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Impact of supplementing of rocket (*Eruca sativa*) extract against foodborne parasite *Trichinella spiralis* experimental infections induced rat tongue muscles toxicity and DNA damage

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Abstract

Trichinellosis is a parasitic disease that is dangerous to humans, the cause is a group of nematodes that are associated with the genus Trichinella. The purpose of this research is to investigate the effects of rocket seed extracts (RSE) on the experimental Trichinellosis-induced damage to the tissue and DNA in the rat's tongue. The current investigation was conducted on 40 male albino rats that were divided into four groups (G1, control; G2, RSE; G3, 35DPI or infected group in which rats were confronted with 1000 larvae of trichinella spiralis; G4, the treated infected group with RSE for a week). The current results showed that; over 97% of the larvae were located in skeletal muscles, and about 2.5% were located in both the diaphragm and the tongue. However, only about 0.5% were located in cardiac muscles. The treatments with 35DIP were significantly less effective than the treatments with 35DPI in reducing the mean number of Trichinella spiralis (78.5± 4.8 versus 291.0 ± 8.5). The muscles of the tongue in 35DPI exhibited severe tissue damage with widespread degenerative changes in the fibers of the muscle along with a large number of T. spiralis larvae encysted in the muscle. Each larva lacked a shell and exhibited inflammation-causing cellular infiltration, widespread hemorrhage, and muscular fibrosis. Tongue section in 5WPI treated with RSE revealed a reduced number of non-encapsulated encysted larvae with mild fibrosis, inflammatory cellular infiltration, and reduction in DNA damage.

Keywords: *Trichinella spiralis*, Eruca sativa, rat tongue, DNA damage, histopathology.

Introduction

Trichinellosis is a parasitic disease that is harmful to humans; the cause is a collective of nematodes associated with the genus Trichinella. [1,2]. Because the heart and respiratory muscles are directly involved, trichinosis is occasionally capable of causing carditis or respiratory failure. It's characterized by fevers, headaches, myalgias, eosinophilia, and periorbital swelling.[3]. It's a parasitic disease that is triggered by worms that are pathogenic to humans via food. Consuming raw or undercooked poultry or fish that contain infectious Trichinella larvae leads to a significant number of human incidents every year [4-6]. The symptoms of Trichinellosis are similar to the different stages of the life cycle, including the migrating, muscular, and intestinal phases [7,8]. These phases are linked to a variety of symptoms. Vomiting, diarrhea, and abdominal pain are all possible effects of the intestinal epithelium being destroyed [9,10]. Fever, increased body temperature, myalgias, rashes, and increased heartbeat are all symptoms that are

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attributed to larval migration. The degree of the infection's severity is directly related to the magnitude of muscular pain experienced, this also affects mobility [9,11]. The encapsulated muscle larvae are primarily responsible for the morbidity [12,13].

The medicinal properties of various plant extracts and their derived products have been researched extensively, this has demonstrated their incredible antioxidant capacity. This attribute is particularly significant because it relates to the potential treatment of numerous debilitating diseases [14-16]. Rocket, which is scientifically known as Eruca sativa and is part of the Brassicaceae family, originally came from the Mediterranean region, but it has since spread across the globe. The mature seeds are referred to as Gargeer, and they are highly esteemed [17-19]. Rocket is typically incorporated into salads, it is also used as a flavoring component or steamed veggie. Additionally, it is an important crop for oil in India, Pakistan, and certain African countries [20-22]. Rocket seeds are used for the production of oil, and it has been used in the traditional pharmacopeia for various purposes antiphlogistic, astringent, depurative, diuretic, digestive, emollient, tonic, stimulant, laxative, and rubefacient [23,24]. Eruca sativa seed extracts were reported to exert antioxidant, antiplatelet, antithrombotic, anticancer, and antimicrobial properties [25,26]. Therefore this study assessed the effects of rocket seeds extracts (RSE) on experimental trichinellosis induce tissue damage, and DNA damage in rat tongue muscles.

Materials and methods

Plant material

Analysis of phenolic compounds by HPLC

Description of an HPLC analysis: Agilent Technologies 1100 series liquid chromatograph was the machine used to separate and detect compounds. It had both an autosampler and a diode-array detector installed. Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) was the stationary phase in the analytical column while a C18 guard column from Phenomenex in Torrance, CA ensured column protection. Instead of eluting components directly acetonitrile (solvent A) and water (v/v) which is a solution of 2% acetic acid (solvent B) were used as mobile phase to investigate the components under consideration. The peaks were identified by comparing their retention times and UV spectra with those obtained from standards characterized earlier on this system using similar analysis conditions that were applied here during the study.

Determination of total flavonoid contents

The measurement of the complete amount of flavonoid present in the extracted seed was initiated as described by Zhishen et al. [27]. Total flavonoid content was determined and expressed in µg/mL of catechin equivalent (CE).

Estimation of DPPH scavenging activity

The stable 1,1-Diphenyl-2-picryl-hydrazyl (DPPH•) was the method used to determine free radical scavenging capacity as reported by Pandey and Rajbhandari [28].

Estimation of total antioxidant capacity

Antioxidant activity has been estimated using a phosphomolybdenum assay which determines the total antioxidant capacity of the compounds [29].

Isolation and infection of Trichinella spiralis muscle larvae

The strain of *Trichinella* used in this research (code: ISS6158 from Istituto Superiore di Sanità) was obtained from rats that were bred in the laboratory of the Department of Parasitology at the Faculty of Medicine, Tanta University. The rats used for this purpose were infected and then sacrificed 5 weeks later. Their skeletal muscle was extracted, chopped, and processed through a standard artificial pepsin-HCl digestion method to acquire Trichinella muscle larvae [30,31]. These larvae were separated using a sieve, and then subjected to repeated precipitation and washing with distilled water before being finally counted using a cell counting chamber. The concentration of the larvae suspended in the fluid was adjusted to 0.25 ml per rat (with each ml containing 1000 live larvae) — this made up the inoculum volume as the recommended dose per rat. Before infection, rats were deprived of food for 12

hours; they were then orally infected with 0.25 ml suspension introduced into the stomach using a tuberculin syringe fitted with an 18-gauge blunt needle [2].

Experimental design and animal groups

The current investigation was conducted on 40 male albino rats that were approximately 150-170 pounds, which were kept in the animal house (Faculty of Science, University of Tabuk, KSA) for two weeks before initiating the experiment. They were maintained on a standard rodent diet and water supply ad libitum, the standard temperature was 23 ± 2 degrees Celsius, and they were maintained in a 12-hour light/dark cycle, with a minimum of 40% relative humidity. Rats were clustered into four groups.

Group 1: Control group, that includes normal, uninfected animals.

Group 2: The RSE group possessed animals that were receiving RSE orally for a period of time of one week.

Group 3: The infected group (35DPI), which comprised rats that were infected with 1000 larvae of trichinella spiralis and then dissected after 35 days (35DPI).

Group 4: The group that received post-treatment comprised rats that were infested with 1000 larvae of trichinella spiralis's muscle larvae (ML) before the RSE was treated (as a dose in G2) for a week.

This research was initiated in January of 2021, and the practical portion was completed in March of 2023, the theoretical portion and writing of the research were initiated, and the research was finally completed in April of 2024.

Sample collection: After 35 days of infection (35DPI), overnight fasted rats will be anesthetized with sodium pentobarbital, and blood will be collected by cardiac puncture. Blood will be placed in EDTA tubes and then centrifuged at 3000 g for 20 minutes. Plasma will be intentionally divided, and each sample will have its label and will be stored at - 20 0C until parasitological, as well as tongue histopathology, analysis.

Histological processing

Samples of the tongue were collected post-necropsy and preserved for 24 to 48 hours by immersion in a 10% neutral buffered formalin solution. The slices, cut at 5 µm thick using a rotary microtome (Litz, Wetzlar, Germany), were stained with hematoxylin and eosin as suggested by Bancroft and Gamble [32].

Comet Assay

Tongue tissue from different groups was used to measure DNA damage using the comet assay method, which was first described by Abd Eldaim et al. [33]. Analysis and measurements were performed as follows: 100 mg of tongue tissue was quickly homogenized and suspended in cold phosphate-buffered saline. The cell suspension was mixed with low melting point agarose and spread on precoated agarose slides. The slides were allowed to solidify and then placed horizontally in an electrophoresis tank filled with buffer for 20 min. Electrophoresis was run at low voltage. After electrophoresis, the slides were gently washed and stained with ethidium bromide. Visualization was done using a fluorescence microscope, and Comet V image analysis software was used to determine both qualitatively and quantitatively the extent of DNA migration (represented by the length of the comet tail) along with other parameters that describe DNA damage.

Statistical Analysis: Data has been provided and analyzed with one-way ANOVA to show the significance of differences. The values are shown as means SE. * and # denote a significant difference between the control group and the 35DPI group at p 0.05 level, respectively.

Results

Eruca sativa seeds extract contents

An excellent resolution was obtained between the standards within 50 minutes. Figure (1) displays the chromatographic profile of both the mixed standard solution and the Eruca sativa seed extract samples, while Table (1) exhibited total phenolic contents and Table (2) total phenols plus total flavonoids as well as antioxidant activity in Eruca sativa seeds extract.

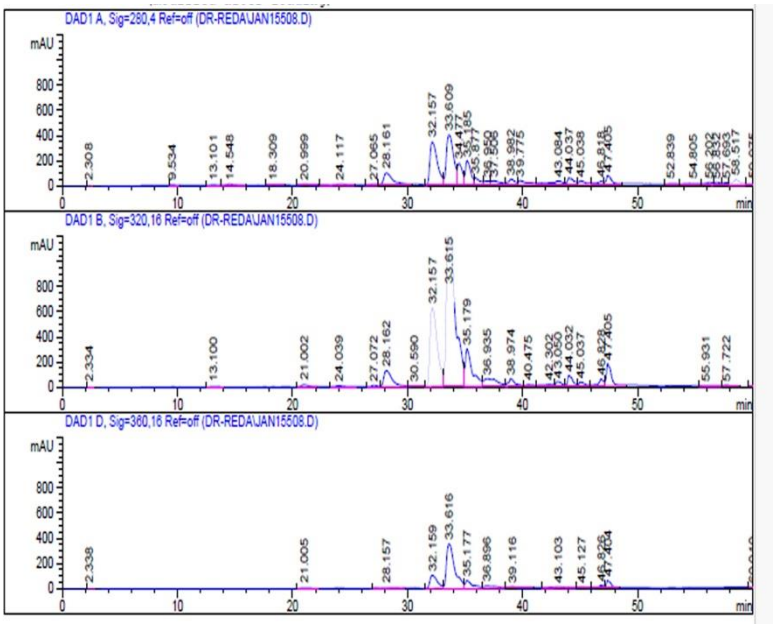

Figure 1: Base Peak Chromatogram (BPC) of RSE.

Table 1: Total phenolic contents: The total phenols content in RSE.

Compound	**RT**	**ug/g**
Gallic acid	5.6	0.000
Protocatechuic acid	9.7	10.750
Gentilic acid	16.7	ND
catachine	18.4	46.878
Chlorogenic acid	20.3	ND
Caffeic acid	21	18.973
Syrngic acid	22.5	ND
Vanillic acid	24.1	13.780
Ferulic acid	32	529.866
Sinapic acid	33.5	1952.023
Rutin	36.1	ND
Coumaric acid	36.7	ND
Rosmarinic acid	40.1	23.905
Cinnamic acid	42.7	9.073
Qurecetin	43.4	29.575
Kaempferol	46.4	21.178
Chrysin	51.7	ND

Black color = 280 nm; red color = 320nm; blue color = 360 nm

Table 2: Estimation of total phenols, total flavonoids and antioxidants activity in Eruca sativa seeds extract

	RSE
Total phenols (mg GAE/g)	**2.803**
Total flavonoids (mg CE/g)	**0.779**
DPPH (mg TE/g)	**3.482**

Parasitological assessment results during the muscular phase of experimental trichinellosis

Results from Table 3 and Figure 2 unveiled the quantity and dispersion of larvae in skeletal muscles, diaphragm muscles, tongue muscles, and heart of the experimentally infected rats at 35DPI. A high proportion (more than 97%) of larvae were found in skeletal muscles while approximately 2.5% were found in both diaphragm and tongue muscles, with only about 0.5% located in cardiac muscles. The treatment of RSE on 35DIP led to a drastic decrease as it induced significant depletion in the mean number of Trichinella spiralis (78.5± 4.8) when compared to that of 35DPI (291.0 ± 8.5).

An analysis of the importance of disparity through one-way ANOVA was conducted to juxtapose the distribution and quantity of larvae. The comparison was made using a computer program. Results are presented as Mean ± Standard Error of Mean (SEM). The one-way ANOVA showed significance at $P < 0.05$.

Table 3. The mean number of *Trichinella spiralis* encysted larvae in the skeletal muscles, diaphragm, and tongue in the 35DPI group.

	No. of T.spiralis larvae/rat (Mean ± SE)
Skeletal muscles	17950 ± 427.0
Diaphragm	214.5 ± 9.2
Tongue	291.0 ± 8.5
Heart	67.0 ± 3.4

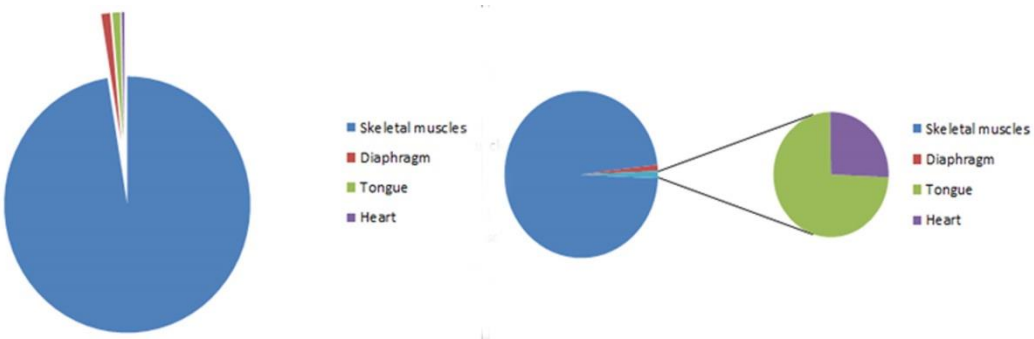

Figure 2: Enclosed larvae inside skeletal muscles, diaphragm, and tongue decreased in the infected group 35 days after pathogen inoculation. The representation of the data is through mean ± standard error of the mean (SEM). There was a significant difference between groups according to one–way ANOVA with $P < 0.05$.

Histopathology of tongue

In Figure 3 and Table 4, the tongue sections of various groups are displayed. Both the control and RSE groups illustrated the existence of keratinized papillae over the connective tissue below which harbored bundles composed of skeletal muscle fibers that run in different directions. The muscle fibers were of a polygonal shape— containing eosinophilic myofibrils with nuclei located at the periphery (as shown in Figure 3A and B). At 5WPI, there was extensive damage noted in the tissue where only muscle fibers exhibited degenerative changes along with a high number of unencapsulated and encapsulated Trichinella larvae; fibrosis was observed in the muscle fibers, mild focal hemorrhage, mild cytoplasmic vacuoles, moderate inflammatory cell infiltration, and obvious areas of coagulative necrosis in the muscle (Figures 3C and D). Tongue sections at 5WPI treated with RSE showed the presence of unencapsulated and encapsulated larvae with mild fibrosis, inflammatory cell infiltration, and mild coagulative necrosis in the muscle (Figure 3E&F).

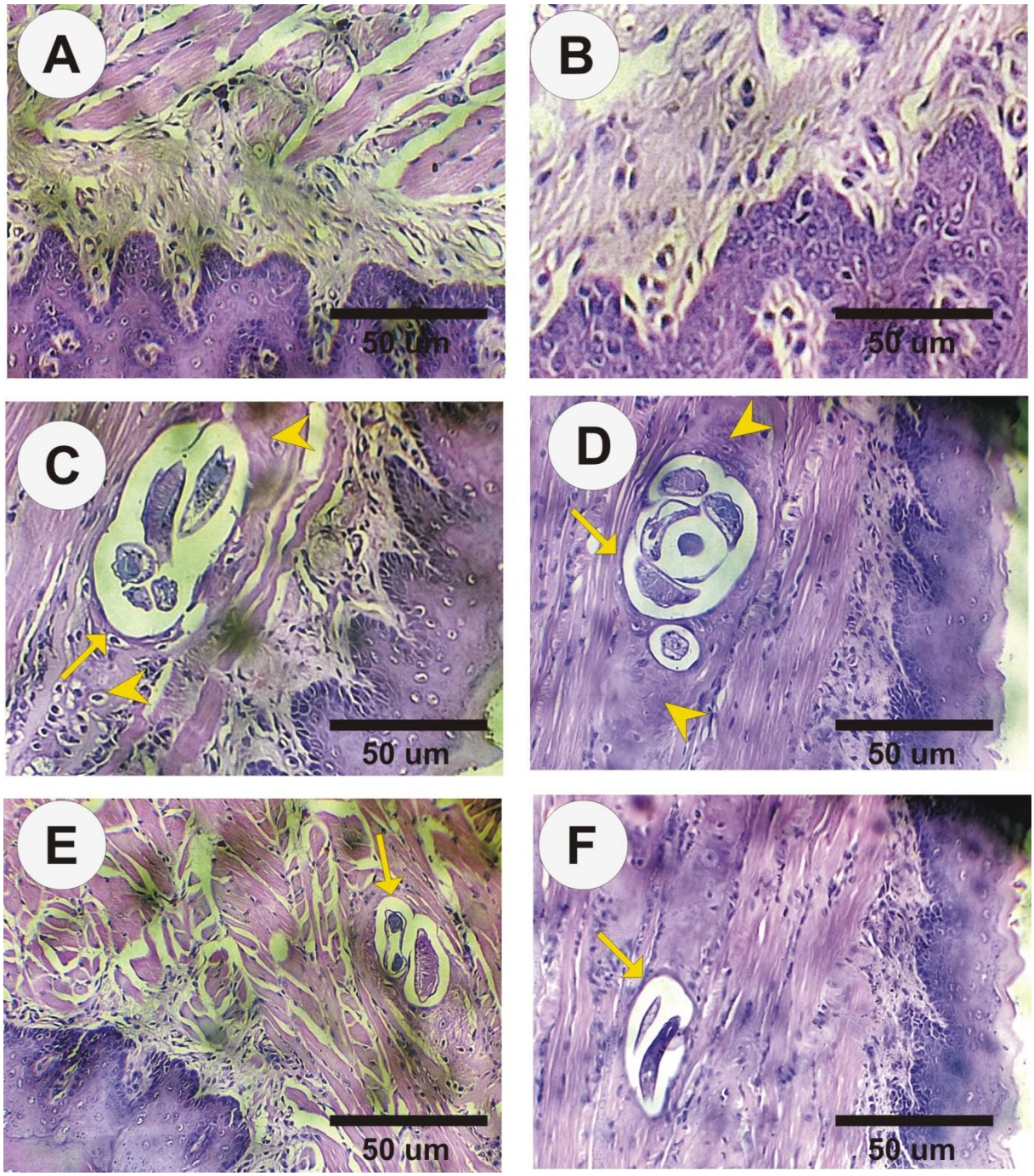

Figure 3: A photomicrograph depicting tongue sections stained with Hematoxylins & Eosin. The control and RSE groups displayed the presence of keratinized papillae in the tongue section which covered the underlying connective tissue comprising bundles of skeletal muscle fibers oriented diversely. At 35DPI, there was an indication of severe tissue damage with diffuse degenerative changes involving all parts of the muscle fibers as well as coagulative necrosis of muscle (shown by arrowheads) and many T. spiralis encysted encapsulated larvae (arrows). The tongue section in 35DPI revealed severe tissue injury with diffuse degenerative changes throughout the muscle fibers, marked coagulative necrosis of muscle (arrowheads) and many *T. spiralis* encysted encapsulated larvae (arrows). E&F: Tongue section in 5WPI+RSE revealed the presence of non-encapsulated encysted larvae (arrows) with mild fibrosis and mild coagulative necrosis of muscle.

Table 4: Histopathological scores of tongue muscles in different groups.

	encapsulated larvae	inflammatory cellular infiltration	fibrosis	focal hemorrhage	Necrosis	cytoplasmic vacuoles
Control	-	-	-	-	-	-
RSE	-	-	-	-	-	-
35DPI	-	++	+++	+	+++	+
35DPI+RSE	-	+	+	-	+	-

Where; -, negative; +, mild; ++, moderate; +++, marked

Tongue DNA damage

The comet assay was used to study DNA damage in rat tongues. Infected rats were compared to a normal control. The results of the comet assay can be seen in Figures (4) and Tables (5). The tongue sample taken at 35DPI showed a substantial increase of DNA damage— indicated by the increase of tail length, tail DNA%, and tail moment— compared to normal control; RSE groups which demonstrated a significant decrease after the application of RSE (35DPI+RSE) for one week did not differ significantly from normal control or RSE about DNA damage (tail length).

Table 5: Comet assay parameters obtained by image analysis in cells of all groups.

Group	Tailed %	Untailed %	Tails length µm	Tail DNA %	Tail moment
Control	2.3	97.7	$1.80^{\#}$±0.33	1.65	1.97
RSE	1.8	98.2	$1.39^{\#}$ ±0.16	1.53	1.75
35DPI	35.5	64.5	4.16^{*}±0.72	5.12	36.09
35DPI+RSE	14.0	86.0	$2.81^{\#*}$ ±0.27	4.38	18.56

* and # signify a significant departure from the control and 35DPI groups respectively at p 0.05.

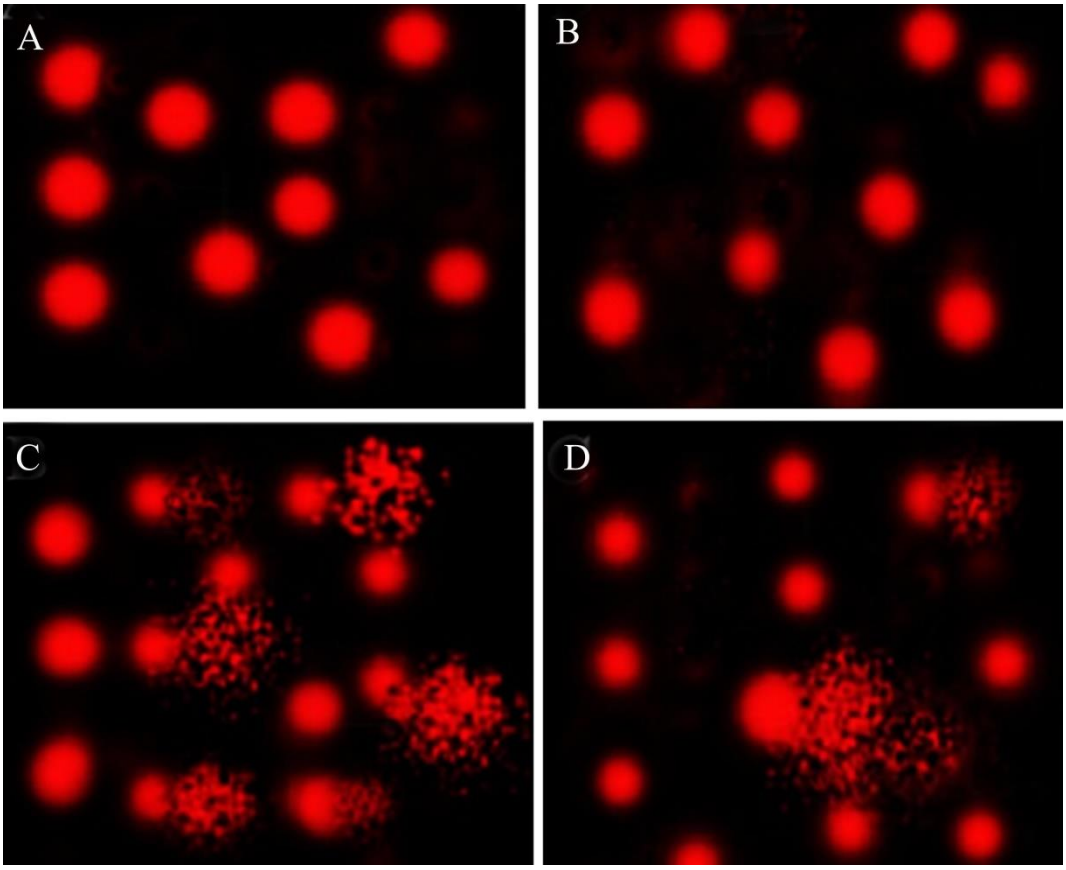

Fig. 4: Photomicrographs representation of DNA damage in tongue tissues, using comet assay, in the normal control group (A), RSE group (B), 35DPI group (C), and 35DPI+RSE group (D).

Discussion

Trichinella spiralis is an internal parasitic worm that has several distinct features that make up the disease referred to as trichinellosis or trichinosis. Trichinellosis is disadvantageous to food quality, detrimental to the human body, and can cause significant negative monetary impacts within the swine production industry [6,34] Other than causing the tissues of the intestines and muscles to suffer, infections with Trichinella spiralis can lead to fevers, headaches, myalgias, eosinophilia, and periorbital edema [2,35]. The results obtained today show that the ethanolic extract from rocket seeds had anti-radical activity plus antioxidants; about 2.803 g of GAE per gram of total Phenols 0.779 g of CE per gram of total Flavonoids, and 3.482 g of TE per gram of DPPH as an antioxidant in the seeds. Rocket seeds are rich in sources 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. Conversely, Quercetin was overrepresented, this suggests that the seeds of rockets have both anti-inflammatory and anticancer properties. Our results concur with Barillari et al. [21] and Koubaa et al. [36] documented those rockets (Eruca sativa L.) possessed anti-inflammatory, antioxidant, and antibacterial properties. As a result, this research sought to investigate the therapeutic effects of rocket (Eruca sativa) seed extract on the tissue damage and DNA damage in the rat's tongue muscles associated with experimental infection with Trichinella.

Recent research has demonstrated that rats that are experimentally infected with Trichinella spiralis have varying levels of muscular damage in their tongues, hearts, diaphragms, and skeletal muscles. This is dependent on the degree of the infection or injury. As a result, the infection primarily harmed striated muscles and occasionally harmed smooth or cardiac muscles. The current results are in agreement with those of Yadav [37]; Basyoni and El-Sabah [38] and Alghabban [2] who documented that muscle cells infected with T. spiralis exhibited deleterious changes in the form of a disarray of the muscle fibers and a significant amount of T. spiralis being diffused across the muscle.

Additionally, the results of the current study demonstrated that approximately 95% of encysted larvae were located in the skeletal muscles, with the remaining 5% being found in the diaphragm, tongue, and heart muscles. The histopathological analysis of the infected rats' tongues' muscle tissue revealed areas of coagulative necrosis as well as significant muscle fibrosis and general degenerative changes in the fibers. Conversely, infected skeletal muscles, the diaphragm, and heart muscles are comprised of T. spiralis larvae that are encysted in a collagen-based capsule except for the tongue. and there was a mild form of inflammation that was primarily composed of lymphocytes, eosinophils, and plasma cells. Other than the direct effects of the parasite itself, several different avenues lead to tissue destruction in trichinellosis.

Recent histology research has demonstrated that the degree of infection has an effect on the pattern of inflammation in the rat's muscles. The response in the skeletal and diaphragm muscles, which have numerous confined larvae, is contrasted with the weaker inflammatory response in the heart and tongue muscles. The treatments of 35WPI with RSE demonstrated a significant enhancement of the tongue relative to the 35WPI group. Tongue sections from 35WPI +RSE showed a decrease in the number of non-encapsulated larvae with mild fibrosis and mild coagulative necrosis of muscle. The current results are in agreement with previous research by Etewa. [39] and Attia et al. [40], documented that in the skeletal muscles of mice with control parasites, multiple deposits of T. spiralis were encysted in the larvae and had mononuclear cells that surrounded the capsule.

It's commonly understood that DNA damage accrues over time and that it's dependent on the time of exposure. The volume of parasitic proteins present also affects DNA damage; in Vietnamese, the production of animal products can cause all kinds of DNA damage. Apoptosis and cell cycle blockage are two separate processes that can be triggered by DNA damage in the germline of a nematode. The latter procedure is identical to the development of genetically programmed cell death and is specifically regulated. When organisms are subjected to stress and have a significant increase in ROS, their DNA is damaged and their processes of oxidative are impaired [42]. The current results indicate that the tongue in the 35DPI group had a higher degree of DNA damage than the normal control and RSE groups, as measured by increases in tail length, DNA%, and moments. Following a week of RSE (35DPI+RSE) exposure, this elevated DNA damage decrease can be attributed to the higher concentration of compounds with antioxidant properties, particularly Phenolic and Flavonoid compounds. These results indicate that increased amounts of free radicals caused by the infection with T. spiralis not only augment the host's defenses against the parasite but also lead to the deleterious effects of the infection on other cells. Additionally, the current results indicate that increased amounts of free radicals caused by the infection with T. spiralis not only augment the host's defense systems against the parasite but also cause damage to other cells via the free radical mechanism. Our results concur with those of Hafez et al. [43] who documented that; T. spiralis's experimental infection causes DNA damage and skeletal muscle apoptosis in mice. This outcome is in agreement with Kucukkurt's study.

[45] reported that; Babesia ovis caused oxidative stress and DNA damage in the Anatolian black goat.

Conclusion

The current results showed that; over 97% of the larvae were located in skeletal muscles, and about 2.5% were located in both the diaphragm and the tongue. However, only about 0.5% were located in cardiac muscles. The muscles of the Tongue in 35DIP exhibited DNA damage and severe tissue injury along with diffuse degenerative changes in the muscle fibers, these changes were accompanied by a large number of T. spiralis encysted larvae. Treatments of 35DIP with RSE resulted in a significant decrease in the average number of Trichinella spiralis, the DNA damage was inhibited, and the Tongue's muscles were improved.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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