



STUDY OF THE EFFECT OF AQUEOUS EXTRACT OF (GINGER) *ZINGIBER OFFICINALE* ROSCO IN THE HISTOLOGICAL STRUCTURE OF PROSTATE GLAND OF WHITE MALE RABBITS *ORYCTOLAGUS CUNICULUS*

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Abstract

The current study was conducted at University of Kufa, The Faculty of Education for Girls, Department of Biology, The Animal House for the period from November 2017 to May 2018, and included the use of (15) adults male of white rabbits *Oryctolagus cuniculus* which were randomly divided into (3) groups equally, they were orally injected by stomach tube and for (60 days), the control group dosed with distilled water, second group dosed with 100 g/kg warm extract of *Zingiber officinale* from body weight, third group dosed with 300 mg/kg warm aqueous extract of *Zingiber officinale* from body weight. After the experiment period was completed (60 days), done sacrifice animals to extract the prostate gland and record its weight and test histological sections and assessing the level of biochemical standards including (testosterone hormone) for serum experimental animal. The statistical analysis results showed that there was no significant change of ($p < 0.05$) in the weight of the prostate gland in the groups treated with warm aqueous extract of *Zingiber officinale*, whereas it showed insignificant increase of $p < 0.05$ in the level of the testosterone hormone in the treated group with 100mg / kg aqueous extract of *Zingiber officinale*, while the group treated with 300mg / kg aqueous extract of *Zingiber officinale* showed a significant increase $p < 0.05$ in the level of testosterone hormone. The aqueous extract of *Zingiber officinale* contains compounds with a biological activity contribute to the development of tissue composition and increase the activity of prostate gland.

Keywords : warm aqueous extract of *Zingiber officinale*, tissue composition, prostate gland, male white rabbits *Oryctolagus cuniculus*

Introduction

The prostate gland is one of the glands attached to the male reproductive system which falls under the urinary bladder neck and surrounds the urinary urethra (Kumar, 2013). The prostate gland consists of a fibrous and muscular floor and filling divided into individual glands that vary in size and shape. The glandular epithelium is lined with different epithelial or cubic epithelial tissue. The glandular epithelium is surrounded by connective tissue and contains spherical structures and with age-related calcification called prostatic prostaticectomy, the prostate produces a watery fluid a low acidity rich in citric acid, prostate phosphatase, amylase, fibrolycin and prostate-specific antigen (PSA). It is useful in the diagnosis of prostate cancer because its concentration is often metastasis during the occurrence of malignant tumor (Eroschenko, 2008). Ginger is known scientifically as *Zingiber officinale* Roscoe belongs to the family Zingiberaceae, and has a long history of medical use more than 2000 years for being one of the most important medicinal plants has a wide spectrum of biological activity and has medical properties because of the presence of gingerols, Shogols and other active compounds (Dhanik *et al.*, 2017). Ginger has been cultivated for thousands of years as a spice and for medicinal purposes in India, China and various parts of the world (Vasala *et al.*, 2004). The

chemical analysis of ginger contains more than 400 different compounds. The main compounds in rhizomes are fat carbohydrates, terpenes and phenolic compounds, and they have effective compounds used in the treatment of many diseases nausea and vomiting, diarrhea, dyspepsia, abdominal pain, constipation, asthma and cough (Grzanna *et al.*, 2005), treatment of sea urchins and sexual dysfunction (Ody, 1997). In addition, ginger shows various biological activities as anti-cancer, anti-inflammatory, anti-oxidant and has antimicrobial properties (Kamtchoung *et al.*, 2002). It also contains proteins, raw fiber ash, vitamins, amino acids and minerals (Langner *et al.*, 1998; Shukla and Singh, 2007). Ginger is characterized by flavor and tasteless taste due to a mixture of volatile oils such as gingerols and Shogaols (Harold 2004).

The Present Study Aimed at: identify the effect of aqueous extract of *Zingiber officinal* in the development of the histological and prostatic structure of the prostate gland and activate the function of testosterone hormone.

Material and Methods

Study Place

The current study was conducted at University of Kufa / Faculty of Education for Girls / the Animal House of the Department of Biology, it contains 15

adult white rabbits *Oryctolagus cuniculus* (4 months old) and their weight ranges 1.532, the animals were brought from farms belonging to Babylon province. The animals were placed in cages prepared for breeding under controlled laboratory conditions (12 hours light / 12 hours of darkness), in an air-conditioned room at 22-28 °C, the cages sprayed with sawdust and take care for cleaning animals and the rabbits were given a meal and water freely all the period of study. The animals were shown to the veterinarian from the Faculty of Veterinary Medicine / University of Kufa to ensure the safety of the animals before the start of the experiment and left the animals for a month and a half for the purpose of adaptation before the start of the experiment.

Preparation of hot aqueous extract of *Zingiber officinale* Rosc

The roots of the ginger plant were purchased from the local markets in Najaf Governorate. The roots of the ginger were cleaned and dried in the shade away from the moisture. In a well ventilated place at room temperature. The dried ginger root was crushed in the electric mill, the powder kept in glass far away from light and heat till usage. The way of (Chakravarty, 1979) was used to prepare the hot aqueous extract of *Zingiber officinale* Rosc. It included taking 20g dry ginger powder with 200ml of boiled distilled water for extraction, then the mixture is placed in an electric mixer and mixed for 15 minutes after that the solution left for 24 hours at room temperature after covering and spray?? with three layers of gauze, then filtering the mixture with filter leaves Watman (No.1) to remove the plankton, the leachate was taken in centrifuge 3000 cycle/min for 10 minutes to separate the residue and obtain a pure extract, the leachate placed in glass containers and the extract is dried with an electric oven at a temperature of (40-45 °C) and it left until become dry and then collect dry powder and preserves the bottle opaque and kept in the refrigerator until use. The original solution was dissolved by dissolving (4 g) dry matter of the water extract in (10 ml) of distilled water to obtain the original solution Stock solution in (0.4 g/ml) concentration and after that it was prepared from the original solution the various concentrations for each extract with pay attention to the animal weights, using the mitigation law

Reagent to detect the active groups in the water extract of ginger root

(i) Alkaloids reagents

Mayer Reagent: It is reported in the detection of all alkaloids. It prepared from dissolving (13.5 g) of mercuric chloride and (5 g) of potassium iodide in (10 ml) of distilled water and then mix the mixture together and added (1-2 ml) of it to (5 ml) of the water and

alcohol extract of the ginger plant roots. A white precipitation to brown was appeared (Harborne, 1984).

(ii) Terpenes Reagents (Saponins)

- **Foam Test:** It is useful in the detection of Saponins, if a tube containing a quantity of water extract showing dense foam over the surface of the extract remains for a long time indicating the presence of turbines (Harborne, 1984).
- **HgCl₂ Reagents:** Prepared by adding (1-2 ml) from (1%) of mercuric chloride to (5 ml) of the extract, a white precipitation was introduced (Harborne, 1984).

(iii) **Resins Reagents:** It was prepared by mixing (50 cm³) from (95%) ethyl alcohol with (5 g) of aqueous and alcoholic extract of *Zingiber officinale* and then placed in boiling water bath for 20 minutes. Then the solution was filtered and 100ml of distilled water was added to the filter. If the precipitation was appeared or turbidity indicates the presence of resin (Harborne, 1984).

(iv) Glycosides Reagents

Molish Reagent: It is prepared by adding two drops of α -naphthol solution to the test tube containing (2 ml) of the water and alcohol extract. Mix the mixture well, then add (2 ml) of concentrated sulfuric acid to the wall of the tube until the two layers appeared the acid layer to the bottom and the extracted layer up and between the two layers violet ring indicate the presence of glycosides (Sheikhly *et al.*, 1993).

(v) **Phenols Reagents : Ferric chloride reagents:** Prepare the detector by dissolving (1 g) of ferric chloride FeCl₃ in (100 ml) of distilled water, moisten the filter paper with vegetable extract, then add drops of ferric chloride reagent and expose the paper to the ammonia vapor. If the blue color appeared indicates the presence of phenols (Adedayo *et al.*, 2001).

(vi) **Tannins Reagents: Lead acetate reagents:** It is useful to detect the tannins by dissolving (1 g) of lead acetate in (100 ml) of distilled water, adding a quantity to the test tube (0.5 ml) of water and showing a white gelatinous precipitate (Ahmed *et al.*, 1989).

(vii) **Flavonoids Reagents : Concentrated sulfuric acid reagent:** it is useful for detecting flavonoids. It is prepared from dissolving (1 ml) of water extract in (1 ml) of concentrated sulfuric acid, showing a dark yellow color (Moussawi, 2014).

(viii) **Furanocoumrns Reagents : KOH reagent (1%):** 10% of the alcoholic potassium hydroxide solution is added to an equal amount of water and alcohol extract,

showing a yellow or green yellow color (Harborne, 1984).

Experimental Design

The Experimental animals were divided into three groups with the equivalent of five rabbits per group:

- **First group I:** Control was dosed with distilled water for 60 days.
- **Second group II:** (100 mg / kg) warm aqueous extract of *Zingiber officinale* of body weight for 60 days (Arash *et al.*, 2009).
- **Third Group III:** (300mg / kg) warm aqueous extract of *Zingiber officinale* of body weight for 60 days (Morakinyo *et al.*, 2008; Shalaby & Mouneir, 2010).

Hormonal Assay

After the trial period (60 days) and then anesthetized with diethyl ether and afterwards the blood was drawn through a heart puncture by a (5 ml) blood syringe, directly the blood transfusion Gel Tube is free of anticoagulant, These tubes were left for a period of time and then placed in the Centrifuge at 3000 rpm for 15 minutes, then the serum free of red blood cells was transferred by Micropipette and transferred to clean, sterile tubes known as Bindrov tubes and kept at (-20 °C) until it is used to measure the concentration of sex hormones. The concentrations of sex hormones, such as the testosterone hormone, was measured in Enzyme - Linked-Immunesorbent Assay (ELISA). The kit was prepared for testosterone hormone was produced by Biocheck / Inc. at a wavelength of 450 nanometers within 15 minutes using the ELISA device manufactured by the Australian company (Organon/ Teknika) (Wisdom, 1976).

Histological examination

The histological sections of the prostate gland were prepared according to the method (Bancroft and Stevens, 2008). The steps included: Dehydration, Clearing, Infiltration, Embedding, Sectioning and Staining by Haematoxyline and Eosin for Mounting, After the tissue sections of the prostate gland have been prepared, the histological segments are examined by the composite microscope Olympus has a camera and examined the slides under the 4x magnification and took pictures of the tissue sections of the gland.

Statistical Analysis: The results of the current study were statistically analyzed using Analysis of variance (ANOVA) by random randomization. In order to test the differences between the parameters of the current study, the least significant difference was used for the least

significance difference (LSD) at the probability level (P <0.05). (Snedecor and Cochran, 1982).

Results

Detection of active compounds in the aqueous extract of ginger plant *Z. officinalis*:

Table 1: Qualitative phytochemical analysis of aqueous extract of Zinger plant (*Z. officinale*)

Active compounds	Reagent type	Result of warm aqueous extract of <i>Zingiber officinale</i>
Alkaloids	Reagent Mayer	+
Turbines	Foam reagent	+
	Mercury chloride reagent	+
Glycosides	mulch reagent	+
Phenolic	Chloride reagent	+
Tannins	Lead acetate reagent	+
Flavonoids	sulfuric acid	+
Potassium	Hydroxide	+
Resins	alcohol ethyl	-

Effect of ginger extract hot aqueous on the weight of the prostate gland

The results of the statistical analysis showed no significant effect of p <0.05 on the weight of the prostate gland in the treated group with warm aqueous extract of *Zingiber officinale* when compared with control group as in table 2.

Table 2: Effect of ginger aqueous extract on the weight of the prostate gland

Groups	Prostate weight
Control	1.5 ±0.2 a
Hot water ginger concentrate 100mg / kg	1.4 ±0.1a
Hot water ginger concentrate 300mg / kg	1.6 ±0.3a
LSD	0.3

The values represent the average of the arithmetic mean ± standard error. Similar letters mean that there are no significant differences level (p <0.05) between group.

The effect of ginger extract hot water on the testosterone

The results of the statistical analysis showed no significant effect of p <0.05 on the weight of testosterone in the treated group with 100 mg/kg warm aqueous extract of *Zingiber officinale* when compared with control group whereas the results of the statistical analysis showed significant increase of p<0.05 in the weight of testosterone of group treated with 300 mg/kg warm aqueous extract of *Zingiber officinale* when compared with control group as in table 2:

Table 2: Effect of water ginger extract on Testosterone

Groups	Testosterone
Control	4.3 ±1.9a
Ginger extract hot water Concentration 100mg / kg	6.1±1.4a
Ginger extract hot water Concentration 300mg / kg	6.7 ±2.5b
LSD	1.9

The values represent the average of the arithmetic mean ± standard error. Similar letters mean there are no significant differences at the level ($p < 0.05$) between groups

The study of histological changes of prostate gland

Control Group: The results of microscopic examination of the tissue of the prostate gland in the control group showed the appearance of fibrous and muscle floor, glandular epithelium, mucous folds and the emergence of amylacea in sachas Fig. (1).

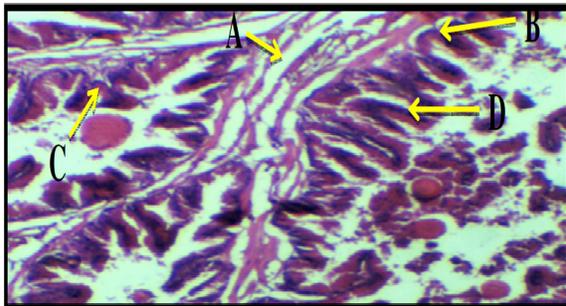


Fig. 1 : Transverse section of the prostate gland tissue in the control group: (A)a fibrous muscular floor, the (B) glandular epithelium (C) Amylacea (D) mucus folds. Stained: Hematoxylin – Eosin Power Zoom (100x).

The structure tissue of the prostate gland in the treated group with 100mg / kg hot water ginger extract:- The results of the microscopic examination in the treatment group with 100 mg/kg warm aqueous extract of *Zingiber officinale* showed appearance of the mucous folds, fibroblasts, the capsule surrounds the prostate gland, and the appearance vessel in such as Fig. (2).

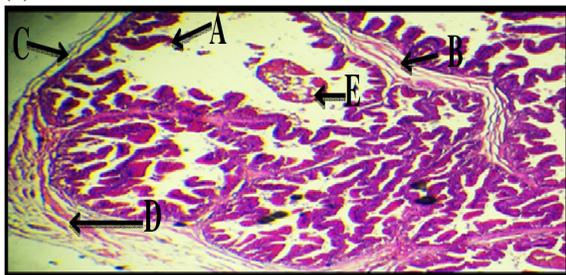
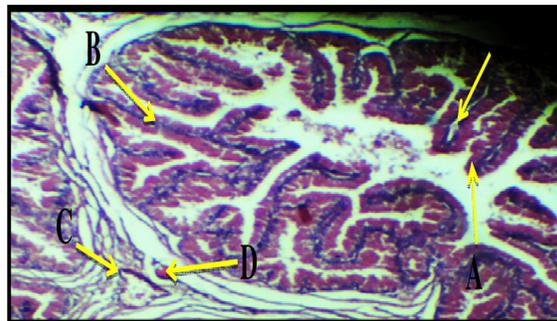


Fig. 2 : Transverse section of the prostate gland tissue in the treatment group with 100mg/kg hot water ginger extract: (A) mucous folds (B) fibrous and muscular floor (C) the capsule of gland (D) the blood vessel (E) Amylacea. Stained: Hematoxylin – Eosin (100x magnification power).

The structure tissue of the prostate gland in the treated group with 300mg/kg hot water ginger extract:-The results of the microscopic examination in the treatment group with 300 mg/kg hot water ginger extract showed increasing the thickness and height of the mucous folds, the clear adrenal epithelium, the fibroblasts and the appearance of the glands and the bloodstream nourishes the prostate gland in such as Fig. (3).



Fig(3): Transverse section of the prostate gland tissue in the treatment group with 300mg / kg hot water ginger extract: (A) increase the thickness and height of the mucous folds (B) The glandular epithelium (C)a fibrous and muscular floor (D) The blood vessel. Stained: Hematoxylin – Eosin Zoom(100x)

Discussion

The treatment group with warm aqueous extract of *Zingiber officinale* did not record affecting in the weight of the prostate gland compared with the control group and agreed with some studies (Morakinyo *et al.*, 2008). We found an insignificant increase in the weight of the prostate gland in the treated group with 300 mg/kg 1.6 ± 0.3 warm aqueous extract of *Zingiber officinale*. The researcher Kim and his group (2011) reported that 6-gingerols, one of the chemical compounds of ginger, is given to patients with prostate cancer at the dose of 6-gingerols and duration of treatment, This may be due to the fact that the dose given 300 mg/kg of water extract resulted to an increase in the weight of the prostate gland, but not noticeable. The tissue test of the prostate gland in the group treated with 100mg / kg extract of water ginger was also shown the thin of glandular mucous folds and unclear adenocarcinoma in addition to mucous in the group dosed with 300 mg/kg extract of water ginger, increased thickness and height of mucous folds, clearness of glands and fibrous and muscular floor. Mescher (2010) reported that the composition and function of the prostate gland depends on the concentration of the testosterone.

The treatment group with 100 mg/kg aqueous *Zingiber officinale* extract showed an insignificant

height of $P < 0.05$ (1.4 ± 1.6) in the concentration of the testosterone hormone, while the treatment group with 300mg / kg water *Zingiber officinale* extract showed a significant increase of $P < 0.05$ (2.5 ± 6.7) and agreed with some studies Khaki *et al.*, 2009; Morakinyo *et al.*, 2010; Riaz *et al.*, 2011; Afzali, 2011 and Ghalehkand. (2018). Sakr and Badawy (2011) indicated that ginger is a medicinal plant that has gained popularity among modern doctors. Ginger is isolated from the main ingredients which active biologically representative by gingerdiol, zingibrene, saponins, and protodioscin, which have a cyclic toxicity of the reproductive system caused by diabetes, Cisplatin and malathion (Riaz *et al.*, 2017). The researchers Morakinyo and his groups (2008) noted that protodioscin and saponins are active compounds of ginger increase the level of testosterone and LH hormones and it is used in traditional medicine to increase sexual desire and treatment of dysfunction and sexual functions. Ginger extract works to increase the activity of androgen hormones and raise the level of testosterone hormone and in turn increases the proportion of sperm and the accumulation of sperm in embiferous tubules of the sperm of mice (Amr and Hamza *et al.*, 2006; Rekha *et al.*, 2010). The researchers Khaki and his groups (2009). pointed out the dose which was given by 50mg/kg and 100 mg/kg ginger powder for the male rat increases the level of testosterone hormone without affecting LH and FSH. That increase the level of testosterone hormone lead to increase the activity of the ovaries of sexual glands, and thus leads to increase the completion of tissue and functional composition of sexual glands, which depend on these glands on androgen hormones and this is confirmed by researcher Chinoy and his groups (1982). Morshedi and his groups 2016 indicated that the time of the removal of male rabbits with the extract of phenol and turbine tubers *Cyperus esculentus* increases the thickness of the glandular epithelial layer of the prostate gland and is due to increase the rise of epithelial layers and increase the activity of the enzyme to the act of electrophoresis of fatty. testosterone and that the chemical components found in the grain of *Cyperus esculentus* similar to the rhizomes components of ginger plant represented by phenols and turbines has increased in the level of testosterone and lactic acid concentrations and in turn has led to the development of tissue structure and increase the activity of the prostate gland, Mainwaring and Wilce (1973) noted that the testosterone hormone has a role in increasing the activity of polyribosomes in the prostate gland leads to increased protein production, thus increasing the level of concentrations of testosterone hormone lead to the development of tissue composition and increase the thickness of epithelium and increase the functional function and activity of the prostate gland. Thus, it is concluded that the water

ginger extract has an androgenic and antioxidant activity at 100 mg/kg and 300 mg/kg and has a beneficial effect on the growth of the tissue and functional composition of the prostate gland.

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