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R E S E A R C H A R T I C L E

Behavioral Studies of Agarwood Smoke in Rats

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A B S T R A C T

In this study the effects of agarwood smoke exposure was evaluated on the general, CNS and reproductive behaviors in male and female rats. The behavioral studies were conducted on both male and female rats by exposing the animals to the smoke of agarwood in a smoke chamber for two durations viz., 30 and 60 minutes. A fourteen-days study plan was designed where the changes in the behavior was recorded using elevated plus maze and light/dark box apparatus. The reproductive behaviors were studied using close circuit camera (CCTV) when the female rat was in estrus cycle. The data from the studies were compared with the control to analyze the influence of agarwood smoke on the behavior in the animals. The data from the study indicated that exposure of agarwood smoke decreased the aggressive, defensive and non-social activities. The CNS studies suggested that exposure of smoke for 30 minutes significantly ($p<0.01$) enhanced some of the parameters for anxiogenic property while 60 minutes duration significantly ($p<0.001$) exhibited anti-anxiety effect. The reproductive behavior indicated that shorter duration of smoke increased the mounts while longer duration did not produce any effect in experimental rats. More studies are suggested in this direction to understand the precise mechanism of action for changes in the behavioral patterns.

Keywords: Agarwood, Smoke exposure, Anxiety, Behavioral pattern, Reproductive study.

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C O N T E N T S

1. Introduction

Aromatherapy is practiced worldwide and it means treating a disease with therapy of smell. Essential oils are the main therapeutic agents that produce the effect of aromatherapy, they are extracted from the fruit, flowers, leaves, seeds, bark and roots of the aromatic plants [1]. These oils are usually administered by inhalation or application on skin. Essential oils provide a feeling of well-being, calmness, relaxation, enhance alertness, and have proven antiviral, antibacterial and antifungal properties, besides being useful in diseases like Alzheimer, cancer, sleep disorders and labor pain[2]. Essential oils produce their effect by the mechanism of their action that causes the release of neurotransmitters in the brain [3]. Agar wood (also known as chenxiang in China; agar in India; oud in the Middle East) is the most expensive resinous wood or essential oil used as incense and is highly rated in perfumery. Agarwood is mostly found in trees of southern Asian countries such as Indonesia, Malaysia, China, Cambodia, India, Myanmar, Vietnam, and Thailand [4]. It is a product found in the trunk or branches of some tree species belonging to the genera Aquilaria and Gyrinops and it appears within the woody tissues of the trees as chunks or dark clumps that contain an aromatic resin, and when it is burned, it produces a fragrant odor [5]. This wood is naturally formed as a defensive response after an injury to the tree caused by microbial infection, insects distressing or lightning strike [6]. Agarwood contain two main chemical constituents; sesquiterpenoids and 2-(2-phenylethyl) chromone derivatives, the percentage of these constituents were found to be higher in a high-graded agarwood compared to low-graded agar wood[4]. Earlier studies have reported the pharmacological activities of agarwood, including anti-inflammatory, anti-cancer, anti-microbial, anti-oxidant and anti-diabetic effects [7]. In-vivo and in-vitro studies of agarwood oil showed significant reduction of inflammation that was comparable to the effect of diclofenac [8]. Another study found that essential oils extracted from agarwood have an anti-neoplastic and anti-angiogenic effects against colorectal and pancreatic cancer cells [9]. However, there are limited studies on agarwood effects on anxiety and other behavioral parameters. Hence, the study was planned to evaluate the effect of agarwood smoke on anxiety, reproductive and the general behavior in rats.

2. Materials and Methods

Animals: Male and female Wistar rats aged 12-14 weeks and weighing 150-200 g were obtained from College of Pharmacy, Qassim University, Saudi Arabia. The experiment was done after obtaining prior permission from the Institutional Animal Ethics Committee (#2017-CP-7). The animals were housed in a temperature controlled room (25ºC), 12 hours' light/dark cycle and access to water and food *ad libitum*. The commercially available agarwood popular among the people of Qassim region, Kingdom of Saudi Arabia was used in this study.

Exposure to smoke:

The rats were held separately to avoid cross exposure to smoke. A fabricated smoke chamber (60×35×28 cm) as described in the earlier study was used to expose the rats to smoke [10]. Animals were divided into three groups comprising of six animals in each as; group-1 served as control kept in a fresh air, whereas group-2 and group-3 were exposed to agarwood smoke in the smoke chamber. Both treatment groups (group-2 and 3) were exposed daily to smoke by burning 0.5g/group of agarwood for 14 days. Smoke duration lasted for 30 min/day for group-1 and 60 min/day for group-2[10]. Animals were observed both during and after the exposure to record the general behavior and at the end of the study period, animals were tested for the CNS and reproductive behaviors as per the standard procedures described below.

Behavioral studies:

A. General behavior

The behavior of animals was observed and recorded during the exposure period of the treatment groups to agarwood smoke and after the exposure for 30 minutes. The behavioral patterns were recorded as per the previous study using a specially designed checklist [11].

B. Light /dark box test

The behavior of animals to dark and light environment was studied using the light/dark box test. This test can be used to evaluate the depressive or anxiety state of the animals. After 14 days of smoke exposure, the animals were individually placed in the brighter chamber and the time spend in the two compartments and the number of crossings between the two compartments were recorded for a period of 5 minutes [12].

C. Elevated plus maze test

Another method to study the anxiety state of the animals was done by using the elevated plus maze test. After 14 days of smoke exposure, the animals were individually placed in the center of the maze facing the open arm. The time spent in open arms and closed arms and number of head-dips were recorded for a period of 5 minutes [13].

D. Reproductive behavior

After 14 days of smoke exposure, female rats were individually checked for their reproductive cycle, called estrus cycle as per the procedure described by Marcondes, et al. 2002[14]. A vaginal secretion was collected by inserting the tip of a plastic pipette filled with 10µL normal saline (NaCl 0.9%) from rats. A one drop of the collected vaginal fluid was placed on a clean glass slide and observed under a light microscope with 10X objective lens for the type of cells. The presence of round and nucleated cells indicated that the animal is in estrus phase (sexually receptive). These animals were left in the cage with the male rat. The observation for the mating behavior was recorded using the close-circuit camera (CCTV) fitted to the computer. The observations such as number of mounts and intromission that took place 30 minutes after sunset and 30 minutes before sun rise were recorded since the nocturnal animals are reported to be most active during this period [14].

Statistics: The data obtained from the study was analyzed by GraphPad InStat software and presented as Mean ± S.E.M. values. The statistical tests used in this study was one-way analysis of variance (ANOVA). $p<0.05$ was considered to indicate the significance of the result.

3. Results and Discussion

The observations from the present study indicated that when the animals were exposed to two durations of agarwood smoke, the general behavior remained almost same as like the control except the parameters such as alertness, aggressive postures, fights, spontaneous movements that were found to be reduced(Table-1). In these groups the animals remained calm, less agitated and confined to one corner that suggests the smoke exposure might have slowed the alert-induced activities. This is one of the characteristics of aromatherapy where exposure to certain chemicals calms the brain functions leading to relaxations of body activities[2]. Similar findings of inhalation of agarwood oil vapor have shown sedative effect is reported by Takemoto et al., 2008 [15].

The CNS activity were studied by light/dark chamber and elevated plus maze apparatus. In the light/dark test, the data from the table indicated that compared to control group, the 30 minutes exposure of agarwood smoke significantly decreased ($p<0.001$) the number of crossing and decreased ($p<0.05$) the time spent in light chamber and on the other hand the 60-minutes exposure significantly increased ($p<0.001$) the number of crossing, decreased ($p<0.05$) the time spent in dark chamber and increased ($p<0.05$) the time spent in light chamber compared to control (Table-2). Similarly, in the elevated plus maze experiment, the observations indicated that 30-minutes exposure of agarwood smoke did not produce any significant change in the time spent in open and close arms but reduced ($p<0.05$) the number of head dips compared to control. The 60 minutes agarwood smoke exposure enhanced ($p<0.001$) the time spent in open arm, close arm ($p<0.05$) and increased ($p<0.001$) number of head dips without affecting the number of vertical rearing compared to control values (Table-3). The data suggests that longer duration of agarwood exposure might enhance the explorative action due to suppression of anxiety and its related parameters and shorter duration has opposite action. Compounds exhibiting both anxiogenic and anxiolytic effects have been reported for centrally acting agents such as diazepam and buspiron. Several factors like dose, duration of exposure, mental condition of the subjects, etc are known to play an active role in such types of actions [16]. Since volatile oils present in the agarwood are found to effect the levels of brain neurotransmitters such as serotonin, endorphin and nor-epinephrine[1,3], similar mechanisms could be responsible for dual action of agarwood on the experimental animals when exposed to two durations of smoke. In the reproductive behavior studies, female rats in the estrus phase were allowed with a male rats and their behavior 30 minutes after sunset and 30 minutes before sunrise is recorded[14]. Our observation indicated that the control animals had an average five number of mounts and one intromission and in comparison, the 30 minutes duration exposure increased the number of mounts to eight ($p<0.001$) compared to control and the intromission remained at one. The 60 minutes exposure rats exhibited decreased number of mounts to three ($p<0.001$) and no intromission took place (Graph-1). Studies in the past have reported that over excitation of brain center can adversely affect the reproductive behavior in both male and female sexual arousal [17,18].The brain neurotransmitters particularly serotonin is reported to play a complex role in the excitation of CNS and sexual-related neuropsychology [19]. Since, agarwood is known to elevate the levels of brain neurotransmitters [1,3], similar mechanisms might have occurred for variation in both the CNS and reproductive behaviors due to differences in the duration of smoke exposure.

Table 1: Effect of agarwood smoke exposure on the general behavior in rats

Behavioral parameters	**Observation**		
	Control	During smoke exposure	After exposure to smoke
Sleeping(Lying or sitting unalert, eyes closed)	+	+	+
Feeding/drinking(Eating food or drinking)	+	–	+
Non-intake maintenance(Grooming, yawning, stretching, sneezing, urinating, defecating)	+	+	+
Exploration(Sniffing air, floor, wall, water bottle, faces, urine or bedding)	+	+	+
Stationary(Alert (eyes open) but no direct attention while lying, sitting or leaning)	+	+	+
Movement(Alert but no direct attention while walking, stretching, climbing or running)	+	–	–
Other non-social behavior(Chewing bedding, digging/scrabbling, jumping)	+	–	–
Aggressive action(Bite, chase, aggressive over (pinning rat on its back), aggressive groom, aggressive sideways, pursuit of fleeing rat)	–	–	–
Defensive action(Defensive over (on back, being pinned), defensive sideways, fight (with and without pursuit))	+	–	–
Social investigation(Sniffing nose, mouth, head,	+	+	+

shoulders, back, flank, anogenital area, belly, tail)			
Other social behavior(Attend, allogroom)	+	+	+

+, observed; −, not observed

Table 2: Effect of agarwood smoke exposure on the CNS activity using light / dark box test in rats

Groups	No. of crossings	Time spent in dark (Sec)	Time spent in light (Sec)
Control	7.00 ± 0.52	237.0 ± 15.60	63.0 ± 5.30
Agarwood smoke (30 minutes)	3.00 ± 0.48**	261.0 ± 18.08	39.0 ± 9.02*
Agarwood smoke (60 minutes)	11.50 ± 0.61**	227.0 ± 10.18*	73.0 ± 2.91*

Values are represented as Mean ± SD.**Statistics**: One-way Anova, *p<0.05, **p<0.001 compared with control group

Table 3: Effect of agar wood smoke exposure on the CNS activity using elevated plus maze test

Grouping	Time spent in OA (Sec)	Time spent in CA (Sec)	No. of head dips	No. of vertical rearing
Control	23.0 ± 3.67	277.0 ± 15.1	3.00 ± 0.02	--
Agarwood smoke (30 minutes)	19.0 ± 3.42	281.0 ± 14.9	1.00 ± 0.02*	--
Agarwood smoke (60 minutes)	85.0 ± 11.70**	215.0 ± 42.60*	9.50 ± 1.34**	1.50 ± 0.03

Values are represented as Mean ± SD. OA – Open arm, CA – Closed arm
Statistics: One-way Anova, *p<0.05, **p<0.001 compared with control group

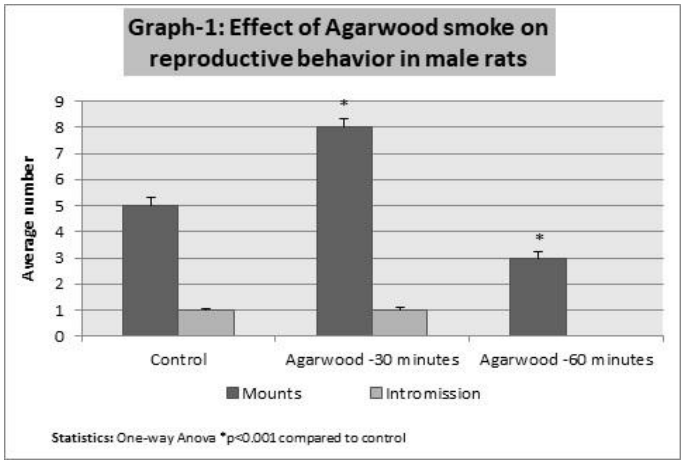

Fig.1: Effect of agarwood smoke on reproductive behavior in male rats

4. Conclusion

The observation from this study suggested that agarwood smoke calmed the general behavior of the animals both during and after exposure. The longer duration of exposure might possess the ability to reduce the anxiety parameters while shorter duration might enhance the reproductive behavior in male rats. Future experiments with more duration of exposure along with biomarkers estimations specific to CNS and reproduction might provide an insight to the mechanism of action of agarwood on the behavioral patterns.

5. Reference

[1] T Dunning. Aromatherapy: overview, safety and quality issues. OA Alternative Medicine. 2013,1(1):1-6.

[2] B Ali, NA Al-Wabel, S Shams, A Ahamad, SA Khan, F Anwar. Essential oils used in aromatherapy: A systemic review. Asian Pacific Journal of Tropical Biomedicine. 2015,5(8):601–611.

[3] G Buchbauer, L Jirovetz. Aromatherapy—use of fragrances and essential oils as medicaments. Flavour and Fragrance Journal. 1994,9(5):217–222.

[4] Y Liu, J Wei, Z Gao, Z Zhang, J Lyu. A Review of Quality Assessment and Grading for Agarwood. Chinese Herbal Medicines. 2017,9(1):22–30.

[5] N Putri, L Karlinasari, M Turjaman, I Wahyudi, D Nandika. Evaluation of incense-resinous wood formation in agarwood (*Aquilaria malaccensis L.*) using sonic tomography. Agricultural and Natural Resources. 2017,51(2):84–90.

[6] H Chhipa, N Kaushik. Fungal and bacterial diversity isolated from Aquilaria malaccensis tree

and soil, induces agarospirol formation within 3 months after artificial infection. Frontiers in Microbiology. 2017,8:1286-1298.

[7] YZH-Y Hashim, PG Kerr, P Abbas, HM Salleh. Aquilaria spp.(agarwood) as source of health beneficial compounds: A review of traditional use, phytochemistry and pharmacology. Journal of Ethnopharmacololgy. 2016,189:331–360.

[8] H Rahman, K Vakati, MC Eswaraiah. In-vivo and in-vitro anti-inflammatory activity of Aquilaria agallocha oil. International Journal of Basic Medical Sciences and Pharmacy. 2012,2(1):2049-2063.

[9] SS Dahham, YM Tabana, MA Iqbal, MBK Ahamed, MO Ezzat, ASA Majid. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of Aquilaria crassna. Molecules. 2015,20(7):11808–11829.

[10] M Ahmed, N Al-Daghri, MS Alokail, T Hussain. Potential changes in rat spermatogenesis and sperm parameters after inhalation of Boswellia papyrifera and Boswellia carterii incense. International Journal of Environmental Research and Public Health. 2013,10(3):830–844.

[11] P Saibaba, GD Sales, G Stodulski, J Hau. Behaviour of rats in their home cages: daytime variations and effects of routine husbandry procedures analysed by time sampling techniques. Laboratory Animals. 1996,30(1):13–21.

[12] SE File, S Pellow. The effects of triazolobenzodiazepines in two animal tests of anxiety and in the holeboard. British Journal of Pharmacology. 1985,86(3):729–735.

[13] S Pellow, P Chopin, SE File, M Briley. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of Neurosciences Methods. 1985,14(3):149–167.

[14] FK Marcondes, FJ Bianchi, AP Tanno. Determination of the estrous cycle phases of rats: some helpful considerations. Brazilian Journal of Biology. 2002,62(4a):609–614.

[15] H Takemoto, M Ito, T Shiraki, T Yagura, G Honda. Sedative effects of vapor inhalation of agarwood oil and spikenard extract and identification of their active components. Journal of Natural Medicine. 2008,62(1):41–46.

[16] NN Kudryavtseva, NP Bondai. Anxiolytic and anxiogenic effects of diazepam in male mice with different experience of aggression. Bulletin of Experimental Biology and Medicine. 2002,133:372-376.

[17] EM Hull, RL Eaton, VP Markowski. Opposite influence of medial preoptic D1 and D2 receptors on genital reflexes: Implications on capulation. Life Sciences. 1992,5:1705-1713.

[18] RR Toledano, JG Pfacis. The sexual arousal and desire inventory: a multidimensional scale to assess the subjective experience of sexual arousal and desire. The Journal of Sexual Medicine. 2006,3:853-877.

[19] TE Kippin, SW Cain, JG Plaus. Estrous odors and sexually conditioned neutral odors activate separate neural pathways in the male rats. Neurosciences. 2003,117:971-979.