Histopathological Reproductive Toxicity Of Methanolic Fruit Extract Of *Citrullus Colocynthis* In Adult Male Albino Rats: Rising Approaches Of Herbal Male Contraceptives.

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Abstract

This investigation on the histopathological reproductive toxicity of *Citrullus colocynthis* fruit extract in adult male albino rats was the goal of the study. To find a reversible male contraceptive, male rats were given the 50% petroleum ether extract of *C. colocynthis* fruits orally at doses of 50, 100, and 200 mg/kg body wt/day for 60 days. For a light microscopic examination, samples from the epididymis, prostate, vas deferens, and seminal vesicles were gathered. All organs' epithelium and connective tissues experienced histological changes as a result of the fruits of *C. colocynthis*. In comparison to controls, interluminar space was increased.

Key Word: histological reproductive toxicity, epithelium, reversible.

Introduction

Due to enduring gender-restrictive norms and inequities, familial arrangements, and other cultural contextual factors, women in India continue to bear the brunt of family planning (Seth *et al.*, 2020). In India and other places, where cultural factors, traditional family roles, gender equality values, and patriarchal gender order tend to undermine women's empowerment, few studies have looked into the role of men and community level factors (Gruber and Szotysek 2016; Cislaghi *et al*. 2020; A. Singh *et al*. 2021). In India, there is evidence of positive associations with the use of contraceptives among uneducated women (McNay...
et al., 2003) for those who have more influence over household decisions and are currently employed, but mixed results when looking at other facets of empowerment (Reed et al., 2016; S. K. Singh et al., 2019). In India, 66.4% of married women reported a need for family planning, and the majority of them (about 70%) utilised contraception and favoured contemporary to traditional methods. Additionally, among women seeking family planning, female sterilisation remained the most preferred strategy (54.2%) (Mejía-Guevara et al. 2021).

Numerous contraceptive techniques have been created for women, but males must also be adequately taken into account (Soni et al., 2015; Thirumalai and Amory, 2021). the need to replace these synthetic agents with effective, safe alternatives based on plants stems from the fact that synthetic contraceptives used for fertility control have a number of negative side effects, including weight gain, hypertension, an increased risk of cancer, and hormonal imbalance (Soni and Mali, 2017).

Many fundamental human requirements, including a range of therapeutic drugs, have been met by plants (Alagawany et al., 2021; Dhama et al., 2021). Therefore, purposeful cultivation efforts are essential for maintaining the supply of certain plant species. The use of medicinal plants in healthcare has a long history, and it is becoming more commonplace globally for both sickness prevention and treatment (Bilal et al., 2021; Reda et al., 2021; Saeed et al., 2021).

Male accessory genital organs play a significant part in the operation of the reproductive system, including the epididymides, prostate, vas deferens, and seminal vesicle (Chughtai et al., 2005). The general appearance and histology of these glands vary significantly among animal species (Thomson and Marker, 2006). The structural variations of these glands among different species might be well described by the anatomical research of accessory genital glands.

_Citrullus colocynthis_ is a desert plant that contains a variety of bioactive substances, including fatty acids, glycosides, flavonoids, alkaloids, and glycoside glycosides. Plant medicines strengthen the immune system. According to Li et al.2022, the dried fruit pulp of _C. colocynthis_ has been used to treat intestinal parasites, gastroenteritis, and indigestion. Excellent pharmacological qualities of _C. colocynthis_ include laxative and purgative effects, as well as anti-diabetic, anti-inflammatory, anthelmintic, and anti-cancerous effects. The fruit’s antibacterial, antioxidant, and anti-inflammatory properties have been the subject of substantial research (Hussain et al., 2014). Antioxidant, anti-inflammatory, anti-diabetic, and antibacterial properties are only a few of its therapeutic traits (Kamran et al., 2018). Its pharmacological attributes include antioxidant, hypoglycemic, antibacterial, anti-bacterial, anticancerous, anti-inflammatory, and analgesic effects. The effects of _C. colocynthis_ include anti-diabetic, anti-hyperlipidemic, anti-antineoplastic, profibrinolytic, anti-allergic, anti-microbial, pest-controlling, and immune-stimulating properties. It also has an impact on fertility and the reproductive system (Meybodi, 2020).
We investigated potential impacts on fertility as a consequence of many traditional usage and the chemical composition of *C. colocynthis* fruits. In order to create a secure and reversible male contraceptive medication, the current study was designed to explore the effects of the methanolic extract of *C. colocynthis* fruits.

**Materials and Methods**

**Identification of plant and preparation of 50 % Methanolic extract**

The Department of Botany, University of Rajasthan, Jaipur, verified the authenticity of the plant *Citrus colocynthis* (Family Cucurbitaceae) using specimen vouchers. Plant matter was pulverised after being shade-dried. According to the WHO procedure (WHO, 1983), this powder was submitted to Soxhlet extraction with 50% methanol for 24 hours (8 hours x 3 days). With a yield of 7.3%, 11 gm of solid material were produced from 150 gm of powdered *C. colocynthis* fruits.

**Animal model**

*Rattus norvegicus* albino males that were healthy adults weighing between 150 and 200 g were employed in the current investigation. The animals were housed in a controlled environment and were given standard maintenance.

**Treatment protocol**

The experiment was carried out to investigate the antifertility effects, potential mechanisms of action, and reversibility of the extract. For the study, five treatment groups of animals, each with ten animals, were needed. Rats from group A were given sterile water to drink on their own, while group B, C, and D animals received various amounts of extract—50 mg, 100 mg, and 200 mg/kg b. wt.—over the course of 60 days. Animals from group E were used as the recovery group; they received a 100 mg dosage daily for 60 days while being held for a 30-day recovery period.

**Histopathological study**

Reproductive organs from the control and treatment groups' contralateral sides were separated and preserved in Bouin's fluid. For histological analysis, the tissues were prepared, and slices of paraffin wax (melting point 55°–62°C) were cut at a thickness of 6 µ, and they were stained with Harris's hematoxylin and eosin to detect histopathological alterations under a light microscope.

**Ethical aspects**

The study was conducted with the approval of the Department of Zoology's ethical committee at the University of Rajasthan in Jaipur, and CPCSEA guidelines were followed (CPCSEA, 2006)
Results and Discussion

There was no significant decrease in body weight after treatment with *C. colocynthis* fruit extract. Serological markers, including SGPT, SGOT, Acid Phosphates, Alkaline Phosphates, Cholesterol, and LDH, did not significantly change in any of the treatment groups compared to the control group in the previous study (Sharma and Chaturvedi 2016).

The epididymis plays a significant role in male fertility, and it can cause azoospermia if its function is disturbed (Soni *et al.*, 2015). Male control rats' caudal epididymis was found to include intertubular compartments and elongated or spherical tubules that were bordered by columnar epithelia that contained primary and basal cells with stereocilia. According to Tolba and Mandour (2018), the stereocilia of epididymal cells have a significant surface area for molecular interaction and display a variety of transport mechanisms and membrane pumps to maintain the vital volume in the epididymides' lumen. In the current study, the epithelial cells were changed from columnar to flat cells and the epithelium of the epididymis' secretory activity decreased, affecting the environment of the epididymal lumen and interfering with the maturation of spermatozoa.

In control groups of mature male albino rats, the cauda epididymis was made up of circular tubules with many sperm in their lumina. The epithelial cells, particularly primary cells in the proximal caudal epididymis, and the size and quantity of clear cells in the area between the proximal and distal caudal epididymis, considerably decreased as a result of *C. colocynthis* fruit extract. Other hazardous drugs like cyclophosphamide and gossypol have also caused these histological alterations in the rats, indicating enhanced phagocytosis inside these epithelial cells. (de Andrade *et al.*, 2006; Mohamed *et al.* 2012).

After extract administration, it was shown that the seminal vesicle's histoarchitecture was negatively affected, with the connective tissue and muscles being disrupted, as well as the lumen's secretion being drastically reduced. According to this study, the *C. colocynthis* fruit extract may improve the quality of the semen in male rats by changing how the seminal vesicle operates (Prashanthi *et al.*, 2006). According to our observations, the seminal vesicle epithelium has undergone significant cell size decrease. The seminal vesicles of the albino rats treated with the extract revealed little secretions within the seminal acini, whereas other acini lacked secretions. The seminal vesicles' acini are bordered with vacuolated epithelium that has very big irregular hyperchromatic nuclei in irregular shapes. According to Afza *et al.* (2017) and Tolba and Mandour (2018), the change is most likely the result of decreased androgen stimulation to these target organs. As a result, the data collected show that the therapy had a negative impact on the functionality of the male reproductive system.

According to histopathological analysis, the vas deferens in control rats seems to be a muscular tube with thick walls. There is a mucosal lining with longitudinal folds and an irregular lumen contour. After extracts were administered, the epithelium of the vas deferens had degenerative changes, and there were no sperm in the lumen, which may have been caused by a drop in testosterone levels. According to Soni *et al.* (2016), the structural change in the vas deferens may cause infertility and carcinogenicity. The anatomy of...
the vas deferens in control group mice displays thick-walled muscle tubules with two longitudinal layers and an intermediate circular layer bordered with pseudostratified epithelium. After administering the plant extract, lower doses caused vas deferens epithelial layer degeneration, but higher doses caused animals to exhibit striking vas deferens degenerative alterations.

Rats from the treated group showed a substantial interstitial oedema and a ventral prostate with considerably lower epithelial height. These results are consistent with another study that found that nicotine treatment for 90 days caused prostate epithelial cells to atrophy in rats (Carvalho et al., 2006). The form and size of the prostatic acini were disturbed in the adult male albino rats treated with *C. colocynthis* fruit extract, according to the ventral prostate's histological structure. When compared to the control groups, the acini exhibited epithelial prostatic hyperplasia and were surrounded by stromal hyperplasia. The prostate plays a significant role in male reproduction, and its secretion is important for sperm to function normally. After treatment, there was little secretion and disorganised, thin cuboidal epithelial cells lined the lumen, demonstrating the antifertility effect of *C. colocynthis* fruit extracts. By controlling blood flow to the prostate, Cristina and a colleague showed that androgen levels affect prostate growth (Cristina et al., 2014).

**Conclusion**

The results of the current investigation indicate that *C. colocynthis* fruits have the ability to reduce male fertility without affecting general metabolism. Because of this, the potential male contraceptive efficacy of the fruit extract from *C. colocynthis* cannot be disregarded, allowing for the smooth growth of clinician interest in clinical studies leading to the creation of a strong herbal male contraceptive.

**References**


CPCSEA (2006) Committee for the purpose of control and supervision on experiments on animals. ICMR, New Delhi.


WHO 1983. Protocol CG-04, Preparation of alcoholic extract for bioassay and phytochemical studies (APJF/IP, 100 1A) Geneva.
Plate: A Histoarchitecture of cauda epididymis
Fig. 1- Control rats
Fig. 2- 50 mg/kg b.wt./day for 60 days treated rats
Fig. 3- 100 mg/kg b.wt./day for 60 days treated rats
Fig. 4- 200 mg/kg b.wt./day for 60 days treated rats
Fig. 5- Recovery group of Rats

Plate: B Histoarchitecture of caput epididymis
Fig. 1- Control rats
Fig. 2- 50 mg/kg b.wt./day for 60 days treated rats
Fig. 3- 100 mg/kg b.wt./day for 60 days treated rats
Fig. 4- 200 mg/kg b.wt./day for 60 days treated rats
Fig. 5- Recovery group of Rats
Plate: C Histoarchitecture of seminal vesicle
Fig. 1 - Control rats
Fig. 2 - 50 mg/kg b.wt./day for 60 days treated rats
Fig. 3 - 100 mg/kg b.wt./day for 60 days treated rats
Fig. 4 - 200 mg/kg b.wt./day for 60 days treated rats
Fig. 5 - Recovery group of Rats

Plate: D Histoarchitecture of vas deferens
Fig. 1 - Control rats
Fig. 2 - 50 mg/kg b.wt./day for 60 days treated rats
Fig. 3 - 100 mg/kg b.wt./day for 60 days treated rats
Fig. 4 - 200 mg/kg b.wt./day for 60 days treated rats
Fig. 5 - Recovery group of Rats
Plate: E  Histoarchitecture of ventral prostate
Fig. 1- Control rats
Fig. 2- 50 mg/kg b.wt./day for 60 days treated rats
Fig. 3- 100 mg/kg b.wt./day for 60 days treated rats
Fig. 4- 200 mg/kg b.wt./day for 60 days treated rats
Fig. 5- Recovery group of Rats