Arab Gulf J. Scient. Res., **8** (1), pp. 183-196 (1990)

The Effect of an Imported Chewing Gum on the Reproductive System in Mice

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ABSTRACT. The effect of an imported chewing gum on the male and female reproductive systems of Swiss albino mice was evaluated. The chewing gum was administered both in drinking water and food at a pooled dose of 2 g/kg/day for a period of 8 weeks. The results revealed that the imported gum lacks the potential to induce spermatotoxicity, sterility or embryonic loss for the exposure period used. The observations on the female reproductive system indicated insignificant changes in fertility and post-implantation loss. However, the total and pre-implantation embryo losses in the treated females increased as compared with the control group. The estrogenic activity of the gum was evaluated using gum extract, suspended in corn oil, and administered (i.p.) in a dose of 250 mg/kg/day for 3 days. The results showed an increase in the uterine weight in experimental groups when compared with control groups. However, a statistically significant activity was evident only in the strawberry-flavoured gum. It is possible that the chewing gum contained some flavouring components with potential estrogenic activity that interfere with the hormonal status and hence the process of ovulation.

There has been a common belief among the public that the use of certain imported chewing gums could lead to sterility. The gum in question came in three different flavours; spearmint, cinnamon and strawberry and is available throughout the Gulf area. In order to practically evaluate the safety of this product for public consumption, the present preliminary investigation was undertaken in order to examine effects the gums might have on the epididymal spermatozoa, male and female fertility, pre- and post-implantation loss and total implantation loss in mice. The study was also extended to include possible estrogenic effects on the uterus of virgin female mice.

Materials and Methods

Animals

The animals used in this study were Swiss albino male and female mice (21-27 g), 5-6 weeks of age. They were caged in air conditioned quarters (23-25°C) with a 12 L: 12 D photoperiod maintained with fluorescent illumination. Food and water were provided *ad libitum*. In some experiments, Swiss albino pre-pubertal female mice (14-16 g) and 23-25 day old were used.

Test substance

Imported chewing gum (Chic) of three different flavours (spearmint, cinnamon and strawberry) was provided and used as the test substance. Ghandour, a locally made chewing gum was used as control. Estradiol was also used as positive control in some selected experiments and was purchased from BDH.

Sample preparation and administration

Powdered chewing gum was administered both in drinking water as suspension in a dose of 1.0 g/kg body weight/day (0.6 per cent) and mixed with food in a dose of 1.0 g/kg body weight/day (0.76 per cent). The animals received a total dose of 2.0 g/kg body weight/day for a period of 8 weeks. Powdered chewing gum was prepared by grinding 60 pieces, each weighing 2.53 g, in a blender (Mounilex). After grinding, the material was soaked in 250 ml. of 95% ethanol, then stirred in an automatic shaker for 24 hours. Filtration and evaporation of the filtrate at 40°C *in vacuo* gave a residue (2.5-3.0 g) that was used for testing.

Quantitative estimation of spermatozoa

Sperm count was carried out according to the method described by Anderson *et al.* (1983). Twenty four hours following the last day of treatment, the animals were sacrificed by cervical dislocation. The epididymides and vas deferens were excised from the reproductive tract and spermatozoa were obtained by making small cuts into the caudae epididymides and vas deferens then washing them into 1.0 ml of Krebs' Ringer bicarbonate buffer, pH 7.4. The spermatozoal count was evaluated with Neubauer hemocytometer after dilution with 19 volumes of the buffer.

Evaluation of morphological abnormalities in sperm heads

The study on sperm head abnormalities was performed according to the method described by Wyrobek and Bruce (1975) and Wyrobek *et al.* (1983). Twenty four hours following the end of exposure to chewing gum, the male mice were killed by cervical dislocation. The caudae epididymides and vas deferens were dissected and placed in a petri dish containing 3.0 ml of 0.9% saline solution

and cut into small pieces. The resulting suspension of sperm was filtered through an 80 μm silk mesh to remove tissue fragments, and 0.5 ml of the filtrate transferred to a centrifuge tube to which 0.05 ml of 1% eosin was added. One drop of the stained solution was placed on each of the coded slides and spread by three passes of another slide. The slides were air dried and mounted with DPX mountant (BDH Chemicals Ltd., Poole, England). The different abnormalities screened were amorphous, flat head, rotated head, microcephali, megacephali and swollen achrosome.

Dominant lethal assay

The dominant lethal assay was conducted according to the method described by Epstein and Rohrborn (1971) and Epstein *et al.* (1972). Twenty four hours following treatment, the male mice were mated to 3 normal virgin females for a period of 2 weeks. In case of females, 2 treated female mice were mated to 1 normal male. Thirteen days following the mid week of their first caging and presumptive mating, the female mice were killed by cervical dislocation. The dissections were continued for 16 days following the date of separation of male mice. After necropsy, the uterine tract was examined and the number of living and dead implants were counted for each pregnant female. From this data base, the following parameters were evaluated:

(1) *Fertility index* was computed as number of pregnant females per number of mated females. (2) *Total loss* was assessed by comparing the number of live implants in the treated and control animals. (3) *Preimplantation loss* was determined by comparing the number of implants per pregnant female in the treated and control groups. (4) *Post-implantation loss,* the measure of dominant lethal mutations, is referred to the number of dead implants per pregnant female.

Evaluation of estrogenic activity

The method used to evaluate the estrogenic activity of the chewing gum extracts was that described by Wirth *et al.* (1981). Ten prepubertal female mice were assigned to each of the test and control groups. The test group received a daily dose (i.p.) of 250 mg/kg body weight for three consecutive days. The animals in the positive control group were treated (i.p.) with estradiol at a daily dose of 50 μg/kg. body weight (0.2 ml) and those in the negative control received the vehicle, corn oil (0.2 ml). Twenty four hours following the last dose, the animals were sacrificed by cervical dislocation and their uteri were weighed. The individual values of the uteri were converted to weight per 100 g body weight.

Statistical analysis

The data on sperm count, sperm abnormalities and embryonic loss was analyzed using Students' t-test and Chi-square test. Chi-square test was used to

analyze fertility in male and female mice. The estrogenic activity was statistically analyzed using the Student's t-test and analysis of variance (Alder and Roessler 1977 and Sokal and Rohlf 1981).

Results and Discussion

The results of this study showed clearly that the imported chewing gum did not have any adverse effect on mean testicular weight, seminal vesicles, sperm count and per cent abnormal spermatozoa in mice for the exposure period used (Table 1). The effect of chewing gum on the fertility of untreated normal female mice mated with treated males was investigated. As shown in Table 2, there was no significant effect on the capability of the treated males to impregnate the females. Any effect that caused induction of embryonic loss in females mated with treated males is presented on Table 3. The results revealed that the gum did not have significant effect ($P > 0.05$) on total, live and dead implants per pregnant female as compared with the control group.

Effect of the chewing gum on female reproductive system

The data obtained in female fertility are the result of mating of chewing gum treated female mice with untreated males (Table 4). The rate of fertility in the treated groups did not differ significantly ($P > 0.05$) when compared with the control group.

The effect of the imported gum on embryonic loss in female mice was studied (Table 5). The results showed that there was a significant reduction in total implants per pregnant female in both spearmint and cinnamon gum groups ($P < 0.05$). Moreover, the reduction in total implants in strawberry gum group was more prominent ($P < 0.01$). Like wise, there was a significant drop in the number of live implants per pregnant female in response to both cinnamon ($P < 0.05$) and strawberry gum ($P < 0.01$) treatments as compared with the control. However, the number of dead implants per pregnant female was not affected by the treatment.

The significance of the reduced count in total implants is possibly due to either loss of fertilized eggs before implantation, loss of unfertilized eggs or interference in the ovulation process. The loss of fertilized eggs may reflect gross chromosomal damage leading to embryonic lethality before implantation (Green *et al.* 1985). The induction of post-implantation loss may also be associated with chromosomal aberrations (Snell *et al.* 1934 and Epstein *et al.* 1970). Since fertility rate (Table 4) and post-implantation loss (Table 5) were not altered by the treatment, the possibility of loss of fertilized eggs can be excluded. On the other hand, the loss of unfertilized eggs or low rate of ovulation reflects either a direct

effect on the ovaries or a central effect via a negative feed back mechanism leading to a decrease in release of FSH and LH (Murad and Haynes 1985). The direct effect may involve blockade of FSH and LH receptors in the ovaries. This may lead to the observed reduction in ovulation.

It is possible that the chewing gum examined contained some flavouring components, that interfere with the hormonal status and hence the process of ovulation. Thus, further experiments were conducted. Pre-pubertal female mice were used to determine whether the imported gum contained any potential estrogenic agents. In the first series of experiments, the estrogenic activity of the three flavours of gum was evaluated using the vehicle, corn oil, as a control. The data revealed that there was a large mean uterine weight in all groups than in negative control (corn oil). The uterine weight in all the gum groups did not exceed the positive control (estradiol) values. However, a significant estrogenic activity was observed only in the strawberry flavoured group as compared with the negative control ($P < 0.05$; Table 6).

It was interesting to find out if the reproductive effects elicited by the strawberry-flavoured imported gum were restricted to this brand or extended to other brands, so, a locally manufactured gum (Ghandour) was similarly subjected to estrogenic evaluation. A comparison of the results obtained with those of the strawberry imported gum are shown in Table 7. There, it can be seen that there is a significant increase in the uterine weight of the strawberry gum treated group compared with the local gum treated group ($P < 0.01$).

In view of the observed data, a central effect of the strawberry gum is more likely. Since the gum showed an estrogenic activity as reflected by an increase in the weight of pre-pubertal uteri. The inability of the gum to affect male gonadotropin release may reflect differences in the sensitivity of male gonadotropin receptors compared with female receptors.

Table 1. Effect of chewing gum on spermatozoal count and morphological abnormalities of the epididymal sperms in mice

Treatment@ dose (g / kg / day)	Number of male mice treated	Mean body* weight ± S.E.	Mean weight* of testis±S.E. (per 100 g)	Mean weight* of seminal vesicles±S.E. (per 100 g)	Mean sperm* count/cu mm (log 10^x) ± S.E.	Number of sperms screened	Mean (%) abnormal sperms ± S.E.
Control# (Ghandour) 2 g/kg/day	10	29.2 ± 1.3	0.71 ± 0.03	0.61 ± 0.03	4.397 ± 0.04	4975	2.5 ± 0.87
Chewing gum - Spearmint, (green colored) 2 g/kg/day	10	35.6 ± 1.03	0.63 ± 0.05	0.69 ± 0.07	4.361 ± 0.04	5187	1.9 ± 0.7
Chewing gum - Cinnamon, (orange colored) 2 g/kg/day	10	32.4 ± 0.81	0.72 ± 0.04	0.66 ± 0.05	4.264 ± 0.07	4977	2.3 ± 0.8
Chewing gum - Strawberry, (yellow colored) 2 g/kg/day	10	33.2 ± 0.86	0.67 ± 0.05	0.72 ± 0.11	4.365 ± 0.04	4363	1.8 ± 0.2

@ The duration of treatment was 8 weeks.
# Chewing gum made in Saudi Arabia.
* $P > 0.05$ (Students' t-test).

Table 2. Effect of chewing gum on fertility of male mice

Treatment[@] dose (g / kg / day) for male mice	Number of male mice mated	Number of[+] untreated female mice mated	Number of females dead/ discarded due to the death of male mice	Total pregnant females/ number of females mated	Percent[*] fertility
Control (Ghandour)[≠] 2 g/kg/day	15	45	10	31/35	88.6
Chewing gum - Spearmint, (green colored) 2 g/kg/day	15	45	4	30/41	73.2
Chewing gum - Cinnamon, (orange colored) 2 g/kg/day	15	45	9	31/36	86.1
Chewing gum - Strawberry, (yellow colored) 2 g/kg/day	15	45	5	32/40	80.0

@ The duration of treatment was 8 weeks.
≠ Chewing gum made in Saudi Arabia.
+ Each male was mated with 3 females.
* $P > 0.05$ (Chi-square test).

Table 3. Induction of embryonic loss in females mated to male mice treated with chewing gum

Treatment@ dose (g / kg / day)	Total Number of pregnant female mice	Total implants*/ pregnant female ± S.E.	Live implants*/ pregnant female ± S.E.	Dead implants*/ pregnant female ± S.E.
Control (Ghandour)# 2 g/kg/day	31	9.9 ± 0.52 (306)	9.03 ± 0.47 (280)	0.8 ± 0.29 (26)
Chewing gum - Spearmint, (green colored) 2 g/kg/day	30	10.9 ± 0.34 (327)	10.50 ± 0.34 (315)	0.4 ± 0.11 (12)
Chewing gum - Cinnamon, (orange colored) 2 g/kg/day	31	10.6 ± 0.34 (328)	10.0 ± 0.31 (310)	0.6 ± 0.16 (18)
Chewing gum - Strawberry, (yellow colored) 2 g/kg/day	32	10.3 ± 0.3 (330)	10.1 ± 0.31 (323)	0.2 ± 0.1 (7)

The total number of male mice mated in each group was 15.
Figures in parenthesis denote the number of implants in each case.
@ The duration of treatment was 8 weeks. # Chewing gum made in Saudi Arabia.
* $P > 0.05$ (Students' t-test).

Table 4. Effect of chewing gum on fertility in female mice

Treatment[@] dose (g / kg / day)	Number of female mice treated	Number of untreated male mice mated	Number of females mice dead/discarded due to the death of male mice	Total pregnant females/ number of females mated	Percent* fertility
Control (Ghandour)[#] 2 g/kg/day	30	15	5	15/25	60.0
Chewing gum - Spearmint, (green colored) 2 g/kg/day	30	15	3	20/27	74.1
Chewing gum - Cinnamon, (orange colored) 2 g/kg/day	30	15	4	21/26	81.0
Chewing gum - Strawberry, (yellow colored) 2 g/kg/day	30	15	3	22/27	81.5

@ The duration of treatment was 8 weeks.
# Chewing gum made in Saudi Arabia.
+ Each male was mated with 3 females.
* $P > 0.05$ (Chi-square test).

Table 5. Induction of embryonic loss in female mice treated with chewing gum and mated to untreated male mice

Treatment@ dose (g / kg / day)	Total Number of pregnant female mice	Total implants/ pregnant female ± S.E.	Live implants/ pregnant female ± S.E.	Dead implants+/ pregnant female ± S.E.
Control (Ghandour)# 2 g/kg/day	15	12.6 ± 0.45 (189)	11.9 ± 0.55 (179)	0.7 ± 0.19 (10)
Chewing gum - Spearmint, (green colored) 2 g/kg/day	20	11.0 ± 0.54* (220)	10.4 ± 0.54 (208)	0.6 ± 0.18 (12)
Chewing gum - Cinnamon, (orange colored) 2 g/kg/day	21	11.0 ± 0.47* (231)	10.4 ± 0.48* (218)	0.6 ± 0.24 (13)
Chewing gum - Strawberry, (yellow colored) 2 g/kg/day	22	10.1 ± 0.7** (222)	9.4 ± 0.7** (206)	0.7 ± 0.17 (16)

The total number of female mice mated to untreated males was 30 in each group
Figures in parenthesis denote the number of implantations in each group.
@ The duration of treatment was 8 weeks. # Chewing gum made in Saudi Arabia.
* $P < 0.05$ ** $P < 0.01$ + $P > 0.05$ (Students' t-test).

Table 6. Estrogenic activity of chewing gum in mice

Treatment[@]/dose (mg/kg/day)	Number of animals treated	Mean body weight ± S.E. (pre-treatment)	Number of animal dissected	Mean body weight ± S.E. (post-treatment)	Weight of the uterus/100 g body weight ± S.E.
Control (1) - (corn oil)	10	15.1 ± 0.23	9	17.2 ± 0.52	0.312 ± 0.02
Control (2) - (Estradiol 50 μg/kg/day)	10	15.6 ± 0.27	10	18.9 ± 0.31	0.581 ± 0.03
Extract of chewing gum - spearmint (green colored) 250 mg/kg/day	10	16.0 ± 0.21	7	18.6 ± 0.2	0.461 ± 0.07
Extract of chewing gum - cinnamon (orange colored) 250 mg/kg/day	10	15.2 ± 0.29	10	17.7 ± 0.4	0.427 ± 0.06
Extract of chewing gum - strawberry (yellow colored) 250 mg/kg/day	10	15.6 ± 0.34	10	18.4 ± 0.34	0.434 ± 0.03*

@ The duration of treatment was three days.

* $P < 0.05$ (Students' t-test)

ANOVA: $t_{.05}$ was used as the tabular value of 't' to calculate Least Significant Difference. Only Estradiol treatment is significantly greater than control.

Table 7. Estrogenic activity of chewing gum in mice

Treatment[@]/dose (mg/kg/day)	Number of animals treated	Mean body weight ± S.E. (pre-treatment)	Number of animal dissected	Mean body weight ± S.E. (post-treatment)	Weight of the uterus per 100 g body weight ± S.E.
Control (1) - (corn oil)	9	14.3 ± 0.26	7	16.9 ± 0.5	0.209 ± 0.02
Control (2) - (Estradiol 50 μg/kg/day)	10	14.9 ± 0.27	9	17.2 ± 0.5	0.503 ± 0.05
Control (3) - (Ghandour[#] 250 mg/kg/day)	10	14.0 ± 0.29	10	16.9 ± 0.4	0.235 ± 0.01
Chewing gum - strawberry (yellow colored) 250 mg/kg/day	9	14.0 ± 0.29	9	17.0 ± 0.5	0.278 ± 0.01*o

@ The duration of treatment was three days.

Chewing gum made in Saudi Arabia.

o Stistically compared to control 1 and control 2.

* $P < 0.05$ (Students' t-test)

ANOVA: $t_{.05}$ was used as the tabular value of 't' to calculate the Least Significant Difference. Estradiol and Strawberry treatments are significantly greater than control.

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(Received 20/12/1988;
in revised form 16/10/1989)

تأثير علك مستورد على الجهاز التناسلي للفئران

عبد الله بن محمد البكيري

قسم علم الأدوية ـ كلية الصيدلة ـ جامعة الملك سعود
ص. ب ٢٤٥٧ ـ الرياض ١١٤٥١ ـ المملكة العربية السعودية

لقد تم تقييم تأثير العلك المستورد على الجهاز التناسلي الذكري والانثوي لفئران التجارب البيضاء. فأعطى العلك لهذه الحيوانات مع مياه الشرب والطعام بجرعة (٢ جم/ كجم وزن) ولمدة ثمانية أسابيع. وفي نهاية المدة وبعد إنتهاء تعاطي العلك تم فحص تأثير العلك على عدد من المتغيرات منها عدد الحيوانات المنوية، تشوهات الحيوانات المنوية وقدرة اخصاب الذكور المعالجة بالإناث غير المعالجة، وعدد البويضات قبل الاخصاب، وعدد البويضات الكلي، وعدد البويضات المخصبة، هذا وقد قورن تأثير العلك المستورد بنظيره العلك «غندور» السعودي على جميع التغيرات السابقة الذكر.

ومن خلال هذه الدراسة تم التوصل إلى النتائج التالية:

١ ـ عدم تأثير العلك على المتغيرات الخاصة بالذكور والتي تشتمل على عدد الحيوانات المنوية وتشوهاتها، وكذلك قدرة اخصاب الذكور للإناث.

٢ ـ وجد أن العلك يقلل عدد البويضات عند الإناث وهذا يعزى لوجود مادة غريبة لها تأثير على معدل الاباضة عند الإناث. هذه المادة قد يكون لها تأثير هرموني على المبايض عند الإناث. لذا تم الكشف على التأثير الاستروجيني للعلك باستخلاص العلك بالكحول ومن ثم تعليق الخلاصة في زيت الذرة وأعطيت حقنا داخل الغشاء البريتوني لعذارى الفئران بجرعة يومية (٢٥٠ ملجم/ كجم وزن) ولمدة ثلاثة أيام متتالية، هذا وقد قورنت هذه مع نتائج خلاصة العلك «غندور» ووجد أن المادة الموجودة في العلك لها تأثير استروجيني واضح وهذا يفسر تأثيره على معدل الإباضة عند الإناث.