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SHORT COMMUNICATION

Antispermatogenic Effect of Chromatographic Fractions Isolated from Petroleum Ether Extract of *Mentha arvensis* Leaf in the Albino Mouse

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The antifertility activity of column chromatographically isolated fractions (petroleum ether:benzene 1:1 v/v and chloroform:methanol 1:1 v/v) of a petroleum ether extract of *Mentha arvensis* leaf were evaluated in the male albino mouse. Administration of the fractions for 20 days at a dose of 1.0 mg/mouse/alternate day reduced the wet weights of genital organs and induced infertility in the male mouse without loss of libido as evaluated by sterile matings with normal proestrus females. Absence of spermatozoa in the cauda epididymes as a consequence of spermatogenic arrest is considered contributory to the antifertility effect. © 1997 by John Wiley & Sons, Ltd.

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INTRODUCTION

In Indian folkloric medicine numerous plant products are used in the regulation of human fertility (Kamboj and Dhawan, 1989). Amongst these, the leaves of *Mentha arvensis* L. (Lamiaceae), the common edible aromatic herb known as field mint, has been described to possess various medicinal properties including an antifertility effect (Satyavati *et al.*, 1987). Hence, the antifertility activity of mint leaf has been evaluated in the female of various laboratory mammals: rabbits (Kapoor *et al.*, 1974), rats (Bodhankar *et al.*, 1971; Kanjanapothi *et al.*, 1981), mice (Garg *et al.*, 1995). However, hitherto there are no reports on the antifertility effect, if any, on the mammalian male. Thus a study of the effect of mint leaf extracts on male reproduction was initiated (Sharma *et al.*, 1995; Sharma and Jacob, 1996) and since in this study promising results were obtained it was considered worthwhile to make a relatively more detailed examination. The present study describes the results obtained with petroleum ether: benzene (1:1 v/v) and chloroform : methanol (1:1 v/v) fractions isolated from the petroleum ether extract of mint leaves on the fertility of the male mouse.

MATERIALS AND METHODS

Fractionation. Air-dried and powdered leaves of *Mentha arvensis* (mint) were Soxhlet extracted with boiling petroleum ether (BP 60°–80°C). The total extract was concentrated *in vacuo* and the residue so obtained chromatographed over deactivated silica gel and eluted with petroleum ether, petroleum ether/benzene (1:1), benzene, benzene/chloroform (1:1), chloroform and chloroform/methanol (1:1) mixtures. This paper describes the evaluation of the petroleum ether/benzene (1:1) fraction, a tawny coloured solid, and chloroform/methanol (1:1) fraction, a dark coloured solid.

Dosage. Adult male Swiss albino mice (26–32 g) of proven fertility were randomly divided into three groups of 10 each. Group 1 animals served as control and received only olive oil vehicle. Group 2 mice received the petroleum ether/benzene (1:1) fraction at a dose 5.0 or 1.0 mg/mouse/alternate day for 20 days, while group 3 males received chloroform/methanol (1:1) fraction at a dose 2.0 or 1.0 mg/mouse/alternate day for 20 days.

Antifertility activity. Animals were minutely observed for any behavioural changes during the treatment period. Before subjecting them to the fertility test the treated mice were cohabited with the cycling females at a frequency of every 4/5 days to exhaust the residual viable spermatozoa in the epididymes. At the termination of the experiment, males from each group were paired individually with two parous proestrus females.

Successful matings in each case were confirmed by the presence of a copulation plug. Mated females were autopsied on day 15 *postcoitum* and the number of implantation sites, if any, were recorded. On day 21, that is 24 h after termination of treatment, males were killed and the reproductive organs, namely, testes, epididymes, seminal vesicle and prostate were excised, cleared of adherent tissue and weighed to the nearest mg.

Sperm counts. Cauda epididymal sperm counts of control and 20 day treated mice were made by using haemocytometric Neubaur chambers (Prasad *et al.*, 1972). The number of spermatozoa was expressed as millions/mL of suspension.

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Table 1. Effect of chromatographic fractions of the petroleum ether extract of *Mentha arvensis* leaf (dose/mouse/alternate day for 20 days) on the fertility of the male mouse and cauda epididymal sperm count

Group	Number of males/ females mated	Number of implantation sites (mean±SEM)	Sperm count (millions/mL±SEM)
Vehicle	5/10	9.98±0.08	11.69±1.46
Petroleum ether/benzene (1:1 v/v)			
1.0 mg	5/10	0.0	0.0
5.0 mg	5/10	0.0	0.0
Chloroform/methanol (1:1 v/v)			
1.0 mg	5/10	0.0	0.0
2.0 mg	5/10	0.0	0.0

Table 2. Effect of chromatographic fraction of the petroleum ether extract of *Mentha arvensis* leaf (dose/mouse/ alternate day for 20 days) on the reproductive organs of the male albino mouse (mg/g body wt±SEM)

		Epididymis			
Group	Testis	Caput	Cauda	Seminal vehicle	Prostate
Vehicle	696.44±7.73	130.37±6.04	87.02±2.06	519.75±28.21	199.35±28.07
Petroleum ether/benzene (1:1 v/v)					
1.0 mg	568.89±18.73[b]	115.29±2.06[a]	80.52±3.01[b]	488.38±22.39[n]	188.12±21.62[n]
5.0 mg	541.28±16.83[c]	110.89±3.21[b]	74.09±3.73[c]	482.81±28.13[n]	187.28±22.81[n]
Chloroform/methanol (1:1 v/v)					
1.0 mg	560.94±20.05[b]	117.89±0.71[a]	76.51±3.34[c]	494.00±23.76[n]	186.98±20.78[n]
5.0 mg	545.44±19.87[c]	115.91±0.69[b]	74.01±2.16[c]	495.00±20.17[n]	181.25±21.61[n]

Significance in relation to control [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$, [n] Nonsignificant

Tissue histology. Histological examination of the testis and cauda epididymis was done. Five μm paraffin sections of the organs were stained with haematoxylin and eosin.

RESULTS

An apparent toxicity was not observed in the male mouse after treatment with the test substances. The mean body weight of the treated groups remained comparable to that of the control. With both the fractions of mint leaf extract successful matings were achieved when treated males were allowed to cohabit with proestrus females of proven fertility, however, no implants were observed in the uteri of such females at doses tested (Table 1). Sperm counts of the cauda epididymes of the treated mice dropped to nil (Table 1). Weights of the seminal vesicle and prostate did not change significantly but the testicular and epididymal weights decreased significantly in comparison with the control (Table 2).

Histological examination of the testis revealed that the two chromatographic fractions of mint leaf extract did not cause any abnormality to the gonial cells but such treatment produced complete spermatogenetic arrest at either the secondary spermatocyte or spermatid stage. The tubular lumina exhibited only desquamated cells and debris (Fig. 1). There was also a marked reduction in the tubular diameter of the cauda epididymis after the 20 day treatment and the tubules appeared either devoid of spermatozoa or contained cellular detritus only (Fig. 2).

DISCUSSION

The results of the present study indicate that chromatographically isolated fractions of mint leaf extract, namely petroleum ether/benzene (1:1) and chloroform/methanol (1:1) can induce sterility in the male mouse even at the very

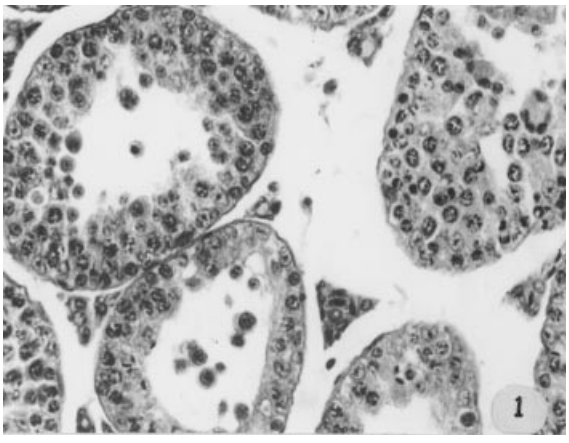

Figure 1. Cross section of the testis of 20 day treated mouse with 1.0 mg mint leaf (petroleum ether/benzene 1:1 v/v fraction of petroleum ether extract) ×200 showing complete spermatogenetic arrest and apparently reduced diameter of the seminiferous tubules.

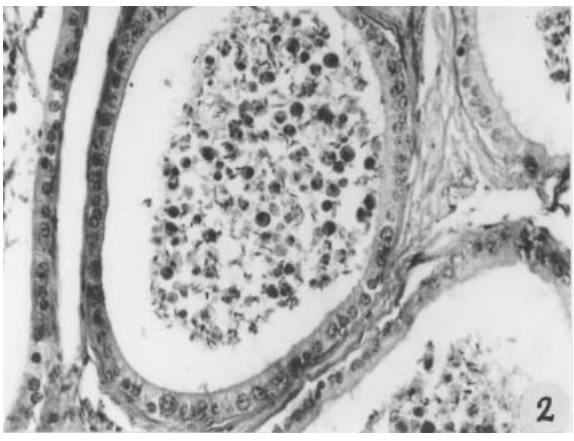

Figure 2. Photomicrograph of the cauda epididymal section of 20 day treated mouse with 1.0 mg mint leaf (petroleum ether/ benzene 1:1 v/v fraction of petroleum ether extract) ×200. Note absence of spermatozoa. Only cellular debri appear in the tubular lumina.

low dose of 1.0 mg. Females mated with such males become either consistently pseudopregnant or returned to their normal cycles soon after *coitus*. The presence of a vaginal plug showed that mating had occurred. Thus, the test substances, while impairing fertility of the male mouse, did not affect the normal sexual behaviour and libido. Failure of nidation could be attributed to the absence of viable sperm reserves in the cauda epididymes as evidenced by sperm counts and histoarchitecture of the organ. Lack of spermatozoa in the cauda epididymis in turn could be attributed to the suppression of spermatogenesis which was brought about by disturbance in the normal testicular function. It is well established that FSH and testosterone are both required by Sertoli cells/germ cells to support spermatogenesis in all its phases (Carr and Griffin, 1992), depletion in the biosynthesis of any one of these hormones, therefore, could block formation of spermatozoa. This conjecture is further supported by the observation that oestrogenic/antiandrogenic substances have an adverse effect on reproductive organ functions (Mann, 1964; Setty, 1979) and *Mentha arvensis* leaf has been reported to possess significant inherent oestrogenicity (Kanjanapothi *et al.*, 1981; Garg and Jacob, 1994). Nevertheless, since spermatogonia did not appear to have been damaged by the treatment, recovery of spermatogenesis would be possible after withdrawal of the treatment (Prasad and Dizfalusy, 1981).

It is well known that synthetic and natural oestrogens can cause infertility in males of several mammalian species including man (Smith *et al.*, 1980) by suppressing spermatogensis. Since administration of oestrogenic compounds to males can invoke sterility as indeed occurs in the female, a similar effect of plant oestrogens but without severe metabolic side effects could be anticipated. In this light, therefore, it can be surmized that fractions of mint leaf extract possibly exert a deleterious but reversible effect on either the testicular or the pituitary gonadotrophin secretions or both.

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