التأثيرات المحسنة لجذور شرش الزلوع على الخصوبة ومعايير المناعة لدى ذكور الفئران

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المستخلص:

كان هدف هذا البحث هو معرفة تأثير مسحوق جذور شرش الزلوع ومستخلصه الإيثانولي على مؤشرات الخصوبة والمناعة لدى الفئران. تم العثور على مجموعتين رئيسيتين من ستة وثلاثين فئران ألبينو ذكر. قبل ذلك، تم تغذية المجموعة الأولى على نظام غذائي أساسي واستخدمت كمجموعة ضابطة سالبة. المجموعة الرئيسية الثانية تم حقنها بجرعة ٠,١مجم / كجم من وزن الفئران في كلوريد الكادميوم لمدة عشرة أيام لإحداث العقم لدى الفئران؛ تم تقسيم هذه المجموعة الرئيسية الثانية إلى ٥ مجموعات، كل منها ستة فئران. أعطيت المجموعة (٢) نظامًا غذائيًا أساسيًا فقط واستخدمت كتحكم (+). تلقت المجموعتان ٣-٤ نظامًا غذائيًا قياسيا يحتوي على (٢,٥، ٥٪) من جذور شرش الزلوع المطحونة؛ تلقت المجموعات ٥-٦ نظامًا غذائيًا قياسيا بالإضافة إلى (٢٥٠، ٥٠٠ مجم / كجم) من مستخلص جذور شرش الزلوع الإيثانولي. تم ذبح الفئران عند انتهاء الدراسة التي استمرت أربعة أسابيع، وتم أخذ السيرم من الدم. تم تقييم المعايير التالية: هرمون تحفيز الجريبات (FSH)، هرمون التستوستيرون، هرمون ملوتن (LH)، المؤشرات المناعية (IgG، IgM)، صورة دهون الدم (TC، TG، TG، LDL-c، VLDL-c، HDL-c)، إنزيمات الكبد (ALP، ALT، AST)، وظائف الكلى (اليوريا والكرياتينين وحمض البوليك). وفقًا للنتائج، ذادت جذور شرش الزلوع بشكل كبير هرمون التستوستيرون، FSH، LH مع خفض دهون الدم ووظائف الكبد والكلوي والعكس صحيح مع HDL-c. كشفت الفحوصات النسيجية عن تحسن في وظائف الخصية. في الختام،

يمكن أن يقلل استخدام جذور شرش الزلوع من الآثار السلبية للسموم في الخصوية ويعزز صحة الخصية من خلال تعزيز قدراتها الإنجابية والمناعية.

الكلمات المفتاحية: الفئران، هرمونات الخصوبة، المناعة، التحاليل الكيميائية الحيوية.

Ameliorative Influences of Ferula Roots on Fertility and Immunity Parameters in Male Rats

Abstract

The aim of this research was once to find out how immunity and infertility in rats had been affected by the powdered Ferula roots and their ethanolic extract. Two major groups of thirty-six male albino rats have been found. Before this, Group 1 was fed a basal diet and used negative control. The second main group received an injection of 0.1mg/kg of rat weight in cadmium chloride for ten days to cause infertility in the rats; the 2nd main group was divided into 5 subgroups (6 rats for each group), Group (2) was given a basal diet only and used as a control (+). Groups 3-4 received a basal diet containing (2.5 and 5%) of powdered Ferula roots; groups 5-6 received a basal diet plus (250 and 500 mg/kg) of Ferula roots ethanolic extract. Rats were slaughtered when the four-week study was finished, and the serum was taken from blood. The following parameters were assessed: Follicle-stimulating hormone (FSH), testosterone, luteinizing hormone (LH), immunological markers (IgG and IgM ranges), lipid fractions (TC, TG, TG, LDL-c, VLDL-c, and HDL-c), liver enzymes (ALP, ALT, and AST), and renal biomarkers (urea, creatinine, and uric acid). According to results, Ferula roots considerably elevated testosterone, FSH, and LH hormone-lowering lipid fractions, liver, and kidney biomarkers, and vice versa on HDL-c. Histopathological examinations revealed improved functioning of the testis organ. In conclusion, using Ferula roots can reduce the negative effects of testicular toxicants and enhance the health of the testis by boosting its reproductive and immunological capabilities.

Keywords: Rats, Reproductive hormones, immunity, biochemical analysis.

INTRODUCTION

The reproduction capability, the capacity to have intercourse and ejaculate viable sperm cells is the definition of normal male fertility **(Engeler *et al.*, 2006)**. A couple is considered clinically infertile if they are unable to conceive after a yr. of trying. About 10–20% of couples worldwide are thought to be infertile **(WHO, 2023)**. According to medical standards, when trying out first begins for infertility, it is encouraged to consider each partner; nevertheless, in as many as 25% of instances, the male partner is no longer evaluated **(Schlegel *et al.*, 2021)**. This difficulty can be linked to a wide range of illnesses, along with extrinsic causes like obesity, pneumonia of the pelvis, infections following parturition or surgery, and intrinsic troubles like anatomical, genetic, hormonal, and immunological diseases **(Larsen *et al.*, 2007)**. The anterior pituitary releases follicle-stimulating hormone (FSH) in response to the hypothalamus' release of gonadotropin-releasing hormone (GnRH). FSH is concerned with developing, regulating, and reproducing the reproductive device in each man and female (**Kaiser and Stamatiades, 2018)**. According to **Kelly and Jones (2013)**, hormone testosterones are involved in many vital human body factors, along with bone density, mental and cognitive function, and sexual function. The excessive concentration of FSH and LH hormones, which are essential for the increase and improvement of the testis, as well as the excessive level of testosterone hormone, which is required for the testis's growth and protection **(Al-Salhie, 2018)**.

The immune system's main function is to protect the body against infections. In this system, lymphocytes, neutrophils, and monocytes/macrophages also referred to as phagocytes are the first line of defense. By influencing different cytokines, ferula herbs, and their components additionally confirmed immunomodulatory effects. According to the literature that is presently available, the numerous active phytochemicals discovered in individuals of the genus Ferula make them effective treatments for oxidative stress,

immunological disorders, and inflammatory illnesses **(Ghasemi *et al.*, 2021)**.

Medicinal herbs are one abundant source of secondary metabolites that improve reproductive function. Hormone homeostasis is essential for reproductive health in each man and women **(Mbemya *et al.*, 2017)**. About 170 species of the genus Ferula, (*Ferula hermonis*) generally called Shirsh-el-Zallouh, are recognized to grow in areas ranging from North Africa to Central Asia and the Mediterranean **(Moran *et al.*, 2002)**. Because of their spermatogenic and aphrodisiac properties, several herbs have been employed. Various species of Ferula have been utilized as aphrodisiacs in ordinary and people medicinal drugs at some stage in several international locations because of historical times. Male sexual troubles have been treated with aphrodisiacs made from numerous types of Ferula root. Male aphrodisiacs still use Ferula additionally known as "Shirsh zallouh" in the Middle East **(Aydogan *et al.*, 2020)**. This species of Ferula has been examined the most out of all the others due to the fact of its potential to enhance sperm health and aphrodisiac properties. The plant's root is regularly referred to as "Lebanese Viagra" or "Lebanese root." According to **Sattar and Iranshahi (2017)**, it has been utilized in regular medicinal drug to decrease plasma cholesterol ranges and total body weight. Plants in this genus consist of a variety of chemicals, which include terpenoids, coumarins, and many sulfide compounds, according to a phytochemical examination of the plants. Among the several substances present in *F. hermonis* are sesquiterpene esters, such as ferritin, tenuferidine, and ferritinol, which are recognized for their estrogenic qualities **(Sayed-Ahmad *et al.*, 2017)**. Ferula has no or little effect when taken sub-chronically and can only have a considerable acute influence on sexual motivation. In traditional medicine, several species of Ferula have been used to increase libido and reproduction **(Bagheri *et al.*, 2023)**. Male rats who had been slow or impotent expanded after receiving an injection of *F. hermonis* extract, as suggested by **Zanoli *et al.*, (2003)**. According to research performed by **Abdul-**

Kader *et al.* (2011), injecting male rats with a 50% aqueous extract of Ferula at a dose of 0.01 and 0.02 mL/100 g/body weight has been found to increase testosterone levels, which may additionally provide an explanation for these effects. Thus, the goal of the current investigation was to find out how rats' immunological and reproductive performance were affected by the powdered and extracted Ferula roots.

Material and methods

Materials

Freula Root of Shirsh-el-Zallouh (*Ferula hermonis*) was bought in Cairo City, Cairo Governorate, from the Haraz herbalist. The cadmium chloride (CdCll2) was once provided by the German chemical manufacturer Merk. Thirty-six adult male albino "Sprague Dawley" rats weighing between 150 and 160 g were used in the investigation. The Research Institute for Medical Insects, located in Doki, Cairo, has bought them. Standard phenolic compounds and Folin-Ciocalteu phenol reagent had been bought from Sigma-Aldrich Inc. (St. Louis, MO). The TC, TG, HDL-c, ALT, AST, ALP, urea, uric acid, and creatinine have been measured in the usage of chemical kits from Techno-gene Chemical Co., Dokki, Cairo, Egypt.

Methods

Preparation of the materials

Ferula root is ground into a powder in accordance with **Rekha *et al.*, (2009),** who state that all herbs must be saved in dry, darker conditions to stop the oxidation process of their contents. After that, it is saved till needed in dark-colored glass bottles in a cool, dry place. Preparing an ethanolic extract from Ferula roots. The combination of 125 g of acetone and twenty-five (25) g of Ferula roots was once prepared in a 1:5 ratio. For the cause of maximizing the extraction process' yield, the resultant solution used to be allowed to rest for sixty minutes. The liquid fraction used to be separated from the solid component rich in residues and contaminants via filtering the solution after the disbursed amount of time. The liquid extract that had been clarified was dried to a

powder in a rotary evaporator that operated in a vacuum at a temperature of 40 ◦C. 2.2 g of dry powder was obtained using the procedure outlined by **Maiuolo *et al.* (2022)**.

Identification and quantification of Ferula roots phenolic compounds by HPLC

Using an HPLC system, the phenolic acid concentration of each extract was determined with a few minor modifications. The analytical column was an ODS column (5 μm, 4.6 mm × 250 mm, Agilent Technologies, Santa Clara, CA, USA). In gradient elution, solvents A (water containing 0.1% (v/v) acetic acid) and B (acetonitrile containing 0.1% (v/v) acetic acid) were utilized. The gradient program was as follows: According to the gradients, B is 92–90% A in 2–7 min; B is 90–70% A in 2–27 min; B is 70–10% A in 27–50 min; B is 10–0% A in 51–60 min; B is isocratic in 51–60 min; and B is 0 to 92% A in 60–70 min. and 60–70 min, 0 to 92% A in B. One milliliter per minute was the steady flow rate, and the injection volume was 20 microliters. The UV detector was calibrated at 280 nm. Various standard phenolic compounds are produced in HPLC quality methanol. To calculate the quantities of phenolic acid, standard curves were developed. The total phenolic acid content was calculated by adding the values of each distinct component of phenolic acid. generated in methanol of HPLC purity as described by **(Radovanović *et al*., 2010)**.

Induced rats' infertility

Cadmium chloride ($CdCl_2$) solution used to be injected intraperitoneally into rats for ten days at a concentration of 0.1 mg/kg of the rat's weight to induce male infertility **(Rekha *et al*., 2009)**.

The design of the test

The study was carried out through the Faculty of Specific Education at Assiut University, and the Ethics Committee approved study protocol **IRB No. 1320240114**. Rats are kept in standard, hygienic environments with metal frames at a temperature of 25°C. Thirty-six adult male white albino "Sprague Dawley" rats weighing between 150 and 160 g had been used in this investigation. To

useful aid adaptation, a preferred diet prepared in accordance with equation **AIN (1993)** used to be as soon as fed to each rat for a period of seven days. Following the length of the modification, eight groups consisting of six rats were created: Group 1: As a negative control group, rats were fed only the standard diet. Group 2: Infertile male rats were fed only the basal diet as a positive control. Group 3: Infertile rats received a basal diet plus 2.5% of the diet's weight in powdered Ferula roots. Group 4: Infertile rats received a basal diet plus 5% of the diet's weight in powdered Ferula roots. Group 5: Infertile rats have been fed a basal diet plus 250 mg/kg of Ferula root extract through a gastric tube containing the diet's weight. Group 6: Infertile rats have been fed a basal diet plus 500 mg/kg of Ferula root extract through a gastric tube containing the diet's weight. Every animal in my test used to be weighed, slaughtered, and given a blood test when the 28-day experiment was finished.

Collection of blood samples

After evaluation, rats that had been placed down with both anesthetics had their abdominal aortas used to draw blood samples. Blood samples have been put in sterile, sanitized centrifuge tubes and left to coagulate for 10 minutes at room temperature to separate the serum. The serum was well separated, placed in sterile centrifuge tubes, and frozen at -20°C to prepare it for analysis. Every serum sample has gone through a biochemical examination **(Schermer, 1967)**.

The biochemical evaluation

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured calorimetrically for this purpose the use of the techniques described by **Fahim *et al.* (1982)**. The **Pradelles *et al.* (1985)** approach was utilized to identify the testosterone hormone via calorimetric methods. Using the approach of **Salauze *et al.* (1994)**, purified myeloma IgM was once received from Serotec (Oxford, United Kingdom) and chromatographically purified rat IgG used to be bought from Cappel (PA, USA). Total cholesterol was measured according to **Allen (1974)**. The techniques for

measuring triglycerides had been described with the aid of **Fossati and Principle (1982)**. The **Lopez (1977)** technique can be used to measure high-density lipoprotein cholesterol (HDL-c). The VLDL-c and LDL-c assessments have been carried out in accordance with **Lee and Nieman (1996)**. LDL-c (mg/dl) is equal to (Total cholesterol – HDL-c) -VLDL-c. Conversely, VLDL-c (mg/dl) = Triglycerides/ (5). According to **Huang *et al.* (2006)**, alkaline phosphatase (ALP) was once formerly examined with the use of enzymatic colorimetry. Aspartate aminotransferase (AST) things have been decided the use of the **Young (1975)** method. We examined the Alanine aminotransferase (ALT) in accordance with **Chawla (2003)**. The enzymatic approach of **Barham and Trinder (1972)** had been used for measuring uric acid. According to **Patton and Croush (1977)**, the enzymatic approach was utilized to determine the urea content. The kinetic technique of **Chromy *et al.* (2008)** was utilized to determine creatinine.

Histopathological examination:

The testicles of each experimental group were divided into small specimens, which were subsequently washed in xylene, embedded in paraffin, dried in increasing ethanol concentrations (70, 80, and 90%), and stored in 10% neutral buffered formalin. According to **Bancroft *et al.* (1996)**, slices of a thickness of (4-6) µm were created using hematoxylin and eosin.

Statistical analysis:

When a significant main effect was found, the data were analyzed using a completely randomized factorial design **SAS, (1988)**; the Student-Newman-Keuls Test was used to separate the meaning. Using the Costat Program, differences between treatments of ($P \leq 0.05$) were considered significant. One-way ANOVA was used to assess biological results.

3. Results and Discussion

Identification of phenolic compounds of Shilish EL Zallouh roots:

The bioactive components of Ferula roots are characterized based on the data proven in Table 1. It is evident to state that

hydroxybenzoic acid, kaempferol, and catchiness have been the three phenolic chemical compounds discovered in the highest concentrations in Ferula roots. The results confirmed that the corresponding amounts were 8360.0, 3827.00, and 3211.00 mg/100g.

Conversely, Ferula roots had the lowest recorded values for daidzin, P-coumaric acid, and ellagic acid. In that order, the levels were 10.78, 20.15, and 25.60 mg/100g DW. These outcomes concur with Vanillic acid (141.35 mg/kg) was once observed to be the most common chemical in the root extract of Ferula, in accordance with **G¨oçeri *et al.* (2022)**. Gallic acid and chlorogenic acid have been previously discovered in the aerial components of Ferula Wild, with portions of 71 and 5.50 mg/g, respectively. Ferula chloroform and methanol extracts had been observed to include ellagic acid in addition to ferulic acid. It is possible to deduce that the roots of Ferula are rich in phenolic chemicals.

Table (1): Characterization of phenolic compounds of Ferula roots by HPLC

Phenolic compounds	Concentration (mg/100g DW)
Vanillic acid	299.34
Catchiness	8360.00
Ferulic acid	877.80
***P*-Coumaric acid**	20.15
Hydroxybenzoic acid	3211.00
Caffeic acid	430.11
Chlorogenic acid	1640.90
Gallic acid	98.85
Syringic acid	26.74
Ellagic acid	10.78
Kaempferol	3827.00
Phenol	2897
Daidzin	25.60
Myricetin	1117.20

Testosterone, LH, and FSH values at different Ferula root concentrations are displayed in Table (2) in infertile rats. There is a considerable difference in the testosterone hormone levels between the control negative group and the positive group. Once, the average levels measured were 2.05 and 1.69 ng/ml. The records obtained related to the infertile groups confirmed that a group receiving 500 mg/kg of Ferula root extract had a considerably higher testosterone hormone value. A drop in value was found in the 2.5% group for Ferula roots powder, with corresponding averages of 3.06 and 2.13 ng/ml.

In regard to LH hormone, the average readings for the control negative group had been 0.87 and 0.64 IU/L, respectively, which was once considerably greater than the readings for the positive control group. All groups' infertile group results, however,

confirmed that the group receiving 500 mg/kg of Ferula roots extract had a significantly greater FSH hormone level. The group that given 2.5% powdered ferula roots showed the lowest value, which were 1.17 and 1.03 IU/L, respectively.

Concerning FSH hormones, the outcomes confirmed that the negative control group had a higher level in contrast to the positive control group, being 1.37 and 1.06 IU/L, respectively. Data on the infertile groups confirmed that the group that received 500 mg/kg of extract from Ferula roots had significantly greater amounts of the hormone FSH. The lowest values were recorded by the group that obtained 2.5% powdered ferula roots, which were 1.71 and 1.58 IU/L, respectively. Our results agree with those of **Al-Salhie and Al-Hummod (2019)**, who observed a statistically significant ($P \leq 0.05$) rise in the levels of LH, FSH, and testosterone hormone in the 200 mg Ferula extract group in contrast to the 100 mg Ferula extract group and the control group. *Ferula hermonis*, which contains a variety of active chemicals, along with ferutinine, feroline, and tenuferidine, may additionally be the cause of these effects **(Abourashed *et al.* 2001)**. Ferutinin's estrogenic action, which raises FSH and LH levels, is the cause of this effect. Like these results, **Zanoli *et al.* (2003)** observed that testosterone levels had been significantly greater in rats given a higher dose of Ferula extract than in animals given a control group.

Additionally, in contrast to control rats, rats that were given Ferula roots showed a considerable increase in serum testosterone levels **(Allouh and Said, 2015)**.

Table (2): Influence of various levels of Ferula roots on reproductive hormones of infertility rats

Parameters / Groups	Testosterone (ng/ml)	LH (IU/L)	FSH (IU/L)
G_1 Control (-v)	2.05±0.11^{c}	0.87±0.05^{c}	1.37±0.11^{c}
G_2 Control (+v)	1.69±0.15^{d}	0.64±0.03^{d}	1.06±0.10^{d}
G3 (2.5% Ferula roots powder)	2.13±0.17^{c}	1.03±0.04^{b}	1.58±0.07^{b}
G4 (5% Ferula roots powder)	2.58±0.14^{b}	1.10±0.06^{a}	1.64±0.27^{a}
G5 (250 mg/kg Ferula roots extract)	2.84±0.16^{a}	1.14±0.05^{a}	1.69±0.14^{a}
G6 (500 mg/kg Ferula roots extract)	3.06±0.18^{a}	1.17±0.07^{a}	1.71±0.13^{a}
LSD (P≤0.05)	**0.420**	**0.103**	**0.122**

Means ±SD in each column that has different superscript letters are considerably different at P≤0.05.

The influence of varying Ferula root dosages on immunological markers in infertile rats was once displayed in Table (3). The average IgG values of the positive control group have been discovered to decreased than those of the control negative group, which had values of 1248.98 and 1219.65 mg/ml, respectively. Concerning the infertile groups, the records gathered confirmed that the group given 500 mg/kg of extract from Ferula roots exhibited a much greater IgG value, whilst the group given 2.5 percent powdered Ferula roots had a decrease in value, which was 1428.03 and 1366.51 mg/ml, respectively.

As for IgM, the maximal value of the negative control group was once previously significantly larger than that of the positive control group, with concentrations of 357.15 and 328.35 mg/ml, respectively. Compared to group three, which received 2.5 percent powdered Ferula roots, the group receiving 500 mg/kg of Ferula

roots extract had an extensively greater IgM value, with means of 420.94 and 384.03 mg/ml, respectively. These outcomes are in line with the findings of **Ghasemi *et al.* (2021)**, who claimed that the immune system's major function is pathogen defense. The first line of defense in this system is made up of neutrophils, monocytes/macrophages, sometimes known as phagocytes and lymphocytes.

Furthermore, according to the nature of their molecules, essential oils extracted from the Ferula plant can stimulate the immune system. Terpenes and other natural agents found in the essential oils of Ferula species are among the substances that have been proven to have immunomodulatory characteristics **(Oüzek *et al.* 2017)**.

Additionally, research in vitro and in vivo demonstrated the immunomodulatory effects of various active chemicals from Ferula root, such as auraptene. Aflatene enhanced the synthesis of IgA and IgG in primary mouse splenocytes, increased the production of IgM in human HB4C5 cell hybridoma, and stimulated the production of IgA and IgM in lymphocytes from mesenteric lymph nodes in the in vitro investigation **(Askari *et al.*, 2020)**.

Table (3): Influence of various levels of Ferula roots on immunity parameters of infertility rats

Parameters / Groups	IgG (mg/ml)	IgM (mg/ml)
G_1 Control (-v)	1248.98±4.25^{d}	357.15±3.45^{e}
G_2 Control (+v)	1219.65±3.91^{e}	328.35±3.60^{f}
G3 (2.5% Ferula roots powder)	1366.51±3.80^{c}	384.03±3.47^{d}
G4 (5% Ferula roots powder)	1394.52±5.11^{b}	389.15±3.53^{c}
G5 (250 mg/kg Ferula roots extract)	1392.53±5.60^{b}	409.12±4.14^{b}

G6 (500 mg/kg Ferula roots extract)	1428.03±5.58[a]	420.94±4.50[a]
LSD (P≤0.05)	**5.370**	**3.750**

IgG= Immunoglobulin G. IgM= Immunoglobulin M.
Means ±SD in each column that has different superscript letters are considerably different at P≤0.05.

The average lipid fraction (total cholesterol, triglycerides, HDL-c, LDL-c, and VLDL-c) of infertile rats with a variety of Ferula levels is shown in Table (4). Total cholesterol (TC) was once considerably greater in the positive control group (157.50 mg/dl) than in the negative control group (93.00 mg/dl). With recognition to the infertile groups, the average values were 96.25 and 113.25 mg/dl for the group given 2.5 percent powdered Ferula roots and the group administered 500 mg/kg of Ferula roots extract, which had a considerably decrease in TC value.

As for triglycerides (TG), the control positive group's values, which measured 143.75 and 70.50 mg/dl, respectively, were obviously far higher than those of the control negative group. According to the data gathered for the infertile groups, the group that received 500 mg/kg of extract from Ferula roots had a much lower TG value, while the group that received 2.5% of powdered Ferula roots had a greater range, with TG values of 121.00 and 86.75 mg/dl, respectively.

When the HDL-c degrees of the positive and negative control groups were compared, the negative control group's value was much higher; the corresponding averages were 51.44 and 32.32 mg/dl, respectively. Concerning the infertile groups, the HDL-c values of the groups receiving 2.5 percent Ferula roots powder and 500 mg/kg of Ferula roots extract were found to be significantly higher, which were 45.99 and 36.53 mg/dl, respectively.

It should also be noted in Table (4) that the group with the positive control had an indicated LDL-c value of 96.43 mg/dl, which was previously significantly higher than the group with the negative control, which had an LDL-c value of 27.46 mg/dl. For the

infertile groups, the statistics provided confirmed that the LDL-c value had previously been significantly higher in the group that received 2.5% of the Ferula roots, and lower in the group that received 500 mg/kg extract of Ferula roots, at 52.52 and 32.91 mg/dl, respectively.

Regarding serum VLDL-c levels, the data confirmed that the control positive group had a much greater average range of VLDL-c than the control negative group, with values of 28.75 and 14.10 mg/ml, respectively. Concerning the infertile groups, the outcomes confirmed that the group that obtained 2.5% of the Ferula roots powder had a considerably greater VLDL-c value than the group that received 500 mg/kg of Ferula roots extract, or 24.20 and 17.35 mg/dl, respectively. These results support the findings of **Rafiee *et al.* (2018)**, who mentioned that, in comparison to the controls, the extract of Ferula roots decreased the levels of blood cholesterol, triglycerides, and LDL in diabetic rats. Furthermore, there was once little weight loss in the animals that received the extract. These effects should be attributed to the extract's content of various flavonoids and terpenoid chemical substances **(Mahendra and Bisht, 2012)**, which reduce blood cholesterol and triglycerides with the aid of regulating fats oxidation and bile cholesterol excretion **(Zeka *et al.* 2017)**. The study's conclusion used to be that the extract from Ferula roots prevented the animals from dropping weight. This result is in line with the earlier study by way of **(Helal *et al.* 2005)**.

Table (4): Influence of various levels of Ferula roots on lipid profile in infertility rats

Parameters / Groups	Total cholesterol mg/dl	Triglycerides mg/dl	HDL-c mg/dl	LDL-c mg/dl	VLDL-c mg/dl
G_1 Control (-v)	93.00±1.54^{e}	70.50±1.65^{f}	51.44±1.45^{a}	27.46±1.12^{f}	14.10±0.52^{e}
G_2 Control (+v)	157.50±2.68^{a}	143.75±2.27^{a}	32.32±1.12^{e}	96.43±2.42^{a}	28.75±0.92^{a}
G3 (2.5% Ferula roots powder)	113.25^{b}±2.45^{b}	121.00±2.10^{b}	36.53±1.47^{d}	52.52±1.60^{b}	24.20±0.64^{b}
G4 (5% Ferula roots powder)	105.55±2.37^{c}	109.25±2.11^{c}	41.01±1.70^{c}	42.69±1.43^{c}	21.85±0.43^{c}
G5 (250 mg/kg Ferula roots extract)	103.50±2.00^{c}	94.75±1.89^{d}	42.23±1.66^{c}	42.32±1.25^{d}	18.95±0.36^{c}
G6 (500 mg/kg Ferula roots extract)	96.25±1.98^{d}	86.75±1.78^{e}	45.99±1.35^{b}	32.91±1.10^{e}	17.35±0.34^{d}
LSD (P≤0.05)	**3.241**	**3.147**	**1.130**	**1.642**	**1.160**

Means ±SD in each column that has different superscript letters are considerably different at P≤0.05.

The influence of different dosages of Ferula roots on liver activity, which includes ALT, AST, and ALP, in male infertile rats were proven in Table (5). The ALT liver enzyme values of the control positive group had been considerably greater than those of the control negative group, which were 75.11 and 34.15 U/L, respectively. Based on the information collected, it was observed that the infertile groups confirmed a significantly decreased ALP value in the group given 500 mg/kg of Ferula root extract than in the group given 2.5 percent of Ferula roots powder, which were 47.41 and 62.71 U/L.

It should be mentioned that the group with control positive had a much greater value for the enzyme AST liver activity than the group with control negative. Averaging reading was 115.20 and

70.50 U/L. On the contrary hand, statistics concerning infertile groups confirmed that ALP levels have been significantly decreased in those groups that obtained 500 mg/kg of Ferula root extract. However, the group that was once given 2.5 percent powdered Ferula roots had a greater value, being, 77.07 and 105 U/L, than the other groups. The group with control positive higher the group with control negative in terms of ALP liver activity. The average amounts were 170.10 and 358.25 U/L. Regarding the infertile groups, the documentation confirmed that the ALP value of the group given 500 mg/kg of extract from Ferula roots was much lower, being, 201.00 U/L, than that of the group given 2.5 percent powdered Ferula roots, being, 266.10 U/L. These outcomes are consistent with those of **Deniz *et al.* (2019)**, who observed that Ferula roots may protect rat liver from oxidative damage caused by way of carbon tetra chloride. The hepatic antioxidant defense system was once restored and lipid oxidation was once reduced, as evidenced by the elevated activity of oxidative enzymes such as glutathione (GPx) and great oxide dismutase (SOD). Ferula roots extract may additionally protect against hepatic injury caused by the aid of CCl_4, which confirms the plant's common use in liver damage treatment.

Furthermore, **Higuchi and Gores (2003)** observed that the synthesis of 8-OHdG in liver DNA was once considerably reduced when Ferula was administered orally as adverse to the CCl_4 to the group, indicating that Ferula may additionally be able to prevent DNA injury prompted by way of CCl_4. The administration of Ferula superior the functions and roles of SOD and GPx, two naturally occurring antioxidants, suggesting that Ferula's advantages may additionally be associated with its antioxidant qualities.

Table (5): Influence of various levels of Ferula roots on infertility rats

Parameters / Groups	ALT U/L	AST U/L	ALP U/L
G_1 Control (-v)	34.15±1.73^{f}	70.50±1.13^{f}	170.10±2.86^{f}
G_2 Control (+v)	75.11±1.10^{a}	115.20±1.35^{a}	358.25±3.97^{a}
G3 (2.5% Ferula roots powder)	62.71±1.26^{b}	105.00±1.13^{b}	266.10±3.17^{b}
G4 (5% Ferula roots powder)	57.62±1.13^{c}	94.34±1.35^{c}	247.13±3.53^{c}
G5 (250 mg/kg Ferula roots extract)	54.36±1.05^{d}	89.53±1.54^{d}	224.53±3.42^{d}
G6 (500 mg/kg Ferula roots extract)	47.41±1.18^{e}	77.07±1.24^{e}	201.00±3.19^{e}
LSD (P≤0.05)	**1.460**	**2.153**	**4.220**

ALT=Alanine aminotransferase. AST= Aspartate aminotransferase. ALP=Alkaline phosphatase. Means ±SD in each column that has different superscript letters are considerably different at P≤0.05.

Table (6) displays the average kidney biomarkers for infertile rats fed different diets. The control positive group's serum area was once recorded at 44.93 mg/dl, significantly greater than the control negative groups. When it came to the infertile groups, the information accrued confirmed that the group that was given 2.5% powdered Ferula roots had a urea value that used to be much greater than the group that received 500 mg/kg of Ferula root extract, with corresponding values of 38.68 and 28.23 mg/dl.

Compared to the control negative group, the average uric acid concentration in the positive control group varied significantly higher, from 8.14 to 4.63 mg/dl. The data for the infertile groups indicated significantly greater uric acid concentrations in the groups that consumed 2.5 percent of the powdered Ferula roots. The levels, which had been 7.03 and 5.00 mg/dl, lowered in the group that obtained 500 mg/kg of Ferula root extract. Table (6) demonstrates

the average blood creatinine content for infertile rats given various diets. When compared to the control negative group, the serum creatinine ranges of the control nice group have been confirmed to have a significantly larger maximal concentration of 1.53 and 0.80 mg/dl. Regarding the infertile groups, the results confirmed that the group obtaining 2.5% of the Ferula roots had a considerably greater creatinine content than the group receiving 500 mg/kg of Ferula root extract, which had been 1.24 and 0.89 mg/dl. Our outcomes are consistent with those of **Nabavi *et al.* (2011)**, who observed that renal functions, such as creatinine and urea, confirmed considerable decreases in all kidney-damaging rats fed different diets. Renal indications extended in rats fed powdered Ferula roots and leaves. These herbs may additionally have anti-inflammatory and antioxidant residences in addition to enhancing glomerular filtration, stopping tissue damage, and enhancing cell potential for reconstruction. Herbal medicines, such as Ferula roots, can be utilized to enhance renal characteristics and prevent or treat renal failure **(Moeini *et al.* 2022)**.

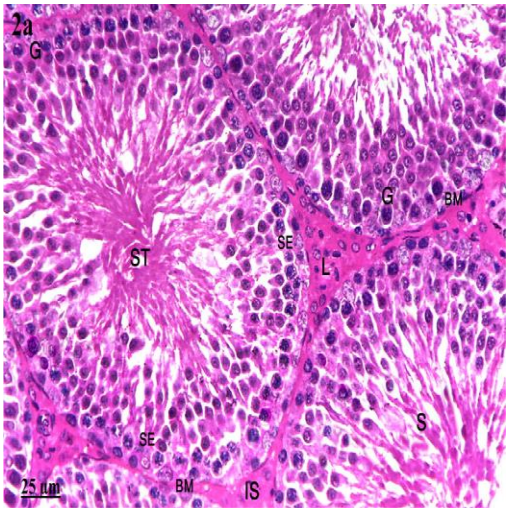
Table (6): Influence of various levels of Ferula roots on infertility rats

parameters / Groups	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl
G_1 Control (-v)	27.41 ± 1.00^{d}	4.63 ± 0.22^{d}	0.80 ± 0.10^{c}
G_2 Control (+v)	44.93 ± 1.01^{a}	8.14 ± 0.13^{a}	1.53 ± 0.41^{a}
G3 (2.5% Ferula roots powder)	38.68 ± 1.04^{b}	7.03 ± 0.24^{b}	1.24 ± 0.25^{b}
G4 (5% Ferula roots powder)	34.33 ± 1.05^{c}	6.34 ± 0.37^{c}	1.10 ± 0.17^{b}
G5 (250 mg/kg Ferula roots extract)	33.40 ± 1.03^{c}	5.20 ± 0.42^{d}	0.99 ± 0.14^{b}
G6 (500 mg/kg Ferula roots extract)	28.23 ± 1.01^{d}	5.00 ± 0.26^{d}	0.89 ± 0.12^{b}
LSD (P≤0.05)	**1.131**	**0.633**	**0.230**

Means ±SD in each column that has different superscript letters are considerably different at P≤0.05.

Histopathological examination of testicular

In a microscale, Normal seminiferous tubules (ST) bordered by germinal epithelium (G) and Sertoli cells (SE) lying on the basement membrane (BM) were visible in a segment of the testicular of the group (1). In the lumen, sperm (S) are visible (picture 1). Group (2)'s testicular slice displayed a significant decrease in the number of germ cells (G); the degenerating and shedding cells had darkly stained nuclei (double arrows) with large gaps (P) between them. Vacuoles (V) and Leydig cells (L) are few (photo1). Group 3's testis revealed a segment containing typical seminiferous tubules (ST), which were surrounded by Sertoli cells (SE) and germinal epithelium (G) and rested on the basement membrane (BM). Within the lumen, sperm (S) are visible. A portion of group 4's testicular tissue demonstrated a small amount of spermatogonia cell necrosis and degeneration lining seminiferous tubules (black arrow), along with an increase in interstitial fibroblasts (red arrow) and an infiltration of inflammatory cells (blue arrow). A segment inside group 5's testicular tissue exhibits a negligible positive α-SMA immunoreaction (arrow) in the tissues surrounding blood vessels and the seminiferous peritubular border. Group 4's testicular slice displays spermatogenic cells (G), Sertoli cells (SE), which sit on the basement membrane (BM), and sperms (S) in the lumen of the seminiferous tubules (ST), which appear to be in normal condition. These findings are consistent with those of **Bagheri *et al.* (2015)**, who reported that a histological investigation revealed that when the dosage was increased, there was an increase in the number of Leydig cells and the spermatogenesis process, but that the Leydig cells became vacuolated. The experimental groups' Johnsen scores were higher than those of the control groups, but the difference was not statistically significant ($P > 0.05$). Despite the histopathological effects on the testicular that were noted, especially at high doses, Ferula roots demonstrated a beneficial effect on spermatic parameters.



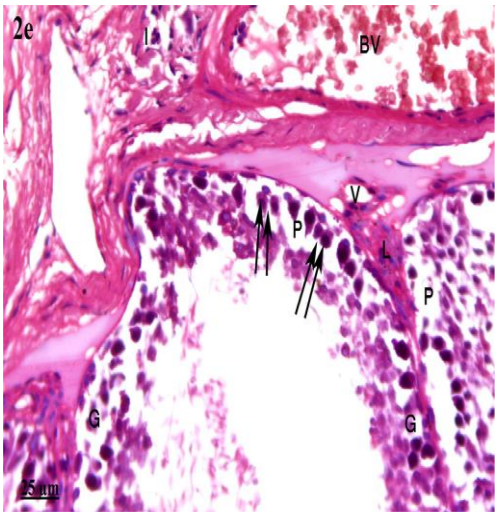
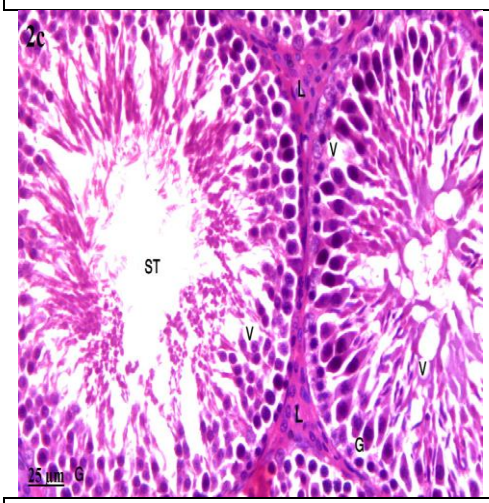


Photo (1): A section in the testicular of group 1 showed normal seminiferous tubules (ST) lined by germinal epithelium (G) and Sertoli cells (SE) resting on the basement membrane (BM). Sperms (S) are seen in the lumen.	**Photo (2): A section in the testicular of group 2 showed marked reduction of the number of germ cells (G); shedding and degenerating cells have dark stained nuclei (double arrows) with wide gaps (P) between them. Few Leydig cells (L) and vacuoles (V).**



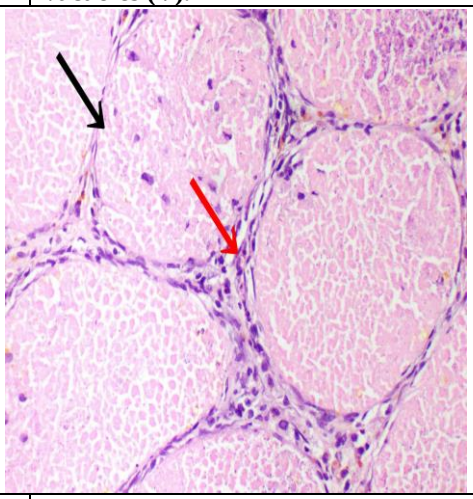
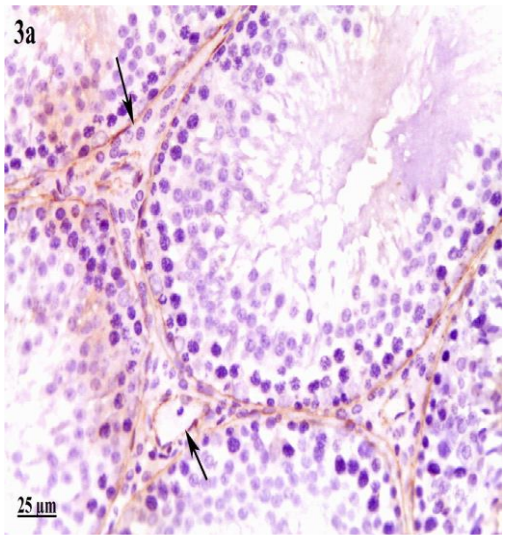


Photo (3): A section in the testicular of group 3 showed normal seminiferous tubules (ST) lined by germinal epithelium (G) and Sertoli cells (SE) resting on the basement membrane (BM). Sperms (S) are seen in the lumen.	**Photo (4): A section in the testicular of group 4 showing slight degeneration and necrosis of spermatogonia cells lining seminiferous tubules (black arrow) associated with interstitial fibroblasts proliferation (red arrow) and inflammatory cells infiltration (blue arrow)**



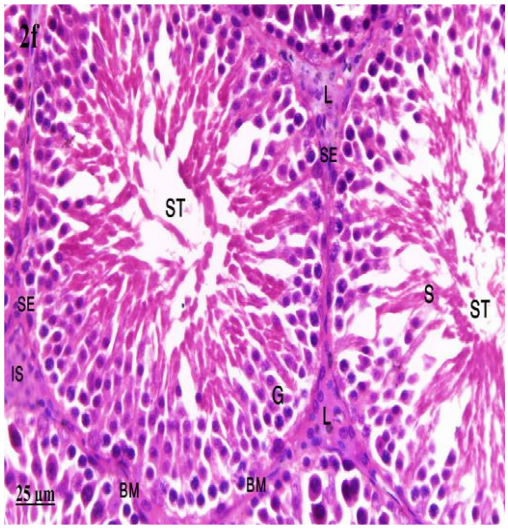


Photo (5): A section in the testicular of group 5 showing minimal positive α-SMA immunoreaction (arrow) in the seminiferous peritubular boundary tissues and around blood vessels.	Photo (6): A section in the testicular of group 4 showing apparently normal seminiferous tubules (ST) with their lining of spermatogenic cells (G), Sertoli cells (SE), which rest on basement membrane (BM), and sperms (S) in the lumen.

4. Conclusion

We can use Ferula roots powder and its extract as a decoction to enhance fertility and immunity due to the fact it has excellent phytochemicals and positive effects on reproductive hormones and the immune system in rats.

5. References

1. **Abdel-Kader, M.M.; Kassem, S.S.; Danial, E.N.; Raouf, A.A. and Hanafi, E.M. (2011):** Valuation of *Ferula hermonis* extract as a growth promoter. Int. J. Acad. Res., 3: 140-147.
2. **Abourashed, E.A., Galal A.M., El-Feraly, F.S. and Khan, I.A. (2001):** Separation and quantification of the major daucane esters of *Ferula hermonis* by HPLC. Planta Med., 67 (7): 681-682.
3. **AIN, American Institute of Nutrition. (1993):** Purified died for Laboratory Rodent Final report. Journal of Nutrition, 123:1939-1951.
4. **Allen, C.C. (1974):** Cholesterol enzymatic colorimetric method. *Journal of Clinical Chem*istry**;** 2 (20): 470.

5. **Allouh, M.Z., and Said, R.S. (2015):** Influence of *Ferula Hermonis* acetonic Extract on exercise adapted rat gastrocnemius. Austin. Cell. Biol., 1(1): 1-4.
6. **Al-Salhie, K.C.K. (2018):** The effect of age on growth and development of the gonads pre- to post sexual maturity of Japanese quail (*Coturnix japonica*). *Kufa J. Agri. Sci*.,10 (3):39-55.
7. **Al-Salhie, KC. and Al-Hummod, S.K. (2019):** *Ferula Hermonis* roots extract on testicular biometry and reproductive hormones in local ducks. Indian Vet. J., 96 (12): 14-17.
8. **Askari, V.R.; Alavinezhad, A.; Rahimi, V.B.; Rezaee, S.A. and Boskabady, M.H. (2020):** Immuno-modulatory effects of methanolic extract of *Ferula szowitsiana* on isolated human Th1/Th2/ Treg cytokines levels, and their genes expression and nitric oxide production. Cytokine,138:1-7.
9. **Aydogan, F.; Baykan, S.; Soliman, G.A.; Yusufoglu, H. and Bedir, E. (2020)**: Evaluation of the potential aphrodisiac activity of sesquiterpenoids from roots of *Ferula hubermorathii* Pesmen in male rats. *J. Ethnopharmacol***;** 257:112868.
10. **Bagheri, S.M.; Shieh, A.; Ghalenoei, J.A.; Yadegari, M. and Alborzi, N. (2023):** Review of potential spermatogenic and aphrodisiac effects of the *Ferula* genus. *The Korean Society for Reproductive Medicine***;** 1: 143-152.
11. **Bagheri, S.M.; Yadegari, M.; Porentezari, M.; Mirjalili, A.; Hasanpor, A. Dashti, M.H. and Anvari, M. (2015):** Effect of *Ferula assa-foetida* oleo gum resin on spermatic parameters and testicular histopathology in male Wistar rats. *Journal of Ayurveda & Integrative Medicine, 6* (3): 175-180.
12. **Bancroft, D.; Steven, A. and Tunner, R. (1996):** Theory and Practices of Histological Techniques, 4th Ed. Churchill Livingstone, Edinburg, London, and Melbourne.
13. **Barham, D. and Trinder, P. (1972):** Determination of uric acid. *Analyst,* 97: 142.
14. **Chawla, R. (2003):** Practical Clinical Biochemistry. Third Edition. Jaypee Broth Medical Publishers (p) LTD, New Delhi.

15. **Chromy, V.; Rozkosna, K. and Sedlak, P. (2008):** Determination of serum creatinine by Jaffe method and how to calibrate to eliminate matrix interference problems. *Clin. Chem. Lab. Med., 46* (8): 1127-1133.
16. **Deniz, Y.; Laloglu, E.; Koc, K. and Geyikoglu, F. (2019): Hepatoprotective** potential of Ferula communis extract for carbon tetrachloride induced hepatotoxicity and oxidative damage in rats. *Biotechic & Histochemistry, 94* (5): 334-340.
17. **Engeler, D. S.; John, H. and Maake, C. (2006):** Prostatitis and male factor infertility: A review of the literature. *Current Prostate Reports, 4* (1): 45-53.
18. **Fahim, M.S.; Fahim, Z. and Harman, J.M. (1982):** Clevenger, T.E.; Mullins, W.; Hafez, E.S. Effect of Panax ginseng on testosterone level and prostate in male rats. *Arch Androl, 8* (4): 261-263.
19. **Fassati, P. and Prencipe, L. (1982):** Triglyceride enzymatic colorimetric method. *Journal of Clinical Chemistry*, 28:2077.
20. **G¨oçeri, A.; Demirtas, ˙I.; Alma, M.H.; Adem, S.; Kasra, Z.A.; Gül, F. and Uzun, A. (2022):** Investigation on chemical composition, antioxidant activity and SARS-CoV-2 nucleocapsid protein of endemic *Ferula longipedunculata* Pes¸men, Grasas Y. Aceites, 73 (450): 1-9.
21. **Ghasemi, Z.; Rezaee, R.; Aslani, M.R. and Boskabady, M.H. (2021):** Anti-inflammatory, antioxidant, and immunomodulatory activities of the genus Ferula and their constituents: A review. *Iran. J. Basic Med. Sci., 24* (12): 1613-1623.
22. **Helal, E.; Mostafa, A.M.; MhMood, A.F. and Kahwash, A.A. (2005):** Hypoglycemic and hyper-insulinemic effects of *Ferula assafoetida* on diabetic male albino rats. *Egypt J. Hosp. Med., 21*:95–108.
23. **Higuchi, H. and Gores, G.J. (2003):** Mechanisms of liver injury: An overview. *Current Molecule Medication 3*: 483-490.
24. **Huang, X.; Choi, Y.; Im, H.; Yarimaga, O.; Euisik, Y, and Kim, H. (2006):** Aspartate Aminotransferase (AST/GOT) and

Alanine Aminotransferase (ALT/GPT) Detection Techniques. Sensors, 6: 756-782.

25. **Kelly, D.M. and Jones, T.H. (2013):** Testosterone: A metabolic hormone in health and disease. *Journal of Endocrinology,* 217 (3): 1-8.
26. **Larsen, S.H.; Wagner, G. and Heitmann, B.L. (2007):** Sexual function and obesity. *Int J. Obes.,* 31: 1189-1198.
27. **Lee, R. and Nieman, D. (1996):** National Assessment. *2nd Ed., Mosby,* Missouri, USA.
28. **Lopez, M.F. (1977):** HDL-cholesterol colorimetric method. *Journal of Clinical Chem*istry, 23: 882.
29. **Mahendra, P. and Bisht, S. (2012):** *Ferula asafoetida*: Traditional uses and pharmacological activity. *Pharmacogn Rev., 6* (12):141-146.
30. **Maiuolo, J.; Musolino, V.; Guarnieri, L.; Macrì, R.; Coppoletta, A.R. and Antonio Cardamone, A. (2022):** *Ferula communis,* L. (Apiaceae) root acetone-water extract: phytochemical analysis, cytotoxicity and in vitro evaluation of estrogenic properties. *Plants,* 11: 1-14.
31. **Mbemya, G.T.; Vieira, L.A.; Canafistula, F.G.; Pessoa, O.D. and Rodrigues, A.P. (2017):** Reports on *in vivo* and *in vitro* contribution of medicinal plants to improve the female reproductive function. *Reprodução & Climatério, 32 (2*): 109-119.
32. **Moeini, R.; Memariani, Z.; Enayati, A.; Gorji, N. and Kolangi, F. (2022):** Nephrotonic and nephroprotective medicinal herbs in traditional persian medicine: Review and assessment of scientific evidence. *Current Traditional Medicine, 8* (3): 1-15.
33. **Moran, J.; van Rijswijk, B.; Traicevski, V.; Kitajima, E.W.; Mackenzie, A.M. and Gibbs, A.J. (2002):** Potyviruses, novel and known, in cultivated and wild species of the family Apiaceae in Australia. *Arch Virol.,* 147:1855-1867.
34. **Nabavi, S.; Ebrahimzadeh M.; Nabavi, S.; Eslami, B. and Dehpour, A. (2011):** Antioxidant and antihemolytic activities of

Ferula foetida regel (Umbelliferae), *European Review Medical Pharmacological Science, 15* (2): 157-164.

35. **Oüzek, G.; Schepetkin, I.A.; Utegenova, G.A.; Kirpotina, L.N.; Andrei, S.R. and Oüzek, T. (2017):** Chemical composition and phagocyte immunomodulatory activity of *Ferula iliensis* essential oils. *Journal Leukoc Biol.,* 101:1361-1371.
36. **Patton, C.J. and Croush, S.R. (1977):** Enzymatic determination of urea. *Journal Analytical Chemistry,* 49: 464-469.
37. **Pradelles, P.; Grassi, J. and Maclouf, J. (1985):** Enzyme immunoassays of eicosanoids using acetylcholine esterase as label: An alternative to radioimmunoassay. *Analytical Chem*istry, *57* (7):1170-1173.
38. **Rafiee, S.; Mojadadi, M.; Molavi, M.; and Nazemi, S. (2018):** Effect of ethyl acetate extract of *Ferula asafoetida* oleo-gum resin on the glucose level and lipid profile in streptozotocin-induced diabetic rats. *Jundishapur J. Nat. Pharm. Prod*., 1: 1-10.
39. **Rekha, D.K.; Nayanatara, A.K.; Ramswamy, C.; Sheila, R.P.; Ramesh, B.M. and Venkappa, S.M. (2009):** Infertility in male Wistar rats induced by cadmium chloride: Role of ascorbic acid. *Journal of China Medical, 41*(11): 616-621.
40. **Salauze, D.; Serre, V. and Perrin, C. (1994):** Quantification of Total IgM and IgG Levels in Rat Sera by a Sandwich ELISA Technique. *Comp. Haematol. Int.* 4:30-33.
41. **SAS (1988):** SAS Users Guide: Statistics version 5th Ed. SAS. *Institute Inc.,* **;** Cary N.C.
42. **Sattar, Z. and Iranshahi, M. (2017):** Phytochemistry and pharmacology of *Ferula hermonis*, Boiss: a review. *Drug Res., (Stuttg)* 67:437-46.
43. **Sayed-Ahmad, B.; Talou, T.; Saad, Z.; Hijazi, A. and Merah, O. (2017):** The Apiaceae: ethnomedicinal family as source for industrial uses. *Industerial Crops Production,* 109:661-71.

44. **Schermer, S. (1967):** The blood morphology of laboratory animal Longmans. Green and Co. Ltd, 350.
45. **Schlegel, P. N.; Sigman, M.; Collura, B.; De Jonge, C. J.; Eisenberg, M. L.; Lamb, D. J. and Zini, A. (2021):** Diagnosis and treatment of infertility in men: AUA/ASRM guideline part I. The Journal of urology, 205 (1): 36-43.
46. **Stamatiades, G.A. and Kaiser, U.B. (2018):** Gonadotropin regulation by pulsatile GnRH: Signaling and gene expression. Mol. Cell Endocrinol., 5 (463):131-141.
47. **World Health Organization (2023):** Infertility prevalence estimates: 1990-2021, Geneva, Switzerland.
48. **Yound, D.S. (1975):** Determination of AST. *Clinical Chemistry Journal,* 22 (5):1-21.
49. **Zanoli, P.; Benelli, A.; Rivasi, M.; Baraldi, C.; Vezzalini, F. and Baraldi, M. (2003):** Opposite effect of acute and sub-chronic treatments with *Ferula hermonis* on copulatory behavior of male rats. International Journal of Impotence Research, 15: 450-455.
50. **Zeka, K.; Ruparelia, K.; Arroo, R.; Budriesi, R. and Micucci, M. (2017):** Flavonoids and their metabolites: Prevention in cardiovascular diseases and diabetes. Diseases, 5 (3):19-25.