

ANTI-FERTILITY ACTIVITY AND HORMONAL PROFILE OF TRANS-ANETHOLE IN RATS

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Abstract: Trans-anethole was studied for antifertility activity in rats at dose levels of 50 mg, 70 mg and 80 mg/kg po. Dose-dependent activity was observed, a 100% anti-implantation activity being achieved at 80 mg/kg, po. The compound showed a significant estrogenic activity and did not possess anti-estrogenic, progestational, anti-progestational, androgenic or anti-androgenic activities. In an earlier study, the compound was found to be safe, its LD₅₀ being more than 3000 mg/kg, po in mice.

Key words : trans-anethole anti-implantation estrogenic

INTRODUCTION

Star anise oil is derived from the fruits of *Illicium anisatum* (Family : Magnoliaceae). The oil has been fully investigated for its chemical composition and has been found to contain trans-anethole as the major constituent (1). In an earlier study, it has shown to be capable of producing a 100% anti-fertility activity in rats at a dose of 100 mg/kg, po, when the treatment was given from day 1 to day 10 of pregnancy (2). This duration covered fertilization, tubal transport, blastocyst formation, implantation and early abortifacient stages. The present study was undertaken to assess the minimum effective dose, the processes that are blocked by trans-anethole and also to investigate its hormonal profile in rats.

METHODS

Adult albino rats of Charles Foster strain (weight 150-160 g each), inbred in the Animal House of this laboratory, were kept on a 14:10 hrs light : dark schedule in an air-conditioned room (25°C; relative humidity 50-60%). The animals were fed a standard

pellet diet (Lipton India Ltd.) with free access to drinking water. The female rats were caged with male rats of known fertility on the evening of proestrus. The presence of copious spermatozoa in the vaginal smear taken the following morning was considered as day 1 of pregnancy. The test material was suspended in aqueous solution with the help of 0.5% carboxymethyl cellulose (CMC) and was administered to pregnant rats orally from day 1 to day 10 of pregnancy in first set of experiments and on specified days in the second set of experiments.

Laparotomy was performed on day 11 under light ether anaesthesia and the number of implantation sites in the uterine horns was recorded. Animal with, at least, one normal foetus was considered as pregnant. The abdomen was sutured and following this, the rats were returned to their respective cages. The treatment was withdrawn after 10th day and the animals were allowed to go to term. After parturition, the number of litters were counted. The delivered pups were observed for at least one month for any gross malformation. A group of pregnant rats, which received the vehicle only served as control.

In the second set of experiment, three groups of 5 pregnant rats each were included. Group I was treated on days 1-2, group II on days 3-5 and group III on days 6-10.

For testing estrogenicity/anti-estrogenicity of trans anethole, immature female rats (weighing 40-50 g each) were divided into four groups of 5 each. The groups received the treatment as follows :

- Group I : Oestradiol valerate 0.1 µg/rat/day sc in ground-nut oil.
 Group II : 0.05 ml of oil sc.
 Group III : Trans-anethole, 80 mg/kg, po.
 Group IV : Oestradiol valerate 0.1 µg/rat/day sc in oil and trans-anethole 80 mg/kg, po.

The treatment was given for 3 days; 24 hours after the last treatment, the rats were sacrificed. The uteri were quickly excised, cleared of the adhering tissue and weighed.

Progestational and anti-progestational activities were assessed by the pregnancy maintenance test in pregnant rats ovariectomized on day 12 of pregnancy (3). After the ovariectomy, the rats which bore normal implantations were regrouped, 6 rats in each group, and treated in the following manner :

- Group I : Control : 0.05 ml ground-nut oil/rat/day sc.
 Group II : Progesterone 2 mg/rat/day sc in ground-nut oil.
 Group III : Trans-anethole 80 mg/kg, po.
 Group IV : Progesterone 2 mg/rat/day sc in ground-nut oil and t-anethole 80 mg/kg, po.

The treatment was given from day 12 to day 19 of pregnancy. Autopsy of the rats was performed on day 20 and number of live foetuses was recorded. The result was expressed as percent of foetal survival.

$$\text{Foetal survival} = \frac{\text{No. of live foetuses}}{\text{Total no. of implantation sites}} \times 100$$

For evaluation of androgenic and anti-androgenic properties of t-anethole, immature male rats, weighing 40-50 g each were divided into four groups of 6 each (4). Treatment of the different groups of rats was carried out as follows :

- Group I : Control : 0.05 ml of ground-nut oil sc.
 Group II : Testosterone propionate 150 µg/rat/day sc in ground-nut oil.
 Group III : Trans-anethole 80 mg/kg, po.
 Group IV : Testosterone propionate 150 µg/rat/day sc in ground-nut oil and trans-anethole 80 mg/kg, po.

The treatment was given for 7 days. The rats were sacrificed 24 hrs after the last treatment. Ventral prostate and seminal vesicles were carefully removed from the animals and quickly weighed.

RESULTS

The result of anti-fertility activity of t-anethole is depicted in Fig. 1a. At the lowest dose (50 mg/kg,

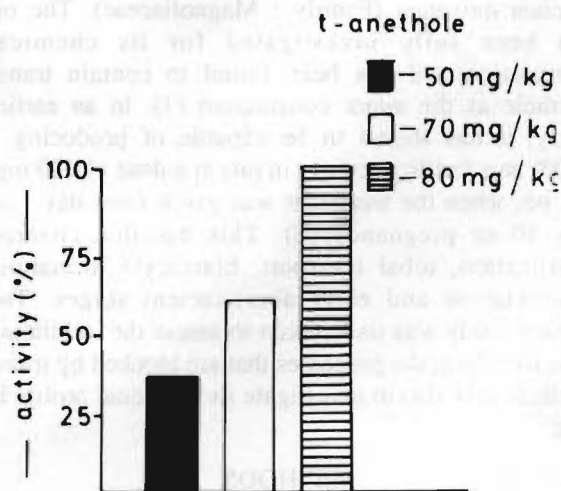


Fig. 1a : Anti - fertility activity

Fig. 1a : Anti-fertility activity of t-anethole at doses 50 mg, 70 mg and 80 mg/kg, po in rats.

po), the compound produced inhibition of pregnancy in only two out of six animals (33.3%). In animals receiving 70 mg/kg, po dose, two out of six delivered pups at the completion of term (66.6%). At 80 mg/kg, po, a 100% anti-fertility activity was produced as no implantation sites were observed at the time of laparotomy on the 11th day of pregnancy. All the control animals delivered normal pups at the term.

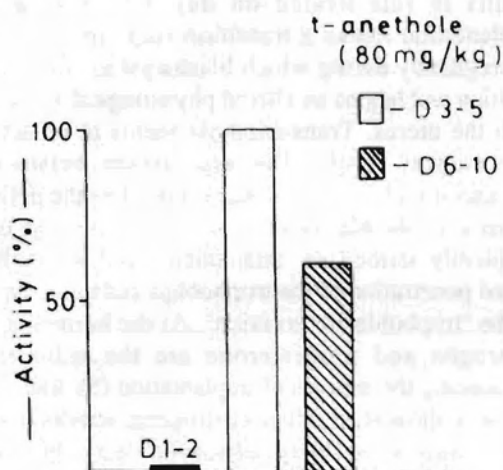


Fig. 1b: Anti-fertility activity

Fig. 1b: Effect of t-anethole (80 mg/kg, po) on different days of pregnancy in rats. Treatment on days 1-2, 3-5 and 6-10.

In the second set of experiment (Fig. 1b), the Group I rats, receiving 80 mg/kg, po on day 1-2 of pregnancy, bore normal implantation sites and delivered the pups at term. In the group II rats, receiving 80 mg/kg, po from day 3-5 of pregnancy, there was successful prevention of pregnancy in all the five rats. Three of the 5 rats of group III, which received treatment from day 6-10, failed to deliver at term.

No gross malformation was observed with pups delivered in any of the groups.

Fig. 2 summarizes the result of estrogenic and anti-estrogenic activities of t-anethole. Estradiol valerate, at a dose of 0.1 µg/rat/day sc produced significant increase in the uterine weight, compared to control (P<0.001). Group III rats, which received an oral dose of t-anethole (80 mg/kg), there was a

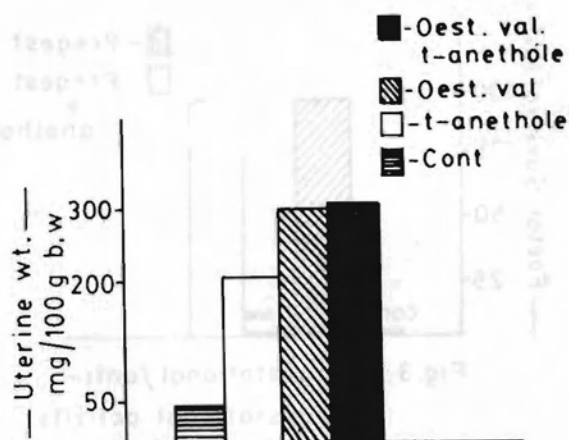


Fig. 2: Estrogenic/anti-estrogenic activity

Fig. 2: Effect on uterine weight of immature rats. t-anethole (80 mg/kg, po), Oestradiol valerate (Standard; 0.1 µg/rat in ground-nut oil sc) and their combination.

significant increase of the uterine weight. Group V rats received both the standard (0.1 µg/rat/day sc in ground-nut oil) and t-anethole (80 mg/kg, po). The uterine weight was not significantly different from that of group II rats.

Trans-anethole was evaluated for progestational and anti-progestational activities in the rats ovariectomized on day 12 of pregnancy and the results of the study are depicted in Fig. 3. Control rats (Group I), which received only the ground-nut oil (0.05 ml, sc), had complete abortion as no pups were delivered at term by any of the rats. Progesterone, 2 mg/rat/day, sc was able to maintain pregnancy and the number of live pups at term were comparable to the number of implantation sites observed on day 12 of pregnancy. T-anethole, 80 mg/kg, po, failed to sustain the gestation on its own; the rats aborted and no live pups were delivered at the completion of the term. When progesterone (2 mg/rat/day, sc) and t-anethole (80 mg/kg, po) were given together to Group IV animals, all the rats again delivered normal pups comparable to the number of implantation sites recorded on day 12 of pregnancy.

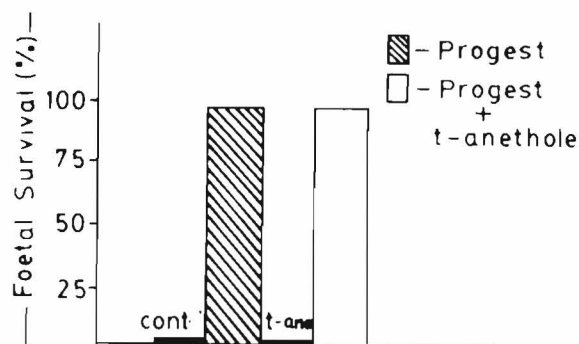


Fig. 3: Progestational /anti-progestational activity

Fig. 3: Progestational/antiprogestational activity of t-anethole (80 mg/kg, po) in pregnant OVX rats. Standard-progesterone (2 mg/rat in ground-nut oil).

Testosterone propionate (150 µg/rat/day, sc) produced a significant increase in weights of both ventral prostate and seminal vesicles ($P < 0.001$) as seen from the Table I. Trans-anethole (80 mg/kg, po) when given along with testosterone propionate failed to produce any significant alteration in the weights of ventral prostate and seminal vesicles compared to control (Group IV).

DISCUSSION

Trans-anethole has shown as dose-dependent antifertility activity. Doses of 50 mg, 70 mg and 80 mg/kg, po produced 33.3%, 66.6% and 100% activity, respectively. It could be acting by blocking one or

more of any of the stages of fertilization, tubal transport, blastocyst formation, implantation or by producing early abortifacient activity. Failure to check pregnancy in rats treated on day 1-2 indicates that trans-anethole does not have any anti-zygotic activity nor does it interfere in the tubal transport of the zygote. The results suggest that trans-anethole has potent anti-blastocystic and/or anti-implantation activity, as indicated by the results in rats treated on day 3-5 of pregnancy. Implantation marks a transition stage in the progress of pregnancy during which blastocyst assumes a fixed position and begins an altered physiological relationship with the uterus. Trans-anethole seems to be acting at two stages; firstly, the association between the blastocyst and the uterus that establishes the definitive position of the blastocyst in relationship to the uterus, frequently termed as 'attachment' and secondly, the actual penetration of the trophoblast cells into the nidus or the 'trophoblastic invasion'. At the hormonal level, oestrogen and progesterone are the main factors influencing the process of implantation (5). Since trans-anethole showed significant estrogenic activity, the anti-implantation activity observed may be due to disturbance in the 'balance' of the optimal ratio of the two hormones. The results indicate that trans-anethole, in the dose employed, has no progestational or anti-progestational activities. The compound does not interfere with the progesterone component of the 'balance'. The anti-implantation activity is due to the relatively higher preponderance of the oestrogen factor.

Trans-anethol has also shown significant early abortifacient activity in the rats treated on day 6-10 of

TABLE I: Effect of testosterone, T-anethole and their combination on the weights of seminal vesicles and ventral prostate in immature male rats (n=6 in each group).

Treatment	Dose	Weight mg/100 g body weight	
		Seminal vesicle	Ventral prostate
Control	Oil only	26.5±0.3	40.3±0.4
Testosterone	150 µg/rat, sc	216.5±1.1	132.7±0.7
T-anethole	80 mg/kg, po	28.1±0.2	39.7±0.3
Testosterone +	150 µg/rat, sc	204.7±1.0	130.8±1.0
T-anethole	80 mg/kg, po		

pregnancy. The explantation could be that though the implantation has taken place, the increased demands of progesterone essential for the sustenance of the gestation (6) were not met, with the result that the pregnancy does not carry to fruition.

It is concluded that trans-anethole, the major

constituent of star anise oil, produces a 100% anti-implantation effect in rats at a dose level of 80 mg/kg, po. It has significant estrogenic activity and is devoid of anti-estrogenic, progestational, anti-progestational, androgenic and anti-androgenic activities.

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