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E: ISSN NO.: 2455-0817 *Remarking An Analisation*

# Pharmacological and Anti-fertility Activities of Some Medicinal Plants

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**Abstract**

Plants and the plant- derived products have been used by humans for various ailments from a very long period of time due to their minimal to no side effects. The present study was conducted for evaluating the pharmacological activity and the anti-fertility potential of fruits of *Citrullus colocynthis* and *Delonix regia* on the reproductive ability of rats. The use of these plants in ethno-medicine and pharmacological purpose have been reported in Indian traditional medicine system (Ayurveda), and several researchers have explored various pharmacological properties like anti-inflammatory, anti-fungal, anti-bacterial, anti-oxidant, analgesic, or anti-proliferative, anti-fertility and abortive functions.

**Keywords:** *Citrullus colocynthis*, *Delonix regia*, Anti-Fertility, Pharmacology.

**Introduction**

Use of medicinal plants have in traditional system has been reported from ancient era for human health care and now-a-days increased due to their minimal side effects. These traditional medicines, much of which is based on the plant based remedies supports over 80% of global population. Research on the medicinal plants ha increased many folds during these recent years. Many researches has been reported that proves the significant potential of medicinal plants in traditional, complementary and alternate system in treatment of human diseases. (Mali *et al.*, 2015).

*Citrullus colocynthis* is a perennial plant belonging to family cucurbitaceae. The common name of the plant is: colocynth, bitter apple, bitter cucumber, bitter gourd. The fruit pulp of the plant has been reported to offers various medicinal properties.(De Smet, 1997). The use in traditional medicine of this plant has been reported for diabetes, bronchitis, asthma, cough, joint pain, leprosy, common cold, jaundice, cancer, toothache, mastitis, wounds and in gastro-intestinal disorders like dysentry, constipation, indigestion, gastroenteritis, colic pain and various microbial infections.(Hussain *et al.*, 2014)

*Delonix regia* is an ornamental semi-deciduous tree belonging to family fabaceae. The tree is found to be distributed throughout Madagascar, Northern Australia, India, and Africa (Indian Medicinal Plants-Dictionary, 2007). The plant has been reported as a traditional medicine used in conditions like rheumatism, inflammation, diabetes, anemia, fever, bronchitis, pneumonia and gynecological disorders (Vargas *et al*. 2010).

**Objective of the Study**

The present study was conducted for evaluating the ethno-medical properties of the methanol extracts of fruits of *Citrullus colocynthis* and *Delonix regia*. These plants were tested for any observing any potential effects on testes and reproductive functions and fertilizing ability of wistar rats.

**Materials and Mathods**

**Animal Model**

The animals used for the conducted investigations were mature male adult wistar rats, weighing between 100-150 gm. All the rats were procured from local animal suppliers, Jaipur and were brought to acclimatization with the laboratory environment beforehand starting experimentations on them. They were kept in the animal house properly caged in polypropylene cages under standard humid conditions, temperature (25 ± 2°C) and required light (12 hr. light/dark cycle). The rats were fed with standard diet of rat pellet of Ashirwad Pvt. Ltd Chandigarh, India and water was provided at repeated time intervals. CPCSEA (INSA, 2000) and Institutional Animal Ethical Committee guidelines were strictly followed in handling the experimented animals.

**Plant Material**

The fruits of both the plants *Citrullus colocynthis* and *Delonix regia* specimens were collected from around the Jaipur district, Rajasthan, India. The collected fruits were washed, shade dried and dried in oven at 40°C as n additional step to get rid of any remaining moisture in the fruit samples, before grinding into a fine powder using the blender.

**Preparation of Crude Extract**

100 gm of powdered fruit extract of each plant was weighed and were then mixed with methanol and distilled water in 50% methanol solution in a beaker. The apparatus is then kept in a water bath at 55°C for 2-3 days. The extract was then filtered using whatmann filter paper (Jain *et al.,* 2011). The extract was then dried using rotary evaporator at 80°C and then is completely dried in oven at 40°C to obtain the dry powder of the extract which was stored at 4°C till further use.

**Experimental Design**

The conducted experiment involves 60 male healthy fertile wistar rats which were divided into three groups for treatment. Each group was marked as follows:

Group-1: Control treated

Group-2: Rats treated at 100 mg/kg body weight of *Citrullus colocynthis* extract for 60 days.

Group-3: Rats treated at 100 mg/kg body weight (b.wt.) of *Delonix regia* extract for 60 days.

Required amount of drug (extracts) was freshly prepared in deionized water (100mg/ml) and administered orally daily at 100 mg/kg b.wt. for 60 days. The drug dose level was calculated according to $LD_{50}$ for *Citrullus colocynthis* (Soufane *et. al*. 2013.), and for *Delonix regia* (*Rajabhau et.al.* 2011.).

**Histological Studies**

The standard technique of double haematotoxylin and eosin (HE) staining was opted for performing the histopathological studies on the experimented set of wistar rats. For autopsy the male reproductive organs and tissues were dissected out blotting free from blood. Fixation was done for 24 hours at room temperature. The organs were cut in slabs of 0.6 mm thick pieces, which were washed thoroughly under running tap water. These tissues were then kept immersed in 70% alcohol with refilling several times. Thereafter the tissues undergo dehydration in ethanol series, clearing in xylene, than embedded in paraffin at 55°C. These embedded tissues were then sectioned to 5 µm sections using rotary microtome for staining. The obtained sections goes through deparaffinization and hydrated through xylene, alcohol and distilled water. These are then immersed in haematoxylin stain for 5 minutes, which were than washed thoroughly under running tap water and rinsed in 70% ethanol which afterwards were counterstained with eosin stain, differentiated and dehydrated in alcohol series, cleared with xylene and were finally mounted in DPX. All the stained slides were observed under microscope and the best chosen were photographed at different magnification in binocular microscope with attached digital camera.

**Parameters**

For releasing the spermatozoa from the epididymal tubules, known amount of caudal epidymis has been tested in definite volume of normal physiological saline. The removal of tissue components was carried out and the sperm suspension was used for evaluating the parameters such as sperm count, sperm motility.

**Body Weight**

The initial and final body weights of the tested animals were recorded. These animals were sacrificed at the end of two, four, or twelve weeks under anaesthetizing 24 hrs after the last dose of the tested treatment duration.

**Sperm Motility**

The parameter of sperm motility was measured using the procedure given by Prasad *et. al.* (1972). The caudal epididymus was dissected, an incission was made and a drop of sperm suspension was squeezed out on a microscopic slide and placed on the neubauer chamber and observed under low magnification (10 X) in a microscope. Sperm counts were made using the hemocytometer. The chamber was focused on the WBC region. Sperm motility was determined by counting both non-motile and motile spermatozoa. A total of minimum 10-12 separate fields were selected and observed for count and the sperm motility was calculated. The Sperm motility was expressed as percentage ratio of motile sperms.

**Sperm Count/Density**

For observing the sperm count/density, the cauda epididyamal sperm suspension was sucked up to the 0.5 marks in WBC pipette. The suspension was then diluted up to the 11 marks with 5% sodium bicarbonate ($NaHCo_3$) and mixed thoroughly. Sodium bicarbonate acts as spermicide and kills the spermatozoa to facilitate counting. Then a drop of suspension was transferred to the Neubauer chamber and gently covered with cover slip. Spermatozoa were counted in 64 sub squares of the WBC counting regions. The sperm density was expressed in term of million spermatozoa /ml of the suspension.

**Fertility Index**

Treated males were cohabitated with normal adult cycling females in the ratio of 1:2 form 55 days of treatment. Thereafter the number of pregnant females was counted to get fertility for 5 successive day index. The fertility index of control and treated groups of animals were calculated by formula (Parker, 2006) mentioned below:

$$\text{Male fertility index} = \frac{\text{Number of males impregnating female}}{\text{Number of males cohabitated}} \times 100$$

**Statistical Analysis**

The values were expressed as Mean ±SEM. The significance of difference among the group was assessed using one students "t"- test. Symbols represent statistical significance as indicated:

$P \leq 0.05 \rightarrow$ a (group-1)

$P \leq 0.01 \rightarrow$ b (group-2)

$P \leq 0.001 \rightarrow$ c (group-3)

**Calculations**

Statistical calculations were based on the biology statistics. Standard error of the mean calculated by following formula:

$$\text{Standard Error (S.E.)}\ \sigma_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

Where

$\sigma_{\bar{x}}$= Standard error

n= no. of set

The significant test was calculated by the formula as given by fischer (1936).

$$\text{Standard Deviation (S.D.)}\ \sigma = \sqrt{\frac{\sum(x-\bar{x})^2}{n}}$$

$\sigma$ = Standard Deviation

x = Sample value

$\bar{x}$ = Sample mean

Sample mean is calculated using below formula:

$$\text{Mean}\ (\bar{x}) = \frac{1}{N}\sum_{i=1}^{N} x_i$$

Where, The mean is often denoted as $\bar{\mathbf{x}}$, pronounced "x bar," and even in other uses when the variable is not **x**, the bar notation is a common indicator of some form of mean. In the specific case of the population mean, rather than using the variable $\bar{\mathbf{x}}$, the Greek symbol mu, or **μ**, is used.

$$\text{Students "t" test (t)} = \frac{M_1 - M_2}{\sqrt{\frac{{SD_1}^2}{N_1} + \frac{{SD_2}^2}{N_2}}}$$

Where

$M_1$ = mean vale of control

$M_2$ = mean vale of treated

$SD_1$ = Standard deviation of first set of values

$SD_2$ = Standard deviation of second set of values

$n_1$ = Total number of values in first set

$n_2$ = Total number of values in second set.

**Ethical Aspects**

The study was approved by Institutional Animal Ethical Committee, School of Basic & Applied Sciences, Department of Zoology, Poornima University, Jaipur, Rajasthan (India), approved the study (2014PUSBAPHDO08403 dated 6 May 2017).

**Result**

**Effects on Body and Weight of Testes**

No changes were observed in final body weight of rats treated with plants extracts in comparison to control treated vehicles (Table-1). The weight of testes was decreased significantly rats treated with plants *Citrullus colocynthis* and *Delonix regia* extracts (Groups-2-3) treatment as compared to control rats Group-1. (Table-1).

**Changes in Sperm Motility in Rats with The Extracts Tretmant**

The sperm motility was decreased significantly in rats treated with plants *Citrullus colocynthis* and *Delonix regia* extracts treatment in comaparison with controls. (Table-1) .

**Table 1: Effect on weight of testes, sperm motility, sperm density and fertility index of Wistar rats treated with *Citrullus colocynthis* and *Delonix regia* at 100 gm /kg b.wt. for 60 days.**

| S.No | Body weight (%) | | Weight of Testes (mg/100g b.wt) | Sperm Motility (Cauda epididymides; %) | Sperm Density million/ml (Testes) | Fertility Index (%) |
|---|---|---|---|---|---|---|
| | Initial (gm) | Final (gm) | | | | |
| **Group–1 Control** | 134.00 ± 3.055 | 166.50 ± 3.337 | 1179.00 ± 2.745 | 68.00 ± 0.258 | 61.00 ± 0.471 | 97.60 ± 0.542 |
| **Group–2 *Citrullus colocynthis*** | 130.50 ± 3.001[a] | 155.00 ± 2.773[a] | 1094.50 ± 3.801[c] | 62.10 ± 0.277[c] | 35.10 ± 0.277[c] | 77.70 ± 0.597[c] |
| **Group–3 *Delonix regia*** | 123.50 ± 2.570[a] | 153.30 ± 2.761[a] | 1138.50 ± 2.668[c] | 55.00 ± 0.715[c] | 40.00 ± 0.715[c] | 87.70 ± 0.597[c] |

Data expressed as Mean ± S. E. and significance at P≤ 0.05 a, P≤ 0.01 b and P≤ 0.001 c

**Effects on Sperm Count**

The sperm density was decreased significantly in rats treated with plants *Citrullus colocynthis* and *Delonix regia* extracts treatment in comaparison to controls. (Table-1).

**Effects on Fertility Index in Rats Treated With Plants Extracts**

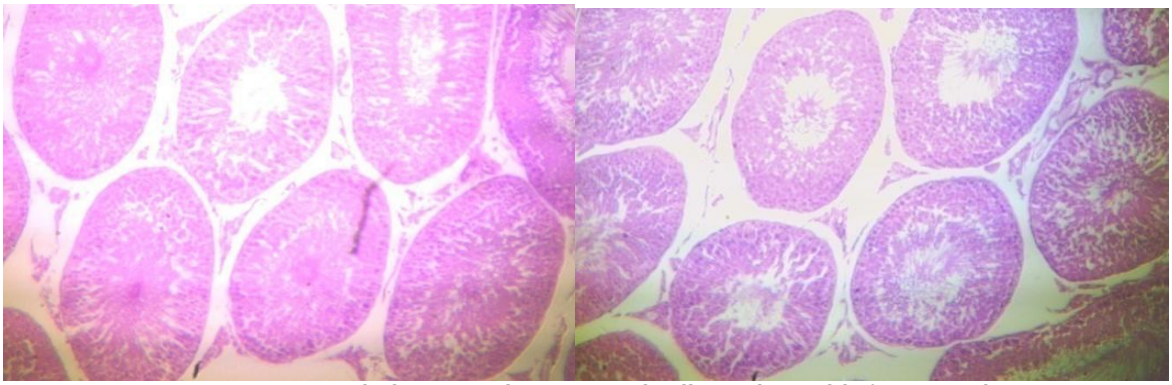
The fertility index was changed significantly in rats treated with plants *Citrullus colocynthis* and *Delonix regia* extracts treatment with comaparison with controls. (Table-1).

**Histopathological Changes in Testes of Rats Treated With Plants Extracts**

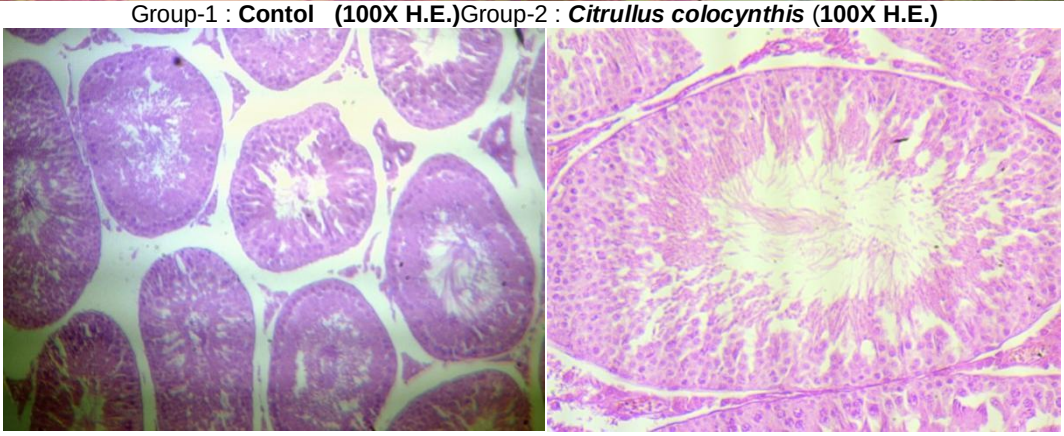
The histopathological changes in testes of rats treted with plants *Citrullus colocynthis* and *Delonix regia* extracts treatment with comaparison to controls. (Table-1).

**Photomicrographs of Testes Of Rats**

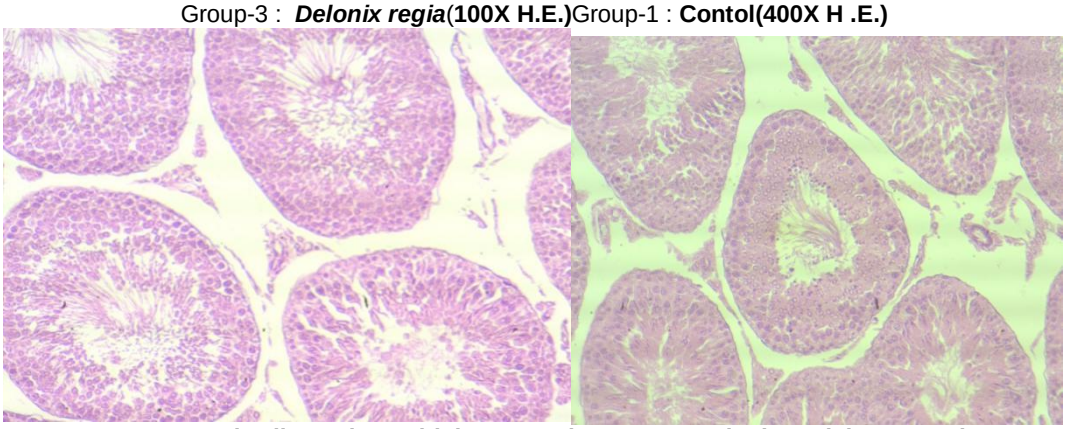
Photomicrographs of testes of rats (Photomicrographs 1-3) histopathology of testes of rats treated with *Citrullus colocynthis* and *Delonix regia* extract showed degeneration changes in germinal epithelium spermatocytes, spermatids and spermatozoa no of sperms decreased significantly after the treatment. Haematoxylin and Eosin (HE).



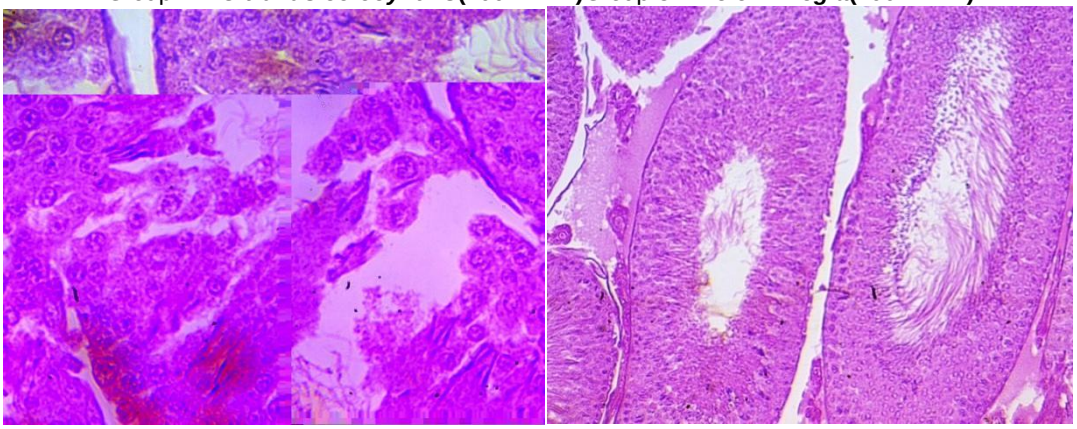
Group-1 : **Contol (100X H.E.)**Group-2 : ***Citrullus colocynthis*** (**100X H.E.)**



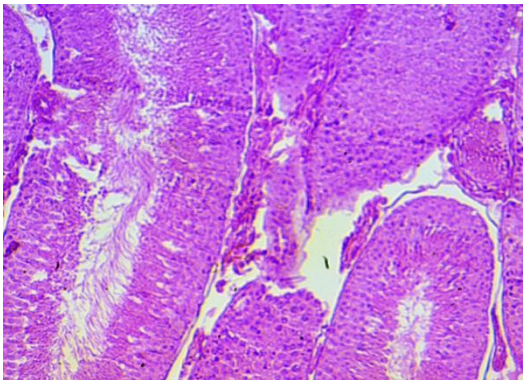
Group-3 : ***Delonix regia***(**100X H.E.)**Group-1 : **Contol(400X H .E.)**



Group-2 : ***Citrullus colocynthis*****(400X H.E.)**Group-3 : ***Delonix regia*****(400X H.E.)**



Group-1 : **Contol** (SCANNER)Group-2 : ***Citrullus colocynthis***(SCANNER)



Group-3 : ***Delonix regia***(SCANNER)

**Table 2 : Independent Samples "t" Test control and C*itrullus colocynthis***

| Independent Samples Test | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Variables** | **Group** | **Mean** | **sd** | **Std. Error Mean** | **t value** | **df** | **P value** |
| **Body weight Initial (gm)** | **Control** | 134.00 | 9.661 | 3.055 | 0.817 | 18 | 0.424 |
| | ***Citrullus colocynthis*** | 130.50 | 9.490 | 3.001 | | | |
| **Body weight Final (gm)** | **Control** | 166.50 | 10.554 | 3.337 | 2.650 | 18 | 0.016 |
| | ***Citrullus colocynthis*** | 155.00 | 8.769 | 2.773 | | | |
| **Weight of Testes (mg/100g b.wt)** | **Control** | 1179.00 | 8.679 | 2.745 | 18.022 | 18 | 0.000 |
| | ***Citrullus colocynthis*** | 1094.50 | 12.021 | 3.801 | | | |
| **Sperm Motility (Cauda epididymides; %)** | **Control** | 68.00 | .816 | .258 | 15.584 | 18 | 0.000 |
| | ***Citrullus colocynthis*** | 62.10 | .876 | .277 | | | |
| **Sperm Density million/ml (Testes)** | **Control** | 61.00 | 1.491 | .471 | 47.375 | 18 | 0.000 |
| | ***Citrullus colocynthis*** | 35.10 | .876 | .277 | | | |
| **Fertility Index (%)** | **Control** | 97.60 | 1.713 | .542 | 24.683 | 18 | 0.000 |
| | ***Citrullus colocynthis*** | 77.70 | 1.889 | .597 | | | |

**Table 3: Independent Samples "t" Test control and *Delonix regia***

| Independent Samples Test | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Variables** | **Group** | **Mean** | **sd** | **Std. Error Mean** | **t value** | **df** | **P value** |
| **Body weight Initial (gm)** | **Control** | 134.00 | 9.661 | 3.055 | 2.630 | 18 | 0.017 |
| | ***Delonix regia*** | 123.50 | 8.127 | 2.570 | | | |
| **Body weight Final (gm)** | **Control** | 166.50 | 10.554 | 3.337 | 3.047 | 18 | 0.007 |
| | ***Delonix regia*** | 153.30 | 8.731 | 2.761 | | | |
| **Weight of Testes (mg/100g b.wt)** | **Control** | 1179.00 | 8.679 | 2.745 | 10.581 | 18 | 0.000 |
| | ***Delonix regia*** | 1138.50 | 8.436 | 2.668 | | | |
| **Sperm Motility (Cauda epididymides; %)** | **Control** | 68.00 | .816 | .258 | 17.103 | 18 | 0.000 |
| | ***Delonix regia*** | 55.00 | 2.261 | .715 | | | |
| **Sperm Density million/ml (Testes)** | **Control** | 61.00 | 1.491 | .471 | 24.523 | 18 | 0.000 |
| | ***Delonix regia*** | 40.00 | 2.261 | .715 | | | |
| **Fertility Index (%)** | **Control** | 97.60 | 1.713 | .542 | 12.279 | 18 | 0.000 |
| | ***Delonix regia*** | 87.70 | 1.889 | .597 | | | |

**Discussion**

Although many compounds have been used to control function of male reproductive systems especially testes to control fertility in male, however, herbal plants extracts have been also practiced in traditional system because they are safe sine ancient tunes many plant either extract or their metabolites have been used for fertility controles. *Daccues carota. Carica papaya, Abrus precatories* etc (Sharma *et al.,* 2017). Plants *Citrullus colocynthis* and *Delonix regia* have been used in traditionally to cure different diseases. Therefore, in present study methanolic extracts of *Citrullus colocynthis* and *Delonix regia*(fruits) was prepared and administered orally in male Wistar rats. The results of the study exhibits that treatment of the extracts in rats caused reduction of weight testes, sperm motility and sperm counts and degenerative changes in testes.

Since androgans, FSH and LH are essential for the production of the normal sperm density, sperm motility (Gupta *et al*., 2018 ; Sharma & Kalla, 1994 ).The treatment caused degereratnie changes in sperm to genesis *Citrullus colocynthis* and *Delonix regia* treatment inhabit spermatogenesis might be due to decreased level male harmones Since testosterone regulates the growth and development of reproductive organs and spermatogenesis Wreford, & Robertson, 1994 Gupta *et al* 2018. Spermatogenesis process the Sertoli cells and Leydig cells cooperation requires for the development seminiferous tubules and germinal cells. Histopathological observations of *Citrullus colocynthis* and *Delonix regia extracts* treated rats showed reducation of the Leydig cells our degenerative changes in spermatogenesis evidence that the treatment of extracts of the plants damage of spermatogenesis (Born *et al.,* 1988). Similar observations have been also reported by the previous researcher in the rat model (El-Dwairi & Banihani, 2007). In the present study, increased androgen production after *Citrullus colocynthis* and *Delonix regia* treatment is reflected by the increased number of mature Leydig cells and their functional status. It was also justified by the enhanced number of spermatocytes and spermatids as these stages are completely androgen-dependent (Agrawal, Chauhan, & Mathur, 1986). Methanolic extrats of *Citrullus colocynthis* and *Delonix regia* treatment significantly reduced sperm density, sperm motility including fertility indices in treated rats might be due decreased androgen levels.

**Conclusion**

In conclusion, our all results Histopathological observations of *Citrullus colocynthis* and *Delonix regia extracts*treated rats showed degenerative changes in seminiferous tubules, decreased number of spermatogenic elements spermatozoa in testes reflects antispermatogenic nature of the treatment, further, decreased weight of testes, sperm motility, sperm dancety and fertility indices support that of the *Citrullus colocynthis* and *Delonix regia* treatment, providing an evidence of the androgen deprivation effects of the extracxts in rats.

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