

Effect of *Mesua ferrea* flower and *Carica papaya* seed extracts on estrous Cycle of female albino rats for Fertility and Anti fertility activity

N. Raja Naik^{1*}, D. Malleswari², P. Indira³

^{1,3} Department of Zoology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India
² Govt. Medical College, Department of Paediatric, Anantapuramu, Andhra Pradesh, India.

*E-mail: rajanaiknenavath@gmail.com

ABSTRACT

Ethanol and Distilled Water extracts of *Mesua ferrea* (*M.ferrea*) flowers and *Carica papaya* (*C.papaya*) seeds were evaluated for fertility and anti-fertility activity in adult female albino rats. Fertility and anti-fertility activity were evaluated by observing estrous cycle. The effect of *M.ferrea* flower & *C.papaya* seed extracts on female reproductive cycle were studied in twenty four sexually matured female albino rats with regular estrous cycle. Rats were randomly divided into four groups of six rats per each group. Experimental groups were treated as follows: Group I (Control), Group II treated with 100 mg/kg, Group III with 200 mg/kg, Group IV with 300 mg/kg of *M.ferrea* flower & *C.papaya* seed extract. The estrous cycle changes were determined by daily observation of vaginal smear. There was an increase in the percentage of rats going into prolong estrous, pro-estrous, met-estrous and di-estrous phases of the estrous cycle in all treated groups. The increase in the duration of the four phases of the cycle has a dose dependent response. Treatment with the highest dose of the extract resulted in a significant increase in the duration of the four phases of estrus cycle compared with the control group. Results showed that *M.ferrea* has the potential for use as a fertility agent for animals in future. Similar experimentation was conducted same for anti-fertility activity by *C.papaya* seed extract. The decrease in the duration of the four phases of the cycle has a dose dependent response. Treatment with the highest dose of the extract resulted in a significant decrease in the duration of the four phases of estrous cycle compared with the control group.

Key words: *Mesua ferrea*, *Carica papaya*, fertility agent, anti-fertility agent and vaginal smear.

Introduction

The reproductive cycle of female rats is called estrous cycle and is characterized as estrous, pro-estrous, met-estrous (di-estrous I) and di-estrous (di-estrous II) (Long & Evans, 1922; Freeman, 1988). The ovulation occurs from the beginning of pro-

estrous to the end of oestrus (Young *et al.*, 1941; Schwartz, 1964). In the female rat the length of the estrous cycle is 4 to 5 days (Long & Evans, 1922; Freeman, 1988; Mandl, 1951), and this short cycle length makes the rat an ideal animal for investigation of changes occurring during the reproductive cycle (Spornitz *et al.*, 1999; Marcondes *et al.*, 2001).

Recently, Marcondes *et al.* (2002) described a fast and simple method to determine estrous cycle phases in unstained vaginal smears, although this method requires more expertise since it is based only on cell shape. Additional information about the stages of the estrous cycle was provided by Hubscher *et al.* (2005), who quantified each cell population found in vaginal smears from rats stained by a modified Papanicolaou method. However, they described only two types of epithelial cells and leukocytes in the smears and did not consider other

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typical features of vaginal smears such as mucus occurrence. Mucus in smears was referred to by Martins *et al.* (2005) after evaluation of the rat estrous cycle by a liquid-based cytology stained with Evans blue. These authors classified each phase by the observation of three types of epithelial cells nucleated keratinized cells, intermediate and deep cells as well as leukocytes, nerve cell quantification was not determined. Therefore, more information regarding the distribution of all cell types found in rat vaginal smears is needed to allow an accurate assessment of the estrous cycle.

The present study was conducted to determine the effect of *M.ferrea* and *C.papaya* on the oestrus cycle of female albino rats and achieve a quantification of the cell types during each phase of the rat estrous cycle, as well as during transitional periods between phases.

Material and Methods

Collection and identification of plant material

The medicinal plant of *Mesua ferrea* flower collected from Anantapuramu super market and *Carica papaya* seeds collected from Anantapuramu surrounding gardens of Rayalaseema in A.P. The identification of plants material have been done by plant Taxonomist, Department of Botany, S.K. University, ATP.

Preparation of Test Drug /Dose

For fertility and ant fertility studies, concentrated ethanolic and D.W extracts of *Mesua ferrea* and Ethanol extract of *Carica papaya* were freshly prepared at different concentrations and administered orally.

Animal model

Sexually matured, healthy female albino rats weighing 120-160g were used for the experiments. The rats were housed in polypropylene cages, under well ventilated animal house conditions (minimum temperature: 28-30°C, photoperiod=12h natural light and 12h dark). The rats were given pelleted feed and tap water *ad libitum*. The experimental protocol was approved by the Institutional Animal ethics committee.

Figure-1. Inserting a gavage needle into a rat



Experimental Design

The animals were divided into 4 groups, consisting of 6 animals in each group. Group-I was maintained as Control and the remaining groups II, III and IV were administered orally 100 mg/kg, 200 mg/kg and 300 mg/kg body weight of *M.ferrea* and *C.papaya* extract by orally using intragastric tube for 21 days daily.

Experimentation I

The animals were divided into 4 groups, consisting of 6 animals in each group.

Group- I: - Control (Received D.w).

Group- II: - Treated with 100 mg D.w Extract /kg b.w.

Group-III: -Treated with 200 mg D.w. extract /kg b.w.

Group-IV: - Treated with 300 mg D.W. extract/kg b.w.

Experimentation II

The animals were divided into 4 groups, consisting of 6 animals in each group.

Group- I: - Control (Received D.w).

Group- II: - Treated with 100 mg Ethanol Extract /kg b.w.

Group-III: -Treated with 200 mg Ethanol extract /kg b.w.

Group-IV: - Treated with 300 mg Ethanol extract/kg b.w.

All the above treatments were given orally by using intragastric catheter for 21 days to cover regular oestrous cycle. The treatment was started from oestrous phase, as the ovarian activities change markedly from one phase to another phase of oestrous cycle. The treatment was given orally every day between 5 to 6 p.m. Vaginal smear using saline solution were taken daily during the entire treatment period to determine the cell type. The duration of estrous cycle together with that of various phases was determined.

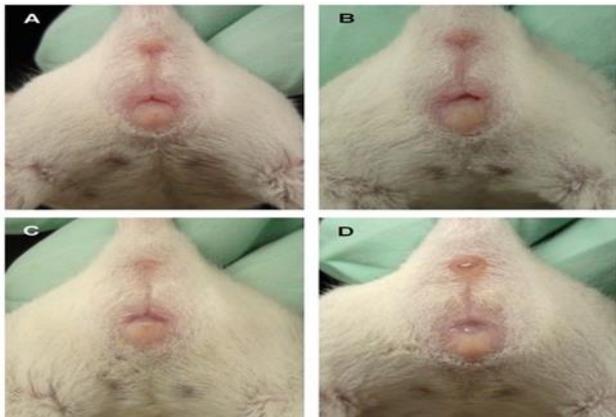
Preparation of Vaginal Smear

During 21 days, every day morning between 8 to 9 a.m. each animal cage was carried to the experimental room. Vaginal secretion was collected with a plastic pipette filled with 0.5 ml of normal saline (NaCl 0.9%) by inserting the tip into the rat vagina, but not deeply. Vaginal fluid was placed on glass slides. A different glass slide was used for each cage of animals. One drop was collected with a clean tip from each rat, unstained material was observed under a lighting microscope, with 10 and 40 x objective lenses.

Results and Discussion

The present study revealed that the D.W extracts of *Mesua ferrea* flowers showed a fertility effect.

Figure-2. Slide with two pipette smears and cover slips. A: Group-I (control), B: Group-II (100 mg/kg), C: Group: III (200 mg/kg), D: Group-IV (300mg/kg).



Effect of *Mesua ferrea* flowers extract on the estrous cycle

Treatment of rats with D.W extract prolonged the estrous cycle significantly characterized by a prolongation of the di-estrous phase. The four phases of estrous cycle observed under the microscope reveal that a positive estrous indicating a quiescent and resting vaginal epithelium, hundreds of large cornified cells generated with nuclei and at the end of estrous the smear becomes cheesy-masses of cornified cells (Fig-3).

Figure-3. Estrous. The smear is much lighter in colour and mucus is rarely seen. The cells are predominately intermediary cells and parabasal cells are rare. Leucocytes are hardly ever observed.

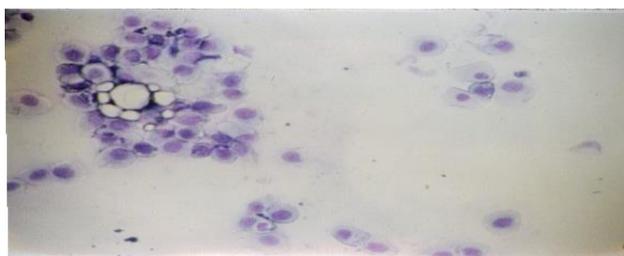
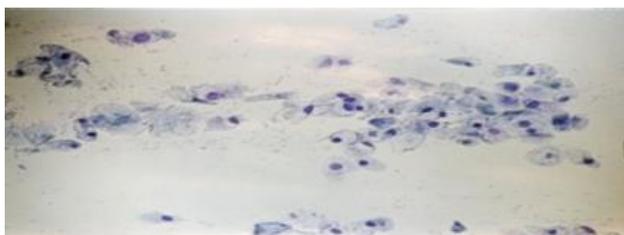


Figure-4. Estrous to Pro-estrous. In this picture few cornified and leucocytes are present.



A pro-estrous smear will have many small epithelial cells with granular cytoplasm, indicating a rapidly growing vaginal epithelium and also the pre-

ovulatory stage (Fig-4). Withdrawal of the treatment showed many significant changes either in the four phases of the estrous cycle or in the duration of the cycle.

Figure-5. Met-estrous. The picture at this stage of the cycle is dominated by leucocytes, often in large numbers and intermediary cells

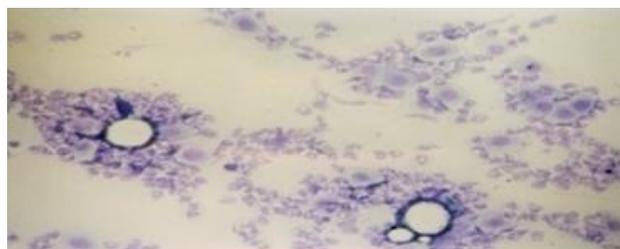
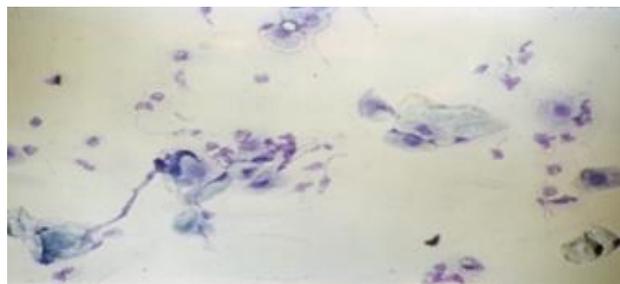


Figure-6. Di-estrous. This stage is characterized by the reduction in cell numbers and the reappearance of mucus often in thin strands



Smear is one in which only large, regular cornified cells are seen indicating maximum growth of the vaginal mucosa. A met-estrous smear will have many leucocytes and few cornified cells, but also some epithelial cells, indicating the post-ovulatory stage and desquamation of the vaginal mucosa (Fig-5). A di-estrous smear will show few nucleated epithelial cells, mucous cells and many leucocytes (Fig-6).

Effect of *Carica papaya* seeds extract on the estrous cycle

The present study revealed that the alcoholic aqueous extract of *Carica papaya* seeds showed an abortifacient effect. Treatment of rats with alcoholic extract prolonged the estrous cycle significantly characterized by a prolongation of the di-estrous phase. The four phases of estrous cycle observed under the microscope reveal that a positive estrous indicating a quiescent and resting vaginal epithelium, hundreds of large cornified cells degenerated with nuclei and at the end of estrous the smear becomes cheesy-masses of cornified cells (Fig-7). A pro-estrous smear will have less epithelial cells with granular cytoplasm, indicating a rapidly growing vaginal epithelium and also the pre-ovulatory stage (Fig-8).

Figure-7. Estrous. In this stage 30 to 40% Epithelial cells and leucocytes are present

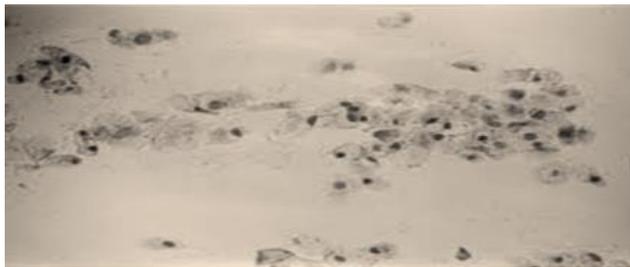


Figure-9. Met-estrous. In this stage 30 to 65-70% Epithelial cells and leucocytes are present

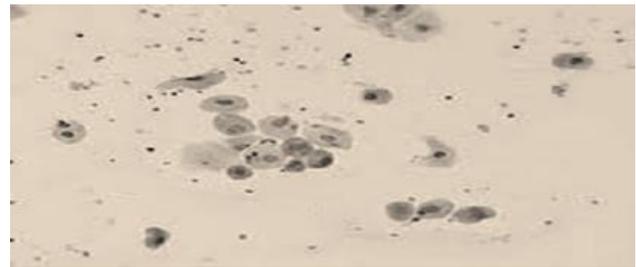


Figure-8. Estrous to Pro-estrous. In this stage 75% few cornified cells and some leucocytes are present.

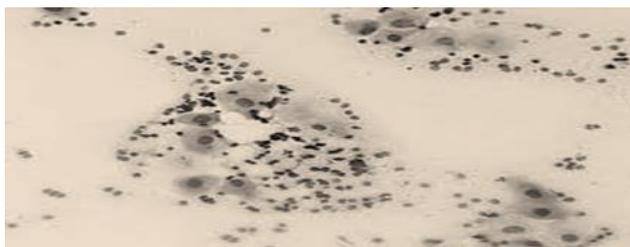


Figure-10. Di-estrous. In this stage 60-70% leucocytes and some epithelial cells are present



Table-1. Effect on the estrous cycle of female albino rats after the administration of 100 mg/kg, 200 mg/kg and 300 mg/kg D.W extract of *Mesua ferrea*

Treatment Groups	Phases			
	Estrous	Pro-estrous	Met-estrous	Di-estrous
Vaginal opening/Cell type obtained in vaginal smear	25% to 40% Leucocytes and cornified cells only	Above 70% Few cornified cells	50% to 70% Cornified cells & many leukocyte	50% to 70 % Leukocytes & epithelial cells
Group I (Control)	1.50 ± 0.78	1.98 ± 0.86	1.50 ± 0.39	1.42 ± 0.35
Group II (100mg/kg)	1.12 ± 0.84	2.21 ± 0.91	1.54 ± 0.44	1.48 ± 0.41
Group III (200mg/kg)	1.14 ± 0.87	2.28 ± 0.94	1.60 ± 0.58	1.57 ± 0.52
Group IV (300mg/kg)	1.19 ± 0.91	2.38 ± 0.98	1.75 ± 0.79	1.69 ± 0.64

Values in Mean ± Standard Deviation, n = 6, P<0.05, When compared with control, respectively

Table-2 Effect on the estrous cycle of female albino rats after the administration of 100 mg/kg, 200 mg/kg and 300 mg/kg ethanolic extract of *Carica papaya*

Treatment Groups	Phases			
	Estrous	Pro-estrous	Met-estrous	Di-estrous
Vaginal opening / Cell type obtained in a vaginal smear	30 to 40% Epithelial cells only	Below 75% Few cornified cells	65to 70% Cornified cells & many leukocyte	60% to 70 % Leukocytes & epithelial cells
Group I (Control)	1.08 ± 0.78	1.98 ± 0.86	1.50 ± 0.39	1.42 ± 0.35
Group II (100mg/kg)	0.89 ± 0.65	1.87 ± 0.74	1.49 ± 0.30	1.36 ± 0.27
Group III (200mg/kg)	0.74 ± 0.43	1.76 ± 0.52	1.37 ± 0.17	1.31 ± 0.19
Group IV (300mg/kg)	0.57 ± 0.32	1.68 ± 0.40	1.22 ± 0.04	1.19 ± 0.02

Values in Mean ± Standard Deviation, n = 6, P<0.05, When compared with control, respectively

Withdrawal of the treatment showed many significant changes either in the four phases of the estrous cycle or in the duration of the cycle.

Smear is one in which only large, irregular cornified cells are seen indicating maximum growth of the vaginal mucosa. A met-estrous smear will have less cornified cells, but also some leukocytes and epithelial cells, indicating the post ovulatory stage and desquamation of the vaginal mucosa (Fig-9). A di-estrous smear will not show epithelial cells, mucous cells and only show few leucocytes (Fig-10).

Conclusion

It was concluded that the D.W and ethanolic extracts of medicinal plants of *M.ferra* and *C.papaya* were experimented with the selected mgs of 100,200 and 300 per kg body weight for fertility and anti-fertility activity on effecting phases of estrous cycle were showed positive results in treated rats. This investigation may be beneficial especially for fertility and anti-fertility activity, in humans also as the experimental is a model.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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