



Effect of adding *Cinnamomum camphora* on the testosterone hormone and reproductive traits of the Awassi rams

Tamara N. Dawood

Department of Public Health – College of Veterinary Medicine / Baghdad University

tamara_algobory@yahoo.com

Abstract

This study was aimed to find out the effect of camphor on sexual behavior in male rams. Eight sexually mature Awassi rams were randomly divided into two groups, the experimental group was received *Cinnamomum camphora* (20 mg/kg/ day) orally with their concentrated diet for four weeks; while the other group (control) were feed on the same diet without adding camphor. Semen was collected from the rams and the results showed that camphor reduced the semen volume, mass activity, testes length and live sperm percentage while there were insignificant differences in the testes circumference, semen concentration, individual motility and testosterone hormone level between the treated and control groups. In addition it was noticed that the abnormal percentage of sperm was significantly higher in camphor group compared with the control group.

Key words: testosterone, Awassi rams , Semen, camphor.

تأثير اضافة الكافور على هرمون التستستيرون والصفات التناسلية في ذكور الاغنام العواسية

تماره ناطق داود

فرع الصحة العامة – كلية الطب البيطري / جامعة بغداد

tamara_algobory@yahoo.com

الخلاصة:

هدفت الدراسة الى معرفة آثار الكافور في السلوك الجنسي في الكباش. تم تقسيم حيوانات التجربة الثمانية بشكل عشوائي الى مجموعتين. اذ تم اعطاء مجموعة التجربة 20 ملغم / كغم / حيوان / يوم من نبات الكافور

مع العليقة المركزة وذلك عن طريق الفم ولمدة اربعة اسابيع في حين غذيت مجموعة السيطرة على نفس العليقة ولكن بدون اضافة نبات الكافور . اظهرت نتائج التجربة انخفاضاً معنوياً في حجم السائل المنوي والحركة الجماعية للحيامن ونسبه الحيامن الحيه وطول الخصية في لوحظ انخفاض غير معنوي في محيط الخصية فضلاً عن انخفاض في تركيز الحيامن والحركة الفردية وكذلك انخفاض في مستوى هرمون التستوستيرون في المجموعه المعامله مقارنة مع مجموعته السيطرةه, في حين لوحظ ارتفاع معنوي في نسبه الحيامن المشوهه في مجموعته المعامله مقارنة مع مجموعته السيطرةه.

Introduction

Cinnamomum camphora (known as Camphor tree or camphor laurel) is a large evergreen tree that grows up to (20–30) meters tall [1,2,3and 4]. The leaves have a glossy, waxy appearance and smell of camphor when crushed which belong to the order Laurales [2and 5]. Camphor is a white waxy flammable and solid with a strong aromatic odor crystalline substance obtained from the tree *Cinnamomum camphora* [4and5]. Camphor laurel has six different chemical variants called chemo types, which are camphor, linalool, 1,8-cineole, nerolidol, safrole, or borneol [6]. Camphor is a terpenoid with the chemical formula $C_{10}H_{16}O$ [7]. Camphor is derived from the Arabic word of 'Kafur', which means chalk [4] and has a long history of herbal use that used in cooking (mainly in India) for medicinal purposes and in religious ceremonies., It is used as analgesic, antihelmintic, antirheumatic; antispasmodic; aromatherapy,cardiotonic; carminative; diaphoretic;

odontalgic; aphrodisiac, antiaphrodisiac, contraceptive, cold remedy, antiseptic and suppressor of lactation [8], also it is used as an inhalant in the treatment of colds and

diseases of the lungs. Recently, investigations have shown that camphor containing compounds have

nicotinic receptor blocking, anti implantation, anti estrogenic as well as

estrogenic activities, and reduced serum triglyceride . Camphor is absorbed through the skin and produces a feeling of cooling similar to that of menthol, and acts as slight local anesthetic and antimicrobial substance[8and9].

Materials and Methods

Eight Awassi lambs rams kept in semi opened shed in the Animal Farm of Veterinary College, Baghdad University, aged 7-8 months with average body weights 33.60 ± 1.02 Kg. The experiment began from April and lasted to the end of June 25th, the animals were fed on a concentrate diet and freely grazed for 3-6 hours/day on the College Fields. Animals were divided randomly and equally into two group (4 each):

The control group lamb rams were daily fed on 2% of the body weight concentrate diet /animal.

The treated group was daily fed on the same amount of the concentrate diet/head containing ground *Cinnamomum camphora* in dose of 20 mg/kg/animal. Parameters and measurements included in this study

Blood samples were taken from the jugular vein once a week and serum separated to estimate testosterone hormone level by radioimmunoassay (RIA). [10]

Semen samples were collected from the animals by using artificial vagina once a week and rams semen quality were examined for physical traits including semen ejaculation volume by the method of Chemineau *et al.* [11] concentration, mass activity by Evans and Maxwell [12] and individual motilities by Colas [13], Sperm viability and sperm morphology abnormalities percentages according to the method of Ollero *et al.* [14]. Also testes dimensions were measured includes the length and width. [15].

The data were collected and statistical analyzed SAS (2010) was used to detect the effect of the different factors (treatments & weeks). Least significant difference (LSD) was used to detect the differences between means [16].

Results

Testosterone hormone levels increased with age in both groups up to the last period of the study (Table,1). The camphor group showed significantly ($P < 0.05$) reduction value compared with the control group at the 4th week of the study.

Table 1. Effect of treatment & period of *Cinnamomum camphora* administration in testosterone level (nmol/L)

Group	Mean $\pm$ SE				LSD Value
	1 st week	2 nd week	3 rd week	4 th week	
Control	0.28 $\pm$ 0.18	0.50 $\pm$ 0.12	0.92 $\pm$ 0.11	1.48 $\pm$ 0.13 a	0.419 *
Camphor	0.15 $\pm$ 0.06	0.26 $\pm$ 0.08	0.52 $\pm$ 0.13	0.96 $\pm$ 0.14 b	0.340 *
LSD Value	0.438 NS	0.355 NS	0.417 NS	0.443 *	---
* ($P < 0.05$) , NS: Non-significant.					

Testis circumference of ram's recorded insignificant differences between the camphor and control groups, but they increased significantly ($P < 0.05$) with advancing of the study (Table,2). While, the length of the treated rams testis were shorter significantly ($P < 0.05$) shorter than of control rams testes at the 1st, 2nd and 4th week (Table,3).

Table 2. Effect of treatment & period of *Cinnamomum camphora* administration on testes circumference (cm)

Group	Mean $\pm$ SE				LSD Value
	1 st Week	2 nd Week	3 rd Week	4 th Week	
Control	10.80 $\pm$ 1.11	13.00 $\pm$ 1.04	15.70 $\pm$ 1.64	18.40 $\pm$ 1.36	3.934 *
Camphor	10.00 $\pm$ 1.38	10.92 $\pm$ 1.43	14.20 $\pm$ 1.77	16.80 $\pm$ 2.39	5.373 *
LSD Value	4.102 NS	4.090 NS	5.567 NS	6.357 NS	---
* (P<0.05) , NS: Non-significant.					

Table 3. Effect of treatment & period of *Cinnamomum camphora* administration on testes length (cm)

Group	Mean $\pm$ SE				LSD Value
	1 st Week	2 nd Week	3 rd Week	4 th Week	
Control	6.86 $\pm$ 0.64 a	8.14 $\pm$ 0.61 a	8.90 $\pm$ 0.60	10.20 $\pm$ 0.47 a	1.761 *
Camphor	4.50 $\pm$ 0.44 b	5.60 $\pm$ 0.40 b	7.38 $\pm$ 0.34	8.02 $\pm$ 0.55 b	1.327 *
LSD Value	1.807 *	1.693 *	1.596 NS	1.681 *	---
* (P<0.05) , NS: Non-significant.					

Awassi rams produced significantly ($P < 0.05$) lower semen volume in the treated group as compared to the control group during all studied weeks (Table,

4) and they recorded 0.70 ± 0.04 and 1.02 ± 0.05 ml for the camphor and control groups at the last studied week respectively.

Table 4. Effect of treatment & period of *Cinnamomum camphora* administration in semen volume (ml)

Group	Mean $\pm$ SE				LSD Value
	1 st Week	2 nd Week	3 rd Week	4 th Week	
Control	0.84 ± 0.05 a	0.86 ± 0.05 a	0.92 ± 0.03 a	1.02 ± 0.05 a	0.142 *
Camphor	0.62 ± 0.06 b	0.68 ± 0.03 b	0.64 ± 0.02 b	0.70 ± 0.04 b	0.128 NS
LSD Value	0.178 *	0.145 *	0.103 *	0.153 *	---
* (P<0.05) , NS: Non-significant.					

Sperm concentration gradually increased in both control and camphor groups with time progress (Table, 5), as well as the camphor group recorded insignificant values in sperm concentration compared with the control groups along all weeks of the studied period.

Table 5. Effect Effect of treatment & period of *Cinnamomum camphora* administration on concentration of spermatozoa (Sperm*10⁶)

Group	Mean $\pm$ SE				LSD Value
	1 st Week	2 nd Week	3 rd Week	4 th Week	
Control	100.80 ± 13.72	104.80 ± 9.28	106.80 ± 9.76	109.60 ± 8.80	31.702 NS
Camphor	80.00 ± 5.46	84.60 ± 4.21	89.20 ± 3.38	92.80 ± 8.69	17.400 NS
LSD Value	34.059 NS	23.505 NS	23.840 NS	28.533 NS	---

NS: Non-significant

Mass activity showed significant ($P < 0.05$) lower value in the camphor group compared with the control group during the 2nd and 4th week of the experimental study, while the individual motility percentage showed insignificant differences between the camphor and control groups along the whole experimental period (Table, 8). i.e. the camphor group showed lower values during the last three weeks of the study.

Table 6. Effect Effect of treatment & period of *Cinnamomum camphora* administration on mass activity (%)

Group	Mean $\pm$ SE				LSD Value
	1 st Week	2 nd Week	3 rd Week	4 th Week	
Control	83.00 $\pm$ 1.22	84.00 $\pm$ 1.00 a	84.00 $\pm$ 1.00	86.00 $\pm$ 1.00 a	3.179 NS
Camphor	86.00 $\pm$ 1.87	76.00 $\pm$ 2.91 b	79.00 $\pm$ 3.67	73.00 $\pm$ 3.00 b	8.804 NS
LSD Value	5.156 NS	7.107 *	8.781 NS	7.292 *	---
* ($P < 0.05$) , NS: Non-significant.					

Table 7. Effect Effect Effect of treatment & period of *Cinnamomum camphora* administration on individual motility (%)

Group	Mean $\pm$ SE				LSD Value
	1 st Week	2 nd Week	3 rd Week	4 th Week	
Control	80.00 $\pm$ 1.58	76.00 $\pm$ 1.87	79.00 $\pm$ 1.00	82.00 $\pm$ 1.22	4.370 NS
Camphor	82.00 $\pm$ 1.22	73.00 $\pm$ 3.00	79.00 $\pm$ 2.44	79.00 $\pm$ 3.67	8.210 *

LSD Value	4.612 NS	8.153 NS	6.101 NS	8.931 NS	---
* (P<0.05) , NS: Non-significant.					

It was indicated that the live sperm concentration of the control group showed significantly ($P < 0.05$) higher values compared with the camphor group at the 3rd and 4th weeks of the study, (Table, 8). In the meantime, the differences in abnormal sperm percentages of the camphor group recorded significantly ($P < 0.05$) higher values than those of the control group during the 1st , 2nd and 4th weeks of the studied period (Table, 9).

Table 8. Effect Effect Effect of treatment & period of *Cinnamomum camphora* adminstration of live sperm (%)

Group	Mean $\pm$ SE				
	1 st Week	2 nd Week	3 rd Week	4 th Week	LSD Value
Control	81.40 $\pm$ 3.41	79.00 $\pm$ 3.06	84.20 $\pm$ 2.03 a	85.40 $\pm$ 1.88 a	8.091 NS
Camphor	74.40 $\pm$ 2.29	77.40 $\pm$ 1.72	78.60 $\pm$ 1.24 b	77.40 $\pm$ 1.07 b	4.701 NS
LSD Value	9.485 NS	8.107 NS	5.505 *	5.009 *	---

Table 9. Effect Effect Effect of treatment & period of *Cinnamomum camphora* adminstration of sperm abnormality (%)

Group	Mean $\pm$ SE				LSD Value
	1 st Week	2 nd Week	3 rd Week	4 th Week	
Control	3.60 $\pm$ 0.74 b	3.20 $\pm$ 0.48 b	3.40 $\pm$ 0.51	4.00 $\pm$ 0.32 b	1.614 NS
Camphor	6.40 $\pm$ 0.67	5.60 $\pm$ 0.81	6.00 $\pm$ 1.09	7.00 $\pm$ 0.89	2.647 NS

	a	a		a	
LSD Value	2.329 *	2.187 *	2.786 NS	2.187 *	---
* (P<0.05) , NS: Non-significant.					

Discussion

The finding of the present study showed that the concentration of the testosterone hormone level in the treated group were significantly lower than the control group and that may be due to that camphor has estrogenic effect which affect on the level of testosterone hormone [8], Nikraves and Jalali [17] showed histopathological changes of the reproductive system in young male mice those received 30 mg /kg camphor orally; treated group showed vascularization and proliferation of sexual cells. The effect of camphor on the male sexual behavior and reproductivity by its effect on serum testosterone level and or sympathetic nervous system that camphor may be inhibited catecholamine secretion by blocking nicotine acetylcholine receptors [9and18].

Testis width showed insignificant difference between treated group and the control group while the testis length showed significant difference, that the treated group testes length were shorter than the control group and these results

agreed with Homady et al. [19] and Durrer et al. [20] which may due to camphor have an effect the internal diameter of seminiferous tubules and the reproductive function of the treated male mice with camphor orally.

The results showed that there were no significance differences in the sperm

concentration and individual motility in rams received camphor at 20mg /kg compared to the control group [19].

The results showed the semen volume of the treated group were significantly ($p<0.05$) lower than the control group with camphor and that may due to that camphor has estrogenic effect which affect the level of testosterone

hormone [8] and may it inhibits the testicular axis in male rats during the prepubertal stage and stimulates it [21], the effect of camphor directly on the testes by its effect on the proliferation cells that affect the semeniferous tubules or indirectly inhibits ICSH and follicle stimulating hormone release [22]. Semen volume that many researchers showed that camphor may not affect in small dose [21].

References

- Rabl; W., Katzgraber; F. and Steinlechner; M.(1997). Camphor ingestion for abortion (a case report). Forensic ScInt, 89: 137-40
- Seidlova-Wuttke; D., Christoffel; J., Rimold; G., Jarry; H. and Wuttke; W. (2005). Comparison of effects of estradiolwith those of octymethoxycinnamate and 4-methylbenzylidene camphor on fat tissue, lipids and pituitary hormones. Toxicol Appl. Pharmacol; 214: 1-7.
- Mirhaydar; H.(1992). Medicinal Plant Facts, Applications in Prevention and Treatment of Diseases. 5(1): pp.85-89.Tehran.

- Usher;G.A.(1974). A dictionary of plants used by man. PMID 201-208.
- Xi-wen; Li., Jie; Li. and Henk van der; W.(20013). *Cinnamomum camphora*. Flora of China. Missouri Botanical Garden, Harvard University Herbaria, Cambridge, MA. PMID 346.
- Hirota; N. and Hiroi; M. (1967). The later studies on the camphor tree, on the leaf oil of each practical form and its utilisation, Perfumery and Essential Oil Record 58, 364-367.
- Lawrence; B. M. (1995). Progress in essential oils. Perfumer and Flavorist, 20, 29-41.
- Akram;M., Jamshidzadeh; J., Sajedianfard; A., Nekooeian; F. and Tavakoli;A. (2006). Effect of Camphor on sexual behaviors in male rats. Iranian journal of pharmaceutical Sci.2 (4):209-214.
- Ng; P.C., Kong; Y., Cheng; K. and Stone; G. (1994). Mixed estrogenic and antiestrogenic activities of yuehchukene a bis-indole alkaloid. Eur J Pharmacol. 264: 1-2.
- Free; L.M. and Blaurock;M.D. (1975). Radioimmunoassay Dept. of Radiology, Albert Einstein College of Medicine Yeshiva University, Bronx New York.
- Chemineau,P.; Cagnie,Y.; Guerin,Y.; Orgeur,P. and Vallet;J.C. (1991). Training manual on artificial insemination in sheep and goats. FAO, Animal Production and Health; 83:1.
- Evans, G. and Maxwell, W.M.C. (1987). Salamons Artificial Insemination of Sheep and Goats. Butter Worths, Sydney, Australia,Pp: 123-194.
- Colas,G.(1983). Factors affecting the quality of ram semen. In:Harsesign, W. (ed). Sheep Production. Butter Worths, London.; Pp:453-465.
- Ollero,M.T.; Muino,M.J.;Lopez, P. and Cebrian,J.A.(1996). Viability of ram spermatozoa in relation to the abstinence period and successive ejaculations. Int.J. Androl.;19:287-292.
- Al-Musawey;J.E.Q.(2009). Effect of using Zingiber officinals and Eruca sativa in some productivity traits, physiological traits and reproductivity characteristics of male Awassi lambs. MSc. Thesis. College of veterinary Medicine Baghdad University.
- SAS. (2010). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
- Nikraves;M.R. and Jalali;M. (2004). The effect of camphor on the male mice reproductive system. Uology J.1(4):201-214.
- Park; T.J., Seo; H.K., Kang; B.J. and Kim; K.T.(2000). Noncompetitive inhibition by camphor of nicotinic acetylcholine receptors. Biochem Pharmacol. 61: 787-93.
- Homady;M.H., Hussain;A.K., Tarawneh;M.J. and Brain;P.F.(2000). Effect of oral application of some medicinal plant extracts used in Jordan on social aggression as well as testicular and preputial gland structures in male mice. Pakistan J.Biol. Sci.3(3):398-402.
- Durrer;S., Ehanes;C., Fuetsch;M., Maerker;K., Schlumpf;M., and Lichtenteiger.(2007). Estrogen Sensitivity of Target Genes and Expression of Nuclear Receptor Coregulators in Rat Prostate Following Pre- and Post-natal Exposure to the UV Filter 4-Methylbenzylidene Camphor. Institute of Pharm. And Toxi. University of Zurich CH-8057 Zurich, PMID.5(2):286-295.
- Carou;M.E.,Szwarcfarb;B.,Deguiz;M. L.,Reynoso;R.,Carbone;S.,Moguilevsky;J. Scacchi; P., and Ponzio;O.(1988). Impact of 4-methylbenzylidene camphor (4-MBC) during embryonic and fetal development in the neuroendocrine regulation of testicular

axis in prepubertal and peripubertal male rats. Pub Med. PMID5(9):97. Aguilar;E.,Bellido;R.,Pinilla;A.,Tejero; A. and Galaz;C.(1987). Mechanisms in

the production of prepubertal reproductive defect in neonatal estrogenized male rats. Andrologia,19:22-31.