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Research Article

Investigation of Fertility Promoting Effect of Typha Capensis in Male Rats

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KEY WORDS:

Typha
Capensis
Infertility

Abstract:

One of the medicinal plants frequently used to manage health with male fertility is Typha capensis. The aim of the present study was to investigate at how it impacted fertility in a rat model of cadmium-induced infertility. In this experiment, 30 male rats weighing 150 to 250 g were employed. The animals divided into five groups, six male rat of each as; group A: was treated with 0.5ml normal saline solution only. Group B: treated with CdCl2 (2.5mg/kg) and 0.5 ml of normal saline ($NaCl_2$ 0.9%) solution served as control group. Group C: treated with $CdCl_2$ (2.5mg/kg) plus T. capensis extract at 120 mg/kg. Group D: treated with CdCl2 (2.5mg/kg) plus T. capensis extract at 200 mg/kg and Group E: $CdCl_2$ (2.5mg/kg) plus T. capensis extract at 400 mg/kg. Gavage was used once a day for 28 days to treat the animals orally. The animals were sacrificed through carbon dioxide sedation after 28 days. The testes and epididymis were harvested through an abdominal midline incision. All clinging tissues were removed, the swollen dried the weights were taken promptly. Histological assessment of the samples was conducted. the absolute weight of testes and epididymis of experimental animals improved by treating with T. capensis significantly ($p<0.05$) differences between group A, D and E when compare with group A that served as control group, whereas group C showed no-significant variance in comparison with group A. The group treated with T. capensis extract demonstrated high sperm counts in comparison to the $CaCl_2$-treated control group, for group treated with extract doses 120, 200 and 400 mg/kg, respectively, with highly significant differences between group D and E in comparison with group B as a control group, whereas the results exhibited non-significant differences between group C treated with 120mg/kg of extract in comparison with group B control. The results also show significantly higher increase in sperm count at group A saline group in comparison with group B control. Our findings showed that T. capensis had significant protective effects with concentration of (200 and 400 mg/kg of TCE) that resulted in optimal sperm production when comparison with control group. The current study's findings demonstrate that T. capensis was able to prevent infertility induced oxidative stress caused on by $CaCl_2$ treatment. Histological examinations show a significant form of protection against cadmium-induced tissue necrosis, which was sufficient to result in increased sperm production.

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INTRODUCTION

Typically, infertility is described as a couple's inability to conceive following a year of frequent, unprotected sexual contact (Kumar and Singh[1]). It affects at least 180 million people worldwide and 15% of all couples in the United States. Male infertility is defined as the inability of a male to successfully become pregnant with a fertile female after at least one year of unprotected sexual activity. About 20% of all cases of infertility are completely the male's fault, while another 30%-40% have the male's involvement. In order to effectively control infertility, both partners must be evaluated because male and female causes frequently coexist. The male component contributes significantly to around 50% of all cases of infertility overall (Jaiswal[2]). There are a number of risk factors for male infertility, including alcohol consumption, medication use, scarring from STDs, obesity, tobacco use exposure to heavy metals as cadmium chloride (CdCl2). Low amounts of cadmium are found naturally in the Earth's crust. It occurs frequently in combination with different elements, such as oxygen and sulfur, as well as carbonate and chloride, to create the compounds cadmium sulfate, cadmium oxide, cadmium carbonateCdCl2, respectively (M Brzóska[3]). Management of male infertility is crucial because it impacts not only the infertile couple but also the phenomena of reproduction as a whole. Male infertility is a global health concern. Understanding the unique contributing factors to male infertility in each location might aid healthcare professionals and policymakers in developing effective management strategies. Planning to completely identify and treat infertile males is not possible without precise and accurate data from the region (Jafari[4]). Exposure to heavy metals has been linked to male infertility. One of the heavy metals is cadmium ion (Cd2+or Cd). Numerous studies using mostly rodent-based animal models and accumulating data from epidemiological studies on humans indicate that cadmium has a negative impact on male fertility (Zhu and Ge[5]). Over 70 million couples are infertile worldwide the majority of them live in developing regions (Vander Borght and Wyns[7]). According to the WHO, infertility is a global public health issue that could endanger the security of people, partnership seven entire communities. In less developed countries, the 12-month prevalence rate of infertility ranges from 6.9-9.3 percent (Inhorn and Patrizio[8]). The bulrush, Typha capensis, is a member of the Typhaceae botanical family. Ibuma (Zulu), bulrush (English)papkuil are some of its other synonyms (Afrikaans). The plant is widespread throughout South Africa, with the exception of the North Western Cape, where it is rare. It often inhabits moist or occasionally damp environments (Musara and Aladejana[15]). This study therefore was conducted to improve the promoting effects of T. capensis in a male of rat model of CdCl2 induced infertility.

MATERIALS AND METHODS

Experimental Design: This study was laboratory based study. The animals divided into five groups, six male rat of each as following:

Group A: was treated with 0.5ml normal saline solution only.

Group B: treated with CdCl2 (2.5mg/kg) and 0.5 ml of normal saline (NaCl2 0.9%) solution served as control group.

Group C: treated with CdCl2 (2.5mg/kg) plus T. capensis extract at 120 mg/kg.

Group D: treated with CdCl2 (2.5mg/kg) plus T. capensis extract at 200 mg/kg.

Group E: CdCl2 (2.5mg/kg) plus T. capensis extract at 400 mg/kg.

Gavage was used once a day for 28 days to treat the animals orally. The animals were sacrificed through carbon dioxide sedation after 28 days. The testes and epididymis were harvested through an abdominal midline incision. All clinging tissues were removed, the swollen dried the weights were taken promptly.

Study Animals: In this experiment, 30 male rats weighing 150-250 g were employed. They were collected from the animal house at the University of Al-Nahrain/College of Medicine. Before the experiment started, the animals were given a week to acclimate to their new surroundings. The rats were kept in grouped cages in an environmentally controlled room with a 12:12 h light/dark cycle and a temperature of around 25 °C. Standard rat pellets were provided to them they were given unlimited access to clean tap water.

Preparation of Aqueous Extract of T. Capensis: T. capensis rhizomes were collected fresh from Iraq's southern marshes (al-Ahwar). The dirt was cleaned out of the newly mined material, which was then air-dried in the shade. Using a laboratory pestle and mortar, the

dried rhizomes were mashed into pulp. For 48 hours, 300 grams of pulverized material were macerated in 1000 mL of distilled water. A hot-air oven at 40 °C was used to filter the mixture and dry the filtrate.

Sperm Collection Method and Analysis: A protocol previously reported by (Sewani-Rusike[9]) was used to collect seminal content of epididymis by cutting of the cauda epididymis, which is the primary sperm storage prior to ejaculation, using surgical blades. Under a light microscope, the sperm count was measured using a Neubaur hemocytometer (Deep 1/10 mm, Labart, Germany).On the hemocytometer, a cover slip was inserted. Using a micro pipette, a drop of caudal sperm solution (10 µL) was loaded under the cover slip. The hemocytometer was then examined under a microscope (Olympus) at a magnification of x40. The number of sperm cells was counted and expressed in millions per milliliter.

Histological Examination: The histological alterations in testicular tissue were assessed using a protocol modified by (Abdel-Magied[10]). The testes were fixed in 10% formalin. The specimens were dehydrated in ethanol at various concentrations before being embedded in paraffin. A rotary microtome was used to cut tissue sections of 5 µm thickness, which were then stained with hematoxylin and eosin (H and E) and viewed under a light microscope.

Statistical Analysis: The results were presented as means±standard error (mean±SE). The data was analyzed using Graph Pad prism program version 9 for Windows. The differences between the means of different groups versus the control group were compared using one way analysis of variance with Dunnett's post-test with $p<0.05$.

RESULTS AND DISCUSSIONS

Sperm Count: (Fig. 1), shows the impact of T. capensis extract treatment on the animals' sperm count. The group treated with T. capensis extract demonstrated high sperm counts in comparison to the CaCl2-treated control group, for group treated with extract doses 120, 200 and 400 mg/kg, respectively, with highly significant differences between group D and E in comparison with group B as a control group (2.41±0.24, 3.63±0.2 Vs. 1.35±0.33x107 mm-3), whereas the results exhibited non-significant differences between group C treated with 120mg/kg of extract in comparison with group B control (1.56±0.36Vs.1.35±0.33x107mm-3). The results also show significantly higher increase in sperm count at group A saline group in comparison with group B control (2.7±0.04 Vs. 1.35±0.33x107 mm-3).

Histopathological Examination:

A: Histological appearance of Group A: was treated with 0.5ml/kg normal saline solution only.

Histological examinations were carried out to determine the structural impacts of daily dosing of T. capensis on testicular tissue. (Fig. 2), demonstrating normal histological appearance of rat's testes with clear cell generation, blood vessels and leyding cells. **B: Histological appearance of Group B:** treated with CdCl2 (2.5mg/kg) and 0.5 ml of normal saline (NaCl2 0.9%) solution.

(Fig. 3), shows histological appearance of rat's testes with completely deterioration of normal structure with heavy infiltration of inflammatory cells and necrosis.

C: Treated Groups with T. Capensis at Different Concentration:

Histological Appearance of Group C: treated with CdCl2 (2.5mg/kg) plus T. capensis extract at 120 mg/kg.

Fig. 4, illustrates distinct but undifferentiated cell generations that are seen within the seminiferous tubules with slightly improvement.

Histological Appearance of Group D: treated with CdCl2 (2.5mg/kg) plus T. capensis extract at 200 mg/kg.

(Fig 5), shows completely cell repair to the normal structure of seminiferous tubules.

Histological Appearance of Group E: CdCl2 (2.5mg/kg) plus T. capensis extract at 400 mg/kg.

(Fig. 6) (3-1 and 3-2), shows apparently cell repair and regeneration of the cells inside the tubules, leyding cells and new blood vessels generation.

Weight of Testes and Epididymis of Experimental Animals: (Table 1), shows the absolute weight of testes and epididymis of experimental animals with highly significance differences between group A, D and E accordance to weight of testes when compare with group A that served as control group, whereas group C showed no-significant variance in comparison with group A. The result also showed statistically difference between group E in comparison with group A control in case of epididymis weight whereas the other groups showed no-significant differences when comparison with control although there are increase in the weight epididymis.

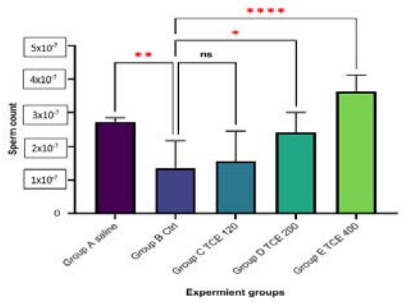

Fig. 1: Effect of T. capensis extract on rat's sperm count. The data presented as mean±SE, N: 6, p<0.001 for five experimental groups

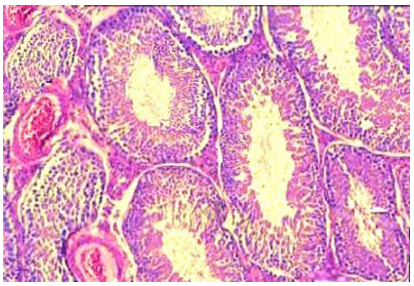

Fig. 2: Cross section in testes of rats treated with normal saline only showing A: sperm forming cells, B: median vacuole (seminal fluid space) and C: Leyding cells (power magnification 100x, H and E stain)

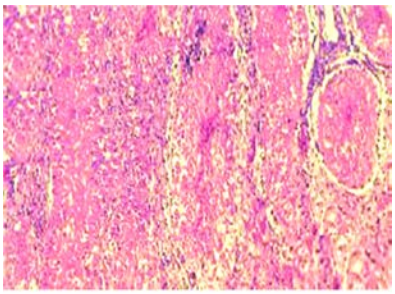

Fig. 3: Demonstrates white blood cell infiltration in the necrotic interstitial tissue along with deteriorated and damaged seminiferous tubule cellular components

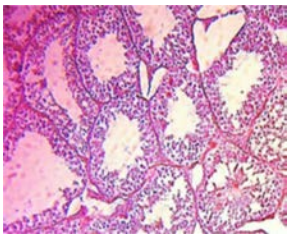

Fig. 4: section revealed, A: Decreased seminiferous tubule diameters, irregular shape disintegration of the layers of spermatogenic cells, B: decrease leyding cells C: broadening of the median gap in the seminiferous tubule (power magnification 100x, H and E stain)

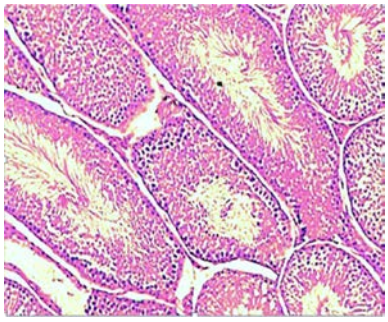

Fig. 5: Section revealed obvious improvement of seminiferous tubules, a: The regularity of the layers of spermatogenic cells and the presence of free sperm inside the lumen of the seminiferous tubules, b: cell repair to the normal structure (power magnification 100x, H and E stain)

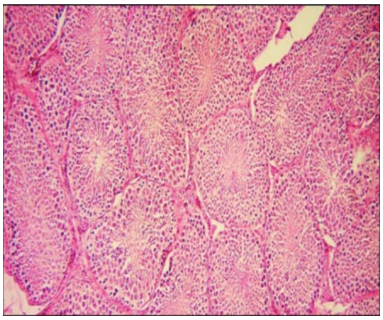

Fig. 6 (3-1): Section revealed, a: increase in the number of layers of spermatogenic cells in the tubular wall, b: evident increase in the number of sperms and a decrease in the lumen (power magnification 100x, H and E stain).

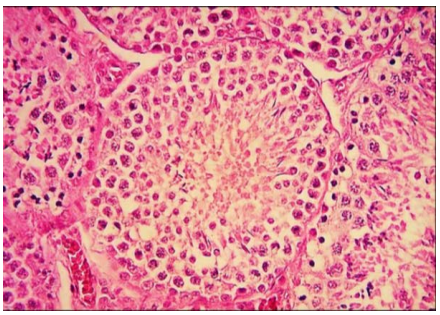

Fig. 6 (3-2): section revealed, A: high density of spermatogenic cells, B: tubules gap filled with sperm (power of magnification 400x, H and E stain).

Table 1: weight of testes and epididymis of experimental animals.

Groups, dose	Testes Mean±SE	Epididymis Mean±SE
CdCl2 (2.5mg/kg)+0.5 ml of saline (ctrl)	±0.3 2.5	±0.1 1.3
Saline 0.5ml/kg only	±0.99a 3.8	±0.03 1.4
CdCl2 (2.5mg/kg)+T. capensis 120 mg/kg	±0.2 2.6	±0.2 1.2
CdCl2 (2.5mg/kg)+T. capensis 200 mg/kg	±0.4a 3.3	±0.4 2.1
CdCl2 (2.5mg/kg)+T. capensis 400 mg/kg	±0.6a 4.01	±0.2a 2.9

Data expressed as mean± standard error, a: p. value significant <0.05 when compared with group A control.

One of the main factors impacting male reproductive potential and many other chronic disorders is oxidative stress (OS), which has been identified (Zhaku[6]). Due to their abundance of unsaturated fatty acids with numerous double bonds and their capacity to produce ROS, particularly superoxide anion and hydrogen peroxide, human spermatozoa are susceptible to oxidative damage. However, by maintaining the proper pro-oxidant antioxidant balance (oxidative stress) and sperm's cellular oxidative stress, antioxidants including vitamins E and C and carotenoids help prevent DNA damage (Abbasi[16]). Natural antioxidants are commonly found in medicinal plants. One of the medicinal plants used in the Eastern Cape of South Africa to treat male reproductive issues traditionally is T. capensis. In this study, a rat model exposed daily to the industrial and environmental toxins cadmium chloride was used to observe the protecting benefits of T. capensis rhizome extract against infertility caused by oxidative stress. Our findings showed that T. capensis had significant protective effects with concentration of (200 and 400 mg/kg of TCE) that resulted in optimal sperm production when comparison with control group as shown on (Fig 1). The tissue necrosis caused on by CaCl2 can be implicated for the reduced sperm count shown in Fig. 1 in the treated animals and these changes improved by seminiferous tubules in the rats'

Testes were damaged and degenerating, as seen by the photomicrograph findings in the CaCl2-treated control group as shown on (Fig 3). One of the essential aspects of fertility is sperm counta decline in sperm count might reduce the probability of a healthy pregnancy (Kooti[11]). Because T. capensis's protective impact from its antioxidant property was sufficient in the animal model confronted with CdCl2 daily for a month, treatment of rats with the aqueous extract of T. capensis rhizome induced a significant increase in sperm count at concentrations of (200 and 400mg/kg) as shown on figures (5, 6 3-1 and 6 3-2 respectively) and modest non-significant improvement in sperm count at concentration (120mg/kg) as shown on (figure 4) when compared with control group. The animal groups that received the extract, however, showed evidence of cell repair at varying doses, with the higher doses (200 and 400 mg/kg) appearing to show signs of apparent cell repair and regeneration of the cells inside the tubules, the generation of leyding cells and new blood vessels and a high density of spermatogenic cells, with the tubules gap filled with sperm as shown on figure (5, 6). This finding raises the possibility that T. capensis might be able to renovate the normal cell architecture, which would enhance the amount of sperm cells in the current study. CaCl2 administration caused reactive oxygen species, which likely lowered spermatogenic cell activity and decreased the density of spermatogenic cells in the seminiferous tubules (Siuet[13]). Excessive free radical production can compromise the anti-oxidant functions of enzymes like peroxidase, superoxide desmidiaceae catalase and result in damage and other deadly cellular effects by oxidizing cellular proteins, membrane lipids, DNAenzymes, impairing cellular respiration (Carocho and Ferreira[12]). The ability of T. capensis to prevent the generation of free radicals has been demonstrated and reported previously (Henkel[14]). Antioxidative protecting effect of T. capensis can also improve by significantly increase in the weight of testes and epididymis of animals treated with TC extract especially at concentration of (200 and 400 mg/kg) when compared with control group as shown on (table 1). These result may explain why there was a statistically significant increase in the level of sperm between the CaCl2-treated control group and all of the T. capensis-treated groups, indicating that the test extract was able to sufficiently protect against infertility induced on by cadmium exposure.

CONCLUSION

The current study's findings demonstrate that T. capensis was able to prevent infertility induced oxidative stress caused on by CaCl2 treatment. Histological examinations show a significant form of protection against cadmium-induced tissue necrosis, which was sufficient to result in increased sperm production.

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