

In vitro effects of terpinoid compounds of *Euphorbia helioscopia* L. extracts on rats epididymal sperm activity rats

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Abstract:

Background:

Many plants have medical importance due to the presence an active compounds in plants extraction acting as a drugs for treatment of several diseases in human and animals. *Euphorbia helioscopia* extract was used to treat diabetes, removal the warts of the skin, stimulate the nervous system and also used against constipation.

Objective:

Study the effect of terpinoid extract of *E. helioscopia* on rat epididymal sperm activity and fertility efficacy of male rats.

Methods:

This study was conducted at Biol. Dept., College of Science/ University of Babylon and Medical College, Mahidol University- Thailand. The study was designed to purify the active compounds of the Terpinoid of *Euphorbia helioscopia* extract by Nuclear Magnetic Resonance (NMR), and studying their effects on sperm motility and grade activity of the epididymal sperms of the rats. Six main active compounds were isolated from the terpinoid extract of *E. helioscopia* due to the differences in color , weight and nature by using NMR. The main terpinoid compounds were named A,B,C,D,E and F.

Two concentration (0.5 mg/ml and 1 mg/ml) of each active compound of *E. helioscopia* extract were added to sperm suspension *in vitro* and incubated for 30 minutes , then sperm motility and grade activity were assessed and the results were compared to those values before adding the active compounds (control).

Results:

The results showed that 0.5 mg/ml of C and D active terpinoid compounds caused significant ($p < 0.05$) decrease in sperm motility compared to before adding the extract, while the same concentration of B,C,D,E and F active terpinoids compound caused a significant ($p < 0.05$) decrease in grade activity. The addition of 1 mg/ml of terpinoid active compounds (C,D and F) caused a significant ($p < 0.05$) decrease in sperm motility, while the grade activity significantly ($p < 0.05$) decreased by adding B,C,D,E and F active terpinoid compounds compared to control. The sub – compounds (C1,C2,D1,D2,D3 and D4) had a powerful decreasing effect on the sperm motility and grade activity compared to control and other active compounds.

Conclusion:

It was concluded that terpinoid compounds of *E. helioscopia* extract have adverse effects on rat epididymal sperm activity.

Key words; *Euphorbia helioscopia*, sperm motility, rats

Introduction

Medicinal plants were used in traditional medicine to treat various diseases in humans and the animals, although the large number of medicinal herbs remains in the world and require verification of their validity and to treat many conditions(1,2). *E. helioscopia* belongs to the family *Euphorbiaceae* (Family Alsosbep Spurge family), which includes about 283 genera and 7300 species. The members of this family distributed in most parts of the world, particularly in the tropics and subtropics and also extend to temperate regions in both hemispheres. *Euphorbia* genus is one of the biggest races of this family in terms of more than 2000 species spread across the world and approximately 44 species in Iraq (3).Five types spread widely in Iraq. The genus *Euphorbia* contains several types, some of them have not contain active compounds free of active substances used or nonmetal plants and others have broad medical uses. Species deployed in Iraq are *E. pilulifera* L. ; *E. pulcherrima* W; *E. heterophylla* L. ;*E. marginata*; and *E. helioscopia* L. (4).

All parts of the plant are toxic, however, the impact of these parts on the animal varies between one part and another, the material secreted by the stem of the plant has toxic effect more than the other parts of the plant. Despite that the plant is considered poisonous it has medical uses, including the use of leaf of *E. helioscopia* in various parts of the world such as Asia, Africa and South America as a popular treatment against diabetes, also used the juice of milk was widely used in removal of warts of the skin, and the plant was used as a stimulator for the nervous system and against constipation (5).

This plant was known as greatly traditional medicine in poor populations in Asia, it was medicinal plants. The dry leaves were the main part of this plant used as a medicine in many disorders of physical chronic conditions such as arthritis and influenza bronchial and skin diseases (6). The studies on the role of this plant *E. helioscopia* on male fertility is almost non-existent , so that this study was performed to determine the effect of terpinoid extract of *E. helioscopia* on sperm activity and fertility efficacy of male rats.

Materials and Methods:

Extraction and Isolation:

Dried and powdered aerial parts of the plant material *Euphorbia helioscopia* L.(2 kg) were placed in Soxhlet apparatus with chloroform solvent and concentrated by rotary evaporator overnight pump. The crude extract (115.06 g) was mixture by using different solvent according polarity (MeOH : CH₂CL₂ : hexane). The column chromatography was used with diameter 15 cm and length 16 cm the number of silica gel was 7734 used(2000 g) and used thin layer of chromatography .

The six fractions of active compound (A-F). Dissolve 0.1 from each fraction in chloroform and take 4 ml in special tube and testing by NMR. These fractions were further submitted to silica gel column chromatography to obtain (D1. D2 . D3

.D5 .C1.C2) all these compounds were different in weight and nature . (7) .

Semen Analysis:

Epididymal semen samples were obtained from Albino rats. Sperm motility was performed as followings:

A drop of well mixed undiluted semen was placed on the surface of a warm dry clean slide. A cover slip was placed on the drop of the semen. The slide is allowed to rest on the microscope until the streaming of the sperm has stopped. Then the drop was viewed at 400X (8). A minimum of around 200 sperm was counted; both motile and immotile sperms were counted in at least 5 separate fields (9).

The motility of spermatozoa in each sample was graded 0, 1, 2, 3, or 4 according to (10,11 and12).

0 = no movement

1 = movement but not forward progression

2 = movement with slow forward progression

3 = movement in an almost straight line with good speed

4 = movement in a straight line with high speed.

The percentage of sperm motility was calculated by:

Motility % = number of motile spermatozoa / total number of spermatozoa (motile and immotile) × 100 .

The above mentioned estimation was considered as control (Before treatment).

Test the effectiveness of separated compounds:

Two concentrations of terpinoid extract were prepared (0.5 mg/ml and 1 mg/ml) and used to study their effects on sperm activity. Test tubes containing phosphate buffered saline (PBS). The tubes containing a mixture of sperms and the compound were incubated in an incubator at a 37C ° for half an hour after which the sperm motility and grade activity were assessed (9).

Six main active compounds were isolated from *E. helioscopia* extract due to the differences in color, weight and nature by using NMR. The main terpinoid compounds were named A,B,C,D,E and F. The weight of six active compound were: A=1.487gm; B=6.77gm; C=17.25;D=29.4gm;E=42.31gm and F=12.99gm. 0.1 gm of each sample was dissolved in chloroform.

Two concentrations (0.5 mg/ml and 1mg/ml) of each active compound of terpinoid extract were added to sperm suspension *in vitro* and incubated for 30 minutes , then sperm motility percent and grade activity were estimated and results were compared to those values before adding the active compounds (control).

Fifteen gm of C and D active compounds were dissolved in different solvent like acetone, hexane, methanol, and dichloromethane, and kept in 4 C° for two days after noticing the presence of crystals. Then these samples were dissolved in methanol and filtered with filter paper. Four sub – compound isolated from D compound (D1,D2,D3 andD4), and two sub –compounds from C (C1 and C2).

Two concentrations (0.5 mg/ml and 1 mg/ml) of each sub - active compound of C&D terpinoid extraction were added to sperm suspension *in vitro* and incubated for 30 minutes , then sperm motility and grade activity were estimated and

the results compared to those values before adding the active compounds (control).

Statistical analysis

Analysis of data was performed by using SPSS (Version 10) in the home computer. Results are expressed as mean $\pm$ S.E. Statistical differences were determined by Least Significance Differences (LSD) test for multiple comparisons between different groups after performing ANOVA (13).

Results:

The results showed that 0.5 mg/ml concentration of C and D active terpinoid compounds caused a significant ($p < 0.05$) decrease in sperm motility percent compared to control (Figure-1-), while the same concentrations of B,C,D,E and F active terpinoid compounds caused a significant ($p < 0.05$) decrease of grade activity (Figure-2-).

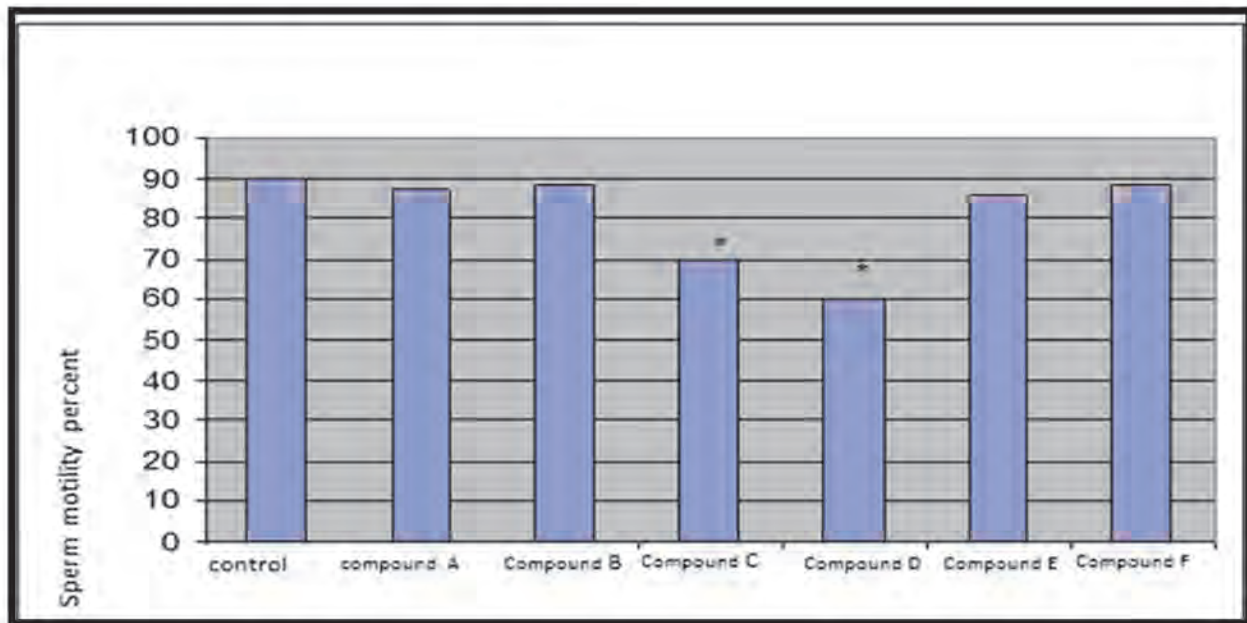


Figure-1- *in vitro* effect of active terpinoid compounds (0.5mg/ml) of *E. helioscopia* on sperm motility rat epididymal sperms after 30 minutes of incubation

*($p < 0.05$) significant decrease

N=4 Rat semen specimens. L.S.D=25.56

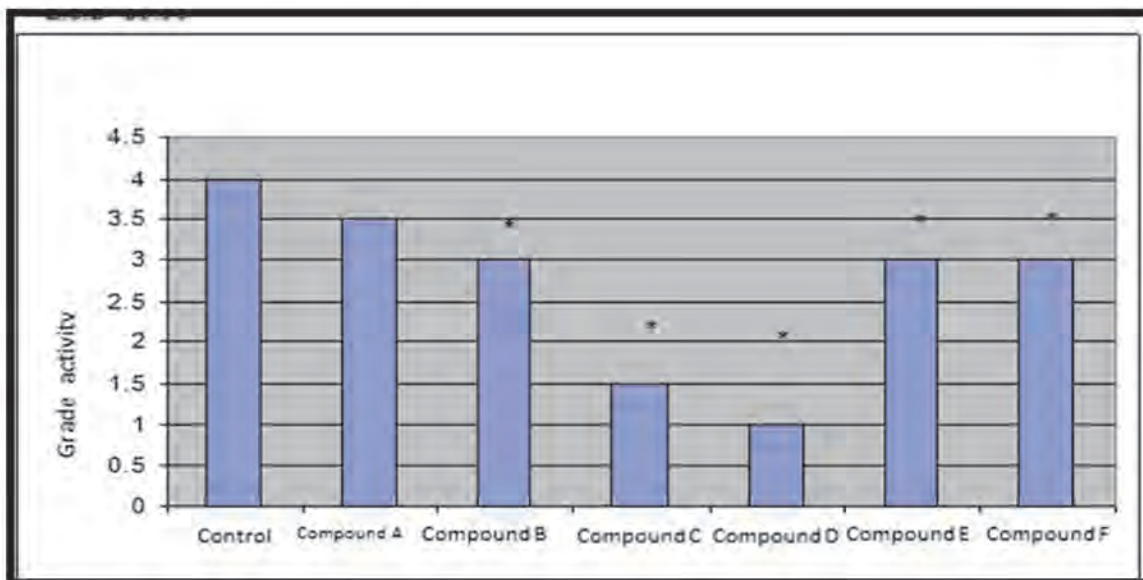


Figure-2- *in vitro* effect of active terpinoid compounds (0.5mg/ml) of *E. helioscopia* on grade activity Rat epididymal sperm after 30 minutes of incubation

*($p < 0.05$) significant decrease

N=4 Rat semen specimens

L.S.D. = 0.95

The addition of 1 mg/ml of terpinoid active compounds (C,D and F) caused a significant ($p<0.05$) decrease in sperm motility percent (Figure-3-), while the grade activity was significantly ($p<0.05$) decreased by adding B,C,D,E and F active terpinoid compounds compared to control (Figure-4-)

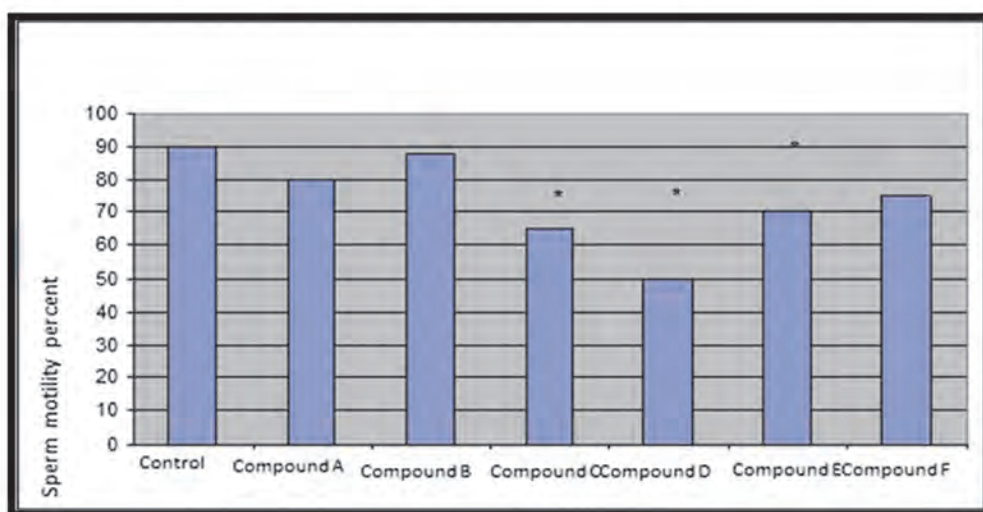


Figure-3- *In vitro* effect of active terpinoid compounds (1mg/ml) of *E. helioscopia* on sperm motility of rat epididymal sperm after 30 minutes of incubation

*($p<0.05$) significant decrease

N=4 Rat semen specimens, L.S.D. = 19.98

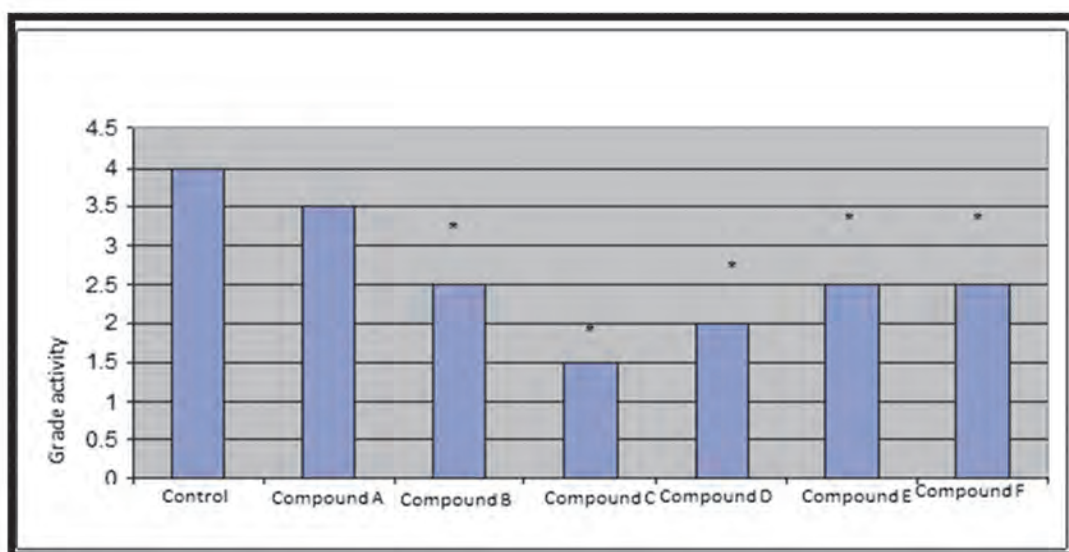


Figure-4- *In vitro* effect of active terpinoid compounds (1mg/ml) of *E. helioscopia* on grade activity of rat epididymal sperm after 30 minutes of incubation

*($p<0.05$) significant decrease

N=4 Rat semen specimens

L.S.D. = 1.15

The incubation of rat epididymal sperm suspension with can D sub-compound each kind of above mentioned sub-compound for both concentrations (0.5mg/ml and 1mg/ml) caused significant ($p<0.05$) decrease in sperm motility percent and grade activity compared to control (Figures 5 to 8).

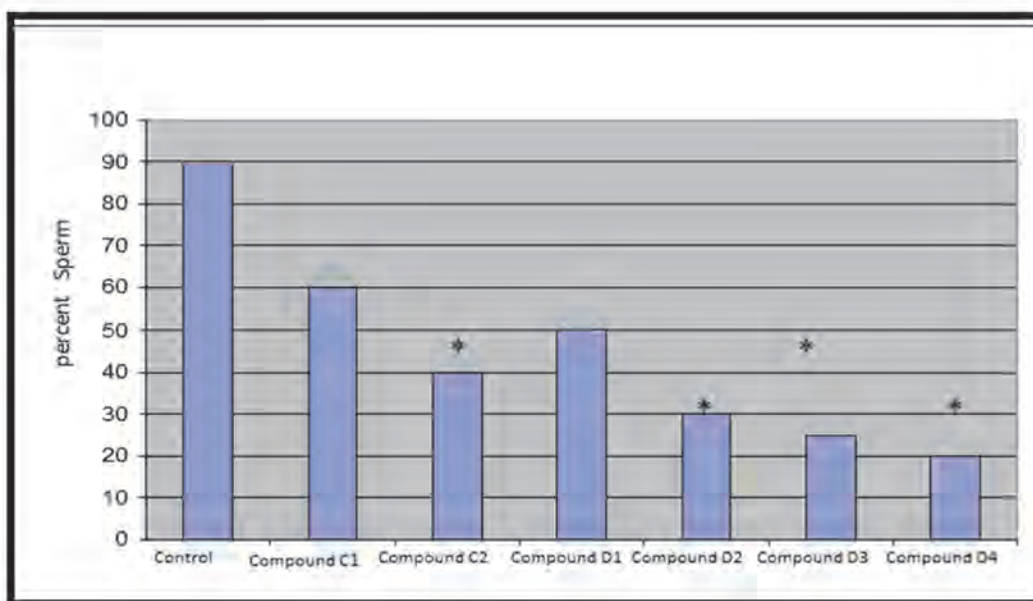


Figure-5- *In vitro* effect of active terpinoid (C&D)compounds (0.5mg/ml) of *E. helioscopia* on sperm motility of rat epididymal sperm after 30 minutes of incubation

*($p<0.05$) significant decrease

N=4 Rat semen specimens

L.S.D. = 22.45

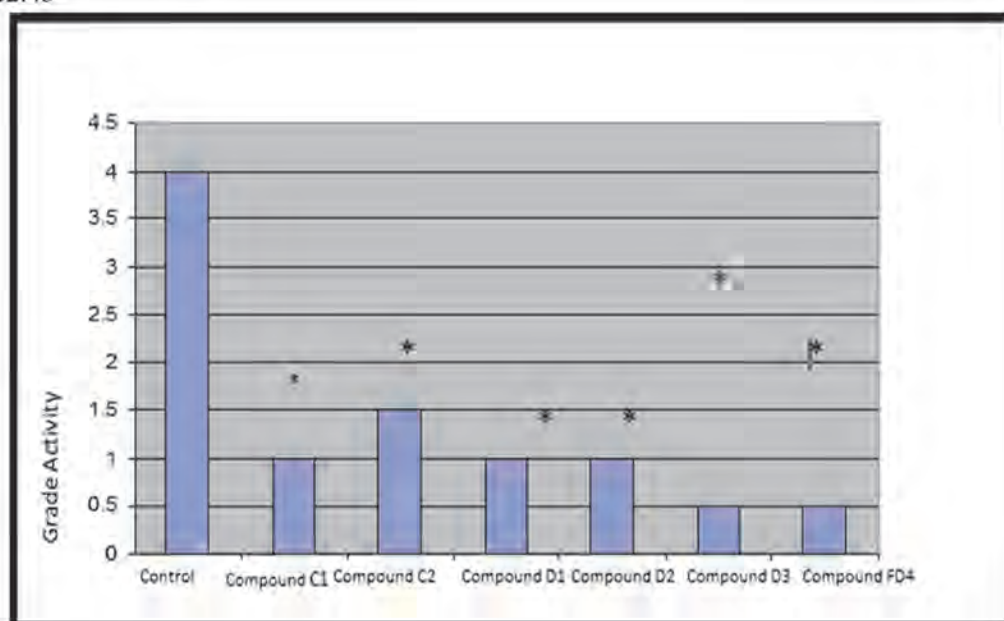


Figure-6- *In vitro* effect of active terpinoid (C&D)compounds (0.5mg/ml) of *E. helioscopia* on grade activity of rat epididymal sperm after 30 minutes of incubation

*($p<0.05$) significant decrease

N=4 Rat semen specimens

L.S.D. 2.55

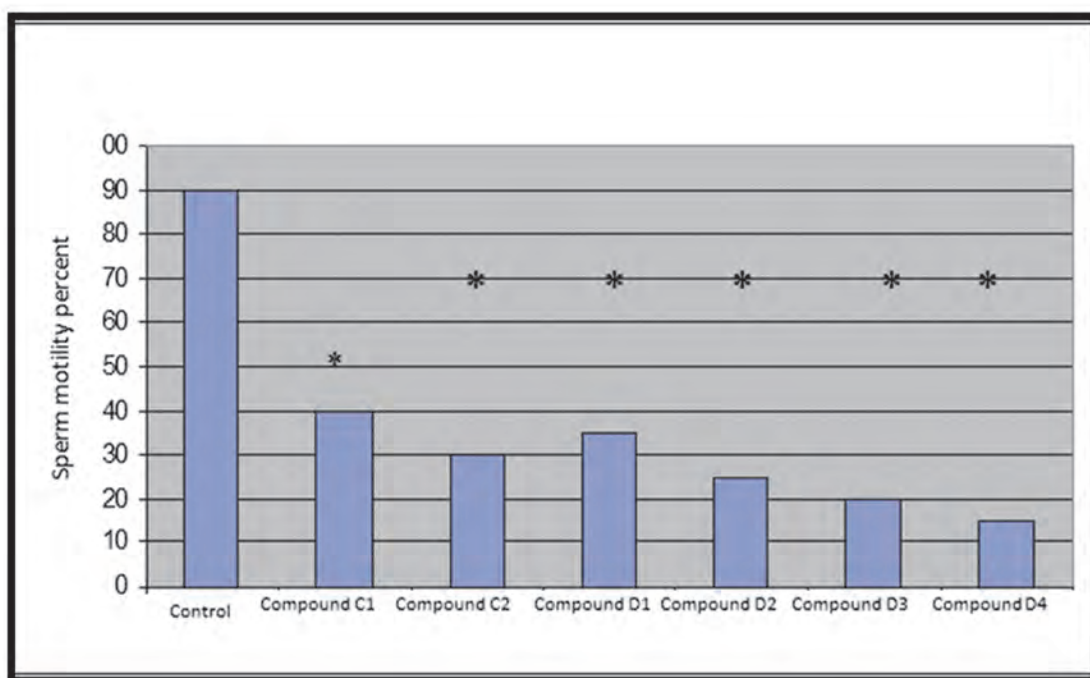


Figure-7- *In vitro* effect of active terpinoid(C&D) compounds (1mg/ml) of *E. helioscopia* on sperm motility of rat epididymal sperm after 30 minutes of incubation

*($p < 0.05$) significant decrease

N=4 Rat semen specimens

L.S.D. = 29.56

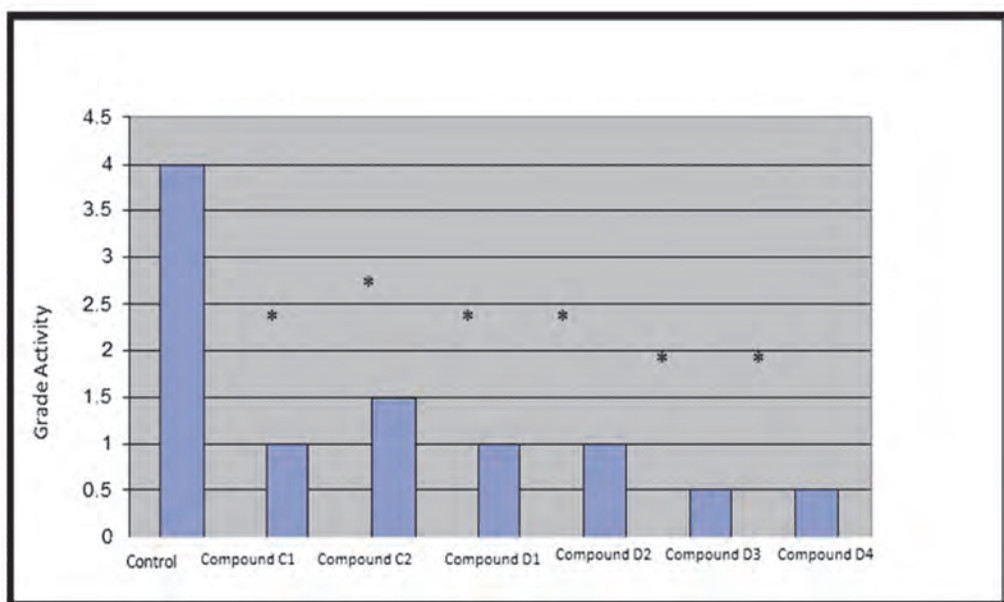


Figure-8- *In vitro* effect of active terpinoid (C&D) compounds (1mg/ml) of *E. helioscopia* on grade activity of rat epididymal sperm after 30 minutes of incubation

*($p < 0.05$) significant decrease

N=4 Rat semen specimens

L.S.D. = 2.34

Discussion:

The sperm motility is the most important sperm parameter because The capacity of human sperm fertilization principally depends on sperm motility and membrane integrity. In this study sperm motility and grade activity has been affected significantly when incubated for 30 minutes with 0.5mg/ml and 1mg/ml of active terpinoid compounds of *E. helioscopia*. This results agree with other study (14), which revealed that concentration of 0.08 g/mL of the extract affected the motility, and at a concentration of 0.16 g/mL, the sperm motility was reduced to 20% immediately (within 20s) when the sperm incubated with extract of *Achyranthes aspera* and *Stephania hernandifolia* plants.

The significant decrease of sperm motility and grade activity as a result incubation with terpinoid compounds of *E. helioscopia* extract may be as a result to increase of oxygen reactive species (ROS). Reactive oxygen species, such as superoxide anion and hydrogen peroxide, are known to impair sperm motility and membrane integrity by inducing membrane lipid peroxidation (LPO),(15). The sperm membrane allows transport of ions and molecules selectively, and this is essential for normal sperm motility.

Most of the herbal spermicidal agents induce spermicidal effects by disrupting the plasma membrane as they act on sperm surface(16). Plant derivatives also cause drastic inhibition in sperm membrane-specific enzymes like acrosin and hyaluronidase, the most important enzymes in the process of fertilization(17).

The treatment of human spermatozoa *in vitro* with 100 mg/ml of *Ruta graveolens* extraction caused significant decline in sperm motility and viability(18), this result agree with our study.

One study(19) demonstrated that the oral administration of phenolics, alkoaloids and terpinoids compounds of *E. helioscopia* extract caused a significant decrease in sperm parameters, sex organs weight, diameter of seminiferous tubules, spermatogenesis cells, Leydig's cells and a decline in several hormones like LH,FSH and Testosterone. Also the fertilization test revealed that the non-treated female mated with male orally treated cannot become pregnant until 6th week after last dose of *E. helioscopia* extract.

According to our results and other studies on *E. helioscopia*, it was concluded that *E. helioscopia* extraction caused a decrease in rat epididymal sperm activity, and it was recommended to study the effect of terpinoid compounds on human sperm activity to use them as a contraceptive.

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