The Pharma Innovation Journal 2021; SP-10(5): 811-815

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(5): 811-815

www.thepharmajournal.com
Received: 15-03-2021
Accepted: 22-04-2021

B Sampath Kumar
Veterinary College, KVAFS, Bangalore Campus, Hebbal, Benguluru, Karnataka, India

Ramesh HS
Veterinary College, KVAFS, Bangalore Campus, Hebbal, Benguluru, Karnataka, India

Nandi S
ICAR-National Institute of Animal Nutrition and Physiology, Audugodi, Bengaluru, Karnataka, India

Girish Kumar V
Veterinary College, KVAFS, Bangalore Campus, Hebbal, Benguluru, Karnataka, India

Corresponding Author:
B Sampath Kumar
Veterinary College, KVAFS, Bangalore Campus, Hebbal, Benguluru, Karnataka, India

Comparative efficacy of *Aegel marmelos* and *Mimosa pudica* leaf extracts on the rat ovarian follicular population and other organs weights

B Sampath Kumar, Ramesh HS, Nandi S and Girish Kumar V

Abstract
The aim of this study is to find out the efficacy of the *Aegle marmelos* (AM) and *Mimosa pudica* (MP) plant extracts on the rat ovarian follicular growth. For this both the plant extracts (AM & MP) were administered orally to the white rats (*Rattus norvegicus*) after being extracted in the soxhlet apparatus with 80 % methanol as a solvent. Before this a total of seven groups of rats (each group consisted of six rats) were maintained, including a control group. Further, both the plant extracts were administered for three successive estrous cycles at the dose of 100, 300, and 1000 mg/kg b.wt. and euthanized. Parameters like body weight (before start & before slaughter), ovarian weight, other organ weights, ovarian follicular number and diameter were measured. The AM @1000, mg/kg b.wt. groups had significantly higher mean surface ovarian follicular number, higher mean preantral follicle number (Small & Large preantral follicel) & higher mean antral follciles (Small & Large antral follicle) than the control group. However, MP @ 1000 mg/kg wt group showed a significantly lower mean number of surface follicles, preantral follicles as well as antral follicles. Likewise, the mean atretic ovarian follicle number was significantly lower in the AM @ 1000 mg/kg b.wt. Group where as higher in the MP @ 300 & 1000mg/kg b.wt. Groups compared to the control. The mean initial and final body weights of all the groups were found to be non-significant. Similarly, no significant differences in mean liver, pancreas, and kidney weights were seen when compared to control. However, higher mean ovarian weight was recorded in the AM @ 1000 mg/kg b.wt. and lower mean ovarian as well as uterine weight was recorded in the MP @ 100,300 & 1000 mg/kg. wt groups compared to the control.

Keywords: Leaf extracts, anti-fertility, follicle, ovary, follicle growth, rats

Introduction
Usage of medicinal plants for augmenting and/or terminating the reproduction in the mammals is been a traditional practice (Hussain *et al*., 2012, Wang *et al*., 2014, Lee *et al*., 2019) [14, 21]. As per the various reports (Sandhya *et al*., 2006, Nath and Deb 2015, Adhami *et al*., 2018) [1] more than 80% of the world's population incorporating the plant extracts in the treatments of various reproductive ailments. However, in order to use them they need to be processed by various methods (heating, drying, boiling, grinding, shading, compressing, and other physic procedures) after extraction (hot/cold) with solvents (organic/inorganic/aqueous) to retain their bioactive compounds (Lee *et al*., 2019). Furthermore, because of the widespread availability and lower cost, usage of plant based medicines increased even more (Hossain *et al*., 2021). In reality, finding their potent dose is a prerequisite for to use them as an alternative to synthetic drugs (Yuan *et al*., 2016) [22]. So also dosage is the most important consideration in determining whether they are beneficial or detrimental (Katiyar *et al*. 2013) [10].
Aegle marmelos is commonly known as *bael* or golden apple and belongs to the family of rutacae (Nigam & Nambiar, 2015) [15]. It is considered as a medicinal plant due to the presence of bioactive compounds in its various parts. For example the leaf extract of the bael having bioactive compounds (Skimmianine, Aegelin, Coumarin, Rutin, β-sitosterol, Marmesinin etc.,) which can alter the reproductive functions in the mammals (Venthodika *et al*., 2020, Dutt *et al*. 2018). Similarly, *Mimosa pudica* is also an another medicinal plant belongs the family of Mimosaceae and commonly known as “Chue Mue” (Rajendran and Sundararajan 2010) [17]. The bioactive compounds present in this plants are (Mimosine, Mimupodine etc.,) majorly responsible for its antifertility activity on the ovarian tissue (Muhammad *et al*., 2016) [14]. Overall, the aim of the present research is to find out the effective & safe dose of AM and MP plant extracts on the rat reproductive system (ovarian follicles, ovarian weight, uterine weight etc.,) as to interpolate the same in other mammals.

Materials and Methods

Leaf extracts preparation

Aegle marmelos and *Mimosa pudica* plant leave were collected in and around the Veterinary college, Hebbal, Bangalore campaus and got validated by Dept. of Forestry and Environemental science, University of Agricultural Sciences, Bengaluru No. F & ES 5/163-6/2/21. Freshly collected fresh leaves were thoroughly washed and dried under shade. Using an electric blender, the dried leave were ground into fine powder and kept at room temperature until extraction. Leaf extracts of *Aegle marmelos* (Dhivya *et al*., 2018) [4], *Mimosa pudica* (Shashikumara *et al*., 2018) [18], were prepared by using hot soxhlet apparatus. In this procedure 80% methanol was used as a solvent. Ten gm of leaves powder was mixed with the 200 ml of methanol (80%) and ran in the soxhlet over the duration of 72 hrs. at the temp. of 64^0C. Finally extracts were dried by oven method (Mediani *et al*., 2013).

Experimental design

Ethical approval for the present experiment was sanctioned form Karnataka Veterinary, Animal & Fisheries Sciences University, Bidar with the IAEC file No: IAEC VCH/IAEC/2019/100).

For this study, mature and cyclical female Wister rats (*Rattus norvegicus*) of 8 weeks of age were selected. Rats were kept in polypropylene cages under normal laboratory conditions (light period: 12:12 h light/dark cycle; temperature: 23 2°C; relative humidity: 55.5%). During the experiments, the animals were fed a commercial diet (Srinivasa feed suppliers, Bangalore, India) and had free access to water (ad libitum). A total of seven groups of six rats in each group were maintained. Leaf extracts of *Aegle marmelos* and *Mimosa pudica* were given at doses of 0 (control), 100, 300, and 1000 mg/kg b.wt. The range of doses were selected based on the LD_{50} (literature survey: Sunitha, 2014) [19] where up to 2000 mg/kg b.wt. dose was reported as safe. The groups were as follows: Group 1: Control group, Group 2: *Aegle marmelos* 100 mg/kg b.wt. (AM 100), Group 3: *Aegle marmelos* 300 mg/kg b.wt. (AM 300), Group 4: *Aegle marmelos* 1000 mg/kg b.wt. (AM 1000), Group 5: *Mimosa pudica* 100 mg/kg b.wt. (MP 100), Group 6: *Mimosa pudica* 300 mg/kg b.wt. (MP 300) and Group 7: *Mimosa pudica* 1000 mg/kg b.wt. (MP 1000).

Collection of samples

Body weight of the rats was measured before starting of experiments and at the end of experiments before slaughter. The above prepared plant extracts were emulsified in the vehicle (Dimethyl sulphoxide) just before administration. Only vehicle dissolved in the water was given to the control group of rats. Rats were dosed for the three consecutive estrous cycles starting from stage of estrous with the plant extracts by oral gavagae with a maximum volume of 0.5 ml. The volume was calculated according to its fasting body weight at the time of dosing (Jondhale *et al*., 2009) [9]. Rats were sacrificed on the day of estrous by ketamine injection after three consecutive estrous cycles. Body weights were measured on the day of slaughter also to find the percent of difference. Ovaries, uterus, liver, pancreas and kidney were also collected in the normal buffer formalin (10% NBF) and mean weights were calculated accordingly according to the procedure described by (Nandi *et al*., 2006).

Follicular dynamic studies

Surface ovarian follicles of both the ovaries were counted under stereo zoom microscope before subjected to the histological sections. To examine the different class of ovarian follicles ovaries from both groups of plant extracts were examined in serial sections of 5µm thickness. Hematoxylin and Eosin was used to stain the histological sections. The average of two perpendicular follicular diameters was used to calculate follicular diameter (Barrends *et al*., 1995). The total number of ovarian follicles various diameter was counted using stereozoom microscope as follows: Small preantral follicles: 50-200 µm; Large preantral follicles: 200-270µm; Small antral follicles: 270-450µm; Large antral follicles: >450µm.

Statistical analysis

Follicular population and other organ weights data between experimental group and control group were analyzed by computer assisted statistical software package (Graph pad Prism, San Deigo, USA). Data was analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. Significance an dnon significance of difference between mena values were determined at 5% level of significance ($P<0.05$).

Results and Discussion

Follicular dynamic studies

The mean ovarian surface follicular number was significantly higher in the AM group extract @ 1000 mg/kg b.wt. (19.0 ± 0.81) compared to the control (11.33 ± 0.49) whereas significantly lower number of surface follicles were recorded in the group of MP 1000 mg/kg b.wt. weight (7.0 ± 0.57) followed by MP @ 300 mg/kg b.wt. (9.0 ± 0.30) compared to the control group respectively.

AM extract @ 1000 mg /kg b.wt. had shown a significantly higher mean number of small as well as large ovarian preantral follicles, whereas MP extract @ 1000 mg/kg b.wt. had shown a significant reduction in the mean preantral follicle number (SPF & LPF) compared to the control group with a values recorded as 78 ± 1.88 (SPF), 52.0 ± 2.2 (LPF) for AM extract @ 1000 group, 30 ± 1.29 (SPF), 22.3 ± 1.14 (LPF) for MP *extract* @ 1000 group and 46.6 ± 0.88 (SPF), 30.0 ± 1.88 (LPF) for the control groups respectively. Similarly, mean antral follicles number was also significantly higher in the AM extract @ 1000 mg /kg b.wt. group with values being as 45.67 ± 2.76 for SAF, 18.8 ± 1.07 for LAF compared to control group (25.5 ± 0.42:SAF, 13.3 ± 0.21: LAF) respectively. Whereas, significantly lowest mean number of antral follicles were recorded in the MP *extract* 1000 mg/kg b.wt. group with a values being as 17.83 ± 0.94 for SAF and 8.16 ± 0.70 for LAF compared to the control group respectively.

The ovarian mean atretic follicle number was significantly lower in the group of AM extract @ 1000 mg/kg b.wt. with a value recorded as 27.17 ± 1.62 compared to the control (48.3± 0.30) respectively. However the MP group @ 300 as well a 1000 mg/kg b.wt. was shown a significantly higher mean atretic follicle number compared to the control with a values being as 54.17±1.13 (MP @300) and 57.17 ± 1.13 (MP@100) compared to the control respectively. The mean number of different classes of follicles were given in the Table no. 1. & Figs 1, 2.

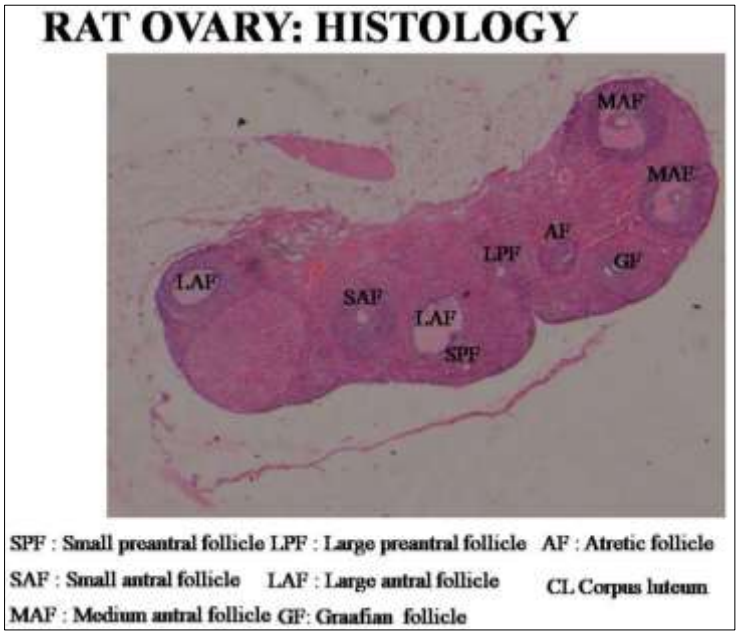
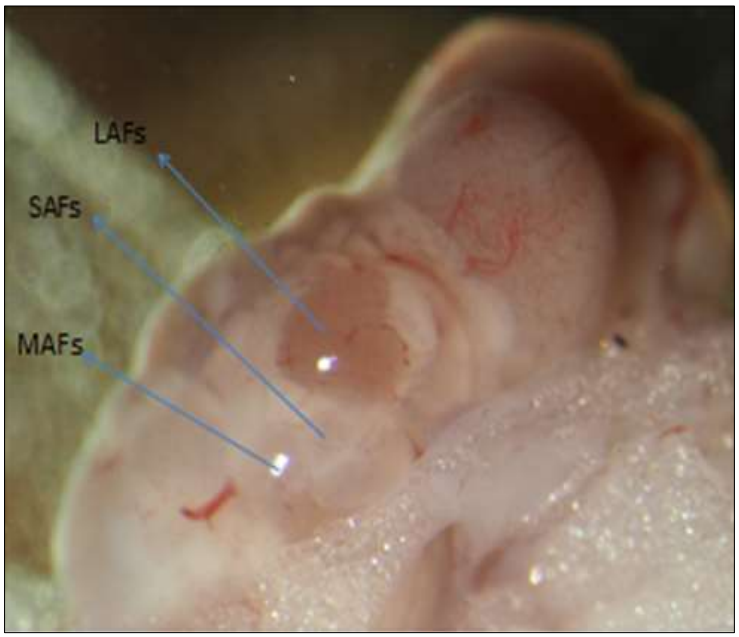


Fig 1: Histological section of the rat ovary



LAF: Large antral follciles, SAF: Small antral follicles, MAF: Medium antral follicles

Fig 2: Surface follicles on the rat ovary

Table 1: Effect of *Aegle marmelos* and *Mimosa pudica* extract on ovarian follicular population of 8 WK old rats. (Values are Mean ± SE of 6 rats in each group)

Follicular population	Control	100 mg/kg b.wt.	300 mg/kg b.wt.	1000 mg/kg b.wt.	100 mg/kg b.wt.	300 mg/kg b.wt.	1000 mg/kg b.wt.
		***Aegle Marmelos* extract (AM)**			***Mimosa pudica* extract (MP)**		
Surface follicles	11.33 ± 0.49a	13 ± 0.48a	10.8 ± 0.50a	19.0 ± 0.81b	11.2 ± 0.40a	9.0 ± 0.30c	7.0 ± 0.57d
Small PF	46.6 ± 0.88a	44 ± 0.88a	50 ± 2.38a	78 ± 1.88b	40 ± 2.33a	38 ± 3.25a	30 ± 1.29c
Large PF	30 ± 1.88a	32 ± 2.0a	33 ± 1.46a	52 ± 2.2b	31 ± 1.34 a	27.17 ± 2.2 a	22.3 ± 1.14c
Small AF	25.5 ± 0.42a	25.3 ± 1.38a	21.6 ± 0.98a	45.67 ± 2.76b	20.17 ± 1.92a	21.33 ± 0.95a	17.83 ± 0.94c
Large AF	13.3 ± 0.21a	12.2 ± 1.47a	13.5 ± 2.16a	18.8 ± 1.07b	10.67 ± 0.66a	10.17 ± 0.60a	8.16 ± 0.70c
Atretic follicle	48.3 ± 0.30a	43.5 ± 1.72a	45.83 ± 2.93a	27.17 ± 1.62b	49.83 ± 2.6 a	54.17 ± 1.13c	57.17 ± 1.13c

PF: Preantral follicle, AF: Antral follicle, P values: <0.05 as compared to control, Values of follicle number were given per ovary, Values with different superscripts in the same row differ significantly

The above results are in accordance with the Dhivya *et al*., 2018 [4], where hydroalcoholic extracts of *Aegle marmelos* leaves orally administered to the rats @ 400 mg/kg b.wt. Resulted in an improvement in the developing follicle number as well corpus luteum which eventually increased the process of folliculogenesis and the ovulation rate. The specific reason

behind this improvement might be due to the presence of coumarins (scopoletin) and/or Aegelin bioactive compounds. Similarly, *Aegle marmelos* leaf extract @ 1000mg/ kg b.wt. Resulted in a significantly higher number of ovarian follicles in the rats after administered orally from diestrous to estorus stage. An increment in the ovarian follicular population (small, medium, large and total) was observed in the anoestrous goats also after receiving a dose (50 % ethanolic extract @ 1000mg/kg b.wt. extrapolated from rats) of combined leaf extracts of *Aegle marmelos* and *Murrayya Koenigii* for 9 days. Likewise, sahiwal heifers fed orally (9 days) with the *Aegle marmelos* extracts + *Murrayya koenigii* leaf extracts (dose extrapolated from 50% ethanolic extract of rats) combination along with the regular concentrate mixture shown a significant improvement in the mean size as well as growth rate of large ovarian follicles (Kumar *et al*., 2016) [13]. However, Rahmawati *et al*., 2018 [16] reported a decline in the ovarian weight, corpus luteum and ovulation rate in the white rats with a reason suggesting the presence of phytosterols (β-sitosterol and stigmasterol) in the extracts which also reduced the FSH levels. Overall the increment behind the different classes of ovarian follicle number by the AM extract @ 1000 mg/kg b.wt. might be due to the bioactive compounds (Aegelin, Coumarin, Skimmianine, Rutin) present in the AM leaf extracts as suggested by various workers (Dhivya *et al*., 2018; Dutt *et al*., 2018, Kumar *et al*., 2016, Venthodika *et al*., 2020) [4, 13]. Moreover, the AM extract was specifically giving positive results @ 1000mg/kg b.wt. rather than other doses compared to the control suggesting a dose dependent action.

Mimosa pudica leaf extracts shown an antifertility effect after receiving a dose of 150 mg/kg b.wt. of root powder in the female white rats for 5 consecutive days. There was a specifically significant reduction in the normal ova number with an increment in the degenerated ova. The reason attributed to this was the due inhibition of steroidogenesis by the active compounds present in the extract (Valsala *et al*., 2002) [20]. Similarly, the methanolic extracts of *Mimosa pudica* root extracts shown to have an antifertility activity in the swiss albino mice after receiving a dose of 300 mg/kg b.wt. for 21 consecutive days. There was an extension in the period of diestrous, alteration in the gonadotropin release and estradiol secretion along with the reduction in the number of litters produced. The reason attributed for this might be suppression of FSH by the compounds present in the root extract powder (Ganguly *et al*., 2007) [8]. However, the whole plant extracts (methanolic) of *Mimosa pudica* @ 2000mg/kg b.wt. Significantly improved the estrogen & progesterone levels in the induced polycystic ovarian syndrome conditions in the rat ovarian tissues (Sunitha *et al*., 2014) [19]. The lower number of ovarian follicles in the present research specifically in the *Mimosa pudica* leaf extracts treated groups compared to the control speculated by the hormone (FSH, LH, estrogen, progesterone) altering actions of bioactive compounds present in the leaf extracts (Valsala *et al*., 2002, Ganguly *et al*., 2007) [8, 20] and/or translational inhibitors (ex; Mimosine inhibit the eIF3: Khanna *et al*., 1993, Dong *et al*., 2003) [5, 11].

Body weight

The mean initial and final body weights (in gms) of AM 100 (151.7 ± 3.5, 171.5 ± 3.12), AM 300 (159.2 ± 1.95, 180.3 ± 2.43), AM 1000 (162.8 ± 4.25, 186.2 ± 3.4), MP 100 (144.8 ± 4.4, 170.3 ± 3.8), MP 300 (154 ± 4.11, 173.7 ± 4.2) and MP 1000 (160.5 ± 3.8, 181.7 ± 5.11) group were found as non-significant compared to control (153 ± 4.61, 176.3 ± 1.43) group with a percentage of change ranged from 13 to 15%.

Organ weights

The mean ovary weight was significantly higher in the AM 1000 (96.2 ± 3.55) group compared to the control group (75.7 ± 1.47). The mean ovarian weight was significantly decreased in the MP 300 (56.7 ± 1.62) & 1000 (52.8 ± 1.57) groups compared to the control. The reason attributed for the higher ovarian weight was the increment in the follicle number (different class) which was due to the bioactive compounds present in the AM leaf extracts (Muhammad *et al*., 2016) [14]. Significantly lower mean uterine weight was observed in the MP 100 (350.8 ± 19.21), 300 (339.7 ± 20.18) & 1000 (314 ± 4.15) groups dose wise compared to the control (406.2 ± 6.08). Whereas no significance difference was observed in the groups of AM for all the concentrations compared to the control. Uterine weight reduction might be due to the inhibitory effect of bioactive compounds present in the MP extracts on the uterine cells.

There was no significant difference was observed between the mean liver, pancreas and kidney weights between the groups compared to control. The mean value for the same was given in the Table No. 2.

Table 2: Effect of *Aegle marmelos* and *Mimosa pudica* extract on ovarian weight and other organ weight of 8 WK old rats, Values are Mean ± SE of 6 rats in each group.

Organ weight	Control	100 mg/kg b.wt.	300 mg/kg b.wt.	1000 mg/kg b.wt.	100 mg/kg b.wt.	300 mg/kg b.wt.	1000 mg/kg b.wt.
		***Aegle marmelos* extract**			***Mimosa pudica* extract**		
Ovarian weight (mg)	75.7 ± 1.47a	77.2 ± 1.99 a	79.5 ± 1.35 a	96.2 ± 3.55 b	67.6 ± 1.83c	56.7 ± 1.62bc	52.8 ± 1.57bc
Uterine weight (mg)	406.2 ± 6.08 a	410 ± 7.57 a	409 ± 8.60 a	412.5 ± 9.58 a	350.8 ± 19.21b	339.7 ± 20.18bc	314 ± 4.15c
Liver weight (g)	6.2 ± 0.79 a	7.8 ± 0.47 a	8.2 ± 0.70 a	8.3 ± 0.33 a	6.8 ± 0.60 a	6.5 ± 0.56 a	5.8 ± 0.70 a
Pancreas weight (mg)	550 ± 18.44 a	559.2 ± 10.15 a	561.2 ± 9.51 a	563.3 ± 8.55 a	544 ± 17.29 a	54.18 ± 8.13 a	539.8 ± 17.03 a
Kidney weight (mg)	670 ± 13.39 a	667 ± 19.57 a	671.8 ± 10.46 a	673.7 ± 13.99 a	668.7 ± 25.28 a	664.3 ± 17.34 a	661.8 ± 18.46 a

P values:<0.05 as compared to control, Values with different superscripts in the same row differ significantly.

Conclusions

AM extracts @ 1000mg /kg b.wt. Can be considered as a safe and effective in the rats for enhancing the ovarian functions as well as rescuing the ovaries form degeneration. However, MP extracts @ 300 & 1000mg/kg b.wt. Can be considered as detrimental which eventually resulted in the antifertility activity.

Acknowledgement

The authors acknowledge the support of the Registrar, KVAFSU, Bidar for providing necessary funds to carry out the research work.

References

1. Adhami S, Siraj S, Farooqi H. Unexplored medicinal

plants of potential therapeutic importance: A review. Tropical Journal of Natural Product Research 2018:2(1):3-11.
2. Ahmad H, Sehgal S, Mishra A, Gupta R. *Mimosa pudica* L. (Laajvanti): An overview. Pharmacogn Rev 2012;6(12):115-124.
3. Cumali K. Medicinal plants and their traditional uses. Journal of advances in plant biology 2018;1(2):8-12.
4. Dhivya C, Dhanalakshmi S, Chitra V, Hawari S. Alleviation of polycystic ovarian syndrome by hydroalcoholic leaf extract of *Aegle marmelos* (L.) Correra in letrozole-induced rat model. Drug invention today 2018;10(7):1246-1250.
5. Dong Z, Zhang JT. EIF3 p170, a mediator of mimosine effect on protein synthesis and cell cycle progression, Mol. Biol. Cell 2003;14:3942-39.
6. Dutt R, Mehrotra S, Hoque M, Shanker U, Singh G, Agrwal SK, Das K, Singh SK. Effect of *Murraya koenigii* and *Aegele marmelos* combination on resumption of fertility in anestrous goats. Journal of applied animal research 2010;38:249-252.
7. Dutt R, Mehrotra S, Shanker U, Singh G. Effect of *M. koenigii* and *A. marmelos* feeding on anestrus buffaloes. Indian Journal of Animal Reproduction 2011;32(1);47-49.
8. Ganguly M, Devi N, Mahanta R, Mridul K, Borthakur. Effect of Mimosa pudica root extract on vaginal estrous and serum hormones for screening of antifertility activity in albino mice. Contraception 2007;76:482-485.
9. Jondhale NS, Shanker U, Mehrotra S, Agarwal SK, Singh S.K, Das G, Deori S. Effect of *Aegle marmelos* and *Ficus religiosa* leaves extracts on the ovarian function in rats. Indian Veterinary Journal 2009;86:1141-1144.
10. Katiyar C, Gupta A, Kanjilal S, Katiyar S. Drug discovery from plant sources: An integrated approach. Ayu 2012;33(1):10-19.
11. Khanna KK, Lavin MF. Ionizing radiation and UV induction of p53 protein by different pathways in ataxia-telangiectasis cells. Oncogene 1993;8(12):3307-3312.
12. Kokane DD, More RY, Kale MB, Nehete MN, Mehendale PC, Gadgoli CH. 651 Evaluation of wound healing activity of root of *Mimosa pudica*. Journal of 652 Ethnopharmacology 2009;124:311-315.
13. Kumar B, Sachan V, Kumar A, Singh V, Das GK, Saxena A. Effect of supplementation of *Aegle marmelos* and *Murraya Koenigii* on the follicular development in delayed pubertal shaiwal Heifer. Journal of Animal Research 2016;6(6):1047-1052.
14. Muhammad G, Hussain MA, Jantan I, Bukhari SNA. Comprehensive reviews in food science and food safety 2016, 15.
15. Nigam V, Nambiar VS. Therapeutic potential of *Aegle marmelos* (L.) Correa leaves as an antioxidant and anti-diabetic agent: A review. International Journal of Pharma Sciences and Research 2015;6(3):611-621.
16. Rahmawati I, Windartik E, Soemah E, Roifah I. Potency of *Aegle Marmelos* extract to the process of infertilization. ARC Journal of Nuring and health care 2018;4(4):13-18.
17. Rajendran R, Sundararajan. Preliminary phytochemical analysis & anti-bacterial activity of *Mimosa pudica* Linn leaves. International journal of pharma and Bio-sciences 2010;6(2):1-8.
18. Shashikumara PC, Amrutheshwari B, Prathima C. Effect of ethanolic extract of *Mimosa pudica* root L. on acute restraint stress-induced anxiety and depression in swiss albino mice. Asian Journal of Pharmaceutical and Clinical Research 2018;11(10):182-6.
19. Sunitha S, Menon S. Evidence based evaluation of medicinal plants used for treating polycytic ovary syndrome. Project proposal submitted to University Grants Commission 2014. F. No. 43-121/2014 (SR).
20. Valsala S, Karpagaganapathy PR. Effect of *Mimosa pudica* root powder on oestrous cycle and ovulation in cycling female albino rat, *Rattus norvegicus*. Short communication. Phytotherapy Research 2002;16:190-192.
21. Wang X, Liu Z. Prevention and treatment of viral respiratory infections by traditional Chinese herbs. Chin Med J (Engl) 2014;127(7):1344-50.
22. Yuan H, Ma Q, Ye Li, Piao G. The traditional medicine and modern medicine from natural products. Molecules 2016;21:559.