OPEN ACCESS
Advances in Animal and Veterinary Sciences
Research Article

Estimation Role of *Spirulina Platensis* Extract in Treatment Experimentally Infertility caused by Metronidazole in Rats

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Abstract | The indiscriminate use of drugs poses a significant health risk. So, the aim of the study is to evaluate the harmful effects of metronidazole in parameters infertility of male reproductive system and treatment by *Spirulina platensis* extract. Thirty two of male rats were used. They were divided into four groups (8/each). The first group administrated distilled water as control group. The second group was received oral dose of metronidazole (18 mg/200 body weight /day) while the third and fourth groups were treated with ethanolic extract of *Spirulina platensis* after receiving metronidazole for (one and two months) respectively. The dose of drug and ethanolic extract was taken orally. When experimental period was ended, prostate was weighted, fertility parameters were studied by assessment of concentration and sperm malformations, fertility index, sex hormones and histological sections of sperm were prepared. The results revealed show that metronidazole intake causes a decline in prostate and body weight, reduction of sperm, fertility index and sex hormones (LH,FSH and Testosterone) with increasing of sperm abnormalities, while all previous indicators of fertility were increased with reduction of abnormal sperm by receiving the ethanolic extract of algae (*Spirulina platensis*). In addition, morphological and structural damages of sperm were involved global, elliptical, lacking of head with quirky and absence of tail as well as sever abnormalities and breakdown of sperm. The conclusion showed that it is terminated metronidazole exposure causes induction of infertility and treated by ethanolic extract of *Spirulina platensis*.

Keywords | Metronidazole, Infertility, *Spirulina platensis,* Sperm, Testosterone, Rats

Received | July 18, 2024; **Accepted** | November 13, 2024; **Published** | January 24, 2025
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Email: fatimaaziz.bio@utq.edu.iq
Citation | Al-Badry FAM (2025). Estimation role of *Spirulina platensis* extract in treatment experimentally infertility caused by metronidazole in rats. Adv. Anim. Vet. Sci. 13(2): 383-388.
DOI | https://dx.doi.org/10.17582/journal.aavs/2025/13.2.383.388
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

Metronidazole is a drug called (Flagyl, Flazole, Metrolag) while the chemical name is 2 methyl-5 nitromidazole 1- ethanol. The drug is administrated by adult and children. It is absorbed by stomach and intestine while it metabolites in liver (Aslam *et al*., 2022). In spite of the role of metronidazole in suppression of pathogens, it has harmful effects associated with reproductive dysfunction, passive characters of and malformations sperm (Al-Timimi, 2021). It is considered as carcinogenetic ability, neuropathy and hepatic tumors when becomes chronic (John *et al*., 2021). *Spirulina* is one of the blue-green algae. It is spiral or filamentous form and it has 15 species in Iraq that recently (Arthrospira) which consumed by human and animals (Gogna *et al*., 2023). *Spirilina* is used largely as a nutrition-

al supplemen. It has been antioxidants, minerals, proteins, vitamins and amino acids (Singh *et al*., 2023). In addition to elevated nutritional value, it is used for flavor and color improvement and as support for immunity (Tongsiri *et al*., 2010).

The aim of the present study is to assess harmful impacts of metronidazole on male reproductive system and treated by ethanolic extract of (*Spirulina platensis*).

MATERIALS AND METHODS

Preparation of Ethanolic Extract

Ethanolic extract of algae (*Spirulina platensis*) was prepared by drying of algae as powder, 20 grams were mixed with 200 mL of ethanol then covering by medical qauze. Soxhelet then evaporating were used for collecting of extract (Harborn, 1984).

Induction of Experimentally Infertility

Metronidazole (500 mg) was used for infertility induction, It was as tablet (Sanofi Aventis Company: France). The oral dose of drug 200 gm/body weight for each animal was (18 mg/day/rat) that prepared according to Paget and Barnas (1964).

Laboratory Animals and Metronidazole Administration

Thirty two of laboratory male rats (11-13) weeks old and weight (210-220) grams. Animals were obtained and reared up animal house of biology department in college of education for pure sciences, university of Thi- Qar. Rats were randomly divided into four groups (n=8) as: First group (Control group) was received distilled water. Second group was administrated oral dose of metronidazole at (18 mg metronidazole /200 grams body weight) for each animal. Third and Fourth group were treated with metronidazole then received ethanolic extract of *Spirulina platensis* for one and two months respectively.

Determination Weights of Body and Prostate

Weights of body and prostate gland were measured for all groups. It was used later for comparing among all groups.

Sperm Parameters

The fertility parameters studied were involved concentration, malformations of sperm and fertility index. Total count of sperm was calculated by method of Soto (1983) while the abnormalities of sperm were determinated according to Wyrobek and Bruce (1975), 100 sperm were counted serially that divided normal and abnormal sperm. While the fertility index was estimated by Oberlander *et al*. (1994), one male was mated with one female then vaginal smears were prepared to fertility assessment.

Hormonal Assay

For sex hormones (LH, FSH and Testosterone) measurement, it must be sera obtaining which collected from blood centrifuged. LH, FSH were assessed by ELISA kits, while the radio immune assay was used for evaluation the concentration of testosterone (Richard and Valbondov, 1974).

Histopathological Examination

Histological sections of sperm were prepared and examined for determination normal and abnormal structures of sperm (Soto, 1983; Wyrobek and Bruce, 1975).

Statistical Analysis

Data were analyzed by SPSS (Version 24) using Less significant difference (LSD), significance was considered at ($p \leq 0.05$) (George and Mallery, 2011).

Table 1: Effect the ethanolic extract of *Spirulina platensis* on body and prostate weight (n=8) (Mean ± Standard deviation).

Groups	Body weight (g)	Prostate weight(g)
Control group	272.66 ±1.76^{a}	0.58 ±0.01^{a}
Metronidazole group	214.84 ±1.69^{d}	0.31 ±0.00^{d}
Ethanolic extract group (One month)	253.74 ±1.63^{c}	0.45 ±0.01^{c}
Ethanolic extract group (Two months)	267.49 ±1.64^{b}	0.51 ±0.00^{b}
LSD	1.38	0.00

Different letters indicate to significant difference (P≤ 0.05) among groups.

RESULTS AND DISCUSSION

Table 1, indicated to a significant decrease (P≤ 0.05) in body and prostate weight of metronidazole group compared with control group. This decreasing may be related to administration of metronidazole. This agreed with Sohrabi *et al*. (2007) who found diminution in weight of sex organs as testis, seminal vesicle and prostate gland after two and six weeks of metronidazole intake orally. Another reason of that may be associated to decreasing in testosterone hormone caused by metronidazole. That result was observed in the current study. This accordance with Grover *et al*. (2001) who reported to reduction of testosterone hormone by peritoneal injection of metronidazole. Chowdhury and Steinberger (1976) revealed to lowering of testis and prostate weight resulted from decreasing of testosterone hormone. While the administration of ethanolic extract of *spirulina* at two periods (one and two months) caused increasing significantly (P≤ 0.05) in these weights compared with metronidazole group, this associated with *Spirulina*. Nikodemusz et al. (2010) investigated that algae have active role in raising of minerals absorption and reduction of diarrhea subsequently elevated of weights. Also, the excess

of body weight may belong to amino acids of *Spirulina*, Duncan and Klesius (1996) focused that essential amino acids as one of the *Spirulina* ingredients. This accordance with Razuki *et al.* (2018) and Shanmugapriya *et al.* (2015) who showed in their results that algae adding to diets of broiler males (Ross 308) enhanced the productive performance.

Table 2, points to significant decreasing (P≤ 0.05) in concentration of sperm and fertility index of metronidazole group compared with control group. This decline may be due to harmful effect of drug on testosterone hormone which has active role in spermatogenesis. Christensen (1975) explained affected of spermatogenesis by testosterone lowering, that identical with Grover *et al.* (2001) showed reduction of testosterone in treated animals with metronidazole. While a significant increasing (P≤ 0.05) in these two parameters is observed by using the extract of *Spirulina platensis*.

Table 2: Effect the ethanolic extract of *Spirulina platensis* in some sperm parameters (n=8) (Mean ± Standard deviation).

Group	Sperm Count× (10^4)	Abnormalities (%)	Fertility index (%)
Control group	206.23±1.12^a	4.54±0.51^d	94.00±0.63^a
Metronidazole group	123.21±1.07^d	85.82±0.84^a	34.00±0.89^d
Ethanolic extract group (One month)	154.44±1.56^c	41.98±1.45^b	73.83±1.16^c
Ethanolic extract group (Two months)	186.94±1.77^b	21.05±1.10^c	90.83±0.75^b
LSD	0.69	0.51	0.43

Different letters indicate significant difference (P≤ 0.05) among groups.

Table 3: Effect the ethanolic extract of *Spirulina platensis* in sex hormones (n=8) (Mean ± Standard deviation).

Group	LH (MIU/mL)	FSH (MIU/mL)	Testosterone (MIU/mL)
Control group	3.51 ±0.01^a	4.92 ±0.01^a	1.96 ±0.02^a
Metronidazole group	1.98±0.00^d	2.06 ±0.03^d	1.21 ±0.01^d
Ethanolic extract group (One month)	2.93 ±0.06^c	3.85 ±0.02^c	1.62 ±0.00^c
Ethanolic extract group (Two months)	3.20 ±0.01^b	4.55 ±0.02^b	1.80 ±0.01^b
LSD	0.00	0.01	0.38

Different letters indicate significant difference (P≤ 0.05) among groups.

Abnormality of sperm occurred increased in metronidazole group while it was reduced when algae exposure. This finding agrees with El-Nahas and El-Shmawy (2004), who found elevated of abnormal sperm in male mice after two weeks of metronidazole exposure. The enhancement in reproductive performance in two algea groups may be related it ingredients as vitamin C of *Spirulina*, Dabrowski and Ciereszko (1996) found that vitamin contribute in protection of sperm and diminishing of abnormalities through spermatogenesis.

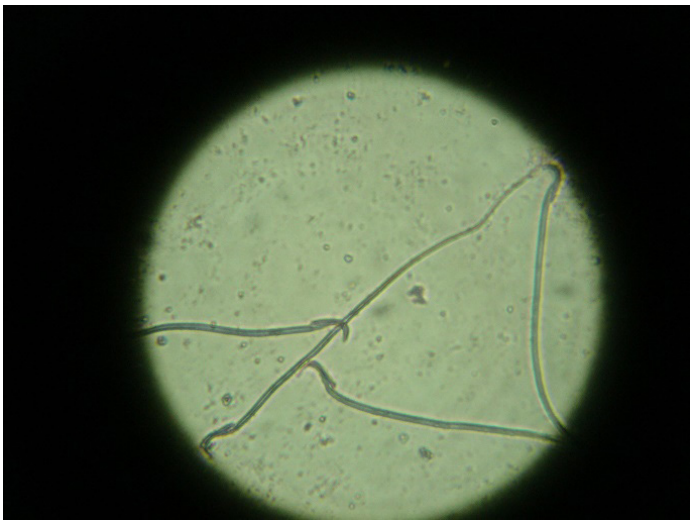

Figure 1: Shows normal sperm of control group (Eosin Stain) (40 X).

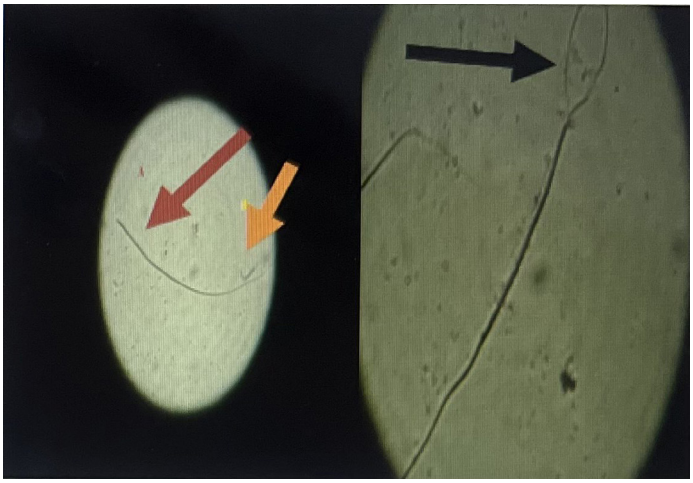

Figure 2: Abnormal sperm of Metronidazole group shows (A): lacking head (red arrow) absence tail (orange arrow) (B) : global head (black arrow) (Eosin Stain) (A) (40 X) (B) (100 X).

Table 3 showed that Sex hormones (LH, FSH and Testosterone) were reduced significantly (P≤ 0.05) by metronidazole administration when comparing with control group, While the levels of these hormones were raised by ethanolic extract of algae receiving. This may be back to role of vitamin E in *Spirulina*, Behn *et al.* (1996) examined the production of testosterone hormone occurred by presence of vitamin E. This agrees with Duncan and Klesins (1996) who confirmed that vitamin E as one *Spirulina* components.

Histological sections of sperm ensured normal architecture

of sperm (Figure 1) with negatively impacts of metronidazole, that included morphological and structural damages such as global, elliptical, lacking of head with quirky and absence of tail as well as sever abnormalities and breakdown of sperm (Figures 2, 3, 4, 5 and 6). Those were regarded to effect of drug on sperm, Mudry *et al.* (1994) pointed to abnormality in head and tail of sperm that resulted from metronidazole. Also, it may be back to leydig cells damaged by drug which considered necessary to secretion of testosterone hormone. This agreed to Pang *et al.* (2005) who noted laceration and tumors in leydig cells caused by metronidazole accompanied by disorders of testosterone subsequently affected of spermatogenesis. Abnormality of sperm may be re lated to injury of prostate gland that resulted from metronidazole. This corresponded to Menedez *et al.* (2002) who mentioned that prostate damaged led to high non active of testosterone that caused disturbances in activity of hormone with interference spermatogenic processes.

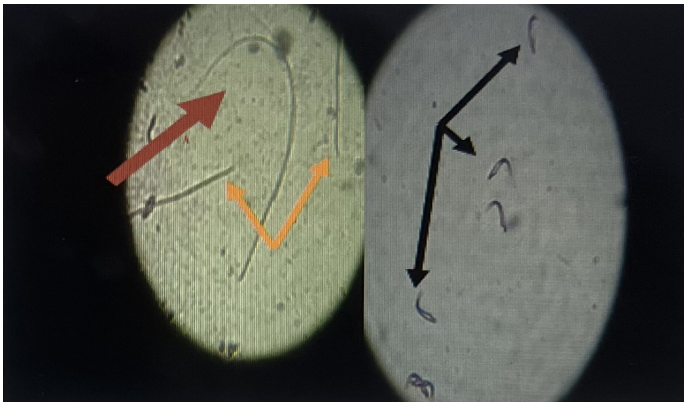

Figure 3: Abnormal sperm of Metronidazole group shows (A) : quirky tail (red arrow) lacking of head (orange arrow) (B) lacking of tail (black arrow) (Eosin Stain) (A) (40 X) (B) (100 X).

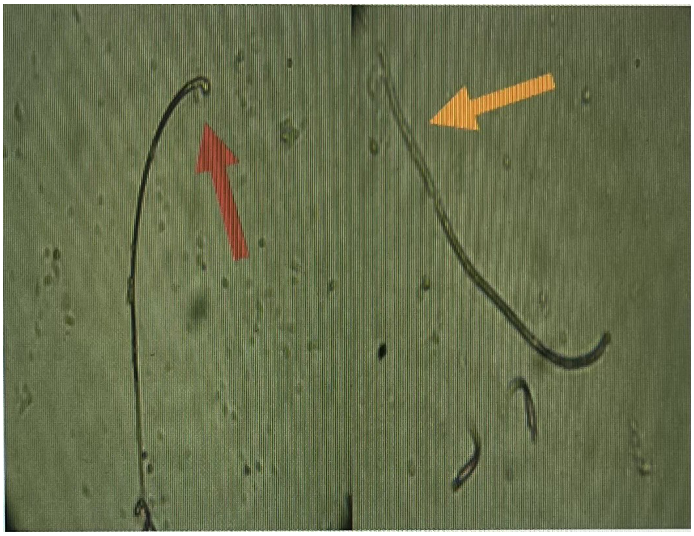

Figure 4: Abnormal sperm of Metronidazole group shows; (A) elliptical head (red arrow) (B) breakdown of sperm (orange arrow) (Eosin Stain) (A) (40 X) (B) (100 X).

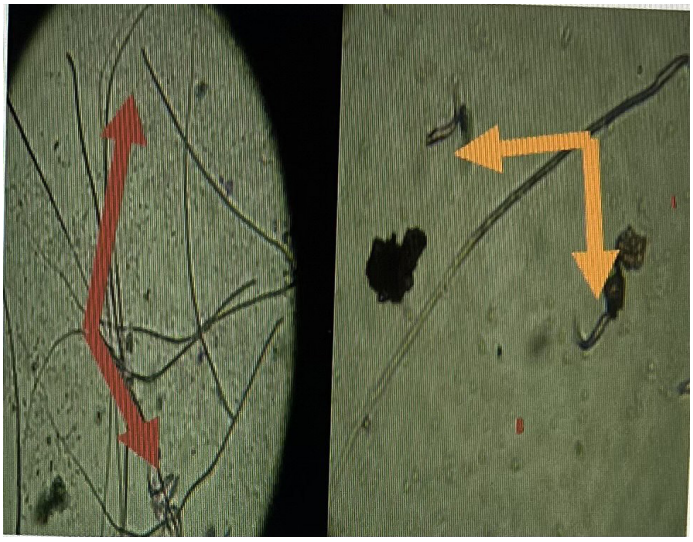

Figure 5: Abnormal sperm of Metronidazole group shows : (A) sever malformations (lacking head and tail) (red arrow) (B) absence tail (orange arrow) (Eosin Stain) (A) (40 X) (B) (100 X).

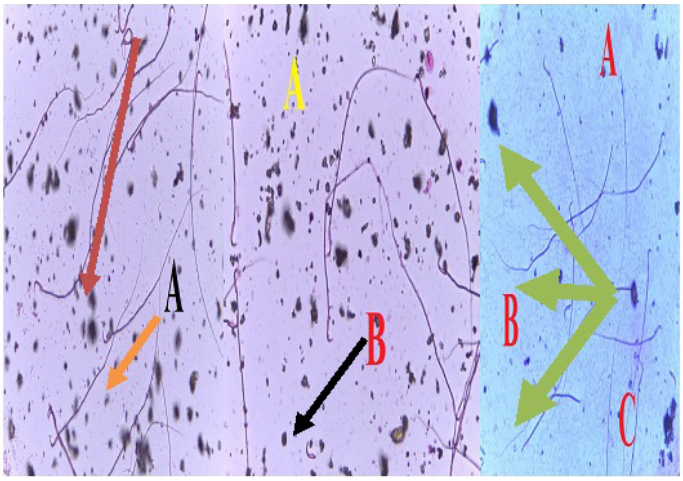

Figure 6: Normal sperm of Spirulina platensis group (One and Two months) shows : (A) absence tail (red arrow) lacking of head (orange arrow) (B) absence tail (black arrow)(C) normal all sperm (green arrow)(Eosin Stain) (A),(B),(C) (40 X).

Consummation of *Spirulina* led to normal sperm occurred and malformations reduction. This may be related to elevated of testosterone which confirmed by results of this current study. Akingbemi *et al.* (2004) found that the production of sperm and abnormalities was controlled by testosterone. Also, there was another reason of that may be due to vitamins (E,C) of algae which helped in repairing the connective tissues of reproductive system and raising of sperm activity. That was identical with Barella *et al.* (2005) who mentioned the role of vitamins in production of testosterone that led to sperm increasing and rehabilitation of seminiferous tubules.

CONCLUSIONS AND RECOMMENDATIONS

It is concluded that metronidazole consummation causes harmful effects in male reproductive system with structural damages in sperm led to infertility. Further, The extract of *Spirulina platensis* is used for decline these damages. According to that, we recommend that non excessive intake of metronidazole and other studies are carrying out for determination of significant role of algea in different diseases treatment.

ACKNOWLEDGMENTS

I want to introduce my sincere thanks to college of education for pure sciences in Thi-Qar university for assisting me during current work achievements.

NOVELTY STATEMENT

The current study confirmed possibility alterations and damages of male reproductive system by metronidazole administration.

AUTHOR'S CONTRIBUTIONS

Author completed experimental design, animal housing and performed the laboratory tests which associated to the present study. In addition to tabulation and analyzed of data as well as preparation and examined sections of sperm.

List of Abbreviations

SPSS, Statistical Package for Social Sciences; LH, Luteinizing Hormone; FSH, Follicle Stimulating Hormone; ELISA, Enzyme-Linked Immunosorbent Assay; gm, gram; LSD, Least Significant Difference.

Ethical Approval

According to committee of animal interest, implementation of current experimental was performed according to ethics committee issued by college of education for pure sciences / university of Thi-Qar (No. 7/54/817 in 3/1/2024).

Conflict of Interest

The author has not declared conflict of interest.

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