DOI: 10.21276/ijabpt.2016.7.3.9

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-7, Issue-3, July-Sept-2016 Coden IJABFP-CAS-USA Copyrights@2016

Received: 11th May 2016 *Revised: 19th June 2016* *Accepted: 24th June 2016*

Research article

ANTIFERTILITY ACTIVITY OF *MANILKARA HEXANDRA* (ROXB.) DUBARD SEED EXTRACT ON MALE ALBINO RATS

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ABSTRACT: The present study involves a standard pharmacological model to test antifertility activity of seeds of *Manilkara hexandra* (Roxb.) Dubard on male albino rats. When the aqueous powdered drug (2gm/body weight) was administered to male albino rats has proved to be an effective antifertility drug. The activity was confirmed by significant decrease in sperm count, biochemical assays so also through histopathological investigations. Hence seeds of *Manilkara hexandra* can be a reliable herbal option after the necessary clinical trials.

Key words: Antifertility, Male contraceptive, *Manilkara hexandra.*

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INTRODUCTION

The global human population has grown from 1 billion in 1800 to 7 billion in 2012. The exponentially growing population has led to food shortages, global warming and other issues of resource scarcity. Therefore, to reduce/control our number has to be the first on a priority list. A good number of synthetic contraceptives are available in market, each one with either a limited success or side effects. Since herbal drugs are easily available and with no side affects the current study was undertaken.

Manilkara hexandra (Roxb.) Dubard a tall tree commonly known as "Khirni" belong to family Sapotaceae (Almeida M R, 2001). The fruit is edible. Seeds are used in the treatment of ophthalmic, leprosy, delirium, ulcers, piles and opacity of cornea. (Kirtikar K R, & Basu B D, 2001). The seed were used for spermicidal by the aboriginals (Khare CP, 2004). To prove this claims of *Manilkara hexandra* seed as male contraceptive, antifertility activity on male albino rats were carried out.

MATERIAL AND METHODS

Collection and preparation of plant extract

Seeds of *Manilkara hexandra* (Roxb.) Dubard were collected in the month of May from Borivili, (Mumbai) Maharashtra. The seeds were selected in a ripened stage from the mature tree. The specimen was identified from Blatter Herbaria at St. Xavier's College, Mumbai, Maharashtra. A voucher specimen was deposited for future use at K.V. Pendharkar College, Dombivli (E), Maharashtra. (Specimen Number: KVP/BOT/0073). Seeds were separated from the ripened fruit and were dried in shade. A fine powder was prepared by grinding the dry deeds in a blender. This powder was stored in airtight container and was used to feed the experimental rats.

Animals: Adult male albino rats, Wistar strain weighing 150-200 gm body weight used for the study were housed under standard laboratory condition. They were fed with standard rodent pellet and water *ad libitum*. The animals were grouped in to two groups of 6 animals each.

a. Control group - Rats receiving 1% gum acacia for 21 days.
b. Group II: Rats receiving *Manilkara hexandra* seed powder dissolved in 1% gum acacia (2gm/ kg body weight) for 21 days.

The study was approved by the ethics committee for animal experimentation, the registration no. 525/02/a/CPCSEA.

Acute Toxicity Study: An acute toxicity study was done to select the dose. The dose up to 2gm/ body weight did not produce any sign of toxicity and mortality. The animals were physically active (Bhagat M, 2007, Meenakshi B and Purohit A, 2004).

Antipyretic test: The crude extract was dissolved in 0.5 ml 1% gum acacia and administered orally to Group II rats for 21 days. On day 22 i.e. 24 hrs after the last dose the animals were sacrificed using ether anesthesia. Reproductive organs were weighed and dissected out (Glover T D and Nicander L, 1971, WHO. Protocol MB-50, 1983).

Bioassay of serum: Blood was collected by cardiac puncture and serum was separated and estimated for protein, cholesterol, alkaline phosphatase and acid phosphatase (Gupta R S, 2001, 2003, Plummer D T, 2004).

Bioassays of testes homogenate: 0.5 mg of tissue was procured from the testis after sacrificing the animals. This was homogenized with 1ml saline using homogenizer. The homogenate was also analyzed for protein, cholesterol, alkaline phosphatase and acid phosphatase (Plummer D T, 2004).

Hormonal assay: Serum testosterone levels were assayed from frozen samples using radio immuno assay method (Allain C C et al, 1974, Bartlett J M S et al, 1990, Dutta B and Mukherjee A K, 1964).

Sperm count: Sperm count was assessed in cauda epididymis by the standard methodology (Prasad M R N et al, 1972). *Histopathological preparation*: The testis was fixed in Bouin's fluid, paraffin sections were made and stained with hematoxylin and eosin. The sections were screened for histopathological effect of the drug.

Statistical analysis: The mean and standard error of mean (SEM) were calculated using Student's t- test (Mahajan B K, 1979).

RESULTS

Weight response – The orally tested *Manilkara hexandra* seed extract caused decrease in the weight of testes, epididymis, seminal vesicle + coagulating gland, vasa deferens and ventral prostrate significantly from the control. (Table 1, Graph 1)

Sperm count – There was a decrease in sperm count in tested rats compared to the control rats. (Table 2, Graph 2)

Hormonal assay – Serum testosterone level of *Manilkara hexandra* seed extract treated animals was decreased significantly in comparison to control group (Table 2, Graph 3)

Biochemical findings- A marked reduction in protein, cholesterol, alkaline phosphatase and acid phosphatase in both serum and testes homogenate was observed in treated rats. (Table 3 &and 4, Graph 4 and 5)

Histopathology of testis – Administration of crude extract caused an effective inhibition of spermatogenesis in male albino rats. Few seminiferous tubules underwent necrosis. Rupturing of basement membrane of seminiferous tubule was observed. There was an increase in the diameter of lumen, compared to control. The treated groups of rat showed agglutination of spermatozoa. (Figs 1 and 2).

Table 1: Effect of *Manilkara hexandra* seed extract on organ weights of male albino rats.

[Values are mean ± SE of six determinations]

Groups	Organ weight				
	Testes mg/100g	E.D. mg/100g	V.D. mg/100g	S.V. + C.G. mg/100g	V.P. mg/100g
Control rats	1.4 ± 0.05	0.28 ± 0.02	0.06 ± 0.004	0.15 ± 0.02	0.07 ± 0.02
Manilkara hexandra seed fed rats	1.28 ± 0.04*	0.25 ± 0.02*	0.05 ± 0.004*	0.01 ± 0.001*	0.02 ± 0.008*

*P<0.1vs Control E.D. = Epididymis, V.P. = Ventral prostrate, V.D. = Vasa deferens, S.V. =Seminal vesicle, C.G. = Coagulating gland

Table 2: Sperm count and Testosterone level of *Manilkara hexandra* seed fed male albino rats
[Values are mean ± SE of six determinations]

Groups	Sperm count (million/ml)	Testosterone (ng/ml)
Control rats	88.3 ± 0.81	2.33 ± 0.01
Manilkara hexandra seed fed rats	30.0 ± 1.69	1.18± 0.05

*P<0.02 vs Control

Table 3: Serum biochemistry of *Manilkara hexandra* seed fed male albino rats
[Values are mean ± SE of six determinations]

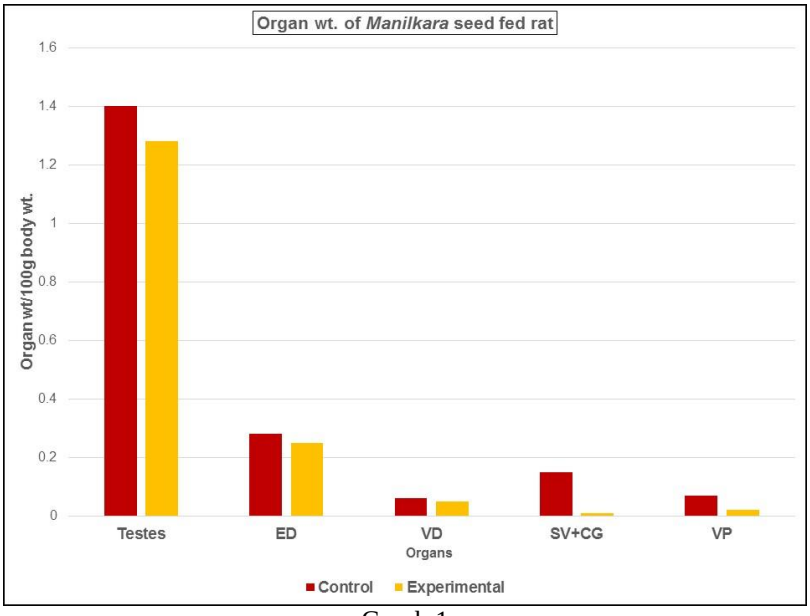
Groups	Protein mg/ml	Cholestrol mg/dl	Alkaline phosphatase u/l	Acid phosphatase u/l
Control	7.3 ± 0.28	61.3 ± 4.9	84.8 ± 29.9	9.31 ± 2.51
Manilkara hexandra seed fed rats	5.8 ± 0.27**	45.8 ± 1**	72.2 ± 0.13*	8.1 ± 1.08*

* P<0.1; **P<0.01vs Control

Table 4: Testis homogenate biochemistry of *Manilkara hexandra* seed fed male albino rats
[Values are mean ± SE of six determinations]

Groups	Protein mg/ml	Cholestrol mg/dl	Alkaline phosphatase u/l	Acid phosphatase u/l
Control	7 ± 0.02	13.1 ± 0.2	88.3 ± 8.2	38.2 ± 2.5
Manilkara hexandra seed fed rats	5.6 ± 0.008**	12.3 ± 0.1*	73.6 ± 7.7***	32.53 ± 1.5*

*P<0.1; **P<0.001; ***P<0.02 vs Control



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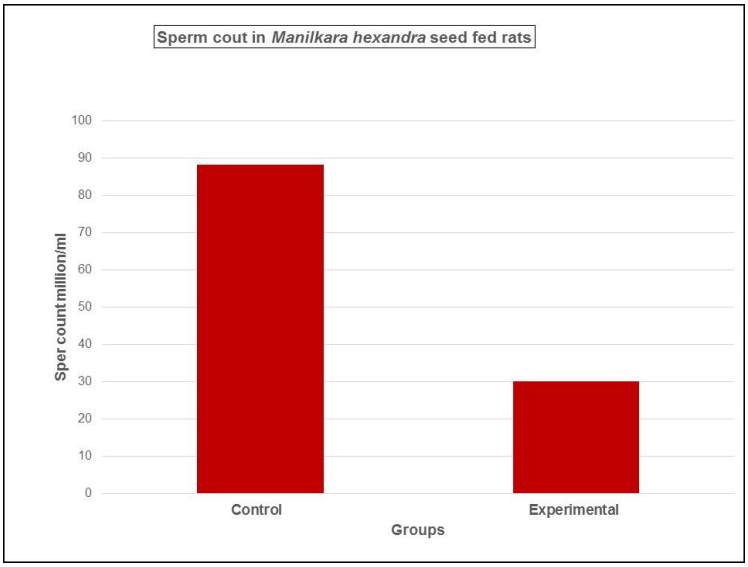

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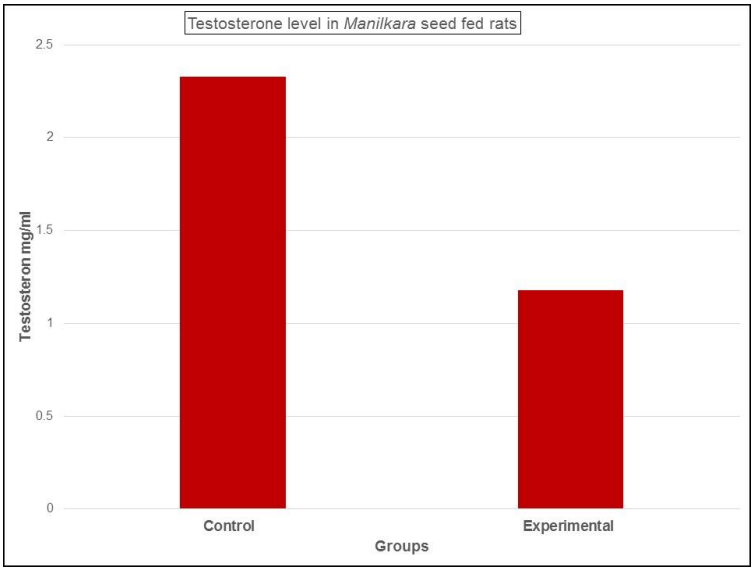
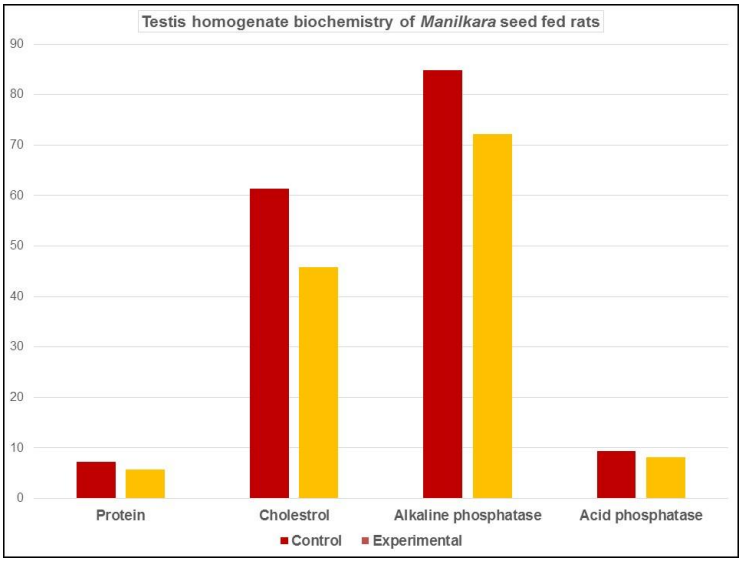


Graph 3



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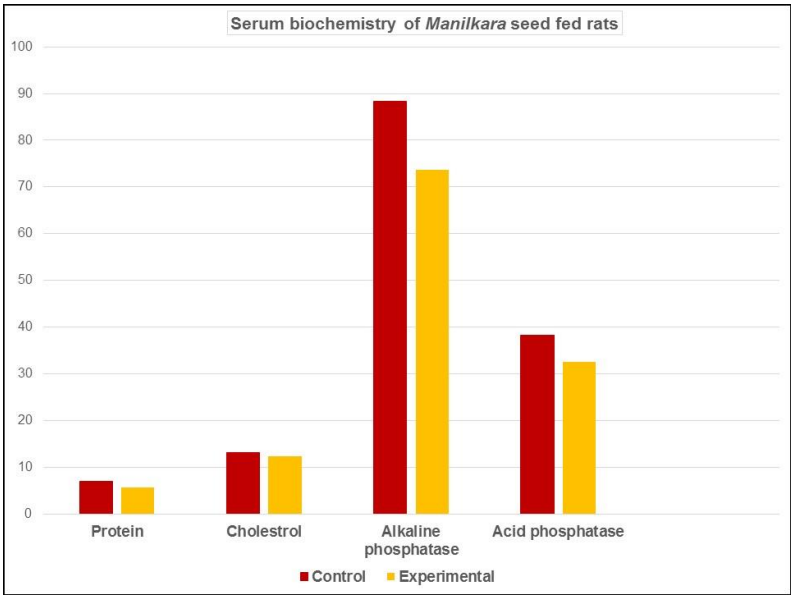

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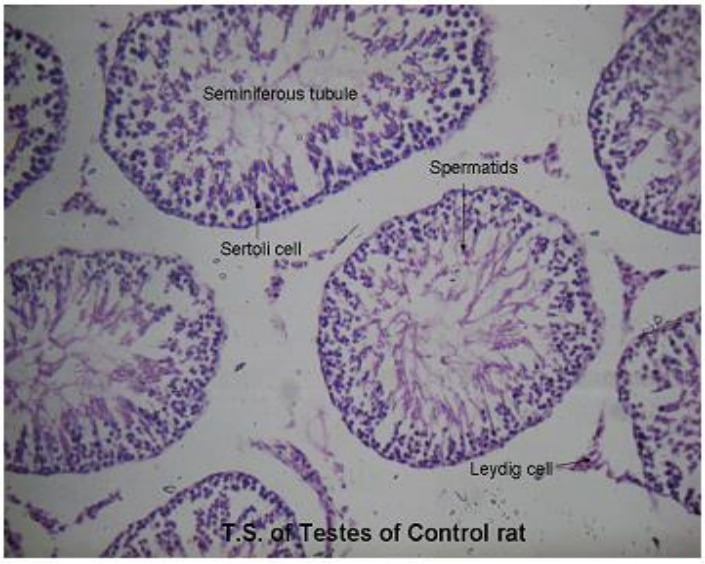

Fig. 1 T.S. of testes of control male albino rat

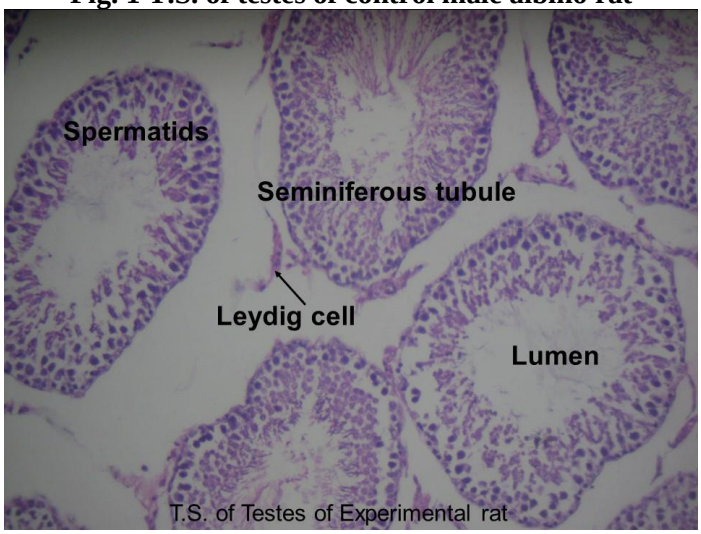

Fig. 2 T.S. of testes of experimental male albino rat

DISCUSSION

In the present study, administration of *Manilkara hexandra* seed extract to rats caused decrease in weight of testes, epididymis, seminal vesicle + coagulating gland, vasa deferens and ventral prostrate which may be due to low plasma level of testosterone. The decrease in weight of accessory sex organs indicates the atrophy of glandular tissue and also reduction in secretory cells thus reflecting the decrease level of testosterone. The decrease in sperm count might be due to partial arrest of spermatogenesis as it was also confirmed by Histopathological findings. A decrease in sperm reserve may be a reasonable cause for reduction in the weight of epididymis.

The effect of the said drug on the sex organ has also affected the biochemical assay of serum and testes homogenate.

Thus, the organ weight, biochemical and hormonal assay along with sperm count go concurrent with histopathological evidences. These results confirm that, the seed of *Manilkara hexandra* have antifertility potentials in male albino rats. Further clinical trials will confirm the safety and efficacy of the said drug of plant origin. The crude seed extracts are given to the rats due to the presence of mixture of bioactive compounds, hence detailed phytochemical analysis will reveal the exact compound responsible for antifertility activity.

ACKNOWLEDGEMENT

The authors are thankful to the mentor Late Dr. Shraddha N Shimpi for her guidance throughout for the completion of this research work.

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ISSN : 0976-4550

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