International Journal of Engineering Research & Management Technology
ISSN: 2348-4039
July-August-2021 Volume 8, Issue-4
Email: editor@ijermt.org www.ijermt.org

Pharmacology and Anti-fertility Activities of *Pongamia pinnata*, a medicinal plant

Dr. Ram Bhajan Kumawat

*Assistant Professor, School of Basic and Applied Sciences, University of Technology, Jaipur - 303903, (Rajasthan, India)

Dr. Renu Singh

#Associate Professor, Seth RL Saharia Govt. PG College, Kaladera, Jaipur (Rajasthan, India)

Abstract

The aim of study is to find out an important source of food and medicine for mankind. Human is usinga group of plants and their natural products for different purpose from ancienttimes.They are easy to use orally, as ailments or in natural forms with no side effects. This use of plant products is a part of traditional medicinal system well known as Ayurveda. *Pongamia pinnata*plant has been used for several pharmacological activities in different parts around the world.

Keywords:*Pongamia pinnata,*Anti-fertility and Pharmacology.

Introduction

Indians use medicinal plants as traditional system of medicine.Plants products have no side effectson humanhealth. All over the world maximum population relies on traditional and natural medicine. In the traditional Chinese system a group of medicine is directly or indirectly made by 5,000 plant species.A large number of folkloric medicinal is being used in the Philippines medicine system, on another side Bangladesh have also use folk medicine. In the recent years, the plant products and their uses in medicinal system has attracted many scientists for research. Group of medicinal plants has attracted a lot of attentions. Their use in various traditional, complementary and alternate plants products are frequently use in medical systems for treatment of human diseases (Chaturvedi*et al.*, 1995; Manohar*et al.*, 2011; Singh*et al.*, 2014; Mali*et al.*, 2015).

Aims and Objectives

The Ethanomedical properties of this plant viz. *Pongamia pinnata* have been reported earlier also by many scientist. In the present study the methanolic extract of these plants were administered orally at the dose level of 100mg/ kg. b.wt. for 60days to evaluate the effect of these plants extract

on testis and reproductive function. The experiment were carried out on male wister rats.

Materials and Methods

❖ Identification of Plant

The plant *Pongamia pinnata* used for experimental purpose was collected from the Jaipur district, Rajasthan and voucher specimen was deposited at Department of Botany, University of Rajasthan, Jaipur (India).

❖ Preparation of crude Extract

Fruits of the Plant*Pongamia pinnata* were washed, shade dried and were also placed in oven at 40°C and was then converted into a very fine powder by using the blender. 100gms of powder of fruits was mixed with distilled water and Methanol in ratio of 1:1 in a beaker and was kept in water bath at 55°C for 12×4 hrs. The extract was then filtered through afine muslin cloth and again using whatman filter paper (Jain *et al.,* 2011). The filtered through whatman filter paper was evaporated using rotary evaporator at 80°C and completely dried to obtain the powder of extract which was stored till further use.

❖ Animals

The present investigation was on mature adult male wister rats (weighing between 100-150 gm/ms) were procured from local animal suppliers, Jaipur and acclimatized before starting the experiment. They were housed in polypropylene cages in the animal house under standard conditions of humidity, temperature (25 ±2°C)and light (12 hr. light/dark). They were fed with standard rat pellet diet obtained from Ashirwad Pvt. Lt.dChandragh, India and water was provided adlibitum. All experimental animals were handled according to the guidelines of CPCSEA (INSA, 2000) and Institutional Animal Ethical Committee.

Experimental Design

A total of 60 male fertile healthyWister rats were purchased and randomly divided into two groups as follows:-

Group-1: Control treated vehicles.

Group-2: Rats treated at 100mg/kgb.wt of *Pongamia pinnata* extract 60 days.

Required amount of drug (extracts) was prepared freshly in double distilled water (100mg/ml) and

administered orally daily at 100mg/ kg b.wt for 60 days. The drug dose level was calculated according to (Lethal dose) LD_{50}*Pongamia pinnata* (Kage *et al.,* 2016).

Histological Studies

Histopathological studies were carried out using the standard technique of double Haematoxylin and eosin (HE) staining. Male reproductive tissues were dissected out and blotted free of bloodand were fixedimmediately after the autopsy. Fixation was carried out at room temperature for 24 hrs. Cut into 0-6mm thick pieces and thoroughly washed overnight under running tap water. These tissues were transferred to 70% alcohol for preservation of tissue. Several changes of 70% were given. The softer tissues were dehydrated in ethanol or alcohol series, cleared in xylene, embedded in paraffin was 55°C and transverse section were cut at 5 µm in rotary microtome for staining. The sectionswere deparaffnized and hydrated through xylene,alcohol series and distilled water and then immersed in haematoxylin. After 5 min sections were thoroughlywashed under running tap water. The section were rinsed in 70% ethanol counterstained with eosin, differentiated and dehydrated in alcohol series, cleared in xylene and mounted in DPX. All the stained slides were observed under microscope and photographs were taken at different magnification in binocular microscope with attached digital camera.

Parameters

A known amount of cauda epididymis was teased gently in a definite volume of normal physiological saline to release the spermatozoa form the epididymal tubules. Thetissue components were removed sand sperm suspension was used for evaluating sperm function parameters such as sperm count, sperm motility.

Testis

The testis are the main reproductive organ in male mice secreting testosterone hormone (spermatogenesis) and also produces sperm or male gamete (spermatogenesis) (Kyung W.C., 1997). Each testis is covered by a membrane called tunicavaginalis.Tunica albuginea and tunica vasculosa layers are made by connective tissues and blood vessels. The Tunicavaginalis consists of inner visceral and outer parietal layers. Inner most layer of the visceral layer of the tunica vaginalis is the tunica albuginea followed by tunica vasculosa (a plexus of blood vessels and connective tissue).

Tunica albuginea is divided into many pyramidal compartments called lobuli of the Testis. At the end, seminiferous tubules straighten to form tubuli recti which continue as rate testis. Each testicular lobule of testis contains one to three highly colied somniferous tubules made up of a single layered germinal epithelium. Testis contain three types of cells population (Tortora. 2014). Inside the seminiferous tubules, two types of cells: Germ cells, Sertoli cells and between the spaces ofseminiferous tubule, interstitial cells called Leydig cells are present.

❖ Spermatogenesis

Spermatogenesis is a process of formation of sperm byspermatogonial cells. In this process two divisional phases mitosis and meiosis occurs in the seminiferous tubule of the testis. spermatogonium is diploid cell and they divide mitotically to produce two diploid primary spermatocytes which are then converted two haploid secondary spermatocytes through meiosisI. These haploid secondaryspermatocytes undergo meiosisII which is a normal equational division not a reduction division.By this process four haploid spermatids are formed which is a pre sperm stage (Amann R.P., 2008). During maturation process (Spermatogenesis) each spermatid losses extra cellular material like cytoplasm and some cell organelles except for mitochondria, nucleus, acrosome centriole etc. Maturation process takes place under the influence of testosterone. Testosterone binds to androgen binding protein (a protein of sertoli cells) present in the seminiferous tubles and initiate maturation of sperms (Eberhard*et al.,* 2012). These spermatozoa are transported to the epididymis where they become active and gain motility.

❖ Body Weight

The initial and final bodyweights of the animals were recorded and the other observations were also recorded because they are correlated with the bodyweight of the rats.

❖ Sperm Motility

A method for sperm motility measured was fond by Prasad *et.al.* (1972). According this method a drop of sperm suspension was placed on the Neubauerchamber (it is an instrument for sperm motility measure) and observed under low magnification power about (10X) in a microscope. The chamber was focused on the WBC region. Sperm motility was determined by counting both type one is motile and another one is non-motile spermatozoa. A total of minimum 10-12 separate fields were scored and the sperm motility was calculated. The Sperm motility was expressed during counting as percent motile sperms.

❖ Sperm Count

The Cauda epeididymal sperm count of all type groups like control and treated or experimental groups of animals; it was determined by the method of Prasad*et, al.* 1972 by using Neubauer chamber of haemocytometer. The caudaepididyamal sperm suspension was sucked up to the 0.5 marks in WBC pipette. The suspension was then diluted up to the 11marks of tube with 5% sodium bicarbonate (NaHCo3) and mixed it very well thoroughly. Sodium bicarbonate acts as spermicidal activity and kills the spermatozoa to facilitate it during counting. Then take a drop of suspension was transferred to the Neubauer chamber for next experimental purpose and gently covered it with a cover slip. Spermatozoa were counted in 64 sub- squares of the WBC counting regions and calculated. The sperm density and count was expressed in term of million spermatozoa /ml of sperm suspension.

❖ Fertility Index

The experiment was conducted with treated males were cohabitated with normal adult cycling females in the ratio of 1:2 from 55thday of treatment. Thereafter numbers of pregnant females were counted to get fertility for 5 successive day index. The fertility indexes of controland treated groups of animals were calculated by formula given by (Parker, 2006) as mentioned below:

$$\text{Male fertility index} = \frac{\text{Number of males impregnating female}}{\text{Number of males cohabitated}} \times 100$$

❖ Statistical Analysis

Thestatistical values were calculated by Mean ±SEM. The significance of difference with the both group was assessed using one side students “t”- test. Symbols represent statistical significance as indicated P ≤ a0.05, b0.01, c0.001 and the non-significance(ns) for Non-significant ≤ 0.05→ a, P ≤ 0.01→ b and P ≤ 0.001→ c.

Results

❖ Effects on Body and Weight of Testis

No significant changes were observed in body weight of rats treated with plant extracts in comparison to control treated vehicles (Table-1-2). The Testis weight wasdecreasedsignificantly in the rats of treated group with plants or experimental group. Table-1 shows comparison between the two groups, the group-1 (controlled) and group-2 (which were treated with plant extract).

❖ Changes in Sperm Motility in Rats with the Extracts Treatment

The sperm motility was **decreased** significantly in experimental group (treated with plant *Pongamia pinnata*extracts) (Table-1).

❖ Effects on Sperm Count

The sperm density and count was shown **decreased**significantly in experimental group (treated with plant *Pongamia pinnata*extracts) (Table-1).

❖ Effects on Fertility Index in Rats Treated with Plant Extracts

The fertility index was changed significantly in rats treated with plant*Pongamia pinnata*extracts treatment with comparison withcontrols (Table-1).

Table 1: Effect shown on weight of Testis, sperm motility, sperm density and fertility index of Wister rats treated with *Pongamia pinnata*at 100 gm /kg b.wt. For 60 days.

S.No	Body weight (%)		Weight of Testis (mg/100g b.wt)	SpermMotility (Caudaepididymides; %)	SpermDensity million/ml (Testis)	Fertility Index (%)
	Initial (gm)	Final (gm)				
Group–1 Control	134.00 ± 3.055^{ns}	166.50 ± 3.337 ^{ns}	1179.00 ± 2.745 ^{ns}	68.00 ± 0.258 ^{ns}	61.00 ± 0.471 ^{ns}	97.60 ± 0.542 ^{ns}
Group–2 *Pongamia pinnata*	110.30 ± 1.415^{c}	130.90 ± 1.574^{c}	1039.50 ± 3.274^{c}	60.00 ± 0.258^{c}	35.00 ± 0.471^{c}	47.30 ± 0.597^{c}

Data expressed as Mean ± Standard error and significance at P≤ 0.05a, P≤ 0.01b and P≤ 0.001 c

Table 2:- Independent Samples "t" Test control and *Pongamia pinnata*

Independent Samples Test							
Variables	**Group**	**Mean**	**Sd**	**Standard Error Mean**	**t-value**	**Degrees of freedom**	**P-value**
Body weight Initial (gm)	Control	134.00	9.661	3.055	7.040	18	0.000
	Pongamia pinnata	110.30	4.473	1.415			
Body weight Final (gm)	Control	166.50	10.554	3.337	9.648	18	0.000
	Pongamia pinnata	130.90	4.977	1.574			
Weight of Testis (mg/100g b.wt)	Control	1179.00	8.679	2.745	32.654	18	0.000
	Pongamia pinnata	1039.50	10.352	3.274			
Sperm Motility (Cauda epididymides; %)	Control	68.00	0.816	0.258	21.909	18	0.000
	Pongamia pinnata	60.00	0.816	0.258			
Sperm Density million/ml (Testis)	Control	61.00	1.491	0.471	39.000	18	0.000
	Pongamia pinnata	35.00	1.491	0.471			
Fertility Index (%)	Control	97.60	1.713	0.542	62.389	18	0.000
	Pongamia pinnata	47.30	1.889	0.597			

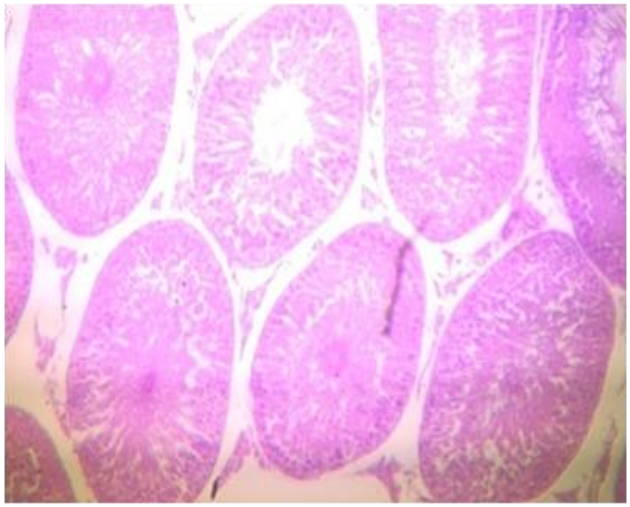
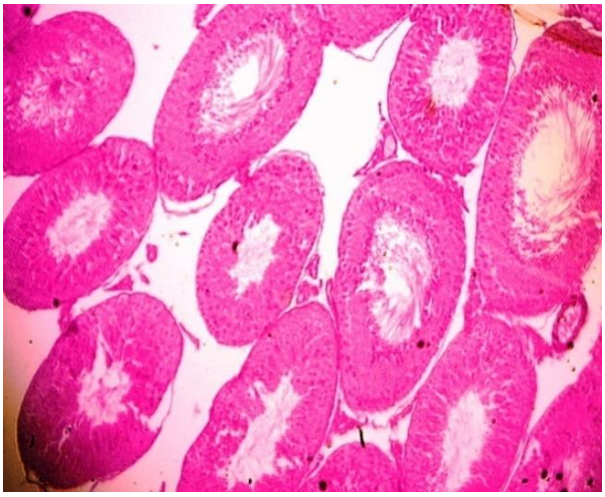
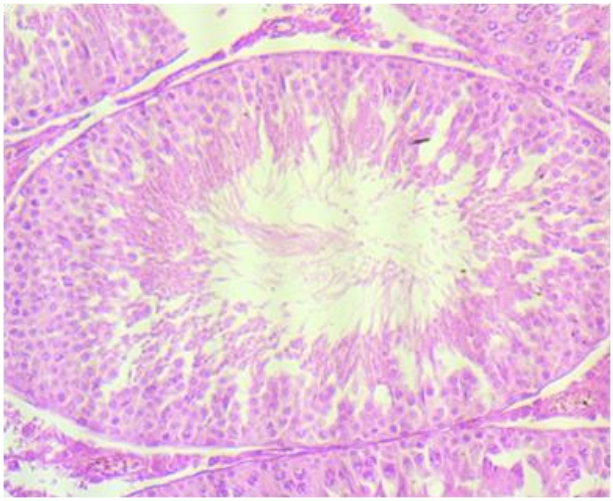
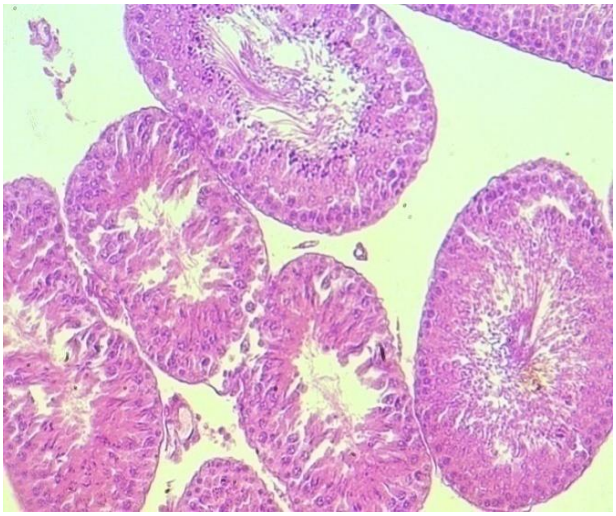
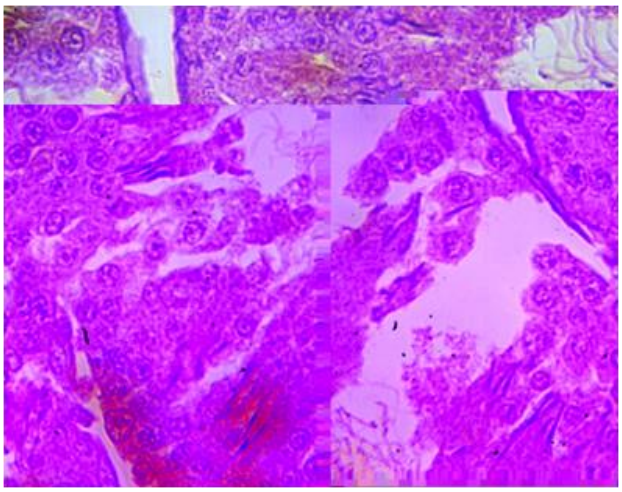
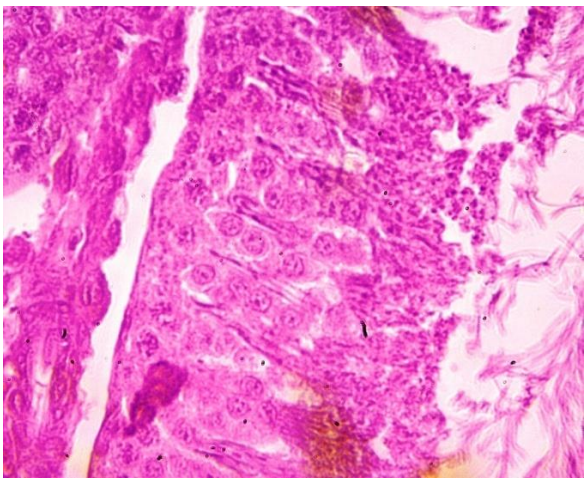
Arrows show a significant change in the testis

❖ **Histopathological Changes in Testis of Rats Treated with Plant Extracts**

Haematoxylin and Eosin (HE) were two stains used for slide preparation of the both experimental and control.

Photomicrographs of Testis of Rats

The photographs of Testis of rats (Photomicrographs 1-6) shows the histopathology of testis of rats treated with *Pongamia pinnata*extract (experimental group). Photographs are showing significant degeneration changes in germinal epithelium of spermatocytes, spermatids and spermatozoa.

	
Group-1: **Control (100XH.E.)**	Group-2: ***Pongamia pinnata*** **(100XH.E.)**
	
Group-1: **Control** (**400XH.E.)**	Group-2: ***Pongamia pinnata*** **(400XH.E.)**
	
Group-1: **Control** (SCANNER)	Group-2: ***Pongamia pinnata***(SCANNER)

Discussion

Although many compounds natural and synthetic have been used to control function of male reproductive systems especially vital organ Testis to control fertility in male.However, herbal plants extracts have been also practiced in traditional medicine system of India because they are safe and no other side effects on living beings.Many plant extract or their metabolites have been used for fertility controls such as*Daucas carota,Carica papaya, Abrusprecatorius* etc (Sharma *et al.,* 2017). Plant*Pongamia pinnata*havebeen used in traditionally to cure different diseases. Therefore, in present study methanolic extracts of *Pongamia pinnata* (fruits) was prepared and administered orally in male Wisterrats. The results of the present study showed reduction of Testis weight, sperm motility and sperm count ability and degenerative changes in Testis. Since androgens, FSH and LH are essential for the production of the normal sperm density, sperm motility (Gupta *et al.*, 2018; Sharma &Kalla, 1994).The treatment causeddegenerative changes in sperm activity during spermatogenesis.*Pongamia pinnata* treatment inhabit spermatogenesis might be due to decreased level of male harmones and their activity since testosterone regulates the growth and development of reproductive organs and spermatogenesisWreford, & Robertson, 1994 Gupta *et al.,*2018. Histopathological observations of the treated group with*Pongamia pinnata*extractsshowed reduction of the Leydig cells our degenerative changes in spermatogenesis(Born *et al.,* 1988). (El-Dwairi&Banihani, 2007) Were also mentioned the reports regarding damage of spermatogenesis by plant extracts. In the present study, increased androgen hormone production after*Pongamia pinnata*treatment is reflected by the increased number ofmature Leydig cells and their functional texture. It was also justified by the enhancement of number of spermatocytes and spermatids as thesestages are completely androgen-dependent (Agrawal, Chauhan, &Mathur, 1986).Methanolicextrats of *Pongamia pinnata*treatment significantly reducedsperm density, Sperm motility including fertility indices intreated rats might be due decreased androgen levels.

Conclusion

We can conclude on the basis of Histopathological observations carried out in extract treated rats showed degenerative changes in seminiferous tubules, decreased number ofspermatogenic elements spermatozoa in testis which reflects antispermatogenic nature of the extract. Further, decreased weight of Testis, sperm motility, sperm density and fertility indices support that ofthe*Pongamia pinnata* treatment, providing an evidence ofthe androgen deprivation effects of the extracts in rats.

References

1) Chaturvedi M, Mali, PC and Dixit VP Fertility regulation in male rats with the help of *Echinopsechinatus* of root extract J Phytological Res 8(1/2) 115-118, 1995.

2) Manohar P., Rajesham V.V., Ramesh M., Kiran KS.,Prasanna K J.Pharmacognostical, Phytochemical and antimicrobial activity of *Bauhinia Racemosa*leavesJ PharmaceuBiol*1(1) 10-14, 2011*.

3) Singh S, Sonia and Kumar N A review: introduction to genus Delonix World J Pharmacy and PharmaceuSci 3(6) : 2042-2055, 2014.

4) Mali PC, Singh A R, Verma M K,Chahar M K and Dobhal MP Contraceptive effects of Withanolide- A in adult male albino ratsAdv. Pharmacol. Toxicol. 16 (1) 31-44, 2015.

5) Ritesh Jain and Sanmati Kumar Jain Total Phenolic Contents and Antioxidant Activities of Some Selected Anticancer Medicinal Plants from Chhattisgarh State, India. Pharmacologyonline 2: 755-762, 2011.

6) Azu, O. O., Duru, F. I. O., Osinubi, A. A, Noronha, C. C., Elesha, S. O. and Okanlawon A. O. Preliminary study on the antioxidant effect of *Kigeliaafricana*fruit extract (Bignoniacieae) in male Sprague- Dawley rats African Journal of Biotechnology Vol. 9 (9), pp. 1374-1381, 2010.

7) J.Vinnarasi, A. AntoArockiaRaj Anti-Muscular Activity of Various Parts of *Martyniaannua*Linn *Ijppr.Human, ; Vol. 9 (4): 250-255, 2017.*

8) *Kyung*-Hoon Shin. Professor of Marine .Journal of Oceanography 53 (1), 41-51, 105, KS Kumar, HU Dahms, JS Lee, HC Kim, *WC* Lee, KH Shin. *1997.*

9) Principles of Anatomy and Physiology by Gerard J Tortora 14e with Lab Manual BIO 301 & 302 Purdue 3e Set. 16 June 2014.

10) The cycle of the seminiferous epithelium in humans: a need to revisit? J Androl. 469-87. doi: 10.2164/jandrol.107.004655. Epub .*Amann RP*. Animal Reproduction 2008.

11) Anton Eberharda,n , Maria Shkaratan b Powering Africa: Meeting the financing and reform challenges$ Energy Policy 42 9–18, 2012.

12) Prasad, M. R. N., Chinoy, N. J., &Kadam, K. M. Changes in succinate dehydrogenase level in rat epididymis under normal and altered physiologic conditions. *Fertility and Sterility*, *23*, 180–190. https://doi. org/10.1016/s0015-0282(16)38825-2, 1972.

13) Abbas, D., G. Simon, R. Ali, N. Hossein, M. Masoud, N. Lutfun and D. Satyajtt,. Flavone Cglycoside and cucurbitacin glycoside from Citrulluscolocynthis. DARA, 2006;14(3): 109-114.

14) Punitha, R. and Manoharan S. Antihyperglycemic and antilipidperoxidative effects of *Pongamiapinnata* (linn.) Pierre flowers in alloxan induced diabetic. J EthonPharmacol; 105: 39-46. 2006.

15) Sandhya B., Thomas S., Isabel W., Shenbagarathai R. Ethnomedical plants used by the Valaiyan community of Piranmalai Hills (reserved forest), Tamilnadu, India - A pilot study. *African Journal of Traditional, Complementary and Alternative Medicines.* 3(1):101–114. 2006.

16) Cos P, Vlietinck AJ, VandenBerghe D, Maes L Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. J Ethnopharmacol 106: 290-292. 2006.

17) *Kyung*-Hoon Shin. Professor of Marine .Journal of Oceanography 53 (1), 41-51, 105, KS Kumar, HU Dahms, JS Lee, HC Kim, *WC* Lee, KH Shin. *1997.*

18) Sharma, M., Arya, D., Bhagour, K., & Gupta, R. S. Natural aphrodisiac and fertility enhancement measures in males: A review. *Current Medicine Research and Practice*, *7*, 51–58. https://doi.org/10.1016/j. cmrp.2017.02.007. 2017.

19) Mahimashama, Dharmendraarya, Kiranbhagour, RadheyguptaModulatory effects of methanolic fruit fraction of *Pedalium murex* on sulphasalazine-induced male reproductive disruption. Wiley andrologiaDOI : 10.1111/and.13190, 2018.

20) Sharma, R. K., &Kalla, N. R. Spermatozoal abnormalities and male infertility in the rat following sulfasalazine treatment. *International Journal of Fertility and Menopausal Studies*, *39*, 347–354. 1994.

21) O'Donnel, L., Mc Lachlan, R. I., Wreford, N. G., & Robertson, D. M. Testosterone promotes the conversion of round spermatids between stages vii and viii of the rat spermatogenic cycle. *Endocrinology*, *135*, 2608–2614. https://doi.org/10.1210/endo.135.6.7988449, 1994.

22) Born, H. J., Poscmann, P. H., Stoll, W., Sandow, J., Taubert, H. D., &Kuhl, H. Investigations upon the mechanism of inhibition of spermatogenesis in the rat by a dimericethynodiol testosterone ester. *ActaEndocrinologica (Copenh)*, *117*, 536–544. https://doi.org/10.1530/acta.0.1170536, 1988.

23) El-Dwairi, Q. A., &Banihani, S. M. Histo-functional effects of *Peganumharmala*on male rat's spermatogenesis and fertility. *Neuroendocrinology Letters*, *28*, 305–310. 2007.

24) Agrawal, S., Chauhan, S., &Mathur, R. Antifertility effects of embelin in male rats. *Andrologia*, *18*, 125–131. https://doi. org/10.1111/j.1439-0272.1986.tb01749.x 1986.