

Effects of Perinatal Exposure of Cardamom (*Elettaria cardamomum*) on the Post-natal Development and Social Behavior of Mice Offspring

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Abstract. Female Swiss-Webster mice were fed on cardamom (*Elettaria cardamomum*) during the pre- and post-partum periods at doses of 5, 10 and 20% (w/w) in Pilsbury's food. Their male and female offspring were respectively subjected to the 'Standard Opponent Test' (SOT) and the 'Tube Restraint Test' (TRT). Morphological observations revealed a decreased post-natal developmental body weight gain and delayed eye-opening in the treated pups. 'SOT' showed a significant increase in defensive, attack, threat and number of fighting behaviors whereas, latencies to first threat and attack were significantly decreased. The 'TRT' showed a significant increase in the latency to first bite and a decrease in the number of bites. Most of these cardamom effects are dose-dependent and it was further observed that the elements of aggressive behavior increased in male and decreased in the female offspring. Thus, results are discussed on the basis of the offspring's sex. The critical period of foetal development *in utero* and the post-natal weaning period are important phases during which cardamom reaches the offspring and induces long lasting effects on their social behavior and morphological developments.

Introduction

The plants of cardamom (*Elettaria cardamomum*) are mainly grown in Sri Lanka and South India. The seeds of their ripe fruits are used medicinally, as a spice, and also as a flavoring agent in curries, coffee and cakes, particularly in the Arab countries [1]. Some is used in the manufacture of liqueurs and a relatively small quantity in pharmacy, chiefly in the form of compound tincture of cardamom [2].

Cardamom seed yields 4% of volatile oils containing a high proportion of Terpinyl acetate and cinole and smaller quantities of other monoterpenes, including alcohols and esters [3]. Govindarajan *et al* [1] reported the presence of over 150 com-

pounds in cardamom aroma. Many of these compounds are commonly found in cardamom oil. Recently, Abo-Khatwa and Kubo [4] reported a total of thirty components from their gas chromatographic and mass spectroscopic analysis of the cardamom oil.

A literature survey reveals that little is known about the effects of cardamom on living biological systems particularly in relation to public health. Mobacken and Fregert [5] reported a single case of allergic contact dermatitis to cardamom elicited by terpenes present in the dried ripe seeds. The present study was designed to investigate the effects on growth and behavior of male and female mice whose mothers were exposed to cardamom during the period around parturition.

Materials and Methods

Swiss-Webster albino mice (*Mus musculus*) were bred and housed under highly controlled conditions [6] in the animal facility of the Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia.

Cardamom administration

Dry and unbleached whole capsules (seeds and pods) of cardamom var. (*Minuscule burkil* (Family: Zingiberaceae) were purchased from the local market for the present study.

Pilbury's food and cardamom were mixed in such a ratio that it provided doses of 5, 10 and 20% of cardamom (w/w). The mixtures were minced finely in an electric grinder and distilled water was added to it. The flour-like paste was recast to the size of Pilbury's pellets in a mould and dried completely at room temperature. These pellets formed the only food for the treated animals, whereas plain Pilbury's food was given to the control group.

Thirty-two pregnant mice (pregnancy estimated from the date of finding the vaginal plug) were allocated to four groups of eight animals. Group I was used as control and Groups II, III and IV received Pilbury's food containing 5, 10 and 20% cardamom (w/w) respectively. Food and water was available to the animal *ad lib* except during the behavioral trials.

The pregnant mice were housed individually in opaque plastic cages measuring 30 × 12 × 11 cm. Initial studies [7,8] have shown that the period around parturition is most suitable for assessing the lasting effects of drug treatments on subsequent behavior in developing rodents. Thus, the treated groups were fed on cardamom during the pre-partum (starting on the day finding vaginal plug) and post-partum period until the weaning (22 days) of their litters. The litters were reduced to eight on the day

of birth and pups were left undisturbed with their mothers until 22 days of age when they were sexed. The males were housed individually for 14 days, whereas the females in groups for 21 days. Subsequently, ten mice from each group (including representatives from each litter) were subjected to a variety of tests.

Morphological measurements

The body weight of the pups were recorded every third day from the day of birth to the age of weaning (to minimize the disturbance of the pups and mothers) on an electronic balance (Mettler PL 3000, Switzerland). The days at which hair appeared and the eyes opened were also recorded. After the completion of various behavioral tests, body weight of the offspring was recorded again.

'Standard Opponent' tests:

These tests were carried out with male experimental offspring only. The male intruders were rendered temporarily anosmic by applying, under ether anaesthesia, 25 μ l of 4% zinc sulphate to the nasal tract. These anosmic 'Standard Opponents' were introduced singly in the home cages of the test animals and the tests were carried out for 500 seconds under dim red lighting (circa 9 lux) with a transparent lid to facilitate visual observations [9]. These opponents were used only once and were approximately of the same age and belonged to the same source as the test animals. The selected "elements" of behavior were based on the descriptions of Brain *et al* [7,8], which involved assessing the total time (in seconds) allocated by subject to the broad behavioral categories of:

- a) Non-social activity (mainly exploration of the cage);
- b) Social investigation of the intruder;
- c) Defensive activity;
- d) Attack;
- e) Threat;
- f) 'Displacement' activity (mainly digging and grooming);
- g) Wall rear;
- h) Latencies to first threat and attack;
- i) Number of fights.

The definitions of all 'elements' are given in Ajarem [9].

Tube restraint tests

These tests were performed only with female offspring. The apparatus for this test (Fig. 1) was based on the equipment described by Wagner *et al* [10,11] and consisted of a cylindrical transparent perspex tube 13 cm in length and with an internal

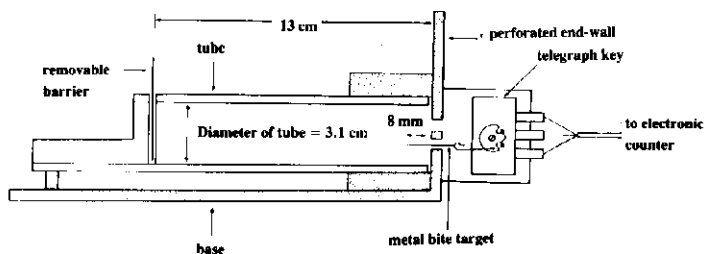


Fig. 1. Diagrammatic side view of the tube-restraint situation employed with female offspring. The perspex tube is fixed to a solid base. The animal is introduced to face the metal bite target which, when activated, operates an electronic counter (not shown: to right). Subjects are prevented from backing out of the apparatus by the removable barrier.

diameter of 3.1 cm. One end of the tube was blocked by a perforated perspex wall through which a 2 cm long metal target was attached to a telegraph key/counter arrangement. This enabled us to record the number of bites directed by the restrained mouse towards the target.

Subjects were held by the tail, allowed to crawl into the restraining tube and a movable perspex barrier was slotted near the rear opening of the tube to prevent the animal backing out. Trials were of 50 seconds duration and carried out under normal laboratory white lights. Biting occurs more frequently under such conditions, indicating, perhaps that increased "novelty" facilitates this response [12]. The behavioral measures used in this test included:

i) the latency (in seconds) to the first bite; and ii) the number of bites activating the counter.

The inside of the tube was cleaned between each test with a 5% Dettol solution (Reckitt and Coleman Ltd., Hull) to minimize potential effects of odors from previous occupants.

Statistical analysis

Student 't' tests were used to make paired comparisons between the body weights and other morphological measures in the different treatment categories. Non-parametric Kruskal-Wallis tests were used to assess the variance on the behavioral measures over different treatment groups. Subsequently, paired comparisons were carried out using Mann-Whitney 'U' test [13] in treatment groups. Behavioral measures are consequently generally presented as median scores with ranges (because of the non-continuous nature of the records).

Results

It is evident from Fig. 2 that the body weights of the treated pups on day 1 after birth were less than their control groups, but the differences were not statistically significant. As the pups grew, the treated pups weighed less than the control group, significantly so at one week of age. This significant decrease in body weight is dose-dependent and was maintained thereafter up to the age of weaning. Higher doses (10% and 20% cardamom) produced highly significant ($P < 0.002$) declines, whereas the lowest dose (5%) was less effective ($P < 0.05$) compared with the control group at 22 days of age.

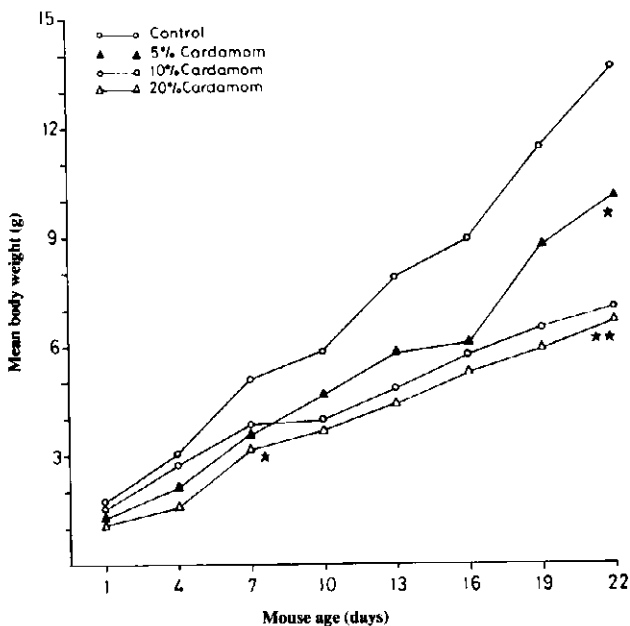


Fig. 2. Effect of perinatal consumption of cardamom by mothers on the body weight gain of the post-natal developing pups.

* ($p < 0.05$)

** ($p < 0.02$)

The data for the cardamom effect on other morphological observations such as eye opening and hair appearance in the pups are presented in Fig. 3. Eye opening was significantly ($P < 0.05$) delayed by 3 days in all treated groups compared to the controls. Hair appearance was delayed by 24 hours in the groups treated with 5 and 10% cardamom whereas in the group treated with 20% cardamom, hair appeared 24 hours earlier than the controls. Thus, the timing of hair appearance did not differ significantly from the control group.

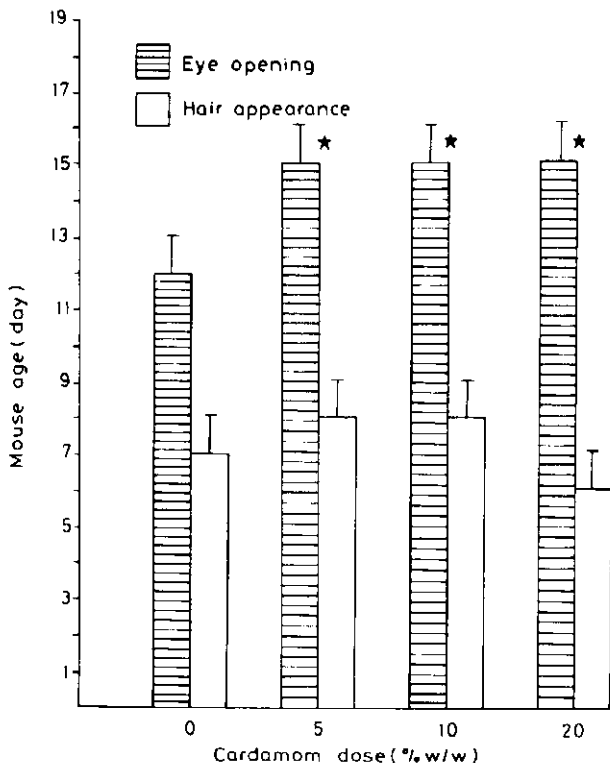


Fig. 3. Effect of Perinatal consumption of cardamom by mothers on the hair appearance and eye opening of their pups.

* ($p < 0.05$)

The results of behavioral observations in the 'standard opponent' test (Table 1) show no significant changes in the overall social and non-social investigations of the cardamom treated groups. Defensive behaviors increased significantly ($P < 0.05$; 0.02) in the groups receiving 10 and 20% cardamom, respectively. The increases in attack, threat and number of fights were highly significant ($P < 0.02$) in the groups receiving 10 and 20% cardamom, respectively. The increases in attack, threat and number of fights were highly significant ($P < 0.02$) in the groups treated with 10 and 20% cardamom. Simultaneously, there was a significant ($P < 0.05$) decrease in the latency to first threat and attack in these treated groups.

The data of 'tube restraint test' in the female offspring (Table 2) showed that in all treated groups, the latency to first bite was extended and the number of bites decreased. These effects were significantly dose-dependent.

Table 3 shows body weights of male and female offspring recorded after the completion of their behavioral tests. The body-weights of both sexes were significantly ($P < 0.05$) decreased as a consequence of cardamom treatment.

Discussion

The results of the present study show that administration of cardamom to pregnant mice during the perinatal period decreases the rate of weight gain of their pups after birth. Furthermore, the loss in body weight is dose-dependent. On the day of birth, the body weight of all treated pups was lower than the control group which could be due to the exposure of embryos to cardamom *in utero*. The significant decrease in the body weight of the pups during weaning period may be due to the presence of cardamom in the milk of the lactating females. It is well-known that significant quantities of compounds that are given in late pregnancy may be transmitted to the offspring during lactation [14]. Coyle *et al* [15] reported that any drug-induced changes in the mother's milk production around the time of parturition might influence behavior of the offspring. In the present study it was observed that exposure of mothers to cardamom during perinatal period induces behavioral changes in their offspring which lasts for a long time. Several reports associated early exposure of pregnant mice to a wide range of compounds with changes in the subsequent behavior of their offspring [9,16,8,17].

The present study revealed that cardamom-induced effects on the offspring behavior was apparently sex-dependent. In male offspring, the elements of aggressive behavior increased whereas in female offspring, a supposedly similar measure decreased significantly. It is clear that threat and attack can be used in a competitive manner (as in the SOT) and in defense (as in the TRT). Consequently, a possible

Table 1. Effects of cardamom consumption by the mothers during parturition on the behavior of their male offspring in a "Standard Opponent Test".

Cardamom Dose	Number of Replicates n	Median (with ranges) number of seconds allocated to behaviors									
		Non-social investigation	Social investigation	Defense	Threat	Attack	Dis-placement	Latency to first Attack	Threat	Number of fights	Wall rear
Control	10	266.8 (210.2-356.7)	145.1 (93.4-284.3)	3.2 (0-12.4)	11.9 (0-159.5)	1.3 (0-72)	0.5 (0-15.5)	468 (186-500)	394 (190-500)	0.5 (0-21)	38.5 (3-57)
5% (W/W)	10	247.4 (185.7-402.3)	135.3 (24.5-315)	8.2* (0-35.6)	0 (0-52.8)	0 (0-119.3)	6.4* (0-39.9)	500 (75-500)	500 (66-500)	0 (0-20)	36.5 (11-61)
10% (W/W)	10	242.1 (118.8-411.1)	130.4 (33.9-278.5)	6.6* (0-71)	34.5* (0-137.4)	14** (0-132.5)	5.1* (0-37.1)	334* (69-500)	287.5* (81-500)	4.5* (0-30)	17.5* (11-41)
20% (W/W)	10	229.5 (14.6-343.5)	173.1 (14.6-343.5)	14.7** (0-58.3)	39.7** (0-147)	12.5** (0-122.4)	5.6 (0-11.4)	384* (87-500)	285* (85-500)	5.0* (0-22)	29.5* (4-44)

* P < 0.05 by the Mann - Whitney U Test.

** P < 0.02 by the Mann - Whitney U Test.

Table 2. Effects of cardamom consumption by the mothers during parturition on the behavior of their female offspring in a tube-restraint test.

Measure	Treatment with Cardamom ^a			
	Control	5% (W/W)	10% (W/W)	20% (W/W)
Latency to first bite (sec) [†]	120 (60-250)	200* (55-500)	365** (77-500)	459** (88-500)
Number of bites	90.5 (5-242)	23* (0-180)	11.5** (0-150)	2*** (0-84)

^a Number of replicates in each group were 10.

[†] Values shown are medians with ranges in parentheses.

* P < 0.05 by the Mann-Whitney U test.

** P < 0.02 by the Mann-Whitney U test.

*** P < 0.002 by the Mann-Whitney U test.

Table 3. Effect of cardamom consumption by the mothers during parturition on the body weight of their offspring after weaning period.

Offspring Sex	Mean body weight \pm S.E.M. ^a after the completion of various tests.			
	Untreated control	Treated with cardamom		
		5% (W/W)	10% (W/W)	20% (W/W)
Males	24.9 \pm 4.7	23.1 \pm 4.6	21.5* \pm 2.4	21.9* \pm 2.2
Females	21.8 \pm 2.1	19.7* \pm 2.6	19.3* \pm 2.3	19.9* \pm 2.1

^a Number of replicates in each group were 10.

* P < 0.05 by the Student's t-test.

reason for the apparent sex difference is that the tests measure different motivations, the effect may calm male aggression and be anxiolytic (fear reducing) in the TRT; perhaps it is this 'calming' effect that makes cardamom popular. However, more specific experiments need to be performed for studying the anxiolytic effects of cardamom. The study provides evidence that cardamom reaches the embryo through the transplacental route and/or through milk during lactation, as reported for other compounds like caffeine, alcohol, nicotine etc. [18,14,9,20]. A cross-fostering experiment may establish whether the effect is mediated via cross-placental passage or via the mother's behavior/lactation? It seems likely that some component of cardamom produces a long-lasting developmental change in the CNS of the offspring. Such exposure to cardamom may bring about the physiological disorders in the offspring, resulting in retarded growth, which was clearly evident in the morphological measures like decreased body-weight and delayed eye-opening.

Since no study was available on the effect of cardamom or its isolated fractions on biological living systems, it is rather difficult to compare the present results. It would be worth investigating the effect of the major fractions of cardamom oil like 1,8-cineol, monoterpenes etc. [4] in experimental models. It is likely that heavy consumption of cardamom may act as chemical signals. Terpenes and terpene derived molecules are known to play important roles as the chemical signals in insects and mammals [21; pp. 61-70].

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دراسة تأثير الهيل على نمو وسلوك صغار الفئران المخبرية

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(استلم في ١٤/١/١٤١٢هـ؛ قبل للنشر في ٢٧/٥/١٤١٢هـ)

ملخص البحث. لقد عرضت الفئران السويسرية أثناء فترة الحمل والولادة لتركيزات مختلفة من الهيل المخلوط مع الطعام بنسبة ٥، ١٠، ٢٠٪ من وزن الطعام. لقد سجلت أوزان الفئران المولودة وكذلك زمن فتح الأعين ووقت ظهور الشعر. وعند بلوغها إثنان وعشرون يوماً عزلت الذكور حيث يوضع كلا منها في قفص صغير لمدة خمسة عشر يوماً، أما الإناث فتعزل في مجموعات لمدة واحد وعشرون يوماً ويتم اختبار سلوك الذكور الاجتماعي عند بلوغها ثمانية وثلاثون يوماً مع ذكور أخرى من النوع والعمر نفسه ولكن حاستها الشمية دمرت مؤقتاً بواسطة ٤٪ من كبريتات الزنك $ZnSO_4$. أما الإناث فدرس سلوكها في الأنبوبة الزجاجية الكابحة للحرية، والتي يوجد بداخلها قضيب معدني (الهدف) ومتصل بجهاز عداد إلكتروني بعد مرات عض الهدف.

النتائج المورفولوجية تشير إلى أن المعالجة بالهيل أثناء فترة الحمل والولادة قد أدت إلى نقص واضح في معدل نمو الفئران المولودة كما أن زمن فتح الأعين قد تأثر وذلك عند مقارنتها بالكنترول.

النتائج السلوكية للذكور تشير إلى أن تعرض الأم الحامل خلال فترة الحمل والولادة للهيل قد زاد من زمن الحركات السلوكية الدفاعية والهجومية والتهددية وكذلك عدد مرات العراك. أما الإناث فقد أحدث الهيل نقصاً في معدل عض الهدف مع زيادة في زمن الحمول (الزمن قبل أول عضه) وذلك عند مقارنتها بالكنترول.

النتائج عموماً تُوضح أن تعاطي الهيل خلال فترة الحمل والولادة قد يؤدي إلى تغيرات مورفولوجية وسلوكية وأن هذه التغيرات قد تكون طويلة الأمد.