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IN VITRO AND IN VIVO EXPERIMENTAL STUDIES ON COMMIPHORA MYRRHA (MYRRH) AS AN INDIGENOUS FERTILITY REGULATING AGENT

(With One Table and 4 Figuers)

By

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دراسات تجريبيه على تأثير نبات المر الحجازى على الخصوبة

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أجريت هذه الدراسة على نبات المر الحجازى الذى يستعمل فى الطب الشعبى كوسيلة لمنع الحمل عن طريق استعماله موضعيا فى المهبل. أظهرت الدراسة التجريبية المعملية أن المر الحجازى له خاصية مبيدات الحيوانات المنويه. عند حقن محلول المر الحجازى فى مهبل الارانب قبل الجماع لم تحدث حالات حمل فى الأرانب التى عوملت بالمر الحجازى بينما حدثت حالة حمل واحدة فى المجموعة الضابطة. تم فحص الغشاء المخاطى المبطن لمهبل الارانب بالميكروسكوب الضوئى والميكروسكوب الالكترونى الماسح لمعرفة تأثير المر الحجازى على الغشاء المخاطى. وقد أظهر الفحص أن التأثيرات التى أحدثها المر يمكن ارجاعها الى تهيج مؤقت فى الغشاء الخاطى.

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COMMIPHORA MYRRHA, INDIGENOUS FERTILITY REGULATING AGENT

SUMMARY

This study was conducted to evaluate the efficacy and safety of a plant product (Myrrh) used for contraception in folk medicine. The in vitro study showed that Myrrh has a spermicidal action. When Myrrh injected into the vagina of rabbits, it prevented pregnancy, while one rabbit among the controls got pregnant. Examination of the vaginal mucosa of rabbits treated with Myrrh by light and scanning electron microscopy revealed that the effect of Myrrh is mainly irritant one and is reversible.

INTRODUCTION

A wide variety of herbal preparations are known in folk medicine to be of benefit as contraceptive agents. Myrrh is a gum resin obtained from the stem of commiphora molmol, family Burseraceae. In China, gum of Myrrh is used for regulation of menstruation *(IBRAGIMOVA and IBRAGIMOVA, 1964)*. Myrrh is also used as emenagoue both in Saudi Arabia *(PERENT, 1972)* and India *(KAMBOJ, 1988)*. In Egypt, dried gum is used as a contraceptive before or after coitus via the vaginal route *(EL-DEEN, 1972)*. This study aimed to evaluate the possible contraceptive effect of Myrrh both in vitro and in vivo.

MATERIAL and METHODS

In vitro study:

The dried gum in its crude form was crushed then dissolved in distilled water (5 gum [powder im 20 mL D.W.). The final solution appeared as a colloidal orange-yellow solution with a pH 4.5.

Five semen samples with normal parameters *(WHO, 1987)* were obtained from five males attending the Infertility Clinic of the Dept. of Dermatology and Veneriology, Assiut University Hospital.

One ml of the prepared solution of Myrrh was added to one ml of the semen samples and incubated at 37°C. Another one ml of the semen samples diluted in one ml of distilled water was incubated at 37°C and served as controls. Immediately and every 5 minutes, one drop of both the treated semen and control ones was examined to assess motility and viability of sperms. Supravital stain was used to differentiate immotile from dead sperms.

Invivo study:

Ten mature female rabbits aged one year were used. They were separated from male rabbits 10 days before the beginning of the experiment to exclude the onset of early pregnancy.

Five rabbits were subjected to vagnial douching with Myrrh solution using plastic catheter with the same concentration utilized in the in vitro study. In the other five rabbits (control cases), normal saline was injected in the vagina instead of Myrrh. A male rabbit of proved fertility was used to inseminate the treated and control cases through natural mating. The day of insemination is considered day one of the study. Rabits kept in separate cages for 10 days after insemination then clinically examined to detect pregnancy. Remating of cases injected with Myrrh was done and then kept separated for another 10 days.

For histopathological and scanning electron microscopic study, tissue specimens from the vagina of the treated as well as control rabbits were taken. The vaginal specimens were routinely prepared as usual and stained with hematoxylin and eosin. For scanning electron microscopy (Sem), the vaginal mucosa was prepared according to the technique described by *ANDERSON (1951).*

RESULTS

In vitro study:

As shown in Table 1, the results were expressed as percentage of sperms revealed different rates of motility.

Adding Myrrh to semen samples led to marked drop in sperm motility, with 95% immotile after 5 minutes, and 100% immotile in all samples after 15 minutes. Supravital staining showed that all immotile sperms were dead.

On the other hand, the control samples showed 85% immotile sperms after 5 minutes raised to 90% after 15 minutes.

The figures in the table were the mean of percentages of sperms in the semen samples examined.

In vivo study:

Among the five rabbits treated vaginally with Myrrh, no cases of pregnancy could be detected during the study, while one case of control animals got pregnant (20%).

When the rabbits treated with Myrrh remated in the 11^{th} day of the study, one case got pregnant.

Light and scanning electron microscopy:

Histologically, the vaginal mucosa of control animals was quite normal. It was formed of intact columnar epithelial cells

together with a few number of golbet cells dispersed between them. The underlying lamina propria was formed of connective tissue fibers and a pronounced vasculature (Fig. 1-A). On SEM, the mucosal surface of the surface of the control animals appeared free from any foreign material. The epithelial cells were regularly arranged, tightly adherent to each other and had numerous microvilli on its surface (Fig. 1-B).

One week post treatment, the epithelial cells showed prominent hydropic degeneration. The mucin-producing cells were highly activated as indicated by their sewlling and increasing numbers. The lamina propria revealed severe congestion of the blood vessels together with oedema and few leucocytic infiltrations (Fig. 2-A). On SEM, the epithelial cells appeared swollen, bulging above the surface and separated from each other. Some of them ruptured and attained a cup-shaped appearance (Fig. 2-B). Few numbers of macrophages attached to the mucosal surface were also present (Fig. 2-C). In addition, a sticky mucous covering was also observed on the epithelial surface (Fig. 3).

Two weeks post treatment, the vaginal mucosa was more or less regularly arranged. However, slight swelling as well as vacuolation of a few number of epithelial cells was also detected. Goblet cells were slightly activated. The lamina propria showed focal lymphoid cell aggregations, while the blood capillaries were insignificantly hyperaemic (Fig. 4-A). On SEM, the mucosal surface retained its regular arrangement in spite of the slight cells swelling and separation of the proximal portions of the epithelial cells. Microvilli were also present (Fig. 4-B).

DISCUSSION

The in vitro study revealed that Myrrh has a spermicidal action, while the in vivo one indicated its contraceptive effect on rabbits. When used vaginally. On clinical and histopathological absis, it was also found that both the contraceptive effect of Myrrh and the changes induced in the vaginal mucosa are reversible.

Similarly, *ANON (1976)* reported that Myrrh has an irritant activity. This could explaine the changes observed in the vaginal mucosa of rabbits which disappeared after two weeks. On the contrary, Ahn (1981) observed that Commiphora Myrrha had an anti-inflammatory and analgesic activity.

In our opinion, the contraceptive effect of Myrrh could be ascribed mainly to its spermicidal action as well as the vaginal mucosal changes. On the other hand, this contraceptive action could not be ascribed due to the effect of distilled

water because on comparing the effect of both Myrrh and distilled water separately in the in vitro study, Myrrh showed a powerful spermicidal action over distilled water. Moreover, in the in vivo study, the distilled water could not be retained in the vagina as the viscous solution of Myrrh.

According to Chaudury (1985) who reported that translation of folklore into scientific application is valuable, and because of prevalence of illiteracy and strong traditions among women in our communities, expanson of the use of such indigenous methods could be achieved with subsequent reduction of the high fertility rates.

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LEGENDS

Fig. 1: Normal appearance of vaginal mucosa of a rabbit (control case):
1-A: Light microscopy (L.M.), H&E stin, x 250.
1-B: Scanning electron microscopy (SEM), x 2000.

Fig. 2: Vaginal mucosa of a rabbit, one week post-treatment with Myrrh:
2-A: L.M. showing degeneration of mucosal cells together with condestion and oedema in lamina propria, H&E stain, x 250.
2-B: SEM showing ruptured mucosal cells with a cup-shaped appearance, x 1500.
2-C: SEM showing few macrophages attached to the mucosal surface, x 1500.

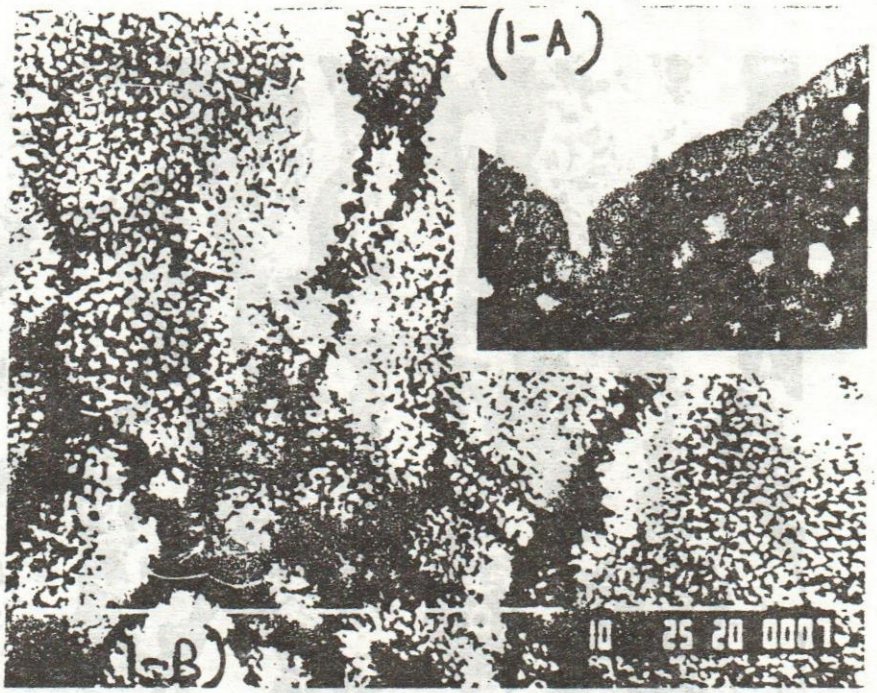
Fig. 3: Vaginal mucosa of a rabbit, one week post-treatment with Myrrh. SEM showing excessive mucous secretion, x 3000.

'Fig. 4: Vaginal mucosea of a rabbit, two weeks post-treatment with Myrrh:
4-A: L.M. showing slight swelling of mucosal cells, slight activation of goblet cells and focal lymphoid cell aggregations in the lamina propria, H&E stain, x 250.
4-B: SEM showing more or less regularly arranged mucosal cells with microvilli, x 3000.

Table 1: Effect of distilled water and Myrrh on sperm motility %.

	Distilled water				Myrrh			
	Excellent	Good	Poor	Imm.	Excellent	Good	Poor	Imm.
Immediate	-	10	10	80	-	-	10	90
5 min.	-	10	5	85	-	-	5	95
10 min.	-	5	10	85	-	-	5	95
15 min.	-	-	10	90	-	-	-	100

n= 5. Imm.= immotile spermatozoa.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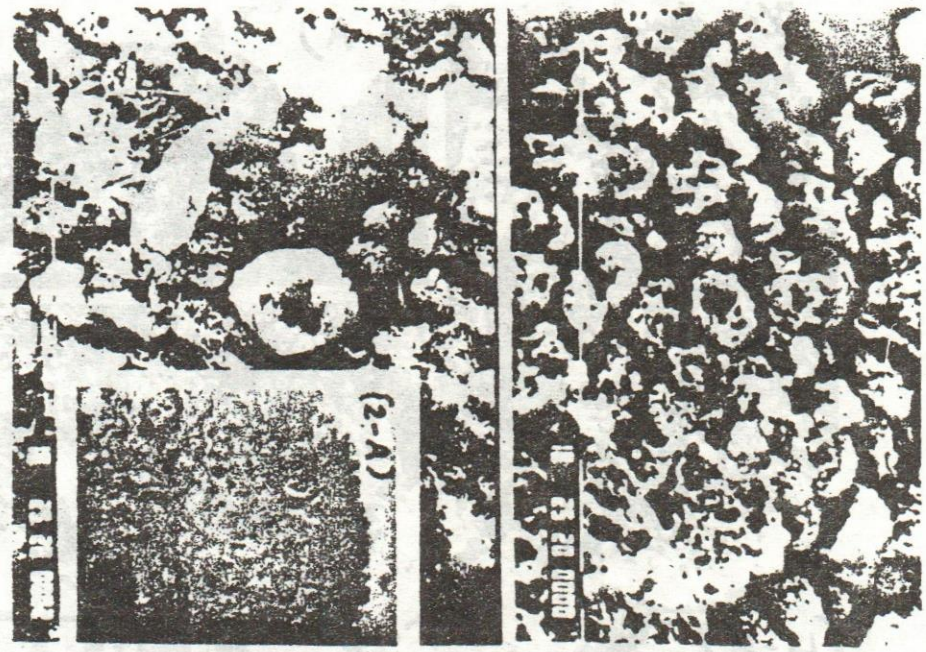
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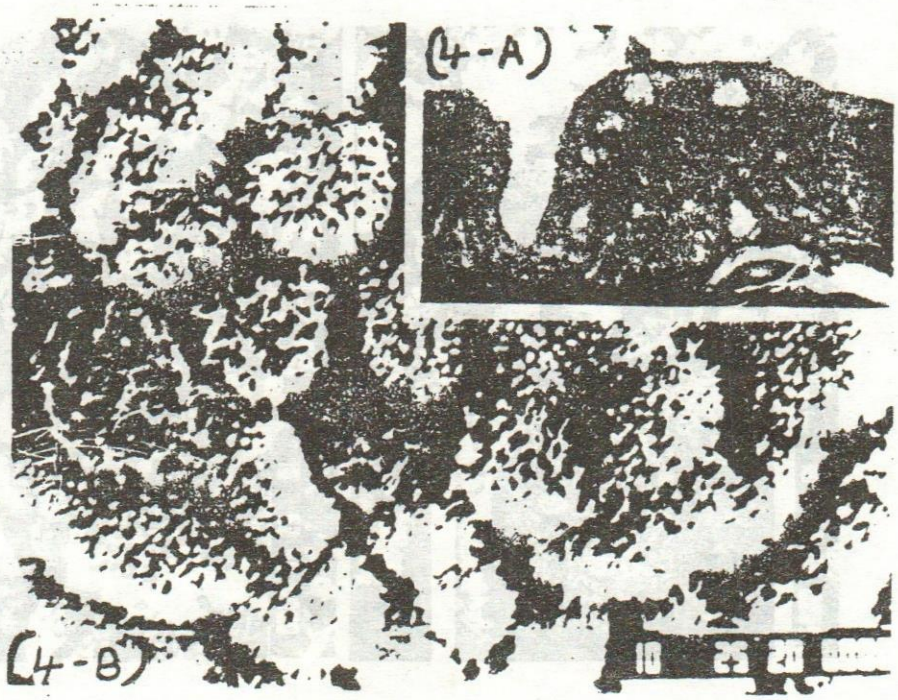
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