

## **Effects of Consumption of Fresh Kola-nut Extract by Female Mice on the Post-natal Development and Behavior of Their Offspring**

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**Abstract.** Female Swiss-Webster mice were given extracts of fresh kola-nuts (*Cola nitida*) mixed in tap water throughout the period preceding and following parturition at concentrations of 8, 16 or 32 mg/liter in their drinking bottles, which were roughly equivalent to the doses of 2.5, 5 or 10 mg/kg body weight. Development was studied in their male and female offspring who were later subjected to 'locomotory behavioral' (LBT), and 'tube restraint' (TRT) tests. Mice whose mothers were exposed to the kola-nut extract showed a significant decline in the rate of post-natal body weight gain but both eye opening and hair appearance occurred earlier than in controls. The numbers of squares crossed, squats, wall-rears and rears in the LBT were significantly affected in male offspring in a dose-dependent fashion whereas these effects appeared biphasic in female counterparts. A significant decrease in the latency to first bite in the 'TRT' was seen at all doses only in males. The number of bites in this test significantly increased following kola-nut extract exposure of dams in both sexes but the effects varied with dose. These effects seem long-lasting and may be induced in the offspring during their *in utero* development and post-natal weaning period.

### **Introduction**

*Cola nitida* is a large tree grown and cultivated in the tropical countries. It is commercially and therapeutically important for its famous "Kola-nuts", which contain caffeine and have a specific flavor [1, 2, 3, 4, 5, 6]. Kola-nuts are reported to contain stimulants that prevent fatigue and dispel sleepiness [2, 7, 8, 9], as well as substances that induce neurotoxicological effects in male albino rats [10]. Moreover, extracts of both kola-seeds and nuts have been reported to induce behavioral changes in rodents such as increasing exploratory, general reactivity and locomotor activity in rats [11]

and producing a dose-dependent, biphasic effects on locomotion in mice [6]. However, the role of kola-nut extracts on pregnant mice and their offspring has yet to be investigated. It is not known whether the effective ingredients present in the kola-nuts can be transferred to the offspring across the placenta and/or via mother's milk. The present study, consequently investigated the effects of administering fresh kola-nut extracts to pregnant mice on the growth, and behavior of their offspring.

## Materials and Methods

### Subjects

Thirty-two pregnant (pregnancy being estimated from the date of finding vaginal plug) laboratory-bred Swiss-Webster albino mice were kept in plastic cages measuring 30×12×11 cm under controlled conditions. Animals were exposed to a reversed white fluorescent lighting schedule on from 22:30 to 10:30 hours (local time) and ambient temperature was regulated between 18 and 22°C, [12, 13]. Subjects were allocated to 4 groups of 8 mice each. Group 1 was a control group receiving tap water only while groups 2, 3 and 4 respectively had daily access to kola-nut extract at an average concentration of 8, 16 or 32 mg/liter in their drinking bottles. The kola-nut extract mixed with tap water formed the only source of drinking fluid for the treated groups of mice. Treatment started on the day of detecting the vaginal plug and continued daily until weaning of the young (day 24 post-partum). Pilsbury's food and the drinking fluid was available to the animals *ad libitum* except during experimental handling.

### Preparation of kola-nut extract

Fresh kola-nuts were purchased from the local markets of Riyadh and prepared for dosing mice as outlined by Ajarem [6], but using tap-water instead of physiological (0.9%) saline. After thorough washing, the nuts were weighed, macerated finely, mixed in a known volume of tap water, stirred vigorously using an electromagnetic stirrer and left undisturbed for overnight at room temperature. The clear supernatant was aspirated and used for treating the mice. The average volume of water consumed per adult mouse per day was known and kola-nuts were weighed and processed as per perinatal body weights of the mice, so as to produce an average concentration of 8, 16 or 32 mg/liter in their drinking bottles and daily ingestions of these concentrations of kola-nuts by the mothers were roughly equivalent to the doses of 2.5, 5.0 or 10 mg/kg body weight respectively.

### Studies on development

The offspring were weighed every fourth day on a Mettler PL3000 electronic, top-loading balance (Mettler Balance Company, Switzerland) from birth until wean-

ing. The days on which the hair appeared and eyes opened, were also recorded. After the weaning period, subjects were exposed to various behavioral tests, and then weighing was resumed.

### **Locomotory behavioral tests**

The experimental mice were individually placed for 300 seconds in a wooden experimental arena measuring 80×80×30 cm and the base divided into 64 equal squares for the open field test and observing various behavioral “elements” such as the number of squares crossed, durations of locomotion and immobility, and numbers of rears, wall-rears, squats and washing as outlined by Ajarem [6, 14]. The observations were carried out under white laboratory lighting and after each test the arena was cleaned to minimize potential effects of odors from previous occupants.

### **Tube restraint test**

The apparatus for this test was based on the equipment described by Wagner *et al.* [15, 16] and consisted of a cylindrical transparent perspex tube 13 cm in length and with an internal 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 one to record the number of bites directed by the restrained mouse towards the target. The test was conducted as outlined by Ajarem and Ahmad [17] for 500 seconds duration under normal laboratory lighting. The inside of the tube was cleaned with 5% Dettol (Reckitt and Coleman Ltd., England) solution between tests to minimize potential effects of odors from the previous occupant.

### **Statistical analysis**

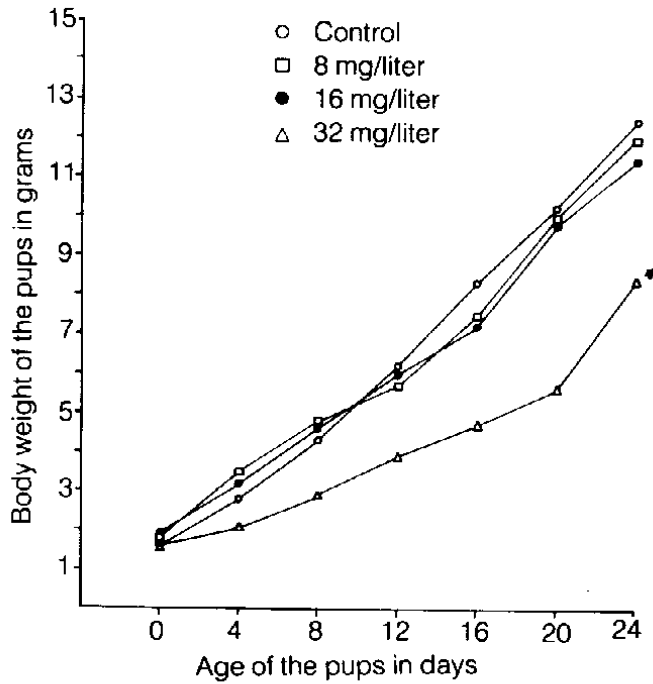
The results were statistically analyzed using Student ‘t’ or Mann-Whitney ‘U’ tests [18] depending on whether the data was normally distributed or not.

## **Results**

Figure 1 presents the body weight changes found in developing mouse neonates. Clearly, the highest dose of kola-nut extract (32 mg/liter) ingested by mothers produced a highly significant ( $P < 0.005$ ) decrease in the body weight of the mice offspring at one week of age. This decrease was maintained thereafter upto the age of weaning. However, the lower doses (8 and 16 mg/liter) of extract resulted in insignificant changes of weight only during the last week prior to weaning.

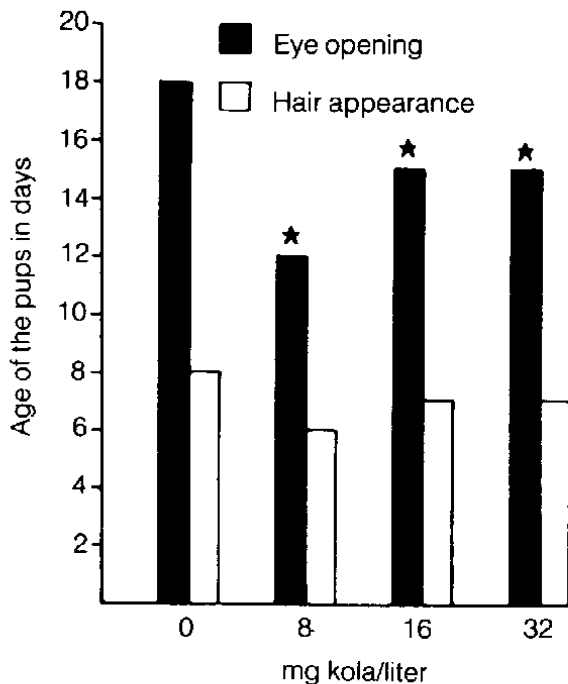
The data of other morphological observations such as eye opening and hair appearance are presented in Fig. 2. Both eye opening and hair appearance occurred

earlier in all treated groups than in controls. These, respectively, occurred 6 and 3 days earlier at 8 mg/liter and 3 and 1 days earlier at both the other two doses.



\* differs from controls ( $P < 0.005$ ) by Student's t-test.

**Fig. 1. Effect of perinatal consumption of kola-nut extract by pregnant mice on the post-natal body weight gain of their developing pups.**



\*differs from controls ( $P < 0.05$ ) by Mann-Whitney U-test.

**Fig. 2. Effect of perinatal consumption of kola-nut extract by pregnant mice on the hair appearance and eye opening of their pups.**

The effects of ingesting kola-nut extract on the locomotor activity and other behavioral elements in male and female offspring are presented in Tables 1 and 2, respectively. The number of squares crossed by male offspring was significantly higher in all treated groups than in controls and the effect appeared dose-dependent. In the female offspring, however, the effect appeared biphasic. The lowest (8 mg/liter) and the highest (32 mg/liter) doses significantly increased the number of squares crossed, while the medium dose (8 mg/liter) was ineffective (Table 2). However, that dose significantly increased the number of squats in both sexes of offspring. On the other hand, the number of wall rears and rears were significantly increased in both sexes at the highest (32 mg/liter) dose.

Table 3 contains the data of 'tube restraint test' of offspring recorded after the completion of the behavioral tests. The latency to first bite was significantly decreased in male offspring at all doses whereas in female offspring there was no effect. The number of bites were significantly increased in male offspring at the medium dose (16 mg/liter) only, but in females, the lowest (8 mg/liter) and the highest (32 mg/liter) doses both produced significant increases.

The body weights of both sexes were significantly lower than in controls only at the highest (32 mg/liter) dose at the termination of the behavioral tests (Table 4).

**Table 1. Effects of making kola-nut extract available to pregnant mothers and their subsequent offspring on the median (with ranges) behavioral indices of their male pups in a locomotory test**

| Treatment groups | Number of acts and postures |               |              |             |             | Durations in seconds |               |
|------------------|-----------------------------|---------------|--------------|-------------|-------------|----------------------|---------------|
|                  | Number of squares crossed   | Wall rears    | Rears        | Wash        | Squats      | Locomotion           | Immobility    |
| Control          | 187<br>( 60-260)            | 15<br>( 4-38) | 0<br>(0-3 )  | 4<br>(2-7 ) | 0<br>(0-2)  | 300<br>(0-300)       | 0<br>(0-90)   |
| 8 mg/liter       | 265<br>(160-333)            | 32<br>(16-40) | 3<br>(0-4 )  | 4<br>(2-12) | 1<br>(0-3)  | 295<br>(0-300)       | 10<br>(0-40)  |
| 16 mg/liter      | 250<br>(160-311)            | 29<br>(0-42)  | 1<br>(0-18)  | 6<br>(3-13) | 2*<br>(1-5) | 265*<br>(0-300)      | 35<br>(15-80) |
| 32 mg/liter      | 290*<br>(100-386)           | 36*<br>(0-50) | 2*<br>(0-14) | 5<br>(2-9)  | 0<br>(0-1)  | 300<br>(0-300)       | 0<br>(0-15)   |

\* differs from controls ( $P < 0.05$ ) by Mann-Whitney U-test.

**Table 2. Effects of making kola-nut extract available to pregnant mothers and their subsequent offspring on the median (with ranges) behavioral indices of their female pups in a locomotory test**

| Treatment groups | Number of acts and postures |                |             |             |             | Durations in seconds |               |
|------------------|-----------------------------|----------------|-------------|-------------|-------------|----------------------|---------------|
|                  | Number of squares crossed   | Wall rears     | Rears       | Wash        | Squats      | Locomotion           | Immobility    |
| Control          | 99<br>( 50-295)             | 12<br>( 2-26)  | 0<br>(0-3 ) | 4<br>(2-6 ) | 0<br>(0-1)  | 300<br>(0-300)       | 0<br>(0-30)   |
| 8 mg/liter       | 233*<br>(193-323)           | 14<br>( 1-22)  | 1<br>(0-4 ) | 3<br>(2-12) | 1<br>(0-2)  | 285<br>(0-300)       | 15<br>(0-40)  |
| 16 mg/liter      | 91<br>( 54-250)             | 17<br>(3-26)   | 1<br>(0-1 ) | 2<br>(1-10) | 3*<br>(2-3) | 241*<br>(0-300)      | 60*<br>(5-90) |
| 32 mg/liter      | 215*<br>(157-307)           | 25*<br>(12-30) | 0<br>(0-11) | 4<br>(2-7)  | 0<br>(0-1)  | 300<br>(0-300)       | 0<br>(0-15)   |

\* differs from controls ( $P < 0.05$ ) by Mann-Whitney U-test.

**Table 3. Effects of making kola-nut extract available to pregnant mothers and their subsequent offspring on the median (with ranges) behavioral indices of their pups in a tube-restraint test**

| Sex of offspring | Measure                               | Concentration (mg/liter) |                |                 |                |
|------------------|---------------------------------------|--------------------------|----------------|-----------------|----------------|
|                  |                                       | Control                  | 8              | 16              | 32             |
| Males            | Latency to first bite<br>(in seconds) | 50<br>(10-120)           | 16**<br>(1-32) | 15**<br>(2-29)  | 15**<br>(6-24) |
|                  | Number of bites                       | 26<br>(3-96)             | 29<br>(2-107)  | 52*<br>(11-146) | 24<br>(7-89)   |
| Females          | Latency to first bite<br>(in seconds) | 21<br>(9-63)             | 20<br>(6-59)   | 22<br>(10-57)   | 21<br>(8-65)   |
|                  | Number of bites                       | 15<br>(5-113)            | 37*<br>(3-168) | 20<br>(11-96)   | 25<br>(1-155)  |

\* differs from controls ( $P < 0.05$ ) and \*\* ( $P < 0.02$ ) by Mann-Whitney U-test.

**Table 4. Effects of making kola-nut extract available to pregnant mothers on the mean body weight (in grams  $\pm$  S.E.M.) of their offspring**

| Sex of offspring | Concentration (mg/liter) |                |                |                             |
|------------------|--------------------------|----------------|----------------|-----------------------------|
|                  | Control                  | 8              | 16             | 32                          |
| Males            | 20.3 $\pm$ 2.5           | 20.5 $\pm$ 2.3 | 22.1 $\pm$ 3.7 | 16.6 $\pm$ 2.1 <sup>a</sup> |
| Females          | 18.5 $\pm$ 1.6           | 17.5 $\pm$ 2.1 | 19.4 $\pm$ 1.7 | 15.6 $\pm$ 1.3 <sup>a</sup> |

<sup>a</sup> differs from controls ( $P < 0.005$ ) by Student's t-test.

### Discussion

Clearly, fresh kola-nut extract administered to pregnant mice may produce the effects on the offspring following passage in the mother's milk, changes in the mother's behavior, transplacental transport to the foetus and/or by self ingestion of material by neonates. The effects of kola-nut extracts in the present studies may have acted through any of these processes or through a combination of several.

The decrease in body weight gain observed in the present offspring appears dose-dependent and this action may be mediated either via the milk [19] and/or, as reported for compounds like caffeine, alcohol and nicotine, transplacentally [12, 20, 21]. Further, drug-induced changes in mother's milk around the time of parturition might influence behavior of the offspring [13, 17, 22, 23, 24, 25]. A modified cross-fostering design soon after birth may be used to confirm whether these effects are mediated via mother's behavior/lactation or via the cross-placental route.

Weaning at 24 days is arbitrary, since pups do eat solid food and use water bottles earlier. In the present study, it is likely that the young may have ingested the kola-nut extract by drinking themselves. Thus, care has to be taken that at weaning the young pups do not self ingest the test material.

It is also likely that kola-nut extract produces developmental changes in the central nervous system of the developing offspring *in utero* as well as post-partum, bringing about physiological disorders resulting in abnormal growth. Such physiological abnormalities are clearly indicated by the abnormal hair appearance and eye-opening in the young as well as by their persistently lagging body weight gain.

The results of 'tube restraint test' clearly show that the stimulants present in the kola-nuts [2, 7, 8, 9] increase the number of bites in male as well as female offspring.

Since the 'tube restraint test' is claimed to measure fear-induced attack, this may represent an anxiogenic effect of kola-nuts. As the results of 'tube restraint test' are controversial [26], further experiments are currently in progress using a plus-maze [27] to examine anxiety in mouse offspring whose mothers are exposed to kola-nut extracts.

Ajarem [6] found that intraperitoneal injections of male mice with kola-nut extract produced biphasic changes in the locomotor activity depending on the dose and the treatment duration. The perinatal administration of the extract carried out in the present study has brought about these effects by low as well as by high doses, and such effects appear to be long lasting as well. However, some biphasic effects have been observed in the number of squares crossed and the amount of target biting by female offspring.

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## دراسة تأثير مستخلص ثمرة الجورو *Cola nitida* على نمو وسلوك صغار الفئران المخبرية

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(سُلِّمَ في ٥ ذي القعدة ١٤١٣هـ، وقَبِلَ للنشر في ١٠ ربيع الآخر ١٤١٤هـ)

ملخص البحث . لقد عرّضت الفئران السويسرية أثناء فترة الحمل والولادة لتركيزات مختلفة من مستخلص ثمرة الجورو الطازجة ٥، ٢، ٥، ١٠ و ٥٠ ملجم / كجم من وزن الجسم . لقد سُجِّلَتْ أوزان الفئران المولودة وكذلك زمن فتح الأعين ووقت ظهور الشعر . وعند بلوغها إثنين وعشرون يوماً، دُرِسَ سلوكها الحركي في المنطقة المفتوحة أما سلوكها العدائي فقد دُرِسَ داخل الأنبوب الزجاجي الكابح للحرية والذي يوجد بداخله قضيب معدني (الهدف) ومتصل بجهاز عداد إلكتروني ليعدّ عدد مرات عض الهدف .

النتائج المورفولوجية تشير إلى أن المعالجة بالجورو أثناء فترة الحمل والولادة قد أدت إلى نقص واضح في معدّل نمو الفئران المولودة ولكن لا يوجد اختلاف في وقت زمن فتح الأعين ووقت ظهور الشعر عند مقارنتها بالكنترول .

النتائج السلوكية تشير إلى أن المعالجة بالجورو قد أحدثت زيادة معنوية في كل من عدد المربعات المقطوعة، عدد مرات الجشوم، عدد مرات الوقوف على الجدار وعدد مرات الوقوف في الهواء مقارنة بالكنترول . أما نتائج الأنبوب الكابح فتشير إلى أن زمن الخمول قبل أول عضه قد نقص في الذكور بينما زاد عدد مرات عض الهدف في كل من الذكر والأنثى .

النتائج عموماً توحي بأن المعالجة بالجورو خلال فترة الحمل والولادة أحدثت تغيّرات مورفولوجية وسلوكية وأن هذه التغيّرات قد تكون طويلة الأمد .