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# Study of Antifertility Effect of Aqueous Extract of *Terminalia bellirica* Leaves on Male Albino Rats

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**Abstract**

Aqueous extract of leaves of *T. bellirica* @ 50mg/100g body weight caused increase in the body weight but decrease in the weight of testes and epididymus after 20 and 30 days of treatment in male albino rats. Increase in the levels of protein, sugar, cholesterol, alkaline phosphatase and lactate dehydrogenase but no change was reported in the level of acid phosphatase in testes and epididymus with oral administration of *T. bellirica* leaves extract. Sperm count showed highly significant decrease.

One group of experimental animals was kept on normal diet for 30 days after 30days of extract feeding. Reversibility was seen in sperm count and the levels of protein, sugar, cholesterol, alkaline phosphatase, acid phosphatase, lactate dehydrogenase came back to normal in testes and epididymus.

**Key words**

Aqueous extract, *Terminalia bellirica,* male albino rats, biochemical parameters

## Introduction

For centuries, plants and plant-based products have been used as a valuable and safe natural source of medicines for treating various ailments. Similarly many plants and plant based products are also used for fertility control.

The roots of *Aristolochia indica* and *Plumbago zeylanica*; the leaves of *Azadirachta indica*, *Catharanthus roseus*, *Vinca rosea* and *Ocimum sanctum*; the flowers of *Hibiscus rosa-*

*sinensis* and *Malvaviscus conzatii*; the seeds of *C. papaya* and *Vitex negundo;* and the fruit of *Momordica charantia* have been identified as candidates for male fertility regulation.

Several commonly used plants have also been reported to adversely affect male reproductive functions in wildlife and humans. The effects observed with most of the plant and plant-based products have been attributed to the antispermatogenic and/or antisteroidogenic properties of one or more active ingredients. *Terminalia bellirica*, is one such plant, said to cause male infertility, in folklore.

In the present paper antifertility activity of aqueous extract of the leaves of *T.bellirica*, has been tried to evaluate.

**Materials and Methods**

**Plant Material**: Leaves of *T. bellirica* were collected from authenticated tress, dried in shade, crushed and powdered using mortar and pestle. The aqueous extract was prepared in double distilled water with the help of Soxhelet apparatus at 95$^{o}$C. Then the extract was dried in oven at 85$^{o}$C and was stored in airtight containers in refrigerator.

**Animal Material**: Mature, male albino rats of proven fertility were acclimatized for laboratory conditions for ten days on standard diet and water was supplied *ad libitum*.

**Experimental Design**: For the present experiment animals were divided into eight groups of six animals each.

Group I- Control Group, 20 days.

Group II- Experimental Group, 20 days (aqueous extract @ 50mg/100g body weight)

Group III- Control Group, 20+30 days.

Group IV- Reversibility Group (extract for 20 days and 30 days more on normal diet)

Group V- Control Group, 30 days.

Group VI- Experimental Group of 30 days (fed on aqueous extract of leaves of *T. bellirica* @ 50mg/100g body weight)

Group VII- Control Group, 30+30 days.

Group VIII - Reversibility Group, 30 +30 days (Animals received extract for more.)

The body weight was taken before starting and after completion of the experiments. The animals were dissected after completion of the experiments, testes and epididymus were taken out and weighed. After processing, part of testes and epididymus was fixed in Bouin Hollenday's

fixative for histological studies and remaining portions were used for the biochemical estimations.

## Results

### Body weight and organ weight

The mean of increase or decrease in body weight and reproductive organ weight is shown in bar diagram 1, 2 and 3.

### Sperm count

Sperm count showed significant decrease after 20 and 30 days treatment with 50 mg/100g dose of aqueous extract of *T.bellirica*.(10-15 x105.normal, 9.167 x105 after 20 days ($p<0.01$)and 5.500 x105 after 30 days ($p<0.001$)treatment) . In respective reversibility groups, sperm count was 11.667 x105 and 11.667 x105, showing normal range.

### Tissue biochemistry

Results of tissue biochemistry are shown in table 1.

**Table 1: Effect of *T. bellirica* leaves aq. extract on biochemical parameters of testes**

| | Mean values | | | | | |
|---|---|---|---|---|---|---|
| Mode of treatment | Protein | Sugar | Cholesterol | Alkaline phosphatase | Acid phosphatase | Lactate dehydrogenase |
| Control 20days | 3.517 ±0.768 | 66.007 ±6.286 | 85.485 ±10.335 | 242.333 ±28.204 | 22.432 ±5.758 | 185.667 ±18.446 |
| Treated 20days | 4.945 [a] ±1.145 | 96.900 [c] ±14.720 | 121.178 [e] ±10.882 | 291.667 [c] ±21.125 | 26.992 ±5.619 | 195.500 ±14.460 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Control | 3.283 | 71.347 | 83.265 | 225.667 | 20.462 | 179.500 |
| 20days (Rev) | ±0.875 | ±8.765 | ±11.271 | ±24.271 | ±6.210 | ±18.588 |
| Treated | 2.850 | 72.993 | 79.752 | 235.333 | 23.868 | 183.333 |
| 20days (Rev) | ±0.723 | ±7.633 | ±9.431 | ±30.949 | ±6.811 | ±20.324 |
| Control | 3.800 | 69.267 | 88.522 | 236.833 | 19.458 | 195.000 |
| 30days | ±1.418 | ±6.059 | ±13.675 | ±30.215 | ±4.637 | ±18.482 |
| Treated | 7.792 [d] | 118.163 [d] | 134.393 [e] | 300.833 [d] | 23.708 | 271.167 |
| 30days | ±0.870 | ±10.412 | ±11.236 | ±24.367 | ±6.626 | ±21.311 |
| Control | 2.517 | 74.702 | 84.943 | 236.833 | 25.773 | 186.333 |
| 30days (Rev) | ±0.714 | ±7.621 | ±9.942 | ±26.317 | ±5.906 | ±16.86 |
| Treated | 4.933 [e] | 85.883 [a] | 111.518 [e] | 229.833 | 26.388 | 230.333 |
| 30days (Rev) | ±0.807 | ±7.618 | ±8.227 | ±23.147 | ±7.895 | ±18.283 |

**Values are mean ± SD. No of rats for each reading = 6.**

**Significance as per Student's "t" test. a=p<0.05; b =p<0.02; c =p<0.01; d =p<0.005; e =p<0.001**

**Discussion**

Slight increase in the body weight of rats fed on aqueous extract of *T. bellirica* indicates normal metabolism. Our observations are in agreement with the findings of Sharma and Jacob (2000) with methanol extract of *Mentha avensis*, Sharma et al (2003) with *Semecarpus anacardium* fruits, Mishra et al (2009) with aqueous extract of *Bouganvillea spectabili* leaves, Sathiyaraj et al (2010) with aqueous leaf extract of *Aegle marmelos* who reported no decrease in total body weight but our findings are in disagreement with Dixit and Joshi (1982) with *Allium*

*sativum* and Mathur et al (2010) with *Tecoma stans* leaves, who reported decreased body weight in rats after treatment.

Weight reduction of the reproductive organs of treated male rats clearly indicates that the drug caused structural and functional alteration in testes and epididymis. Administration of the aqueous extract of leaves of *T.bellirica* caused a decrease in the weight of testes and epididymus, thereby indicating that extract must have damaged the germinal components substantially. Decrease in testes and epididymus weight was also reported by Dixit et al. (1978) with *Momoridica charantia* aqueous extract. Tyagi et al. (1989, 94) with acetone extract of *Trigonela foenum graecum (Linn)* also reported decrease in testes weight after 30 days. Seetharam et al. (2003) with *Amalakyadi churna* on male albino rats also reported decrease in testes and epididymis weight.

Most research groups have reported reduction in reproductive organ weights with different plant extracts such as Chauhan et. al. (2007) with *A. marmelos,* Mishra et al (2009) with aqueous extract of *Bouganvillea spectabilis*, Kachhawa et. al. (2010) with methanol extract of *Momordica dioica* root, Mathur et al (2010) with *Tecoma stans* leaves, Sathiyaraj et al (2010) with aqueous leaf extract of *Aegle marmelos*. Similar results have been observed with *Semecarpus anacardium* fruits (Sharma et al 2003) and *Carica papaya* seeds (Lohiya et al , 2002, Udoh and Kehinde, 1999). According to Gupta et. al, (2004), methanolic pod extract of *A. lebbeck* affected the male reproduction and caused reduction in testicular and accessory sex organ weights significantly.

Increased level of proteins in testes with aqueous extract of *T. bellarica* showed non-utilization of proteins in the testes indicating that no new cells are being formed. As protein synthesis and concentration in the sex organs are androgen dependent, increased level of proteins indicate that androgen levels are not being affected by the aqueous extract of *T. bellarica*. This fact is proved by Brooks (1980) who reported restoration of normal levels of proteins after testosterone therapy.

Joshi et al (1996) administered leaf extract of *Azadirachta indica* to male albino rats and reported decreased level of protein and suggested a general disturbance of protein anabolism, which may be due to androgen deficiency. The reduced protein content was observed in reproductive organs by Sarker et. al. (2000) with extract of *Piper betle* on Swiss albino male

mice. Significant decrease in testicular protein level was reported by Chauhan et. al. (2007) with ethanolic extract of leaves of *A. marmelos.* Shivabasavaiah et. al. (2011) also reported reduction in the level of proteins of testes and epididymis with extract of *Madhuca indica* leaves but no significant change in protein level of testes was reported after long-term treatment with the methanol subfraction of *Carica papaya* seeds by Manivannan et. al. (2009).

The increased level of sugars in testes reflects accumulation of sugar in the Sertoli cells due to arrest of spermatogenesis and absence of sperms in seminiferous tubules. Our findings are in agreement with Joshi et al (1996) who worked on aqueous extract of leaves of *Azadirachta indica* and reported increased level of sugar in male albino rats and gradual recovery after withdrawal of treatment and reported spermatogenic arrest.

Gupta et. al, (2004) reported low glycogen content in the testes after *A. lebbeck* administration. It is probably due to the inhibition of phosphorylase activation or the depletion of certain other enzymes which could block androgen synthesis. Chauhan et. al. (2007) with *A. marmelos* reported significant reduction in glycogen level. Shivabasavaiah et. al. (2011) also observed reduction in the level of glycogen of testes and epididymis, which affects spermatogenesis and sperm maturation.

Cholesterol is required for normal testicular activity as it is the precursor in the synthesis of steroid hormones. Increased level of cholesterol in testes is due to disruption of spermatogenesis. Cholesterol is the precursor of androgen hormones, so increased level of cholesterol indicates the cessation of hormone synthesis necessary for spermatogenesis. Our findings are in agreement with Anjali et al (1996) who reported increase in cholesterol level in testes with *A. indica*. Seetharam et al (2003) also reported increased level of cholesterol with Amalakyadi churna in male albino rats. A highly significant increase in testicular cholesterol was observed by Chauhan et. al. (2007) with ethanolic extract of leaves of *A. marmelos.* On the other hand Manivannan et. al. (2009) reported no significant change in cholesterol level of testes after long-term treatment with the methanol subfraction of *Carica papaya* seeds.

Alkaline phosphatase is primarily of testicular and epididymal origin. Increased level of ALP indicates the deficiency in androgen production which may cause impairment of the process of spermatogenesis and damage to germinal components. Our findings are in complete

agreement with Achal Garg (1979) who reported increase in level of alkaline phosphatase in gerbils after feeding with *Calotropis procera* extract, on the other hand non-significant change was observed by Chatterjee et al (1994) in testes ALP level with *Piper betle* extract.

Acid phosphatase activity is associated with lysosomes. The reason for increased level of acid phosphatase might be due to the tissue damage. As a rule the more drastic would be the damage to the overall germinal elements, the higher will be the acid phosphatase activity. Our findings are in agreement with many workers e.g. Reddy and Subodha (1967) using WIN 18446, Kar et al (1968) using busulfan for 10 days, Seetharam et al (2004) using Amalakyadi churna for different durations in male albino rats.

Lactate dehydrogenase (LDH) is associated with the maturation of germinal epithelial layer of seminiferous tubules and associated with post meiotic spermatogenic cells as it is found in spermatocytes, spermatids and spermatozoa **(Sinha *et al.* , 1997)**. The increased level of LDH may be due to the spermatogenic arrest. Our findings are in agreement with Nair et al (1989) who reported increase in level of LDH after administration of gossypol acetic acid for 10 weeks on male albino rats. Manivannan et. al. (2009) reported no significant change in LDH level in testes after long-term treatment with the methanol subfraction of *Carica papaya* seeds.

The decreased number of sperms suggests alteration in sperm production in testes. Oral administration of alcohol extracts of the seeds of *Momordica charantia* to male albino rats at a dose of 25 mg per 100 g b.w. for 35 days caused a decrease in the number of spermatocytes and spermatids, with the effects being more significant when administered through the intraperitoneal route (Kachhawa et. al. 2010). The crude extract of garlic (*Allium sativum*) when administered to male rats at varying concentrations (5%, 10%, 15% and 30%) for 30 days caused an increase in the percentage of empty seminiferous tubules and brought about a decrease in serum testosterone levels, with the effects being invoked at a dose as low as 10% (Dixit et al 1982).

In the end we may conclude that aqueous extract of leaves of *T. bellirica* has definite effect on the male reproductive organs. The results of the present study appear promising but before saying anything with certainty, further investigations are required.

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