

Protective Effect of *Eruca sativa* Leaves Extract on Sperm Abnormalities in Mice Exposed to *Hypericum triquetrifolium* Aqueous Crude Extract



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ABSTRACT

This study was carried out to evaluate the effect of *Eruca sativa* on the cytotoxic effect of *Hypericum triquetrifolium* on sperm abnormalities in albino mice. Leaves of *E. sativa* and aerial parts of *H. triquetrifolium* were dried in shade and grinded and their aqueous extracts were used for the treatments to study their effect on sperm morphology. Treated groups were injected with a single dose of 38 mg/kg body weight (BW) of *Hypericum* subcutaneously, while the *Eruca* groups were orally administered with 250 mg/kg BW twice/week for 2 weeks. After the exposure of *H. triquetrifolium*, the frequency of abnormal sperms showed a highly significant induction of sperm abnormalities; separated head from tail sperms, swollen head, hookless, defective head, and hook. However, the *Eruca* group showed no obvious abnormalities in sperm morphology, while in cotreatment with both *Eruca* and *Hypericum* (H/E) groups, there was an extremely significant decrease ($P < 0.0001$) in the abnormal sperms. In conclusion, it appeared that *E. sativa* could prevent or at least minimize the damages that *Hypericum* toxins would make on the sperm morphology significantly.

Index Terms: *Eruca sativa*, *Hypericum triquetrifolium*, Sperm morphology

1. INTRODUCTION

There has been a growing interest in the study of medicinal plants and their traditional use in different parts of the world over the past few decades as this can lead to new discoveries about plant agents. Therefore, traditional medicine remains as an integral part in the most Arabic countries and the flora of these countries with their use somehow is similar [1]. *Hypericum* species are herbaceous plants known to have medicinal properties and are widely used in phototherapy in many countries. The *Hypericum* genus

is known to contain a wealth of secondary metabolites and many of which are biologically active. The main constituents are naphthodianthrone (hypericin, pseudohypericin, protohypericin, and protopseudohypericin), phloroglucinols (hyperforin, adhyperforin, hyperfirin, and adhyperfirin), and a broad range of flavonoids (hyperoside and rutin) [2]. It has been used in Traditional Arab Herbal Medicine to treat various inflammatory diseases and as sedative, astringent, antispasmodic, for intestine and bile disorders and poisonous antioxidant, antiviral, antimicrobial, and antinociceptive activities have also been reported in the literature for *Hypericum triquetrifolium* [3]. Along with the medicinal plants, Iraq also has a rich and diverse flora including a wide variety of plants with the potential to cause animal and human poisoning [2]. Animal-plant poisoning is usually accidental and occurs most often due to unfavorable conditions when pasture is poor in drought, overstocking, and trampling of grazing. Plant poisons consist of toxic compounds that can actually be fatal even in small doses while others may cause a

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reduction in performance as weight loss, weakness, diarrhea, or rapid pulse rate [3].

As previous studies have shown, *Hypericum* is one of the most common poisonous plants in Iraq and the genus has more than 400 species [3], but only sixteen is observed in Iraq, and the most abundant types are *H. triquetrifolium* and *Hypericum perforatum* [4].

H. triquetrifolium Turra are a perennial herbaceous plant and one of the Iraqi wild species of *Hypericaceae* distributed in the North and Northwest of the country, the local Arabic name of the species is Roja and the Kurdish name is Swrnatik [5]. It contains a mixture of poisonous pigments referred to as hypericin that is able to cause many deleterious effects in livestock including hyperthermia and acute photodermatitis when consumed by grazing animals [2]. Animals that are most likely to be poisoned are sheep, goats, horses, cattle, and swine. Symptoms that result of poisoning are first to appear in unpigmented or lightly pigmented areas of skin that damaged and may become necrotic, and never recover and regrowth of hair in those areas are uncommon [6]. Then, lactating animals suffering from decline or shutoff milk completely, even in severe case animals loss appetites and die from starvation and dehydration [7].

Many researchers conducted to review the effect of *H. perforatum* and compared its toxicity to *H. triquetrifolium* in each rabbit and sheep [8]-[10]. However, an investigation accomplished in 2010 in Iraq/Kurdistan, studied the cytotoxic and genotoxic effect of *H. triquetrifolium* tested on male albino mice to come up the result with that, indeed in a particular dose, *Hypericum* is noxious to both sperm cells and chromosomes that influence sperm morphology and chromosomal aberrations [11]. A similar study performed in 2012 in Iraq/Kurdistan, using different doses and duration revealing the same result of cytotoxicity and genotoxicity of *H. triquetrifolium* on albino mice [12].

However, there is no study shows an effect of a medicinal plant in animals that have been exposed to *H. triquetrifolium* as a common toxic plant on sperm morphology; thus, this study is aimed to achieve exactly that goal to compare both results before and after the treating with both plants, toxic, and medicinal.

Eruca sativa known as Jarjir, Rocket, or Arugula plant belongs to the Brassicaceae family, is a minor oil crop and medicinal plant in several parts of Middle East since ancient it has been used in traditional medications as remedies for different

diseases [13]. Hence, phytochemical composition and corresponding biological activities are crucial to apprehending the therapeutic potential of medicinal plants. Numerous studies infer that pharmacological action of any medicinal plants is attributed to the presence of secondary metabolites; these generally consist of the phenolic compounds, alkaloids, tannins, saponins, carbohydrates, glycosides, flavonoids, steroids, etc. Among the others, phenolic compounds are the universally discovered phytochemicals for the sake of therapeutic potential in a different medicinal plant [14]. All of these secondary metabolites and particularly phenolic compounds have been reported as scavengers of free radicals and also have been considered as good therapeutic candidates for free radical related pathologies as it has been reported that *E. sativa* seed extracts are potent antioxidants, exhibit diuretic effects, and provide renal protection [14]. The previous phytochemical studies of *E. sativa* showed that leaves and seeds contain glucosinolates. Three new quercetins have been isolated and identified from *E. sativa* leaves [15]. However, there is not sufficient information in the form of scientific analysis about detailed phytochemical composition of *E. sativa* and their respective bioactivities [16]. On the other hand, many studies revealed the intense aphrodisiac effect of Jarjir since ancient Roman times [17], [18], in a way that the seed oil enhance increasing fertility and sexual activity through dilation of seminiferous tubules, proliferation of spermatogenic cells, increasing mitotic activity, number of sperms, epididymis weight, elevating level of testosterone, and hyperplasia of interstitial Leydig cells have also been noticed [19]. In addition, Barillari *et al.* [20] proposed that the presence of saponin and alkaloid extract is responsible for increasing sperm activity.

2. MATERIALS AND METHODS

The aerial parts of *H. triquetrifolium* were harvested by hand at Moghagh/Piramagrwn/Sulaimanya during the early stage of flowering time in June since the plant shows its most toxicity at this stage while *E. sativa* was obtained from a local market in Sulaimanya. The same procedure was used for both plants, as they aqueous extracted. The plants were air-dried indoors at room temperature for about 1 week, then ground to obtain a powder using an electric grinder. The powder suspended in distilled water for about 24 h at the rate 50 g/400 ml, and then the solution was filtered twice using Whatman Filter Paper. The final crude extract was dried using an oven at 42°C temperature for about 12 h to obtain powder crude extract, then kept in dark bottles at 4°C until preparing the treatment doses [10].

2.1. Solution Preparation

The powder of the plants crude extracts was used for preparing the solution as follows:

- –35 mg/kg body weight (BW) of *H. triquetrifolium* were mixed with 1 ml of distilled water shaking until dissolving the powder completely and injected subcutaneously to the mice
- –250 mg/kg BW of *E. sativa* powder was mixed with 1 ml of distilled water that administered orally using gavage needle.

2.2. Experimental Design

Twenty-five albino male mice were divided into five groups designated as C, T1, T2, T3, and T4. Each group consisted of 5 mice and subjected to the following treatments:

Control: Mice were treated with 1 ml of distilled water.

- T1: Mice were treated with a single dose of 1 ml *Hypericum* extract subcutaneously at the dose 38 mg/kg BW
- T2: Mice were orally treated with 1 ml *Eruca* extract using gavage needle at the dose 250 mg/kg BW twice a week for 2 weeks
- T3: Mice were treated with a single dose of 1 ml *Hypericum* extract then injected with 1 ml of *Eruca* by oral administration twice a week for 2 weeks
- T4: Mice were treated with 1 ml of *Eruca*
- Then, injected with 1 ml of *Hypericum*.

2.3. Collection and Preparation of Sperms

After sacrificing the animals with cervical dislocation, the sperm morphology of the treated mice was examined 2 weeks after the first treatment. Vas deference of each mouse of the five groups was removed from the testes and put into a small Petri dish filled with normal saline. Using a scissor and disposable blades, vas deference was cut from the testes and sperm were transferred (semen extracted) on to a clean slide, preparing a smear for each. The smear was stained with hematoxylin for 15 min and washed, then stained with eosin and washed again. At the final step, the slides left to dry, then the results were read, counting about 100 sperm from each slide/animal (500 sperm for each treatment) to determine sperm morphology abnormalities [11].

2.4. Statistical Analysis

The values of the investigated parameters were analyzed using a statistic program GraphPad (Prism 2019). The experimental results were expressed as mean \pm standard error of the mean. Groups were compared by analysis of variance using one-way, two-way ANOVA, and Dunnett's

test for multiple comparisons test. $P < 0.05$ was regarded as statistically significant.

3. RESULTS AND DISCUSSION

Table 1 summarized the results of sperm morphology observations among treated groups and the control group. The data obtained from 100 sperm/replication (500 sperm/group) in semen samples collected from vas deferens of each mouse. The data show that there is a significant difference between control and *Hypericum* treated group (T1). The most frequent aberrant shapes were sperm without a head, sperm without a tail, swollen head, hookless, defective head, and defective hook. While in the *Eruca* treated groups (T2), there was no significant difference in the aberrant types comparing to control group. However, in the creating groups, (groups treated with both *Eruca* and *Hypericum*), in compare with T1 (*Hypericum* treated group) and the results exhibited that all forms of sperm abnormalities were significantly lowered in T3 and T4 comparing to T1, and the total number of normal sperm increased significantly. While T3 and T4 comparing to each other were not significantly different, mentioning that the (*Hypericum-Eruca*) treated groups (T3 and T4), differed in the subsequent of the treatments, wherein T3 the injection started with *Hypericum* and ended with *Eruca* while in T4 the reverse was applied; hence, the results showed no significant difference in none of the parameters comparing to one another. Fig. 1 showed the aberrations resulted in this study.

The whole process of developing spermatogonia to haploid spermatids takes around 35 days in mice [13], [14] through a complex process is known as spermatogenesis. Three crucial events are the major steps of this process: (i) Mitosis which is the multiplication of spermatogonia; (ii) reducing chromosome number from diploid to haploid by meiosis and begins with the entry of Type B spermatogonia into the prophase of the first meiotic division. These cells now called primary spermatocytes, divide to form secondary spermatocytes and next to form round spermatids; and (iii) the successful transformation of the round spermatid into the complex structure of the spermatozoon is called spermiogenesis.

Each of these steps represents a key element in the spermatogenic process, defects in any of them can fail in the entire process and lead to the production of defective spermatozoa or reduction in sperm production [17]. As previous researchers have confirmed its genotoxic effect on sperms and the male reproductive system in general, *Hypericum* toxicity attributed to the active principles

TABLE 1: Cytogenetic effect of *Eruca sativa* and *Hypericum triquetrifolium* with their interaction on sperm

Treatments	Aberrant							
	Normal sperm	Sperm without tail	Sperm without head	Defective head	Hookless	Defective hook	Swollen head	Two-tail
Control	87.00±3.162 ^a	3.200±0.4899 ^a	1.600±0.4000 ^a	0.2000±0.2000 ^a	0.4000±0.2449 ^a	0.200±0.200 ^a	0.4000±0.2449 ^a	0.00±0.00 ^a
Hypericum (T1)	57.00±1.449 ^b	5.200±0.8602 ^a	9.400±2.293 ^b	4.800±0.7348 ^b	4.400±1.122 ^b	2.600±0.6782 ^b	4.800±1.393 ^b	0.6000±0.4000 ^a
Eruca (T2)	90.80±1.241 ^a	3.600±0.6000 ^a	3.2000±0.5477 ^a	1.200±0.3742 ^a	1.000±0.4472 ^a	0.00±0.00 ^a	0.2000±0.2000 ^a	0.000±0.000 ^a
H/E (T3)	92.80±1.497 ^a	4.800±1.068 ^a	0.8000±0.5831 ^a	1.000±0.4472 ^a	0.4000±0.2449 ^a	0.000±0.000 ^a	0.2000±0.2000 ^a	0.000±0.000 ^a
E/H (T4)	93.60±0.509 ^a	2.600±0.5099 ^a	0.6000±0.4000 ^a	0.4000±0.2449 ^a	0.6000±0.2449 ^a	0.600±0.400 ^a	0.000±0.000 ^a	0.000±0.000 ^a

Using Dunnett test analysis, we compared the morphological abnormalities that have been observed by microscopic examinations among the treatment groups. E: *Eruca*, H: *Hypericum*, letters, ^a and ^b represent significant difference value (P<0.05), same letter: No significant difference, different letter: Significant difference

(hypericin, hyperforin, quercetin, and flavonoids) [18]. This study attempted to repair or reduce its effect on germ cells. It is believed that any abnormalities in sperm morphology may be due to a change in the genetic component and these are classified as defects in the head, midpiece, and tail.

The results in this study agreed with Mohammed and Kheravii [19] as they stated that the frequency of abnormal mice sperms examined for 35 days injected with *H. triquetrifolium* but using different doses revealed a significant induction of sperm abnormalities in all concentrations of plant extract comparing with untreated animals. The most frequent types of sperm abnormalities of the treated groups were irregular head defect, pseudodroplet defect, bent midpiece defect, and corkscrew midpiece defect.

Mohammed and Mohammed [19] illustrated that the mixture of the compounds found in the aqueous extract of *Hypericum* caused cytotoxicity and induced different cytogenic effects in both somatic and germ cells of male albino mice. Similar to this conclusion, Mohammed and Ali [21] have also verified that at dose 17 mg/kg BW, the total abnormal spermatozoa has increased compared with the control group, indicating that the most frequent aberrant occurrence was bent midpiece and coiled tail as sperm morphology was always an indicator of toxicity and mutagenicity in mammals [21].

The results in this study revealed that *Eruca* has reduced the toxicological effects of *Hypericum* on sperm morphology in a marvelous way as it's obvious in T3 and T4 groups, this is in agreement with many researches as [22] reported that exposed rats to cadmium chloride treated with *E. sativa* seeds extracts, improved the hormonal profile concentrations, serum testosterone, follicle-stimulating hormone, and luteinizing hormone (LH) and number of Leydig cells by increasing them parallel with alleviating the testicular toxicity induced by cadmium chloride [22]. They accredited the result to its antioxidant and free radicals scavenging activity [20] of many phytochemical compounds including glucosinolate and flavonoids [23] against oxidative damage induced by Cd and thereby improving the pituitary- testes axis and fertility [24], [12].

E. sativa seeds extract exhibited an evidence for a stimulatory effects on reproductive gonadal system through androgenic activities through increasing the number of Leydig cells could be due to the free radical scavenging ability through excluding the Fe³⁺ [15] or may be due to an increasing the number and/or the sensitivity of receptors

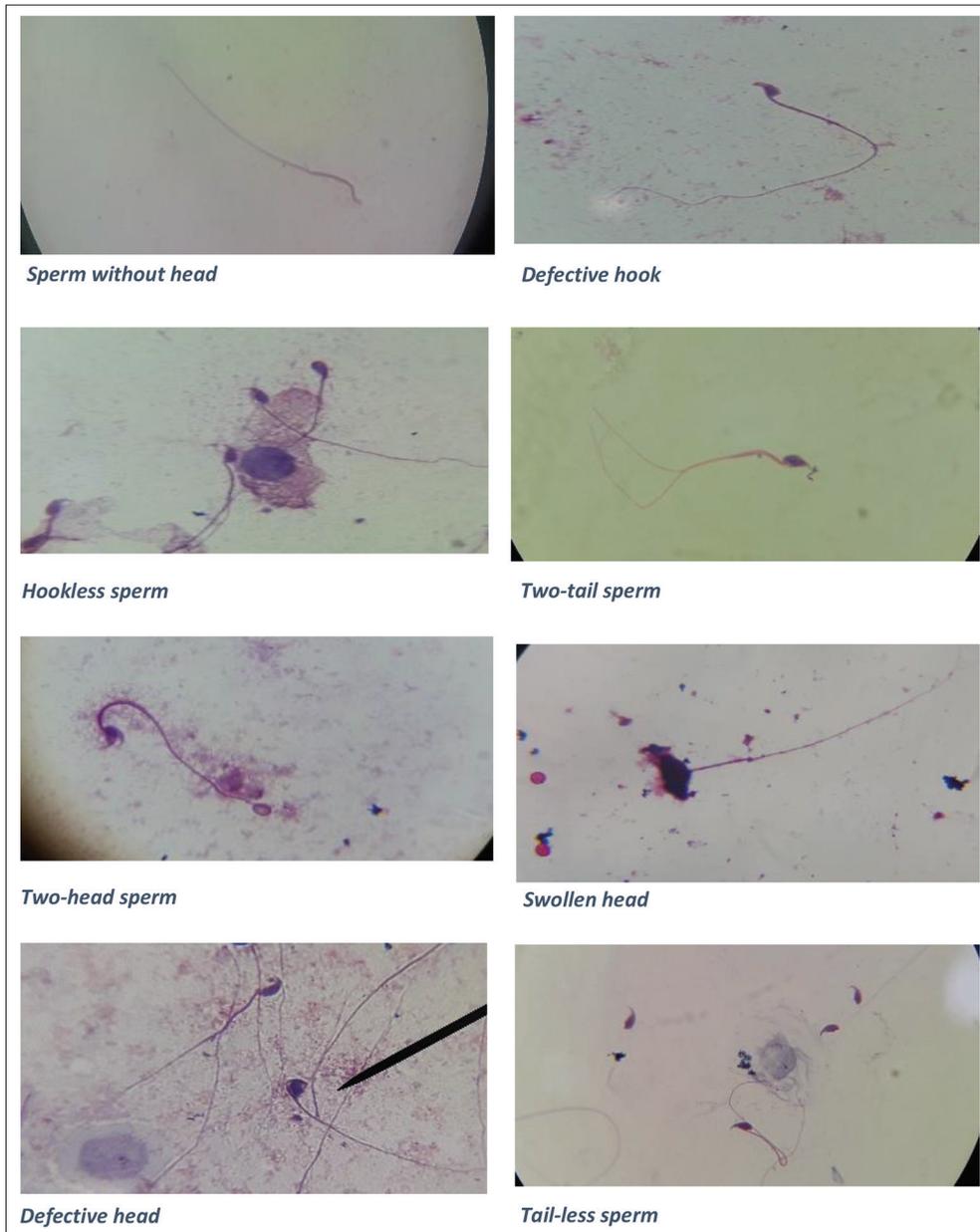


Fig. 1. Sperm abnormality types, depending on their morphology ($\times 1000$).

of the Leydig cells to LH which led to increase testosterone biosynthesis [16].

These findings cooperate with the researches of Mona and Nehal [25] and [26] that concluded the capability of *E. sativa* to improve healthy sperm characteristics and fertility. The increase of abnormal sperm morphology and decrease in viability may serve as a useful indicator of potential damage to the sperm by intubation of H_2O_2 [25] or may be due to impaired of Leydig's cell functions that can lead to enhances

alteration of testosterone synthesis [12], [27]. Thus, in the present study, an improvement in male spermatogenesis in the cotreatment group has been documented, we suggest that *Eruca* protects and reduces the cytotoxic effects of *Hypericum*.

4. CONCLUSION

we concluded that Eruca could lower most of the sperm abnormalities significantly, and highly prevent the action of the Hypericum's toxins.

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