



# The cytogenetic effect of *Euphorbia tirucalli* stems methanolic extract on sperm head morphology in male albino mice

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## Abstract

This study was carried out to investigate the effect of the methanolic crude extract of *Euphorbia tirucalli* Linn (Aveloz) stems on sperm head morphology in male balb/c albino mice. A total of 55 male mice were divided into 5 groups. Each of the Control- (D.W.) and Control+ (MMC) groups consisted of 5 animals, while each of the three treatment groups which injected intraperitoneally with (0.07, 0.14, 0.28) mg/kg doses consisted of 15 animals, and the results were investigated after (7, 21, 35) days of daily injection using 5 animals per each period. The results revealed that the effect of methanolic crude extract of *Euphorbia tirucalli* stems was dose and time dependent and had significant ( $P < 0.01$ ) toxic effects on sperms of male mice that increased with the increasing of dose and time. The sperm head morphological changes that investigated were: the sperms with (swelling head, twisty hook, no hook, abnormal terminal body and irregular head shape). From these results, it can be concluded that in spite of the *Euphorbia tirucalli* medical effects and uses in traditional medicine, the caution and the choice of the appropriate dose and period of treatment must be taken into account. Also, the Gas chromatography-Mass spectrometry (GC-MS) analysis was carried out to detect the chemical content of the extract; the results revealed that it contains 15 chemical compounds which are hydrocarbons, fatty acids and steroids, alcohols and one terpenoid compound.

**Keywords:** *Euphorbia tirucalli*, sperm head morphology, mice, GC-MS

## Introduction

Using the plants as remedies for diseases is very old, and most of traditional medical systems using plants as medications for many maladies [1], but in spite of the numerous medicinal effects of the plants, they may contain toxic, mutagenic, carcinogenic and teratogenic constituents, which may not be of easiness to be detected by traditional medicine experts [2].

*Euphorbia tirucalli* L. (Aveloz) belongs to the family Euphorbiaceae and is a chubby (succulent) plant from which a white viscous latex exudes, it has pencil-like smooth branches without any thorns, the arbor has a distinctive shape that is easy to remark with a tall of up to 10-15 m [3]. The plant is used to treat gonorrhoea, whooping cough, asthma, leprosy, enlargement of spleen, jaundice, tumors and bladder stones. Stem latex is used to treat warts, tooth ache, cough, asthma, ear ache, leprosy, abdominal pain, tumors, rheumatism, skin diseases and intestinal worms. Root is used for colic pains in traditional medicine [4].

The basic of sperm is a stream of genetic information, intuitively the change in the chromosome content may cause variation in the size of sperm, and this refers to relation between genetic and morphology of sperm [5]. Sperm abnormalities may return to environment, genetics or both of them, but the environment is more influential, and this may associate with infertility in male and the fail of pregnancy [6]. Sperm abnormalities are classified into two groups, primary and secondary abnormalities depending on their presumptive origin, that the primary one is the disorder occurring during spermatogenesis process and the secondary one is that developing subsequent to spermiation process [7]. Sperm abnormalities classified as defects in the sperm (head, middle piece or tail) [8]. Sperm head abnormalities test is one of the most rapid, the easiest and the lowest cost tests for the detection of mutagens and carcinogens [9]. Till now, there are no studies that deal with the side effects of this plant extracts on sperms although its

broad use in traditional medicine. For this reason, this study aimed at the investigation of the effect of *Euphorbia tirucalli* stems methanolic extract on sperm head morphology in male albino mice.

## Materials and methods

### Collection of plant material

The stems of *Euphorbia tirucalli* were collected from the University of Technology, Baghdad, Iraq garden in September, 2015.

### Preparation of extract

According to [1], fresh plant stems were washed with tap water and dried in shade. The dried stems were crushed into powder, 50 gm of powder was extracted with 300 ml of absolute methanol by soxhlet apparatus for 24 hrs., then the extract was dehydrated with rotary evaporator, then the doses were prepared from the produced powder.

### Animals

Male albino mice which were mature sexually (6-8) weeks age of the genus *Mus musculus*: Balb/c with weight of (20-30) gm were bought from both of the National Center for Drug Control and the Iraqi Center for Cancer Research and Medical Genetics, Baghdad, Iraq. The animals were kept in the animal house of Biotechnology division, University of Technology under constant temperature ( $25\pm 1$ )°C along the period of experiment, and received the standard rodent diet and water ad libitum.

### Experimental design

The animals were divided into 5 groups:

**Group 1:** This group consisted of 5 animals which were intraperitoneally injected with 4mg/kg of Mitomycin-c drug before 24hr. from the dissection (control+) [10].

**Group 2:** This group consisted of 15 animals which were injected with 0.07mg/kg of the methanolic extract of *Euphorbia tirucalli* stems inside the peritonium. This group was subdivided into 3 subgroups: **G2A:** 5 animals were dissected after 7 days, **G2B:** 5 animals were dissected after 21 days and **G2C:** 5 animals were dissected after 35 days of treatment.

**Group 3:** This group consisted of 15 animals which were intraperitoneally injected with 0.14mg/kg of *Euphorbia tirucalli* stems methanolic extract. This group was subdivided as the previous group into 3 subgroups (**G3A**, **G3B** and **G3C**) in which equal numbers of animals were dissected after (7, 21 and 35) days of treatment respectively.

**Group 4:** This group consisted of 15 animals which were intraperitoneally injected with 0.28mg/kg of *Euphorbia tirucalli* stems methanolic extract. This group was also subdivided as the previous groups into 3 subgroups (**G4A**, **G4B** and **G4C**) in which equal numbers of animals were dissected after (7, 21 and 35) days of treatment respectively.

**Group 5:** This group consisted of 5 animals which injected with 0.1ml of distilled water (control-).

### Sperm head abnormalities test

The method of [9] with few modifications was used, that the suspension of sperms of animals was obtained by the cutting of testis caudal epididymis in plate contained drops of mammalian saline. The suspension was spread and extended on glass slides, the slides were air dried and then stained with haematoxylin stain (10 min.), washed and then stained with eosin stain (15min.). 1000 sperms for each group and subgroup were examined directly under the optical microscope to detect the sperms head regions morphological abnormalities.

### Statistical analysis

The results were explicated as  $\mu\pm SE$  for all groups. The differences between the mean values of each group and the control-group were evaluated by t-test, and  $P<0.05$  was considered statistically significant.

### Gas chromatography-Mass spectroscopy analysis of the extract

The analysis was carried out using GC-MS-QP 2010 Plus Shimadzu system, by Elite-1 silica non polar moderate size column (5ms $\times$ 30m $\times$ 0.5 $\mu$ m) fused with 100% dimethyl polysiloxane, and the system ionic electronic energy was 70eV, Helium gas (99.999%) was used as carrier gas with constant current mean (1ml/min.) and injection volume of 2 $\mu$ L, and split ratio of 1:10, with injection temperature of 250°C, and the ionic source temperature was 200°C, and the thermal system was started as 60°C for 2min. and graduated to 300°C (10°C/min.) to be total of 14min., the range of mass spectro. Was (40-600) Da, by using National institute of standards software/ version NIST 8-2010.

## Results

### Sperms heads morphology

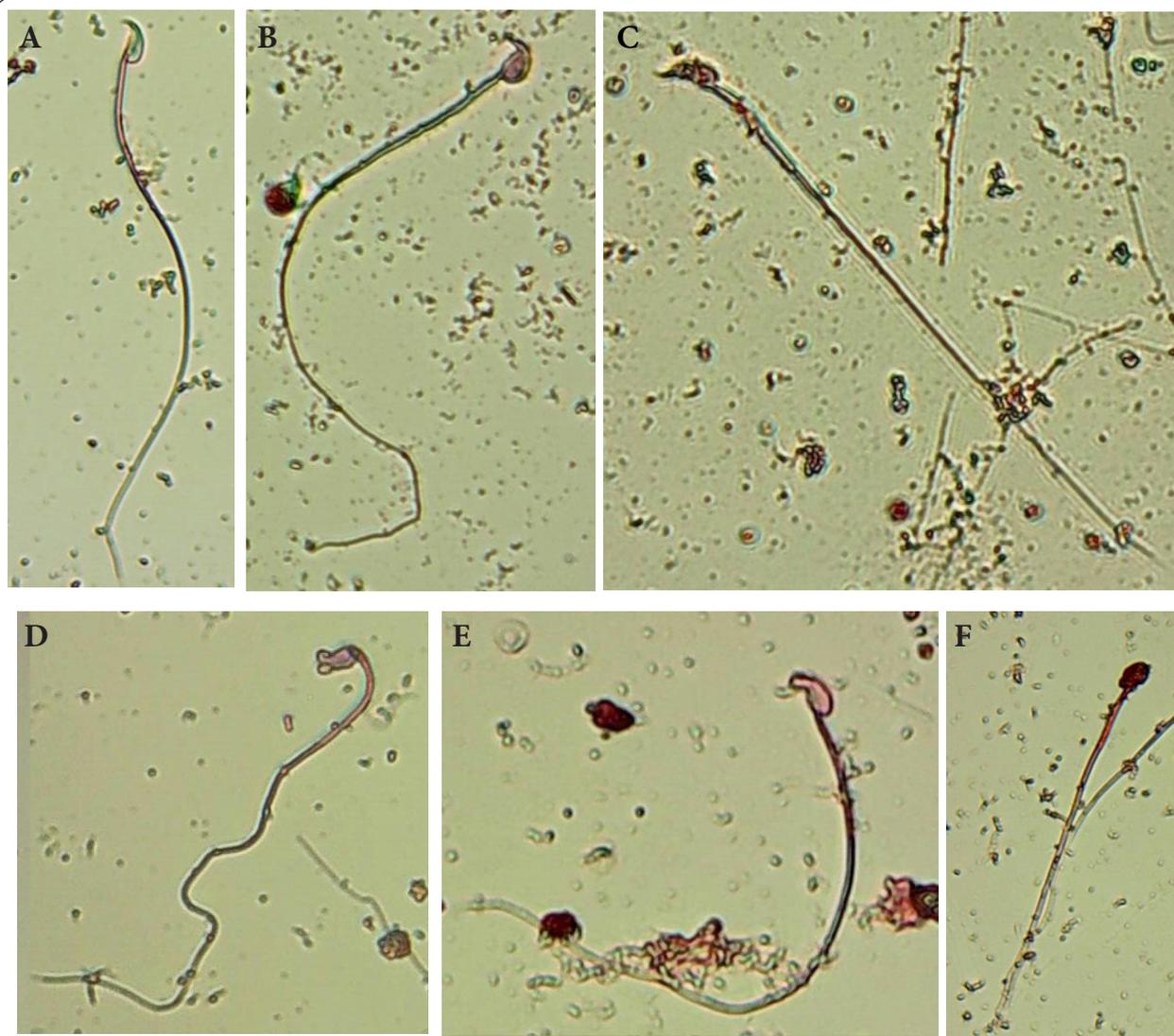
After the detection of sperms heads shapes under the optical microscope, the results revealed that the extract caused significant ( $P<0.01$ ) changes in the morphology of sperms heads, and there were sperms with (swelling head, twisty hook, no hook, abnormal terminal body and irregular head shape). The **Tables 1-3** show the means and standard errors of these changes after (7, 21 and 35) days of treatment in compare to the control- group, and the **Figure 1** show the morphological changes of sperm heads.

### GC-MS analysis

The results had shown that *Euphorbia tirucalli* methanolic extract contains hydrocarbones, Steroids and fatty acids, Alcohols and one Terpenoid compound, as in **Table 4**, and **Figure 2** shows the chromatogram as TIC.

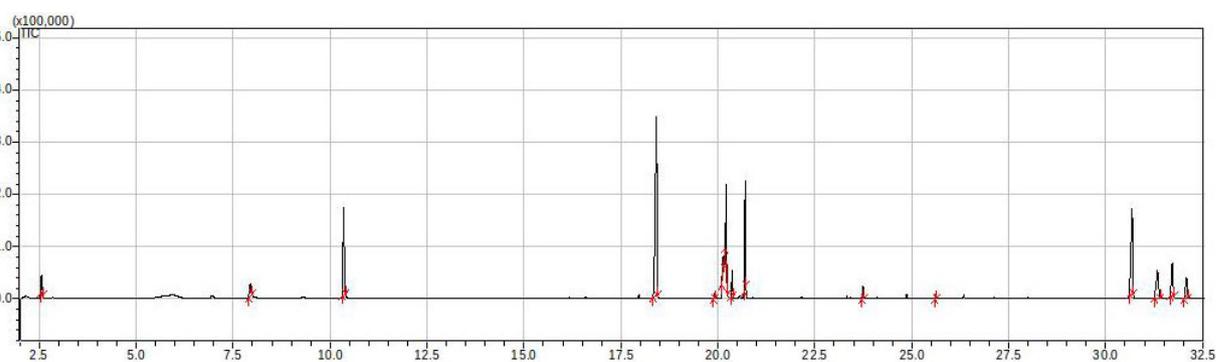
## Discussion

The results revealed that the MMC drug has a significant ( $P\leq 0.01$ ) toxic effect on the sperms of mice when compared with control-group, and this agrees with the result of [11], this effect of MMC may return to the chemical structure



**Figure 1. Sperm head abnormalities after intraperitoneal treatment with *Euphorbia tirucalli* stems methanolic extract.**

- A: Normal sperm; B: Sperm with swelling head;
- C: Sperm with twisty hook; D: Sperm with no hook;
- E: Sperm with abnormal terminal body
- F: Sperm with irregular head shape.



**Figure 2. GC-MS analysis for the *Euphorbia tirucalli* stems methanolic extract.**

**Table 1. The effect of *Euphorbia tirucalli* stems methanolic extract on the sperm head shape of male albino mice (M±SE) after 7 days of intraperitoneal treatment.**

Group	Swelling head	Twisty hook	No hook	Abnormal terminal body	irregular head shape	Abnormalities summation
Control-	2.7±0.51	0.8±0.32	1.6±0.33	0.1±0.1	0.9±0.34	6.1±0.32**
Control+	8.8±0.86	7.5±0.88	3.3±1.22	0.7±0.15	4.3±0.7	24.6±0.76
0.07mg/kg	3.6±0.6	1.8±0.03	2.2±0.37	1.8±0.2	1.6±0.21	11±0.28**
0.14mg/kg	4.6±0.33	3±1	2.22±0.12	2±0.33	2.3±0.33	14.12±0.42**
0.28mg/kg	7.2±0.69	3.5±0.68	2.4±0.60	2±0.44	6±0.97	21.1±0.67**

\*\*means P≤0.01

**Table 2. The effect of *Euphorbia tirucalli* stems methanolic extract on the sperm head shape of male albino mice (M±SE) after 21 days of intraperitoneal treatment.**

Group	Swelling head	Twisty hook	No hook	Abnormal terminal body	irregular head shape	Abnormalities summation
Control-	2.7±0.51	0.8±0.32	1.6±0.33	0.1±0.1	0.9±0.34	6.1±0.32**
Control+	8.8±0.86	7.5±0.88	3.3±1.22	0.7±0.15	4.3±0.7	24.6±0.76
0.07mg/kg	7.33±1.45	3.33±0.34	3.34±0.32	2.33±0.33	2.66±0.37	18.99±0.56**
0.14mg/kg	7.5±0.47	3.5±0.37	3.9±0.48	2.8±0.38	3.8±0.38	21.5±0.41**
0.28mg/kg	10.8±0.48	4.7±0.30	4.8±0.44	3.9±0.36	6.1±0.26	30.3±0.36**

\*\*means P≤0.01

**Table 3. The effect of *Euphorbia tirucalli* stems methanolic extract on the sperm head shape of male albino mice (M±SE) after 35 days of intraperitoneal treatment.**

Group	Swelling head	Twisty hook	No hook	Abnormal terminal body	irregular head shape	Abnormalities summation
Control-	2.7±0.51	0.8±0.32	1.6±0.33	0.1±0.1	0.9±0.34	6.1±0.32**
Control+	8.8±0.86	7.5±0.88	3.3±1.22	0.7±0.15	4.3±0.7	24.6±0.76
0.07mg/kg	15.33±0.66	4±0.57	3.33±1.2	3.66±1	4±0.57	30.32±0.8**
0.14mg/kg	16.33±1.45	5.66±0.7	4.33±0.88	3.78±0.15	4.33±0.45	34.43±0.72**
0.28mg/kg	19.8±1.04	7±0.59	5.9±0.54	4.5±0.84	8±0.49	46.04±0.7**

\*\*means P≤0.01

**Table 4. The chemical compounds in *Euphorbia tirucalli* stems methanolic extract detected by GC-MS analysis.**

Compound	Molecular formula	Peak area	Retention time (min.)	Molecular weight
Toluene	C <sub>7</sub> H <sub>8</sub>	77796	2.557	92
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	70784	7.940	144
Tetradecane, 1-iodo-	C <sub>14</sub> H <sub>29</sub> I	399045	10.354	324
n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	1092769	18.418	256
Butanoic acid, 2-propenyl ester	C <sub>7</sub> H <sub>12</sub> O <sub>2</sub>	15778	19.910	128
Bicyclo[3,2,1]octane	C <sub>8</sub> H <sub>14</sub>	87816	20.142	110
Linolenic acid	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	303951	20.216	278
Nonadecanoic acid	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	85601	20.366	298
2-Hexyl-1-octanol	C <sub>14</sub> H <sub>30</sub> O	321634	20.702	214
Phthalic acid, 4-cyanophenyl nonyl ester	C <sub>24</sub> H <sub>27</sub> NO <sub>4</sub>	37798	23.742	393
3,4-Hexanedione, 2,2,5- trimethyl-	C <sub>9</sub> H <sub>16</sub> O <sub>2</sub>	11486	25.613	156
Lanosterol	C <sub>30</sub> H <sub>50</sub> O	513639	30.679	426
A-Norcholestan-3-one, 5-ethenyl-, (5 beta)-	C <sub>28</sub> H <sub>46</sub> O	228429	31.338	398
Tetrahydrosmilagenin	C <sub>27</sub> H <sub>48</sub> O <sub>3</sub>	199721	31.715	420
Alpha-Bisabolo	C <sub>15</sub> H <sub>26</sub> O	116381	32.084	222
<b>Total peak area</b>		<b>3562628</b>		

of Quinone in MMC molecule which through a series of bio-reductive processes generates OH<sup>-</sup> radicals that of high reactivity which was supposed to have the potential to damage the DNA directly [12,13], as well as other biomolecules of the cell, since, free radicals are highly reactive which is called reactive oxygen species (ROS), they are susceptible to undergo reduction by the oxidation of cell molecules (DNA, proteins and lipids) [14,15]. Since, MMC is a bio reductive alkylating agent, it also cause DNA damaging by causing the cross-linking of bases in the same or adjacent DNA strands at the Guanine N2 position which result in monofunctionally and biofunctionally alkylated G-MMC monoadducts and G-MMC-G interstranded and intrastranded cross-links at CpG and GpG sites respectively [16], that may lead to cell death by apoptosis [17]. Also some reports mentioned that MMC is able to activate caspase-3, caspase-8 and caspase-9 mediated apoptosis and also necrosis [18].

The male mice treated intraperitoneally with the methanolic extract of *Euphorbia tirucalli* stems showed significant (P≤0.01) toxic effects on the sperms directly proportional to the dose and period of treatment, and the sperm head morphological changes that investigated were: the sperms with (swelling head, twisty hook, no hook, abnormal terminal body and irregular head shape), and this agrees with many studies that detected the toxic effects of some medicinal plants on the sperms of male rodents, such as [19] which referred to the toxic effect of methanolic extract of *Ximenia americana* leaves, bark and roots on male rats sperms, and [20] which referred to the toxic effect of chloroform extract of *Citrullus colocynthis* seeds on the sperms of mice on high doses. This may be because that the extract of *Euphorbia tirucalli* contains Alkaloids, Flavonoids, Terpens, Saponins [21], flavonoids, saponnins, anthraquinones, alkaloids and terpenoid which are pro-oxidant molecules may facilitate the causing of oxidative damage due to free radicals (FR) and reactive oxygen species (ROS) generation, and also the oxidative stress that accompanied by the generation of ROS and FR involoved in the causing of "perceptual block" to the stimulation of pheromones [22]. Besides the pro-oxidant effects, defects in the morphology of sperms were detected in mice administered with some extracts revealed that some extracts may have direct effects on the sexual glands and sperm cells, and this may expressed as the ROS and FR in the extract may have destroyed testicular germ cells with membrane damage or may be macromolecule degradation, which resulted in sperm abnormalities that is significantly recognized [23]. Also, it should be referred to that the Spermatogenesis process taking five weeks to complete during which the sperm cell passes through three shapes (spermatid, spermatocyte and spermatogonia) before becoming a mature sperm cell [9].

In the current study, the GC-MS results revealed that the extract contains 3 hydrocarbons (Toluene, Tetradecane, 1-iodo- and Bicyclo[3,2,1]octane), and there are number of studies which revealed the toxic effect of hydrocarbons, such as

the study of [24] which revealed the causes of increasing in the cases of spontaneous abortion or miscarriage which has been linked to paternal exposures to hydrocarbons and other chemical substances. Also, the study of [25] which revealed that polycyclic aromatic hydrocarbons (PAH) bind to the aryl hydrocarbon receptor (AhR), and the AhR ligands induce the proliferation, differentiation, and apoptosis of cell, and it is known that human sperms possess AhR and may be susceptible to those substances. Also, polycyclic aromatic hydrocarbon-DNA adducts are early genotoxicity markers to sperms in spermatozoa phase [26]. Another study [27] revealed that the hydrocarbons (kerosene and petrol) were toxic to the sperm parameters and testis of the rats, these may promote infertility in male by altering functions of the testis or lead to humans and animals health hazards in reproduction process whom may be exposed to this environmental pollutant, especially in oil spillage areas.

## Conclusion

From this study, it could be concluded that although the broad using of traditional medicine and herbalism, there may be many side effects in medicinal herbs which should be studied, and the chemical content of any medicinal herbal should be accurately analyzed.

## List of abbreviations

Ahr: Aryl hydrocarbon receptor  
DW: Distilled water  
Da: Dalton  
DNA: Deoxyribonucleic acid  
eV: Electron Volte  
FR: Free radical  
GC-MS: Gass Chromatography- Mass Spectroscopy  
MMC: Mitomycin- C  
PHA: Polycyclic aromatic hydrocarbons  
ROS: Reactive oxygen species

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

Authors' contributions	RJA	AAA
Research concept and design	--	✓
Collection and/or assembly of data	✓	--
Data analysis and interpretation	✓	✓
Writing the article	✓	--
Critical revision of the article	--	✓
Final approval of article	✓	✓
Statistical analysis	✓	✓

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