Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Effects of some alcoholic extracts of propolis in ovulation and fertility rate of the ovary and oviduct in quail

Mohammed Hayder Hamad*, Mazin Eidan Hadi, Marwa Fadhil Alsaffar

Al Mustaqbal University College, Babylon, Iraq

** Corresponding author's E-mail: Haidermohammed031@gmail.com*

ABSTRACT

This research was carried out in quail in a special place of the laboratory from 6/9/2018 through 23/11/2018 to detect effects of adding the alcoholic extract of propolis on the ovulation and fertility rate in the ovary and oviduct of quail. Eight groups and repetitions for each group were employed. Groups were homogenous in weights and were placed in 13 cages. Each group consists of 10 quail. In the first treatment (T_1), quails were fed on regular feed without supplementation. Quails were fed in the second treatment (T_2) by adding the alcoholic extract of propolis in an amount of 200 mg kg^{-1}. The third group (T_3) was the same as normal food after adding propolis alcoholic extract at a concentration of 400 mg kg^{-1}. The results of the histological study showed that T_3 (adding 400 mg kg^{-1}) led to a physiological reproductive activity and an increase in the ovulation rate (%) than the normal limit and an elevation in the fertility rate without the appearance of unpleasant side effects or pathological or macroscopic or histological changes. The alcoholic extract of propolis led to the growth and increase of the ciliated and ciliated epithelial layer cells in their numbers and shapes in the oviduct, despite the rapid growth and rapid ovulation, unless there was no change in the shape of the egg or a change in its components.

Keywords: Quail, Cholesterol, Ovary tissue, Alcoholic extract, Propolis.
Article type: Research Article.

INTRODUCTION

The cultivation of medicinal and aromatic plants and herbs has spread in most parts of the world and has been used for its medicinal effectiveness and quick cure for diseases which are used as whole herbs, powders, or aqueous or aquatic or oily extracts (Shalmany & Shivazad 2006). Propolis is a resinous material collected by honey bee workers from the buds and bark of some types of trees, including oak, birch, willow, chestnut, young elm, pine, fir, eucalyptus and other varieties. Due to the contrast of the sources of propolis, its color tends gradually with all possible colors between yellow and black, and sometimes its color may tend to red or green, in addition to having a strong and aromatic smell (Martos *et al.* 2008). There are some reports about quail reproductive system around the world (Angel Daniel *et al.* 2022; Hussein & AL-Bayar 2022). However, there is no report about the effects of alcoholic extracts of propolis on the ovary of this bird. Hence, this study aimed to determine these effects in order to enhance the reproductive success in quail.

MATERIALS AND METHODS

This study was carried out in Al Mustaqbal University College, Babylon, Iraq, from 6/9/2018 through 23/11/2018 to detect effects of adding the alcoholic extract of propolis on the ovulation and fertility rate in the ovary and oviduct of quail. Eight groups and repetitions for each group were employed. Groups were homogenous in weights and were placed in 13 cages. Each group consists of 10 quail. In the first treatment (T_1), quails were fed on regular feed without supplementation. Quails were fed in the second treatment (T_2) by adding the alcoholic extract of

Caspian Journal of Environmental Sciences, Vol. 21 No. 2 pp. 317-323 Received: Sep. 12, 2022 Revised: Dec. 19, 2022 Accepted: Feb. 29, 2023
DOI: 10.22124/CJES.2023.6496

Publisher: University of Guilan,

propolis in an amount of 200 mg kg^{-1}. The third group (T_3) was the same as normal food after adding propolis alcoholic extract at a concentration of 400 mg kg^{-1}.

Preparation of the alcoholic extract of propolis

Propolis was provided from the local markets and then cut the raw into very small pieces. We solve 30 g of raw material in alcohol to get the propolis extract in 70 mL ethyl alcohol at 96% concentration and placed in a clean glass jar in a dark place, 4 times daily for at least two weeks. The solution was then filtered with Whatman filter paper (Shalmany & Shivazad 2006) and placed in the rotary evaporator, for the purpose of extracting the solution. The solution was then placed in an electric oven at 45 °C for 20 minutes to dispose the remaining alcohol. After extracting the extract, it was weighed by a sensitive balance and stored in clean containers (Ziaran *et al.* 2005).

Histological study by light microscopy

For the purpose of studying the histological structure of the ovary and oviduct of quail, the following chemicals and colours were used:

Chemicals and dyes used

Formalin solution with 10% formalin fixative concentration was prepared the installer according to the method of Vacca (1985).

Subject	Quantity (mL)
Formalin 40%	100%
Distilled water	900

We used this solution in the installation and accreditation described by Bancroft & Stevens (1982)

Aqueous Bouin's solution

This solution was used in the installation and accreditation described by Bancroft & Stevens (1982).

Subject	Quantity (mL)
Saturated aquatic acid	75
Formalin 40%	25
Acetic acid	5

Alcoholic alcohols

Progressive concentrations of ethanol alcohol including 30%, 50%, 70%, 80%, 90% and 95% using distilled water were prepared according to Luna (1968).

Alcohol Eosin stain

It was prepared according to Bancroft & Stevens (1982).

Subject	the quantity
Eosin Y	1 g
Ethyl alcohol concentration 95%	99 mL

Harris Hematoxylin Stain

This is a basal colour that are generally used for all animal tissues, especially when using the colour of the Eosin, according to Bancroft & Stevens (1982).

Subject	the quantity
Hematoxylin powder	1 g
Potassium Chloride	20 g
Mercury Oxide	0.2 g
Alcohol Absolute 100%	100 mL
Distilled water	200 mL
Acetic acid	8 mL

Dissection of animals

At the fifth week of the quail lifetime, 8 rats were taken from each treatment. The total number of quail was 16 and then the animal was explained after anaesthesia based on
Evans & Delhunta (1996) as follows:

1-Placing the animal in a Dissecting Tray.

2- Removing the skin, and then the sternum as well as caudal appendage until the area separated with the gravitational bone (Coracoid bone Cranial).

3- Making a cut in the skin in the lower abdominal region.

4- Elevation of the liver after cutting the suture that connects it to the transverse septum barrier separating the pericardial cavity and the abdominal cavity.

5- The samples were transferred to formalin.

Preparation of histological slides

Paraffin slices were provided based on Luna (1968).

Fixation

A section of samples was placed in a 10% formalin solution for 24 hours.

Washing

Samples installed with a formalin solution (10%) were washed using tap water for half hour.

Dehydration

The samples were passed with an ascending sequence of ethyl alcohol for the purpose of drawing water from the sample, starting from 70%, 80%, 90%, 95% and 100% for half an hour per concentration.

Clearing

Samples were cleared using xylene for 15 minutes to make samples more transparent.

Infiltration

Before the leakage, the samples were transferred to a mixture of xylene and paraffin wax, melting 58-56 ºC at 1: 1 for half an hour, then placed in molten paraffin wax and repeated three times for half an hour each.

Embedding and making blocks

The samples were immersed in the same type of wax used for filtration. The molten wax was poured into special molds for this purpose. The samples were then transferred to the air bubbles to remove hot bubbles around the sample and leave the mold to harden.

Trimming and Sectioning

The molds were waxed using a sharp scalpel and mounted on a wooden stand, then placed on the Rotary Microtome and cut into serial sections with a thickness of 7 µm (Seven *et al.* 2009). The sections were then placed on clean glass slides coated with a thin layer of Mayer's aluminium and distilled water. Hot plate temperature was 37 ºC to dry according to Bancroft & Stevens (1982).

Staining

The slides were coloured with their own colours as follows:

Harris Haematoxylin and Eosin

The sections were coloured with haematoxylin Harris-eosin

- Histological sections were put in the xylene and in two stages for ten minutes for each stage.
- The syllables underwent a downward chain of concentration of ethyl alcohol.
- Rinsing the sections with Haematoxylin Harris for 15 minutes and then washing with tap water for 2 minutes. Afterward, washing the sections in distilled water for 2 minutes.
- The sections were painted with the eosin coating for 3-4 minutes, transferred to ethyl alcohol 70% concentration for 2 minutes.
- Drying the sections with a series of progressive concentrations of ethyl alcohol 70-100% and for 2 minutes per concentration.
- Raising sections using xylene in two phases and for 2 minutes for each stage.

Mounting

The plates were placed using a Dextrin Plasticizer Xylene (D. P. X), then covered with glass cover and no bubbles, the glass slides were transferred to a 37 °C hot plate and left to dry.

Microscopy

Microscope photography

Microscopic slides were examined using a light microscope and various magnification powers to suit the current study requirements. The microscopic slides were selected with a digital microscope equipped with a digital camera and a standard 12-megapixel Canon camera was used to visualize prototypes (Tekeli *et al.* 2011).

RESULTS AND DISCUSSION

The ovary

The results of the current study showed the effect of alcoholic extract of propolis in the morphological description and tissue composition of the quail liver then comparing with control as follows (Abd El-Hady 2002)

Evaluating the histological sections of the ovaries and oviducts treated with alcoholic extract of propolis at a concentration of 400 mg kg^{-1}

The results of the histological study showed that feeding the quail on the standard diet supplemented with alcoholic extract of propolis at a concentration of 400 mg kg^{-1} led to an increase in the number and size of mature follicles, early ovulation and improved fertility, as well as an increase in the number of uterine cells, a small increase in ovarian diameter and a rise in cells, the epithelial lining of the ovary, as well as the oviduct, especially in the uterine region, and a little congestion in the blood vessels (Dogan *et al.* 2006**)**. The reason for the increase in the number of ovarian follicles, as well as an increase in the height of the epithelial cell and the early maturation of the ovaries and genitals, can be attributed to the fact that propolis contains triterpenic-acid methyle substances. The esters of these substances act as estrogen that cause these changes and may cause reactions to the positive action directed to activate and improve the gonads, as well as the epithelial cells and others (Kujumgiev 1999). The egg follicles are more mature than the control, and the tissue forming the ovarian stroma is more than control, with little congestion in the cortex, the pulp of the ovary, and this is due to the fact that propolis contains oils that work as a special mechanism to stimulate the pituitary gland to secrete quantities of hormones, especially the follicle-stimulating hormone (FSH) and the hormone LH to stimulate the growth of follicles and the formation and maturation of eggs. This result was in agreement with Marsh (1993). Using the extract in the diet, from the first day of breeding through the day of marketing (Abd El-Hady *et al.* 2002), as a long period of time was sufficient for the secretion of additional quantities of the follicle-stimulating hormone (FSH) and the hormone LH. Thus, we observed an increase in ovarian growth and in the number of cells lining the ovary and oviduct which is in agreement with Francis *et al.* (2004).

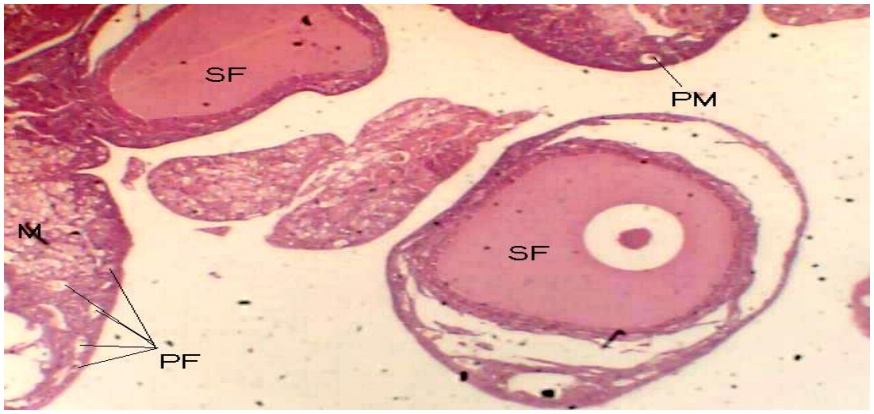

Fig. 1. A cross section of an ovary of a female quail treated with alcoholic extract of propolis at a concentration (400 mg kg^{-1}), the growth of immature primary ovarian follicles is widely spread in the ovary, growth of mature secondary ovarian follicles, enlargement of the nucleus, and cell differentiation due to treatment with the extract (Hematoxylin & Eosin stain, 400X).

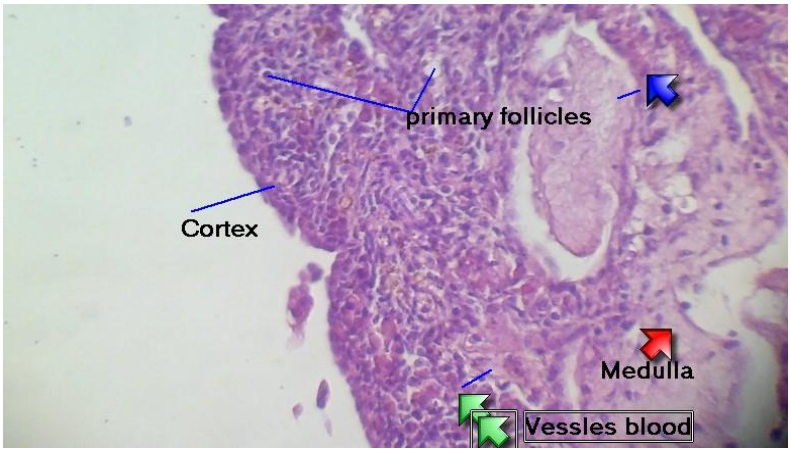

Fig. 2. A cross-section of the ovaries of a female quail treated with alcoholic extract of propolis at a concentration (400 mg kg^{-1}). He noted the ovary and the areas it consists of, the cortex, which increased in the height of its cells. As for the pulp, it was noted that there was a contraction in its cells and a little congestion in the ovarian blood vessels, and the differentiation of the primary follicles Premature unripe (Hematoxylin & Eosin stain, 400X).

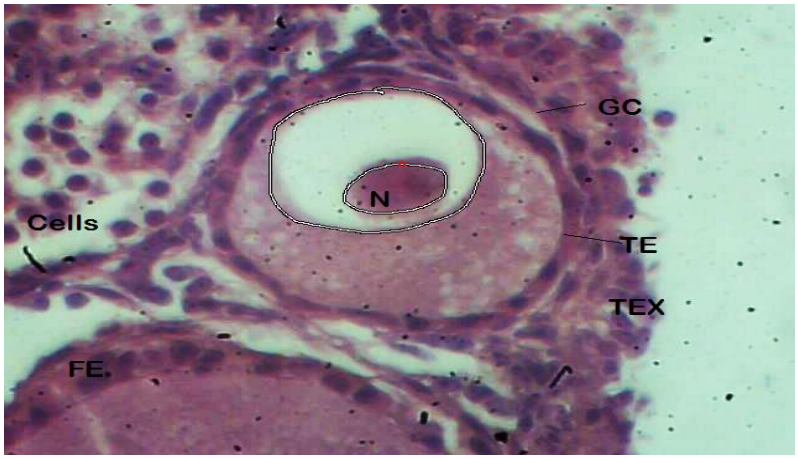

Fig. 3. A cross-section of the ovary of a female quail treated with alcoholic extract of propolis at a concentration (400 mg kg^{-1}), note the increased growth of the ovarian vesicle and the parts that make up the vesicle are the internal theca, the external theca, the surrounding granulosa cells, the epithelial cells that surround the vesicle from the outside, and the surrounding cells (Haematoxylin & Eosin stain, 400X).

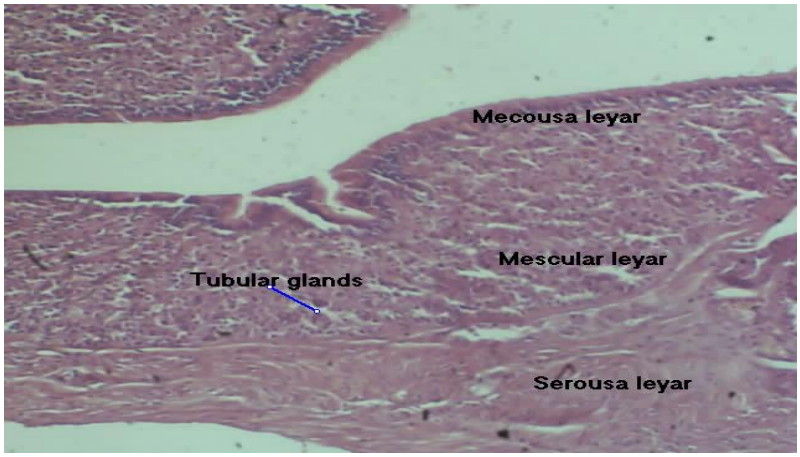

Fig. 4. A cross section of the oviduct of a female quail treated with alcoholic extract of propolis at a concentration of (400 mg kg^{-1}). Note the layers that make up the oviduct, which are the mucous layer, the muscle layer and the serous layer, where there was an increase in the height of their cells, and note the tubular glands (Haematoxylin & Eosin stain, 400X).

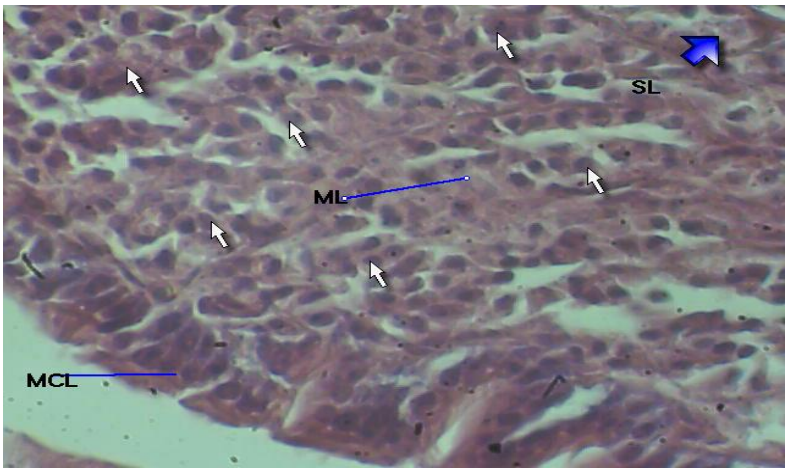

Fig. 5. A cross-section of the oviduct of a female quail treated with alcoholic extract of propolis at a concentration (400 mg kg^{-1}), this image shows the areas of the oviduct more carefully, where he noticed an increase in the number of cells lining the ovary canal, as well as an increase in multinucleated cells and an increase in oviduct tissue (Hematoxylin & Eosin stain, 400X).

REFERENCES

Abd El Hady, FK & Hegazi, AG 2002, Egyptian propolis: 2. composition, antiviral and antimicrobial activities of east Nile propolis. *Zeitschrift für Naturforschung*, 57: 386-394.

Angel Daniel, PU, Jhonn Lenon, CJ, Laura Candelaria, CH, Alabdallah, Z & Nikishov, AA 2022, Alterations in the mass

of quail eggs of different densities during storage. *Caspian Journal of Environmental Sciences*, 20: 1163-1166.

Bancroft, J & Stevens, A 1982, Theory and practice of histological techniques. 2nd ed., Churchill Livingstone, London: 662 p.

Dogan, M, Silici, S, Saraymen, R & Ilhan,IO 2006, Element content of propolis from different regions of Turkey. *Turkey Acta Alimentaria Journal*, 35: 127-130.

Evans, HE & Delahunta, A 1996, Miller's guide to the dissection of the dog. 4th ed. W.B. Saunders Company, USA, 359 p.

Hussein, SS & AL-Bayar, MA 2022, Effects of different levels of dietary zinc supplementation on the testis parameters of the Japanese quail males. *Caspian Journal of Environmental Sciences*, 20: 799-803.

Luna, LG 1968, Manual of histological staining methods ,3rd Edition, McGraw-Hill book Co. Inc., New York, 258 p.

Kujumgiev, A, Tsvetkova, I, Sevkedjieva, Y, Bankova,V, Chiristov, R & Popov, S 1999, Antibacterial, antifungal, and antiviral activity of propolis of different geographic origin. *Journal of Ethnopharmacology*, 1: 235-240.

Martos, MV, Navajas, YR, Lopez, JF & Alvarez, JAP 2008, Functional properties of honey, propolis, and Royal jelly. *Journal of Food Science*, 73: 117-124.

Seven, PT, Seven, I, Yilmaz, M & Simsek, UG 2009, The effects of Turkish propolis on growth and carcass characteristics in broiler under heat stress. *Animal Feed Science and Technology*, 146: 137-148.

Shalmany, SK & Shivazad, M 2006, The effect of diet propolis supplementation on ross broiler chicks performance. *International Journal of Poultry Science*, 5: 84-88.

Tekeli, A, Kustu, H & Geli , L 2011, Effects of *Z. officinale* and propolis extracts on the performance ,carcass and some blood parameters of broiler chicks. *Current Research in Poultry Science*, 1: 12-23.

Vacca, L 1985, Laboratory manual of histochemistry. Raven Press, New York, 328 p.

Ziaran, HR, Rahmani, HR & Pourreza, J 2005, Effect of oil extract of propolis on immune response and broiler performance. *Pakistan Biological Sciences*, 8: 1485-490.

Bibliographic information of this paper for citing:

Hamad, M, H, Hadi, M, E, Alsaffar, M, F 2023, Effects of some alcoholic extracts of propolis in ovulation and fertility rate of the ovary and oviduct in quail. Caspian Journal of Environmental Sciences, 21: 317-323.