INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Volume: 2: Issue-3: July-Sept -2011 IJABPT *ISSN 0976-4550*

ISOLATION AND EVALUATION OF ANTIFERTILITY ACTIVITY OF TOTAL ALKALOIDS FROM LEAVES OF *AEGLE MARMELOS* IN MALE ALBINO RATS (*RATTUS NORVEGICUS*)

B. Satheesh Kumar[1], K. Madhukar Rao[1], K. Madhusudhan[3], M. Krishna Reddy[2*], MSK Prasad[1*]

[1]Dept of Biochemistry, [2]Dept of Zoology, [3]Dept of Botany, Kakatiya University, Warangal- 506 009.

ABSTRACT : *Aegle marmelos* (Rutaceae) is being used in traditional medicine treatments, such as for intermittent fever, intestinal ailments, fertility control etc. It has been proved to be effective against several major diseases including cancer, diabetis and cardiovascular diseases. Although the plant is a well known male antifertility plant, till today only the crude extracts of the plant were screened for antifertility activity in male rats. The plant is rich in alkaloid content and Aegelenine, Marmeline and Skimmianine, are some of the alkaloids isolated so far, showed variety of pharmacological activities. In view of these facts, in the present study, total alkaloids have been isolated from leaves of *A. marmelos* and their effect on fertility of adult male albino rats (*Rattus norvegicus*) was investigated. Three different doses 20, 40, 80 mg/kg body weight of total alkaloids were orally administered to mature male albino rats (Wistar strain) of proven fertility (235-2450gr) for 60 days. On day 61, all the animals were sacrificed and the fertility and safety parameters were studied. Weights of all the major reproductive organs, accessory glands and sperm counts were significantly decreased in dose dependent manner suggesting the antifertility activity and serological parameters showed no significant changes in treated animals at the tested dose levels indicating the safety of long-term use of total alkaloidal fraction of *A. marmelos.*

Keywords: *Aegle marmelos*, Alkaloids, Antifertility, male rats.

INTRODUCTION

Despite the availability of modern (orthodox) medicine, many developing countries, especially in the rural areas, still rely heavily on traditional healers and medicinal plants to meet their primary health care needs and that of their domestic animals. This has been attributed to easy accessibility and low cost of herbal medicine. Evaluation of the importance and the effects of plant derived drugs on fertility of laboratory animals have long been recognized. The information obtained from extracts of medicinal plants makes pharmacological studies possible. In modern times, the active ingredients and curative actions of medicinal plants were first investigated through the use of European Scientific methods (Harborn, 1998). The most important ingredients present in plant communities turn out to be alkaloids, terpenoids, steroids, phenols glycosides and tannins (Abayomi, 1993).

Aegle marmelos (Rutaceae) is commonly known as bael tree which is considered as a sacred tree by the Hindus. The bael tree has great mythological significance and abounds in the vicinity of temples. The leaves of the tree are traditionally used as sacred offering to Lord Shiva, the God of health. It is one of the most useful medicinal plants of India. Its medicinal properties have been described in the ancient medical treatise in Charaka Samhita. All parts of this tree-stem, bark, root, leaves and fruit at all stages of maturity have medicinal virtues and have been used as medicine for a long time. It has been claimed the leaf of *Aegle marmelos* posses contraceptive efficacy (Bhattacharyay, 1982). The ethanolic extract of *Aegle marmelos* leaf possesses anti-spermatogenic activity (Sur *et al,*. 1999) and aqueous extract of the leaf has anti-motility action on spermatozoa in rats (Sur *et al.,* 2002).

The leaves of Bael are astringent, laxative and expectorant and are useful in treatment of ophthalmia, deafness, inflammations, cataract, diabetes, diarrhoea, dysentery, heart palpitation and asthmatic complications (Kirtikar and Basu, 1993). Fresh aqueous and alcoholic leaf extracts of *Aegle marmelos* were reported to have a cardio tonic effects in mammals (Haravey, 1968) and (Nadkarni, 2000). *Aegle marmelos* leaf extract has been reported to regenerate damaged pancreatic beta cells in diabetic rats (Das *et al.,* 1996), increased the activities of peroxidase in the liver tissues of Isoproterenol treated rats (Rajadurai *et al.,* 2005) and it was found to be a potential antioxidant drug, which reduces the blood sugar level in alloxan induced diabetic rats (Sabu and Ramadasan, 2004). An aqueous decoction of the leaves has been shown to possess a significant hypoglycemic effect (Karunanayeke *et al.,* 1984). Previous studies revealed that some compounds including cinnamic acid and coumarins derivates and alkaloids have been isolated from *Aegle marmelos* (Basu and Sen 1974, Govindachari and M.S.Premila 1983 and Sharma *et al.,* 1981). skimianine and aegelin are the alkaloids present in the leaves (Sugeng Riyanto *et al.,* 2001). The phytochemical screening of the crude extract revealed the presence of Alkaloids, Cardiac glycosides, Terpenoids, Saponins, Tannis, Flavonoids, and Steroids (Venkatesan *et al.,* 2009).

Considering the diverse medicinal properties of *Aegle marmelos* and presence of pharmacologically active alkaloids of the plant, the present study was under taken to evaluate the contraceptive efficacy of alkaloids of *Aegle marmelos* in male rats.

MATERIAL AND METHODS

Collection of plant material

Leaves of *A. marmelos* were freshly collected during Sep-Dec in Warangal district of Andhra Pradesh, India and were cleaned with distilled water and shade dried at room temperature. The plant was authenticated and a voucher specimen was preserved in our laboratory, Department of Zoology, Kakatiya University, Andhra Pradesh.

Isolation of total alkaloids

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms (International Union of Pure and Applied Chemistry, 1995). They can be extracted by acid-base extraction with ease. Powdered sample of the air-dried raw material was wetted with aqueous ammonia (10%) and then completely dried at room temperature. The sample was packed in soxhlet apparatus and extracted with $CHCl_3$ for 12hrs to extract alkaloidal bases. After the completion of extraction process, the extract was concentrated to a 1/10 volume using rotary evaporator *in vacuo*, and extracted with aqueous H_2SO_4 (5%). The acidic solution of bases was washed with ether and made basic with ammonia (25%). Alkaloids from the basic solution were fractionated (3X) with $CHCl_3$ in separating funnel. The solvent was removed from chloroform fraction to get the total alkaloids.

Treatment of animals

Wistar strain male albino rats of proven fertility (235-245gr) were brought from National Institute of Nutrition (NIN), Hyderabad. They were acclimatized for laboratory conditions for one week under controlled temperature of 21±1°C and 12:12 hr light/dark cycles. Food and water were available *ad libitum*. The animals were equally divided into four groups each containing six animals. Known amount of total alkaloids were dissolved in distilled water. The treatment groups T1, T2 and T3 were subjected to oral administration of total alkaloids about 20, 40, 80 mg/kg body weight/day respectively for 60 days using Gastric gavage. The control group animals received vehicle only (distilled water).

Body and organ weights and Fertility parameters

Initial and final body weights of the animals were recorded. After 24 hours of the last dose, the rats were weighed, anesthetized and blood was collected by puncturing the retro-orbital venous plexus and serum was separated.

The rats were sacrificed by cervical dislocation, the reproductive tract was taken out trimmed free of fat and each organ was weighed separately on an electronic balance. The relative weights of the organ per 100 g of body weight were calculated. The male reproductive organs used for the study included testes, epididymides, ventral prostrate and seminal vesicle. To determine sperm density, 100 mg of cauda epididymides was minced in 2 ml of physiological saline. One drop of an evenly mixed sample was applied to a Neubauer's counting chamber under a cover slip and observed under light microscope. Cauda epididymal sperm counts were made by routine procedure and expressed as million/ml of suspension (Prasad *et al.,* 1972).

Serum biochemical parameters

The safety of total alkaloids has been evaluated at the tested dose levels by comparing the serological parameters of treated group animals with the control group. The serum was analyzed to estimate blood glucose (Astoor and King, 1954), Urea (Varley, 1969) and total protein (Lowry *et al.,* 1951). Albumin was measured by colorimetric estimation. Globulin was obtained from the difference between total protein and albumin. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were determined using a photoelectric colorimeter (Duncan *et al.,* 1994 and Toro and Ackermann, 1975). Serum urea and creatinine levels were determined using photoelectric colorimeter as described by (Coles, 1986 and Toro and Ackermann, 1975).

STATISTICAL ANALYSIS

Results were expressed as mean ± standard deviation (S.D.). Where applicable, the data were subjected to one way analysis of variance (ANOVA). P values at 5% were regarded as significant and the comparison between the control and experimental groups was done using the Dunnett's test.

RESULTS AND DISCUSSIONS

Body weight and Fertility parameters

During the period of experiment the rats were healthy, growing at normal growth rate. Their body weight gain was similar to that of control animals (Table 1). Histopathology showed that no significant lesions were observed in this study and this may point to the fact that this plant is relatively safe for use nutritionally and medicinally.

Table-1: Weights of reproductive organs

Group	Average body weight (Avg±SD)		Weights of organs (%)			
	Treatment		Testes (GSI)	Cauda epididymis	Ventral Prostate	Seminal vesicles
	Before	After				
Control	242±5.37	273±6.32	0.992±0.18	0.212±0.03	0.214±0.05	0.305±0. 05
T1 (20mg/Kg)	243±6.61	269±8.18	0.712±0.19	0.184±0.04	0.184±0 07	0.273±0. 07
T2 (40mg/Kg)	239±7.25	267±9.34	0.632±0.13	0.149±0.06	0.151±0.08	0.226±0. 09
T3 (80mg/Kg)	241±7.01	273±9.94	0.524±0.17	0.102±0.05	0.127±0.09	0.185±0.06

GSI = Gonado somatic index, $p<0.05$, 6 animals per each group.

In rats, the whole spermatogenic process requires 53 days out of which spermatozoa spend the last 6 to 7 days in the final transit through epididymides (Ke and Tso, 1982). Alkaloids of *A. marmelos* were administrated for one complete spermatogenic cycle. The present study shows that, administration of these alkaloids resulted in a decrease of all fertility parameter in male albino rats. The weights of testes and accessory glands are shown in table-I. The weight of all reproductive organs was markedly reduced when compared to that in the control group. The GSI (Gonado Somatic Index) and weights of accessory glands were significantly ($p<0.05$) decreased in dose dependent manner. In a similar study *piper betle* linn (stalk) extract caused significant reduction in fertility at dose 1.25 g/kg and higher doses. A reduction of relative weight of testis and other accessory sex organs was also recorded (Adhinkary *et al.,* 1990). In another similar study oral administration of seed oil of *Melia azadrach* L at 100mg/kg body weight caused a significant decrease in fertility parameters like GSI and weights of accessory glands (Parandin *et al.,* 2008). Hence the results of the present study indicated the correlation of reduction in weights of testes and accessory glands with the antifertility effect on rats.

Long-term administration of alkaloids of *A. marmelos* caused a significant decrease ($p<0.05$) in sperm density about 58.90%, 78.39% and 86.19% in treatment groups T1, T2 and T3 respectively in comparison with control group animals (Table-2).

Table-2: Sperm concentration

Group	Sperm concentration (millions/ ml) (Avg±SD)	Percentage change
Control	56.86 ± 3.18	NIL
T1 (20mg/Kg)	23.37±2.12	-58.90
T2 (40mg/Kg)	12.29±1.16	-78.39
T3 (80mg/Kg)	7.85±1.32	-86.19

$p<0.05$, 6 animals per each group.

In a similar study, it was found that feeding 50% ethanolic extract of *A. aspera* to male rats resulted in reduced sperm counts (Sandhyakumary *et al.,* 2002). Based on the present study it could be concluded that the active principle could be an alkaloid.

Serum biochemical parameters:

All the serological parameters (Table-3) showed no significant changes in treated animals in comparison with the control group animals. In a similar study administration of methanolic stem extract of *Sarcostemma acidum* (Roxb) reduced the sperm concentration and serum parameters were within the normal range indicating no toxic side effects on normal metabolism at the tested dose levels (Pramod kumar *et al.,* 2002). Hence the present study shows that the total alkaloids of *A. marmelos* are safe without any toxic side effects at the tested dose levels.

TABLE-3: Serum parameters (Average ±SD)

Serum values	Control	Treatment		
		T1(20mg/Kg)	T2(40mg/Kg)	T3(80mg/Kg)
Glucose (mg/dl)	155.65±9.13	172.32±18.22	125.95±15.18	182.49±17.52
Total protein(g/dl)	7.28±0.11	7.13±0.22	6.62±0.35	6.21±0.38
Albumin(g/dl)	3.51 ± 0. 11	3.68 ± 0.12	3.28 ± 0.25	3.32 ± 0.24
Globulin (g/dl)	3.77±0.17	3.55±0.22	3.44±0.19	2.89±0.15
Urea (mg/dl)	11.3±1.5	9.5±1.0	5.1±0.9	10.2±1.3
Creatinine (mg/dl)	0. 81 ± 0. 18	0. 89 ± 0. 22	1.26 ± 0.35	1.41 ± 0.42
ALT (U/L)	392.7±5.8	411.7±7.7	421.2±8.2	452.2±8.6
AST (U/L)	193.1±3.0	204.3±4.3	253.1±6.6	277.8±6.4
ALP (U/L)	77.1±4.5	79.1±4.5	87.7±5.8	92.4±6.7

ALP= Alkaline phosphatase**; ALT =** Alanine Serum Transaminase**;**
AST = Aspertate Serum Transaminase**.** $p<0.05$, 6 animals per each group**.**

CONCLUSION

The results revealed that the total alkaloidal fraction of *A. marmelos* shows antifertility activity in male albino rats on dose dependent manner. Hence the total alkaloidal fraction needs to be studied further to identify and isolate the active compounds to develop new effective and safe antifertility compound.

ACKNOWLEDGMENT

The author B. Satheesh Kumar (CSIR-SRF) gratefully acknowledges Council of Scientific and Industrial Research (CSIR), New Delhi, for awarding research fellowship through joint UGC-CSIR National Eligibility Test.

REFERENCES

1. A.K. Nadkarni (2000). Indian materia medica, IIIrd Edn. Popular press, Mumbai, India, pp. 45 – 49.
2. A.V. Das, P.S. Padayatti and C.S. Paulose (1996). Effect of leaf extract of *Aegle marmelos* (L) Correa ex Roxb. On histological and ultrastructural changes in tissues of streptozotocin induced diabetic rats. Indian J Exp Boil, 34: 341 – 345.
3. A. Astoor and E.J. King (1954).Simplified colorimetric blood sugar method Biochem J; 56: XLIV.
4. B.R. Sharma, R.K. Rattan and P. Sharma (1981). Marmeline, an alkaloid, and other components of unripe fruits of *Aegle marmelos*, Phytochemistry, 20(11), 2606-2607.
5. D. Basu and R. Sen (1974). Alkaloids and coumarins from root-bark of *Aegle marmelos*, Phytochemistry, 13, 2329-2330.
6. D. Venkatesan, C.M. Karrunakarn, S. Selva Kumar and P.T. Palani Swamy (2009). Identification of Phytochemical Constituents of Aegle marmelos Responsible for Antimicrobial Activity against Selected Pathogenic Organisms. Ethnobotanical Leaflets 13: 1362- 1372,
7. E.H. Coles (1986). Veterinary Clinical Pathology. W.B. Saunders' Co. Philadelphia; 5-87.
8. E.H. Karunanayeke, J. Welihinda, S.R. Sirimanne and G. Sinnadorai (1984). Oral hypoglycemic activity of some medicinal plants of Sri Lanka. J Ethno Pharmacol, 11: 223 – 231.
9. G. Toro and P. Ackermann (1975). Practical Clinical Chemistry. Little Brown and Company: Boston. 26-29.
10. H. Varley (1969). Determination of blood urea by urease nesslarization method. In: Practical Clinical Biochemistry. 4th ed. White Herrers Press Ltd :London; 158.
11. International Union of Pure and Applied Chemistry (1995). "Alkaloids". Compendium of Chemical Terminology, Internet edition.
12. J.B. Harborn (1998). Phytochemical methods, A guide to modern techniques of plant analysis, 2nd edition Hall, New York. pp. 5-11
13. J.R. Duncan, K.W. Prasse and E. A. Mahaffey (1994). Veterinary Laboratory Medicine (Clinical Pathology). Iowa State University Press: Ames. 94-96.
14. K. Sandhyakumary, R.G. Boby, M. Indira (2002). Impact of feeding ethanolic extracts of *Achyranthes aspera* Linn. on reproductive functions in male rats. Indian J Exp Biol, 40(11):1307-1309.
15. K.R. Kirtikar, B.D. Basu (1993). Indian medicinal plants IInd Edn. Periodical Experts Books Agency, New Delhi, pp. 499 – 505.

16. M. Rajadurai, M. Padmanabhan and P.S.M. Prince (2005). Effect of *Aegle marmelos* leaf extract and tocopherol on lipid peroxidation and antioxidants in isoproterenol induced myocardial infarction in rats. Cardiol, 1: 40 – 45.
17. M.C. Sabu and K. Ramadasan (2004). Antidiabetic activity of *Aegle marmelos* and its relationship with its antioxidant properties. Indian J Physiol Pharmacol, 48: 81– 88.
18. M.R.N. Prasad, N.J. Chinoy and K.M. Kadam (1972). Changes in succinate dehydrogenase levels in the rat epididymis under normal and altered physiological condition. Fert Ster, 23:186–190.
19. O.H. Lowry, D.J. Rosenburg, A.L. Farr and R.J. Randall (1951). Protein measurement with folin-phenol reagent. J Biol Chem,193: 265-275.
20. P. Adhinkary, J. Banerji, D. Choudhary, A.K. Das, C.C. Deb, S.R. Mukherjee and A..Chatterjee (1990). Effect of *piper linn* (stalk) extract on male rat fertility. Indian pharmacy, 22: 145-149.
21. Pramod kumar Verma, Anita sharma, Annu Mathur, Prachi Sharma, R.S. Gupta, S. C. joshi and V.P. Dixit (2002) "Effect of sarcostemma acidum stem extract on spermatogenesis in male albino rats" ,Asian J Androl, 4(1): 43-47
22. R. Parandin, H.R. Sadeghipour, S.A. Haeri Rohani (2008) "Evaluation of antifertility effect and recovery of the seed oil constituents of Iranian species of *Melia azadrach* L. in male rats" Journal of Reproduction & Contraception, 19(3):161-166
23. S. Abayomi (1993). Historical review of traditional medicine in Africa, Spectrum Book ltd, Ibadan pp: 9-25.
24. S. Bhattacharyay (1982). Medical importance of *Aegle marmelos*. In: Chiranjib Banushadi. Ananda publisher, Kolkata, India, pp. 341 – 346.
25. S.K. Haravey (1968). A preliminary communication of the action of *Aegle marmelos* (Bael) on heart. Indian J Med Res, 56: 327 – 331.
26. Sugeng Riyanto, Md. Aspollah Sukari and Mawardi Rahmani *et al.,* (2001). Alkaloids from *Aegle marmelos* (Rutaceae). Malaysian Journal of Analytical Sciences, 7 (2),463-465
27. T.K. Sur, S. Pandit and T. Pramanik (1999). Antispermatogenic activity of leaves of *Aegle marmelos*, Corr. in albino rats: A preliminary report. Biomedicine, 19: 199 – 202.
28. T.K. Sur, S. Pandit and T. Pramanik and D. Bhattacharyya (2002). Effect of *Aegle marmelos* leaf on rat sperm motility: An in vitro study. Indian J Pharmacol, 34: 276-277.
29. T.R. Govindachari and M.S. Premila (1983). Some alkaloids from *Aegle marmelos*, Phytochemistry, 22 (3), 755- 757.
30. Y.B. Ke and W.W. Tso (1982). Variations of Gossypol susceptibility in rat sper-matozoa during spermatogenesis. Int J Fert, 27 (1):42–46.