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Effect of ethanolic leaf extract of *Calotropisprocera* and *Terminalia chebula*on reproductive organs of male albino rats (*Rattusnorvegicus*).

D.N.Choudhary[1]andRicha Sharma[2]
1. Associate Professor , University Department of Zoology, TMBU, Bhagalpur
2. Research Scholar, University Department of Zoology, TMBU, Bhagalpur
Bihar, India

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ABSTRACT: Plants have historically been utilised to treat variety of diseases. 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. In the present study the effect of oral administration of leaf extract of *Calotropisprocera*and fruit extract of *Terminalia chebula* (100mg/Kg Body.Wt) on male reproductive organs of male albino rats was investigated. Various reproductive end points such as organ weight, Acid phosphatase activity , fructose content, Total protein , Sperm count were assessed. Histology of testes in treated rats showed degenerative changes in the seminiferous tubules. The decreased sperm count , reproductive organ weight , fructose in coagulating gland (CG) and protein content in seminal vesicle (SV) including changes in the spermatogenic elements of testis suggesting the antifertility activity of both the testplants.

Keywords: Antifertility ,*Calotropisprocera* , *Terminalia chebula* , Reproductive organs , Biochemical parameters , Sperm count.

I. INTRODUCTION

In emerging nation, the population growth is one of the main causes of poverty and population. It is becoming a major problem ie. facing a significant strain on economic, social and environmental resources[1]. It is creating so many obstructions worldwide day by day. This overpopulation can be checked through biological means with special reference to modulation in the human fertility ability[2].

Plants have historically been utilised to treat variety of diseases. Plants have been used globally across varied cultures as a safe natural source of medicines. From time immemorial, humans have relied on plants that could meet their basic necessities such as food, shelter, fuel and health. Search for male antifertility factor of medicinal plants remained a potential area of investigation[3][4][5]. It has been noted that many phytochemicals are becoming more significant in man[6]. In numerous animal models plants have been found to have therapeutic and contraceptive properties[7]. With reference to adjusting the human reproductive rate , this over population can be controlled biologically[8]. Though, different hormonal contraceptive tablets are being developed alongside advances in reproductive biology, however, they all have negative effects. Hence, there is an urgent need for an efficient drug to oppose this problems. In that attempts the use and effects of common medicinal plants in reproduction may be an important tool in the direction of population control[6][7]. Pharmacists are also trying for the familiar options by studying medicinal plants[9][10]. Though, anti-spermatogenic activity of some medicinal plants have been reported by many workers[4][11][2] few plants also possess male antifertility activity[12]13][14]. A number of medicinal plants have already been studied for their effect on the fertility such as *Andrographispaniculata* [7]*Aegelmarmelos* , *Tinosporacordfolia* , *Murrayaannua*[13] and *Gossipiumharvesium*[15]. In the present investigation , the attempt has been made to study the antifertility activity of two common medicinal plants *Calotropisprocera*(Aak) and *Terminalia chebula*(Hadad or Harre) in male albino rats.

II. MATERIAL AND METHODS

I.Plant extract preparation

The plant extract was prepared by the method adopted by[16]. Fresh mature leaves of *Calotropisprocera*and fruits of *Terminaliachebula* were taken for investigation. The parts of the test

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plant were washed and dried properly at room temperature.The dried plant parts were powdered by using grinder machine. 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 settled, and kept covered. At the end of second day the extract was filtered with no.1 whatman filter paper. The filterate was taken on a petri dish and evaporated at room temperature. The residue remained in the petri dish was ready for experiment.

II.Experimental animal

Male Sprague Dawley rats (160-180 gm of 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 groupwas containing 6 animals. The experimental group of rats were administerd orally with suspension of test plant extracts at a dose of 100 mg /kg body weight for 21 days .The control group was fed with distilled water for the same period of treatment[4][17].

Fertility performance of individual rat was done from day 16^{th} to $21^{st}$of treatment . Each male rat was caged separately with 2 coeval females for mating. Presence of sperm in the vaginal smear indicated that the females had mated to the particular male and the day of mating was considered to be the day 1^{st} of pregnancy.Laprotomy was done on 8^{th} day of pregnancy to examine and record the corpora lutea and Implantation sites. Litters were examined and litters size was recorded at term. Male rats were sacrificed on 22^{nd} day and different tissues were collected and weighed on Torsion balance. Serial sections of testis were prepared for microscopic observations[18][19].

III. Biochemical parameters :

Fructose estimation in coagulating gland(CG) was evaluated bystandardcolometric method[20]. Acid phosphatase activity in ventral prostate (VP) was evaluated by the method adopted by Sigma Technical Bulletin no. 104 [21]. Protein estimation in seminal vesicle (SV) was estimated by standard colorimetric method[22].

Spermatozoa collected from Caput, Corpus and Cauda epididymis and vas were examined under compound microscope and their number and morphology were recorded[23][24][4].

The data were analysed statistically using student t-Test.

III. RESULT AND DISCUSSION:

The male reproductive system consists of testis as the main reproductive organ and other accessory structures, with a primary responsibility of sperm production. Agents that alter testicular function will ultimatelyaffect the quality and quantity of spermatozoa, which depends on several reproductive factors[11].

In the present study, the effects of ethanolic leaf extract of *Calotropisprocera*and fruit extract of *Terminalia chebula* on the reproductive performance of male albino rat were investigated.

BODY WEIGHT

The body weight of male albino rats were found significantly increased($P<0.001$) by oral administration of ethanolic leaf extract of *Calotropisprocera* whereas significantly decreased ($P<0.001$) in case of male rats treated with ethanolic fruit extract of *Terminaliachebula* (Table 1 & graph 1). The decreased body weight may be due to suppression or less secretion of growth hormone(GH) from the pituitary gland [25][26]. Increased body weight showed by rats treated with *Calotropisprocera*(Table 1 & graph1) may be due to absence of toxic elements in the plant extract, not affecting the normal functioning of GH [4].

REPRODUCTIVE ORGAN WEIGHT

Result showed that the weight of various reproductive organs such as Testis, Seminal vesicle(SV), Coagulating gland(CG), Ventral prostate (VP) and Epididymis of male rats treated with both ethanolic leaf extract of *Calotropisprocera*and fruit extract of *Terminalia chebula*were found significantly decreased ($P<0.001$) with compared to control group (Table 1& graph 2.).Significant reduction in the weight of various reproductive organs of treated male rats may be due to low gonadotrophic activity [3][27][28] or due to reduced level of androgen causing interference in the formation and maturation of spermatozoa[29][30][31][32][33]. The structural and functional integrity of reproductive tissues depend upon the circulating androgen[34] and therefore, any small change in testosterone content may result in reduction in the weights of the reproductive organs[35][36].

BIOCHEMICAL PARAMETERS

There was a significant reduction in fructose content in coagulating gland (CG), protein content in seminal vesicle (SV) and acid

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phosphatase activity in Ventral prostate (VP) observed in male rats treated with both the testplants*Calotropisprocera* and *Terminaliachebula* compared with control (Table 2 Graph 3).

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[36][17][31]. 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 productionto meet the energy requirement[5].

SPERM COUNT

Significant reduction($P< 0.01$ & $P <0.05$) was observed in sperm count of male albino rats treated with both the test plants *Calotropisprocera*and *Terminalia chebula*(Table 1 &Graph 5).

The reduced sperm count observed in treated male rats may be due to insufficient amount of Testosterone productionand degeneration of Seminiferous tubules(fig-2&3) and leydig cells[37][5]. It is well known that testosterone plays an important role in spermatogenesis and affects the epididymalmilieu[38][26]. So it must have affected spermatogenesis[39]. Hence it clearly indicated that both *Calotropisprocera* and *Terminalia chebula* possess antiandrogenic activity which may lead to antifertility in male rats [26].

FERTILITY PERFORMANCE TEST

Female mated by males treated with leaf extract of *Calotropisprocera*and fruit extract of *Terminalia chebula* showed a significant reduction($P<0.01$) in corpora lutea and implantation sites whereas significant reduction($P<0.05$) in litter size was observed in females mated by males treated with leaf extract of*Calotropisprocera*only(Table2 & Graph 4). Out of six males treated with fruitextract of*Terminalia chebula* only 3 treated males could mate 3 normal females.

The decrease in implantation sites were obviously due to changes in endocrine activity of luteastructures[40]. Many plants have been reported to be antiovulatoryand anti-implantational[3]. 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[4]or may be due to either inhibition of implantation or increased resorption of fetuses[41] or due to some toxic components reaching the female genital tract with semen [42][3]. In addition , the number of decreased implantations may also be due to the decreased sperm count and motility[43]may be another important reason.

HISTOLOGICAL STUDY OF TESTIS :

The histological studies of testis of male rats treated with both ethanolic leaf extract of *Calotropisprocera*(fig-2)and fruit extract of *Terminalia chebula*(fig-3)showed significant degeneration in seminiferous tubules and Leydig cells as compared with control group. In both the experimental groups seminiferous tubule showed shrunken or ruptured spermatogenic elements indicated wide spread damage of testicular structure. Some of the tubules showed negligible or very less number of spermatozoa in the lumen. Seminiferous Tubular diameter (STD) of the testis were decreased. Loosening and sloughing of germinal epithelial in testis of treated rats were also observed[11].

The reduced testicular weights and ruptured seminiferous tubule with decreaed tubular diameter indicated wide spread damage[44]. A reduction in the tubular diameter with less number of spermatozoa could be due to a destructive effect of ethanolic extract of *Calotropisprocera*(fig-2)and *Terminalia chebula* (fig-3). 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[36][5][37][26].

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Table 1 : showing the effects of ethanolic leaf extract of *Calotropisprocera* and fruit of *Terminaliachebula* on Body weight , reproductive organ weight , Sperm count and biochemical constituents.

S.V= Seminal Vesicle C.G= Coagulating Gland V.P= Ventral Prostate

Treatment (No. of animals)	Change in body weight (g)	Weight of organs,				Fructose in (CG) mg/100mg of tissue		Protein in (SV) mg/100mg of tissue	Acid phosphatase in (VP) mg/hr/100mg of tissue	Sperm count(millions/ml)
		Testis	S.V	C.G	V.P	Epidi-dymis.				
Control (6)	8.3±2.26	0.18±0.12	0.46±0.04	0.16±0.01	0.44±0.06	0.11±0.05	0.51±0.02	31.83±0.91	42.23±0.45	$192X10^4$ ±4.21
Calotropis procera(6)	9.8±2.74	0.04±0.01	0.37±0.02	0.09±0.01	0.11±0.00	0.08±0.04	0.36±0.04	12±2.55	22.04±3.32	133.4X10 4
Terminalia chebula(6)	7.57±2.49	0.02±0.01	0.33±0.02	0.1±0.00	0.10±0.01	0.09±0.02	0.33±0.02	18.67±1.56	9.82±0.49	67.13X10 4

Table 2: Showing the effect of ethanolic leaf extract of *Calotropisprocera*and fruit extract *Terminaliachebula* on Fertility performance of male rats.

Effects of plant extract on fertility of male rats					
	No. of successful males	No. of Mated females	Corpora lutea site	Implantation site	Litter size
Control	6	6	4.33±0.28	2.42±0.29	4.17±0.48
Calotropis procera	6	5	3.1±0.46	2±0.37	1.8±0.97
Terminalia chebula	6	3	3.8±0.49	2.5±0.29	2±0.58

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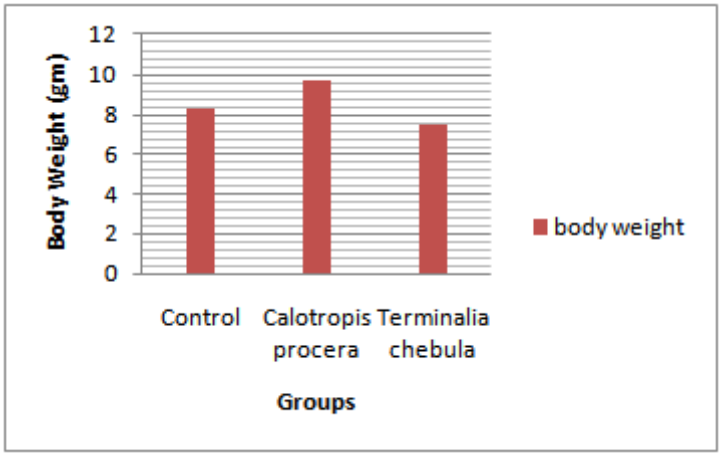
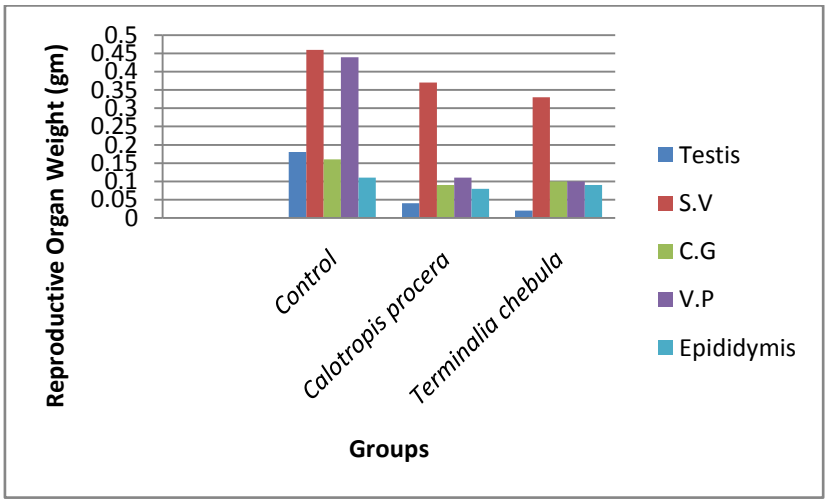
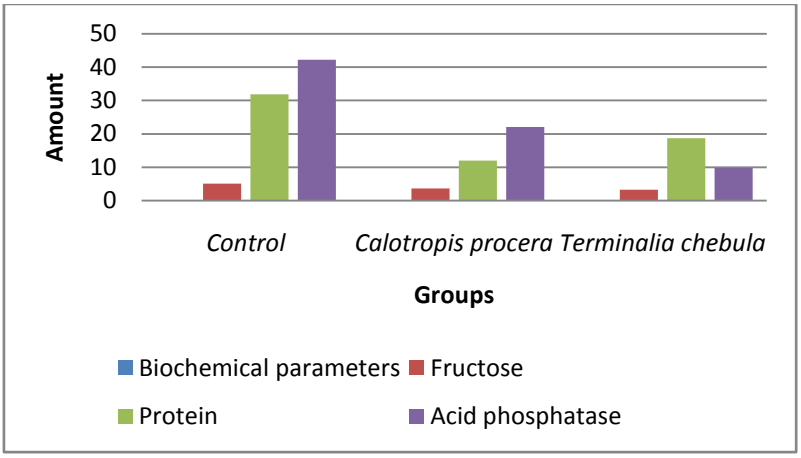


Graph 1: Showing the effect of plant extract on body weight in male albino rats.



Graph 2: Showing the effect of plant extract on reproductive organ weight in male albino rats.



Graph 3: Showing the effect of plant extract on biochemical test (Fructose, Protein, Acid phosphatase) in male albino rats.

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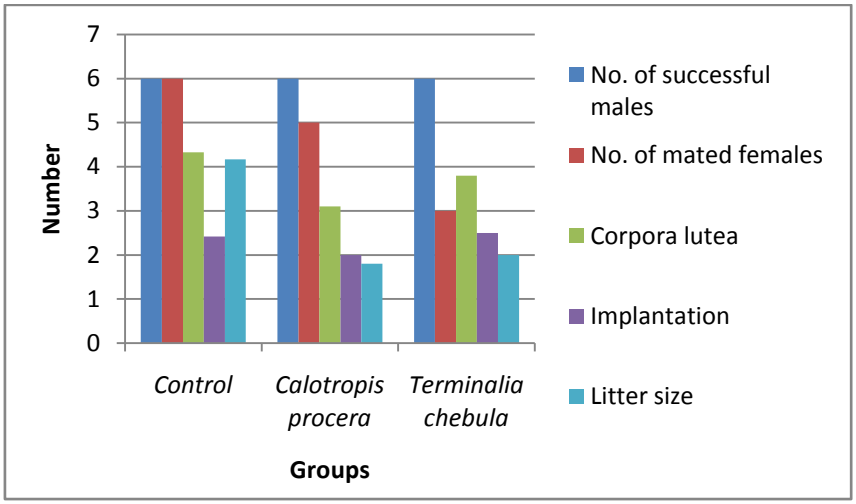
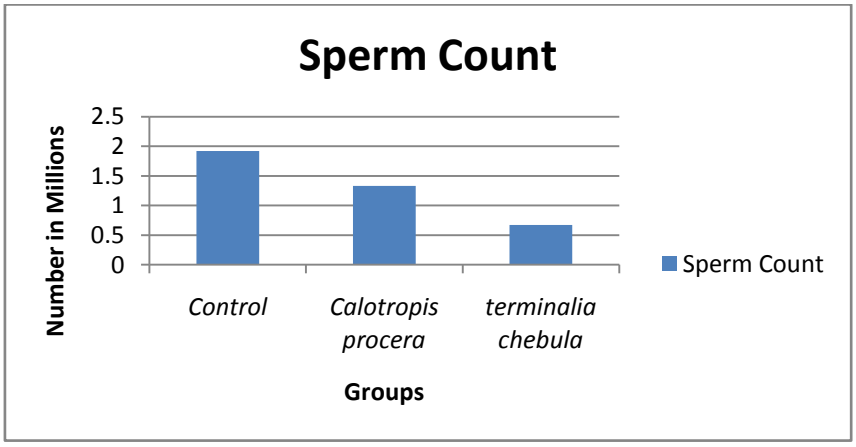


Graph 1: Showing the effect of plant extract on Fertility performance in male albino



Graph 5: Showing the effect of plant extract on Sperm Count in male albino rats.

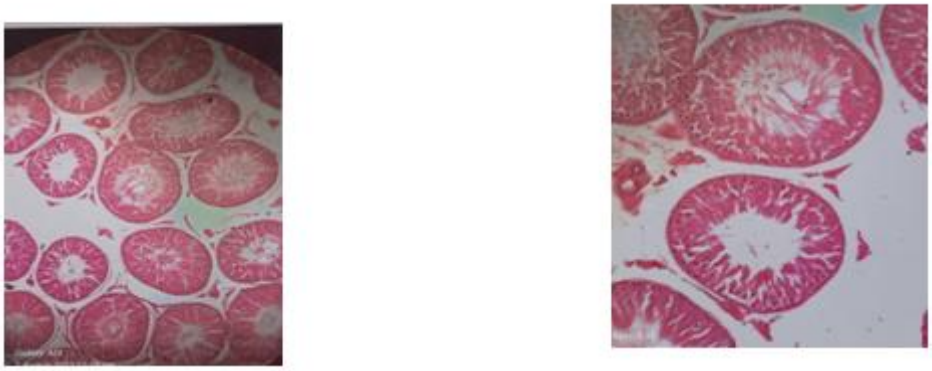


Figure 1: Photomicrograph of rat testis administered orally with *Calotrophicprocera(*ethanolic leaf extract).

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Figure 2: Photomicrograph of rat testis(Control)

Figure 3: Photomicrograph of rat testis administered orally with *Terminalia chebula*(ethanolic fruit extract).

IV. CONCLUSION :

The present investigation revealed that the ethanolic leaf extract of Calotropisprocera and fruit extract of Terminalia chebula has potent effect on fertility rat of experimental rats due to intervening androgen levels and reduced sperm count. The drug can be proved to be a potent herbal agent for reduced spermatogenic activity and can endorse the participation of males in family planning. Therefore, based on overall findings in the present study, it can be concluded that ethanolic leaf extract and fruit extract of Calotropisprocera and Terminalia chebula holds the potential to be exploited as a male contraceptive in future.

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Authors contribution : First author designed the work and prepared the manuscript. The second author did the experimental work.

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

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