Pheromonal compounds and Histological Alteration of Castrated Mose Preputial Gland in Relation to *Eruca sativa* Extract

Merza H. Homady

Keywords: ***Eruca sativa***, **acini, melanocortin, pheromone, histology**

This study included the effects of 3mg/kg/day of *Eruca sativa* on:

* Melanocortin-5-receptor (MC5R);

* Total lipid compounds,

* Essential pheromonal compounds and

* The Histological structure of the castrated mice preputial glands for 42 days of treatment were investigated.

This treatment is resulted in:

A significant increase of MC5R level in preputial gland of castrated treated animals with *Eruca sativa* as compared with the control group ($P<0.01$).

Also it has marked effects on the: total lipid compounds, essential pheromonal compounds and histological restoration of the preputial Gland structures in castrated treated subjects.

Moreover this treatment was able to restore four compounds (out of Six) of the essential pheromones which are:

1- α.-Farnesene and (E)-α.-Farnesene

2- (1-Tetradecanol)

3- Tetradecanol acetate)

4- (1- Hexadecanol acetate).

Histological sections of mouse preputial glands from castrated subjects treated with *Eruca sativa* revealed that:

The gland restore its activity and structure, with acini at different stages of maturation.

Introduction

.* Preputial glands are exocrine modified sebaceous glands, that are located subcutaneously in pubic area of many rodents such as mice and rats .

* The gland is surrounded by a capsule of connective tissue, from which trabeculae extend to the interior to divide the parenchymatous tissues into acini, the acini are at different stages of maturation .

* The acini of the preputial glands consist of basal cells with a basophilic cytoplasm and secretory cells containing an oily secretion.

*The gland has a long excretory duct lined by stratified squamous epithelium.

Why we chosed the preputial gland as a model of the study?

The preputial glands are the source of chemical signals (pheromones producing glands) that promotes reproduction and behavior of mice .

What is a Pheromone?

The word pheromone means , to carry or transfer chemical factors that triggers or inhibit a social response in members of the same species.

The pheromones are chemical compounds that act outside the body to impact the behavior of the receiving individual.

Generally there are different types of pheromones , that refer to different functions, e.g. :

* Alarm pheromones,
* Food trail pheromones,
* Sex pheromones and many others that affect behavior or physiology of the recipient.

Pheromones are used, from basic unicellular prokaryotes to complex multicellular eukaryotes.

The molecules are emitted by an individual and received by a second individual of the same species, that may cause a specific reaction.

Mice use pheromones , to regulate social behaviors of the same species such as pup, suckling , aggression and other activities.

- Pheromones have been characterized to generate either:

* signalling activity:

that result in a rapid change in motor activity by the recipient , such as aggression or fear or

* Priming activity:

that trigger rather slower neuroendocrine or endocrine changes in the receipient,

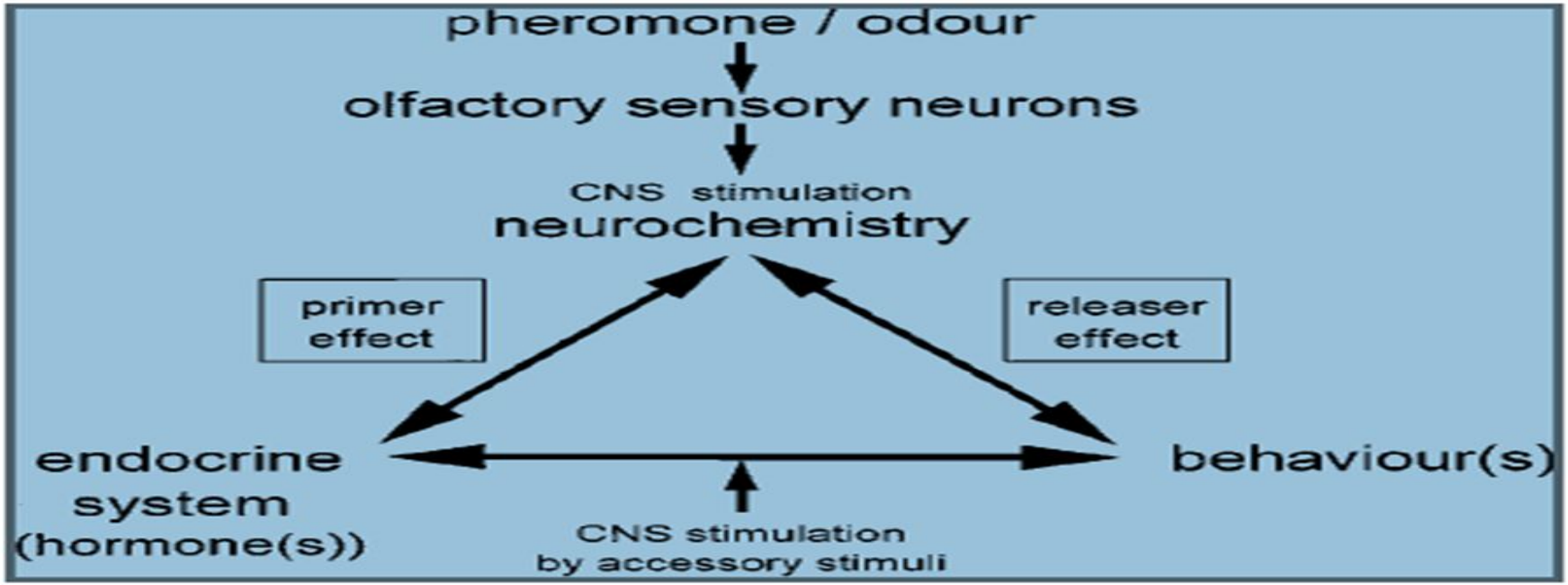


Figure: Showing the interaction and feedback loops in the sequence from odor to behavior and endocrine effects (Sachs ,1999).

- In the house mouse, there are about **40** identified pheremonal compounds in preputial glands secretions:

* 31 were esters ,
* 2 sesquiterpens, and
* 7 alcohols . (Zhang *et al*, 2007).

- Numerous studies have revealed that preputial glands of male mice are the main sources of pheromones .

The preputial glands are androgen- dependent organ, usually steroid hormones , that stimulates or controls the growth and development of the gland in rodents.

Many studies have suggested that the activity of preputial glands depended on the Melanocortin 5- Receptor (MC5R) , which is one of the family of five G protein- coupled receptors (GPCRs; MC1R–MC5R) expressed in different tissues, which serve diffrent physiological functions (Ramachandrappa *et al* , 2013).

Medicinal plants play a role in physiological and behavioural activates of animals. Crude extracts from some medicinal plants are widely utilize for their antifertility potentials in rats and mice.

Homady *et al*., (2000), have reported that reduction in the seminiferouse tubules diameter, impairments in the testicular germinal cells, preputial glands weight and histological changes , in addition to social aggression was observed in treated mice with some medicinal plants.

In contrast, other plants such as *Eruca sativa* have potent effects on the sex accessory organs and fertility of male mice (Homady , 2000, 2003).

Eruca sativa also have anticancer effects for inhibition of melanoma tumor growth (Khoobchandani *et al.*,2011), also it has a potent anti-inflammatory in the prevention of inflammatory skin diseases, such as soriasis , and possess a potent antioxidant to renal protective activity.

Arugula (*Eruca sativa*)

Up to our knowledge there was no work has done on both MC5R or GC-Ms analysis of the preputial pheromones of mice treated with many drugs or medicinal plants, so the present study is aimed to:

Evaluate the effect of 3mg/kg/day of *Eruca sativa* on:

* MC5R level;
* Total lipid components;
* Essential pheromonal compounds and
* Histological structure of preputial gland of castrated mice.

Materials and Methods:

Tuck Ordinary (TO) strain albino mice were used in this experiment. Thirty grouped-housed animals of comparable age (3 weeks) were allocated as to two groups A and B (N= 15):

* Group A intact male mice received tap-water as control,
* Group B castrated male mice treated with 3mg/kg/day of *E. sativa* for 42 days.

Daily administration :

Intra-gastric application of plant extract was made and the fluids wer administered for 6 weeks.

In the course of the experiment (3 g) of the extract residue was dissolved in 100 ml of distilled water immediately before administration to the animals and 3mg/kg/day of *Eruca sativa* was used .

At the end of the experiment, the mice were killed by cervical dislocation and samples taken for the study.

The Results

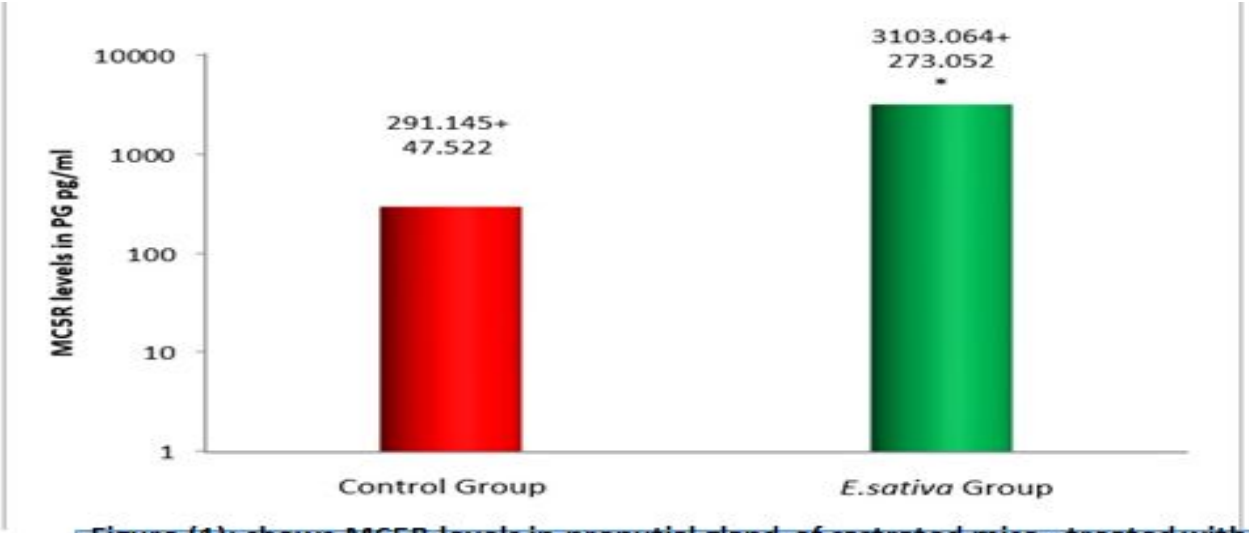


Figure (1): shows MC5R levels in preputial gland of castrated mice treated with 3mg/kg/day of *E. sativa* .
This treatment resulted in significant increase of MC5R levels in the serum ($P<0.01$) as compared with the control subject.

By using a combination of both :

Gas Chromatography and Mass Spectrometry (GC-MS), to analysis the total lipid compounds (volatiles), found in the preputial gland of intact control group, it appeared that there are fifty five (55) compounds in the gland as appeared in the following table.

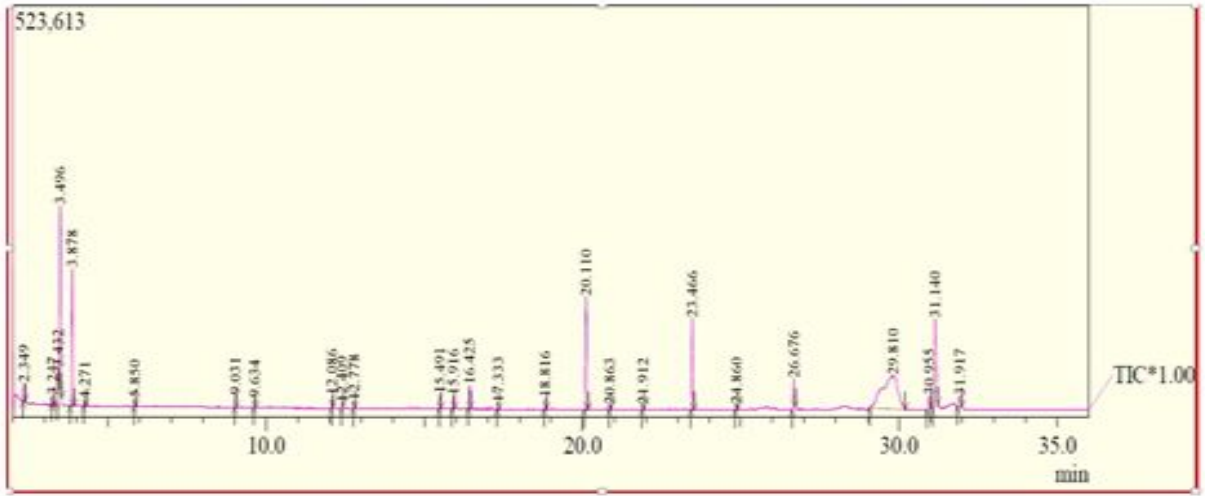


Figure (2): showing the peaks of volatile compounds found in the preputial glands of intact control group.

		GC peak	RetentionTime	Compounds
		1	3.267	Bis (2-hydroxypropyl) ether
		2	3.517	Cyclobutane, 1,2-bis(1-methylethenyl)-, trans
		3	3.758	Decane, 3-ethyl-3-methyl
		4	3.892	7-Octen-2-ol, 2,6-dimethyl
		5	4.283	1,6-Octadien-3-ol, 3,7-dimethyl
		6	4.575	4-[2-(2-Propenyl)-1,3-dioxolan-2-yl]-1H-pyrazole
		7	4.650	1,3,3-Trimethylbicyclo[2.2.1]heptan-2-ol
		8	4.892	3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)
		9	5.067	Cyclohexanol, 1-methyl-4-(1-methylethenyl)
		10	5.242	2-Propenoic acid, 2-methyl-, ethenyl ester
		11	5.367	exo-2-Hydroxy-1,7,7-trimethylnorbornane
		12	5.500	endo-2-Hydroxy-1,7,7-trimethylnorbornane
		13	5.667	(-)-Terpinen-4-ol
		14	5.858	alpha.-Terpineol
		15	5.975	.gamma.-Terpineol
		16	7.525	Sulfurous acid, 2-ethylhexyl hexyl ester
		17	8.500	2,2,5-Trimethylhexane-3,4-dione

		18	[illegible]	alpha.-Terpineol acetate
		19	[illegible]	Acetic acid, geraniol ester
		20	[illegible]	2,4,6-Trimethyl-1-nonene
		21	[illegible]	3,5-Xylenol, 2,4-dichloro
		22	[illegible]	N-Tosyloxy-2,2-bis(trifluoromethyl)aziridine
		23	[illegible]	1,5-Heptadiene, 2,5-dimethyl-3-methylene
		24	[illegible]	β- Farnesene
		25	[illegible]	Oxalic acid, cyclobutyl nonyl ester
		26	[illegible]	alpha. Isomethyl ionone
		27	[illegible]	5-Acetyldihydro-2(3H)-furanone
		28	[illegible]	3,8-Dimethylundecane
		29	[illegible]	p-Aminophenyl trifluoromethyl ether
		30	[illegible]	α.-Farnesene and (E)- α.-Farnesene
		31	[illegible]	Carbamic acid, methyl-, 2,6-di-tert-butyl-p-tolyl ester $$ Azak
		32	[illegible]	6-Heptenoic acid, 4-isopropenyl-6-methyl-2-oxo-, ethyl ester$$3-Tetradecen-5-yne
		33	[illegible]	Phthalic acid, 4-bromophenyl ethyl ester
		34	[illegible]	6-Methyl-1-octene
		35	[illegible]	Decane, 6-ethyl-2-methyl
		36	[illegible]	Pyruvic acid, 3-hexenyl ester or 1-Octanol, 2-nitro
		37	[illegible]	Natural rhodinol, acetylated or Propanoic acid, 2-methyl-, 3,7-dimethyl-6-octenyl ester
		38	[illegible]	3-Hexanone, 2,5-dimethyl, or Pivalic anhydride
		39	[illegible]	Oxalic acid, cyclohexyl dodecyl ester , Cyclopenteneacetic acid, 3-oxo-2-pentyl-, methyl ester
		40	[illegible]	3,9-Dodecadiene

		42	16.575	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl-, trans
		43	17.125	n-Hexyl bromide
		44	17.333	Dodecane, 2,7,10-trimethyl
		45	18.275	Sulfurous acid, 2-ethylhexyl hexyl ester
		46	20.108	Hexadecanoic acid, 16-hydroxy-, .omicron.-lactone
		47	20.433	1- Tetradecanol
		48	20.867	1-Hexadecanol
		49	22.525	Pentadecanoic acid or Palmitic acid
		50	23.467	1-Hexadecanol, acetate
		51	25.958	Tetradecanol, acetate
		52	26.392	Octadecanoic acid or Eicosanoic acid
		53	30.942	9-Octadecen-1-ol, (E) or 1-Eicosyne
		54	31.142	3-Dodecanol
		55	34.233	1,13-Tetradecadiene

No.	Essential Pheromones
1	β- Farnesene
2	(E)- α.-Farnesene and α.-Farnesene
3	1- Tetradecanol
4	1-Hexadecanol
5	1-Hexadecanol, acetate
6	Tetradecanol, acetate

This table showing the essential pheromone compounds found in the preputial gland of the normal group .

- The treatment of castrated subject with *E. sativa* was able to restore **26** compounds of volatile lipid components in the preputial gland, (out of 10 compounds as appeared in castrated subjects , previous study) .

- Additionally this treatment has dramatic effects for restoration **4** of the essential pheromonal compounds (out of 6 for the control group and was 0 in castrated group as compared).

Figure (4): Showing the total lipid contents in the preputial gland of *E. sativa* treated group as compared with the control.

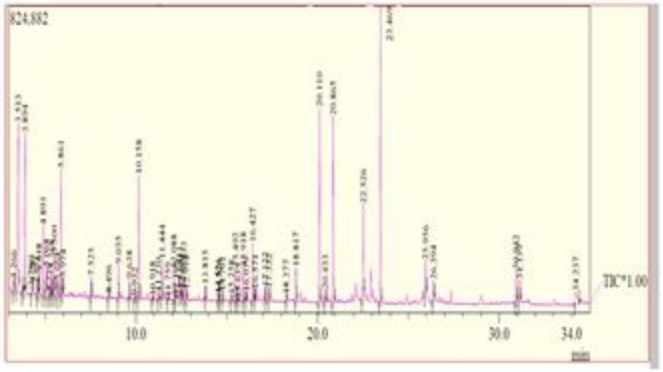


Figure (3): Showing the chromatogram of the peaks of **volatile compounds** found in the preputial glands of castrated treated with 3mg/kg/ of *E. sativa*

This table showing the essential pheromonal • compounds found in the preputial gland of castrated treated groups.

GC peak	Retention Time	Compounds
1	2.350	Benzene-1,2-dimethyl
2	3.250	Bis(2-hydroxypropyl) ether
3	3.433	Benzene, 1-ethyl-2,4-dimethyl-
4	3.500	Cyclohexene, 1-methyl-4-(1-methylethenyl)-
5	3.875	2,6-Dimethyl-7-octen-2-ol
6	4.275	Pentane, 3-bromo
7	5.850	4-[(7-Hydroxy-4-oxy)heptoxy]acetophenone
8	9.033	2-Oxopropanoic acid, (2'-acetylphenyl) ester
9	9.633	1-Penten-3-one, 2,4-dimethyl
10	12.083	3-Penten-2-one, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)

11	12.408	Butanoic acid, (tetrahydro-2-furanyl)methyl ester
12	12.775	α.-Farnesene and (E)- α.-Farnesene
13	15.492	Bicyclo[2.2.1]heptane, 2,2,3-trimethyl-, endo
14	15.917	3-Methyl-2-butenoic acid, cyclobutyl ester
15	16.425	1,2-Pentanediol, 5-(6-bromodecahydro-2-hydroxy-2,5,5a,8a-tetramethyl-1-naphthalenyl)-3-methylene-, 1,2-diacetate
16	17.333	Butane, 2,2-dimethyl
17	18.817	1H-Tetrazole-1,5-diamine
18	20.108	1,3-Propanediol, 2-dodecyl
19	20.867	1- Tetradecanol
20	25.467	1-Heptadecanol, acetate
21	24.858	Oxalic acid, allyl hexyl ester
22	26.675	Tetradecanol, acetate
23	29.808	Cyclopropanemethanol, .alpha.,2-dimethyl-2-(4-methyl-3-pentenyl)-, [1alpha.(R*),2.alpha.
24	30.958	Pyruvic acid, 3-hexenyl ester
25	31.142	9-Octadecenamide (Oleic acid amide)
26	31.917	1-Hexadecanol, acetate

No. of compounds	Essential Pheromonal Compounds	Intact Control Group	Castrated Treated Group
1	β- Farnesene	+	—
2	(E)- α.-Farnesene and α.-Farnesene	+	+
3	1- Tetradecanol	+	+
4	1-Hexadecanol	+	—
5	1-Hexadecanol, acetate	+	+
6	Tetradecanol, acetate	+	+

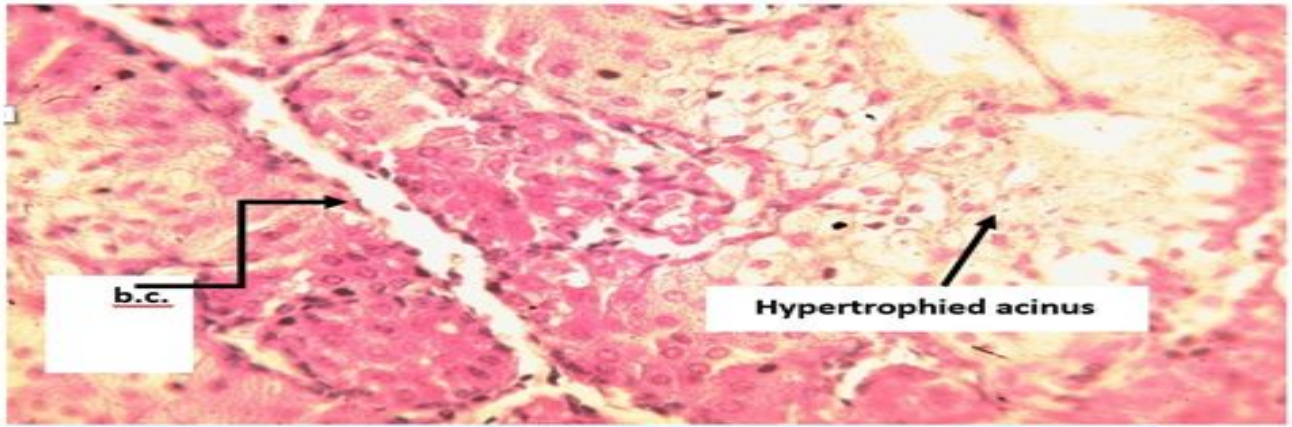
Table (1) Showing the essential pheromonal compounds found in the preputial gland of both intact control and castrated treated groups.

* The histological examination of preputial gland from intact control group, showed the normal structure of the gland .

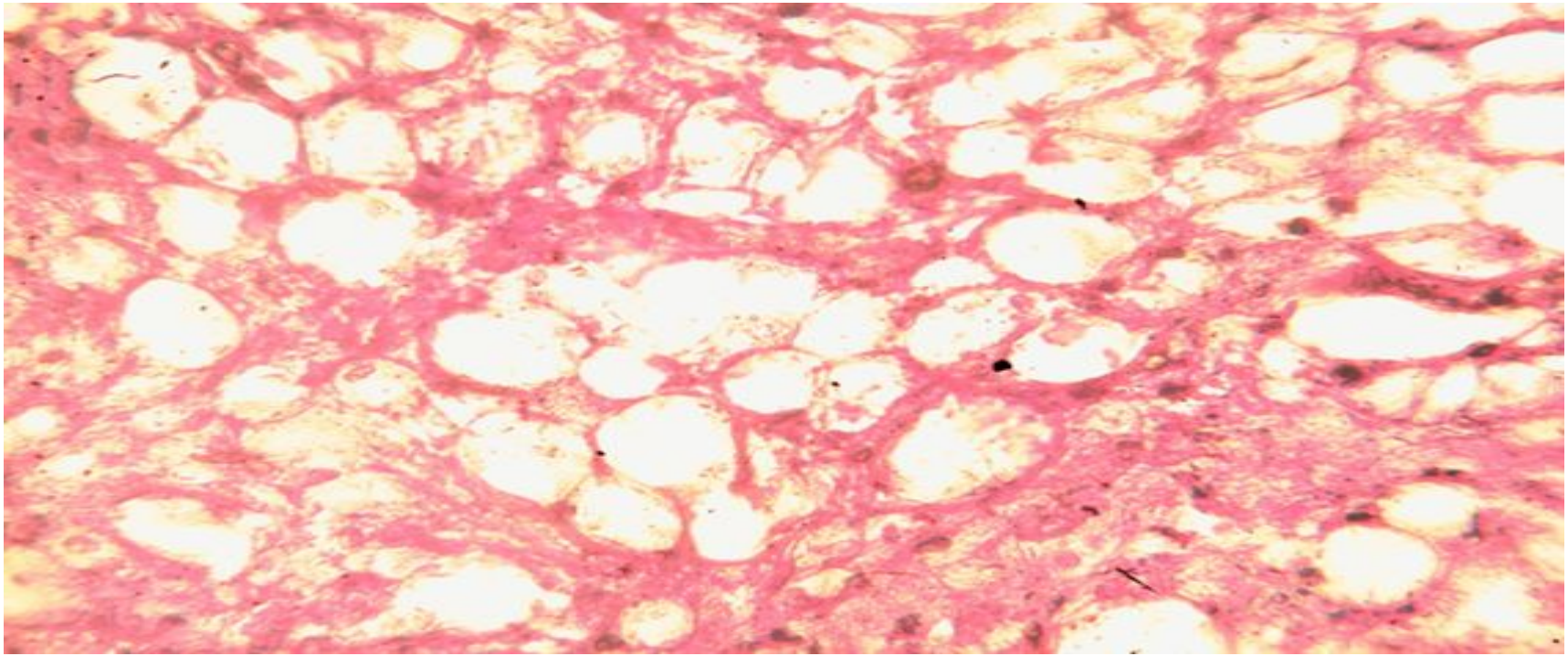
The gland is surrounded by a capsule of dense connective tissue, from which trabeculae extend to the interior to divide the parenchymatous tissues into alveoli which are at different stages of maturation and many of them are hypertrophied with numerous normal cytoplasmic organelles and healthy oval-shaped nuclei.

* Basal cells those surrounding the acini are more obvious.

* The gland has a long excretory duct with a wide lumen lined by stratified squamous epithelium and the ducts empty into the preputial cavity.



Cross histological section of the preputial gland from intact mouse stained with H&E, showing the acinus and basal cells (b. c.). (400X).

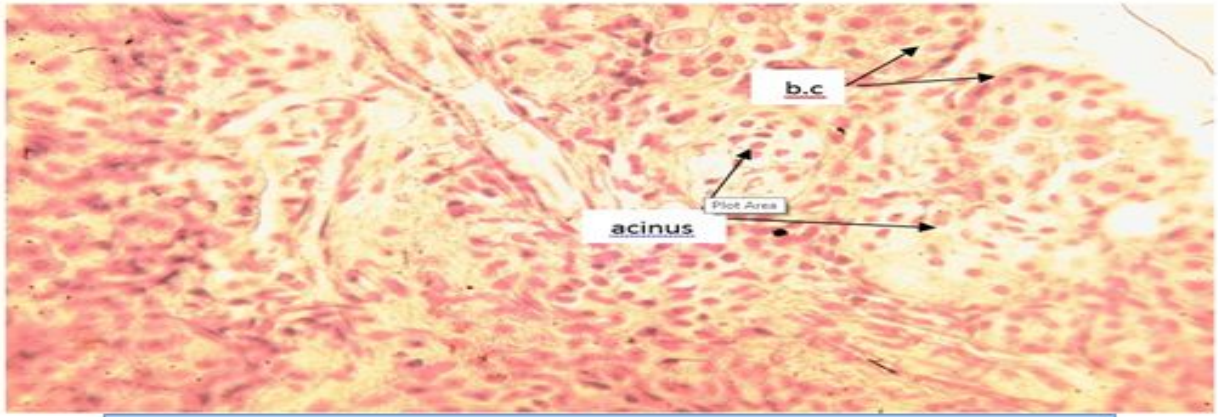


Histological sections of preputial glands from castrated subjects treated with *Eruca sativa* revealed that:

*The gland was well developed with many acini at different stages of maturation.

Most are saccular with covering of basement membrane and well-developed connective tissue.

* Acinar cells appeared in good condition with variable shapes, nuclei in such acini assumed different shapes.



Cross histological section of the preputial glands from castrated mouse treated with *E.sativa* stained with H&E, showing the arrangement of numerous basal cells (b.c) around acini and (400X)

Discussion:

- ***Eruca sativa*** has an enhancement factors on both MC5R level and essential pheromonal compounds of the mouse preputial gland.
- ***Eruca sativa*** was able to restore both glandular activity and MC5R level in preputial gland of castrated subjects.

* Such treatment also has marked effects on the total lipid compounds (26 out of 55 compounds are restored) as well as it was able to restore four essential pheromonal compounds (α.-Farnesene and (E)-α.-Farnesene); (1-Tetradecanol); (Tetradecanol, acetate) and (1-Hexadecanol, acetate) .

* These dramatic effects may be resulted from the presence of some trace elements (Cr, Cu, Fe, Mn and Zn) in the *Eruca sativa* extract (Bukhashi *et al* 2007) .

* Copper (Cu) has been shown to be an important factor for immune system function based on evidence that Cu metabolism affects the function of several classes of immune system cells particularly those involved in producing antibodies.

- Cu is also vital for the activity of an enzyme responsible for removing toxic free radicals from the body (Cu-Zn superoxide desmotase) as well as for the activity of phagocytes (Ahola *et al.*, 2008).
- * The histological restoration of preputial gland and maturation of many acini with most of them are hypertrophied as to the normal structure indicate that the presence of orally- active agents in extracts of *E. sativa* which suggest the presence of hormone influencing compounds with varied effects.

* Moreover this can be attributed to effect on either cellular respiration or due to accumulation of lipid droplets or glycogen in the cytoplasm of secretory cells.

* Other substances produce cytoplasmic vaculation of tissue act by accumulation of phospholipids and concentration membranous structures within secondary lysosomes of these cells (Yano *et al.*, 2001).

* Phytochemical analysis of *Eruca sativa* indicates that alkaloids, cardiac glycosides, flavonoids, phenolics, ascorbic acid, saponins and tannins are present in the seed, leaves of the *E. sativa* (Gulfraz *et al*., 2011).

* All these compounds are responsible for different bioactivities including antimicrobial activity against various pathogenic microorganisms (Ettebong and Nwafor, 2009).

*Alkaloids and their synthetic derivatives are being used as basic therapeutic agents for their analgesic, antispasmodic and bactericidal effects.

It has been reported that: •

* Natural ascorbic acid is vital for the body • performance (Okwu and Josiah, 2006; Aiyelaagbe and Osamudiamen, 2009).

* The presence of phenolic compound in the *E. sativa* • indicates its antimicrobial properties against pathogenic bacteria (Khoobchandani *et al.,* 2010).

* Cardiac glycosides are helpful to overcome various • human diseases.

* Saponin has the property of precipitating and coagulating red blood cells (Okwu and Josiah, 2006).

Solvent extracts from *E. sativa* were assayed for anticancer activity against melanoma cells, that inhibited melanoma growth and angiogenesis in mice without any major toxicity. (Khoobchandani, *et. al.*, 2010).

- Further studies are needed to identify the active principals of this plant on the pheromonal compounds of this gland.

Thank you