

# Antifertility Effect of Aqueous and Alcoholic Extract of *Momordica Dioica* and *Melia Azedarach* on Male Albino Rats

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**Abstract:-** Plants have long been utilized to treat a variety of illnesses. Search for male antifertility factor of medicinal plants remained a potential area of investigation. The present investigation was aimed to study the effect of oral administration of two medicinal plants, *Melia azedarach* (leaf) and *Momordica dioica* (fruit) at a dose of 100mg/kg body weight for 21 days on reproductive organs of male albino rats. Body weight, Reproductive Organ weight, Fructose content in Coagulating gland (CG), Acid phosphatase activity in Ventral prostate (VP), Total protein in Seminal vesicle (SV) were investigated in both control and treated groups. Histology of testis in treated rats showed degenerative changes in the seminiferous tubules. The decrease in sperm count with wider lumen, organ weight, fructose content in CG, protein content in SV, suggesting the antifertility activity in both tested plants. Corpus luteal sites, Implantation sites, and Litter size were also found significantly reduced.

**Keywords:-** Antifertility, Biochemical Parameters, *Melia Azedarach*, *Momordica dioica*, Sperm Count, Testis.

## I. INTRODUCTION

Plants have historically been utilised to treat variety of diseases. From time immemorial, humans have relied on plants that could meet their basic necessities such as food, shelter, fuel and health[1][2]. Plants have been used globally across varied cultures as a safe natural source of medicines.

Search for male antifertility factor of medicinal plants remained a potential area of investigation[1][3][4]. For thousands of years Indian traditional medicine has been performed through the use of Ayurveda. Many studies on the pharmacology and clinical therapies of Ayurvedic medicinal herbs have been conducted. It has been noted that many phytochemicals are becoming more significant in man[5]. In numerous animal model plants have been found to have therapeutic and contraceptive properties[6]. The practice of using contraceptive is not new. Contraceptive use has been expanded dramatically over last 40-50 years among women of all reproductive ages. Numerous plant and plant derived items have been used as contraceptives and abortifacient

from ancient times[7]. Numerous expert have looked at the contraceptive qualities of hers and plant components in both men and women[8]. It has been noted that many phytochemicals are becoming more significant in man [5].

In emerging nation, the population growth is one of the main causes of poverty and population. It is becoming a major problem i.e., facing a significant strain on economic, social and environmental resources. It is creating so many obstructions worldwide day by day[2][9]. With reference to adjusting the human reproductive rate this overpopulation can be controlled biologically[11]. Though different hormonal contraceptive tablets are being developed alongside, however, they all have negative effects, hence there is an urgent need for an efficient plant based drugs to oppose these problems. In this context use of common medicinal plants in reproduction meaning may be an important tool to prevent population explosion[2][5]. Though anti spermatogenic activity of some medicinal plants have been reported by many workers[3][7]. Few plants also possess male anti fertility activity[12][13]. The present work has been carried out to study the antifertility activity of two common medicinal plants i.e., *Momordica dioica* (fruit) and *Melia azedarach* (leaf) on reproductive organs of male albino rats. To know the contraceptive strength of these two considered or selected medicinal plants rats were treated with both aqueous as well as alcoholic extract.

## II. MATERIAL AND METHOD

### A. *Momordica dioica*

*Momordica dioica*, or spiny gourd, is a perennial climber belongs to the Cucurbitaceae family. Traditional medical practitioners employ the fruit, leaves, and roots of *Momordica dioica* as medication. Plant extracts are thought to provide antidiabetic[14], hepatoprotective[15], renoprotective[16] and anti-hyperglycemic[17] properties. Strong antioxidant properties have been observed for the alcoholic extract of *Momordica dioica*. According to a study, the root of *M. dioica* extracted in ethanol has potent abortifacient properties[18]. The present study was conducted to assess the antifertility activity of alcoholic and aqueous extract of fruit of *Momordica dioica*.

### B. *Melia Azedarach*

The plant *Melia azedarach* Linn, or mahanimba, is a member of the Meliaceae family[19]. It is a big, neem-like evergreen tree that grows all over India. It has historically been used as an astringent, stomachic, emmenagogue, anthelmintic, and antilithic diuretic[20]. The study was conducted to assess the antifertility activity of ethanolic and aqueous extract of leaf of *Melia azedarach*.

### C. Experimental Animal

Male Sprague dawleyrats (170-200gm) body weight of proven fertility were selected for the experiment. Three separate groups ( One for control and two for experimental) of male rats were selected . Each group containing 7 animals. The experimental group of rats were administered orally with suspension of test plant extracts at a dose of 100mg /kg Body weight for 21 days .The control group was fed with distilled water for the same period of treatment[2][4].

### D. Plant Extract Preparation

#### ➤ Alcoholic Extract

The plant extract was prepared by the method adopted[21]. Whole fruit of *Momordica dioica* and leaves of *Melia azedarach* were taken for investigation. The fruit and leaf of the test plant were washed and dried at room temperature . The dried plant parts were powdered by using mixer grinder. 20gm of plant powder of each test plant was poured into a conical flask containing 150ml of 50% of ethanol. The mixture was stirred, allowed to settle, and kept covered. At the end of second day the extract was filtered with no.1 what man filter paper. The filtrate was taken on a petri dish and evaporated at room temperature. The residue remained in the petri dish was ready for experiment.

#### ➤ Aqueous Extract

Whole fruit of *Momordica dioica* and leaf of *Melia azedarach* was taken. These parts were washed and air dried. For extract preparation, 10g of dried plant parts will be dissolved in 100ml of distilled water and left for 12hrs, filtered and diluted with distilled water for required dose (0.1ml/rat/day). After 12 hrs the solution will be filtered. The filtrate is now ready for dilution and required dose preparation[2][22].

Fertility performance of individual rat was done from day 16th to 21st of treatment . Each male rat was caged separately with 2 coveal female of proven fertility. Presence of vaginal smear indicated that the females had mated to the particular male and the day of mating was considered to be the day 1st of pregnancy.

Laprotomy was done on 8th day of pregnancy to examine the record of Corpora lutea and Implantation sites. Litters was examined and litters size was recorded at term[3][23].

Male rats were sacrificed on 22nd day and different tissues were collected and weighed on Torsion balance. Serial sections of testis were prepared for microscopic observations [23].

Fructose estimation in Coagulating gland (CG) was evaluated by colometry[24]. Acid phosphatase activity in Ventral prostate (VP) was evaluated by method adopted Sigma Technical Bulletin no. 104[25]. Protein estimation in seminal fluid was estimated by colorimetry[26].

Spermatozoa collected from Caput, Corpus and Cauda epididymis and vas were examined under compound microscope and their number and morphology were recorded [2][22].

The data were analysed statistically using student t-Test.

## III. RESULTS

### A. Body weight

When *Momordica dioica* fruit extract (aqueous and alcoholic) was administered orally, there was a significant drop ( $P < 0.01$ ) in body weight when compared to the control group. Similarly, rats showed significant reduction ( $P < 0.01$ ) in the body weight after oral administration of *Melia azedarach* (both aqueous and alcoholic) compared to control (Table 1 and graph 1). The pituitary gland may be suppressed or has secreted less growth hormone (GH), which would explain the cause of lower body weight[2][27][28].

### B. Reproductive Organ Weight:

There was a significant reduction in weight ( $P < 0.01$ ) detected in the reproductive organs like Seminal vesicle (SV), Coagulating gland (CG), Ventral prostate (VP) Testis and Epididymis (Table 1 and graph 2). Significant weight loss of multiple reproductive organs in treated male rats may be due to decreased gonadotrophic activity or low androgen levels that interfere with spermatozoa formation and maturation[29][30].

### C. Biochemical Parameters

When compared to the control group, there was a significant decrease ( $P < 0.01$ ) in the biochemical parameters such as Protein, Fructose, Acid Phosphatase activity following the oral administration of both the test plants i.e., *Momordica dioica* (fruit) and *Melia azedarach* (leaf) (aqueous and alcoholic (Table 2 and Graph3). Decline in fructose content in Coagulating gland (CG) and acid phosphatase activity in Ventral prostate (VP) were possibly due to decrease secretion of endogenous androgen [2][24]. Reduced protein content in SV of treated rats compared with control group was due to toxic manifestation which lead to the breakdown of protein and impaired source of ATP production to meet the energy requirement [31].

#### D. Haematological Parameters

After the oral administration of *Momordica dioica*(fruit) and *Melia azedarach*(leaf) (aqueous and alcoholic) though, there was reduction found in blood glucose and haemoglobin level as compared to the control group but it was not significant (Table 3 , Graph 5&6).

#### E. Sperm Count

Sperm count was also found significantly decreased ( $P < 0.001$  and  $P < 0.05$ ) in rats treated with both the plants *Momordica dioica*(fruit) and *Melia azedarach*(leaf) respectively compared to control group (Table 2 and Graph 4).The lowered sperm count in the treated male rats could be the consequence of disintegration of seminiferous tubule and leydig cell as well as decreased testosterone synthesis[30][32]. It is commonly known that testosterone plays a critical role in spermatogenesis and affects the epididymal environment. It must have therefore affected spermatogenesis. Therefore, it was clear that alcoholic and aqueous extracts of both the test plants have antiandrogenic property that may lead to antifertility in male rats[27][30][33].

#### F. Fertility performance test

After oral administration of *Momordica dioica*(fruit) extract *Melia azedarach*(leaf) extract(both alcoholic and aqueous) a significant decrease ( $P < 0.01$ ) in corpus luteal sites, implantation sites and litter size, was observed (Table 4 and Graph 7).

- ***Momordica dioica*** : Out of seven, Only five male rats treated with an aqueous fruit extract of *Momordica dioica* could be able to mate with only five normal female rats. In contrast, only three male rats out of seven treated with alcoholic fruit extract of *M. dioica* were able to mate with four normal female rats.
- ***Melia azedarach*** : Out of seven, only three male rats treated with an aqueous leaf extract of *Melia azedarach* could be able to mate with only three normal female rats whereas only four male rats out of the seven rats treated with alcoholic leaf extract of *Melia azedarach* were able to mate with only four normal female rats.

The decrease in implantation sites were obviously due to changes in endocrine activity of luteal structures. Many plants have been reported to be anti-ovulatory and anti-implantational[34]. However, the extracts showed abortifacient effects as there was reduction in viable litter size. These abortifacient effects were indicative of either changes in maternal estrogen/progesterone ratio or may be due to either inhibition of implantation or increased resorption of fetuses[35] or due to some toxic components reaching the female genital tract with semen[2][3]. In addition, the number of decreased implantations may also be due to the decreased sperm count and motility[37] may be another important reason.

## IV. HISTOLOGY OF TESTIS

A microscopic histological analysis of control group testes revealed normal germinal epithelium maturation and cytoarchitecture. Every stage of spermatogenesis, including primary and secondary spermatocytes and germinal epithelium producing spermatogonia divided by sertoli cells, is indicative of a healthy testis. Mature sperm ready for release were visible in the lumen.

Testes of rats treated with an aqueous and alcoholic extract of *Momordica dioica*(fruit) showed sign of deterioration of spermatogonial layer in the seminiferous tubules and a decrease in the number of sperm in the lumen. Though the tubules showing the trend of degeneration but it was not significant. Only one or two tubules were found ruptured in the focus. Wider lumens were present with less spermatozoa and degeneration in spermatogonial layer were observed(fig. 4&5).

The testes of rat treated with an alcoholic extract of *Melia azedarach*(leaf) showed comparatively higher degeneration in histology as well as number of sperms. The seminiferous tubules showed wider lumen and less spermatozoa. Rupture in spermatogonial lining were also observed under the microscope. The testis of rat treated with aqueous extract of *Melia azedarach* though showed spermatozoa in the lumen but there are certain abnormalities(breakage) found in the spermatogonial lining. Spermatozoa in the lumen were also found clumped in the lumen of the tubule in comparison to testis of rats treated with an alcoholic extract of *Melia azedarach*(fig.1&2).

The reduced testicular weights and ruptured seminiferous tubule with decreased tubular diameter indicated wide spread damage[38]. A reduction in the tubular diameter with less number of spermatozoa could be due to a destructive effect of ethanolic extract of *Momordica dioica*(fruit) and *Melia azedarach* (leaf). Degeneration of seminiferous tubules and Leydig cells, less diameter of tubular lumen with insufficient number of spermatozoa could be due to insufficient production of androgen or antiandrogenic property of both the test plants[32][39]. Seminiferous tubules with reduced or ruptured spermatogenic components in both experimental groups suggested widespread injury to the testicular structure. There were extremely few or no spermatozoa in the lumen of some of the tubules. The testis's seminiferous tubular diameter (STD) shrank. In the testis of treated rats, loosening and sloughing of the germinal epithelium were also noted[40].

Table 1: Showing the effect of aqueous and Alcoholic Extract of *Momordica dioica*( fruit)and *Melia Azedarach* ( leaf ) on Body Weight and Reproductive Organ Weight in Male Albino Rats

Male rats (Control/traeated)	Initial body weight	Final body weight	Wt. of reproductive organs				
			Testis	S.V	V.P	C.G	E.P
Control	190.6±3.5	194.4±4.09	0.18±0.12	0.50±0.02	0.53±0.01	0.17±0.007	0.06±0.014
<i>Momordica dioica</i> (aqueous.)	179.4±1.2	180.6±2.83	0.03±0.01	0.30±0.01	0.11±0.01	0.1±0.01	0.01±0.028
<i>Momordica dioica</i> (alcoholic)	184.4±3.48	188±4.32	0.04±0.008	0.28±0.018	0.11±0.010	0.09±0.005	0.08±0.01
<i>Melia azedarach</i> (aqueous)	187.17±4.13	193.63±3.23	0.04±0.01	0.48±0.11	0.08±0.01	0.20±0.01	0.09±0.02
<i>Melia azedarach</i> (alcoholic)	175±1.37	176.8±1.39	0.02±0.008	0.38±0.03	0.08±0.008	0.18±0.02	0.09±0.21

Abr. : SV- Seminal vesicle VP- Ventral prostate CG- Coagulating Gland EP- Epididymis.

Table 2: Showing the Effect of Aqueous and Alcoholic Extract of *Momordica Dioica*( fruit)and *Meliaazedarch* ( leaf) on Sperm Count and Biochemical Parameters

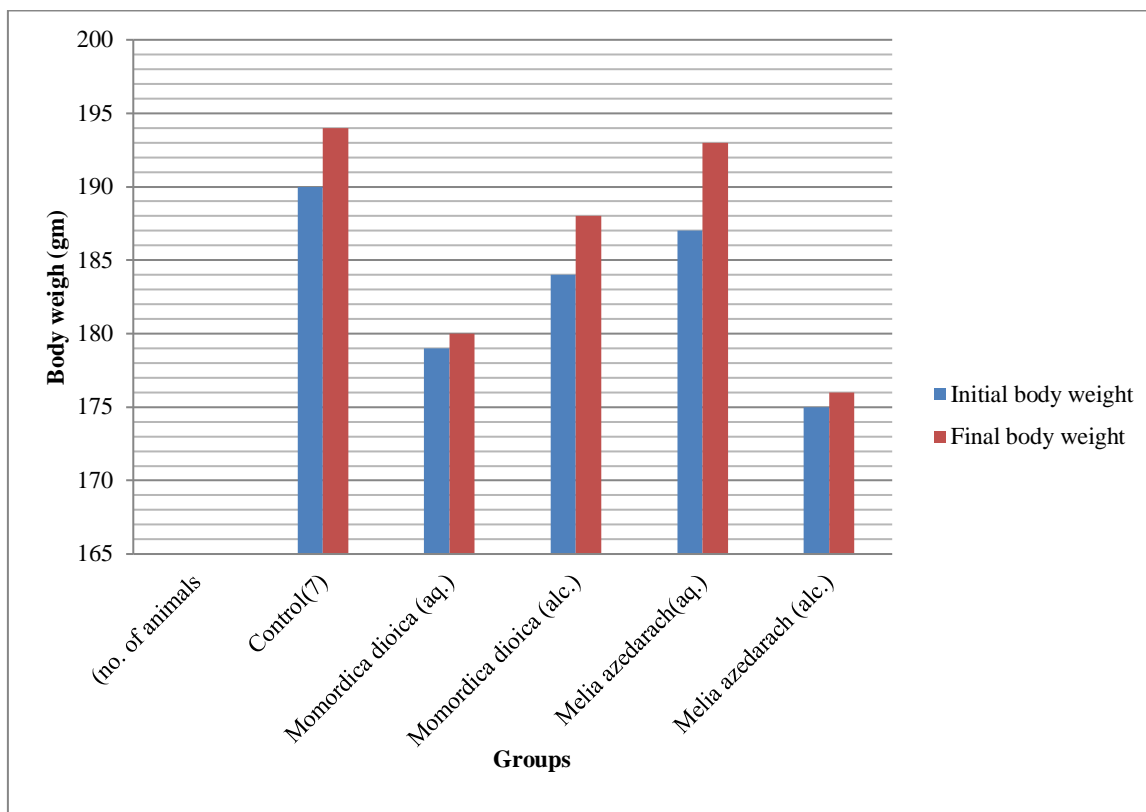
Male rat (Control/Treated)	Sperm count	Fructose(CG) mg/100mg of tissue	Protein(SV) mg/100mg of tissue	Acid phosphatase(VP) mg/hr/100mg of tissue
Control	196.9±3.001	0.53±0.01	32.8±0.86	42.26±0.56
<i>Momordica dioica</i> (aqueous)	90.8±1.42	0.16±0.008	13±0.94	15.44±0.66
<i>Momordica dioica</i> (alcoholic)	79±1.41	0.14±0.015	10.2±0.91	10.53±0.967
<i>Melia azedarach</i> (aqueous)	89.92±1.49	0.2±0.02	8.67±1.76	17.66±2.52
<i>Melia azedarach</i> (alcoholic)	94.6±1.80	0.02±0.0008	8.6±1.20	12.49±2.12

Table 3: Showing the Effects of Aqueous and Alcoholic Fruit and Leaf Extract of *Momordica Dioica* and *Meliaazedarch* on Haematological Parameters

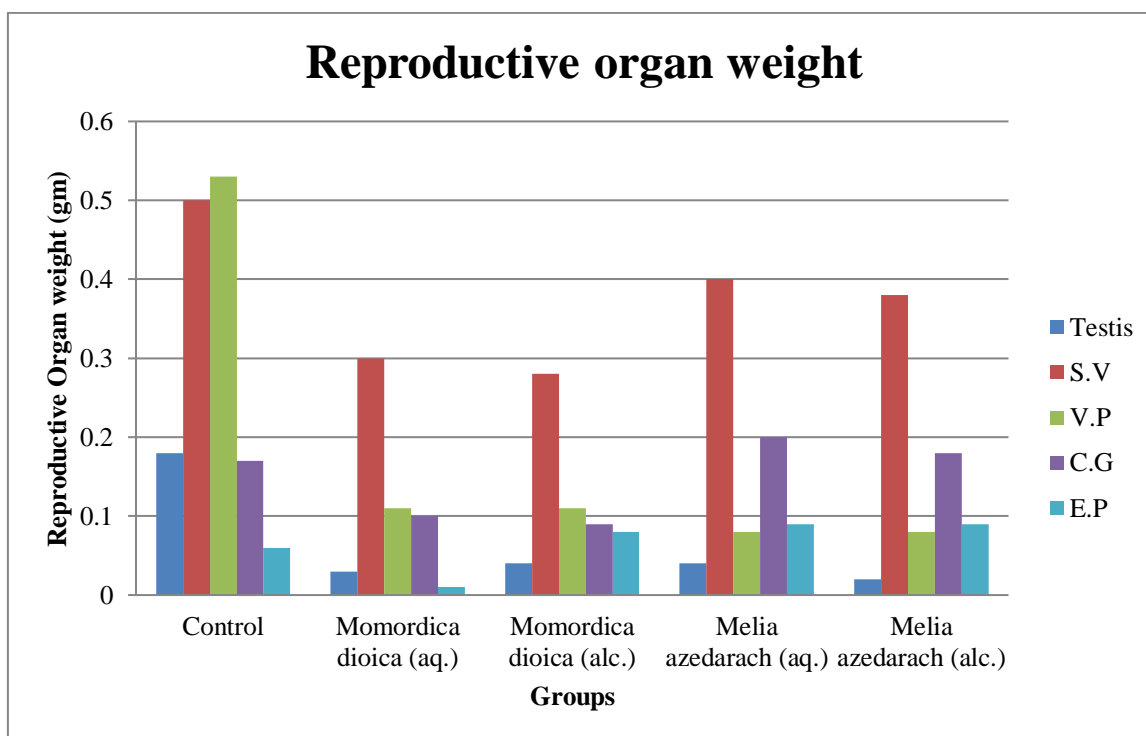
Male rats (control/Treated)	Blood glucose(mg/dl)	Haemoglobin(gm%)
Control	109±1.18	10.3±0.3
<i>Momordica dioica</i> (aqueous)	104.4±1.96	10.64±0.89
<i>Momordica dioica</i> (alcoholic)	104.4±1.93	8.8±0.33
<i>Melia azedarach</i> (aqueous)	109±2.32	6.07±0.77
<i>Melia azedarach</i> (alcoholic)	103.6±1.69	8.0±1.02

Table 4: Showing the Effects of Aqueous and Alcoholic Extract of *Momordica Dioica*(Fruit)and *Melia Azedarach*(Leaf) on Fertility Performance Test

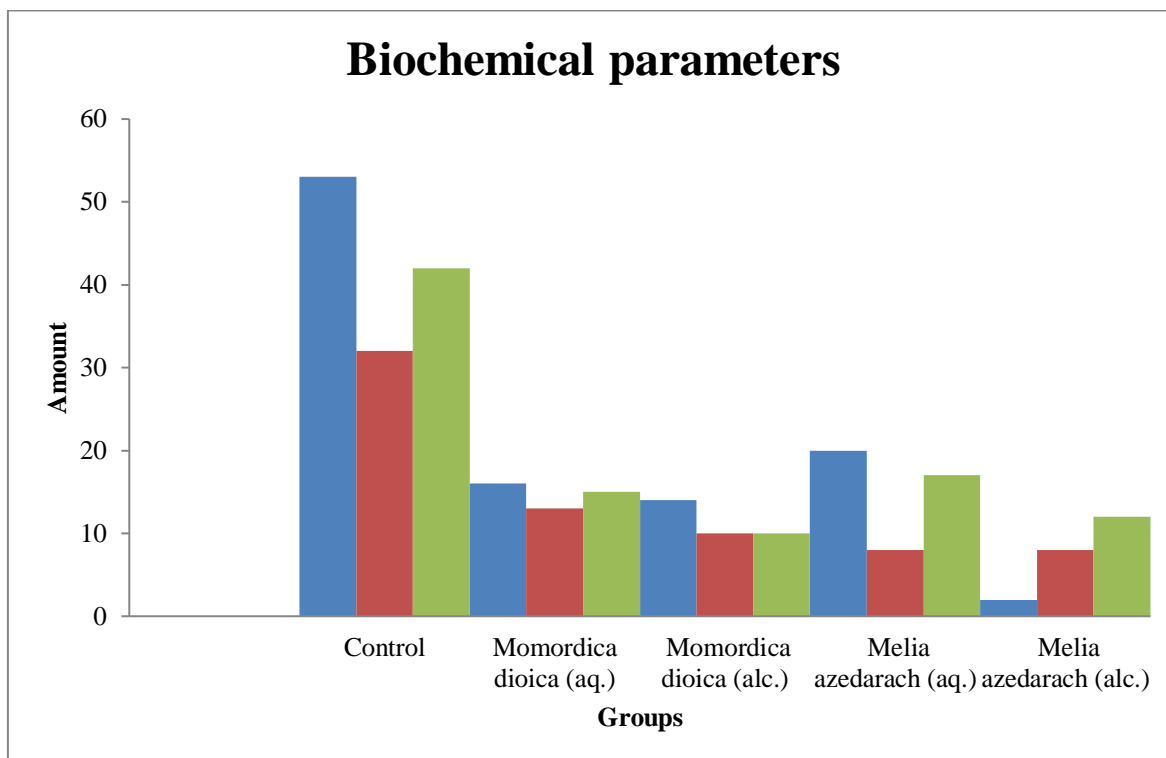
Effects of plant extract on fertility of male rats						
	No. of breeder males	No. of successful males	No. of Mated females	Corpora lutea	Implantation site	Litter size
Control	7	5	7	3.9±0.27	2.8±0.29	5±0.31
<i>Momordica dioica</i> (aqueous)	7	5	5	2±0.42	1±0.25	2.5±0.28
<i>Momordica dioica</i> (alcoholic)	7	3	4	2.88±0.39	1±0.26	2±0.40
<i>Melia azedarach</i> (aqueous)	7	3	3	4±0.68	2.33±0.67	3.25±1.25
<i>Melia azedarach</i> (alcoholic)	7	4	4	1.67±0.42	1±0.25	2±0.57



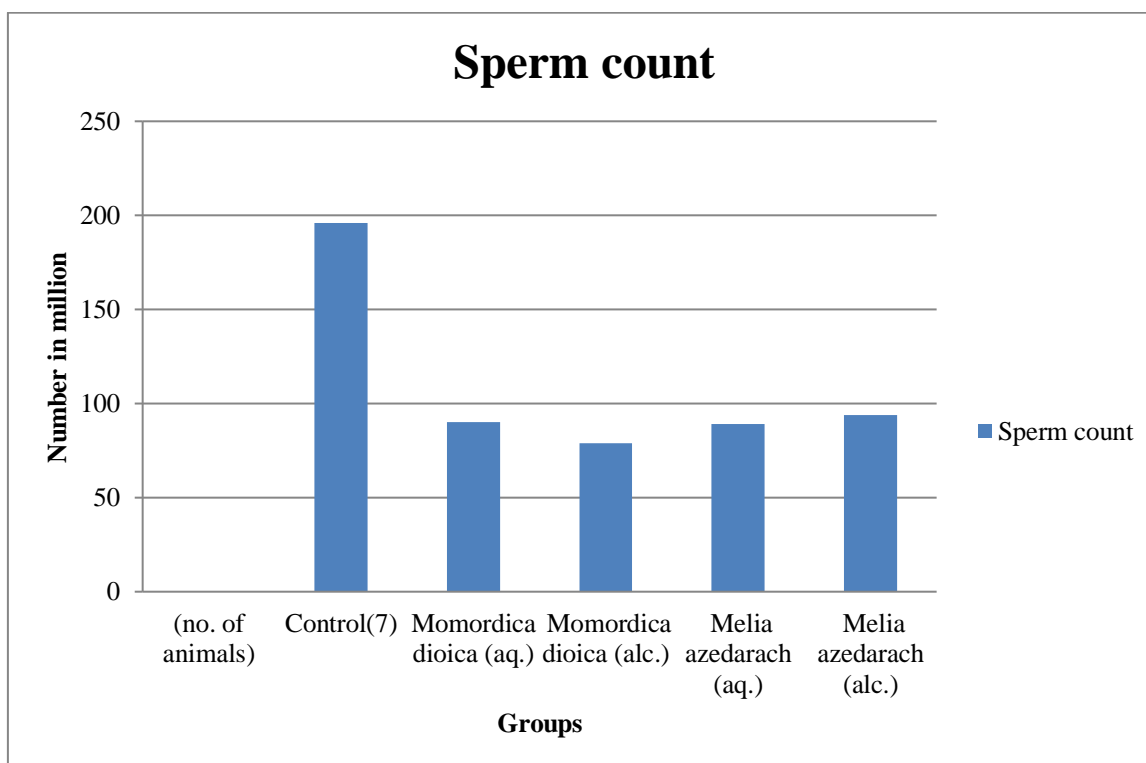
Graph 1: Showing the Effect of Both Plant Extracts On Body Weight In Male Albino Rats



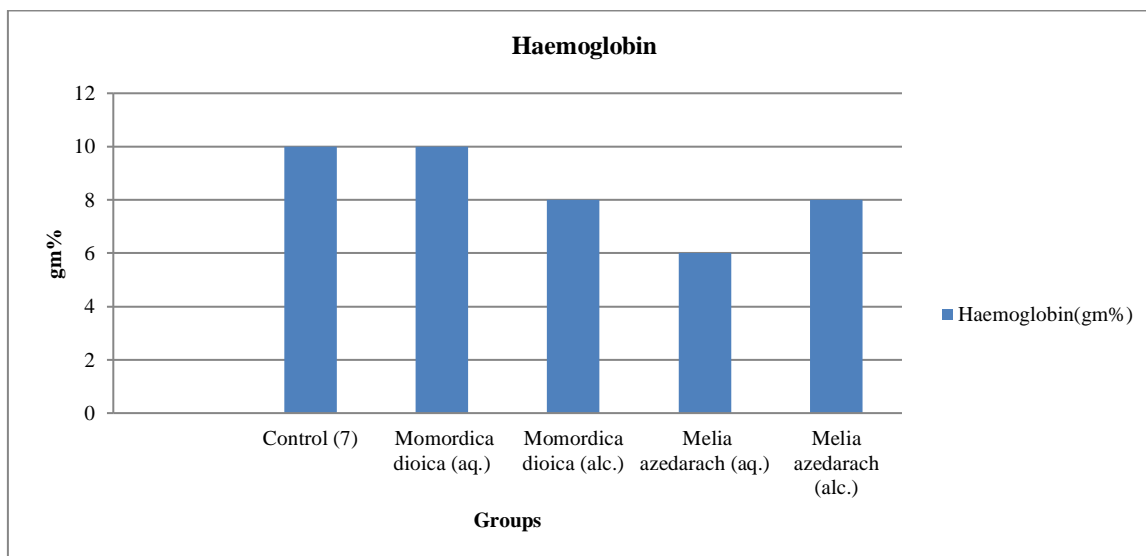
Graph 2: Showing the Effect of Both Plant Extracts On The Reproductive Organ Weight.



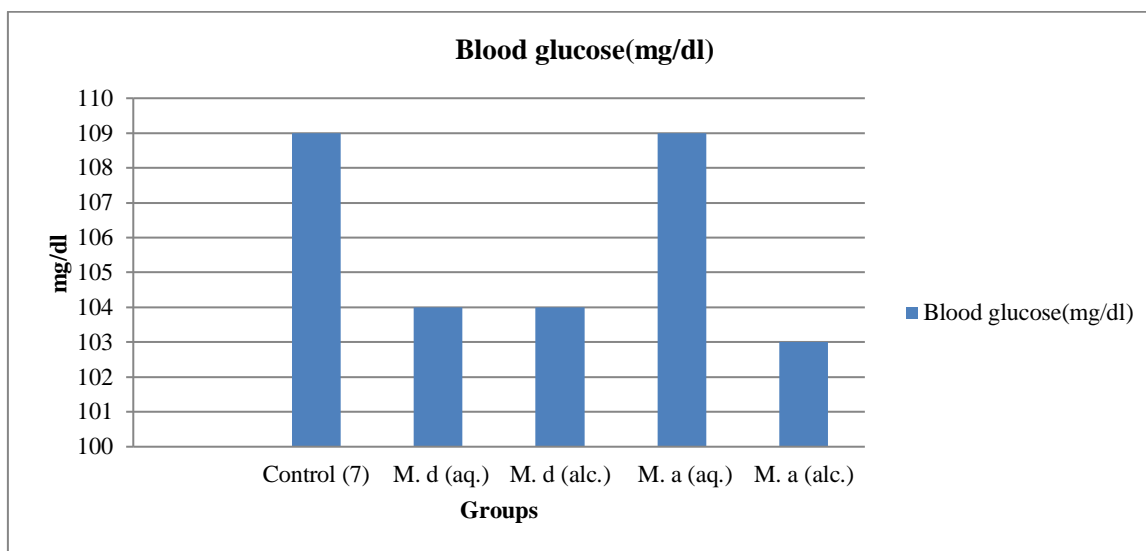
Graph 3: Showing the Effect of Both Plant Extracts on the Biochemical Parameters (Protein, Fructose, Acid Phosphatase)



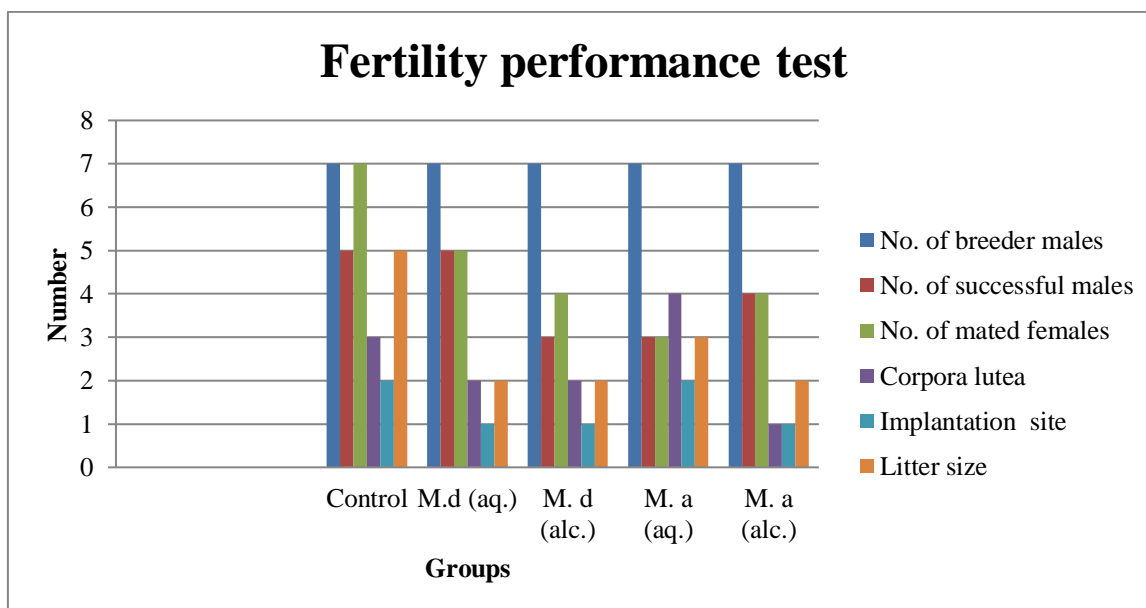
Graph 4: Showing the Effect of Both Plant Extracts On The Sperm Count



Graph 5: Showing the Effect of Both Plant Extractson Haemoglobin Test



Graph 6: Showing the Effect of Both Plant Extracts on the Blood Glucose



Graph 7: Showing the Effect of Both Plant Extracts on the Fertility Performance Test of Male Rats



## V. DESCRIPTION OF HISTOLOGICAL PHOTOGRAPH

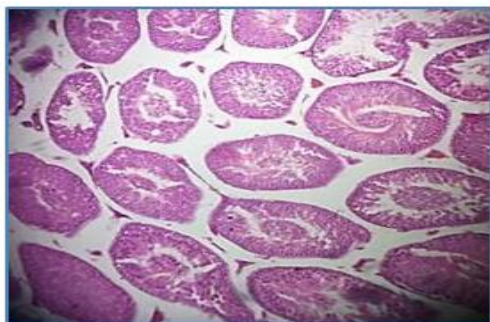


Fig. 1: Histology of Testis treated with Aqueousextract of *Melia azedarach*(10X)

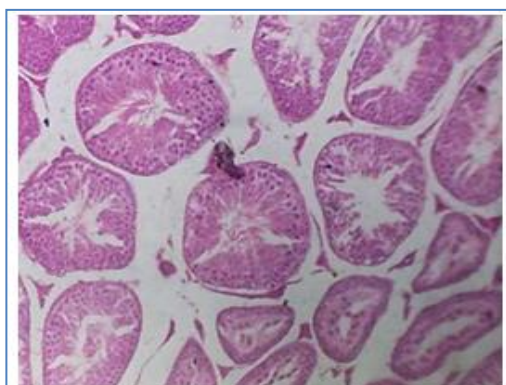


Fig. 2: Histology of Testis treated with Alcoholic extract of *Melia azedarach*(10X)



Fig. 3: Histology of Testis of Control Rat



Fig. 4: Histology of Testis Treated with Aqueousextract of *Momordica dioica*(10X)

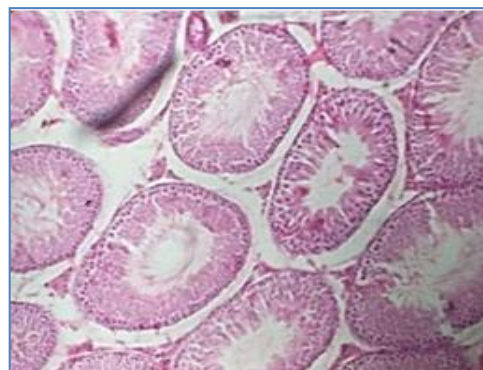


Fig 5: Histology of Testis treated with Alcoholic extract of *Momordica dioica*(10X)

## VI. CONCLUSION

The present study indicated that oral administration of both alcoholic and aqueous extract of *Momordica dioica* (fruit) and *Melia azedarach* (leaf) extract significantly affects reproductive function of experimental rats because it altered androgen levels and causes less sperm count and testes cytoarchitecture. Thus, it may be concluded that both the tested plants were found to have potential antifertility agent for decreasing spermatogenic activity.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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