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The Effect of the Alcoholic Extract of *Laurus Nobilis* Leaves on LH Levels in White Male Rats

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ABSTRACT

This research was carried out on adult male albino rats at the animal facility of the College of Education for Girls, University of Kufa, from September 2020 to August 2021. The aim was to explore the protective effects of the ethanolic extract of Laurus nobilis leaves on luteinizing hormone levels in male albino rats subjected to oxidative stress. The study involved 24 male rats of the species Rattus, each 12 weeks old and weighing between 150 and 300 grams. These animals were allocated into four groups, with each group containing six rats. The first group, which served as the negative control, received only water. The second group, acting as the positive control, was given hydrogen peroxide (1 mL per 200 grams of body weight). The third group was administered the ethanolic extract of Laurus nobilis leaves (300 mg per kg of body weight). The fourth group received both the ethanolic extract of Laurus nobilis leaves (300 mg per kg of body weight) and hydrogen peroxide (1 mL per 200 grams of body weight). All groups of rats were dosed orally using a stomach tube for 60 days. Body weights were recorded before and after the treatments. Upon conclusion of the experiment, the rats were euthanized using diethyl ether anesthesia, and blood samples were drawn directly from the heart via a syringe. Blood was collected through cardiac puncture for the purpose of biochemical and hormonal analysis. The results indicated a significant reduction ($P<0.05$) in luteinizing hormone (LH) levels in the positive control group as opposed to the negative control group. Conversely, administering the ethanolic extract of Laurus nobilis leaves caused a notable increase ($P<0.05$) in the average levels of this hormone in all treated groups compared to the positive control group. The findings of this research suggest that the ethanolic extract of Laurus nobilis leaves, due to its antioxidant capabilities, can shield against the damaging effects and oxidative stress caused by hydrogen peroxide, aiding in the preservation of tissue structures and preventing pathological changes.

INTRODUCTION

Chemical pharmaceutical drugs are often associated with toxic side effects and reduced or no efficacy after prolonged use, which has encouraged the exploration of herbal medicine. Medicinal plants are considered excellent alternatives as they are often readily available, cost-effective, and less toxic(Khan *et al.*,2012). Many modern drugs are directly or indirectly derived from plants and have proven effective in addressing various disorders (Rathnakar *et al.*, 2011).Among these plants is *Laurus nobilis* (bay laurel), which has been positively evaluated by numerous researchers who have explored its wide range of pharmacological activities. Studies have specifically highlighted the antioxidant and wound-healing properties of the ethanolic extract of *Laurus nobilis* leaves (Vardapetyan et al.,2013).The noble laurel (*Laurus nobilis*) belongs to the Lauraceae family and is commonly known as bay leaf or laurel leaf, with the most widely recognized name being bay leaf. It is an aromatic evergreen tree rich in phenolic compounds that provide it with therapeutic properties, such as antioxidant, anti-inflammatory, and antiviral effects. Native to the Mediterranean region, bay leaves have been used since the times of ancient Greeks and Romans as a fragrant herb in cooking to add a distinctive

flavor to food (Costa *et al.,* 2015; Reis *et al.,* 2018).Luteinizing hormone (LH), secreted by the anterior pituitary gland, stimulates Leydig cells to produce testosterone (Guyton & Hall, 2021). LH binds to specific receptors on the surface of Leydig cells, triggering the production of enzymes that catalyze the synthesis of steroid hormones. The end result is the conversion of cholesterol into testosterone, which then diffuses out of Leydig cells—part of it entering the circulatory system and the rest reaching neighbouring Sertoli cells. (Preston & Wailson, 2013).The amount of testosterone secreted generally increases with the availability of LH(Guyton & Hall, 2021).

METHODS

Experimental animals

This study was conducted from September 2020 to August 2021 in the animal house of the College of Education for Girls, University of Kufa. The experiment utilized 24 adult male albino rats (Rattus rattus), aged 12 weeks and weighing 180–300 g. The animals were obtained from external sources and transported to the study site. They were housed in appropriately sized plastic cages to ensure free movement, with the cage floors covered with wood shavings that were regularly cleaned and disinfected with an alcohol-based sanitiser once a week to maintain hygiene.The rats were kept under controlled laboratory conditions, including proper ventilation, a 12-hour light/dark cycle, and a temperature of 25 ± 3°C. They were provided with water and feed regularly throughout the experiment. The animals were acclimatized to the laboratory environment for two weeks before the start of the experiment.

Preparation of the Experimental Plant

The plant samples were obtained from herbal shops in the markets of Najaf Governorate. The plants were cleaned thoroughly and ground into a fine powder using an electric grinder. The resulting powder was stored in clean, dry containers at room temperature until use.

Preparation of the Alcoholic Extract of *Laurus nobilis* Leaves

The leaves of *Laurus nobilis* were ground into a fine powder using an electric grinder. The extraction process was carried out using a Soxhlet extractor. For each extraction cycle, 30 g of the powdered leaves were placed in a thimble, and 450 mL of 70% ethanol was added. The extraction was performed at 45°C for 24 hours. This sequential extraction process continued until the required amount of powder was processed for the experiment.After extraction, the solution was filtered and placed in clean, opaque glass containers. The extract was then dried using a spray dryer. The resulting dried powder was collected, stored in opaque containers, and kept in a refrigerator until use.

Animal Groups

A total of 24 male Rattus rattus rats were divided into four groups, each comprising six rats. The animals were treated for 60 days as follows:

Group 1 (Negative Control): This group was given only regular drinking water and served as the negative control.

Group 2 (Positive Control): This group was administered 0.5% hydrogen peroxide (H_2O_2) orally and served as the positive control.

Group 3: This group was given 300 mg/kg of body weight of the ethanolic extract of *Laurus nobilis* leaves. After 30 minutes, the animals were administered 0.5% hydrogen peroxide (H_2O_2) orally.

Group 4: This group was administered 300 mg/kg of body weight of the ethanolic extract of *Laurus nobilis* leaves only.

The treatments were administered using a thin metal gavage tube connected to a medical syringe. The syringe was used to draw the substance (extract or H_2O_2), which was then delivered through the gavage tube into the animal's esophagus, ensuring full ingestion of the substance.

RESULTS

Effect of Different Treatments on Luteinizing Hormone (LH) Levels

The results presented in Figure (1) indicated a significant decrease ($P<0.05$) in the average LH levels in the positive control group (1.05 mIU/mL) compared to the negative control group (2.05 mIU/mL). Conversely, a significant difference ($P<0.05$) was observed in the group treated with ethanolic extract + hydrogen peroxide compared to the negative control group. Moreover, the results showed a significant increase ($P<0.05$) in LH levels in the group treated with the ethanolic extract of *Laurus nobilis* leaves (300 mg/kg) and the group treated with the ethanolic extract + hydrogen peroxide compared to the positive control group. The LH levels in these two groups were 1.59 mIU/mL and 1.48 mIU/mL, respectively.

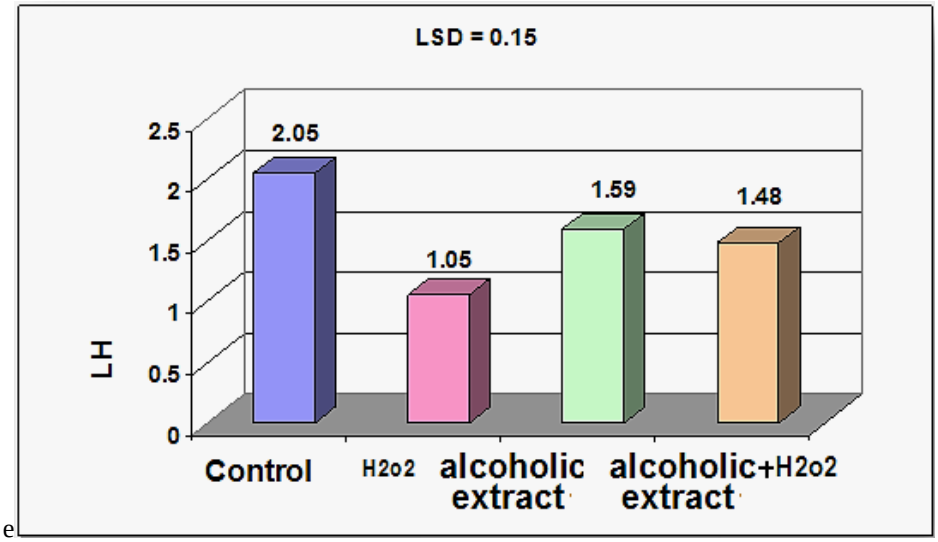

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Figure (1): Effect of different treatments on luteinizing hormone (LH) levels in male rats orally dosed for 60 days.

DISCUSSION

The results revealed a significant decrease in LH levels in the positive control group compared to the negative control group. These findings are consistent with those reported by Darbandi *et al*. (2018) and can be attributed to oxidative stress (OS) caused by an increase in reactive oxygen species (ROS). This oxidative stress leads to cell apoptosis and impaired spermatogenesis (Han *et al.*, 2016). Gonadotrope cells secrete gonadotropin-releasing hormone (GnRH) as well as LH and FSH hormones (Guyton & Hall, 2021). FSH receptors are located on the membranes of Sertoli cells, while LH receptors are present on the membranes of Leydig cells. Together, these receptors regulate testosterone synthesis, maintain normal spermatogenesis, and ensure the health and density of sperm (Patton & Battaglia, 2007).On the other hand, the group of animals treated with the alcoholic extract of bay leaves at a concentration of 300 mg/kg for 60 days exhibited a significant increase in LH levels. These results align with the findings of Teixeira and de Araujo (2019), who studied the effects of certain plants in treating male infertility. These plants demonstrated beneficial effects by reducing oxidative stress, regulating hormone levels, and detoxifying the body, in addition to their antioxidant properties.The primary impact of medicinal plants on male reproductive functions lies in the antioxidants they contain. These plants are rich in phytochemicals capable of inhibiting lipid peroxidation of sperm membranes. Among these compounds are polyphenols, flavonoids, and tannins, which perform various functions in the male reproductive system, such as supporting spermatogenesis and steroidogenesis (Lohiya *et al.,* 2016; Mansouri *et al.,* 2016).

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