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Ameliorative Effect of The *Moringa Pterygosperma* Extract Against Cyclophosphamide on the Reproductive System

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Abstract

Infertility is one of the major health problems in couples since was impacted their families and communities. Chemotherapies treatment that is used in cancer diseases can lead to infertility as cyclophosphamide. Cyclophosphamide is used for the treatment of different diseases as leukemia, lymphoma, and multiple myeloma. Recently scholars' direction for using the natural product to decreased the negative effect of chemotherapies on testicular and reproductive parameters. The *Moringa Pterygosperma* is a tree spread in India that has medicinal and medicinal properties as well as has an antioxidant effect on the body. Thus, our studies aimed to the investigation of the product effect of aquatic extract of *Moringa Pterygosperma* against the adverse effect of cyclophosphamide on the reproductive parameters including sperm motility, sperm viability, sperm morphology, acrosomal integrity, and testicular degeneration. Finally, our study found that is significant improvement in the treatment group with aquatic extract of *Moringa Pterygosperma*.

Keyword: fertility, chemotherapy, testis, sperm parameters and antioxidants

Introduction

Infertility affects all circles and societies, males and females, furthermore is not considered a serious physical disease, but the problem lies in its side effects, which may affect one of the pillars of the family (Al-Mousaw et al., 2022; Bustani et al., 2022). In the last few years found the medication as chemotherapy used to treat cancer causes different pathogenic strass on various body organs and decreased in sperm quality and hormonal disturbance, all those factors lead to infertility and sexual problems (Alabedi et al., 2021). Cyclophosphamide is chemotherapy used for the treatment of different types of cancer as ovarian cancer (Zsiros et al., 2021), breast cancer (Yu et al., 2021), and sarcoma (Leavey et al., 2021), by suppressing the immune system (Al-Mousaw et al., 2022). Last studies illustrated that cyclophosphamide has different side effects as testicular degeneration, hormonal disturbance and decreased the white blood cell counts, but the most effective was infertility (Fusco et al., 2021) due to that adverse effect on spermatogenesis and decrease in sperm quality (Liu et al., 2021). Generally, infertility is due to cyclophosphamide instructs to two reasons; first due to oxidative effect of cyclophosphamide on the soft tissue as tests, since induced testicular degeneration (Ebokaiwe et al., 2021). The other reason due to induced sperm DNA damage on the spermatogenesis

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stages (van den Boogaard et al., 2022). Previous studies suggested used the natural antioxidant foe decreased the adverse effect of the cyclophosphamide (Al-Mousaw et al., 2022; Alabedi et al., 2021; Ali Hameed et al., 2021). Moringa Pterygosperma may be one of the latest trends in the nutritional supplement market and considered as the most prominent food alternative in recent times, as it has become a superfood due to its alternative medicinal and therapeutic properties (Bennett et al., 2003). Previous Studies proved that aquatic extract of *Moringa Pterygosperma* has anti-inflammatory (Al Zoubi et al., 2022; Rao and Mishra, 1998), anticancer (kumar Bargah, 2015) and antioxidant (Luqman et al., 2012) properties. Last studies used the natural products as lycopene (Al-Mousaw et al., 2022), curcumin (Bustani et al., 2022) and Ocimum tenuiflorum (Alabedi et al., 2021) for decreased the adverse effect of different threptic drugs used in humans as Filgrastim (Alabedi et al., 2021).

Thus, our study aimed to evaluate and determine the productive effect of the *Ocimum tenuiflorum* against Cyclophosphamide on the reproductive parameters.

Material and methods:

Animals:

Forty male albino Wistar rats were used in this study, animal bodyweight 150–200 g that selected for the study. The animals were obtained from the physiology department, faculty of veterinary university of Bagdad, Iraq, and hosed in international cages in faculty of science, Kufa. Iraq, at temperature of 25–30 °C and supplemented by a pellet diet foot for nutrition and water.

Experimental design: -

Animals was divided into four groups each group contains 10 rats. That groups included; 1st group control group (Cg), 2nd group is negative control group (Ncg) since that intraperitoneal injection of cyclophosphamide at (200 mg/kg) in a signal dose on the initial first day of the experiment (Al-Mousaw et al., 2022; Oyagbemi et al., 2016), 3rd (T1) and 4th (T2) group was treatment group which besides the intraperitoneal injection of cyclophosphamide that treated with aqueous extract of *Moringa Pterygosperma* administered by gavage at 100 and 200 mg kg^{-1} respectively for 10 days (Farid and Hegazy, 2020; Jaiswal et al., 2009). Finally, all animal was sacrificed after 41 days of experimental (Mohammadi et al., 2014). Immediately the testes and tail of the epididymis of testis was taken for histological and sperm evaluation for determined the reproductive fertility parameters.

Sperm parameters

Sperm parameters included Sperm general and progressive motility, viability and acrosomal integrity percentage, and sperm concentration.

The tail of the epididymis was rinsed and incubated in two ml of normal saline at 37°C and cut into about 200 pieces using an anatomical micro-scissor to leak the spermatozoa from the

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epididymal tubules for further tests (Alabedi et al., 2021). The motility of sperm estimation under microscopic after placing 10 microliters of the semen on a dry and warm slide then covered by coverslip (Bustani and Baiee, 2021). While Morphology, viability and acrosomal integrity were measured by using eosin nigrosine stain which mixed 20 microliters by 20 microliters of sperm and evaluated under microscopic.

Extraction of aqueous extract: -

The extract was prepared by using the Soxhlet extractor tools by using hot water. The extract was concentrated under reduced pressure and then lyophilized. Later, this *Moringa Pterygosperma* extract was used for experimental.

Cyclophosphamide

The Cyclophosphamide was purchased from Al-Faiha Company-Najaf, Iraq.

Statistical analysis

The statistical analysis was performed using Graphpad Prism 8® software repeated measure two-way ANOVA test was conducted Tukey's multiple comparison test was applied to determine differences among the effect of the cyclophosphamide and to investigate the protective effects of treatment concentration on sperm quality during the study period.

P-values < 0.05 were used considered as statistically significant. Data are presented as mean ± standard error of the mean (SEM).

Results and discussion:

Fertility disorder is one of the world problems unsolved, as well as that, is different way causes decreases on the rats of fertility. Acquire reasons due to adverse effects of cancer chemotherapy. The result at the table 1 showed the productive effect of Moringa against the adverse effect of the *Cyclophosphamide* at the single dosage 200 mg/kg since induced the testicular degeneration as proved by a previous study (Ebokaiwe et al., 2021; Oyagbemi et al., 2016). Furthermore, the outcomes in the table 1 illustrated the change in sperm parameter since the sperm parameters of rats in the negative group decreased significantly lower than control in the all terms (motility, viability, morphology, acrosomal integrity and sperm concentration), as well as the testicular degeneration, is showed in the histology section in figure (1- B) comparative with the control in figure (1-A). Moreover, that histological section of the negative group showed tissue damage with the inability to distinguish the stages of sperm formation or Sertoli cells and Leydig cells as described in the previous studies the *Cyclophosphamide* has a negative effect on the soft tissue (Abdelfattah-Hassan et al., 2019; Bramwell et al., 1987). Generally, chemotherapy lead to an increase the reactive oxygen species in the cell including the spermatozoon cells and sperm germ cell (Al-Mousaw et al., 2022) and ovary cell (Yu et al., 2021). All the study result outcomes regarding to effect of

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Cyclophosphamide on sperm parameters are in agreement the previous studies (Al-Mousaw et al., 2022; Çeribaşi et al., 2010; Ebokaiwe et al., 2021).

Finally, the previous studies showed that natural antioxidant causes decreased the adviser effect of the *Cyclophosphamide* (Al-Mousaw et al., 2022; Mohammadi et al., 2014) as our result that was significant promoting of Moringa Pterygosperma against the Cyclophosphamide in both sperm parameter evaluation (Table 1) and in the histological section of the testis (figure 1-C) which the histological showed section shows the stages of spermatogenesis with clarity of lamina propria and lumen. All the study results were proved our suggested which the concentration value in table 1 which treatment groups showed non-significant different with the control, moreover the T2 group is superiority among the all groups, which *Moringa Pterygosperma* has anti-inflammatory properties as proved by the previous researcher (Rao and Mishra, 1998) which illustrated the anti-inflammatory and antioxidant effect of the *Moringa Pterygosperma* on the body tissue.

Table 1: Effect of *Moringa Pterygosperma* on sperm parameters against *Cyclophosphamide*

Group	Motility		Viability	Morphology	Acrosomal integrity	Sperm Concentration
	General	Progressive				
C	84.5 ± 0.2 a	77 ± 0.5 a	86 ± 1.5 a	97.5 ± 1 a	99.6 ± 0.2 a	$12.5 \times 50 \times 10^6$ a
Nc	49.5 ± 2.5 b	40 ± 4 b	63 ± 2 B	92.5 ± 0.5 b	92.6 ± 0.3 b	$6.4 \times 50 \times 10^6$ b
T1	75.5 ± 0.2 c	71 ± 0.2 a	77 ± 0.5 c	96.5 ± .5 a	99.2 ± 0.3 a	$11.5 \times 50 \times 10^6$ a
T2	81.5 ± 0.2 ac	75 ± 0.4 a	84 ± 0.25 a	97.6 ± 1 a	99.6 ± 0.1 a	$13.5 \times 50 \times 10^6$ a

Values are means ± SEM of 40 adults male Wistar rats
[abcd] Means values between groups with different superscripts are significantly different at ($P < 0.05$).
C: control group
Nc: negative group: intraperitoneal injection of cyclophosphamide
T1: -**Administered of** *Moringa* ***Pterygosperma*** **by gavage at 100mg/kg after intraperitoneal injection of cyclophosphamide**
T2: - Administered of *Moringa* ***Pterygosperma*** **by gavage at 100mg/kg after intraperitoneal injection of cyclophosphami**

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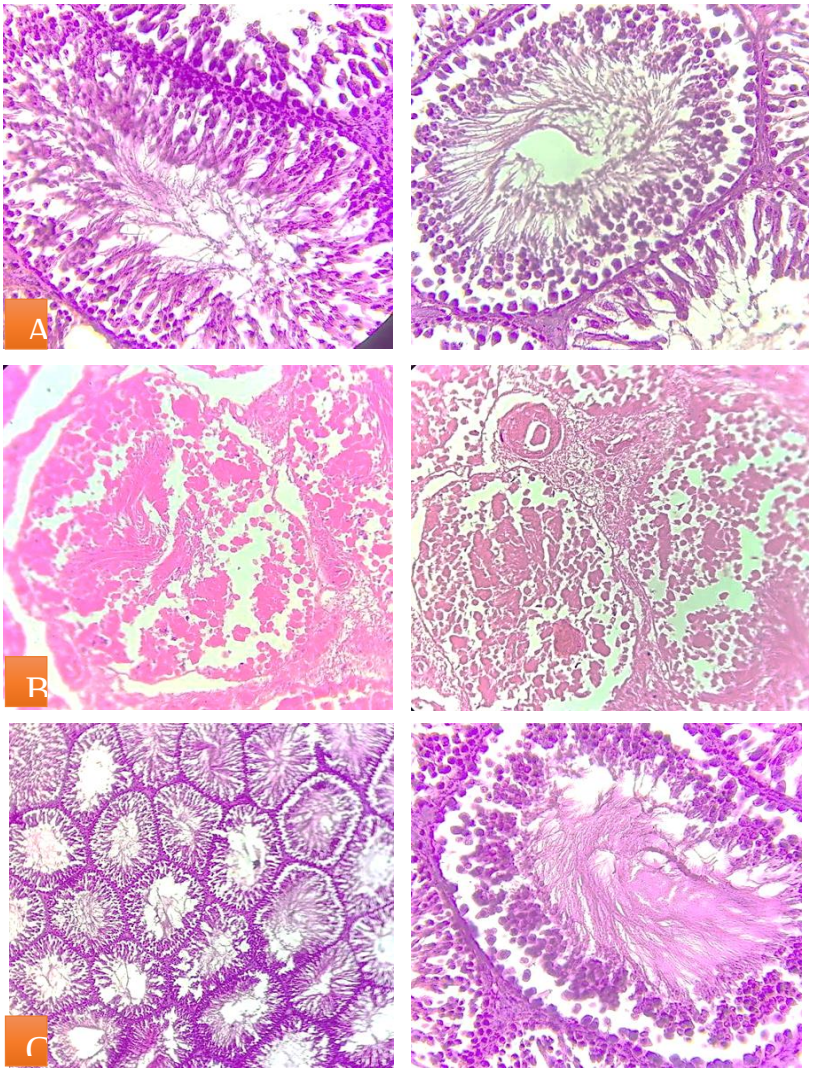

The histopathological section of testis

A- histopathological section of control rats

B- histopathological section **negative group: intraperitoneal injection of cyclophosphamide**

C- histopathological section of the animal that was **administered of** *Moringa* ***Pterygosperma*** **by gavage at 100mg/kg after intraperitoneal injection of cyclophosphamide**

D- histopathological section of the animal that **administered of** *Moringa* ***Pterygosperma*** **by gavage at 100mg/kg after intraperitoneal injection of cyclophosphamide**

Summary

Finally, our study found that is significant improv of the treatment group with aquatic extract of *Moringa Pterygosperma* and have product effect of the adverse effect of

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cyclophosphamide on the reproductive parameters included sperm motility, sperm viability, sperm morphology, acrosomal integrity, and testicular degeneration

Ethics

All the procedures in this study, including animal husbandry, handling, and scarifying were performed according to the guidelines instructed by the Animal Ethics Committee of the University of Kufa, Najaf, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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