WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 8, 31-46. Research Article ISSN 2277– 7105

PHYTOCHEMICAL INVESTIGATION AND EVOLUATION OF THE BIOLOGICAL EFFECT OF CYPERUS SECONDARY METABOLITES ON FERTILITY IN MALE MICE

Wid Ibrahim Kadm* and Prof. Dr. khulood. W. Abood

Aldora City, Baghdad State, Baghdad Country, Iraq.

Article Received on
23 May 2017,

Revised on 14 June 2017,
Accepted on 05 July 2017

DOI: 10.20959/wjpr20178-8867

***Corresponding Author**
Wid Ibrahim Kadm
Aldora City, Baghdad State,
Baghdad Country, Iraq.

ABSTRACT

The present study was designed to detect the effect of *Cyperus esculentus* extract on male albino mice fertility. *C. esculentus* extract was prepared by maceration of 50 gm of *C. esculentus* tubers with 90% methanol, then chemical detection of flavonoids, alkaloids, tannins, saponins, terpenes and steroids was carried out. The use of high performance liquid chromatography technique helped in detection of querciten, rutin, kaempferol, Myricetin and Luteolin. The effect of *C. esculentus* methanolic extract on the sperm including sperms concentration, percentage of viable sperms, percentage of morphologically abnormal sperms and an assay of serum testosterone were studied. Twenty five mice were divided equally into five groups, including negative and positive controls while other groups were treated with different concentrations of *C. esculentus* extract. The extracts were administered orally for 3 weeks. The results showed a significant increase ($p \leq 0.01$) in sperms concentration after 3 weeks of treatment with the *C. esculentus* extract at doses 200, 400 and 600 mg/kg when compared with controls. A significant decrease ($p \leq 0.01$) in dead sperm and percentage of morphologically abnormal sperms was observed after treatment with *C. esculentus* extract at doses (200, 400 and 600) mg/kg when compared with controls. A significant increase ($p \leq 0.01$) in Serum testosterone was observed in mice treated with 200 and 400 mg/kg when compared with controls and with another group treated with *C. esculentus* extract at dose of 600 mg/kg.

KEYWORD: Male fertility, tiger nut, *Cyperus esculentus* antioxidants.

INTRODUCTION

Herbal medicine is a growing area of health care that demands attention. Herbal medicines have been widely utilized as effective remedies for the prevention and treatment of multiple health conditions for centuries by almost every known culture. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species.[1] According to the WHO more than 80 % of the world's population relies on traditional herbal medicine for their primary health care.[2] Chemical components of the medicinal plant are the most important for pharmaceutical companies. People are interested in medicines prepared from plants due to their little side effects, cheap and almost available compared with synthetic drugs. This may be because of the low concentrations of the active compounds found in plants which the human body would need.[3] Medicinal plants and herbal medicine are one of the current areas of investigation in which various drugs have been identified which affect fertility, both in a positive and a negative sense but some of which have side effects that are undesirable,[4,5] *Cyperus esculentus* is a perennial plant species that belongs to the Cyperaceae family and grows abundantly in the Mediterranean region.[6,7] Tubers of this plant are considered one of the earliest food sources known to humanity, where they have been documented to be cultivated by ancient Egyptians since 5000 BC.[6,8] These tubers are commonly known by several names such as chufa, earth almond, and tiger nut. *C. esculentus* is a potentially valuable food source for humans and animals due to its rich nutritional contents of fat, carbohydrates, and minerals.[9,10]

Analysed tigernut tuber for the presence of phytochemicals, it was observed that alkaloids, cyanogenic glycosides, resins, tannins, sterols and saponins were present in the raw tuber, however only alkaloids, sterols and resins were present in the roasted sample. In addition to being a food source, *C. esculentus* tubers have several other purposes. According to Ayurvedic medicine, *C. esculentus* tubers can be used for their aphrodisiac properties.[11] In the Middle East, they are known to the public as "Hab Al-zulom" (Arabic), which translates to "the seeds of men", owing to their apparent ability to improve male sexual activity; thus, they are frequently given to grooms during their honeymoons as a sexual invigorator. However, there has been no scientific evidence to date on the influence of *C. esculentus* tubers on male sexual behavior. A previous study.[12] reported protective effects of *C. esculentus* on testicular weight and spermatogenesis process in mice treated with lead acetate.

They speculated that these effects could be due to either the antioxidant ability of *C. esculentus* or its positive influence on sex hormones. In addition, it has been claimed that treatment with *C. esculentus* methanolic extract improves sperm count and motility in male rats, which is associated with increased gonadotropins and testosterone serum levels.[13] *C. esculentus* has many biological potentials and pharmaceutical applications including the treatment of measles and fever, colon cancer, coronary heart disease, obesity, diabetes, flatulence, indigestion, diarrhea, dysentery and arteriosclerosis, alkaloids, saponins and tannins found in this plant are known to have antimicrobial activity, as well as other physiological activities.[14,15] Alkaloids are known for their toxicity, they inhibit certain mammalian enzymic activities such as those of phophodiesterase, prolonging the action of CAMP, they also affect glucagons and thyroid stimulating hormones, while some forms have been reported to be carcinogenic.[16] Some have been used either as an analgesic, antispasmodic, bactericidal agents.[17] Saponins have been reported to be useful in reducing inflammation of upper respiratory passage and also chiefly as foaming and emulsifying agents and detergents.[17] Tannins have astringent properties that hasten the healing of wounds and prevention of decay. Tannin compounds have antimicrobial activities and are responsible for preventing and treating urinary tract infections and other bacterial infections. The result of the determination of phytochemical test indicated that the tuber possess some biologically active compounds which could serve as potential source of vegetable drugs in herbal medicine. These phytochemicals exhibit diverse pharmacological and biochemical actions when ingested by animals.[18]

METHODS

Plants Collection

C.esculentus was obtained from a local markets (Herbs shope) in Baghdad and identified by prof. Dr. Khulood Al_sammarraie.

Plants Extraction

The dried tubers of *C. esculentus* was powdered using a blender for 10 minutes, and then extracted with methanol (90%), 50 grams of the processed plant were extracted in 250 ml of the solvent and left in shaking water bath (40ºC) for 24hrs. Extract was then filtered with gauze and then with filter paper. The obtained extract was then evaporated at (55ºC) using a rotary evaporator, and the resultant crude extract was dried using lyophilizer. Dried extract was collected, weighed and stored in frozen at (-20ºC) until used to prepare the required

doses and concentrations.[19] The weight of residue of *C. esculentus* tuber extracts was 5.5g which represents 11% of the original tubers sample weight and the appearance of the residue was yellow in color.

Measurement of the Extract Acidity

An aliquot of 10g of *C.esculentus* powder was mixed with 50 ml of absolute methanol for 10 minutes using a magnetic stirrer. The suspension was filtered and the acidity of the filtrate was measured using a pH meter.[20]

Chemical Detection of Plants Extracts

Detection of Terpenes and Steroid

An aliquot of 1 ml of methanol extract was mixed with few drops of chloroform, then a drop of acetic anhydride and drop of concentrated sulphuric acid were added, brown precipitate appeared which representing the presence of terpenes, the appearance of dark blue color after few minutes would represent the presence of steroids.[21]

Detection of Flavonoids

The detecting solution was prepared by mixing 10 ml of ethanol (50%) with 10 ml of potassium hydroxide (50%), and then 5 ml of this solution was added to 5 ml of the plant extract. The appearance of yellow color was an indicator of the presence of flavonoids.[22]

Detection of Resins

An aliquot of 10 ml of distilled water acidified with 4% hydrochloric acid were added to 5 ml of the plant extract, the appearance of turbidity indicated the presence of resins.[20]

Detection of Tannins

An aliquot of (25ml) of methanolic extract was mixed with ferric chloride solution (FeCl2) (1%; w/v), the appearance of greenish-blue color was an evidence for the presence of tannins.[23]

Detection of Alkaloids

An aliquot of 10 ml of the plant extract was acidified by adding HCL, test it by Mayer's reagent, appearance of white precipitate indicates the presence of alkaloids.[24]

Detection of Saponins

This method was done according to the method described by.[25] Saponins were detected by two methods:

A. A solution of plant powder was shaken vigorously in a test tube. The formation of foam standing for a time indicates a positive result.

B. An aliquot of 5 ml of the plant extract was added to 1-3 ml of 3% ferric chloride solution, a white precipitate was developed indicating the presence of saponins.

Detection of Flavonoids Compound by HPLC

HPLC application for qualitative detection of flavonoids standards rutin, quercetin, Myricetin, Luteolin and for kaempferol of the *C.esculantus* was done in Iraqi Ministry of Science and Technology. The condition for detection of qurciten, rutin, kaempferol, Myricetin and Luteolin as follow:

Mobile phase: were 0.1% phosphoric acid: acetonitrile (20:80, V/V), Column: C_18 (25cm), Flow rate: 1.5ml/min, Injected volume: 20µl, Temperature: 25ºC, Wave length: 285nm, Instrument: refractive index detector RF Shimadzu.

Laboratory Animals

Albino male mice were the laboratory animals that employed in carrying out the experiments of the study. They were supplied by the Drug Control Center (Iraqi Ministry of Health), and their age at the start of the experiment was 8-10 weeks, their weight was 21-44 grams. They were divided into groups; each group was kept in a separate plastic cage. The cages were put in a room with optimal temperature (25 ºC). The animals were given water and fed throughout the experimental work.

Experimental Design

The experiment was designed to assess fertility, three doses (200, 400 and 600 mg/kg) of *C.esculentus* extract (methanolic extract), as well as, proviron (positive controls) and water (negative controls) were used Therefore, the animals were divided into five groups (each group contains 5 mice). Just before treatment, the extracts were dissolved in distilled water to facilitate oral administration to the mice. Then the mice were sacrificed after 3 weeks and showed the results.

Group1 (negative control): mice were treated with water.

Group2 (positive control): mice were treated with 0.36 mg/kg Mesterolone (Proviron).

Group3: mice were treated with 7 mg/ ml of *C.esculents* extract (200mg/kg).

Group4: mice were treated with 14.8 mg/ ml of *C.esculents* extract (400mg/kg).

Group5: mice were treated with 23.4 mg/ ml of *C.esculents* extract (600mg/kg).

Collection of Blood Samples and Determination of Testosterone levels

At the end of the experiment, blood was drawn from the heart directly by stab the heart (using syringe) to get the largest amount of blood and collected into micro centrifuge tubes. Blood samples were centrifuged at 3000 rpm for15min to get serum, then the serum had frozen (-20°C) in refrigerator until the testosterone assay. The testosterone concentration was determined using the Testosterone Enzyme Immunoassay kit.

Semen Preparation

Soon after killing mice and dissection, the epididymes and testes were removed for study the sperm concentration, morphological and viability.

Sperm viability and morphology

The epididymes minced with small scissors in Petri dish containing phosphate buffer saline (PBS) 1ml. A drop of semen suspension was mixed with a drop of eosin stain (1%) a thin smear of semen -eosin was put on the slide and then mixed by others slide which used to make a thin smear in a third slide and the third slide left to dry at room temperature, the slides were examined under light microscope at (40x). The dead sperms stain pink color while the live one is bright without color. Also the morphology of abnormal sperm was determined according that . The sperm viability was estimated according to the following equation.[26]

Percentage of dead sperm % = (NO. of dead sperm /total NO. of sperm) X 100

The percentage of sperm abnormality was estimated according to the following equation.[27]

Abnormality % = (No. of abnormal sperms/ total NO. Of sperm) X 100

Sperm Concentration

Sperm concentration was calculated according to the following steps:

The sperm suspension prepared was pulled by RBC pipette till "0.5" mark, and then diluted with the diluting solution till "101" mark, so the dilution rate is 1:200, the content of the

pipette was mixed; the coverslip was placed over the counting chamber of hemocytometer, a small amount of the diluting solution containing sperms was placed at the edge of the coverslip and drawn by the capillary action under the coverslip, the slide was placed under the microscope and the number of sperms was counted in five large squares.

$$No.= n\times D\times 400\times 1000 /N\times 1/10$$

The number of sperm / ml was calculated using the following formula.[28]

n: number of sperms in the five squares, D: inversion of the dilution rate (200), 400: inversion of the small square size, N: number of small squares in the hemocytometer (80),1/10: depth of the hemocytometer.

Statistical Analysis

The Statistical Analysis System- SAS (2012).[29] program was used for the effect of difference factors in study parameters. Least significant difference –LSD test (ANOVA) was used to significant compare between means in this study.

RESULTS AND DISCUSSION

Methanolic Extract of C.esculentus

Fifty grams of *C.esculentus* powdered tubers was used for the preparation of the extract, the pH value of the methanolic extract was 7. The weight of the residue obtained after lyophilized was 5.5g, which represents 11% of the original roots sample weight. The appearance of the extract was light yellow in color.

Chemical Detection of *C. esculentus* Active Compounds

The active constituents of methanolic extract of *C. esculentus* were determined using different specific reagents. The results indicated the presence of different active compounds which are (flavonoids, alkaloids, steroids, tannins, saponins, terpenes and resin) (Table 1). Previous pharmacological and chemical studies indicated the presence of several active compounds, including (alkaloids, flavonoids, steroids, tannins, resins and saponins).[10,30] Another study indicated that *C. esculentus* contained terpenes.[31]

Table 1: Chemical detection of some active compounds in *C.esculentus* methanolic extract. (Note) + indicates the presence of the active compounds.

Secondary Metabolites	Reagent	Indication	Result of detection
Alkaloids	Mayer's reagent	White ppt.	+
flavonoids	Ethanol with KOH	Yellow color	+
Tannins	Ferric chloride	Greenish-blue	+
Resins	Hydrochloric acid	Turbidity	+
Terpenes and Steroids	Chloroform, acetic anhydride and sulphuric acid	Brown ppt. Blue color	+ +
Saponins	Extract shaking Ferric chloride solution	Foam White ppt	+ +

HPLC Analysis of *C. esculentus.*

Detection of flavonoids in *C.esculentus* methanolic extract obtained from dried tubers by HPLC analysis indicated the presence of:

A. Myricetin, with retention time (1.892) minutes, figure (1) in comparison with myricetin standard (1.793) figure (2).
B. Quercetin, with retention time (3.233) minutes, figure (1) in comparison with quercetin standard (3.122) figure (2).
C. Kaempferol, with retention time (4.163) minutes, figure (1) in comparison with kaempferol standard (4.022) figure (2).
D. Rutin, with retention time (5.05) minutes, figure (3-3) in comparison with rutin standard (4.977) figure (3-2).
E. Luteolin, with retention time (5.988) minutes, figure (1) in comparison with luteolin standard (5.875) figure (2).

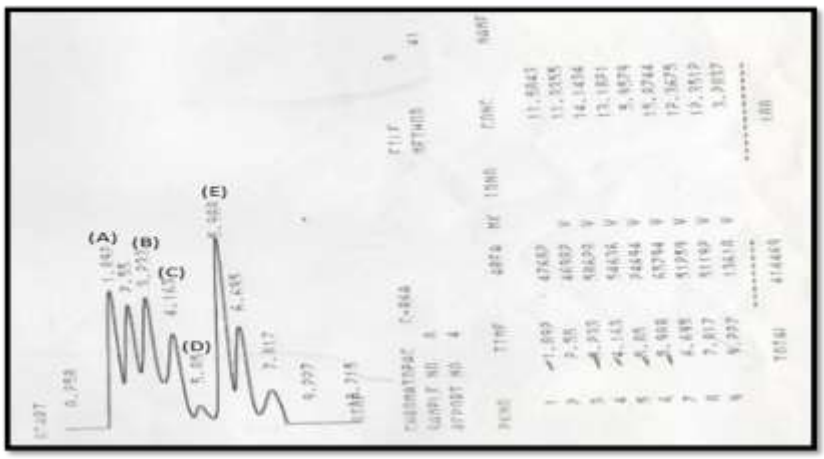


Figure 1: HPLC analysis of the *C. esculentus* dried tubers methanolic extract. A: myricetin, B: quercetin, C: kaempferol, D: rutin, E: luteolin.

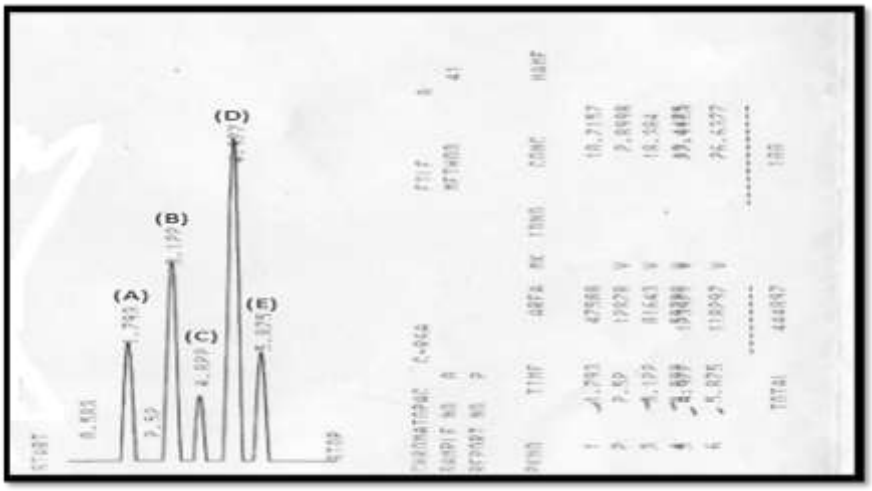


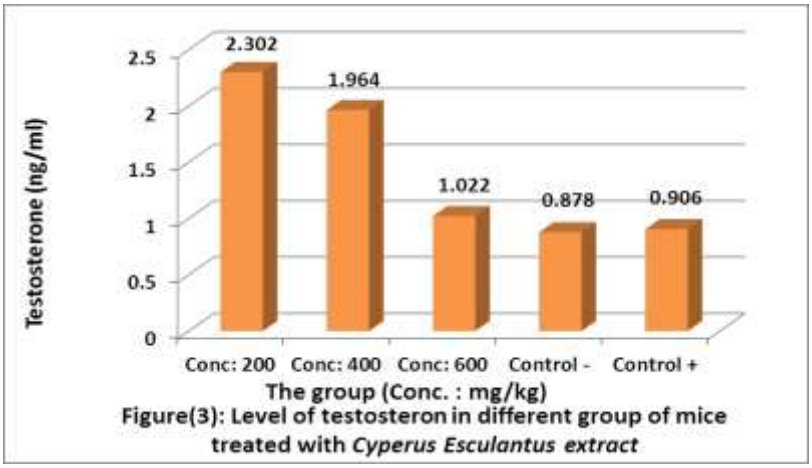
Figure 2: HPLC analysis for flavonoid standard. A: myricetin, B: quercetin, C: kaempferol, D: rutin, E: luteolin.

Testosterone serum concentration after 3 weeks of treatment

Results in table (2) indicated a significant increase ($p<0.01$) in serum testosterone concentration after 3 weeks in mice treated with *C. esculantus* extract compared with negative and positive controls-treated mice. serum testosterone in *C. esculantus* treated mice with concentrations 200, 400 and 600 mg/kg were elevated to (2.302± 0.008, 1.964± 0.046 and 1.022± 0.011 ng/ml) while in the negative control, water treated mice was (0.878±0.006 ng/ml) and in positive control group, mesterolone drug treated mice was (0.906±0.005 ng/ml). The levels of testosterone in all treated groups were measured (figure 3). Testosterone concentration were elevated in doses (200 and 400) mg/kg compared with the dose (600) mg/kg. So, there is a significant increase ($p<0.01$) in serum testosterone concentration as compared with the dose 600 mg/kg.

Table 2: Effect of *C. esculentus* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *C. esculentus* on serum testosterone concentration in mice (mean ± SD).

Mice groups	Mean ± SD of Testosterone concentration (ng/ml)
200 (mg/kg)	2.302 ± 0.008 a
400 (mg/kg)	1.964 ± 0.046 b
600(mg/kg)	1.022 ± 0.011 c
Negative Control (water)	0.878 ± 0.006 d
Positive Control (mesterolone)	0.906 ± 0.005 d
LSD value	0.0646 **
P-value	0.0001
** ($P<0.01$). Means having with the different letters in same column differed significantly.	



Figure(3): Level of testosteron in different group of mice treated with *Cyperus Esculantus extract*

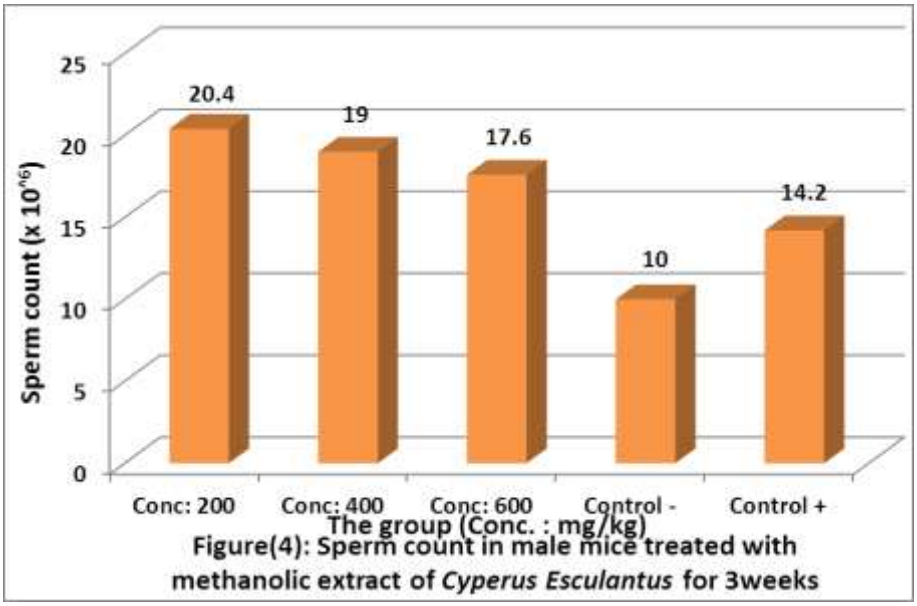
Esculentus contained flavonoids compounds such as qureceine and rutin. Qurecetine could increase serum testosterone levels in male and found to improve the action of sex hormone (LH). This hormone stimulates male testicles to produce greater levels of testosterone, which in turn helps increased sexual drive.[32] Androgenic effect is attributable to testosterone levels in blood; *C. esculentus* extract has a role in testosterone secretion confer best availability of hormone to gonads. The testes, epididymis and other reproductive organs are structurally and physiologically dependent upon the testosterone.[33]

Sperms concentration

The results of sperms concentration in table (3) and figure (4) showing a significant increase ($p>0.01$) in sperms concentration after treatment with the *C. esculentus* extract at doses 200, 400 and 600 mg/kg (20.40±0.51, 19.00± 0.54 and 17.60± 0.51 sperm/ml) when compared with negative control, water (10.00+ 0.71 sperm/ml), positive control, mesterolone drug (14.20+ 0.58 sperm/ml). The *C. esculantus* extract contained many active compounds especially flavonoids that contributed in an increasing sperms concentration. The mechanism for increased sperms concentration may be due to the presence of quercetin. It was known that quercetin can increase the numbers of spermatogonial cells by reduceing the oxidative damage in the testes (Mi and Zhang, 2005).[34] Other study found that quercetin increases the testosterone level so that quercetin led to boost sperm quality and fertility(35).[35]

Table 3: Effect of *C. esculentus* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *C esculentus* on sperms concentration in mice (mean ± SD).

Mice groups	Mean ± SD Sperm count (x 10^6)
200 (mg/kg)	20.40 ± 0.51 a
400 (mg/kg)	19.00 ± 0.54 ab
600(mg/kg)	17.60 ± 0.51 b
Negative Control (water)	10.00 ± 0.71 d
Positive Control (mesterolone)	14.20 ± 0.58 c
LSD value	1.699 **
P-value	0.0001
** (P<0.01). Means having with the different letters in same column differed significantly.	



Figure(4): Sperm count in male mice treated with methanolic extract of *Cyperus Esculantus* for 3weeks

Morphologically Abnormal Sperms

Morphological study of sperms is an important aspect in the assessment of sperm functions (Katz et al.,1982).[36] Results in table (4) and Figure (5) revealed a significant decrease ($p \leq 0.01$) in percentage of morphologically abnormal sperms after treatment with *C. esculentus* extract at doses 200, 400 and 600 mg/kg (11.00 $\pm$ 0.71, 12.60 $\pm$ 0.40 and 13.60 $\pm$ 0.08) when compared with negative control (water treatment) (20.40 ± 0.51) and positive control (mesetorlone drug) (17.60 ± 0.67).

Table 4: Effect of *C. esculentus* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *C. esculentus* on percentage of morphologically abnormal sperms in mice (mean ± SD).

Mice groups	Mean ± SD
	Abnormal sperm (%)
200 (mg/kg)	11.00 ± 0.71 d
400 (mg/kg)	12.60 ± 0.40 dc
600(mg/kg)	13.60 ± 0.08 c
Negative Control (water)	20.40 ± 0.51 a
Positive Control (mesterolone)	17.60 ± 0.67 b
LSD value	2.102 **
P-value	0.0001
** (P<0.01). Means having with the different letters in same column differed significantly.	

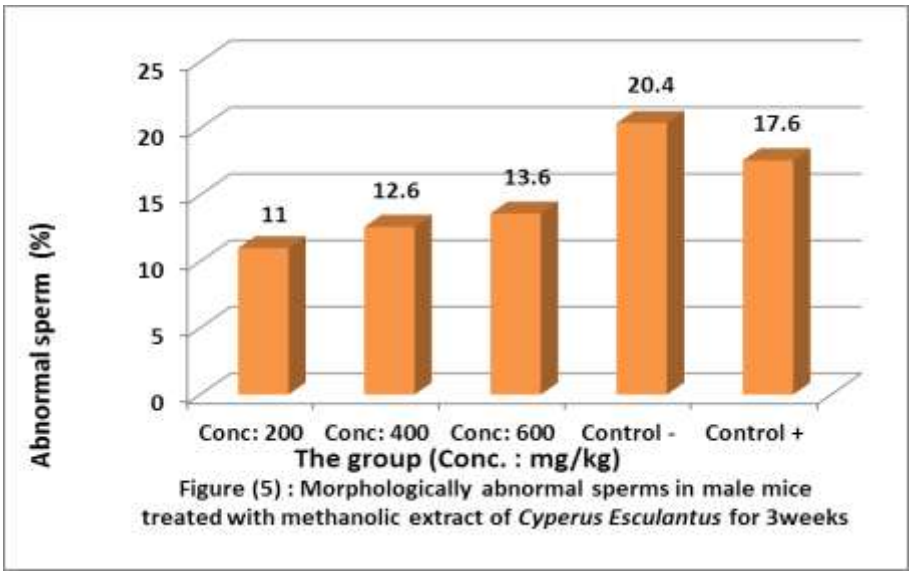


Figure (5) : Morphologically abnormal sperms in male mice treated with methanolic extract of *Cyperus Esculantus* for 3weeks

Results in table (4) and Figure (5) revealed a significant decrease ($p \leq 0.01$) in percentage of morphologically abnormal sperms after 3 weeks treatment with C. esculentus extract at doses 200, 400 and 600 mg/kg when compared with negative control (water treatment) and positive control (mesetorlone drug). The activity of plant extract can be referred to the presence of flavonoids and oleic acid, linoleic acid and palmitic acid that act as antioxidant.[10,30] These compounds protected the plasma membrane of the sperm against the influence of oxidative stress.

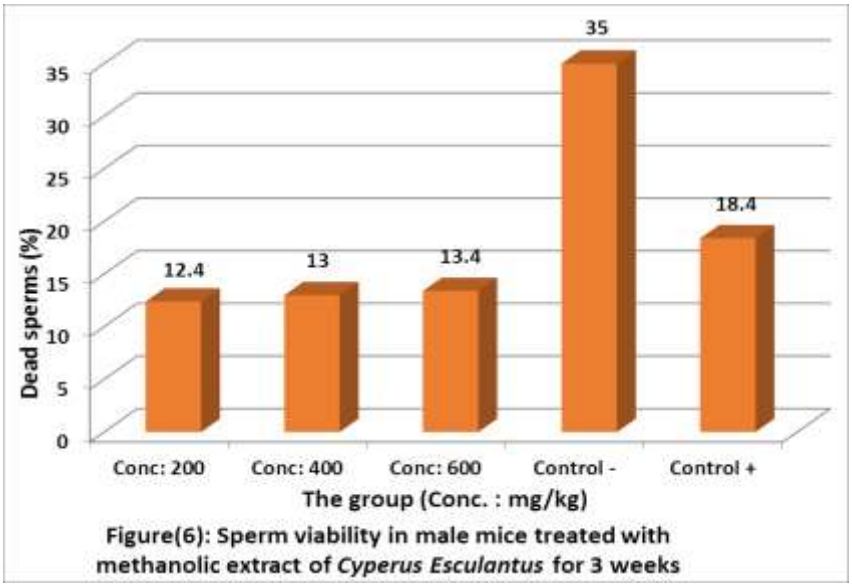
Sperms viability

In table (5) and figure (6) there is a significant decrease ($p>0.01$) in percentage of dead sperms after treatment with *C. esculentus* extract at doses (200, 400 and 600) mg/kg, with a percentage of dead sperms (12.40 ± 0.51, 13.00 ± 0.71 and 13.40 ± 0.67) respectively when

compared with negative control (water treatment) (35.00 ± 0.71) and positive control (mesterolone drug) (18.40 ± 0.51).

Table 5: Effect of *C. esculentus* methanolic extract (200,400 and 600 mg/kg) for 3 weeks treatment with *C. esculentus* on percentage of sperms viability in mice (mean ± SD).

Mice groups	Mean ± SD
	Dead sperms (%)
200 (mg/kg)	12.40 ± 0.51 c
400 (mg/kg)	13.00 ± 0.71 c
600(mg/kg)	13.40 ± 0.67 c
Negative Control (water)	35.00 ± 0.71 a
Positive Control (mesterolone)	18.40 ± 0.51 b
LSD value	1.856 **
P-value	0.0001
** (P<0.01). Means having with the different letters in same column differed significantly.	



Figure(6): Sperm viability in male mice treated with methanolic extract of *Cyperus Esculantus* for 3 weeks

The results showed significant decrease (p>0.01) in percentage of dead sperms after treatment with *C. esculentus* extract at doses (200, 400 and 600) mg/kg, when compared with negative control (water treatment) and positive control (mesterolone drug). Flavonoids, like Rutin has shown a significant stimulating effect on sperm parameters like sperm count, sperm morphology and sperm viability, these results were in agreement with other studies on flavonoids effect on male reproductive system, flavonoids including (quercetin) showed positive effect on the function of prostate.[37] In this study, it has found that oral administration of *C. esculentus* extract significantly enhanced certain sperm function parameters such as sperm concentration, sperms viability and percent of abnormal sperms.

REFERENCES

1. Srivastav, S.; Singh, P.; Mishra, G.; Jha, K. K.; Khosa, R. L. Achyranthes aspera-Animportant medicinal plant: A review. Journal of Natural Product and Plant Resources, 2011; 1(1): 1-14.
2. Vijayan, A.; Liju, V. B.; John, J. V.; Reena; Parthipan, B.; Renuka, C. Indian Journal of Traditional Knowledge, 2007; 6(4): 589-594.
3. Mackin, J. Cornell Book of Herbs and Edible Flowers. Cornell Cooperative Extension Ihaca, New York, U.S.A., 1993; 78- 99.
4. Delaszlo, H. and Henshaw, P. S. Plant materials used by primitive people to affect fertility. Science, 1954; 199: 626-631.
5. Ahmad, F.; Khan, R. A. and Rashid, S. Pharmacological evaluation of medical plants for their analgesic activity in mice. Medical Journal of the Islamic Republic of Iran, 1998; 10(2): 149-152.
6. Ezeh, O. and Gordon, M. H. Niranjan K. Tiger nut oil (*Cyperus esculentus L.*): a review of its composition and physic-chemical properties. European Journal of Lipid Science and Technology, 2014; 116: 783–794.
7. Pascual, B. ; Maroto, J. V. ; Lopez-Galarza, S. ; Sanbautista , A. ; Alagarda , J. Chufa (*Cyperus esculentus* L. var*sativus*Boeck.): an unconventional crop. Studies related to applications and cultivation. Econ Bot, 2000; 54: 439–448.
8. Defelice, M. S. Yellow nut sedge *Cyperus esculentus* L.- Snack food of the gods. Weed Technol, 2002; 16: 901–907.
9. Tunde-Akintunde , T. Y. and Oke, M. O. Thin-layer drying characteristics of tiger nut (*Cyperus esculentus*) seeds. Journal of Food Process and Preserve, 2012; 36: 457–464.
10. Chukwuma, E. R.; Obioma, N. and Cristopher, O. I. The phytochemical composition and some biochemical effects of Nigerian tiger nut (*Cyperus esculentus* L.) tuber. Pakistan Journal of Nutrition, 2010; 9(7): 709–15.
11. Caius, J. F. The medicinal and poisonous plants of India. Jodhpur: Scientific Publishers, 1998; 167–168.
12. Al-Shaikh, M. N.; Abdul Wahab , T. A. ; Abdul Kareem, S. H. ; Hamoudi, S. R. Protective effect of chufa tubers (*Cyperus esculentus*) on induction of sperm abnormalities in mice treated with lead acetate. International Journal of Drug Development and Research, 2013; 5: 387–392.
13. Agbai, E. O. and Nwanegwo, C. O. Effect of methanolic extract of *Cyperus esculentus*L. (Tigernut) on luteinizing hormone, follicle stimulating hormone, testosterone, sperm

count and motility in male albino wistar rats. Journal of Medicine and Biosciences, 2013; 5: 52–61.

14. Sofowora, E.A. Medicinal plants and Traditional Medicine in Africa. Ibadan-Owerri-Kaduna-Lagos Spectrum Books Limited, 1993; 159-238.
15. Evans, N.S. Trease and Evans. Pharmocognosy, 15th Edn., Elsevier, India, 2005; 1-24.
16. Okaka, J.C.; N.J. Enoch, and N.C. Okaka, Human nutrition: an integrated approach. Enugu, ESUT Publications, 1992; 57-58.
17. Frantisek, S.S. The natural guide to medicinal herbs and plants. Tiger Barks Cast, Twinkemhan, United Kingdom, 1991; 1-5.
18. Amadi, B.A.; C.O. Ibegbulem, and A.C. Egbebu, Assessment of the effect of aqueous extract of (*Asimina triloba*) root on organ weights and liver function of albino rats. International Journal of Natural and Applied Sciences, 1991; 2: 79-81.
19. Arokiyaraj, S.; Perinbam, K.; Agastian, P. and Balaraju, K. Immunosuppressive effect of medicinal plants of kolli hills on mitogen-stimulated proliferation of the human peripheral blood mononuclear. Indian Journal of Pharmacology, 2007; 39: 180-183.
20. Shihata, I. M. A pharmacological study of anagalis arvensis. M.D. Vet. Thesis, Cairo University, 1951; 43.
21. Al-Abid, M. R. Zur *zusammen* setrung der Absschlu B memtrame in phoenix dactily Frawuzburg University, Wurzburg F. R. of Germany, 1985.
22. Jaffer, H.; Mahmoud, M.; Jawad, A.; Naji, A. and Al Naib, A. Phytochemical and biological screening of some Iraqi plants. Fitoterapia, 1983; 59: 229-238.
23. Harborne, J. B. Phytochemical Methods (A guide to Modern Technique of Plant Analysis), Chapman Hall, London, 1984.
24. Trease, J. E. and Evans, W. C. Pharmacognocy. 13th ed., Balliere. Tindall, 1987, 62-68.
25. Stahle, E. Thin layer chromatography a laboratory hand book, 2nd ed., translated by Ashworth, M. Spring-verag, Berlin, heidelberty, New York, 1969.
26. Hafez, E. S. E. Artificial insemination. In Reproduction in Farm Animals (Ed. Hafez E. S. E), 5th edn, Lea & Fediger, Philadelphia, PA, 1987: 481-497.
27. Dale, B. and Elder, K. Sperm oocyte interaction, sperm parameters and sperm preparation techniques. in vitro, 1997; 20-120.
28. Dale, B. and Elder, K. Sperm oocyte interaction, sperm parameters and sperm preparation techniques. in vitro, 1997; 20-120.
29. SAS. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA, 2012.

30. Siqun Jing; Saisai wang; Qian Li; Lian Zheng; Li Yue; Shaoli Fan; Guanjun Tao. Dynamic high pressure microfluidization-assisted extraction and bioactivities of *Cyperus esculentus (C. esculentus L.)* leaves flavonoids. Food Chemistry, 2016; 192: 319–327.
31. Klein, B.; Gallardo-Chacón, J.-J.; Codina-Torrella, I.; Trujillo, A.-J. and Juan, B. Evaluation of Volatile Compounds of "Tiger Nut Beverage" (Orxata de Xufla) Headspace by Optimized Solid-Phase Micro-Extraction. Open Access Library Journal, 2014; 1: e1015.
32. Ma Z, H. N. T.; Hoa H. T.; Tien, D. P.; Huynh, H. Reproduction of rat prostate weight by combined quercetin finasteride treatment is associated with cell cycle deregulation, Journal of Endocrinology, 2004; 181: 493-495.
33. Amini, A. The effects of gossypol on spermatogenesis in NMRI mice. Iranian Journal of Science and Technology (Sciences), 2005; 29(1): 123-133.
34. Mi Y, Z. C. Protective effect of quercetin on Aroclor 1254 –induced oxidative damage in cultured chicken spermatogonial cells. Toxicological Sciences, 2005; 88(2): 545-550.
35. Taepongsorat, L.; Tangpraprutgul, P.; Kitana, N. & Malaivijitnond, S. Stimulating effects of quercetin on sperm quality and reproductive organs in adult male rats. Asian journal of andrology, 2008; 10(2): 249-258.
36. Katz, D. F; Diel, L. and Overatreet, J. W. Difference in the movement of morphologically normal and abnormal human seminal spermatozoa. Biology of reproduction., 1982; 26: 556-576.
37. Shuk-mei ho; Viccione, T.; Yu, M. In-vitro and in-vivo inhibition of nuclear type II estrogen binding sites in the dorsolateral prostate of noble rats. Journal of steroid biochemistry and molecular biology, 1993; 46: 489–495.