.9914	0.9937	0.99897	0.9981	1	

	Post - Aroma therapy	0.9996	0.9782	0.9834	0.9927	0.9950	0.99959	0.9995	0.9995	1
	Post	0.9982	0.9692	0.9740	0.9851	0.9886	0.99679	0.9971	0.9985	0.9985

Discussion

In this study, We hypothesize that essential oils have positive effects on helping lonely, captivated carnivores to cope with stress, namely, the Common Leopard, Asiatic Lion, Asiatic Wolf, Jungle cat, and Stripped Hyena, which has resulted in significant changes in behavioral (increased frequency of social behaviors, decreased frequency of stress-related behaviors) and physiological (decreased fecal glucocorticoid concentrations or FGCs) indicators of well-being when comparing before (pre-enrichment phase), during (enrichment phase), and 10 days after (post-enrichment phase). The current hypothesis is also correlated with Newberry (Post, 2007) study, which stated that aromatherapy is a technique to improve the biological functioning of captive animals through modifications to their environment and continuous use of environmental enrichment can reduce stress, improve the welfare of captive animals, and increase the complexity of controlled environments, which can positively impact the reproduction of the captive animals (Amaya et al., 2020) also stated in his work that olfactory enrichment appears as a promising aid in improving the well-being of wild animals as the given chemical stimuli may induce changes in physiology, including in reproduction.

The carnivores under study were of high ecological importance: Asiatic Wolf (Endangered), Jungle Cat (Least concerned), Common Leopard (Vulnerable), Asiatic Lion (Endangered), and Stripped Hyena (Near threatened). These mammals were housed in a zoo within a small enclosure without any enrichment and complexities, as shown in Table 1. The Asiatic Wolf, Jungle Cat, and Asiatic Lion were zoo-born, the Common Leopard was wild confesticated, and the Stripped Hyena was from a circus. All animals suffered from stress and boredom as per behavior observation by the focal method of behavior monitoring. In that situation, current short term aromatherapy was approved as very effective to reduce the stress. We also identified a significant reduction in rates of stress-related behaviors after the Asiatic Wolf and lar Stripped Hyena were exposed to the series of essential oils, which is the most promising conclusion of this early investigation about the possible good effect of such essential oils. Similarly, when (Uccheddu et al., 2018) tested the effects of several essential oils on domestic dogs, they discovered that some scents increased the frequency with which dogs displayed signs of calm. In contrast, others reduced the frequency with which dogs displayed signs of stress, such pacing, and excessive grooming. Studies on cheetahs

and Sumatran tigers (Panthera tigris sumatrae) indicated that a hay ball infused with cinnamon (Damasceno et al., 2017) significantly reduced stereotypical pacing behavior. Our series of essential oils had a substantial impact on stress-related behaviors in both Asiatic Wolves and Stripped Hyena, supporting our hypothesis that exposure to pleasant odors would have a calming effect on the animals' demeanor.

Asiatic wolf is considered Endangered according to (List, 2004), The IUCN Red List of Threatened Species. It faces many threats like habitat loss, biotic pressure by humans, i.e., grazing, fuel wood collection, and human-wolf conflict due to the depredation of their livestock (Rafacz & Santymire, 2014). So, it is an important species that must be protected and conserved. In Lahore Zoo, the Asiatic wolf is found to be the most stressed animal out of the 5 carnivore species because it is confined solitarily in a smaller cage, which is not suitable for the wolf to perform its normal activities through which it can get rid of boredom.

The cortisol level of Asiatic Wolf before aromatherapy was found to be between 82.6±0.927 ng/ml, which is quite high as compared to the research done on solitary wolves by (Post, 2007).This may be due to the reason that the cage of the solitary wolves on which he experimented was so gracious and vegetated with proper space for the wolves to perform their daily life activities. In contrast, the cage Wolf in Lahore Zoo is so confined and narrow, with an area of 486 sq ft only so that captivity itself has become a cause of stress for the Wolf. As (Donald et al., 2023) found that providing natural area and social structure were the two most critical aspects of housing and husbandry for Asiatic wolves. A primary enclosure for the long-term holding of a large canid should be at least 5,000 square feet, that is 164% larger than the Lahore zoo's wolf enclosure, as noted here. This finding is in agreement with the recommendations of the AZA Canid Taxon Advisory Group in conjunction with the AZA Animal Welfare Committee. So these should be the primary consideration whenever gray wolves are kept captive. Of all four oils, Lavender oil had the most positive effects on the wolf behavior. The results of the current study agree with the already done work (Wells & Egli, 2004). (Ellis & Wells, 2010) suggests that captive animals, including giant felids, primates, reptiles, and canids, benefit from olfactory stimulation as a type of enrichment. Cats have a complex sense of smell and have been seen to have adverse reactions to various scents due to their inability to metabolize them (Fernández-Sepúlveda & Martín, 2022). We gave the 4 oils to the jungle cat. It showed the most positive results with the lavender oil. When we poured the oil on the wooden logs in the cage, it got attracted to the lavender oil odor. It properly sniffed and licked it, showing the sign of liking. There was an obvious reduction in the frequency of stereotypic behaviors like pacing. Lavender's ability to alleviate both the mental and physical

manifestations of anxiety was confirmed in a clinical trial conducted by (Sayorwan et al., 2012) (Fischer & Romero, 2019), lending credence to these findings.

There was an increase in passive behavior in jungle cats, such as lying and sleeping. The current result is supported by (Koulivand et al., 2013; Quirke & O'Riordan, 2011). They reported that massage with lavender oil resulted in maximum absorption of linalool and linalyl acetate from the skin and central nervous system depression. The calming effects of linalool and the narcotic effects of linalyl acetate have both been observed in the jungle cat.

However, the ELISA results showed that the overall cortisol level was not significantly decreased in the jungle cat as only lavender oil reduced the cortisol level from 155±0.707 ng/ml to 115.2±0.583 ng/ml while for all oils, the cortisol values were quite close to the pre values. It is due to the reason that its cage is extremely pathetic with no vegetation, no means of activity, not even a single tree with an area of only 51.75 sq ft, which is 171% smaller than the recommended cage size for a cat by (Fay & Miller, 2015; MELLEN & SHEPHERDSON, 1997). According to him, the recommended minimum space per cat is 338 sq ft for a single 10kg cat and 676 sq ft for a single 20 kg cat. As this adult jungle cat is about 12 to 15kgs in size, the present cage is extremely small, which induces captivity stress of 159ng/ml. High levels of chronic stress, as indicated by FGCs (Sapolsky, 2002) and FGC concentrations, have been shown to increase when animals are exposed to high-stress levels. The small-sized enclosure is achronic stress, which is why the aromatherapy could not do the much effect that was expected. However, lavender oil showed positive results that must be considered and applied for long-term and better concentrations.

Asiatic Lion and Common Leopard have been declared endangered and vulnerable, respectively, and the Hyena is near threatened by (List, 2004). So they need great attention to be conserved and well managed in captivity. These animals need spacious and natural habitats to perform their normal life activities, which are important for their well-being. In Lahore Zoo, they are confined solitarily in pathetic cages that are not according to their natural habitat needs. So they face captivity stress while being confined in such cages. As per the results of the current study, Leopards, Lion, and Hyena were less stressed than the wolf and jungle cats. Lavender oil showed the maximum decrease in stress levels, which agrees with the study of (Ogata et al., 2020) that lavender oil is most effective in relieving stress and anxiety. The work of (Ali et al., 2015; Ogata et al., 2020)on lavender oil properties also supports these results. Furthermore, the correlation coefficients between different phases and treatments involving various essential oils, including Lavender oil, Eucalyptus oil, Lemon oil, and Peppermint oil, suggest that the animals consistently exhibited normal passive behavior after aromatherapy with these essential oils.

Focal observations and ELIZA results favor Lavender oil to be the most effective in reducing stress and stereotypic behavior by inducing a positive relaxing effect on the central nervous system of the animals. While (Greenfield, 2013; Kritsidima et al., 2010) claimed that the effects of using lavender oil on people were only temporary, the results of this study seem to corroborate the idea that doing so may alleviate anxiety in cats. It also accords with (Gök et al., 2015; Kasper et al., 2010) that both clinical and preclinical investigations have found that lavender essential oil reduces anxiety. Anxiety was reduced in preclinical investigations of people with generalized anxiety disorder and it had an anxiolytic impact on people with subsyndromal anxiety. (Koulivand et al., 2013; Quirke & O'Riordan, 2011) corroborated our findings by reporting that linalool and linalyl acetate are rapidly absorbed by the skin during massage, leading to CNS depression. The calming effects of linalool and the narcotic effects of linalyl acetate have both been observed in the jungle cat. Lavender oil has high concentrations of linalool and linalool acetate (Table 7), two chemicals that have been linked to a variety of health benefits, including the alleviation of insomnia, enhancement of mood and cognitive function, and reduction of aggressive tendencies and anxiety(Han & Parker, 2017; Janda et al., 2019).

The (Koulivand et al., 2013) also supports this result by suggesting that only some of the major compounds of the EOs contribute significantly to their anxiolytic and antidepressant effects, including linalool, limonene, and pinene. This result gets further support by (Han & Parker, 2017; Kasper et al., 2010), suggesting the same point that EOs with high content of compounds like linalool, limonene, and pinene are expected to have anxiolytic and antidepressants. Multiple investigations point to the significance of chemical communication. In particular, it is well-documented that olfaction, in addition to vision and hearing, plays a crucial role in the survival of certain species (Janda et al., 2019).

So, in the light of the current study results, 4 oils would be arranged in the following order based on their role in reducing stress in solitary captive animals: Lavender > Eucalyptus > Peppermint > Lemon is very much clear from Table 7 as the best anti-depressant quality is found in Lavander Oil.

ESSENTIAL OIL	COMPOSITION	APPLICATIONS
LAVENDER OIL	Linalyl acetate 39.10%, linalool 29.7%, alpha terpineol 4.35%, trans caryophyllene 3.76%	Anti depressent, anti oxidant, anti inflamatory, treat headaches, relieve painful muscles, skin problems
EUCALYPTUS OIL	Eucalyptol 76.81%, terpinene 7.75%, alpha terpineol 1.44%	Anti-oxidant, anti inflamatory, treat hedaches, Throat infections, catarrh, coughs, bronchitis, asthma, skin infections
LEMON OIL	Limonene 71.81%, γ-terpinene 8.72%, β-pinene 6.61%, myrcene 1.55%, sabinene 1.21%,sabinene 1.21%, p-cimene 0.87%	Boost immune system, improves digestion, relieve labour pain, control vomiting and nausea
PEPPERMINT OIL	Menthol (45.34%), menthone (16.04%), menthofuran (8.91%), cis-carane (8.70%), 1,8-cineole (4.46%), neo-menthol (4.24%), and limonene (2.22%)	Antipruritic, astringent, rubefacient, antiseptic, and antimicrobial purposes and for treating neuralgia, myalgia, headaches, and migraines

Table 7. Chemical composition and applications of essential oils

Although lavender oil has shown positive results, its effects did not last after the enrichment phase. When the cortisol level was measured after 10 days of the enrichment phase, it was again raised to its initial level. This indicates that aromatherapy can be used continuously as a short-term sensory enrichment plan. The short-term effects of essential oils are supported by(Greenfield, 2013; Kritsidima et al., 2010) , who tested lavender oil for reducing dental anxiety and concluded that lavender does reduce affective aspects of anxiety, but it does not affect cognitive components and, hence, the processes that are likely to maintain anxiety-provoking thoughts and behaviors. Finally, we should note that this is only a pilot study, so there are bound to be caveats. Our study is ambitious in many ways (we worked with multiple species, applied multiple oils, and tracked behavior). Still, we found the issue of limited data due to a small sample size, mostly due to the availability of only 5 study animals at the zoo. However, as the first study of its sort, the results of this investigation will be invaluable in promoting the health and safety of ecologically significant captive carnivores worldwide.

Conclusions

The results of this pilot study are promising, and they indicate that our innovative, short-term sensory enrichment program may reduce stress in zoo-housed carnivores, particularly in the case of carnivore species for whom odor plays a significant role in their life. Aromatherapy is effective in stress management and increases the well-being of captive animals by inducing positive effects on their behaviors. The study findings are promising. The survey results coincide with the hypothesis that aromatherapy effectively reduces stress among captive animals so that it can be used as a short-term enrichment plan. The essential oils showed beneficial effects via the olfactory

system on the nervous system in animals and humans and its chemical properties, impacting pathophysiology and physiology. Focal observations and ELISA results favor Lavender oil as the most effective in reducing stress and stereotypic behavior by inducing a positive relaxing effect on the central nervous system of the animals. So, in light of current study results, lavender oil is highly recommended for aromatherapy. Future work into the impact of our aromatherapy on the well-being of carnivores should be expanded in future studies to include a bigger sample size and additional species. Also, it would be necessary to explore further types of fragrance enrichment by considering the ecological/biological relevance of the scent enrichment to the study species.

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