

## Evaluation of Effect of *Momordica Dioica* Extract on Reproductive System of Male and Female Rats

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<http://dx.doi.org/10.13005/bpj/1248>

(Received: August 25, 2017; accepted: September 14, 2017)

### ABSTRACT

To determine the effect of ethanolic extract of *Momordica Dioica* on the fertility of male and female albino rats. 36 healthy Wister Rats (18 male and 18 female) weighing between 150-200g were selected, divided into Male and Female groups. Each category (male and female groups) contains 3 groups of 6 each. First group received distilled water and considered as control. The second, and third groups of animals were received ethanolic extract of *Momordica Dioica* orally at a dose of 250, 500, mg/kg body weight respectively for a period of 30 days. In male rats group, there was a substantial decrease in the sperm count, motility and a substantial reduction in sex hormones and fertility capacity reduction were observed. In female rats group, estrogen and progesterone hormone levels were reduced significantly. The decreased levels of sperm count, fertility capacity, in male rats, serum hormonal levels in female rats reveals the antifertility activity of *Momordica Dioica* in dose dependent manner.

**Keywords:** *Momordica Dioica*, Antifertility, spermatogenesis, estrogen, testosterone, progesterone.

### INTRODUCTION

Population control is an issue of global and national public health concern. Birth control is an essential part of our life. A variety of synthetic contraceptive agents are available in the market only for women, and their use is associated with severe side effects, the progress and possibilities on male are still slow and limited<sup>1</sup>. The World Health Organization suggested that practice of usage of traditional medicine for the control of fertility, instead of synthetic drugs, as cost effective management for Birth control<sup>2</sup>. 80% population in the world opting for plant products to treat the diseases<sup>3</sup>. 90% of African countries people depends on the plant products for treating health problems<sup>3, 4</sup>. Traditionally, plant medicines were used for the regulation of fertility in the past<sup>5</sup>. Since then, many numbers of medicinal plants have been screened for their antifertility effect

and their use in female fertility regulation<sup>6, 7</sup>. Many plants have been examined for their antifertility effect previously. *Momordica Dioica* is one of the valuable medicinal plant with potent anti-fertility activity. It contains the various Phytochemicals such as alkaloids, tannins, flavonoids. *Momordica Dioica* commonly known as teasel gourd and used as vegetable belongs to family Cucurbitaceae and under the genus *Momordica*. It is a climber plant commonly known as Kakrol, Kantola, Kantroli, Ban karola or Small bitter-gourd is a relatively small oval to ovoid vegetable. It is also called as jangle Karela. It is often cultivated for its fruits, which are used as vegetable.

The Fruits are reported to show anti-inflammatory, anti-ulcer, anti-oxidant, and hepatoprotective<sup>8</sup>. It is traditionally used as astringent, febrifuge, antiseptic, antihelminthic, and

spermicidal. Also Used in bleeding piles, urinary infection and as a sedative. Its roots have been studied for its anti fertility effect in rats<sup>9</sup>. Previous studies indicate that it possesses antioxidant, hepatoprotective, antibacterial, anti-inflammatory, anti-lipidperoxidative, hypoglycemic and analgesic properties. Its contraceptive activity in Rats has been evaluated by its root extract previously but not with whole fruit extract. In the present study, we intended to evaluate its anti-fertility effect in male and female rat's reproductive hormones and was compared its potency in male and female rats.

## MATERIALS AND METHODS

### Collection of *Momordica Dioica* fruits

Fresh fruits of *Momordica Dioica* popularly known as jangle Karela, they were collected from Nallamalla forest in Kurnool district in April 2017. They were thoroughly cleaned to remove dust particles and other foreign materials and cut into small pieces dried in shaded areas. After complete drying of pieces, fine powder was prepared with grinder. Its medicinal properties and uses for health were explained and certified by Dr.Ramesh Chandra, PhD. Rtd.Professor of Botany-Hyderabad.

### Procedure for Ethanolic extract

The completely dried fruits of *Momordica Dioica* (MD) were prepared as fine powder with grinder. 30g of powder was weighed and filled in the sac like cloth material and arranged in the Soxhlet basket. The Soxhlet flask was filled with 300 ml of ethyl alcohol and it was considered as solvent. Tap water was running through the inlet and outlet of the condenser. Due to the heating mantle at the bottom of the Soxhlet, The solvent was evaporated. The vapour was condensed in the condenser and fallen into the sac which was filled with mace powder in the



**Fig. 1: *Momordica Dioica***

basket. After filling of the basket, the solvent fallen from the basket into flask, which was dark in color. Many cycles were repeated in 24hrs duration and when it becomes clear, that indicates the procedure was completed. Finally the extracted solvent was placed on the water bath for proper evaporation to get solid extract. This mass was measured and calculated<sup>10</sup>. After complete evaporation of ethanol, 6g of solid extract was remained out of 30grams of dry powder. The percentage of yield extract was (20%). This extract was dissolved in 5 ml distilled water and administered orally by gastric intubation.

### Acute Toxicity study

Toxicity study was conducted as per OPPTS regulations in different set of healthy albino rats like up and down procedure<sup>11</sup>. The rats were divided into five groups of six rats in each group. Each group was treated with different doses of ethanolic extract of *Momordica Dioica* (10mg/kg, 50mg/kg, 100mg/kg, 200mg/kg, and 500mg/kg). The animals were observed for over all behavioral changes and death rate. No abnormal changes were found in any group up to 500mg/kg. So, *Momordica Dioica* extract was found to be safe and doses were decided 250mg/kg, 500mg/kg for the present study.

### Experimental design

This study was conducted in Kurnool Medical College (KMC)-Kurnool. Before conducting this study, Institutional Animal Ethical Committee (IAEC) permission was taken. This study was conducted strictly according to CPCSEA guidelines. The rats were fed with commercial pellet diet and water and maintained under standard laboratory conditions with 12:12 h light: dark cycle<sup>12</sup>.

### Grouping of rats

36 healthy Wister Rats (18 male and 18 female) weighing between 150-200g were selected and taken from the central animal house of Kurnool Medical College (KMC) for the present experimental study. Male groups and Female groups were separated. Simultaneously both the groups were treated with *Momordica Dioica* extract for 30 days.

### Male groups

Male rats were randomly divided into 3 groups and 6 rats for each group. Group-I- Control: treated with distilled water.

Group-II- Test -1: treated with Low dose of MD-Extract 250mg/kg.

Group-III- Test -2: treated with High dose of MD-Extract 500mg/kg.

### **Female groups**

Female rats were randomly divided into 3 groups and 6 rats for each group.

Group-I- Control: treated with distilled water.

Group-II- Test -1: treated with Low dose of MD-Extract 250mg/kg.

Group-III- Test -2: treated with High dose MD-Extract 500mg/kg.

### **Mode of treatment with Test drugs**

Ethanollic extract of *Momordica Dioica* was given according to body weight of the rats and administered orally with oral feeding tube daily for 30 days.

### **Assessment of parameters**

After treatment with *Momordica Dioica* extract for 30 days, various parameters were assessed in Male and Female groups.

### **In male groups**

#### **Sperm concentration, motility and abnormality**

The caput and cauda regions of epididymis was chopped separately in two petridishes and 1 ml of normal saline at (36°C ) was added to the semen to increase sperm survival (in-vitro). The semen mixture was taken into the red blood pipette up to the 0.5 mark, and mixed with hot saline, taken up to the 101 mark. The normal saline at the stem of the pipette was disposed and the content was mixed completely. A drop of the semen mixture was placed on the Neubauer counting chamber which spread under the cover slip by capillary action. The chamber was mounted on the stage of microscope, observed under the magnification of  $\times 40$  and counted and expressed in million per ml<sup>13</sup>. A drop of the sperm saline mixture was taken in a separate glass slide. One slide was covered with a cover slip and observed under the microscope. Sperm motility at the caudal epididymis was then assessed by calculating the motility spermatozoa per unit area. A smear was made on another slide and total morphological abnormalities were observed<sup>14</sup>.

### **Fertility of male albino rats**

Fertility was assessed in adult male rats treated with *Momordica Dioica* extract and in the control male counterparts. Each male rat was placed in an individual cage with two virgin untreated female rats of the same strain. They were left together for 10 days during which two oestrone cycles had passed in female rats. And after 10 days Male and Female rats were separated. In the female groups, number of pregnant rats were identified and killed by cervical dislocation by giving anaesthesia, implantation sites, and the number of foetuses was recorded<sup>15</sup>.

### **Sex hormones and pituitary gonadotropins in Male albino rats**

Blood was collected from retro-orbital puncture. After separation of serum from the blood sample, the serum was assessed for Testosterone, LH, Oestrogen and FSH. The quantitative determination of hormones was done by using Enzyme Immuno Assay method (EIA).

### **In female groups**

Blood was collected from retro-orbital puncture by giving light ether anaesthesia. Samples were centrifuged at 3000 rpm for 10 min to obtain serum. Estimation of Estrogen and Progesterone levels was done as described by Lilaram and Nazeer Ahmed R<sup>16</sup>.

### **Statistical analysis**

The results obtained were expressed as Mean $\pm$ SEM and were analyzed by the application of one way analysis of variance (ANOVA), followed by Dunnett's t-test. Results were considered to be statistically significant at  $p < 0.05$ .

## **RESULTS**

### **In male groups**

Administration of ethanolic extract of *Momordica Dioica* in male rats for 30 days, showed significant decrease in sperm count, sperm motility and also observed sperm abnormality according to extract dose in T-1 and T-2 groups when compared with the group-I. (Tab: 1)

After treatment with ethanolic extract of *Momordica Dioica* in male rats for 30 days, showed

**Table 1: Effect of Momordica Dioica extract on sperm concentration, motility and abnormality in the epididymis of adult male albino rats.**

Group	Sperm concentration (counts $\times 10^6$ mil)		Sperm motility (FMI) at Cauda	Sperm abnormality (%)	
	Caput	Cauda		Head	Tail
Control(DW)	265.000 $\pm$ 1.623	383.282 $\pm$ 5.053	102.683 $\pm$ 3.296	6.525 $\pm$ 1.245	6.253 $\pm$ 1.653
low Dose- 250mg/kg	230.000 $\pm$ 2.663	356.689 $\pm$ 6.547	89.193 $\pm$ 1.685	10.325 $\pm$ 3.981	8.233 $\pm$ 0.923
High Dose- 500mg/kg	198.000 $\pm$ 2.284	335.253 $\pm$ 2.532	64.289 $\pm$ 4.658	13.652 $\pm$ 2.035	11.000 $\pm$ 0.635*

\*: Statistically significant,  $P < 0.05$  compared with the control.

**Table 2: Effect of Momordica Dioica extract on the fertility of male albino rats**

Group	No. of Males	No. of Females	No. of Pregnant females	No of implantation
Control(DW)	6	12	9	9.452 $\pm$ 1.312
low Dose-250mg/kg	6	12	5	5.000 $\pm$ 0.693
HighDose-500mg/kg	6	12	2	3.005 $\pm$ 0.458*

\*: Statistically significant,  $P < 0.05$  compared with the control.

reduced in fertility capacity which is observed in Female rats (conceived and non conceived rats) according to dose given in T-1, and T-2 groups when compared with the group-I (Tab: 2)

After 30days treatment with Momordica Dioica extract, sex hormones were assessed. Estrogen levels slightly increased and LH, FSH, Testosterone levels are significantly decreased according to extract dose in T-1 and T-2 groups when compared with the group-I. (Tab: 3)

#### In female groups

After giving of ethanolic extracts of Momordica Dioica for 30days, Progesterone levels are reduced effectively according to dose of extract when compared with Group-I rats. (Table- 4)

After giving of ethanolic extracts of Momordica Dioica for 30days, Estrogen levels are

reduced effectively according to dose of extract when compared with Group-I rats. (Table- 5)

#### DISCUSSION

Evaluation of new drugs with lowest side effect which can act both on male and female reproductive system is necessary in the present era. Because, the present available anti fertility drugs are causing many unwanted effects on long term usage. Male contraceptives are not available for clinical use. So, to produce herbal drugs without side effects in Male and Female reproductive systems, the present study was undertaken. The main purpose of the present study was to evaluate the anti-fertility effect of Momordica Dioica extract in Male and Female rats simultaneously. In Male rats, sperm concentration, motility and abnormality in the epididymis, fertility capacity, and male hormone levels were assessed. In Female rats,

**Table 3: Effect of Momordica Dioica extract on sex hormones and pituitary Gonadotropins in male albino rats**

Group	Testosterone (ng/dL)	LH (mIU/mL)	FSH (mIU/mL)	Estrogen (pg/mL)
Control(DW)	2.968±0.244	3.400±0.260	0.260±0.0221	28.000±1.255
low Dose-250mg/kg	2.058±0.254	2.422±0.221	0.242±0.0256	30.000±0.445
High Dose-500mg/kg	1.558±0.243*	1.243±0.021	0.143±0.0253*	31.000±1.148*

\*: Statistically significant,  $P < 0.05$  compared with the control.

**Table 4: The effect of administration of ethanolic extracts of Momordica Dioica on Progesterone levels in adult Wistar rats**

Groups	Dose	No. Of Rats	Progesterone values Nmol/l
Control	Distilled Water	6	62.53±22.45
low Dose	250mg/kg	6	56.28±19.65
High Dose	500mg/kg	6	48.54±28.16*

\*: Statistically significant,  $P < 0.05$  compared with the control.

**Table 5: The effect of administration of ethanolic extracts of Momordica Dioica on Estrogen levels in adult Wistar rats**

Groups	Dose	No.of Rats	Estrogen values Nmol/L
Control	Distilled Water	6	49.63±21.36
Low Dose	250mg/kg	6	36.41±10.11
High Dose	500mg/kg	6	31.15±3.46*

\*: Statistically significant,  $P < 0.05$  compared with the control.

plasma estrogen and progesterone, levels were determined after treatment with extract. Our study revealed that ethanolic extracts of Momordica Dioica effectively reduced the plasma levels of estrogen and progesterone in the female rats according to dose, and reduced the sperm count, motility, fertility and testosterone levels in Male rats. After treatment with Momordica Dioica in Male groups for 30 days, found that there is a significant decrease in sperm count of epididymis, increase motility and abnormality of sperm. The evolution of matured sperm is important to male fertility. The growth of spermatozoa and

testosterone in testis are mainly controlled by FSH and LH which are released from anterior pituitary gland<sup>17</sup>. FSH activate spermatogenesis in the steroli cell, while the LH enhance the production of testosterone in the leydig cells of testis<sup>18</sup>. The present study revealed that, treatment with ethanolic extract of Momordica Dioica for 30 days may modify the physiological role of the steroli and leydig cells. The extract treated group II, and III (250, and 500 mg/kg body weight) produced a significant reduction in sperm count at caudal region 383.282±5.053, 356.689±6.547, 335.253±2.532 in Control, Test-1

and Test-2 groups respectively. This may be due to Momordica Dioica extract at given doses, to either interfere with spermatogenetic process in the seminiferous tubules, epididymal function or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropin which may result in modification of spermatogenesis production (19,20). In Males, reduction of testosterone levels  $2.968 \pm 0.244$ ,  $2.058 \pm 0.254$ ,  $1.558 \pm 0.243$  in Control, Test-1 and Test-2 groups respectively. It may impair spermatogenesis and cause male infertility. This study further observed significant increase in the estrogen level  $28.000 \pm 1.255$ ,  $30.000 \pm 0.445$ ,  $31.000 \pm 1.148$  in Control, Test-1 and Test-2 groups respectively. This increase might be due to transformation of testosterone in to estrogen<sup>21, 22</sup>. When male rats combined with females rats (6 male rats, 12 female rats) during treatment with Momordica Dioica for 30days, the conceived rats number was decreased when compared with control group. In addition, the number of implantations decreased due to the decreased in sperm motility and sperm count.

Administration of Momordica Dioica in Female group rats for 30 days reduced Estrogen levels by 13.22 nmol/L, 18.48nmol/L in the T-1, and T-2 groups respectively. The pituitary-gonadal axis having main role for the maintenance of the female reproductive system, so any disturbance to this axis can leads to abnormal function<sup>23</sup>. FSH stimulates development of the Graafian follicle while LH causes to produce testosterone which is then converted to estrogen by aromatase enzyme. Thus, the decreased estrogen levels noticed in the present study. It may

be due to suppressant effect of Momordica Dioica on pituitary gonadotropins, and direct toxic effect on follicular cells as seen with the seminiferous tubules in male rats. Administration of Momordica Dioica decreased the serum Progesterone levels of female rats in the T-1, and T-2 groups by 6.25nmol/L, and 13.99 nmol/L respectively. More estrogen concentrations are required for the LH surge that enhances ovulation. The subsequently developed corpus luteum secretes progesterone that helps in implantation and confirmation of pregnancy<sup>24</sup>. Reduced in Estrogen levels prevents ovulation so, it leads to reduced progesterone levels also. Another possible mechanism for the reduction of progesterone levels may be due to direct toxic effect on the corpus luteum. Presence of Saponins, flavonoids, tannins, Terpenoids and alkaloids in the Momordica Dioica. Literature shows that presence of Saponins, flavonoids, tannins, alkaloids possess antifertility activity<sup>25</sup>. The conclusion of the present was, this plant extract have showed antifertility activity in Male and Female reproductive system. Therefore use of herbal medication should be encouraged according to its scientific evidence and activity.

#### ACKNOWLEDGEMENT

The author Rajesh Pusuloori Expressing extreme pleasure and thanks to staff and technicians of Pharmacology dept. of Kurnool Medical College, Kurnool who support and helped throughout this research work.

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