Bulletin of Faculty of Science ,Zagazig University (BFSZU) **2023**
Bulletin of Faculty of Science, Zagazig University (BFSZU)
e-ISSN: 1110-1555
Volume-2023, Issue-3, pp-77-86
Research Paper DOI: **10.21608/bfszu.2023.187732.1242**

Control of Theba pisana land snails using pharmaceutical mono-hormonal contraceptive drug at Sharkia Governorate.

Mahmoud, S. Abd El-Atti[1], Mahmoud, A. Desouky[1] , Ali, A. Elsheakh[2], Wesam, S. Elgohary[2]

1 Zoology Department, Faculty of Science, Zagazig University, Zagazig, 44519, Egypt.
2 Plant Protection Research Institute, Agriculture Research Center, Doki, Giza, Egypt.
Corresponding author. mahmoudatti1@yahoo.com

ABSTRACT: ***Theba pisana is considered one of the most prevalent and dangerous land snails in the Mediterranean region especially in Egypt. Owing to their small sizes, climbing tendency, capability of producing hazardous effects on plants and, high reproductive rates, all these make difficulties of their effective control. The goal of the current study was to control this harmful snail by applying The Ovunhipita contraceptive pills. Three different concentrations of the drug (0.75, 1.5, and 3 µg/g) were tested on the reproductive biology, antioxidant enzymes and the histological structures of both hermaphroditic and digestive glands after 6 weeks of treatments using baits technique. Results indicated that egg masses laid / snail were decreased significantly (0.25, 0.15, and 0.15 egg mass/snail) after treatment with 0.75, 1.5 and 3 µg/g, respectively compared to control (1.65 egg mass/snail). Egg numbers /mass were significantly reduced to 56, 53, and 15.9 eggs/mass respectively compared to control (91.12 eggs/ mass). In addition, the number of eggs laid by snails, the rate of hatchability was significantly decreased with increasing concentration of the drug. The tested antioxidant enzymes (CAT, SOD, and MDA) activities were increased, while GST levels were decreased significantly compared to control. Histological investigations of treated snails for 6 weeks showed alterations in the normal structures of both ovotestis and digestive glands. The application of a mono-hormonal contraceptive drug is an environmentally safe method for controlling harmful land snails in spite of using chemical pesticides.***

Date of Submission: 19-01-2023 Date of acceptance: 29-01-2023

I. INTRODUCTION

Worldwide, land snails are considered one the most serious agricultural pests. Vegetables, grains, decorative plants, orchard trees and diverse plant species can be targeted by sails at various growth phases (El-Okda, 1980; Godan, 1983). Snails can leave an unpleasant odor on plants as a result of their mucous secretions during their movements causing contamination of plants and preventing animals from eating (Sallam et al., 2009). The most efficient way of control for mollusks is still chemical molluscicides, although several risks for people, animals, and almost all terrestrial organisms (Gabr et al., 2006; Gad, 2022)). Snails are hermaphrodites that sperms mature before eggs and have a reciprocal exchange of sperms during mating (Routray and Dey, 2016). The white garden snail Theba pisana (Gastropoda: Helicidae) is an invasive terrestrial snail and spread in many countries in the Mediterranean region (Neubert, 1998; Herbert 2010). This species is widely distributed in various regions at Sharkia Governorate, Egypt. T. pisana is particularly dangerous due to its small size, capacity to survive long and explosive reproductive rates, which can result in up to 3,000 snails on a tree in less than five years (Cowie 2009, Deisler et al. 2015). During one breeding season, T. pisana produced up to 4566 eggs / couple (Cowie, 1984; Baker, 1991). In nests that the snails have dug out, eggs are laid a centimeter or two below the surface (Hodel et al., 2018). The control of land snails has historically relied on chemical methods. The use of poisonous molluscicides and the prevalence of harmful snails may be reduced by treating snails with safe drugs and naturally toxic products (Abd El-Atti, et al., 2020a). Synthetic hormones may interfere with the reproductive biology of snails and may be used as an efficient method for minimizing land snails population densities. Estrogen and progestin hormones inhibited the growth of ovarian follicles by reducing of follicle-stimulating hormone secretion in monkeys (Koering et al., 1994). The combination of progesterone and

estrogen hormones elevated their ovulation-inhibitory effects (Curtis et al., 2013). Ovunhipita (Desogestrel 75µg) is a commercial drug used as a mono-hormonal contraceptive drug and has the power to inhibit female ovulation **(Scala *et al.*, 2013).** The present study deals with studying the effect of different concentrations of synthetic progesterone hormone on the reproductive biology, antioxidants and the histology of ovotestis and digestive glands of *T. pisana* land snails. This research hopes also to use a safe technique for controlling these deleterious agricultural pests and reduce their great populations in fields of Sharkia Governorate.

. II.Materials and Methods

2.1. Materials:

2.1.1. Collection and habituation snails

Mature white garden snails *T. pisana* (15-18 mm length and 12-15 mm width) were collected from El-Mohammadia village, Menia Al-Qamh district, Sharkia Governorate, Egypt, in October 2021. The collected snails were transferred to the laboratory and kept in glass cages (40 x 30 x 30 cm) containing moist soil about 10 cm high and covered with muslin cloth and tied with a rapper band to prevent them from escaping. They fed daily on fresh lettuce leaves (*Lactuca sativa* L.) and the rearing cages were also cleaned daily by removing the traces of the decayed leaves and feces of snails for 2 weeks. Snails were kept at 20 ºC ± 2 ºC and a relative humidity of 80–90 % **(El- Okda, 1984**).

2.2. Methods:

2.2.1. Baits preparation

The Ovunhipita contraceptive drug is a progestin mono hormonal drug manufactured in Egypt. Each tablet contains 75µg of the synthetic hormone Desogestrel. Three different concentrations were prepared (0.75, 1.5, and 3µg/g). Baits were formulated by adding 5g sugarcane syrup to 95g of moist wheat bran. Snails were divided into 4 groups; the first is untreated (control) and three groups were fed on baits containing three different concentrations of the drug and 5 replicates were applied. Each replicate was prepared by adding four snails and left to feed on 10g of the baits for six weeks.

2.2.2. Biological studies

Egg numbers were counted weekly during the period from 15 November to the end of December. Egg masses were removed weekly from the soil and put in a Petri dish then counted and left to be hatched. Juveniles were counted and the incubation period and hatching rate were determined for 6 weeks.

2.2.3. Enzymatic activities measurements

Control and treated snails were dissected and soft tissues were dissected out and homogenized in distilled water using a Teflon homogenizer. The resulting homogenates were centrifuged at 4000 r.p.m. for 10 minutes in a refrigerated centrifuge. Deposits were discarded and supernatants were kept in a deep freezer until use. The activities of catalase (CAT), superoxide dismutase (SOD), glutathione-S- transferase (GST) and Malondialdehyde (MDA) were analyzed spectrophotometrically using kits obtained from Bio Diagnostic Company.

2.2.4. Histological investigations

The hermaphrodite and digestive glands of both control and treated snails (3µg/g Desogestrel) were dissected out and fixed in alcoholic Bouin. Specimens were dehydrated in an ascending series of ethyl alcohol, cleared in Xylene and finally embedded in paraffin wax. Sections (4–5 µ m thick) were mounted on glass slides and stained with Hematoxylin and Eosin.

2.2.5. Statistical analysis

Data were expressed as means (±SE) and analyzed by one-way analysis of variance (ANOVA). Duncan test was used to calculate significant differences ($P < 0.05$) between treatments **(Costat, 2005).**

III.RESLUTS And DISCUSSIONS

3.1. Reproductive biology of *T. pisana* snails treated with 3 different concentrations of Desogestrel

Table (1) indicated that the reproductive biology of treated snails such as egg mass/ snail, egg number/ mass, egg number/ snail, incubation period/ day and the hatching rate of eggs were dramatically decreased with increasing the concentration of Desogestrel. The highest concentration (3µg/g) induced the greatest decrease of all measured parameters.

Table (1): Effect of 3 different concentrations of *Ovunhipita* contraceptive drug on the reproductive biology of *T.*

parameters		Egg Mass/Snail	Egg No./ Mass	Egg No./Snail	Inc. period(day)	Hatch. Rate %
Control		$1.65^{a}\pm0.05$	$91.12^{a}\pm0.98$	$145.7^{a}\pm4.4$	$11.78^{a}\pm0.05$	$96.94^{a}\pm0.14$
Drug conc. µg/g	**0.75**	$0.25^{b}\pm0.0$	$56^{ab}\pm2.15$	$14^{b}\pm0.54$	$13.2^{a}\pm0.17$	$80.8^{ab}\pm3.3$
	1.5	$0.15^{b}\pm0.03$	$53^{ab}\pm10.2$	$13.25^{b}\pm2.5$	$6.8^{a}\pm1.28$	$41.33^{bc}\pm8.32$
	3	$0.15^{b}\pm0.04$	$15.9^{b}\pm4.5$	$5.5^{b}\pm1.52$	$5.5^{a}\pm1.5$	$25.09^{c}\pm6.88$
LSD 0.05		0.23	38.18	17.96	6.64	37.86
P		0.0000 ***	0.0069 **	0.0000 ***	0.0701 ns	0.0032 **

pisana snails after 6 weeks of treatment**.**

Variables were presented as mean ± S.E. Mean values with the same alphabetical superscripts are statistically insignificant. Values are statistically significant at $P < 0.05$.

Egg masses/snail decreased significantly with increasing the drug concentrations (0.75, 1.5, and 3 µg/g) and the percentage of decreases were 84.9%, 90.9%, and 90.9% respectively (Fig.1). The egg number / mass and egg number / snail were significantly reduced with increasing the concentrations of progesterone synthetic hormone. The highest concentration of Desogestrel (3 µg/g) caused decreasing the number of eggs by 96.23%. In addition, the hatching rate percentage of the fertilized eggs was gradually decreased with increasing concentration of progesterone by 80.8%, 41.33%, and 25.09 % respectively compared to control(Fig. 1).

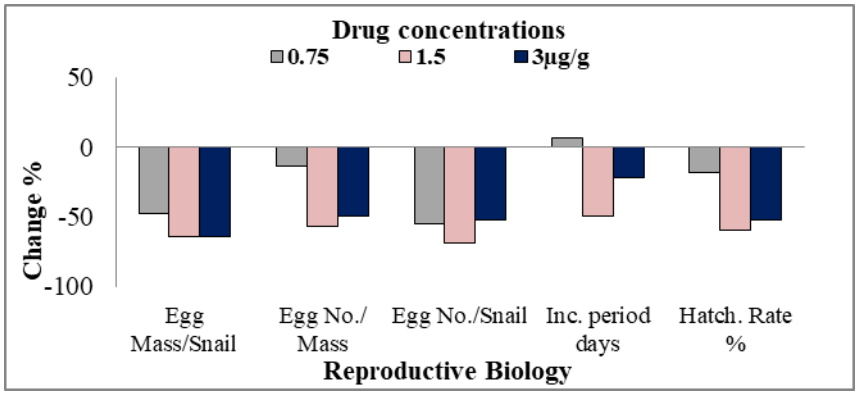

Figure 1: Changes % of some reproductive aspects of *T. pisana* snails treated with 3 different Concentrations of the synthetic progesterone hormone (Desogestrel) for 6 weeks.

3.2. Antioxidant enzymes assay

The highest concentration of Desogestrel (3 µg/g) caused a highly significant increase ($P<0.05$) of catalase, superoxide dismutase and Malondialdehyde enzymes while glutathione-S- transferase (GST) enzyme levels were decreased after 6 weeks of treatment (Fig. 2). Catalase enzyme (CAT) activity increased up to 15.15 U/g with an increasing percentage 66.4% compared to control (9.1U/g).

The activity of superoxide dismutase (SOD) was elevated to (156.7 U/g) with a percentage of (26.9%) compared to control (123.6 U/g). Malondialdehyde(MDA) levels were elevated up to 4.92 nmol/g. Contrary, glutathione-S-transferase (GST) was decreased (1.8 U/g) and the percentage was -59.2% compared to control snails (4.44 U/g).

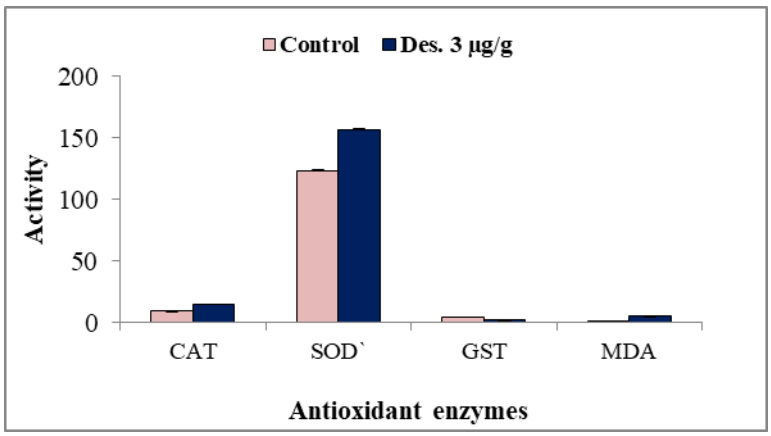

Figure 2: The activities of some antioxidant enzymes in T. pisana snails treated with 3µ/g Desogestrel

3.3. Histological investigations

3.3.1. Histological structure of the normal hermaphrodite gland of untreated *T. pisana* snails

An embedded hermaphrodite gland can be seen in the digestive gland of *T. pisana*. This gland has many acini with oval, flattened nuclei and irregular chromatin patches that are surrounded by squamous follicular epithelium.

Description of male acini for Spermatogenesis

Sertoli cells; with ovoid nuclei, inserted between follicular cells and have an abundance of euchromatin (Plate 1B). The stages of spermatogenesis can be concluded as the following:-

1) Spermstogonia; are small, semi-rounded cells and nuclei are strongly basophilic. By progressively dividing by mitoses near to the inner acinial wall, these cells form a cluster around Sertoli cells (Plate 1A&B). **2) Primary spermatocytes**; the first stage of spermatocyte development are somewhat large cells, rounded or pear-shaped, and have large nuclei (Plate1A). **3) Secondary spermatocytes;** are cylindrical and longer than primary spermatocytes and they contain apical nuclei (Plate1B). **4) Spermatid;** Three stages of spermatids can be determined. The early spermatids (ESd) are cylindrical and have small centrally located oval nuclei.

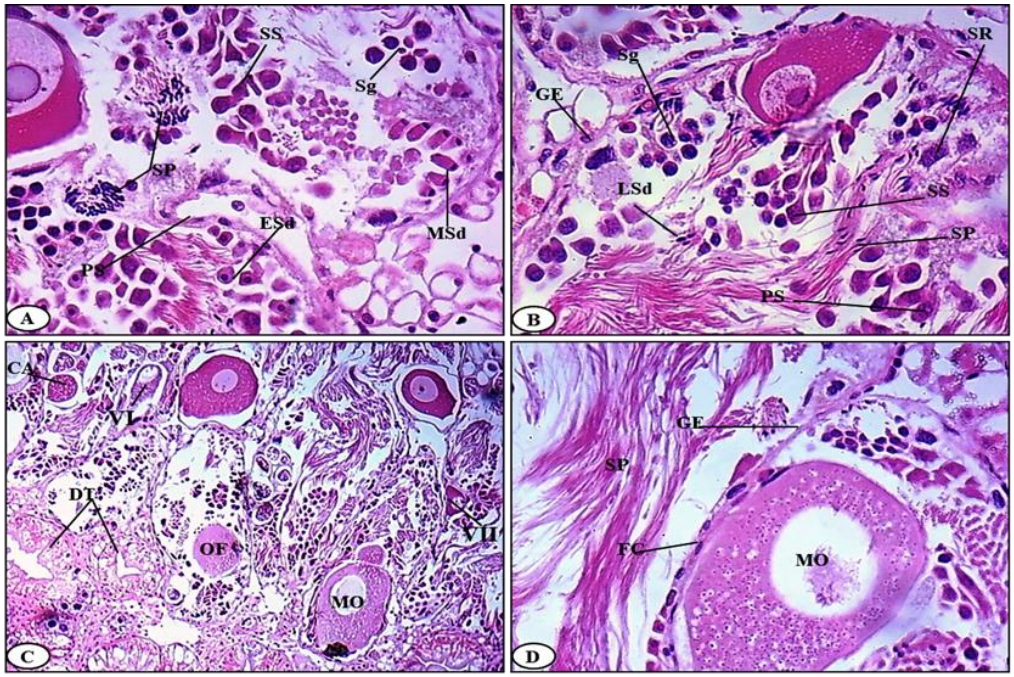

Plate 1: T.S. of the hermaphrodite gland of untreated *Theba pisana* stained with H&E showing different stages of spermatogenesis and Oogenesis. (A&B): show different stages of spermatogenesis (X=400). (C): show different stages of oogenesis (X=100). (D): T.S showing higher magnification of female acinus containing mature ovum (X=400). *CA: Corpus albicans; DT: Digestive tubule; ESd: Early stage spermatid; FC: Follicular Cell; GE: Germinal epithelium; LSd: Late stage spermatid; Mo: Mature ovum; MSd: Middle stage of spermatid; OF: Ovulated follicle; Ps; Primary Spermatocytes; Sg: Spermatogonia; SR: Sertoli cell; SP: Spermatozoa SS: Secondary spermatocytes; VI: Vitellogenic oocyte I; VII: Vitellogenic oocyte II.*

The intermediate spermatids are elongated with kidney-shaped nuclei and are attached to Sertoli cells. The late spermatids have kidney-shaped nuclei, much more elongated axoneme and the spermatids continue to expand, causing the entire cell to enlarge (Plate A&B). 5) Mature sperm; newly produced spermatozoa are concentrated on Sertoli cells and finally clustered in the male acinial core. They have much longer tails, mid-pieces, and fusiform-shaped heads (Plate 1A-D).

Description of female acini for Oogenesis

The germinal epithelium surrounding the acini divided to produce female germ cells. The younger cells are found near the proximal part of the acinus, whereas the older, bigger cells finish differentiating there at the bottom. Oocytes were developed through successive previtellogenic and vitellogenic stages (Plate 1).
Vitellogenic oocytes I; are large rounded cells with central rounded eosinophilic nuclei and a few tiny follicular cells.
Vitellogenic Oocytes II; have enormous nuclei with nucleoli and a lot of yolk is present in their granular cytoplasm.
Vitellogenic oocytes III or mature oocytes; have a big number of follicular cells and a lot of yolk (Plate 1C&D).

3.3.2. Histopathological alterations in hermaphrodite gland of snails treated with 3 µg/g desogestrel.

All stages of gametogenesis in the hermaphroditic acini of snails treated with 3µg/g Desogestrel were histologically disrupted (Plate 2 A-D). Desogestrel highest concentration. caused abnormalities in all stages of spermatogenesis. Most of the germinal epithelial layer was swollen. It was discovered that few spermatogonia developed into spermatocytes, which had severe deteriorations. The majority of spermatidstages were not present in the acinus; nevertheless, the early stage could be distinguished and displayed abnormalities, and only a small number of them developed into spermatozoa, which seemed diminished (Plate 2 A&B). Oogenesis exhibited significant deformations at all stages. From the germinal epithelium, which seemed vacuolated, and the follicular layer, which had

deformations, the female acinus displayed some degeneration until it reached the mature state. Oogenesis' vetillogenic stages showed significant abnormalities, and mature oocytes were also negatively impacted (Plate 2 C&D).

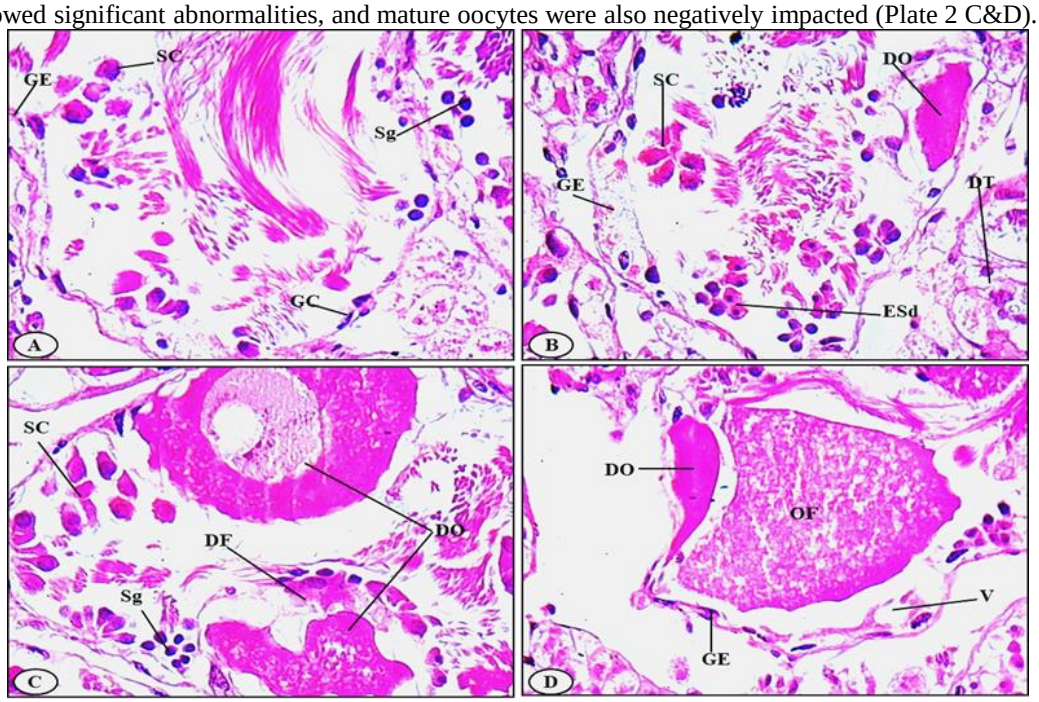

Plate 2: T.S. of hermaphrodite gland of *T. pisana* (Stained H&E) treated with 3 µg/g Desogestrel (X=400). (A & B) indicate alterations of stages of spermatogenesis. (C&D) show the deteriorations in the follicular layer and oocytes (X=400). *DT: Digestive tubule; DF: Deformed Follicular layer; DO: Deteriorated oocyte; ESd: Early Stage spermatid; GC: Germinal Cell GE: Germinal Epithelium; OF: ovulated Follicle; SC: Spermatocytes; Sg: Spermatogonia; V: Vacuole.*

3.3.3. Histological structure of the digestive gland of *T. pisana*.

a. Histological structure of the digestive gland of untreated *T. pisana* snails.

The digestive gland of *T. pisana* snails is composed of digestive tubules; the intertubular connective tissue is present between the digestive tubules. Each tubule is encircled by a layer of circular muscle. The epithelium lining the digestive gland tubules is made up of three main cell types. The cells are divided into digestive cells (DC), excretory cells (EC), and calcium cells (CC).

Digestive Cells: They are the most abundant type, have several vacuoles in their cytoplasm and are columnar in shape with slightly rounded apical surfaces. Their spherical or oval basally situated nuclei.

Calcium Cells: Conical cells found at the corners of the digestive tubules, found singly or in groups and contain rounded calcium spherules that appear as illuminated bodies and have a conical appearance. Apical secretory granules and big, spherical, central nuclei are characteristics of calcium cells.

Excretory Cells: Rounded and have a single, sizable vacuole that sometimes contains a sizable amount of secretory granules. Their basally situated, tiny, spherical nuclei are circular (Plate 3A).

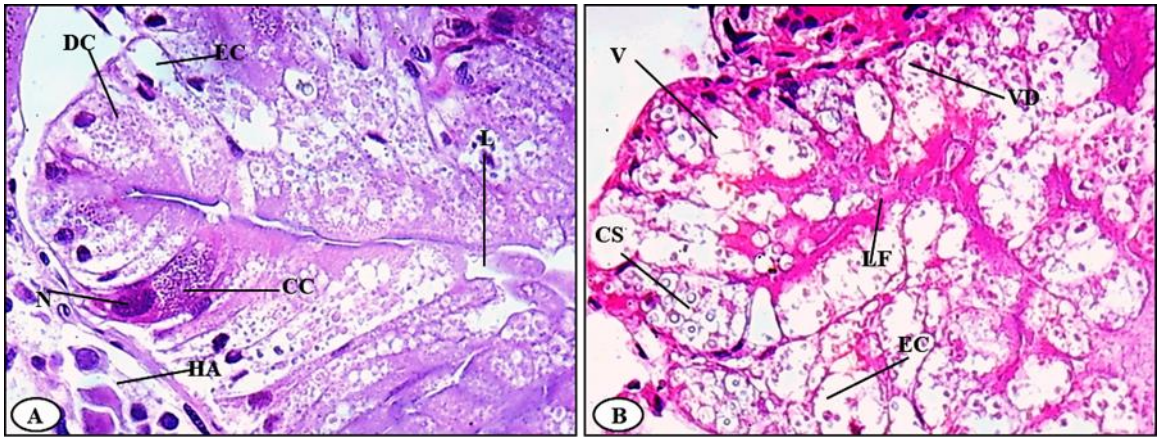

Plate 3: T.S. of the digestive gland of *T. pisana* snail stained H&E. (A): T.S. in the digestive tubule of untreated snails (X=400). (B): shows the histological alterations occurred in treated snails (X=400). *CC: Calcium Cell; CS: Calcium spherules; DC: Digestive Cell; EC: Excretory Cell; HA: Hermaphrodite Acinus; L: Lumen; LF: Excessive Lumenal Fluid; N: Nucleus; V: Vacuole; VD: Vacuolated Digestive Cell.*

b. Histopathological alterations in digestive gland of treated *T. pisana* snails.

Treatment of snails with 3 µg/g for 6 weeks produced few histological alterations inside the digestive tubules in the form of mild tubular deformations, vacuolation and excessive luminal fluid inside the digestive tubule. The digestive cells showed high vacuolated cytoplasm. Calcium cells showed decreasing number of calcium spherules and nucleus disappeared. The excretory cell was highly vacuolated with dark granules (Plate 3B).

IV. DISCUSSION

Land snails are very harmful pests to various kinds of crops at nearly all their parts. (**Hussein and Sabry, 2019).** Therefore, control of them was necessary in order to reduce the economic loss of crops. Chemical molluscicides were used for a long time in pest control but the excessive use of these harmful materials caused highly lethal effects on non-target organisms, so looking for other safe solutions was necessary. Ovunhipita is a mono-hormonal (progestogen) drug tested on *T. pisana* snail. It caused a significant decrease in Egg number/ snail and also reduced hatching rates with increasing concentrations. Their efficacy may be due to decrease the FSH and LH levels, leading to the suppression of follicular activity and ovulationas the active ingredient "Desogestrel of Ovunhipita contraceptive drug binds to the progesterone receptor found in the pituitary gland and female reproductive system **(Aronson, *et al.* 2015)**. This binding may dull the pre-ovulatory prosses and binds to the receptor of Luteinizing hormone **(Sitruk-Ware, 2008)**. Desogestrel is a third-generation progestogenic steroid, used as a female contraceptive. Ovulation is significantly inhibited by desogestrel due to its inhibitory effect on stopping femal's egg oviposition **(Scala *et al.* 2013)**. on the same trend, **Abd El-atti *et al.* (2020a)** used a combination of estrogen and progesterone represented in Yasmin® pills in affecting the egg production of *Eobania vermiculata* snail and found that it reduces the egg number laid. Also, results were in accordance with **Mubarak (2016**) who showed that the complete lack of ova in the ovotestis of *E. vermiculata* was produced by sublethal concentrations of chlorfluazuron (IGR). However, when estrogen and progesterone were given to *Achatina fulica* snails, the size of the ovotestis significantly increased **(Kruatrachue *et al.*, 1996)**.

A biological oxidant-to-antioxidant ratio imbalance is thought to cause oxidative stress, which can lead to oxidative damage to lipids, proteins, carbohydrates, and nucleic acids. Typically, an essential indicator of oxidative damage is the aberrant creation of ROS, which can seriously harm cell structure **(El-Demerdash, 2007).** When organisms are exposed to a chemical stress, the antioxidant system activity may either increase or decrease **(Ojha *et al.*, 2011)**. In the current study all the tested antioxidant enzymes (CAT, SOD, and MDA) were decreased significantly by treatment with the highest concentration of Desogestrel medication except GST is decreased. The increase in antioxidant enzymes may be due to oxidative stress induced by the drug and some disturbances in the digestive gland of treated snails. As informed the breakdown of hydrogen peroxide occurrs by CAT and SOD, which are mainly found inside peroxisomes **(Halliwell and Gutteridge, 1999)**.while Catalase is an essential enzyme in the detoxification

mechanisms and catalyzes the conversion of H2O2 to molecular water and oxygen **(Sanchezcasas *et al*., 1994). So,**the significant induction of CAT and SOD activities seen in this study is an indicator of cellular lesions and can be explained by the activation of an anti-oxidant mechanism to prevent the accumulation of ROS **(Radwan *et al*., 2010**). Similarly, **Khene *et al*. (2017**) found a significant increase in CAT activity in *Helix aspersa* snail after exposure to TiO2 nanoparticle. MDA also was significantly increased and this is in accordance with **Ugokwe *et al*. (2020)** who reported a significant increase in the activities of CAT, SOD, and MDA in the digestive gland of giant African land snail exposed to waste leachate. GST in the present study had been reduced significantly and this could be because of damages in the digestive gland which represents the main site of antioxidant formation (**Ferrari *et al*., 2007; Zarai *et al*., 2011**).

Histological structure of the hermaphrodite gland of the tested snail was highly affected by treatments of Desogestrel highest concentration. This could be a result of the decreases in luteinizing hormone (LH) and follicle-stimulating hormone which restrict the growth of ovarian follicles and so prevent ovulation **(Tafurt *et al*., 1980).** Similar to this, injecting vertebrate hormones such estradiol, testoviron, progesterone, and gonadotrophin into the gastropod of *Achatina fulica* led to significant histological changes in its ovotestis **(Kruatrachue *et al*., 1996**). Changes in the ovaries appear to be a result of the suppressive effect of contraceptive steroids on pituitary gonadotropin secretion **(Abd El-atti *et al*. 2020a)**. In Broiler chicken treated with Microgynon birth control pills, light microscopy revealed severe histopathological changes in the pituitary, thyroid, and adrenal glands that were represented by necrosis, cytoplasmic vacuolation, and hyperplasia **(Layth and Majdy, 2018)**. Numerous histological changes linked to oral contraceptive use have been found in women's ovaries and blood vessels, supporting this study. These changes include glandular hyperplasia, ovarian size reduction, suppression of follicle growth, reduced luteinization, and fibrosis in blood vessels **(Bernard, 1976).** The male acini of treated snails showed histopathological changes particularly in decreasing spermatogenesis stages. Desogestrel has an androgenic effect as it decreases the level of Testosterone hormone **(Stone, 1995)**. It has a considerable impact on the plasma levels of SHBG. As a result of this elevation, the levels of total and free Testosterone in circulation significantly decline, lowering their androgenic potency. This characteristic was helpful as an anti-androgenic treatment for women with hyperandrogenic symptoms **(Kaplan, 1995).** Given that Testosterone stimulates spermatogenesis in the testes **(Smith & Walker, 2014; Zhou *et al*., 2019),** it is possible that Desogestrel adverse effects on spermatogenesis stages are caused, by the interruption of Testosterone production. Similar results were results were observed in male mice treated with oral contraceptive pills (**Wafeeq, 2013**). Also, histological alterations in the ovotestis may be due to the oxidative stress occurred by the drug and this was confirmed by **Alchalabi *et al*. (2016)** who indicated the histopathological changes in ovarian and uterine tissues of rats associated with oxidative stress induced by electromagnetic waves.

In the current study on the digestive gland of *T. pisana*, histological analyses established the presence of three primary cell types: excretory, calcium, and digestive cells composing the epithelial wall of digestive tubules **(Lopes *et al*. 2001**; **Ismail *et al*. 2013; Sharaf *et al*. 2015; Abd El-atti *et al*. 2020b).**

Several mild alterations were observed in the structure of the digestive gland of *T. pisana* snails exposed to the highest concentration of Desogestrel for six weeks. Deformations appeared in the digestive, calcium, and excretory cells in addition to excessive fluid in the lumen of some tubules. Deformations induced might be due to the oxidative stress induced by the drug on the snail. High production of ROS and its direct interaction with biological tissues are the major mechanisms exerted by any strange introduced to biological systems **(Ma and Diamond, 2013)**. Also, the time of treatment may induce the oxidative stress that affected the tissue and the prolonged hyperoxia caused an inflammatory response resulting in inflammation and led to histological damages **(Nagato *et al*., 2012; Ibtissem *et al*., 2017)**.

V. CONCLUSION

The Ovunhipita contraceptive pill strongly affected the reproductive biology and antioxidant enzymes of T. pisana snails. This drug also induced many histopathological alterations in the hermaphrodite and digestive glands of treated snails. This environmentally safe baits technique was efficient as a control method and ensure the survival of non-target organisms harmed by using chemically synthetic molluscicides. In Egypt, using contraceptive medications to control snails will prove easy successful, applicable, environmentally safe, and effective enough to reduce high populations of land snails destroying commercial crops.

REFERENCES

Abd El-Atti, M. S., El-Sayed, A. S. A. and Said, R. M. (2020a): Usage of pharmaceutical contraceptive drug for controlling *Eobania vermiculata* snails by baits technique. Heliyon, 6 (12): 1-8.

Abd El-Atti, M.S., Khalil, A.M., Elsheakh, A.A. and Elgohary, W.S. (2020b): Biological control of *Monacha cartusiana* "glassy clover land snails" by microbial biopesticides Biozed and Biogard, using bait technique. Biocatalysis and Agricultural Biotechnology, 25: 1-6.

Alchalabi, A.S., Rahim, H., Aklilu, E., Al-Sultan, I.I., Malek, M.F., Ronald, S.H. and Khan, M.A. (2016): Histopathological changes associated with oxidative stress induced by electromagnetic waves in rats' ovarian and uterine tissues. Asian Pacific Journal of Reproduction, 5(4): 301-310.

Aronson, J.K. ed. (2015): Meyler's side effects of drugs: the international encyclopedia of adverse drug reactions and interactions. Elsevier.

Baker, G. H. (1991): Production of eggs and young snails by adult *Theba pisana* (Müller) and *Cernuella virgata* (da Costa) (Mollusca: Helicidea) in laboratory cultures and field populations. Austral. J. Zoo. 39(6): 673-679.

Bernard, G.M.D. (1976): Histologic changes associated with oral contraceptive usage. Ann. Clin. Lab. Sci. 6 (4): 291–299.

Cowie, R. H. (1984): The life cycle and productivity of the land snail *Theba pisana* (Mollusca: Helicidae. J. Animal Ecol. 53: 311-325.

Cowie, R. (2009): *Theba pisana* (white garden snail). CABI Invasive Species Compendium. On-line: https://www.cabi.org/isc/datasheet/62094.

Costat (2005): Version 6.311, copyright (c), CoHort Software, 798 lighthouse Ave. PMB 320, Monterey, CA, 93940, USA.

Curtis, K.M., Tepper, N.K., Jamieson, D.J., Marchbanks, P.A. (2013): Adaptation of the World Health Organization's selected practice recommendations for contraceptive use for the United States. Contraception. 87 (5): 513–516.

Deisler, J. E., Stange, L. A. and Fasulo. T. R. (2015): White Garden Snail, *Theba pisana* (Müller) (Gastropoda: Helicidae). Univ. Florida/IFAS Extension Publ. EENY-197.

El-Demerdash, F.M. (2007): Lambda-cyhalothrininduced changes in oxidative stress biomarkers in rabbit erythrocytes and alleviation effect of some antioxidant. Toxicol in Vitro., 21: 392-397.

El-Okda, M.K. (1980): Land snails of economic importance on vegetable crops at Alexandria and Neighboring regions. Agric. Res. Rev. Egypt. 58:79 – 86.

El-Okda, M. M. (1984): Land Mollusca infestation and chemical control in El-Ismaelia Governorate. Agric. Res. Rev., 62(1): 87-92.

Ferrari, A., Venturino, A. and Pechen, P.M. (2007): Effects of carbaryl and azinphos methyl on juvenile rainbow trout (*Oncorhynchus mykiss*) detoxifying enzymes. Pest Biochem Physiol., 88:134-142.

Gabr, W.M.; Youssef, A.S. and Khidr, F.K. (2006): Molluscicidal effect of certain compounds against two land snail species *Monacha obstructa* and *Eobania vermiculata* under laboratory and field conditions. Egyp. J. Agricul. Res. 84(1):43–50.

Gad, A. (2022): Emamectin benzoate as a potential molluscicide against white garden snail, *Theba pisana* in association with biochemical defects. Pest Manag. Sci. 78(4):1657-1664.

Godan, D. (1983): Pest slugs and snails. Springer-Verlag, Berlin, 445 pp.

Halliwell, B., Gutteridge, J. (1999): Free radicals in biology and medicine, Oxford Univ. Press, New York. pp. 105–245.

Herbert, D.G. (2010): The introduced terrestrial mollusca of South Africa. SANBI Biodiversity Series 15. South African National Biodiversity Institute, Pretoria, p 108.

Hodel, D. R., Arakelian, G. and Wilen, C. A. (2018): The white garden snail an old but potentially serious pest of landscape ornamentals. PALMARBOR 3: 1-20.

Hussein, M. A. and Sabry, A. K. (2019): Assessment of some new pesticides as molluscicides against the adult and eggs of chocolate banded snail, *Eobania vermiculata*. Bulletin of the National Research Centre. 43:75.

Ibtissem B.A, Hajer B.S, Ahmed H, Awatef E, Choumous K, Ons B, Mounir ZK, Najiba Z. (2017): Oxidative stress and histopathological changes induced by methylthiophanate, a systemic fungicide, in blood, liver and kidney of adult rats. Afri. Health Sci.; 17(1): 154-163.

Ismail, S. A.; Rashed, A. A.; Abou-senna, F. M. and Abed, M. (2013): Physiological and histochemical studies on the land snail, *Monacha cartusiana* in Sharkia Governorate. Egypt. J. Agric. Res., 91 (1): 207-216.

Kaplan, B. (1995): Desogestrel, norgestimate, and gestodene: the newer progestins. Annals of Pharmacotherapy, 29(7-8), 736-742.

Khene, L., Berrebbah, H., Yahyaoui, A., Bouarroudj, T., Zouainia, S., Kahli, H. and Bourayou, C., (2017): Biomarkers of oxidative stress, lipid peroxidation and ROS production induced by TiO2 microparticles on snails *Helix aspersa*. Studia Universitatis" Vasile Goldis" Arad. Seria Stiintele Vietii (Life Sciences Series), 27(2): 127-133.

Koering, M.J., Danforth, D.R., Hodgen, G.D. (1994): Early follicle growth in the juvenile macaca monkey ovary: the effects of estrogen priming and follicle-stimulating hormone. Biol. Reprod. 50: 686–694.

Kruatrachue, M., Songmuang, K., Suchart, E.U., Sretarugsa, P., Chavadei, I. (1996): Effect of vertebrate hormones on the reproductive system of *Achatina fulica* (gasrtopoda: stylomatophora). J. Sci. Thiland. 22: 249–265.

Layth, M.M., Majdy, F.M. (2018): A Study of physiological and histopathological effect of oral contraceptive (Microgynon) overdose on female Broiler chicken. Basrah J. Vet. Res. 17 (1): 1–13.

Lopes, M.; Rodrigijes, A. and Marigomez, I. (2001): Morphology and histology of the digestive gland of *Oxychilu*s (Dt-ouetia) *atlarzticus* (Morelet&Drouet) (Gastropoda: Pulmonata). Arquipélago, Life and Marine Sciences. Supplement 2 (Part B): 7 1-76.

Ma, H. and Diamond S.A. (2013): Phototoxicity of TiO2 nanoparticles to zebrafish (*Danio rerio*) is dependent on life stage. Environ. Toxicol. Chem. 32: 2139–2143.

Mubarak, S.A. (2016): The malformation effect of chlorfluazuron on the reproductive system of land snail Eobania vermiculata. The J. Bas. & Appl. Zoo. 74, 51–55.

Nagato, A.C., Bezerra, F.S., Lanzetti, M., Lopes, A.A., Silva, M.A.S., Porto, L.C. and Valença, S.S. (2012): Time course of inflammation, oxidative stress and tissue damage induced by hyperoxia in mouse lungs. International journal of experimental pathology, 93(4):269-278.

Neubert, E. (1998): Annotated checklist of the terrestrial and freshwater molluscs of the Arabian Peninsula with descriptions of new species. Faun. Arab. 17: 333–461.

Ojha, A., Yaduvanshi, S.K., Sivastava, N. (2011): Effect of combined exposure of commonly used organophosphate pesticide on lipid peroxidation and antioxidant enzymes in rat tissues. Pesticide Biochem Physiol., 99:148-156.

Radwan, M.A., El-Gendy, K.S., Gad, A.F. (2010): Biomarkers of oxidative stress in the land snail, *Theba pisana* for assessing ecotoxicological effects of urban metal pollution. Chemosphere, 79, 40–46.

Routray, S. and Dey, D. (2016): Snails and slugs as crop pests. Rashtriya Krishi, 11(1): 40-41.

Sallam A.A.A., El-Massry S.A. & Nasr I.N. (2009): Chemical analysis of mucus from certain land snails under Egyptian conditions. Archives of Phytopathology and Plant Protection, 42: 874–881.

Sanchezcasas, P. and Klessig, D.F. (1994): A salicylic acid-binding activity and a salicylic acid inhibitable catalase activity are present in a variety of plant-species. Plant Physiol., 106:1675-1679.

Scala, C., Leone Roberti Maggiore, U., Remorgida, V., Venturini, P.L. and Ferrero, S. (2013): Drug safety evaluation of desogestrel. Expert opinion on drug safety, 12(3): 433-444.

Sharaf, H.M.; Salama, M. A. and Abd El-Atti, M.S. (2015): Biochemical and histological alterations in the digestive gland of the land snail *Helicella vestalis* (locard, 1882) exposed to methiocarb and chlorpyrifos in the laboratory. Int. J. Sci. and Res., 4(7): 334-343**.**

Sitruk-Ware R. (2008): Pharmacological profile of progestins. Maturitas, 61: 151–157.

Smith, L. B., & Walker, W. H. (2014): The regulation of spermatogenesis by androgens. Seminars in Cell & Developmental Biology, 30, 2–13.

Stone, S.C. (1995): Desogestrel. Clinical obstetrics and gynecology, 38(4):821-828.

Tafurt, C.A., Sobrevilla, L.A., de Estrada, R. (1980): Effects of progestin-estrogen combination and progestational contraceptives on pituitary gonadotropins, gonadal steroids and sex hormone-binding globulin. Fertil. Steril. 33: 261–266.

Ugokwe, C.U., Okafor, F.C., Okeke, P.C., Ezewudo, B.I. and Olagunju, T.E. (2020): Induction of genetic alterations and oxidative stress in giant African land snail (*Limicolaria aurora*) exposed to municipal waste leachate. Rev. toxicol, 19-25.

Wafeeq, N.H.A. (2013): Histological and biochemical study on effect of the oral contraceptive pills in experimental male mice. AL- Taq. 26 (3): 37–45.

Zarai, Z., Boulais, N., Karray, A., Misery, L., Bezzine, S., Rebai, T., Gargouri, Y., Mejdoub, H. (2011): Immunohistochemical localization of hepatopancreatic phospholipase A2 in *Hexaplex trunculus* digestive cells. Lipid. Heal. Dis., 1 (1): 10–91.

Zhou, R., Wu, J., Liu, B., Jiang, Y., Chen, W., Li, J., He, Z. (2019): The roles and mechanisms of Leydig cells and myoid cells in regulating spermatogenesis. Cellular and Molecular Life Sciences: 76: 2681–2695.