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Characterization of the *Urtica Dioica* Leaf Extracts and Studies Its Effects on DNA and Sperm in Rats

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A B S T R A C T

This study was carried out to test the activity of the methanol-water (20:80 V/V) extract of the leaves of *Urtica dioica L*. on the DNA and sperms in Rat. The antioxidant activity of the extract was tested using two systems: the β-carotene spray method and measuring the coupled oxidation of β-carotene and linoleic acid. The rats were treated with the extract at 100, 500, and 1000 mg/kg body weight, and the extracted DNA from white blood cells. The results showed the presence of good antioxidant activity in the extract through the appearance of 3 positive bands (Rf= 0.4, 0.94, 0.96) that kept the yellow color of β-carotene. This activity was also confirmed from the ability of the extract to keep the of absorbance (at 470 nm) stable, relatively high, and close to the positive control curve for BHT that shows antioxidant activity when using the coupled oxidation of β-carotene and linoleic acid. Treating the rats with the extract at concentrations of 100 and 500 mg/kg did not have the effect on the DNA, while the third concentration (1000 mg/kg) caused DNA destruction. This was clear from the appearance of DNA smear with a molecular size approximately 0.3-20.5 Kbps**.** The study of the effect of the extract on rat sperm, the result shows different abnormalities in sperm, especially in high dose.

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Introduction

Due to increasing trend in recent times toward medicinal plants, whether natural or extracted extracts towards their preventive or therapeutic uses, *Urtica dioica* plant, which is used in many countries in traditional medicine, has been selected and a number of major modern researches have alerted to the importance of going to study This plant at the level of its various biological activities [1] has been noted for the presence of various active antioxidant compounds [2] and anti-mutation and inhibitory cancer, especially prostate cancer [3].

Urtica dioica L. belongs to the Urtieaceae family and has more than a common name such as Stinging nettle and Great nettle. The length of the stem is 2-3 feet, sometimes up to 4 feet, and the branches can branched underground so that the vegetative groups multiply above them. The air parts are small, but they are especially abundant in the lower side of the leaf and legs, as they contain histamine and formic acid, the main cause of skin allergies when contact with this plant. This plant grows well in the soil rich in nitrogen [4].

Many compounds and elements present in fruits and vegetables play an important role in preventing and inhibiting mutation and thus preventing cancer according to the theory that the occurrence of cell mutation due to the toxic genetic effect leads to the initiation of cancer. This risk is the final expression of a series of events, including the taking of the compound or the carcinogen and its proliferation or metabolism and then the transfer of its metabolites into the cell and its end in the nucleus and thus damage the DNA and the stability of the damage [5]. all of these events are affected by opposite forces such as inhibitors of the elements and compounds present in fruits and so on, where lead these inhibitors do through multiple mechanisms or interfere with other inhibitors to play their role by reverse interaction towards mutagens and carcinogens depending on the type of change that gets in the defensive means of the body and thus prevention first and preventive boom and diseases related to them.

Many studies have included the design of appropriate life systems to test the susceptibility of chemicals to the development of mutations and carcinogenesis and to detect possible changes in DNA molecules. These systems include bacteria, yeast, fungi, plants, insects, fish and some mammals. The *urtica dioica* plant has an excellent anti-oxidant effect which is attributed to the presence of phenolic compounds in it Due to the presence of phenolic compounds [4] indicated that the water extract of the *Urtica dioica L.* contains high antioxidant efficacy calculated directly by ABTS-Radical Scavenging Abitations, Kidney stones are one of the oldest known and common diseases in the urinary tract system. Various human studies have suggested that diets with a higher intake of vegetables and fruits play a role in the prevention of kidney stones. In this review, we have provided an overview of these dietary plants, their main chemical constituents, and their possible mechanisms of action. Camellia sinensis (green tea), *Rubus idaeus* (raspberry), *Rubia cordifolia* (common madder), *Petroselinum crispum* (parsley), *Punica granatum* (pomegranate), *Pistacia lentiscus* (mastic), *Solanum xanthocarpum* (yellow-fruit nightshade), *Urtica dioica* (stinging nettle), *Dolichos biflorus* (horse gram), *Ammi visnaga* (khella), *Nigella sativa* (black-cumin), *Hibiscus sabdariffa* (roselle), and *Origanum vulgare* (oregano) have received considerable interest based on scientific evidence. Beside these dietaryplants, phytochemicals—such as catechin, epicatechin, epigallocatechin-3-gallate, diosmin, rutin, quercetin, hyperoside, and curcumin—as antioxidant dietary phyto-phenols were found to be effective for the prevention of urolithiasis (the process of stone formation in the urinary tract). The main underlying mechanisms of these dietary plants and their isolated phytonutrients in the management of urolithiasis include diuretic, antispasmodic, and antioxidant activity, as well as an inhibitory effect on crystallization, nucleation, and aggregation of crystals [24]. This activity was found to be directly proportional to phenol content in the extract, which was found to be directly proportional to the content, Pieroni *et al*. pointed to the strong anti-oxidant effect of the fire plant in the detection of diphenyl-2-picrylhydrazil radical through the use

of two tests. The first test included inhibition of non-enzymatic efficacy of non-enzymatic inhibitory of bovinebrain lipid peroxidation, and the second inhibition of the enzyme Xanthine oxidase and in both antioxidant tests showed a strong effectiveness of this plant extract where the inhibitory activity more than 50% [6].

The present study aimed to examine the antioxidant potential of the methanolic water extract of the leaves of the *Urtica dioica* plant and to study its effect in the genetic material, based on DNA analysis. In study indicate that UD protected DXR-induced testicular toxicity and improve semen parameters, thus suggesting its co-administration as a supportive agent during doxorubicin treatment. Further studies could be aimed to determine protective effect of UD against chemotherapeutic agents such as DXR [25].

Materials and methods

Preparation of Urtica dioica plants extract

A specific weight was taken from the leaves and washed with tap water and then with distilled water, then dried and cut by scissors into small pieces. Small pieces of leaves were mixed at a rate of 1 g of leaves: 3 ml of extraction solution (extract solution representing 20 methanol: 80 distilled water, V / V) by blender and for half an hour at room temperature. Serve the solution with a gauze cloth to get the water leachate. The leachate was distributed on 10 mL test tubes and centrifuged at 3500 rpm for 20 minutes. Collect the upper fluid for the tubes and concentrate using rotary evaporator to obtain the dried extract. Keep the extract in the refrigerator until use)Sato et al, 1990).

Experimental animal

The white mice (Balb / C) were used approximately 6 weeks age after they were brought from the Faculty of pharmacy - University of kerbala and raised inside metal cages specially designed for the purposes of education, which was exposed 12 hours lighting and 12 hours, dark and was observed at the level of feed transactions, The least to adapt to the conditions of the laboratory before conducting experiments, with knowledge used male only.

Characterization of plant extracts by thin layer chromatography (TLC)

100µl of the extract was placed at the base of the plate (repeated 3 times with a 5-minute interval). The plate was placed in a vertical position in a glass basin (10 × 24 × 40) $cm^3$and the same extraction solution was used (distilled water: 20, V / V) as a liquid phase of the separation process, monitors the process of liquid phase rise on the plates until it reaches near the top edge approximately The plates are extracted and left at room temperature until drying and the examination of the spots formed by visible light as well as ultraviolet [7], where the Rd Retardation Factor (Rf) was determined for the formed packets as well as the color and number of these beams, with the knowledge that :

$$\mathbf{Rf} = \frac{\textbf{distance traveled by the package}}{\textbf{The distance traveled by the liquid phase}}$$

Antioxidant efficacy test

A) Method of spraying with beta-carotene: β-carotene

The anti-oxidant efficacy test was performed on TLC plates using the Beta carotene spray method mentioned by Pratt and Miller [8]. It dissolved 9 mg of beta-carotene in 30 ml of chloroform and added two drops of pure linoleic acid and 60 ml of ethanol to the beta-carotene solution - chloroform where this mixture is sprayed onto the plates. After spraying, the plates are exposed to normal light until the earth color is shortened (2-6 hours). The beams that hold the yellow color represent antioxidant components so that their color density is proportional to the effectiveness.

B) Method of measuring the comparative oxidation of beta carotene and linoleic acid:

The antioxidant activity was determined by dissolving approximately 1 mg of beta-carotene in 10 mL of chloroform, and then 1 mL of beta-carotene chloroform dissolved in a glass flask containing 20 mg of pure linoleic acid and 200 mg of 40-Twen under boiling conditions and after chloroform Using rotary evaporator (at 50 °C) add 50 mL of distilled water with strong circulation. Take 5 ml of this emulsifier and add 0.2 ml of the test extract (1000PPM) where the absorbance is measured at a 470 nm wavelength (taking into account the zeroing of the device on a sample of emulsion of beta-carotene), as well as the two negative same as the previous amount of emulsifier plus 1 ml of distilled and positive water added to 1 ml of BHT (100 PPM). The tubes are placed in a water bath at 50 ° C and the absorbance is read every 15 minutes to 105 minutes (so that the reading rate is taken for three replicates).

DNA extraction and analysis test

This test was performed using white albino rats at 8 weeks of age. The project was done with consideration of ethical issues and obtaining license from the ethics committee of local institute. The extract concentrations for animal treatment were 100 mg/kg, 500 mg/kg and 1000 mg/kg. The final concentration was consistent with the average lethal dose determined by reported method by Badilla et al. [9]. The dose was given once orally after dissolving the extract in the drinking water and then collecting the blood from the animals killed after 24 hours of treatment. The Lambda virus was used as a volumetric guide. 30 µl (0.5 µg/µl) was cut by the Eco RI and according to the guidelines the company is equipped with enzyme.

The total DNA was extracted from the white blood cells after experimental animals were treated with the progressive concentrations of the extract using the DNA extraction kit supplied by promega. Electrophoresis of DNA extracted from white blood cells was performed on the agarose gel [10].

Study of abnormalities of sperm cells in treated animals

For sexual cells, the Coles method was followed by cutting the skin down the ventral cavity of the animal [11] The testes were extracted and the epididymis was removed. After the removal of the white tunic of the testicle, the epidermis was grafted in a drop of PBS (Phosphate buffered saline).

Statistical analysis

All values were shown as the mean ± standard error of mean (SEM). For statistical evaluation, one-way analysis of variance (ANOVA) followed by Tukey post hoc test was used (SPSS software version 18.0). Differences with a value of $P<0.05$ were considered to be significant.

Results

Characterization of plant extracts by thin layer chromatography (TLC)

Table 1 shows the pattern of TLC migration of the methanol-water extract of the leaves of the *Urtica dioica* plant when examined with visible light and ultraviolet light (Fig. 1). The results showed that TLC was relatively versatile in the number of beams, (Rf = 0.4, 0.94, 0.96).

Table 1. Characterization of the packages formed on the TLC plates of the methanolic water extract of the fire plant

Bands properties			Method of examination
Number	color	RF	
3	Violet-reddish	*0.4	Visible light
	Green - yellowish	*0.94	
	Build	*0.96	
6	Purple	*0.4	UV
	Blue	0.65	
	Blue	0.8	
	Blue	0.9	
	Green – yellowish	*0.94	
	Brown - Reddish	*0.96	

*** Joint beams when tested with conventional light and ultraviolet radiation.**

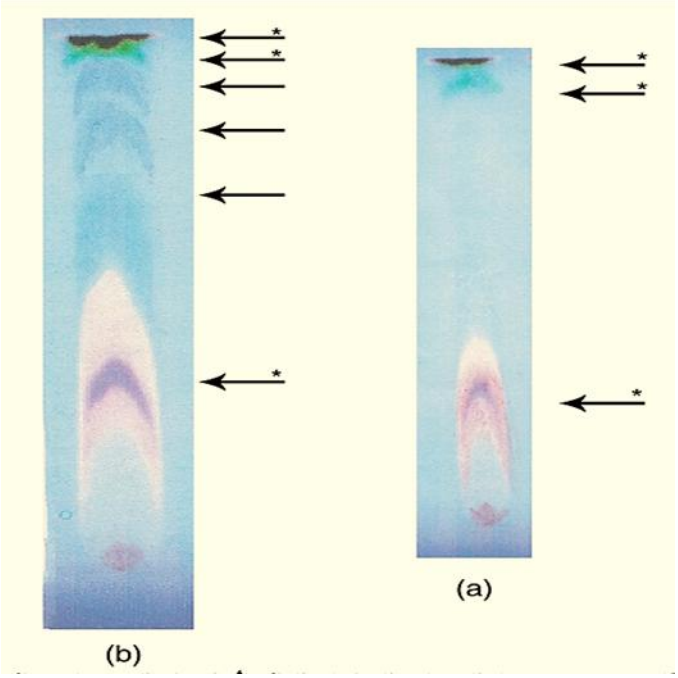

Fig. 1. The mode of migration of the methanolic water extract for leaves of the fire plant on the TLC plates. a) when the visible light test; b) when UV scans are performed; Share: Suggest sites packages) *Indicates common packets.

Antioxidant efficacy test

Table 2 of beta-carotene and linoleic acid, which showed a relatively high and stable effect of the curve up to the period of 105 minute, compared to the grants Z negative control (Fig. 2, 3).

DNA extraction and analysis test

Table 3 shows the characteristics of the DNA extracted from the white blood cells of the rats treated with graduated gradients of the methanolic water extract of the urtica plant, which showed the non-degradation of the DNA at the treatment of concentrations 100 and 500 mg/kg, which led to the formation of a similar package to the control sample at a larger molecular size From

21.2 Kb, while the treatment with a concentration of 1000 mg/kg resulted in the degradation of the DNA in a swab with a molecular size ranging from 0.3 to 20.5 kg base pair (Fig. 3).

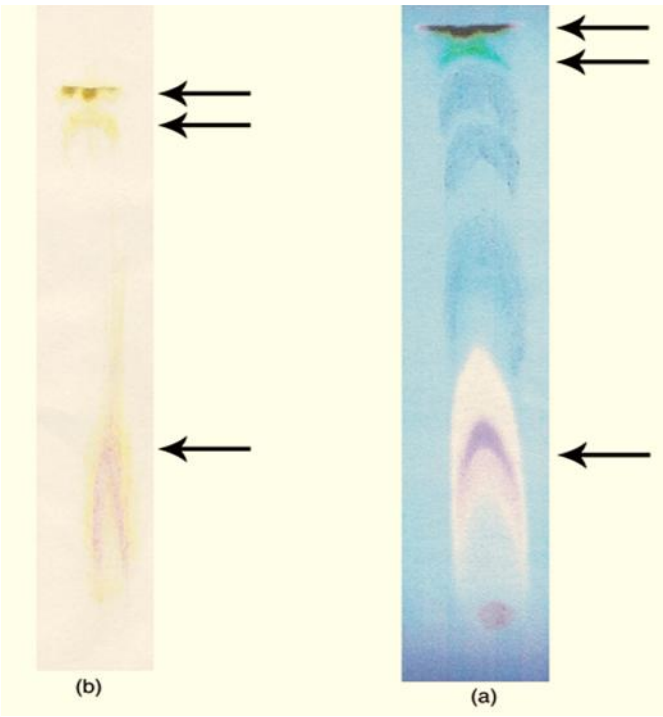

Fig. 2. Examination of the anti-oxidative effect of beta-carotene spraying of the methanolic water extract of the fire leaf. (a) Before spraying; (b) After spraying. Stocks: refers to packages with antioxidant efficacy (which retained the yellow color).

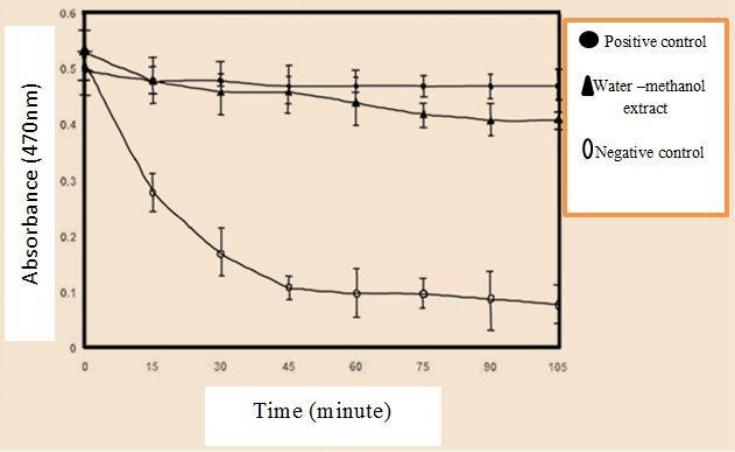

Fig. 3. Test the antioxidant efficacy of the oxidative oxidation method of beta carotene and linoleic acid for the methanolic water extract of the leaves of the fire plant. Where each reading represents an average of three replicates ± standard deviation (SE).

Table 2. Test results of the antioxidant potential of the methanolic water extract of the fire plant using the beta-carotene spraying test. (+: The presence of antioxidant efficacy (packages that retained the yellow color); -: Lack of antioxidant efficacy)

Examination test	BAND (Rf)
+	0.4
-	0.65
-	0.8
-	0.9
+	0.94
+	0.96

Table 3. Properties of DNA extracted from white blood cells of mice treated with graduated gradients of methanolic water extract (20: 80, V / V) for fire plant.

Field number figure (4-4)	Properties of DNA		Concentration Kg/mg
	SIZE (Kb)	Shape of electrophoresis DNA	
1	<21.2	Band	Negative control
2	<21.2	Band	100
3	<21.2	Band	*500
4	0.3-20.5	Smear	1000

***Selection concentration**

Results from the study of abnormalities of sperm cells in treated rats

Table 4 shows the rate of total deformities in the sperm of the rats treated at the highest dosage of the extract at different time intervals. The highest rate of these abnormalities is the loss of head hock (418.1) and the tail loss (410.5) head loss (296.6), then lobed head (126.6). Table 5 shows the mean number of abnormalities in the sperm.

Table 4. The rate of total deformities in the sperm of rats treated with a dose of 1000 mg/kg of extract and at different time intervals.

Summation	Loss head	Tail loss	Hock head loss	Deviant head hock	Lobed head	Time (day)
69.9	11	22.6	21	15.3	0	Negative control
258.9	50	61.1	78.6	45	24	7
279.9	52	63.3	85.6	53	26	14
307.9	58	72.3	88	60.6	29	21
342.9	60.3	101	78.3	82	21.3	28
387.8	71.3	112.6	87.6	90	26.3	35
	291.6	410.5	418.1	330.6	126.6	Abnormalities via (5 weeks)

Table 5. Mean total deformities in the sperm of rats treated with a dose of 1000 mg/kg of extract and at different time intervals (X :mean; SD: standard devasion; SE: standard error).

X± SD	SE	Summations
4.00 ± 69.9	2.30	Negative control
4.58 ± 258.9	2.64	Day 7
5.47 ± 279.9	3.15	Day 14
6.12 ± 307.9	3.53	Day 21
7.59 ± 342.9	4.38	Day28
9.16 ± 387.8	5.29	Day35

Discussion

The data from TLC migration of the methanol-water extract of the leaves of the *Urtica dioica* plant when examined with visible light and ultraviolet light shown in the table and Figure 1. Which can be explained in the light of the containment of the *Uurtica dioica* plant on many components, especially since the alcoholic water extracts of this plant contain various compounds such as multiple sugars [12]. Schottner et al. pointed out that the polar extracts of this plant contain Materials for fetal including (+) - neoolivil and secoisolariciresinol (-) - [13]. Akbay et al. demonstrated that the methanolic extract of air parts of fire plants contains glycosidic-flavinoid compounds when using chromatographic and optical methods. The most important of these compounds Include: quercetin-3-O-ratinoside, kaempherol-3-O-rutinoside, isorhamnetin-3-O-glucoside[14].

The water extract of this plant contains phenolic compounds in good quantities with a concentration of 68 - 4162 mg / L, Many of the phenolic acids and flavonoid compounds were separated from the water and methanol extracts of this plant [15]. [26] Showed that Phenolic compounds like flavonoids, phenolic acids, and polyphenols are the most important in Tragopogon genus constituents. The HPLC method was applied to evaluate four phenolic acids and one flavonoid including caffeic acid, gallic acid, *ρ*coumaric acid, ferulic acid, and catechin in root and aerial part of T. graminifolius. Figure 1 shows the chromatogram extract of aerial part was *ρ*-coumaric (6.357 ± 0.014 mg·g of phenolic compound in aerial part and also in standards. The high amount phenolic compound in 70% ethanolic followed by ferulic acid (1.24 ± 0.018 mg·g−1), while the most phenolic compound in root was *ρ*-coumaric acid (2.685 ±0.031 mg·g)) followed by catechin (2.067 ± 0.021 mg·g)

Gulcin et al. reported that the water extract of the fire plant contains phenolic compounds similar to pyrocatechol [16] Karakayas et al. concluded that the water extract of the fire plant contains a high antioxidant effect and that this activity is directly proportional to the content of the plant phenols (r = 0.95) [4]. Other studies have also revealed the ability of the plant extracts to increase the effectiveness of antioxidant enzymes in the bodies of the labs, especially liver enzymes, which included: cytochrome b5, NADH-cytochrome b5 reductase, Gluathione S-transferase, DT-diaphorase, Superoxide dismutase, Catalase [17].

Gulcin et al. pointed to the effectiveness of antioxidants, microbes, ulcers and analgesia. It was found in this study that concentrations 50, 100 and 250 µg of water extract of this plant lead to inhibition of 39, 66 and 98% respectively in the process of composition of peroxide (Peroxidation of linoleic acid). This indicates the good ability of this plant as an antioxidant as well as its ability to reduce free radicals [16].

The results of this study show that the DNA is not affected by moderate concentrations of 500 mg/kg, while DNA is negatively affected by high concentrations of up to 1000 mg/kg. This indicates the effectiveness of the extract of DNA either by its effect on single and/or double tapes. This indicates that these effects should be taken into account when dealing with the high concentrations of fire plant extract as a proposed therapeutic substance in the future, and this is

consistent with the findings of many researches regarding the possible toxic effects of extracts of urtica plant given high dose.

Study was confirmed by Huang et al., which showed that there are toxic effects of the extract of this plant when given with a high dose ranging between 1.0226-1.2022 g/kg body weight [18]. The DNA analysis has been used in many studies as a function of the toxicity or toxicity of environmental factors, whether physiological or chemical. DNA studies have been shown to understand the mechanisms of apoptosis within the organism's body and glass [19]. This criterion was adopted, for example, in studying the effect of cadmium in the induction of cell death and DNA degradation on liver cells in Rainbow trout [20] as well as on the effect of helium-ray radiation or gamma radiation on DNA in glass [21,22].

This finding indicates that the uritca does not have toxic and mutagenic effects at concentrations not exceeding 500 mg/kg. The process of ascertaining the absence of such effects before interfering with the incinerator is consistent with that of a number of researchers [23]. The higher concentrations of root extract and wheat leaves do not have any toxic effect. Another study indicated that root juice and *Thevetia peruviana* seeds obtained by boiling with water did not affect partial DNA damage and no chromosomal fractures were detected. In the study by Sylianto et al. when he examined toxic and mutagenicity effects of akapulko plant leaves and the results showed not having extracted any partial effects on the DNA in terms of the fraction chromosomal or any toxic effects (Fig. 4, 5, 6, 7).

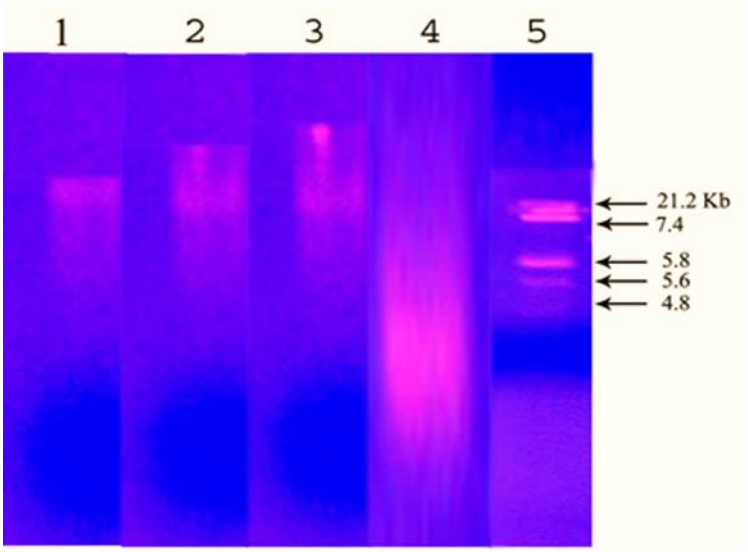

Fig. 4. The electrical transfer of the extracted DNA from the white blood cells of the rats after treatment with three gradient concentrations of the methanolic water extract of the fire plant. 1: negative control (without treatment) 2, 4, 3: concentrations 100 1000, 500, mg/kg, respectively; 5: LAMDA virus after cutting with the cutter Eco RI (volumetric guide)

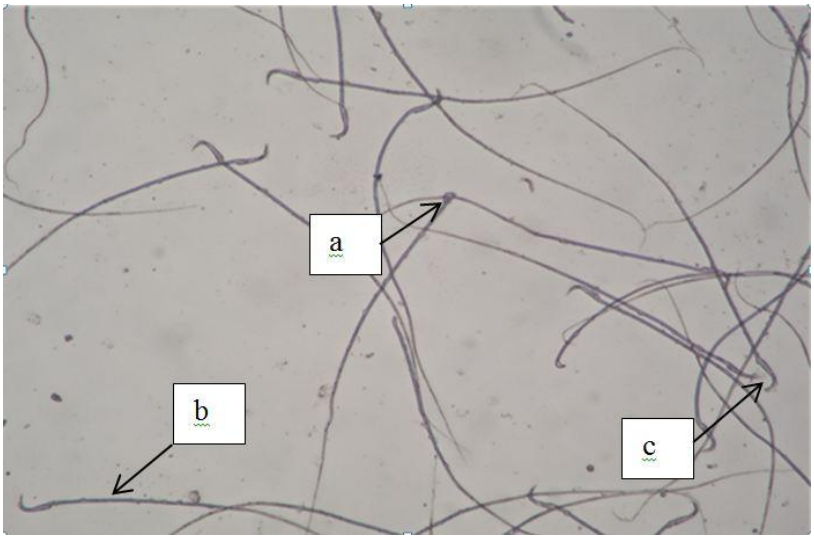

Fig. 5. Head-shaped sperm of male rats treated with a dye extract used in the form of hematoxylin. a) lobed head; b) sperm of headless dog head; c) intact sperm.

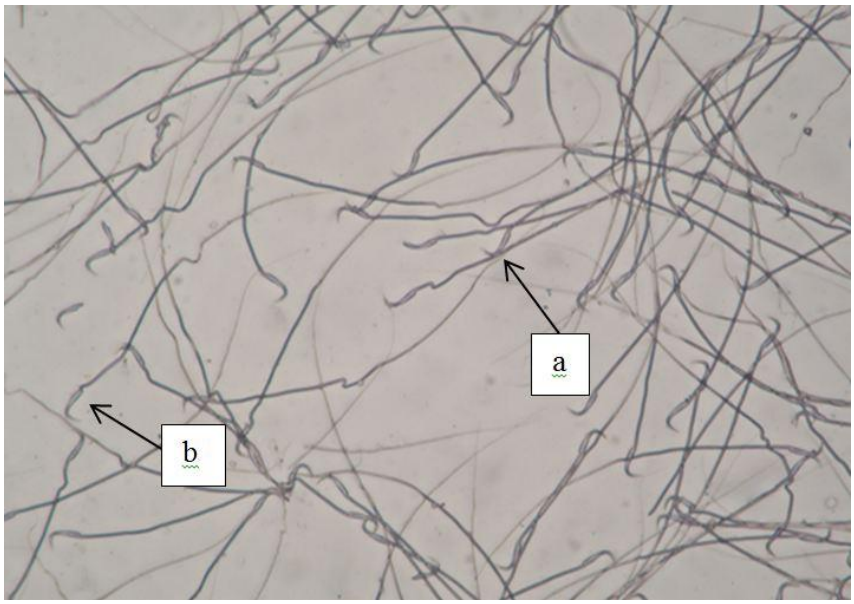

Fig. 6. Headless sperm of male rats treated with extract. Dye used is hematoxylin. a) sperm of the headless dog; b) sperm less tail.

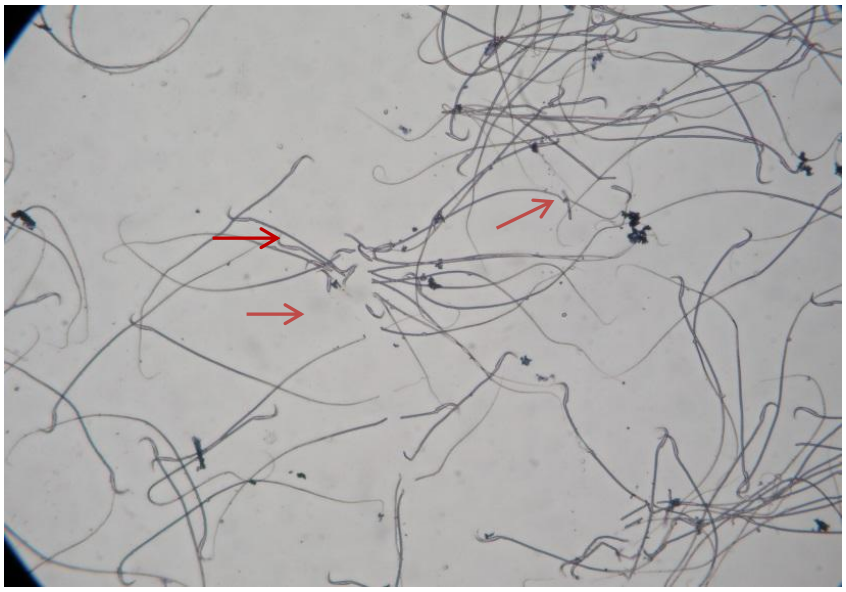

Fig. 7. Seedless tail of male rats treated with extract (dye used is hematoxylin).

Furthermore, Results from the study of abnormalities of sperm cells in treated rats are generally consistent with the results of other studies in terms of a positive relationship between the incidence rate and these distortions have a length of time for exposure to the mutant. In a study on the effect of long-term administration of triptolide (diterpene tripoxide isolated from some chines plants and used as a contraceptive by preventing sperm formation in men) in the formation of sperm and fertility of male rats, the apparent appearance of abnormalities on the sperm, *Urtica dioica* have been used in folk medicine and has several pharmacological properties including antioxidant, anti-apoptotic and anti-fibrotic activities. *Urtica dioica* showed protective effect against DXR-induced changes on epididymal sperm parameters such as sperm count, sperm motility, sperm viability and rate of abnormal sperm cells [24].

Tail and occurrence of chromatin non-condensation prior to maturation of sperm nuclei and incomplete absence in the plasma membranes of the mid-section of the sperm with regulatory imbalances in mitochondrial membranes and sperm collection which decreases sperm cells motility by activating CB1 receptors in mature sperm cells, by U. dioica. The activity of these factors is probably increased by nicotine and another study on the effect of administration of marine leaves to male white mice after time periods Graduated a Per week it noted that there is almost a direct correlation between the percentage of those distortions and the length of the time period. The extract also affects the female sex cells of the rat, where it binds DNA to two intra-strands cross-link and DNA-DNA strands in the sex cells of the rat ovary while simultaneously altering the structure and function of the ovary through its effect On granular cells that are a good target, leading to the small diameter of ovarian follicles [25]. study confermed by [27]showed that that U.dioica caused a significant change in these indices and inhibited the harmful effects induced by nicotine in reproductive parameters. These days, medicinal plants have numerous applications and one of the target tissues for plant extracts is reproductive organs such as testis and sperm parameters ,on the other side administration of the hydroalcoholic extract of *U. dioica* leaves, before induction of diabetes has a possible protective effect against histomorphometric alterations in seminiferous tubules of streptozotocin-induced diabetic rats [28].

Conclusion

It was shown that there are several important component in the methanolic-water extract of the *urtica dioica* plant. There are harmful and varied effects at the molecular level of DNA and the genetic expression of proteins and chromosomes is due to the relatively high concentrations of fire plant extract also there effects on sperms.

Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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