ORIGINAL RESEARCH ARTICLE

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Purified Neem (*Azadirachta indica*) Seed Extracts (Praneem) Abrogate Pregnancy in Primates

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The use of neem (Azadirachta indica) seed extracts (Praneem) given orally for abrogation of pregnancy in subhuman primates is described. Oral administration of Praneem was initiated after confirmation of pregnancy using Leydig cell bioassay estimating rising levels of chorionic gonadotropin (CG) in the blood from day 25 onwards of the cycle and continued for six days. Termination of pregnancy was observed with the appearance of blood in the vaginal smears and decline in CG and progesterone. Pregnancy continued in the control animals treated with peanut oil at the same dose. The effect was observed in both baboons and bonnet monkeys. The treatment was well tolerated; blood chemistry and liver function tests had normal values. The animals regained their normal cyclicity in the cycles subsequent to Praneem treatment. CONTRACEPTION 1996;53:375–378

KEY WORDS: *Azadirachta indica,* fertility studies, primates, pregnancy termination

Introduction

Over fifty million abortions are carried out each year around the globe. There is continuing need to develop additional methods, administrable preferably by oral route, to enable the termination of an unwanted pregnancy. A major step in this direction occurred with the introduction of RU486, a progesterone receptor blocking steroid which in combination with prostaglandins could bring about abortions in 96% of cases.[1] Plant products have been employed for pregnancy interruption in traditional medicine in many countries. However, objective scientific studies on their efficacy and safety are lacking. We reported recently the abrogation of pregnancy in rats following oral administration of neem (*Azadirachta indica*) seed extracts;[2] the treatment was effective in 100% of animals tested. The treatment was well tolerated and the animals regained fertility in subsequent cycles. The chemical composition of these extracts has been mostly delineated and these contain a number of chemically defined terpenoids and limonoids, besides fats and fatty acids.[3] Although common mechanisms exist in reproduction of rodents and primates, there are also distinct differences. As, for example, the pituitary hormones continue to act as gonadotropins in rodents and no gonadotropin of chorionic origin is made in the species; whereas the pituitary is silent in primates and gonadotropic stimulus is provided by chorionic gonadotropin of trophoblastic origin. In order to gauge the potential application of the neem extracts as abortifacients, it was considered appropriate to test their action in subhuman primates. This article describes the results obtained in baboons (*Papio anubis*) and bonnet monkeys (*Macaca radiata*).

Material and Methods

Praneem

Purified neem seed extracts (Praneem) were prepared from taxonomically characterized neem seeds. The oil was extracted in a table-top electrically driven machine (Komet oil expeller, IBG Monforts, Germany), followed by a two-step refining procedure by which the suspended materials were removed. The oil was kept at room temperature (25°C) overnight, the supernatant was decanted and centrifuged at 1500 g for one hour, followed by ultracentrifugation at 65,300 g for another 1 hr. The pellet was discarded and the supernatant frozen at 4°C for further study. The preparation was screened to be free of aflatoxins B_1, B_2, G_1 and G_2 by TLC analysis on precoated Silica gel G plates using chloroform:isoamyl:alcohol:methanol (90:32:3) as solvent system. Pure aflatoxins from a kit (Sigma Chemical Company) were used as reference standards. The specifications of a batch (PV006) of

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Praneem were: specific gravity 0.905 g/ml; pH 5.7; saponificiation value 206.7; acid value <24; iodine value 24. The free fatty acid composition of the preparation as determined by gas chromatographic analysis was as follows: palmitic acid (19.6%); stearic acid (17.2%); oleic acid (41.2%); linoleic acid (0.82%); and other undetected minor acids (1.65%). The bitter principles include proto-meliacins, meliacins, limonoids, pentanortriterpenoids and norterpenoidal constituents as reported elsewhere.[4]

Animals

The study was conducted in two primate species, the adult baboons (*Papio anubis*) and bonnet monkeys (*Macaca radiata*). They were maintained in semi-natural conditions in the Primate Research Facility of the National Institute of Immunology. Animals of reproductive age only were employed for the study.

Fertility Studies

Female baboons were mated repeatedly with males of proven fertility during the estrous phase indicated by perineal sex swelling. For female bonnets, matings were set from day 8 to day 13 of the cycle.

Sample Collection for Estimation of Chorionic Gonadotropin (CG) and Progesterone Measurement

Blood samples were collected on alternate days from day 25 of the cycle for measuring CG by bioassay and twice weekly for measuring progesterone by RIA. Each time, 2 ml of blood was drawn via the femoral vein; the serum was collected and stored at -20°C.

Bioassay for Estimating Chorionic Gonadotropin CG

Chorionic gonadotropin was estimated in the serum samples by Leydig cell bioassay as per method described by Van Damme et al.[5] The method's basis is that short-term cultures of Leydig cells produce assayable quantities of testosterone in the presence of CG and the effect of the hormone is dose-dependent.

Radioimmunoassay for Progesterone

Progesterone levels in the sera samples were determined according to the method described by Brenner et al.[6] employing WHO matched reagents.

Treatment Regime

Neem seed extracts (Praneem) were given orally using a catheter tube after confirming pregnancy by measuring high levels of CG in blood. Animals were anesthetized with Ketamine (0.4 ml Ketamin hydrochloride, Themis Pharmaceutical Ltd, India) before treatment to avoid struggle and stress. Parallel controls received peanut oil.

Results

Pregnancy Termination

The study was conducted in five baboons (*Papio anubis*) and three bonnet monkeys (*Macaca radiata*). The pregnancy was monitored by appearance of CG bioactivity in the serum which normally increases over an eight-to-ten-day period before declining. The occurrence of conception was further confirmed by serum progesterone levels which in pregnant animals did not decline. After establishing that the animals were pregnant by these two criteria, they were administered purified neem seed extracts daily for six days by oral feeding tube. Three out of four baboons given the treatment experienced abrogation of pregnancy (Table 1). This was indicated not only by bleeding but also by both progesterone and CG levels falling to near zero levels (Figure 1). The baboon and monkey given an equal amount of peanut oil instead of Praneem continued to maintain pregnancy with sustained progesterone serum levels. Baboon 68, in which no termination of pregnancy took place following administration of purified neem seed extracts, had vomited the oil on the second day of the treatment. It is possible that this baboon did not receive an adequate dose of Praneem.

Figure 1 gives the data and kinetic changes in CG and progesterone profiles of the treated and the control baboons. The treatment was totally effective in the bonnet monkeys tested. The treatment was well

Table 1. Abortifacient action of Praneem given orally in primates

Animal #	Treatment and Dose	Days of Treatment Since LMP	Day of Onset of Bleeding and Its Duration (Days)
Pan 67	Praneem	37–42	48–50 (3 days)
Pan 52	6 ml for	35–40	42–45 (4 days)
Pan 32	six days	37–42	44–46 (3 days)
Pan 68*		39–44	Pregnancy continued
Pan 63	Peanut oil 6 ml for 6 days	38–43	Pregnancy continued
MRA 526	Praneem	43–48	52–54 (3 days)
MRA 672	3 ml for 6 days	42–47	52–54 (3 days)
MRA 638	Peanut oil 3 ml for 6 days	36–41	Pregnancy continued

*Vomiting observed on day 2 of treatment.

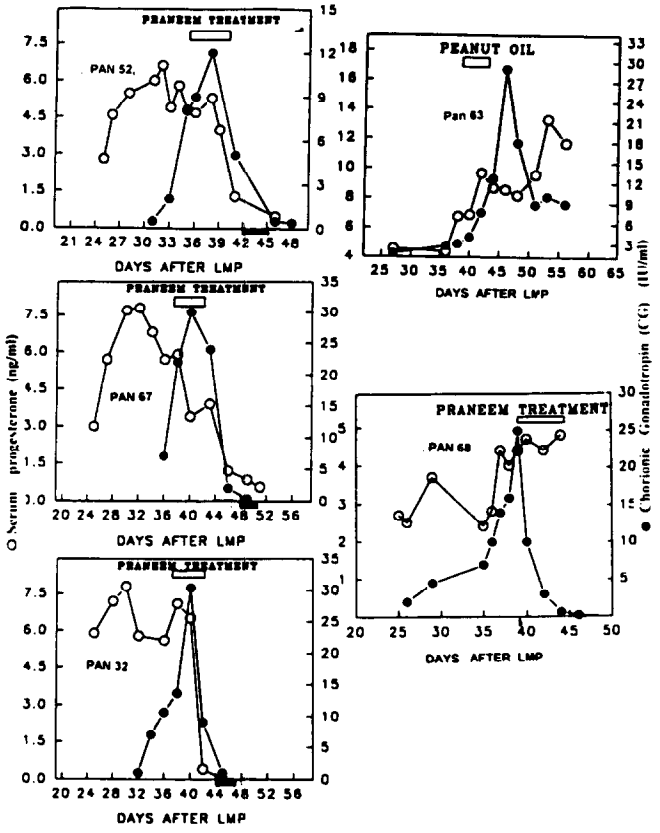

Figure 1. Effect of administration of neem seed extracts (Praneem) given orally in pregnant baboons. Treatment given after confirming CG in the blood as illustrated by (□). Termination of pregnancy observed by decline in CG (●--●) and progesterone; (○--○); (■) represents vaginal bleeding. Pan 63 shows the control baboon treated with peanut oil; Pan 68, the baboon where abrogation of pregnancy was not observed after Praneem treatment.

tolerated except for one baboon where vomiting took place after the administration of Praneem on day 2 of the treatment. No other behavioral change or alteration in food intake was noted. Blood chemistry and liver function test parameters before and after treatment were not altered (Table 2).

Reversibility

The reversibility of the effect of purified neem seed extract (Praneem) was manifested by the observation that baboons whose pregnancy was terminated by this treatment developed perineal sex swelling (due to estrogens) in the subsequent cycles. Normal cyclicity was regained after one irregular cycle. The animals mated with males of proven fertility. On becoming pregnant, the pregnancy proceeded to term. Pups born to these mothers (previously treated with Praneem) were normal. Figure 2 gives the case history of two such baboons. The treatment had no apparent residual effects on reproductive functions.

Effect of Praneem Oral Treatment on Progesterone

Data in Figure 1 show that soon after institution of the treatment with Praneem, progesterone decline commences. On the other hand, no consistent kinetic correlation is seen with CG levels. One could, thus, hypothesize that the treatment may be causing the lysis of the corpus luteum, the ovaries being the source of progesterone in these species at this stage of pregnancy. To test this hypothesis, normally cycling baboons were given oral treatment with Praneem for six days at the same dose from day 18 to 23 of the cycle. However, no shortening of the menstrual cycles was noted. Thus, Praneem does not appear to impair the corpus luteum function of the nonpregnant female baboon. The mechanism by which abrogation is caused may be similar to those identified during the previous study[2] in rodents.

Discussion

This study demonstrates that Praneem administered orally for six days after confirming pregnancy by the rising levels of CG in the blood, brought about termination of pregnancy; peanut oil given by the same route at the same dose did not show this effect. The effect was reversible and fertility was regained in the cycles subsequent to Praneem treatment. The treat-

Table 2. Hematological and clinical chemistry parameters as studied in baboons treated with Praneem

Parameter	Praneem (n = 3) Before Treatment (Mean ± SEM)	Praneem (n = 3) After Treatment (Mean ± SEM)
Hb (g %)	11.7 ± 0.26	11.6 ± 0.1
TLC/mm^3 (thousands)	7.3 ± 0.44	7.0 ± 0.26
DLC (%)		
Neutrophils	55.3 ± 1.76	56.0 ± 2.3
Lymphocytes	42.3 ± 2.02	41.3 ± 2.9
Monocytes	1.0 ± 0.01	1.3 ± 0.33
Eosinophils	1.3 ± 0.33	1.3 ± 0.33
Bilirubin (mg%)	0.43 ± 0.03	0.46 ± 0.06
SGPT (IU/lit)	22.0 ± 1.15	22.6 ± 1.3
SGOT (IU/lit)	25.3 ± 1.3	26.0 ± 1.15
Urea (mg%)	27.3 ± 0.33	29.0 ± 0.01
Creatinine (mg%)	1.06 ± 0.03	1.1 ± 0.04
Glucose (mg%)	64.3 ± 2.3	63.0 ± 2.51
Total	7.1 ± 0.2	7.23 ± 0.14
Albumin	4.0 ± 0.17	4.16 ± 0.08
Globulin	3.1 ± 0.033	3.13 ± 0.06

n = number of baboons; TLC, total leucocyte count; DLC, differential leucocyte count; SGPT, serum glutamate oxaltate transaminase; SGPT, serum glutamate pyruvate transaminase.

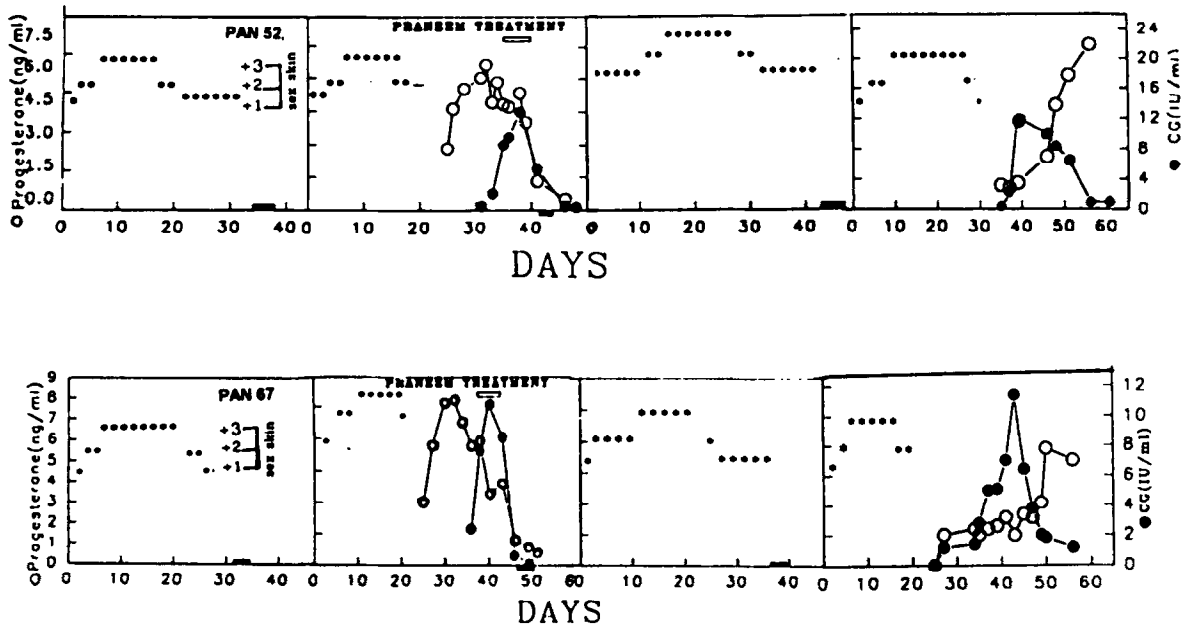

Figure 2. Perineal sex swelling (***) pattern of baboons Pan 52 and Pan 67 before and after treatment with Praneem in pregnant cycle. Both animals regained fertility in a subsequent cycle.

ment was well tolerated with no residual effect compromising the future fertility of the animals.

Neem (*Azadirachta indica*) extracts have strong immunomodulatory properties.[7] Evidence has been gathered to show that immunological mechanisms play a role in maintenance of pregnancy.[8] Cytokines secreted by T-helper 1 cells, i.e., gamma interferon and TNF alpha, have detrimental effects on fetal survival, whereas cytokines IL-3 and GM-CSF help in gestation.[9] Our previous work has shown that oral administration of purified neem seed extracts (Praneem) caused an alleviation of both the immunoreactive and bioactive TNF alpha and gamma interferon in the sera. The draining mesenteric lymph node cells synthesized and secreted these cytokines and Th 1 type of cytokines were also present in the feto-placental cultures. These transitory changes were presumably the basis of termination of pregnancy in rodents. Similar mechanisms may be responsible for termination of pregnancy in primates following ingestion of the neem seed extracts.

Acknowledgments

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