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Effects of Oral Applications of Some Medicinal Plant Extracts Used in Jordan on Social Aggression as Well as Testicular and Preputial Gland Structures in Male Mice

Merza. H. Hornady[1], H. H. Hussain[2], K. A. Tarawneh[1], J. M. Shakhanbeh[1], I. A. Al-Raheil[3] and P. F. Brain[4]
[1]Department of Biology, [2]Department of Chemistry, [3]Department of Physics, College of Science, Mu'tah University, P.O. Box 7-Alkarak-Jordan
[4]School of Biological Sciences, University of Wales Swansea, Swansea, SA2 8PP, U. K.

Abstract: The effects of ingestion of freshly prepared ethanolic extracts of four medicinal plants much used in Jordan were investigated on social aggression as well as on the histology of the testes and preputial glands of intact male mice. Intra-gastric application of *Cinnamomum camphora* extract significantly inhibited attack on subjects by aggressive residents. This treatment in these mice also reduced preputial activity and resulted in aspermatozoia. In contrast, the administration of *Eruca sativa* or *Nigella sativa* extracts dramatically increased the attack to which the mice were subjected as well as enhancing the maturation and differentiation of testicular spermatozoa and augmenting the activity of the preputials. The latter histological effects were most evident with *E. sativa* extracts.

Key words: Medicinal plants, mice, pheromones, preputial gland, social aggression, testes

Introduction

The roles of plants and their products in treatment of male and female infertility and sexual aggression have attracted researchers for over a century. Many plants possess spermicidal and semen-coagulating properties in male laboratory animals whereas others possess anti-ovulatory, receptivity blocking and abortifactant activities in laboratory mammals (Alkofahi *et al*., 1996). Several authors suggest that many indigenous plants have antifertility activities (Al Khamis *et al*., 1988; Elbetieha *et al*., 1996). No single unmodified plant product has, however, yet been established as an effective, safe contraceptive agent and herbal aphrodisiacs remain in the realm of speculation.

Odours from sex accessory glands are very important in aggression as well as other social activities of rodents (Homady and Brain, 1982). The male rodent's preputial gland is a modified sebaceous gland, frequently implicated in the production of behaviour modulating "pheromones". The structure and activity of the gland is dependent on hormonal factors with androgens, oestrogens, adrenocorticotrophic hormone (ACTH), melanocyte stimulating hormone (MSH) and prolactin all being shown to influence its size and activity (Homady *et al*., 1986). Preputial-derived odours may certainly change rodent behaviour but it is difficult to identify the complex specific chemicals underlying such effects and the response often depends on the nature and experience of the recipient animal (Brain *et al*., 1987). The gland's structure can be certainly used to assess whether sex hormones are stimulated or suppressed.

It was thought interesting to investigate the effects of various stepwise extracts of medicinal plants used in Jordan on a variety of histological and behavioural measures reflecting altered gonadal activity in individually housed male mice. The present work evaluated the effects of ingestion of ethanolic extracts of four skich medicinal plants used in folkloric medicine. They are *Eruca sativa* (used to treat sterility and to augment sexual desire in males); *Cinnamomum camphora* (used to calm sexual activity, including in some cases of sexual crime); *Nigella sativa* (used to encourage increase in body weight as well as in the treatment of respiratory and urinary tract infections and headaches) and *Salvia fruticosa* (used in the treatment of male sterility). The effects of the plant extracts were studied on social aggression; testicular function and structure of preputial glands in laboratory mice. It was thought that such a study would at least partially confirm or refute the claimed benefits of the herbal treatments and might suggest directions for future therapeutic investigations.

Materials and Methods

Plant material extracts: Dried material from each plant was obtained from a local market in Al-Karak (Jordan) and pharmacognostically identified in our laboratory before use. The ethanolic extracts were prepared by boiling each material in 97 percent ethanol for 5 minutes, filtering after 2-3 h, drying over $MgSO_4$ and removing the solvent at 30°C/14 mm Hg to give oils. These extracts were stored at 4°C and used within 24 hours. Fresh solutions of the extracts were prepared in distilled water immediately before administration to the animals (see below). Naturally, the modes of administration to humans are not so complex but an attempt was being made here to standardize application.

Animals: Tuck Ordinary strain albino mice were bred and maintained in the animal house unit in the Faculty of Science'at Mu'tah University. They were maintained at a controlled temperature 21 ± 1°C using 2-h light: 12 h-darkness schedule (white lights on 06.00-18.00 h local time). Subjects were housed in type M1 plastic cages (North Kent Plastics, Erith, Kent, U.K.) measuring 30 x 12 x 11 cm with wire grid tops. Sawdust bedding was used and food and water were available *ad libitum.* Forty intact male "resident" mice were individually housed at 7 weeks of age for three days before behavioural tests to induce e moderate level of aggressiveness (Goldsmith *et al*., 1976). A further 40 group-housed (N = 8) intact male mice were allocated to 5 categories treated, from 3 weeks of age, with 0.1 ml of the following daily (Table 1);

1. *E. sativa* extract.
2. *C. camphora* extract.
3. *N. sativa* extract.
4. *S. fruticosa* extract.
5. Normal saline (controls).

Daily Administrations: Intra-gastric application of plant extracts or normal saline were made using animal feeding needles (Popper and Sons, New York) at 10.00 h each day. The fluids were administered for 30 days before behavioural testing.

Aggression tests: Treated mice were individually introduced for 10 minutes into the home cages of residents. Tests were conducted under dim red light and encounters were repeated for three consecutive days (McKinney and Christian, 1970). A series of electromechanical counters were employed to obtain routine measures of attack (Goldsmith *et al*., 1976). The aggressive resident mouse's behaviour was observed and monitored the following parameters:

1. Number of animals showing overt attack.
2. Latency of attack (in seconds) from the time of the introduction of the opponent.

Table 1: Phytochemical screening of some medicinal plants used in Jordan.

Species and Family	Plant part	Alkaloids	Anthraq-uinones	Colimarins	Flavonoids	Saponins	Sterols and/or Terpenes	Tannins
E. sativa (Eruciferae)	W	+	-	+	-	+	-	-
C. camphora (Lauraceae)	L+S	+	-	+	-	-	+	-
N. sativa (Ranunculaeae)	Sd	++	-	+	+	-	+	-
S. fruticosa (Labaitae)	W	-	-	-	+	++	+	+

W : whole plant, Sd : seeds, L : leaves, S : stems + present, + + present in quantity, -absent

Table 2: Mean (±S. E.) body (g) and relative organ weights (mg/100 g) in intact male mice treated daily with saline or the following ethanolic plant extracts for 30 days (N = 8)

Treatment	Body weight	Right testis	Right preputial gland	Right preputial gland minus sebum	Right seminal vesicle	Right seminal vesicle without fluid
Normal saline	22.97±0.67	18.55±1.88	8.59±0.96	5.99±0.35	15.87±1.19	8.25±0.66
*E. sativa**	20.75±0.4.7*	25.90±1.10^{+++}	16.04±1.11*	6.78±0.45	17.96±1.25	10.33±1.12
C. camphora	21.94±1.41	14.47±1.07	6.12±0.82	3.70±0.53*	12.45±1.30	8.30±1.11
N. sativa	27.81±0.59^{+}	27.53±0.57^{++}	16.61±1.88^{++}	10.08±1.22^{+++}	20.94±1.17^{+++}	9.99±1.02
S. fruticosa	26.49±1.10*	23.44±0.50**	14.41±1.12*	8.54±0.85*	20.63*1.80***	13.89±1.81*

* Differs from normal saline treated group ($p<0.01$) on Student's t -test.
** Differs from normal saline treated group $p<0.02$) on Student's t -test.
*** Differs from normal saline, treated group ($p < 0.04$) on Student's t -test.
+ Differs from normal saline treated group ($p < 0.001$) on Student's t -test.
++ Differs from normal saline treated group ($p < 0.002$) on Student's t -test.
+++ Differs from normal saline treated group ($p < 0.005$) on Student's t -test.

Table 3: Mean (± S.E.) seminiferous tubule diameters tu) in wax sections from intact mice treated daily with saline or extracts of some medicinal plants used in Jordan

Treatment	Diameter
Normal saline	253.47+1.56
E. sativa	346.95+3.47*
C. camphora	217.80+1.54*
N. sativa	277.82+1.69*
S. fruticosa	250.55+1.55

*Differs from normal saline treated group $p<0.0001$ on Student's t-test

3. Number of bouts of biting attacks directed towards the opponent.
4. The accumulated attacking time (AAT).

Body and Organ weight determinations and histology: At the end of the experiment, the treated mice were killed by cervical dislocation. The body, right testes, right preputial glands (with and without sebum) and right seminal vesicles (with and without fluid) were weighed. Organ weights were eventually expressed in relative terms (mg/100 g body wt). Mean values±SE were obtained for each experimental category. The left testes and preputial glands were used for histological investigation under light Microscopy. The histological methods used have been described earlier (Homady *et al.,* 1986). In addition, seminiferous tubtile diameters were measured in paraffin sections of testicular material using a micrometer microscope with a x 40 objective. Five samples were used for each treatment and 20 sections were taken from eath. Three random transverse-section measurements were taken from each of the 100 sections. The total of 300 measurements per treatment was meaned to give an average value in micrometers.

Statistical treatments: Body and organ weights as well as seminiferous tubule diameters were analyzed using Student's 't' test and behavioural data were analyzed using Mann Whitney U-test and the analysis of proportions test (Siegel, 1956).

Results

Body and organ weights: The results for body and relative organ weights are presented in Table 2. Significant differences were found' for mean' body weights between controls and categories treated with *N. sativa* ($p<0.001$), *E. sativa* and *S. fruticosa* ($p<0.01$) extracts. *E. sativa* produced suppression and the others increases in weight. Mean body weight was not significantly altered by *C. camphora* treatment. Relative testis weights were unaltered by treatment with *C. camphora* but were highly augmented c.f. controls in categories treated with *E. sativa* ($p<0.005$), *N. sativa* ($p<0.002$) and *S. fruticosa* ($p<0.02$). Relative preputial gland weights were elevated in categories treated with *N. sativa* ($p<0.002$), *E. sativa* and *S. fruticosa* ($p<0.001$). *C. camphora* suppressed the relative preputial gland (minus sebum) weights ($p<0.001$) whereas these weights were elevated in groups treated with *N. sativa* ($p<0.005$) and *S. fruticosa* ($p<0.01$). Relative seminal vesicle weights were significantly elevated in both *N. sativa* ($p<0.005$) and *S. fruticosa* ($p<0.04$) treated subjects. In addition, the relative seminal vesicle (without fluid) weight was elevated ($p<0.01$) in subjects treated with *S. fruticosa* c.f. controls.

Histology

Testes: The normal structure of the mouse testis (Fig. 1a and b) is confirmed in the control sections. The histology of the testes in animals treated with *N. sativa* and *S. fruticosa*, did not differ from that in the control group (not shown). The testes of mice treated with *E. sativa* (Fig. 1c and d) had well-defined seminiferous tubules lined by spermatogonia, primary and secondary spermatocytes, spermatids (in various phases of spermatogenesis) and the spermatozoa were easily identified. The interstitial cells were highly developed, being relatively large and irregular in shape. Thin cytoplasmic prolongations were seen when these cells were closely apposed. *E. sativa* treatment seemed to stimulate the growth of testes and enhance the proliferation, maturation and differentiation of spermatozoa as compared with the control groups. Histological examination of the testes in group-treated with *C. camphora* (Fig. 1e and f) showed no signs of cycles of spermatogenesis, there being marked collapse of the seminiferous tubules, which had low germinal epithelia. A number of Sertoli cells showed pyknosis and there was general tendency for nuclear shrinkage. Leydig cells were decreased in number and generally atrophied. The mean seminiferous tubule diameters (Table 3) broadly confirmed the above histological picture with animals dosed with *E. sativa* having markedly greater mean diameters than the saline-treated controls whereas subjects receiving *C. camphora* had smaller diameters. Mice receiving *S. fructicosa* extracts did not differ from controls and those receiving *N. sativa* had a modest increase.

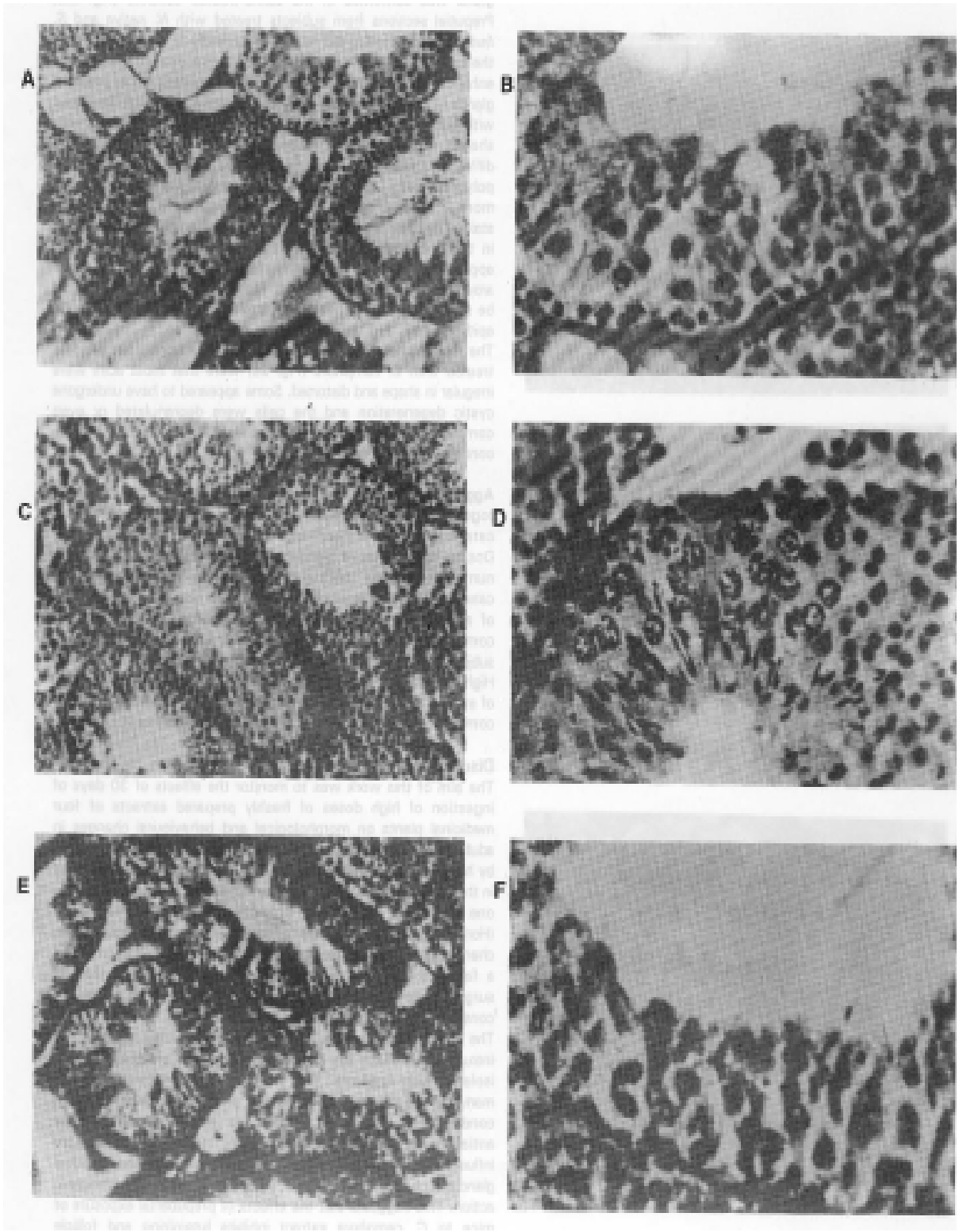

Fig. 1: Thick (6μ) paraffin sections of mouse testes. a, b Control (x50 and x100, respectively). c, d *Eruca sativa* - treated animals (x50 and x100, respectively). Note the well-defined seminiferous tubules with enhanced proliferation and differentiation of spermatoza. e, f *Cinnamomum camphora* - treated animals (x50 and x100, respectively). Note the condensation of seminiferous tubules with low germinal epithelia and the presence of numerous degenerating spermatids

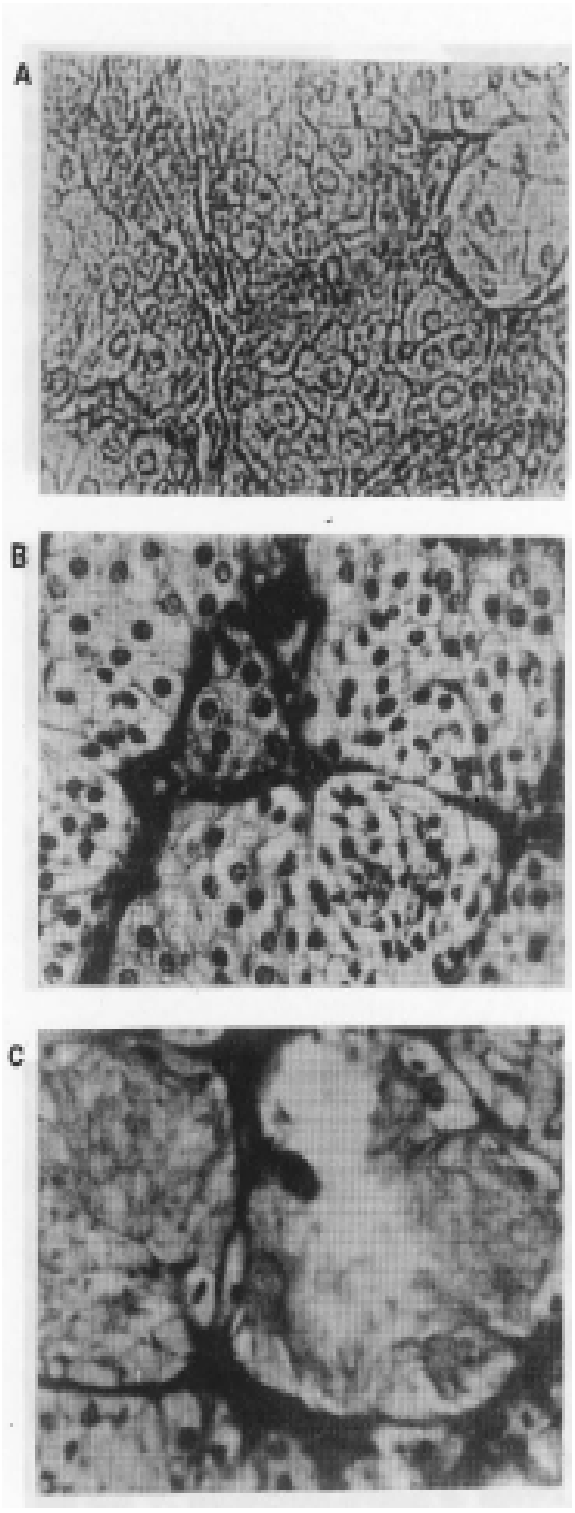

Fig. 2: Thick (6μ) paraffin sections of mouse preputial gland (all x100). A. Control B. *Eruca sativa* - treated animals. Note the well- developed structure with numerous acini of variable shapes and at different stages of maturation. C. *Cinnamomum camphora*- treated subjects. Note the progressive degeneration and formation of cystic acini

Preputial glands: The normal histology of the mouse preputial gland was confirmed in the saline-treated controls (Fig. 2a). Preputial sections from subjects treated with *N. sativa* and *S. fruticosa* (not shown) did not differ markedly in any respect from the control group. Treatment with *E. sativa* extract appeared to enhance preputial maturation and lipid content. The preputial glands of intact animals treated with *E. sativa* were well developed with acini at different stages of maturation (i.e. they had variable shapes and many were fused). Nuclei in such acini assumed different shapes and were peripherally located in the roughly polygonal cells. Acini in peripheral parts of the gland appeared more active than those located more centrally having darker-stained cytoplasm and nuclei. Hypertrophied acini (generally those in the centre of the gland) had a relatively lighter cytoplasmic appearance and some degenerated nuclei. Connective tissue around acini was well developed. Blood vessels and nerves could be seen in the connective tissue stroma and stratified squamous epithelial cells (Fig. 2b) lined ducts.
The most obvious features.of histological sections from subjects treated with *C. camphora* (Fig. 2c) were that most acini were irregular in shape and distorted. Some appeared to have undergone cystic degeneration and the cells were degranulated or even completely degenerated. The nuclei were often pyknotic and the connective tissue stroma was thin.

Aggression test data: The data for categories 1-5 is represented together with a statistical analysis in Table 4. None of the categories showed significant differences in attack latencies. Dosing with *E. sativa* significantly increased the AAT and the number of attacks (both $p<0.001$) compared to the control category. Significant declines ($p<0.05$) on both AAT and number of attacks were evident in subjects treated with *C. camphora* compared with the controls. Intruders dosed with *N. sativa* were subject to longer AATs and more attacks ($p<0.05$) c.f. controls. Highly significant increases in both AAT ($p<0.003$) and number of attacks ($p<0.002$) were evident in subjects given *S. fruticosa* compared with counterparts receiving saline treatment.

Discussion

The aim of this work was to monitor the effects of 30 days of ingestion of high doses of freshly prepared extracts of four medicinal plants on morphological and behavioural changes in adult male mice after prolonged prepubertal exposure. As indicated by herbalists, *N. sativa* extracts produced a very marked increase in the body weights of treated mice. The preputial gland is clearly one source of "aggression-promoting factor" in the mouse (Homady, 1982). Indeed, this 'pheromone' has been implicated in changes of aggression (Homady *et al.*, 1992). This gland produces a factor that is "aggression-promoting" in the sense that its surgical removal reduces a resident mouse's ability to perceive a conspecific as a suitable object for attack.
The present results show that *C. camphora* extract ingestion by intruders decreases the attack to which mice are subjected by isolated male residents. This treatment concomitantly reduced many indices of preputial activity and produced an aspermatozoic condition of the testes, similar to that often seen after antiandrogen treatment (Homady *et al.*, 1986). These inhibitory influences on social aggression, as well as testicular and preputial gland structure/activity suggest an antiandrogenic or oestrogenic action. This suggests that the effects of prepubertal exposure of mice to *C. camphora* extract inhibits luteinizing and follicle stimulating hormone release (Aguilar *et al.*, 1987). It is not, however, clear from the present work whether *C. camphora* extract acts directly on the testes or indirectly via the hypothalamic structures regulating gonadotrophin secretion. Ingestion of ethanolic extracts obtained from *E. sativa*; *N. sativa* and *S. fruticosa* all increased the attack to which intruders were subjected by aggressive male conspecifics.

Table 4: Summed data (proportions or medians with ranges) over 3 days for individually-housed male mice encountering intact male mice "intruders" ingesting daily the following ethanolic plant extracts for 30 days

Treatment of intruders	Proportion of animals attacking in at least 2/3 tests	Latency to attack (secs)	AAT (secs)	Number of attacks
Normal saline	5/8	601.0 (61-1800)	48.0 (0-96)	35.0 (0-92)
E. sativa	8/8	282.5 (83-776)	134.0 ** (54-185)	108.5 ** (45-136)
C. camphora	2/8	1462.0 (733-1800)	7.5* (0-34)	5.0 * (0-26)
N. sativa	8/8	465.5 (157-911)	82.5 * (57-132)	68.5 * (41-112)
S. fruticosa	7/8	619.5 (15-1357)	156.0 + (27-221)	133.5 + + (19-195)

* Differs from category treated with normal saline $p<0.05$ (Mann Whitney U-Test).
** Differs from category treated with normal saline $p<0.001$ (Mann Whitney U-Test).
\+ Differs from category treated with normal saline $p<0.003$ (Mann Whitney U-Test).
\+ + Differs from category treated with normal saline $p<0.002$ (Mann Whitney U-Test).

It is well known that androgens are responsible for enhancing the growth of the preputial gland and stimulation of testicular spermatogenesis (Brain and Homady, 1985a). Dihydrotestosterone injection has similar (but more rapid) effects on the attack to which castrated mice are subjected (Brain and Homady, 1985b) than these oral applications of oils. Subjects treated with *E. sativa* showed marked enhancement of the preputial gland as well as increased spermatogenesis in the testis. Both suggest that this extract contains a specific and selective compound(s) which influence(s) spermatogenesis and preputial activity and hence pheromone production in this species. The most parsimonious explanation is that *E. sativa* extracts have androgenic activity or stimulate testicular steroid production. It is less easy to explain the behavioural effects of administering *N. sativa* or *S. fruticosa* extracts. *N. sativa* did significantly increase the mean diameter of the seminiferous tubules (suggesting an androgenic stimulation) but did not markedly change histology. *S. fruticosa* was not associated with any obvious changes in structure or activity of the preputial or the testis.

In conclusion, the present studies indicate the presence of orally active agents in extracts of *C. camphora*, *E. sativa* and *N. sativa* which suggest the presence of hormone-influencing compounds with varied effects. Further studies of dose-responsiveness of these extracts are needed. It would also be useful to attempt to identify the active principles of these medicinal plants on fertility and reproduction of laboratory animals. The phytochemical screening provides no clues concerning the classes of compounds accounting for the different effects of these extracts. However, the effects of the herbal treatments for *E. sativa* (seemingly increasing an androgen-dependent activity); *C. camphors* (having a calming effect) and *N. sativa* (increasing body and organ weights) are broadly reflected their claims. The present results with *S. fructose* provided no evidence for a stimulatory effect on the gonadal system over and above the behavioural change.

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