مجلة التربية النوعية والتكنولوجيا (بحوث علمية وتطبيقية)
Journal of Specific Education and Technology (Scientific and Applied Research)
KSU Kafrelsheikh University جامعة كفرالشيخ

Potential Preventive Roles of *Eruca Sativa* Leaves Extract Against Acrylamide-Induced Biochemical and Histological Alterations in Rat Testis

Mohamed R. Elkabary[*1], Hisham H. Saad [2] and Adel A. Badr[3]

[1,2]Deopartment of Nutrition and Food Science, Faculty of Home Economics, Menoufia University, Shebin El kom, Egypt.
[3]Fellow Lecturer of Nutrition Department, Menoufia University Hospitals, Shebin El kom, Egypt.
[*]***Corresponding author:*** *Email: m_elkabary@yahoo.com*

Abstract:

Processing/cooking starchy foods at high temperatures such as French fries and other baked goods produces toxic chemicals, including acrylamide. Acrylamide (ACR) is known to cause many toxic effects in various organs of the body, including the testicles. Therefore, the present study aimed to investigate the potential protective effects of *Eruca sativa* leaves ethanolic extract (ESLE), against acrylamide-induced biochemical and histological alterations in rat testis. Rats (n=30), were randomly assigned to five equal groups as follow: Group 1, served as normal control; Group 2, model control, received 50 mg/kg BW/day of ACR to induce infertility; groups 3 to 5 received ESLE-1, ESLE-2 and clomid (Clo, infertility drug reference) at concentrations of 250, 500 and 1.2 mg/kg BW/day by oral gavage for 28 days, respectively followed by ACR. Treatment of rats with ACR caused a significant increased ($p \leq 0.05$) in sperm abnormalities and testis malonaldehyde (MDA) by 471.32 and 292.1%, respectively compared to the normal animals. Also, sperm count, sperm motility, serum testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) content were significant ($p \leq 0.05$) decreased by -93.78, -68.95, -80.84, -76.98 and -89.16%, respectively. Furthermore, testis antioxidant enzymes (Superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) activities were decreased by -79.29, -81.09 and -80.74%, respectively, compared to the normal control rats. Additionally, liver dysfunction and harmful histopathological changes in testicular tissue were observed as ACR treatment. However, intervention with ESLE (500 mg/kg) significantly ($p \leq 0.05$) restored most of these parameters to levels close to normal. In conclusion, these observations serve as a foundation for using ESLE to treat and/or prevent ACR toxicity. Therefore, we suggested including fresh *Eruca Sativa* leaves and their extracts into our daily meals, beverages, and pharmaceutical formulations.

Keywords: *infertility, oxidative stress, clomiphene citrate, sperm quality, testicular toxicity, bioactive compounds.*

Introduction

The global expansion of fast food chains and quick service restaurants has enhanced the demand for baked and fried starchy foods, especially French fries, cakes, potato chips and biscuits in various countries both developing and developed alike due to their attractive taste and speed of preparation **(Dağoğlu et al., 2024).** Since the preparation of these foods requires high heat treatment, they have become a serious risk to public health **(Rifai and Saleh, 2020).** Many studies have confirmed that these heat treatments of starchy foods produce unwanted heat-induced toxins as a by-product, known as "thermally processed contaminants" **(Radad *et al.*, 2020).** Acrylamide is one of the most toxic chemicals found in these foods and has aroused a lot of curiosity in recent years **(Alturki *et al.*, 2022 and Govindaraju *et al.*, 2024)**.

Acrylamide, whose chemical name is 2-propenamide (C_3H_5NO), is a white, odorless, water-soluble, crystalline solid that is extremely reactive. Its molecular weight is 71.08 g mol^{-1}. **(Semla *et al.*, 2017)**. Moreover, according to a large body of research, ACR is also a potent chemical inducer of neurotoxicity, hepatotoxicity, nephrotoxicity, genotoxicity, muscle damage, and developmental and reproductive abnormalities in animal models **(Kandemir *et al.*, 2020 and Radad *et al.*, 2020)**. In addition, accumulating evidence suggests that acrylamide is one of the chemicals that exert harmful impacts on the biological system causing infertility in humans **(Saleh *et al.*, 2024)**. In this context, **Farag *et al.*, (2021)** study demonstrates that ACR causes decreased sperm quality, testicular degeneration, epididymal weight loss, and abnormal steroid signaling. However, Systematic research has mainly pointed to oxidative stress in the molecular mechanism of ACR-infected testicular toxicity **(Gül *et al.*, 2021; Kucukler *et al.*, 2020; Radad *et al.*, 2020)**. ACR is metabolized in the liver via glutathione conjugation or oxidation, which yields glycinamide, which encourages membrane oxidation and the production of reactive oxygen species (ROS) **(Radad *et al.*, 2020; Sengul *et al.*, 2021)**. Thus, acrylamide induces a decrease in the antioxidant defense environment to trigger reproductive disorder and DNA damage in sensitive organs. Furthermore, deleterious impacts of ACR on Follicle-stimulating hormone (FSH), luteinizing hormone (LH), serum testosterone (T) and the testicular antioxidant system have been indicated **(Saleh *et al.*, 2024)**.

Hence, this research highlights the infertility-causing testicular toxicity of acrylamide, as infertility is highly prevalent worldwide, with approximately 48.5 million couples (15%) experiencing difficulty conceiving **(UCLA Health, 2023; WHO, 2023)**. Infertility is often caused by problems with ejaculation, absent or low sperm counts, or abnormal sperm shape (morphology) and movement (motility) **(WHO, 2023)**. Despite this reality, infertility in the developing world remains a poorly researched and neglected topic, affecting approximately 15% of all married pairs, but ~50% of these deformities are obvious in males **(Thoma *et al.*, 2021).** In light of the above, it strongly suggests that natural products with antioxidant activity may play a beneficial role against ACR toxicity.

Medicinal plants have potential therapeutic impacts for many human diseases, so they have received great attention because they are natural medicines that contain safe phytochemicals, in addition to being healthier than synthetic medicines **(Chaachouay & Zidane 2024)**. Folk medicine widely uses watercress (*Eruca sativa*), an edible medicinal plant belonging to the *Brassicaceae* family **(Yaniv *et al.*, 1998)**. It has numerous pharmacological properties and a strong free radical scavenging effect in vitro and in vivo **(Ramazzina *et al.*, 2022)**. Previous experimental studies have shown that *Eruca sativa* contains a wide range of phytochemicals, which have been associated with a wide range of pharmacological activities such as anticoagulant and antiplatelet activities **(El-Gayar *et al.*, 2022)**, antimicrobial and anti-inflammatory **(Pagnotta *et al.*, 2022),** antioxidant **(Algreiby, 2024)**, antidiabetic and antilipid **(Raouf *et al.*, 2024)**. In addition, *Eruca sativa* contains calcium, manganese, potassium, sodium, iron, copper, zinc, and secondary metabolites, and its glucosinolates (GSLs) have anticancer properties **(Crescenzi *et al.*, 2023; Ramazzina *et al.*, 2022 and El-Gayar *et al.*, 2022)**. *Eruca sativa* has also been known to have an aphrodisiac impact since roman times **(Wells, 2024)**. Recently, *Eruca sativa* leaves have been reported to improve sperm health and fertility parameters by increasing testicular, epididymal and seminal vesicle weights, increasing serum testosterone levels and testicular GSH levels associated with improved semen quality and quantity **(Abd-Elsalam e*t al.*, 2021 and Grami *et al.*, 2024)**.

Male hypogonadism can also be treated with clomiphene citrate (Clomid), a selective estrogen receptor modulator (SERM) that was first created to treat female infertility. Clomiphene blocks estrogen receptors in the hypothalamic arcuate nucleus and competitively inhibits 17β-estradiol. **(Dickey *et al.*, 1996)**. By increasing gonadotropin synthesis and blocking

the hypothalamus' and pituitary gland's negative feedback of estrogen, this action causes endogenous testosterone release. **(Scovell & Khera, 2018)**. Furthermore, clomiphene can preserve high follicle stimulating hormone (FSH), luteinizing hormone (LH), and intratesticular testosterone levels, therefore boosting spermatogenesis and increasing sperm concentration and motility. **(Huijben e*t al.*, 2023)**. Given the high prices of infertility drugs in addition to their side effects, most patients turn to safe alternative methods such as herbal and medicinal plant treatments. Thus, the goal of the current study was to compare the ethanolic extract of *Eruca sativa* leaves with clomid (Clo, a reference infertility medicine) and assess the preventive effect of this edible medicinal plant against acrylamide-induced testicular damage. In addition, to explain the previous probable roles, this study will look at the proximate composition, bioactive component concentration, and antioxidant activity of the leaf extract.

Materials and Methods:

Materials:

Plant:

Fresh *Eruca sativa* leaves were procured for the current study from the local market in Sheibin El-Kom city, Menoufia governorate, Egypt, and were verified by a taxonomist from the Faculty of Agriculture at Menoufia University in Shebin El-Koum, Egypt.

Drug used:

Clomiphene citrate tablets (Clo 50 mg tab.) were used and procured from El-Gomhorya company for trading drugs, chemicals, and medical requirements in Cairo, Egypt.

Chemicals and reagents:

Acrylamide (ACR) was purchased from Biodiagnostic Co. (Dokki, Giza, Egypt). Casein, serving as the primary protein source for rats' diet preparation, was supplied by Morgan Company for Chemicals in Cairo, Egypt, in addition to vitamins, salt mixtures, choline chloride, cellulose, L-methionine. All biochemical assay kits and reagents used in this study were procured from El-Gomhorya company for trading drugs, chemicals, and medical requirements, and from Alkan Medical Company in St. El-Dokki, Giza, Egypt. Every chemical and reagent utilized in this investigation was analytical grade or as pure as could be purchased commercially.

Animals:

Thirty male albino Sprague-Dawley rats aged 8 weeks and weighing 150-160 g, were acquired from the National Research Center's animal

housing unit in Giza, Egypt. The animals' weight was performed weekly during the experiment to record any sign of toxicity.

Ethical approval:

All the experimental and animal care protocols were ethically approved by the Institutional Animal Care and Use Committee (IACUC) at Menoufia University, Sheibin El-Kom, Egypt (Approval No. **MUFHE/F/NFS/38/24**). All biological experiments were performed in compliance with the policies of the IACUC for the use and care of laboratory animals.

Methods:

Basal Diet:

According to **Reeves *et al.*, (1993)**, the basic diet is made using the following formula: 465.692g of corn starch, 140g of casein-85% protein, 155g of dextrinized corn starch, 100g of sucrose, 40g of soybean oil, 50g of fiber, 35g of mineral mixture, 10g of vitamin mixture, L-cystine (1.8g), 2.5g of choline bitartrate, and 0.008g of tert-butylhydroquinone. Additionally, components of vitamin and mineral mixes were created in accordance with **Reeves *et al.*, (1993)**.

Preparation of the *Eruca sativa* leaves ethanolic extract (ESLE):

Samples of dried rocket leaves were brought to the laboratory. After sorting the leaves to remove damaged and foreign bodies, they were ground to a reduced powder (20 mesh) in a high-speed grinder (Moulinex Egypt, El Araby Company, Egypt) and mixed to create uniform samples. ESLE was prepared using the **Banso, (2009)** methodology. Fifteen liters of 95% ethanol were used to extract 2.2 kg of shade-dried rocket leaves. The mixture was then crushed using a magnetic stirrer for three hours each day and left for 21 hours for three days. The mixture was then filtered on Wattman #45 filter paper, so it was extracted again with 9 liters of 95% ethanol and filtered. The soluble ethanol extract was dried under decreased pressure at 60ºC.To compute the percentage yield of green fatty crude ESLE, use the formula (weight of extract/original weight × 100), which yields 12.16%. The plant extract was refrigerated at 4°C until using.

Analysis of phenolic compounds by high-performance liquid chromatography (HPLC):

An Agilent 1260 series was used for the HPLC analysis. A Zorbax Eclipse Plus C8 column (4.6 mm x 250 mm i.d., 5 µm) was used for the separation. Water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a

flow rate of 0.9 ml/min made up the mobile phase. The following is the sequential programming of the mobile phase in a linear gradient: 82% A for 0 minutes, 82% A for 0–1 minutes, 75% A for 1–11 minutes, 60% A for 11–18 minutes, 82% A for 18–22 minutes, and 82% A for 22–24 minutes. At 280 nm, the multi-wavelength detector was investigated. Each sample solution had a 5 µl injection volume. The column temperature was kept at 40°C.

Total phenolic content (TPC):

In accordance with the method described by **Jaćimović *et al.*, (2022)**, the TPC of *Eruca sativa* samples was evaluated using the conventional spectrophotometric folin–ciocalteu reagent. Using a gallic acid standard curve, the TPC was calculated as milligrams of gallic acid equivalents per 100 grams of fresh weight samples (mg GAE/100g).

Total flavonoid content (TFC):

The TFC of *Eruca sativa* samples was identified spectrophotometrically using the standard aluminum chloride method, as outlined by **Jaćimović *et al.*, (2022)**. Results were expressed as milligrams of rutin equivalents per 100 grams of fresh weight samples (mg RE/100g).

Determination of total antioxidant activity by DPPH:

The free radical scavenging capability was assessed using the technique of **Hwang and Thi (2014)**.

Experimental design:

Every biological studies was carried out in compliance with the guidelines established by the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council **(NRC, 1996)**. Rats (n=30) were kept in wire cages in separate rooms with normal healthy conditions, including a 12-hour lighting cycle, a temperature of 25 ± 30°C, and a relative humidity of 56±4%. Prior to the trial, all rats were given BD for a week in order to acclimate them. After one week period, the rats were divided into five equal groups as follow: group 1 (Normal control), healthy rats without intervention; group 2 (Model control), given by gavage 50 mg/kg bw/day ACR for 28 days to induce infertility; groups 3 to 5 received ESLE-1, ESLE-2 and Clo (infertility drug reference) at concentrations of 250, 500 and 1.2 mg/kg bw/day by oral gavage for 28 days, respectively followed by 50 mg/kg bw/day ACR. Based on the results of several previous studies, the concentrations of ESLE extract were selected for the experiments. **(Abd-Elsalam *et al.*,2021)**. The doses of ACR and Clo. were selected according to **Mehri *et al.*, (2016)** and **Al-sagheer and Kaabo (2022)**, respectively.

Biological Evaluation:

Daily food intake and weekly body weight were recorded during the 28-day experiment, and final body weights were collected at the conclusion of the study. The body weight gain (BWG,), food intake (FI) and food efficiency ratio (FER) were determined according to **Chapman *et al.*, (1959)** using the following equation: FER= grams gain in body weight (g/28 day)/ grams feed intake (g/28 day).

Samples:

At the end of the 4-week experimental period, by diethyl ether the rats were anesthetized and slaughtered, and Blood was obtained from the portal vein and by centrifugation at 3000 g for 10 minutes, the serum was separated stored at -20°C until use for further biochemical assays. Organs (testis, epididymis, seminal vesicles, and ventral prostate) were dissected, washed in ice-cold saline, and weighed. The gonadosomatic index (GSI) was derived using the formula (organ weight/body weight) × 100 **(Ansari and Ahmed 2014).** The testes were then separated into two halves. Part one was rinsed with 50 mM (sodium phosphate buffer saline pH 7.4) in ice containing 0.1 mM EDTA to eliminate any RBCs or clots before being stored at -20°C for the testicular oxidative stress marker assays. The second was preserved in 10% formaldehyde for sectioning and histological investigation **(Abd-Elsalam *et al.*, 2021)**.

Sperm quality analysis:

Each animal's epididymis was cut with a scalpel so that the sperm could be distributed in 3 mmol/L of Hanks' balanced salt solution (HBSS) at 37°C. Following the procedure outlined by **Tekin and Çelebi (2022)**, the suspension was analyzed for motility and viability, then sperm concentration and morphology.

- ***Sperm count***. The sperm count was calculated by diluting it by a factor of 1:50 in HBSS. Using a micropipette, this solution was added to the two grids of a Neubauer counting chamber. Using an optical microscope at high power (×40), the number of sperm in each of the four primary corner squares was counted.
- ***Sperm motility***. Two drops of epididymal sperm suspension were poured into a warmed slide and placed on a microscope stage heated to 37°C before being inspected under a light microscope. Motility was measured by counting at least 100 sperm from five separate squares based on the percentage of motile sperm. The sperm concentration and motility rate were determined using the following formulae.: **Sperm motility rate** = (The number of motile sperm/ the total number of sperm) ×100.
- ***Sperm morphology and viability***. For morphological assessment, two slides were prepared from epididymal sperm suspension. A specimen of 10

µl was placed on a slide, smeared with another slide, and the preparation was left to dry in air and then stained with eosin-nigrosine stain (1% eosin and 10% nigrosine). This stain can pass through the sperm membrane. The stain penetration was prevented If the membrane was intact, as in the case of viable spermatozoa. At the same time, the dye could penetrate the sperm cytoplasm if the membrane was damaged, like dead spermatozoa **(Kalaivani *et al.*, 2018)**. By preparing an eosin-nigrosine smear at an optimum temperature of 37°C and assessing at least 100 sperm under a bright-field microscope using magnification 1000X, sperm viability was determined **Rato *et al.*, (2013).**

Determination of serum reproductive hormones concentration:

Follicle-stimulating hormone (FSH), serum testosterone (T), and luteinizing hormone (LH) were measured by ELISA reader using commercial assay kits according to **Teerds *et al.*, (1989) , Sakuma (2009), Shioya and Wakabayashi (1998)** procedures, respectively.

Determination of serum liver function:

The modified kinetic method of **Yound (1975) and Tietz (1976)** was used to quantify the activities of serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST). The modified kinetic approach of **Vassault *et al.*, (1999)** was used to measure the activity of alkaline phosphatase (ALP).

Assessment of testicular oxidative stress markers:

In 10 volumes of 0.1 M Tris-EDTA solution (pH 7.4), tissue homogenates from testicular samples were prepared and centrifuged at 3200 × g for 20 min at 4 °C, and the upper juice fractions were used for spectrophotometric assessment of the levels of the following according to the method of **Abd-Elsalam *et al.*, (2021)**:

Superoxide dismutase (SOD) testicular activity was measured using the **Masayasu and Hiroshi (1979)** technique. The method of **Jollow *et al.*, (1974)** was used to assess glutathione activity (GSH). The **Aebi (1983)** approach was used to estimate the catalase (CAT) activity. The method of **Ohishi & Yagi (1979)** were used to estimate the amounts of malondialdehyde (MDA).

Histological evaluation:

Testis specimens were preserved in a 10% buffered formalin solution for 48 hours. Dehydration was carried out using graded ethanol, which was then imbedded in paraffin. The testis was sectioned using a rotary microtome and stained with haematoxylin and eosin (H and E) to detect microscopic histological changes. **(Bancroft & Gamble, 2002).**

Statistical analysis:

The collected data was analyzed using the Statistical Package for Social Sciences (SPSS, version 22). The means ± standard deviation (SD) were used to express the results. Duncan's multiple-range test and a one-way analysis of variance (ANOVA) were used to compare the experimental groups. P-values ≤ 0.05 were regarded as statistically significant. The percentage (rate) of changes between infertility control rats (model control) and normal control rats was determined to assess the severity of Biochemical and Histological Alterations in Rat Testis caused by ACR treatment. Likewise, to further evaluate the protective effects of the tested experimental diets(ESLE) , the rate of changes between the infertility rats treated with ESLE, Clo and model control rats was calculated as follows:

% of change compared with normal control (%)=

$$\frac{\text{(model control rats-Normal control rats)}}{\text{(Normal control rats)}} \text{x100}$$

% of change compared with model control (%)=

$$\frac{\text{(infertility groups treated with ESLE and Clo - Model control rats)}}{\text{(Model control rats)}} \text{x100}$$

Results and discussions:

Bioactive compounds, total phenolic (TPC), total flavonoid (TFC) and antioxidant activity (AOA) by 1,1-diphenyl-2-picrylhydrazyl (DPPH) of *Eruca sativa* leaves ethanolic extract (ESLE):

Bioactive compounds contents in ELSE:

Table 1 displayed the phenolic and bioactive component concentrations of the *Eruca sativa* leaves ethanolic extract (ESLE). According to this statistics, the greatest compound was sinapic acid, which was followed by cinnamic acid, ferulic acid, rosmarinic acid, quercetin, catechin, kaempferol, coffeic acid, vanillin, and protocatechuic acid. Sinapic acid contains antioxidant, antibacterial, anti-inflammatory, anticancer, and anti-anxiety properties. **(Ramazzina *et al.*, 2022)**, there is a reason for using *Eruca sativa* leaves to treat oxidative stress-related diseases, including infertility.

Also, such data concur with **Alghabban, (2024)** and **Crescenzi *et al.*, (2023)** who found that *Eruca sativa* is a rich source of flavonols and other typical compounds such as catechin, gallic acid, sinapic acid, ferulic acid, quercetin, and rosmarinic acid, which have some biological properties, particularly regarding their antioxidant properties. in addition to, these results agreed with **Raouf *et al.*, (2024)**, which showed that *Eruca sativa* has greater importance for biological activity through the availability of glucosinolates (GSL) and flavonoids in its leaves composition. It's

interesting to note that glucosinolates are the precursors of bioactive substances like erucin and sulforaphene, which are thoroughly researched for their potential to improve health. **(Connolly *et al.*, 2021)**.

Additionally, the current study's data are consistent with those acquired by **Abd-Elsalam *et al.*, (2021), Awadelkareem *et al.*, (2022) and Algreiby,(2024)** who showed that *Eruca sativa* has positive effects on human health because it contains many biologically active compounds, such as vitamins, fatty acids, alkaloids, flavonoids, terpenoids, phenols, antioxidants, and glucosinolates (GSLs).

Table 1. Bioactive constituents of *Eruca sativa* leaves ethanolic extract (ESLE)

Bioactive constituents of ESLE	
Compound	**Conc. (µg/g)**
Protocatechuic acid	14.30 ± 3.05
Catechin	49.38 ± 3.92
Coffeic acid	23.75 ± 3.52
Vanillin	14.55 ± 1.06
Ferulic acid	933.13 ± 12.56
Sinapic acid	1815.68 ± 17.05
Rosmarinic acid	63.91 ± 4.68
Querectin	51.31 ± 4.37
Cinnamic acid	10.51 ± 1.09
Kaempferol	25.81 ± 2.94

Total phenolic (TPC), total flavonoid (TFC) and antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) of ELSE:

Table 2 shows the total phenolic (TPC), total flavonoid (TFC) as well as antioxidant activity by DPPH. The results obtained show that the ethanolic extract from *Eruca sativa* had anti-radical activity plus antioxidants; about 14.5 mg of GAE per gram of total Phenols 10.941 mg of CE per gram of total Flavonoids, and 3.81 mg of TE per gram of DPPH as an antioxidant in the leaves.

These results are completely in line with those of **El-Gayar *et al.*, (2022)**, who found that *Eruca sativa* leaf extract is a good source of flavonoids and phenolic compounds, with 12.522 mg GAE/g and 9.938 mg CE/g extract, respectively, and that the antioxidant activity of the plant demonstrated a decrease in the DPPH and ABTS with a 63.2% and 90.3% scavenging activity. Additionally, these results concur with **Barillari *et al.*, (2005)** and **Koubaa *et al.*, (2015)** documented those rockets (*Eruca sativa L.*) possessed anti-inflammatory, antioxidant, and antibacterial properties.

Moreover, **Awadelkareem *et al.*, (2022)** showed that the antioxidant activity of hydrogen peroxide (H_2O_2) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) in *Eruca sativa* leaf extract was determined to be IC50 = 76.05 µg/mL and 66.16 µg/mL, respectively. According to **El-Nwehy *et al.*, (2023)**, a variety of extracts of *Eruca sativa* have been regarded as a plentiful source of antioxidants since they contain high levels of GSL, flavonoids, and carotenoids. The methanolic extract of *Eruca sativa* leaves included a TFC of 14.03 mg of CE/g, a TAC of 0.25 mg of cyanidin-3-glucoside/g, 0.36 mg of β-carotene/g, and 1.49 mg of L-ascorbic acid/g, a methanolic extract of *Eruca sativa* leaves with a DPPH scavenging ability of 71.70 percent with an IC50 of 0.15 mg/mL and a low FRAP (123.16 mM of Fe2+/g) demonstrated 80% antioxidant activity when tested using DPPH and FRAP procedures according to **Keyata *et al.*, (2021).**. As a result, this research sought to investigate the therapeutic effects of *Eruca sativa*.

Table 2. Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AOA) by DPPH of ESLE.

TPC (mg GAE/100g)	TFC (mg RE/100g)	AOA DPPH (mg TE/g)(%)
14.5±0.98	10.941±0.64	3.81(88.8%)

GAE: gallic acid equivalent; RE: rutin equivalent

Biological Studies of *Eruca sativa* leaves ethaolic extract (ESLE):

Effect of *Eruca sativa* leaves ethaolic extract (ESLE) and Clomid (Clo) on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of infertility rats:

Table 3 displayed the effects of ESLE and Clo on the infertile rats' body weight gain (BWG), feed efficiency ratio (FER), and feed intake (FI). These findings showed that, in comparison to the normal group, the ACR-treated rats had significantly ($p\leq0.05$) lower BWG (-128.3%), FI (-49.9%), and FER (-156.3%) at the end of the experiment (4 weeks). But after four weeks of treatment with ESLE (250 and 500 mg/kg BW/day) and Clo (1.2 mg/kg BW/day), the infertility rats' BWG, FI, and FER increased significantly ($p\leq0.05$) at rates of 17.35, 26.7, and 162.7%, 331.03, 70.8, and 235.1%, and 358.24, 78.2, and 247.9%, respectively, in comparison to the model control group. The rate of increasing in BWG, FI and FER of the infertility rats were exhibited a dose-dependent manner.

The data in the same table showed that the ELSE-2+ACR (500 mg/kg body weight/day) and Clo.+ACR groups recorded the best results for BWG, FI and FER and there were non-significant ($p\leq0.05$) differences

between them. These findings are in line with **Yildirim *et al.*, (2024)**, who demonstrated that ACR toxicity leads to weight loss due to several factors such as oxidative stress, where cells receive less oxygen leading to fatigue and decreased physical activity in addition to muscle weakness, decreased muscle mass, decreased appetite, and impaired ability of the body to absorb nutrients, leading to decreased food intake and weight loss. Furthermore, acrylamide, due to its high toxicity and its oxidative stress, can cause muscle weakness, weight loss, according to **Perera *et al.*, (2021).**

On the contrast, intervention with ESLE showed positive results, these findings are in line with **El-Gayar *et al.*, (2022)**, who found an increase in body weight in rats treated with doses of 1, 3, and 5 g/kg body weight ESLE. At the 7th week, administering *Eruca sativa* extract significantly raised body weight ($p<0.01$). Because of the presence of a significant amount of GSL, flavonoids, and carotenoids, various extracts from *Eruca sativa* leaves were considered as an abundant source of antioxidants, which helps prevent oxidative stress and hence restore cell viability and weight gain, according to **El-Nwehy *et al.*, (2023) & Javed *et al.*, (2024).**

Table (3): Impact of ESLE/Clo interventions on changes in BWG, FER, and FI(g/day) among infertility rats

Groups		BWG (g/day)		FI(g/day)		FER (g/ day)	
		Mean ±SD	Change %	Mean ±SD	Change %	Mean ±SD	Change %
Normal control		37.43±2.60^{a}	---	22.36±2.01^{a}	---	1.67±0..13^{a}	---
Infertility groups	**Model control**	-10.61±0.85^{c}	-128.3	11.20±0.98^{d}	-49.9	-0.94±0.03^{d}	-156.3
	ESLE-1+ ACR	8.421±2.55^{c}	17.35	14.20±0.95^{c}	26.7	0.59±0.18^{c}	162.7
	ESLE-2+ ACR	24.48±1.70^{b}	331.03	19.13±0.32^{b}	70.8	1.27±0.07^{b}	235.1
	Clo.+ ACR	27.40±3.32^{b}	358.24	19.96±0.32^{ab}	78.2	1.39±0.30^{ab}	247.9

Each value represents mean ± SD (n=6). Values under the same column with different superscripts mean significant difference at P≤0.05. ESLE-1+ ACR, ESLE-2+ ACR and Clo.+ ACR, infertility groups received orally 250,500 mg/kg bw/day of ESLE and 1.2 mg/kg bw/day of Clo, respectively followed by 50 mg/kg bw of ACR. BWG: Body Weight Gain; FI: Food Intake and FER: Food Efficiency Ratio

Effect of *Eruca sativa* leaves ethaolic extract (ELSE) and Clomid (Clo) on final body weight (FBW), organs weight and gonadosomatic index (GSI) of infertility rats:

The impact of ESLE and Clo on final body weight (FBW), organs weight and gonadosomatic index (GSI) of infertility rats was shown in Table 4. The results showed that the FBW in model control group was significantly ($p\leq0.05$) lower than normal control group with means of 113.23±2.65 and 162.04±3.04 respectively. While the mean values of FBW in ESLE-1+ACR, ESLE-2+ACR and Clo+ACR groups were significantly

($p \leq 0.05$) higher than model control group with means of 132.64±1.92, 149.48±1.70 and 152.40±3.31, respectively.

At the same table, these data found that the mean values of weights and GSI of testes, epididymis, seminal vesicles, and ventral prostate at model control group were significantly lower than the normal control group. Also, the intervention with ESLE extract and Clo led to a significant ($p \leq 0.05$) decrease in the weights and GSI of these organs. In addition to, group 4 which received orally 500 mg/kg BW/day of ESLE had the best results. The present study shows that the ACR induced toxicity led to decrease testicular and epididymal weights of infertility rats. These results agreed with **Saleh *et al.*, (2024)** who found a significant decrease in both relative testicular and epididymal weights in ACR-intoxicated rats compared to the control group.

Also, results of the current study were in agreement with the study of **Abd-Elsalam *et al.*, (2021)** which reported that acrylamide-induced toxicity led to a significant decrease in the relative epididymis weight but were in contrast to these results for the testicular weight as the results of this study did not show significant differences in the relative testicular weight. Furthermore, rats treated with ACR had lower testicular weights in the other groups than in the control group, but this difference was not statistically significant, according to **Yildirim *et al.*, (2024)**. It's interesting to note that the current study shows that ESLE (500 mg/kg) caused a significant ($p \leq 0.05$) increase in final body weight, as well as weights and GSI of the testes, epididymis, seminal vesicles, and ventral prostate. These findings are in line with a study by **Ansari and Ahmed (2014)** who showed that rats given *Eruca sativa* had significantly higher organ weight and GSI when compared to the diabetic control group. Also, **Grami *et al.*, (2024) & Algreiby, (2024)** reported that treatment with ethanolic extract of *Eruca sativa* significantly increased the weight of testes, epididymis, seminal vesicles, and prostate in rats at the end.

For Clo, the present data showed improvement in testes weight after treatment with Clo but this result was in disagreement with **Elbana (2023)**, who reported that atrophy and decreased testicular weight in rats was associated with treatment with clomiphene citrate; they attributed these changes to suppression of gonadal activity.

Table (4): Impact of ESLE/Clo interventions on changes in FBW, organs weight and GSI among infertility rats

Parameters		Normal control	Infertility groups			
			Model control	ESLE-1+ ACR	ESLE-2+ ACR	Clo.+ ACR
FBW (g)		162.04^{a}±3.04	113.23^{d}±2.65	132.64^{c}±1.92	149.48±1.70^{b}	152.40±3.31^{b}
Organ weights(g)	**Testes**	3.56±0.21a	1.13±0.25e	1.73±0.21d	2.28±0.20c	2.86±0.15b
	Seminal vesicles	1.53±0.25a	0.52±0.07c	0.81±0.10b	1.27±0.14a	1.40±0.10a
	Epididymis	0.56±0.12a	0.16±0.07b	0.22±0.10b	0.45±0.06a	0.51±0.09a
	Prostate	0.51±0.13a	0.18±0.06b	0.20±0.01b	0.41±0.08a	0.49±0.07a
GSI (%)	**Testes**	2.20±0.15a	1.01±0.24d	1.31±0.15c	1.52±0.15c	1.88±0.08b
	Seminal vesicles	0.95±0.15a	0.46±0.06b	0.61±0.08b	0.85±0.10a	0.92±0.05a
	Epididymis	0.35±0.07a	0.15±0.06b	0.16±0.08b	0.29±0.04a	0.34±0.06a
	Prostate	0.31±0.08a	0.09±0.04b	0.15±0.01b	0.25±0.06a	0.33±0.04a

Each value represents mean ± SD (n=6). Values under the same column with different superscripts mean significant difference at P≤0.05. ESLE-1+ ACR, ESLE-2+ ACR and Clo.+ ACR, infertility groups received orally 250,500 mg/kg bw/day of ESLE and 1.2 mg/kg bw/day of Clo, respectively followed by 50 mg/kg bw of ACR. FBW: Final Body Weight and GSI: Gonadosomatic index

Effect of *Eruca sativa* leaves ethaolic extract (ESLE) and clomid (Clo) on sperm parameters (sperm count, sperm motility %, and sperm abnormalities %) of infertility rats:

The impact of ESLE and Clo on the sperm count, sperm motility percentage, and sperm abnormalities percentage of infertile rats was displayed in Table 5. According to these data, the ACR-treated rats showed a significantly (p≤0.05) lower sperm count (-93.78) and sperm motility percentage (-68.95%) at the end of the experimental (4 weeks) than the normal group. In contrast, the ACR-treated rats exhibited significantly (p≤0.05) increased in sperm abnormalities % by (471.32) compared to the normal group. However, intervention with ESLE (250 and 500 mg/kg BW/day) and Clo (1.2 mg/kg BW/day) for 4 weeks led to significantly (p≤0.05) increase on sperm count and sperm motility % by 530.9, 1093.4 and 1105.2 % and111.76, 177.4 and 191.2% respectively and led to significantly (p≤0.05) decrease in sperm abnormalities % by -44.35, -72.7 and -72.2% compared to the model control group.

These results also showed that the groups treated with 500 mg/kg BW/day of ESLE and 1.2 mg/kg BW/day of Clo recorded the best results for these markers and there were non-significant (p≤0.05) differences between them. These investigation found that ACR application led to an increase in aberrant sperm morphology and a decrease in sperm count and viability, which is consistent with the results obtained. Research has

indicated that ACR lowers sperm quality and quantity. These effects may be related to the direct toxic effects of ACR on sperm cells, the disruption of the hypothalamic-pituitary-gonadal axis, and increased production of reactive oxygen species **(Govindaraju *et al.*, 2024; Alturki *et al.*, 2022; Abd-Elsalam *et al.*, 2021; Farag *et al.*, 2021; Kucukler *et al.*, 2020)**.

According to **Triningsih *et al.*, (2021)**, reactive oxygen species (ROS) are known to damage nuclei, prevent enzyme processes, hinder the synthesis of sulfhydryl antioxidants, and start lipid peroxidation in cell membranes, which is necessary to give the plasma membrane the fluidity necessary for sperm movement. However, a prior study suggests that ACR-induced reductions in testosterone hormone may lead to hypospermatogenesis, which is in line with these findings **(Farag *et al.*, 2021)**. Additionally, this data supports the findings of **Saleh *et al.*, (2024) and Yildirim *et al.*, (2024)**, who discovered that, in comparison to the control rats, ACR exposure significantly decreased the number of sperm and the percentage of sperm motility and live sperm while also significantly increasing the percentage of abnormal sperm. According to **Saleh *et al.*, (2024)**, the primary causes of these detrimental effects of ACR on sperm count and quality in the epididymis are the breakdown of seminiferous tubule epithelial cells and the inhibitory action of ACR on kinesin and dynein, the motor proteins of the cytoskeleton of sperm flagella, which results in impaired sperm motility. On the other hand, 4 weeks of ESLE treatment successfully reversed the negative effects of ACR on sperm count and quality, as shown by a notable increase in sperm number, motility, and viability and a decrease in aberrant sperm. These findings show that ESLE positively affects sperm count and morphology by suppressing ACR-induced oxidative stress and inflammation in testicles with its antioxidant and anti-inflammatory activity.

These findings are in line with a study by **Grami *et al.*, (2024)** that discovered that *Eruca sativa* has a protective impact on sperm parameters by sustaining sperm cell density and sperm count through its antioxidant capability. These findings also concur with those of **Raouf *et al.*, (2024) and Algreiby (2024)**, who demonstrated a notable improvement in the occurrence of typical sperm morphology in the *Eruca sativa*-treated groups when compared to the control group. They also explained that this improvement was caused by the rocket leaves' high concentration of saponins, alkaloids, terpenes, flavonoids, glycosides, and steroids, all of which promote sperm supply. Additionally, **Abd-Elsalam *et al.*, (2021)** previously found that ESLE improved sperm quantities, morphology,

vitality, and motility in healthy rodents. For Clo, these data showed a significant (P≤0.05) increase in sperm count, sperm motility % and a significant (P≤0.05) decreased in sperm abnormality % after oral administration of Clo with ACR for 28 days . This finding was consistent with that of **Wahab *et al.*, (2019)**, who showed that the administration of Clo enhanced sperm motility and viability in rats exposed to lead acetate. Clo may increase the anterior pituitary gland's production of follicle-stimulating hormone and luteinizing hormone, which could be a mechanism for increasing sperm count and quality **(Chehab *et al.*, 2015; Ring *et al.*, 2016)**. It has been found that higher levels of luteinizing hormone promote spermatogenesis and the production of testosterone **(Chehab *et al.*, 2015; Ring *et al.*, 2016)**.

These results concurred with those of **Huijben *et al.*, (2022)**, who discovered that clomiphene citrate treatment causes functional alterations in sustentacular cells, which are linked to an increase in the number of sperm. However, these findings contradicted those of **Elbana (2023)**, who demonstrated that following the administration of clomiphene citrate, the number of total sperm fell and aberrant sperm morphology rose.

Additionally, according to **Elbana (2023)**, the medication Clo should be stopped for at least three cycles before being taken again and should not be utilized for more than three to six cycles. Therefore, intervention with ESLE in these study is considered better than the drug because it is safe and effective in improving sperm quality.

Table (5): Impact of ESLE/Clo interventions on changes in sperm count x106, sperm abnormal morphology(%), sperm viability (%) among infertility rats.

groups		Sperm count (million/ml)		Sperm Motility (%)		Sperm Abnormality (%)	
		mean±SD	Change%	mean±SD	Change %	mean±SD	Change %
Normal control		24.45^{a}±2.11	---	49.29^{a}±1.14	---	10.60^{d}±0.85	---
Infertility groups	**Model control**	1.52^{d}±0.48	-93.78	15.30^{d}±1.96	-68.95	60.56^{a}±0.86	471.32
	ESLE-1+ ACR	9.59^{c}±0.65	530.9	32.40^{c}±2.26	111.76	33.70^{b}±2.46	-44.35
	ESLE-2+ ACR	18.14^{b}±1.32	1093.4	42.45^{b}±2.42	177.4	16.53^{c}±2.66	-72.7
	Clo.+ ACR	18.32^{b}±1.24	1105.2	44.56^{b}±0.58	191.2	16.83^{c}±2.62	-72.2

Each value represents mean ± SD (n=6). Values under the same column with different superscripts mean significant difference at P≤0.05. ESLE-1+ ACR, ESLE-2+ ACR and Clo.+ ACR, infertility groups received orally 250,500 mg/kg bw/day of ESLE and 1.2 mg/kg bw/day of clo, respectively followed by 50 mg/kg bw of ACR.

Effect of *Eruca sativa* leaves ethaolic extract (ESLE) and clomid(Clo) on serum testosterone (T), luteinizing hormone (LH) and follicle stimulating hormone (FSH) of infertility rats:

Data in Table 6 were shown the impact of ESLE and Clo. on serum testosterone, LH, and FSH of infertility rats. Such data indicated that rats treated with ACR (model control) had decreased levels of the testosterone, LH, and FSH compared to the normal control group by -80.84, -76.98 and -89.16%, respectively. However, ELSE-1, ESLE-2, and Clo intervention at doses of 250, 500, and 1.2 mg/kg BW/day, respectively followed by 50 mg/kg BW/day of ACR for 4 weeks resulted in a significant ($p \leq 0.05$) increase in testosterone, LH, and FSH levels by 175, 260.22 and 281.62% and 188, 268.6 and 260.1% and 259.65, 455.86 and 545.38%, respectively, compared to the model control group.

From the previous results, it can be considered that the effect of 500 mg of ESLE was similar to the effect of Clo, as there was non-significant ($p \leq 0.05$) difference between them in serum testosterone, LH, and FSH levels. These results established that ACR triggered endocrine disruption underscored by the altered levels of testosterone, LH and FSH which has been reported in studies of **Abd-Elsalam *et al.*, (2021) and Farag *et al.*, (2021)** who found that the hormonal imbalance mentioned here is due to the harmful effect of ACR on testosterone synthesis in the testicles. Also, **Saleh *et al.*, (2024)** found a significant reduction in serum testosterone (71%), FSH (75.95%) and LH (76.83%) levels has been observed in ACR-intoxicated group, compared to the control rats. On the other hand, ESLE improving activity on testosterone, LH and TSH concentrations in the present study results was consistent with previously reported results in cadmium exposed rats **(Nowfel and Al-okaily 2017)**, nicotine intoxicated rats **(Abd El-Aziz *et al.*, 2016)**, and healthy rats **(Al-Qudah, 2017)**.

Extract of *Eruca sativa* leaves is considered for the rise of testosterone level and increased sperm activity as well as it reduced sperm death and its abnormalities **(Hadi, 2017).** As **Raouf *et al.*, (2024)** pointed out the higher rise in testosterone and a slight increase in luteinizing hormone (LH) in the treated groups with *Eruca sativa* leaves extract are associated with the presence of polyphenolic flavonoid compounds in the leaf of *Eruca sativa*. In corollary, it could be suggested that the improvement of reproductive function, sperm quality and testis histology observed in rats treated with ESLE may be attributed to the antioxidant activity of ESLE which has ameliorating effect on the hypothalamus–pituitary–testis axis leading to restorative levels of T, LH and FSH.

For Clo, the present study showed an improvement in serum testosterone, LH and FSH levels in infertile rats after Clo treatment. This result was in agreement with **Wahab *et al.*, (2019)** who found that taking Clo with lead acetate resulted in a significant increase ($p<0.05$) in serum levels of luteinizing hormone and testosterone. But the current data was in disagreement with **Elbana (2023)** who found that Clo inhibited the secretion of gonadotropins FSH and LH in rats.

Table (6): Impact of ESLE/Clo interventions on changes in serum T, LH and FSH among infertility rats.

groups		T (ng/ml)		LH(ng/ml)		FSH(ng/ml)	
		Mean ±SD	Change %	Mean ±SD	Change %	Mean ±SD	Change %
Normal control		27.56±0.13^{a}	---	19.25±0.10^{a}	---	135.88±3.17^{a}	---
Infertility groups	**Model control**	5.28±0.55^{d}	-80.84	4.43±0.02^{d}	-76.98	14.72±0.63^{d}	-89.16
	ESLE-1+ ACR	14.52±0.27^{c}	175	12.67±0.44^{c}	186	60.73±2.73^{c}	259.65
	ESLE-2+ ACR	19.02±0.36^{b}	260.22	16.33±0.21^{b}	268.6	98.50±2.24^{b}	455.86
	Clo+ ACR	20.15±1.40^{b}	281.62	15.95±0.18^{b}	260.1	95.00±5.96^{b}	545.38

Each value represents mean ± SD (n=6). Values under the same column with different superscripts mean significant difference at P≤0.05. ESLE-1+ ACR, ESLE-2+ ACR and Clo.+ ACR, infertility groups received orally 250,500 mg/kg bw/day of ESLE and 1.2 mg/kg bw/day of Clo, respectively followed by 50 mg/kg bw of ACR. FSH, Follicle Stimulating Hormone; LH , Luteinizing Hormone.

Effect of *Eruca sativa* leaves ethaolic extract (ESLE) and clomid (Clo) **on liver enzymes of infertility rats:**

The effect of *Eruca sativa* leaves ethaolic extract (ESLE) and clomid (Clo) on liver functions (ALT, AST and ALP) of infertility rats are shown in Table 7. Such data indicated that at the end of the experiment (4 weeks), the ACR-treated rats exhibited significantly ($p\leq0.05$) increased in ALT (153.18%), AST (115.28%) and ALP (277.41%) compared to the normal group. However, intervention with ELSE-1 and ELSE-2 at doses of 250 and 500 mg/kg BW/day, respectively followed by 50 mg/kg BW/day of ACR for 4 weeks led to significantly ($p\leq0.05$) decrease on the ALT, AST and ALP by-24.31and -38.06%, and -22.8 and -34.16%, and -23.07 and -45.7% compared to the model control group, respectively. Also, the study showed that the levels of AST, ALT and ALP in infertility rats decreased in a dose-dependent manner. In addition to, these results in the same table showed non-significant($p\leq0.05$) differences between the group treated with Clo and the model control group, which explains the presence of side effects of the Clo drug for treating infertility, represented by an increase in liver enzymes (ALT,AST and ALP) by 2.43, 3.72 and 5.84%, respectively compared to the model control group.

Regarding the activities of the enzyme, the results of the current study showed that ACR significantly increases the concentrations of ALT, AST and ALP in the serum. These results were consistent with those of **Cerrah *et al.*, (2023) and Erfan *et al.*, (2021)**, who found that ACR caused elevated levels of, ALT, AST and ALP in addition, diffuse degenerative changes and necrosis in hepatocytes, and moderate inflammation in the portal area were detected in liver tissues in the ACR group while ESLE prevented the biochemical changes induced by acrylamide, it also alleviated the damage in tissue structure. Again, in ACR-related hepatotoxicity, apart from oxidant and antioxidant parameters, pro-inflammatory cytokines have been reported to play a role and demonstrated that acrylamide caused an increase in the expression of interleukin-1beta (IL-1β), interleukin-6, tumor necrosis factor-alpha (TNF-α) and nuclear factor kappa b (NF- κB) in liver and kidney tissues **(Kandemir *et al.*, 2020)**.

This literature information shows that antioxidant and anti-inflammatory plants can be used in acrylamide hepatotoxicity. Therefore, the data of the present study confirmed that the ESLE prevented the biochemical changes induced by acrylamide and reduced AST, ALT and ALP levels which demonstrating that it could prevent the live cells damage and alleviated the damage in tissue structure. Additionally, the present data are in agreement with those of **Ybañez-Julca *et al.*, (2022) and Rahbardar *et al.*, (2021)** who reported that the protective role of *Eruca sativa* ethanolic extract may be achieved by the noticeable improvement in serum ALP, AST, γ-GT, and LDH, which goes in parallel with the suppressive effect on ACR due to the effect of various antioxidant compounds such as flavonoids, Zn, and Cu present in *Eruca sativa* extract, which are thought to be an essential component of free radical scavenging SOD and also impair lipid peroxidation. In line with current data, **Zangana and Erdeni (2020)** showed that liver enzymes were improved by use different doses of *Eruca sativa* extract.

For Clo, the present data indicated that Clo intervention caused an increase in liver enzymes and this result was consistent with **Al-Amoudi (2012)** who reported an increase in the levels of liver function enzymes ALT, AST, ALP in rats that were given clomiphene citrate. Furthermore, **Zhang *et al.*, (2018)** study reported cases of liver disease associated with the administration of clomiphene citrate to male patients. From the above, we can say that Clo is not recommended for use by patients with liver disease due to its side effects on elevated liver enzymes. This is what

distinguishes the ethanolic extract of *Eruca sativa* leaves at a dose of 500 mg/kg, the results of which showed an improvement in liver enzymes compared to the Clo group.

Table (7): Impact of ESLE/Clo interventions on changes in ALT, AST and ALP among infertility rats.

groups		ALT (u/ml)		AST(u/ml)		ALP(ng/ml)	
		Mean ± SD	Change%	Mean ± SD	Change%	Mean ± SD	Change%
Normal control		32.81±2.80^{e}	---	43.76±2.93^{e}	---	1.86±0.26^{e}	---
Infertility groups	**Model control**	83.07±4.79^{ab}	153.18	94.21±2.14^{ab}	115.28	7.02±0.52^{ab}	277.41
	ESLE-1+ ACR	62.87±3.19^{c}	-24.31	72.73±4.33^{c}	-22.8	5.40±0.62^{c}	-23.07
	ESLE-2+ ACR	51.45±3.53^{d}	-38.06	62.02±2.29^{d}	-34.16	3.81±0.11^{d}	-45.7
	Clo+ ACR	85.09±4.05^{a}	2.43	97.72±2.14^{a}	3.72	7.43±1.08^{a}	5.84

Each value represents mean ± SD (n=6). Values under the same column with different superscripts mean significant difference at P≤0.05. ESLE-1+ ACR, ESLE-2+ ACR and Clo.+ ACR, infertility groups received orally 250,500 mg/kg bw/day of ESLE and 1.2 mg/kg bw/day of Clo, respectively followed by 50 mg/kg bw of ACR.. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase.

Effect of *Eruca sativa* leaves ethaolic extract (ESLE) and clomid (Clo) on changes in the activity of oxidative and antioxidant enzymes in the testicular tissues of infertility rats:

The effect of ESLE and Clo intervention on changes in SOD, GSH, CAT and MDA levels profile in the testicular tissues of infertility rats were shown in tables 8 and 9. The data indicated that at the end of the experiment (4 weeks), the ACR-treated rats exhibited significantly (p≤0.05) decreased in SOD, GSH and CAT by-79.29, -81.09 and -80.74 %, respectively whereas, the MDA increased by 292.1% in testicular tissues compared to the normal control group. However, intervention with ESLE (250 and 500 mg/kg BW/day) for 4 weeks led to significantly (p≤0.05) increase in SOD, GSH and CAT by 68.29, 239.02 and 138.7%, and 254.95, 104.4 and 261.1%, respectively as well as the MDA level in testes tissue was significantly (p≤0.05) declined by -39.89 and -63.77%, respectively compared to the model control group.

In the same table, the data showed a non-significant (p≤0.05) difference between the Clo+ACR group and the model control group, as the results showed a significant decrease (p≤0.05) in the levels of SOD, GSH and CAT in the testicular tissues of the Clo+ACR group by-81.31, -75.46 and -81.01%, respectively as well as the MDA level in testes tissue was significantly (p≤0.05) rose by 244.6% compared to the normal control group. Overall, the present data found that ESLE treatment at a dose of 500

mg recorded the best results for antioxidants and oxidative enzymes in testicular tissues of infertility ratsACR was found to significantly increase MDA levels in testicular tissue and reduce GSH, SOD, and CAT activities in experimental studies of a testicular injury model it induced in rats **(Yildirim *et al.*, 2024)**.

Additionally, ACR-induced liver oxidative stress lowered SOD, GSH, and CAT activity and increased lipid peroxidation, according to **Ybañez-Julca *et al.*, (2022) and Saleh *et al.*, (2024)**. These investigation found that ACR treatment significantly reduced SOD, GSH, and CAT activities and raised MDA levels in testicular tissue, which is in line with earlier research. At the same time, ESLE intervention prevented oxidative stress caused by ACR, and these results come in harmony with **Ramazzina *et al.*, (2022)** who demonstrated that the ethanolic extract increased cell viability and was able to reduce cytotoxicity and ROS and attributed this to its high content of glucosinolates which led to a significant decrease in intracellular ROS production. where the endogenous antioxidant system detoxifies reactive oxygen species (ROS) from physiological metabolisms to preserve cellular redox homeostasis **(Abd-Elsalam *et al.*, 2021; Awadelkareem *et al.*, 2022 and Raouf *et al.*, 2024).**

Furthermore, *Eruca sativa's* high concentration of carotenoids, fibers, minerals, glucosinolates, isothiocyanates, flavonoids including kaempferol, quercetin, and isorhamnetin, flavanols, and phenolic compounds is thought to be responsible for its antioxidant qualities **(El-Gayar *et al.*, 2022; El-Nwehy *et al.*, 2023; Algreiby, 2024 and Grami *et al.*, 2024)**. GSLs found in watercress leaves break down during stress to produce biologically active compounds called isothiocyanates (ITCs), which have anti-inflammatory and antioxidant properties **(Bell *et al.*, 2020)**. This illustrates how the phenolic chemicals in *Eruca sativa*, which influence the modification of antioxidant enzymes by enhancing their function, contribute to its antioxidant properties **(Nowfel and Al-Okaily 2017)**. According to **Pagnotta *et al.*, (2022)**, *Eruca sativa* has the ability to protect cells from oxidative stress and decrease the release of inflammatory mediators.

As for Clo, these results did not show any improvement in antioxidant levels and agreed with **Wahab *et al.*, (2019)**, who found that giving clomiphene citrate to mice exposed to lead acetate, a chemical similar in effect to acrylamide, did not affect the activity of catalase, superoxide dismutase, or malondialdehyde concentrations. This observation suggests that a possible mechanism of Clo in non-improving antioxidant

enzymes may be its non-ability to prevent lipid peroxidation, free radical generation, or oxidative stress induced by ACR. Therefore, we recommend eating *Eruca sativa* leaves rich in antioxidants, glucosinolates and erucin to prevent testicular toxicity caused by acrylamide.

Table (8): Impact of ESLE/CLO interventions on changes in SOD, GSH, and CAT in the testicular tissues among infertility rats

Groups		SOD (U/mg tissue)		GSH (ng/mg/ tissue)		CAT (U/mg/ tissue)	
		Mean ±SD	Change%	Mean ±SD	Change %	Mean ±SD	Change%
Normal control		3.96±0.09^{a}	---	5.87±0.08^{a}	---	3.74±0.217^{a}	---
Infertility groups	**Model control**	0.82±0.03^{d}	-79.29	1.11±0.08^{d}	-81.09	0.72±0.036^{d}	-80.74
	ESLE-1+ ACR	1.38±0.17^{c}	68.29	2.65±0.49^{c}	138.7	1.47±0.213^{c}	104.4
	ESLE-2+ ACR	2.78±0.08^{b}	239.02	3.94±0.43^{b}	254.95	2.60±0.273^{b}	261.1
	Clo.+ ACR	0.74±0.17^{d}	-81.31	1.44±0.35^{d}	-75.46	0.71±0.087^{d}	-81.01

Each value represents mean ± SD (n=6). Values under the same column with different superscripts mean significant difference at P≤0.05. ESLE-1+ ACR, ESLE-2+ ACR and Clo.+ ACR, infertility groups received orally 250,500 mg/kg bw/day of ESLE and 1.2 mg/kg bw/day of Clo, respectively followed by 50 mg/kg bw of ACR. SOD: Super oxide dismutase; GSH: Glutathione and CAT: Catalase.

Table (9): Impact of ESLE/Clo. interventions on changes in MDA in the testicle tissue among infertility rats

groups		MDA (nmol/g tissue)	
		Mean ±SD	Change %
Normal control		7.367±0.45^{e}	---
Infertility groups	**Model control**	28.9±1.17^{a}	292.1
	ESLE-1+ ACR	17.37±1.4^{c}	-39.89
	ESLE-2+ ACR	10.47±0.6^{d}	-63.77
	Clo.+ ACR	25.39±2.5^{b}	244.6

Each value represents mean ± SD (n=6). Values under the same column with different superscripts mean significant difference at P≤0.05. ESLE-1+ ACR, ESLE-2+ ACR and Clo.+ ACR, infertility groups received orally 250,500 mg/kg bw/day of ESLE and 1.2 mg/kg bw/day of Clo, respectively followed by 50 mg/kg bw of ACR. MDA: Malondialdehyde.

Effect of *Eruca sativa* leaves ethaolic extract (ESLE) and clomid (Clo) on rats testes histological disorders induced by acrylamide:

Effect of *Eruca sativa* leaves ethaolic extract (ESLE) and clomid (Clo) on rats testes histological disorders induced by acrylamide were shown in Figure 1. Under a microscope, the testes of rats in group 1 (normal control) displayed full spermatogenesis, normal lining spermatogoneal cells, and the typical histological structure of a

seminiferous tubule (Photos A, B, and C). In contrast, testes of rats from group 2 (model control) exhibited degeneration of spermatogoneal cells lining seminiferous tubules with formation of spermatid giant cell as well as interstitial edema (Photos. D, E & F). On the other hand, some testicular sections of rats from group 3 (ESLE-1+ACR) showed degeneration of spermatogoneal cells lining seminiferous tubules and interstitial edema (Photos. H and i), whereas other sections revealed normal seminiferous tubules and complete spermatogenesis with sperm production in their lumen (photo. G).

Furthermore, testes of rats from group 4 (ESLE-2+ACR) exhibited no histopathological alterations and histologically normal seminiferous tubules (Photos. J, K & L). Additionally, testes of rats from group 5 (Clo+ACR) showed degeneration of spermatocytes lining the seminiferous tubules and interstitial edema (photos. M, N& O).

These findings concurred with those of **Saleh *et al.*, (2024)**, who discovered that the control group's testes had typical seminiferous tubules with neatly arranged sperm chains. In contrast, the testes of the ACR group showed increasing testicular degeneration with substantial germ cell death, particularly spermatocytes as well as a decrease of round and elongated spermatocytes.

ACR toxicity results in significant testicular insults, including swelling, necrosis, spermatid vacuolization, and irregular multinucleated giant cell formation in seminiferous tubules. These can cause apoptosis and atrophy, as well as sperm chromosomal aberration, low sperm numbers, and poor sperm viability, all of which can ultimately hinder spermatogenesis, according to previous studies on male rodents **(Abd-Elsalam *et al.*, 2021)**. The results of this study regarding the effect of Clo were in conflict with the result of **Elbana (2023)** who reported the success of a modified clomiphene citrate regimen in increasing sperm count without any risks to testicular tissue.

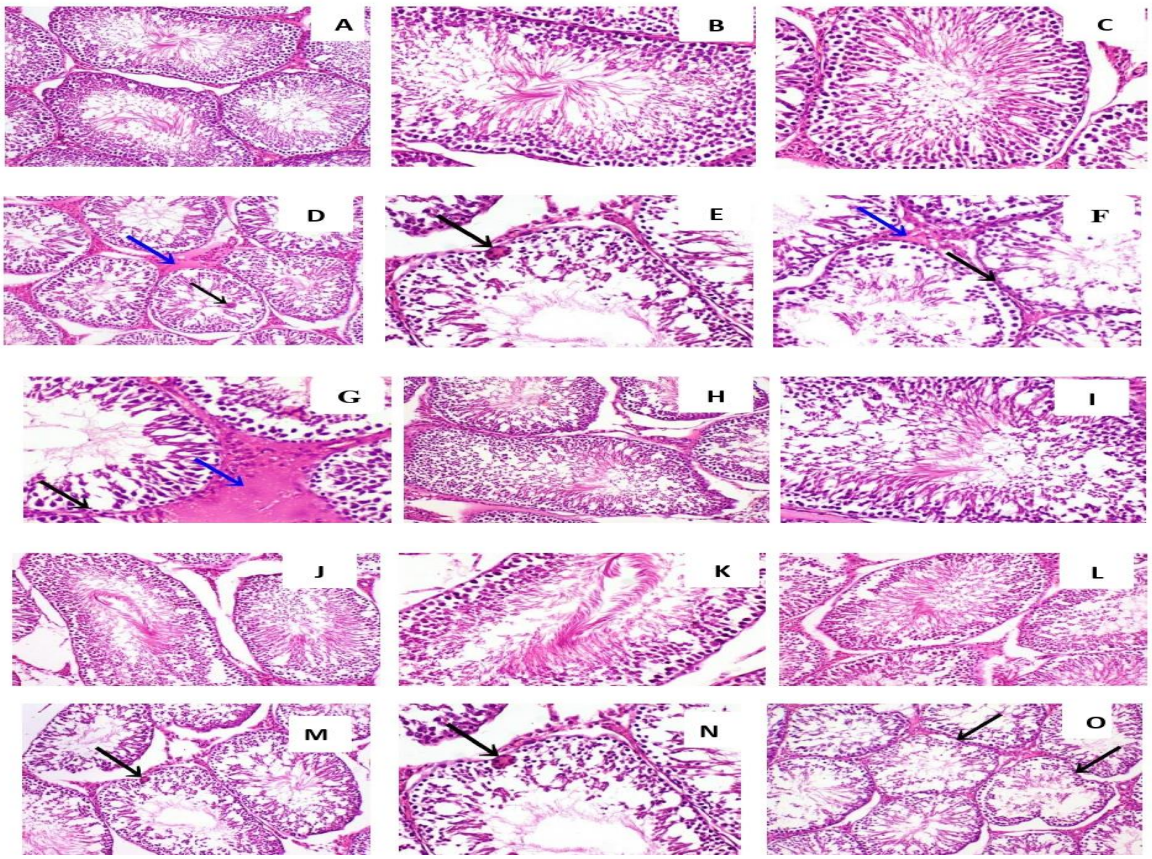
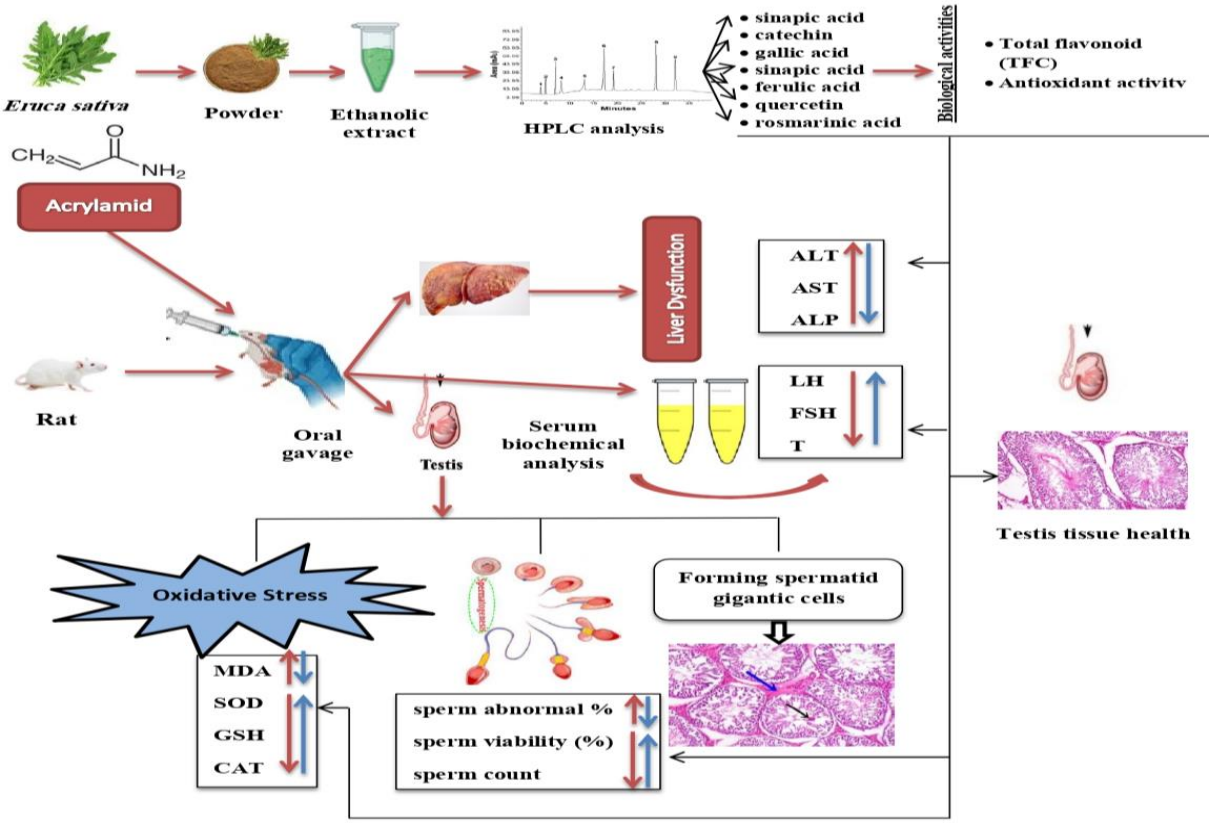


Figure 1. Effect of *Eruca sativa* leaves ethaolic extract (ESLE) and clomid (Clo) on rats testes histological disorders induced by acrylamide. (H & E X 100&200). **Photo A,B and C**, normal control group exhibiting normal spermatogoneal cells, full spermatogenesis, and normal seminiferous tubule histological structure; **Photo D&F**, Spermatogoneal cells lining seminiferous tubules in the model control group are degenerating, becoming spermatid gigantic cells (black arrow) and interstitial edema (blue arrow); **Photo E,** model control group demonstrating the development of spermatid gigantic cells and the degradation of spermatogoneal cells lining seminiferous tubules (black arrow); **Photo G,** ESLE-1+ACR group displaying interstitial edema (blue arrow) and spermatogoneal cell degeneration lining seminiferous tubules (black arrow); **Photo H,** ESLE-1+ACR group displaying full spermatogenesis, with sperm generation in their lumen, and typical seminiferous tubules; **Photo I,** ESLE-1+ACR group showing no histopathological alterations; **Photo J,** ESLE-2+ACR group displaying full spermatogenesis, with sperm generation in their lumen, and typical seminiferous tubules; **Photo K&L,** ESLE-2+ACR group showing no histopathological alterations; **Photo M&N,** Clo+ACR group demonstrating the development of spermatid gigantic cells and the degradation of spermatogoneal cells lining seminiferous tubules (black arrow); **Photo O,** Clo+ACR group displaying interstitial edema (blue arrow) and spermatogoneal cell degeneration lining seminiferous tubules (black arrow).

Conclusion

In conclusion, In the current investigation, ACR, a food-borne toxin, caused liver dysfunction, testicular oxidative stress, endocrine dysregulation, and decreased sperm quality. The manipulation process, as illustrated in Figure 2, involves enhancing the activities of liver enzymes, serum testosterone, serum LH, and FSH, sperm quality analysis (serum count, sperm motility percentage, sperm morphology and viability percentage), oxidative and antioxidant enzymes in testicular tissue, and histological alterations of testicular tissue, all of which have a negative impact on the process of injuries to the biological system. The abundance of bioactive chemicals and their associated biological activities may be the reason behind these biological enhancements brought by ESLE. Based on



the concentrations assessed, we have suggested the use of *Eruca sativa* leaves in our regular meals, beverages and pharmaceutical preparations.

Figure 2. Graphical summary showing the potential preventive roles of *Eruca sativa* leaves extract against acrylamide-induced biochemical and histological alterations in rat testis

Author Contributions

Mohamed R. Elkabary took part in the study protocol development, conceptual information retrieval, results validation, statistical analysis, and

paper preparation. He also performed a critical revision to intellectually structure the content and approved the publication of the final version. Data collection, compilation, analysis, and interpretation, as well as the retrieval of conceptual knowledge and the writing of draft papers, were all carried out by Hisham H. Saad. Adel A. Badr contributed significantly to the work's conception, design, and draft paper production.

Conflict of Interest

In releasing this work, the authors affirm that they have no conflicts of interest.

References

Abd El-Aziz G, El-Fark M, Hamdy R (2016) Protective effect of *Eruca sativa* seed oil against oral nicotine induced testicular damage in rats. *Tissue Cell* 48:340–348

Abd-Elsalam, R. M.; El Badawy, S. A.; Ogaly, H. A.; Ibrahim, F. M.; Farag, O. M. & Ahmed, K. A. (2021). *Eruca sativa* seed extract odulates oxidative stress and apoptosis and up-regulates the expression of Bcl-2 and Bax genes in acrylamide-induced testicular dysfunction in rats. *Environmental science and pollution research international*, *28*(38), 53249–53266.

Aebi, H.E. (1983) Catalase. In: Bergmeyer, H.U., Ed., Methods of Enzymatic Analysis, Verlag Chemie, Weinhem, 273- 286.

Al-Amoudi, W. M. (2012). Biochemical and Histological Effects of Clomiphene Citrate on Liver of Female Albino Rat. *Journal of American Science*, 8(5):646:651.

Alghabban, A. (2024). Impact of supplementing of rocket (*Eruca sativa*) extract against foodborne parasite Trichinella spiralis experimental infections induced rat tongue muscles toxicity and DNA damage. *Journal of Bioscience and Applied Research*, 10(3), 407-420.

Algreiby, A.A. (2024). Substantiable *Eruca sativa* Biomass: From Mass Spectrometry to Insights for Biopharmacotherapy. *Quality Assurance and Safety of Crops & Foods* 16(4):57-79.

Al-Qudah, M. (2017) Effects of *Eruca sativa* leaves extracts on testes, fertility potential and testosterone concentration in male rats. *Annual Research & Review in Biology* 16:1–7

Al-sagheer, A. E and Kaabo, H. (2022). Effect of Clomiphene Citrate on the Kidney of Female Albino Rat and the Possible Protective Effect of Ascorbic Acid (Light and Electron Microscopic Study), *Al-Azhar International Medical Journal*, 3(3), 20.

Alturki, H. A.; Elsawy, H. A. & Famurewa, A. C. (2022). Silymarin abrogates acrylamide-induced oxidative stress-mediated testicular toxicity via modulation

of antioxidant mechanism, DNA damage, endocrine deficit and sperm quality in rats. *Andrologia*, 54(9), e14491.

Ansari, M,N. and Ahmed, G.M. (2014). Ameliorative Effect of Rocket Leaves on Fertility in Streptozotocin-Induced Diabetic Rats. *International Research Journal of Biological Sciences*, 3(8), 89-97.

Awadelkareem, A. M.; Al-Shammari, E.; Elkhalifa, A. E. O.; Adnan, M.; Siddiqui, A. J.; Snoussi, M.; Khan, M. I.; Azad, Z. R.; Patel, M. & Ashraf, S. A. (2022). Phytochemical and In Silico ADME/Tox Analysis of *Eruca sativa* Extract with Antioxidant, Antibacterial and Anticancer Potential against Caco-2 and HCT-116 Colorectal Carcinoma Cell Lines. *Molecules (Basel, Switzerland)*, 27(4), 1409.

Bancroft, D., & Gamble, M. (2002). The theory and practice of histology technique (5th ed., p. 75). Churchill Living Stone.

Banso, A. (2009). Phytochemical and antibacterial investigation of bark extracts of Acacia nilotica. *Journal of Medicinal Plants Research*, *3*, 082-085.

Barillari, J., Canistro, D., Paolini, M., Ferroni, F., Pedulli, G. F., Iori, R., & Valgimigli, L. (2005). Direct antioxidant activity of purified glucoerucin, the dietary secondary metabolite contained in rocket (*Eruca sativa* Mill.) seeds and sprouts. *Journal of agricultural and food chemistry*, 53(7), 2475–2482.

Bell, L., Chadwick, M., Puranik, M., Tudor, R., Methven, L., Kennedy, S., & Wagstaff, C. (2020). The *Eruca sativa* Genome and Transcriptome: A Targeted Analysis of Sulfur Metabolism and Glucosinolate Biosynthesis Pre and Postharvest. *Frontiers in plant science*, 11, 525102.

Cerrah, S., Ozcicek, F., Gundogdu, B., Cicek, B., Coban, T. A., Suleyman, B., Altuner, D., Bulut, S., & Suleyman, H. (2023). Carvacrol prevents acrylamide-induced oxidative and inflammatory liver damage and dysfunction in rats. *Frontiers in pharmacology*, 14, 1161448.

Chaachouay, N., & Zidane, L. (2024). Plant-Derived Natural Products: A Source for Drug Discovery and Development. *Drugs and Drug Candidates*, *3*(1), 184-207.

Chapman, D. G., Castillo, R., & Campbell, J. A. (1959). Evaluation of protein in foods. I. A method for the determination of protein efficiency ratios. *Canadian journal of biochemistry and physiology*, 37(5), 679–686.

Chehab, M., Madala, A., & Trussell, J. C. (2015). On-label and off-label drugs used in the treatment of male infertility. *Fertility and sterility*, 103(3), 595–604.

Connolly, E. L., Sim, M., Travica, N., Marx, W., Beasy, G., Lynch, G. S., Bondonno, C. P., Lewis, J. R., Hodgson, J. M., & Blekkenhorst, L. C. (2021). Glucosinolates From Cruciferous Vegetables and Their Potential Role in Chronic Disease: Investigating the Preclinical and Clinical Evidence. *Frontiers in pharmacology*, 12, 767975.

Crescenzi, M. A., Cerulli, A., Montoro, P., & Piacente, S. (2023). Metabolite Profiling for Typization of "Rucola della Piana del Sele" (PGI), *Eruca sativa*, through UHPLC-Q-Exactive-Orbitrap-MS/MS Analysis. *Foods*, *12*(18), 3384.

Dağoğlu, I., Keskin A. Z., Korkak, F. A., Kazdal, S. M., & Dağ, A. (2024). Acrylamide in heat-treated carbohydrate-rich foods in Turkey. Food additives & contaminants. *Part B, Surveillance*, 17(2), 122–128.

Dickey, R. P., & Holtkamp, D. E. (1996). Development, pharmacology and clinical experience with clomiphene citrate. *Human reproduction update*, 2(6), 483–506.

Elbana, A. l. (2023). Effect of Clomiphene Citrate on the Ultrastructure of Testis in Albino Rats. *Int. J. Morphol* ; 41(5): 1492-1500.

El-Gayar, H. A., Salem, E. T., & Edrees, G. M. (2022). The protective effect of *Eruca sativa* against lipid metabolic abnormalities induced by dioxin in male rats. *Egyptian Journal of Basic and Applied Sciences*, 9(1), 383–392.

El-Nwehy, S.S., Abd-Rabbu, H.S., El-Nasharty, A.B. and Rezk, A.I., (2023). Improvement of (*Eruca sativa* Mill) yield, oil, chemical constituents and antioxidant activity utilizing a by-product of yeast production (CMS) with zinc and boron under salinity stress conditions. *Oil Crop Science* 8(4): 218–227.

Erfan O. S., Sonpol H. M.,&Abd El-Kader M. (2021). Protective effect of rapamycin against acrylamide-induced hepatotoxicity: The associations between autophagy, apoptosis, and necroptosis. *Anat. Rec*. 304 (9), 1984–1998.

Farag, O.M.; Abd-Elsalam, R.M.; Ogaly, H.A.; Ali, S.E.; El Badawy, S.A.; Alsherbiny, M.A.; Li, C.G.; Ahmed, K.A.(2021). Metabolomic Profiling and Neuroprotective Effects of Purslane Seeds Extract Against Acrylamide Toxicity in Rat's Brain. *Neurochem. Res*. **2021**, *46*, 819–842.

Govindaraju, I., Sana, M., Chakraborty, I., Rahman, M. H., Biswas, R., & Mazumder, N. (2024). Dietary Acrylamide: A Detailed Review on Formation, Detection, Mitigation, and Its Health Impacts. *Foods*, 13(4), 556.

Grami, D., Selmi, S., Rtibi, K., Sebai, H., & De Toni, L. (2024). Emerging Role of *Eruca sativa* Mill. in Male Reproductive Health. *Nutrients*, 16(2), 253.

Gül, M., Kus¸tepe, E. K., Erdemli, M. E., Altınöz, E., Ba_g, H. G., Gül, S., & Göktürk, N. (2021). Protective effects of crocin on acrylamide-induced testis damage. *Andrologia*, 00, e14176.

Hadi, M.A., Almamoori, A.M., Al-Hassnawi, A.T. & Hameedi, E.H. (2017). Oxidative response associated with treatment of male Albino rats with *Eruca sativa* Mill leaves extract and correlations with complete blood picture. *Journal of Pharmaceutical Sciences and Research* 9(11): 2278–2285.

Huijben, M., Huijsmans, R. L. N., Lock, M. T. W. T., de Kemp, V. F., de Kort, L. M. O., & van Breda, J. H. M. K. (2023). Clomiphene citrate for male infertility: A systematic review and meta-analysis. *Andrology*, 11(6), 987–996.

Huijben, M., Lock, M. T. W. T., de Kemp, V. F., de Kort, L. M. O., & van Breda, H. M. K. (2022). Clomiphene citrate for men with hypogonadism: a systematic review and meta-analysis. *Andrology*, 10(3), 451–469.

Hwang, E.S. & Thi, N.D.(2014). Effects of extraction and processing methods on antioxidant compound contents and radical scavenging activities of laver (porphyra tenera). *Prev Nutr Food Sci*.;19:40–48.

Jaćimović, S., Popović-Djordjević, J., Sarić, B., Krstić, A., Mickovski-Stefanović, V., & Pantelić, N. Đ. (2022). Antioxidant Activity and Multi-Elemental Analysis of Dark Chocolate. *Foods*, *11*(10), 1445.

Javed, S., Shahzadi, Z., Yousaf, Z., Anjum, I., Aftab, A., Hanif, S., Maqbool, Z., Ullah, R., Raza, M. A., & Iqbal, Z. (2024). Anti-anemic potential of *Eruca sativa L*. in iron-deficient rat model; network pharmacology profiling. *Food science & nutrition*, 12(10), 7331–7346.

Jollow, D. J., Mitchell, J. R., Zampaglione, N., & Gillette, J. R. (1974). Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology*, 11(3), 151–169.

Kalaivani, M., Saleena, U. V., Kamath Katapadi, K. G., Praveen Kumar, Y., Nayak, D., & Nilima (2018). Effect of acrylamide ingestion on reproductive organs of adult male wistar rats. *Journal of Clinical and Diagnostic Research*, 12(11).

Kandemir F. M., Yıldırım S., Kucukler S., Caglayan C., Darendelioğlu E., Dortbudak M. B. (2020). Protective effects of morin against acrylamide-induced hepatotoxicity and nephrotoxicity: A multi-biomarker approach. *Food Chem. Toxicol*. 138, 111190.

Keyata, E. O., Tola, Y. B., Bultosa, G., & Forsido, S. F. (2021). Phytochemical contents, antioxidant activity and functional properties of Raphanus sativus L, *Eruca sativa L*. and Hibiscus sabdariffa L. growing in Ethiopia. *Heliyon*, 7(1), e05939.

Koubaa, M. ., Driss, D., Bouaziz, F. ., Ellouz Ghorbel, R. ., & Ellouz Chaabouni, S. . (2015). Antioxidant and Antimicrobial Activities of Solvent Extract Obtained from Rocket (*Eruca sativa* L.) Flowers. *Free Radicals and Antioxidants*, 5(1), 29–34.

Kucukler, S., Caglayan, C., Darendelio_glu, E., & Kandemir, F. M. (2020). Morin attenuates acrylamide-induced testicular toxicity in rats by regulating the NF-κB, Bax/Bcl-2 and PI3K/Akt/mTOR signaling pathways. *Life Sciences*, 261, 118301.

Masayasu, M., & Hiroshi, Y. (1979). A simplified assay method of superoxide dismutase activity for clinical use. *Clinica Chimica Acta*, 92(3), 337–342.

Mehri, S., Dadesh, Q., Tabeshpour, J., Hassani, F. V., Karimi, G., & Hossein, H. (2016). Evaluation of the neuroprotective effect of silymarin on acrylamide-induced neurotoxicity. *Jundishapur Journal of Natural Pharmaceutical Products*, 11(14), e37644.

Nowfel, A.J. & Al-Okaily, B.N. (2017). Oxidative stress: Role of *Eruca sativa* extract on male reproduction in rats. *Adv. Anim. Vet. Sci.* 5(1): 39-46.

NRC, National Research Council (1996). Guide for the Care and Use of Laboratory Animals Washington: National Academy Press. Obtained from hypertensive-diabetes rats. *Fundam Clin Pharmacol*, 10, 329–336.

Ohishi, N. & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95:351–358

Pagnotta, E., Ugolini, L., Matteo, R. & Righetti, L. (2022). Bioactive compounds from *Eruca sativa* seeds. *Encyclopedia* 2(4): 1866– 1879.

Perera, D. N., Hewavitharana, G. G., & Navaratne, S. B. (2021). Comprehensive Study on the Acrylamide Content of High Thermally Processed Foods. *BioMed research international*, 23:2021:6258508.

Radad, K., Amir, Y. E., Al-Emam, A., Al-Shraim, M., Bin-Jaliah, I., Krewenka, C., & Moldzio, R. (2020). Minocycline protects against acrylamide-induced neurotoxicity and testicular damage in Sprague-Dawley rats. *Journal of toxicologic pathology*, 33(2), 87–95.

Rahbardar, M., Cheraghi Farmad, H., Hosseinzadeh, H., & Mehri, S. (2021). Protective effects of selenium on acrylamide-induced neurotoxicity and hepatotoxicity in rats. *Iranian journal of basic medical sciences*, 24(8), 1041–1049.

Ramazzina, I., Lolli, V., Lacey, K., Tappi, S., Rocculi, P., & Rinaldi, M. (2022). Fresh-Cut *Eruca Sativa* Treated with Plasma Activated Water (PAW): Evaluation of Antioxidant Capacity, Polyphenolic Profile and Redox Status in Caco2 Cells. *Nutrients*, 14(24), 5337.

Raouf, C. G., Noori, A. O., Salih, K. Y., Mohammad, T. H., Mohammed, A. R., & Hadi, J. M. (2024). Effect of *Eruca sativa* on Spermatogenesis in Rats Exposed to Cigarette Smoke. *Cureus*, 16(8), e67662.

Rato, L., Alves, M. G., Dias, T. R., Lopes, G., Cavaco, J. E., Socorro, S., & Oliveira, P. F. (2013). High-energy diets may induce a pre-diabetic state altering testicular glycolytic metabolic profile and male reproductive parameters. *Andrology*, 1(3), 495–504.

Reeves, P., Nielsen, F. & Fahey, G. (1993). AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. *Journal of Nutrition*, 123(11), 1939-1951.

Rifai, L. and Saleh, F.A. A. (2020) Review on Acrylamide in Food: Occurrence, Toxicity, and Mitigation Strategies. *Int. J. Toxicol*, 39,93–102.

Ring, J. D., Lwin, A. A., & Köhler, T. S. (2016). Current medical management of endocrine-related male infertility. *Asian journal of andrology*, 18(3), 357–363.

Sakuma, Y. (2009). Gonadal steroid action and brain sex differentiation in the rat. *Journal of Neuroendocrinology*, 21(4), 410–414.

Saleh, D.O., Baraka, S.M., & Jaleel, G.A.A.(2024). Eugenol alleviates acrylamide-induced rat testicular toxicity by modulating AMPK/p-AKT/mTOR signaling pathway and blood–testis barrier remodeling. *Sci Rep* 14, 1910.

Scovell, J. M., & Khera, M. (2018). Testosterone Replacement Therapy Versus Clomiphene Citrate in the Young Hypogonadal Male. *European urology focus*, 4(3), 321–323.

Semla, M., Goc, Z., Martiniaková, M., Omelka, R. &Formicki, G. (2017). Acrylamide: A Common Food Toxin Related to Physiological Functions and Health. *Physiol. Res*. 2017, 66, 205–217.

Sengul, E., Gelen, V., Yildirim, S., Tekin, S., & Dag, Y. (2021). The Effects of Selenium in Acrylamide-Induced Nephrotoxicity in Rats: Roles of Oxidative Stress, Inflammation, Apoptosis, and DNA Damage. *Biological trace element research*, 199(1), 173–184.

Shioya, N., & Wakabayashi, K. (1998). In vivo bioactivities and kinetic parameters of rat luteinizing hormone components: Discrepancy between in vitro and in vivo assays. *Endocrine Journal*, 45, 307–314.

Teerds, K. J., Closset, J., Rommerts, R. F. G., De Rooij, D. G., Stocco, D. M., Colenbrander, B., Gwensing, J., & Hennen, G. (1989). Effects of pure FSH and LH preparations on the number and function of Leydig cells in immature hypophysectomized rats. *Journal of Endocrinology*, (120), 97–106.

Tekin, S., & Çelebi, F. (2022). Investigation of the effect of hesperidin on some reproductive parameters in testicular toxicity induced by Bisphenol A. *Andrologia*, 54(10), e14562.

Thoma, M.E., Fledderjohann, J., Cox, C.M., & Kantum Adageba, R. (2021). Biological and Social Aspects of Human Infertility: A Global Perspective. *Oxford Research Encyclopedia of Global Public Health*.

Tietz, N.W. (1976). Fundamental of Clinical Chemistry. Philadelphia, W.B. Saunders, 243.

Triningsih, D., Yang, J. H., Sim, K. H., Lee, C., & Lee, Y. J. (2021). Acrylamide and its metabolite induce neurotoxicity via modulation of protein kinase C and AMP-activated protein kinase pathways. Toxicology in vitro : *an international journal published in association with BIBRA*, 72, 105105.

UCLA, University of California, Los Angeles Health (2023). Infertility: Symptoms, treatment, diagnosis. https://www.uclaheal th.org/obgyn/infertility. *Veterinary Sciences* 5:39–46

Vassault, A., Grafmeyer, D., Graeve, J., Cohen, R., Beaudonnet, A. & Bienvenu, J. (1999). Quality specifications and allowable standards for validation of methods used in clinical biochemistry. *Ann Biol Clin* (Paris)., 57(6), 685-95

Wahab, O. A., Princely, A. C., Oluwadamilare, A. A., Ore-Oluwapo, D. O., Blessing, A. O., & Alfred, E. F. (2019). Clomiphene citrate ameliorated lead acetate-induced reproductive toxicity in male Wistar rats. *JBRA assisted reproduction*, 23(4), 336–343.

Wells, R. (2024). Origins and diversity of brassica and its relatives. In: Vegetable brassicas and related crucifers. *CABI*, *GB*, pp. 1–41.

WHO, World Health Organization (2023). Infertility. https://www.who.int/news - room/fact-sheets/detail/infertility.

Yaniv, Z., Schafferman, D., & Amar, Z. (1998). Tradition, uses and biodiversity of Rocket (*Eruca Sativa*, Brassicaceae) in Israel. *Economic Botany*, 52(4), 394-400.

Ybañez-Julca, R. O., Palacios, J., Asunción-Alvarez, D., Quispe-Díaz, I., Nwokocha, C. R., & de Albuquerque, R. D. D. G. (2022). Lepidium meyenii Walp (red maca) Supplementation Prevents Acrylamide-Induced Oxidative Stress and Liver Toxicity in Rats: Phytochemical Composition by UHPLC-ESI-MS/MS. *Plant foods for human nutrition* (Dordrecht, Netherlands), 77(3), 460–466.

Yildirim, S., Sengul, E., Aksu, E. H., Cinar, İ., Gelen, V., Tekin, S., & Dag, Y. (2024). Selenium reduces acrylamide-induced testicular toxicity in rats by regulating HSD17B1, StAR, and CYP17A1 expression, oxidative stress, inflammation, apoptosis, autophagy, and DNA damage. *Environmental toxicology*, 39(3), 1402–1414.

Yound, D. S. (1975). Determination of GOT. *Clin. Chem*., 22 (5), 21-27.

Zangana, A.J and Erdeni, A.A.H.(2020). Therapeutic attempt of amoebic dysentery with water extract of *Eruca sativa L*. in laboratory mice and its histopathological effect*. Journal of Physics Conference Series* 1664(1):012135.

Zhang, H. M., Zhao, X. H., He, Y. F., & Sun, L. R. (2018). Liver injury induced by clomiphene citrate: A case report and literature reviews. *Journal of clinical pharmacy and therapeutics*, 43(2), 299–301.

مجلة التربية النوعية والتكنولوجيا (بحوث علمية وتطبيقية)
Journal of Specific Education and Technology (Scientific and Applied Research)

الأدوار الوقائية المحتملة لمستخلص أوراق نبات الجرجير ضد التغيرات البيوكيميائية والنسيجية الناجمة عن مادة الأكريلاميد في خصية الفئران

[*1] محمد رأفت القباري ، [2] هشام حمدي سعد ، [3] عادل عبد الرسول بدر

[2,1] قسم التغذية وعلوم الأطعمة ، كلية الاقتصاد المنزلي ، جامعة المنوفية ، شبين الكوم ، مصر.
[3] زميل بقسم التغذية ، مستشفيات جامعة المنوفية ، شبين الكوم ، مصر.
[*] *المؤلف الرئيسي:* m_elkabary@yahoo.com

الملخص:

إن معالجة/طهي الأطعمة النشوية في درجات حرارة عالية مثل البطاطس المقلية وغيرها من المخبوزات تنتج مواد كيميائية سامة، بما في ذلك مادة الأكريلاميد. ومن المعروف أن مادة الأكريلاميد تسبب العديد من التأثيرات السامة في مختلف أعضاء الجسم، بما في ذلك الخصيتين. لذلك، هدفت الدراسة الحالية إلى التحقيق في التأثيرات الوقائية المحتملة لمستخلص أوراق نبات الجرجير الإيثانولي، ضد التغيرات البيوكيميائية والنسيجية الناجمة عن مادة الأكريلاميد في خصية الفئران. تم توزيع الفئران (ن = ٣٠) عشوائيًا على خمس مجموعات متساوية على النحو التالي: المجموعة ١، عملت كمجموعة تحكم طبيعية؛ المجموعة ٢، مجموعة التحكم النموذجية، تلقت ٥٠ مجم/كجم من وزن الجسم/يوم من مادة الأكريلاميد لإحداث العقم؛ تلقت المجموعات من ٣ إلى ٥ مستخلص أوراق نبات الجرجير الإيثانولي-1 ومستخلص أوراق نبات الجرجير الإيثانولي-٢ والكلوميد (مرجع دواء العقم) بتركيزات ٢٥٠ و٥٠٠ و١.٢ مجم/كجم وزن الجسم/يوم عن طريق التغذية الأنبوبية عن طريق الفم لمدة ٢٨ يومًا، تليها الأكريلاميد على التوالي. تسبب علاج الفئران بـالأكريلاميد في زيادة كبيرة ($p\leq0.05$) في تشوهات الحيوانات المنوية ومالونالدهيد الخصوي بنسبة ٤٧١.٣٢ و٢٩٢.١٪ على التوالي مقارنة بالحيوانات الطبيعية. كما انخفض عدد الحيوانات المنوية وحركتها وهرمون التستوستيرون في المصل والهرمون اللوتيني ومحتوى الهرمون المنبه للجريب بشكل كبير ($p\leq0.05$) بنسبة -٩٣.٧٨ و-٦٨.٩٥ و-٨٠.٨٤ و-٧٦.٩٨ و-٨٩.١٦٪ على التوالي. علاوة على ذلك، انخفضت أنشطة إنزيمات مضادات الأكسدة في الخصية (سوبر أكسيد ديسميوتاز ، والجلوتاثيون والكاتالاز) بنسبة -٧٩.٢٩، -٨١.٠٩ و-٨٠.٧٤٪، على التوالي، مقارنة بالفئران الضابطة الطبيعية. بالإضافة إلى ذلك، لوحظ خلل في وظائف الكبد وتغيرات نسيجية ضارة في أنسجة الخصية المعالجة بالأكريلاميد. ومع ذلك، فإن التدخل باستخدام مستخلص أوراق نبات الجرجير الإيثانولي (٥٠٠ مجم / كجم) أعاد بشكل ملحوظ ($p \leq 0.05$) معظم هذه المعلمات إلى مستويات قريبة من المعدل الطبيعي. وفي الختام، تعمل هذه الملاحظات كأساس لاستخدام مستخلص أوراق نبات الجرجير الإيثانولي لعلاج/أو منع سمية الأكريلاميد. لذلك، اقترحنا ادراج أوراق الجرجير الطازجة ومستخلصاتها في وجباتنا اليومية والمشروبات والمستحضرات الصيدلانية.

الكلمات الرئيسية: *العقم، الإجهاد التأكسدي، سترات الكلوميفين، جودة الحيوانات المنوية، سمية الخصية، المركبات النشطة بيولوجيًا.*