OESTROUS CYCLE MODULATING EFFECT OF HYDROMETHANOLIC EXTRACT OF *AZADIRACHTA INDICA* (A. JUSS) LEAVES IN WISTAR RATS

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ABSTRACT
The study aimed to explore the potential of Azadirachta indica leaves as a natural alternative for regulating the oestrous cycle in animal production. Eighteen mature Wistar rats were randomly assigned to three groups of six rats and treated per os with 0, 500, and 1000 mg/kg body weight of A. indica hydromethanolic leaf (AIHL) extract for 15 days. AIHL extract extended the average oestrous cycle period, increased dioestrus frequency, and decreased oestrus and metoestrus frequencies. Additionally, the ovarian and reproductive tract weights relative to body weight were significantly reduced ($p < 0.05$). The extract contained saponins, tannins, cabohydrates, terpenes, steroids, alkaloids, and flavonoids. Azadirachta indica leaves have the potential to modulate the mammalian oestrous cycle.
Keywords: *Azadirachta indica* leaves, Oestrous cycle, Ovarian weight, Reproductive tract weight, Dioestrus

INTRODUCTION
Oestrous synchronisation improves reproductive management, but traditional hormonal methods raise safety concerns. This study investigates the effect of *Azadirachta indica* leaf or Neem extract on the oestrous cycle, reproductive organ morphometry, and hormone and lipid profiles in female Wistar rats. This study aimed to explore the potential of *A. indica* leaves as a natural alternative for regulating the oestrous cycle in animal production

MATERIALS AND METHODS
Plant collection, extraction and phytochemical analysis
A. indica leaves were harvested and authenticated. After air-drying and pulverisation, 6 kg of leaves were extracted with 70% hydromethanol for 48 hours, yielding the *Azadirachta indica* hydromethanolic (AIHL) leaf extract. The extract was screened for saponins, tannins, and other compounds through standard protocols, including spectrophotometric and gravimetric analyses.

Thirty mature female Wistar rats were housed under controlled conditions for acclimatization and received routine prophylaxis. Median Lethal Dose was determined using Lorke's (1983) method across various doses to establish safety limits for treatment. Following ethical approval, 18 rats with confirmed cyclicity were randomly divided into three groups of six rats per group for treatment with 0, 500, and 1000 mg/kg body weight of AIHL. During treatment average oestrous cycle duration, number of cycles, and frequency of oestrous stages were measured by vaginal cytology (Marcondes *et al.* (2002). At the end of treatment, paired ovaries and reproductive tracts were weighed relative to body weight. Serum was analysed for hormone (FSH, oestrogen, progesterone) levels, and lipid (Total cholesterol, triglycerides, low density and high-density lipids) levels.

Data was analyzed using one-way ANOVA, with means separated via Tukey's test ($p < 0.05$). Results are presented as mean ± SD.

RESULTS AND DISCUSSION
Hydromethanol extraction of *Azadirachta indica* leaves yielded 4.88% AIHM extract, lower than the 19% yield reported by S-Prateek *et al*. (2023) using a more vigorous ethanol extraction method. The non-toxic effect of AIML aligns with findings from Tepongning *et al*. (2018), who observed no toxicity in rats. AIHL contains phytochemicals such as flavonoids, saponins, phenols, tannins, alkaloids, and sterols, which have been shown to exhibit hormone-like activities (Tables 1 – 2).

Table 1: Qualitative phytochemical contents of AIHL

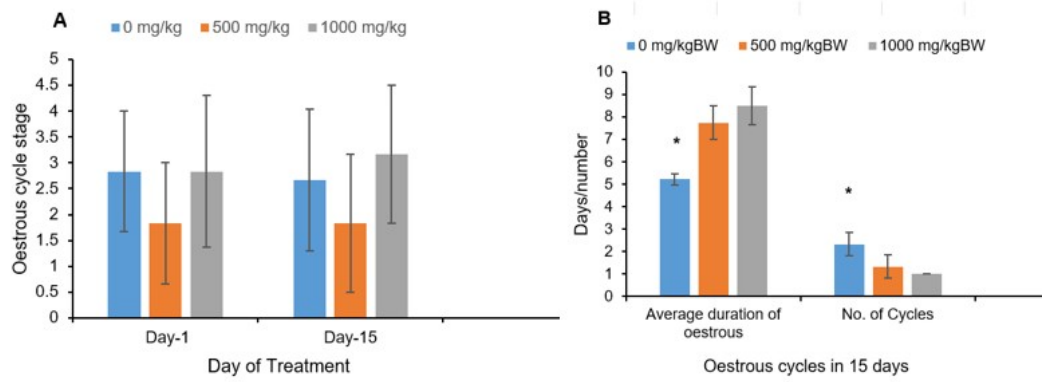
Phytochemicals	Result
Saponins,	+
Tannins,	+
Cabohydrates	+
Terpenes	+
Steroids	+
Anthraquinone derivatives/glycosides	-
Flavonoids	-
Alkaloids	+

+= present; - = absent

Table 2: Quantitative phytochemical contents of AIHL

S/N	Phytochemicals	Method (Calibration curve/ formula)	Quantity (Mean ± SD)
1	Total Flavonoid mg/g (Quercetin Equivalent)	Spectrophotometric analysis y = 0.001x - 0.0047 (0.997)	34.07 ± 1.64
2	Total Saponins (%)	Gravimetric analysis Weight of saponin/weight of sample x 100	25.53 ± 0.41
3	Total Phenols (mg/g Gallic acid equivalent)	Spectrophotometric analysis y = 0.0088x + 0.0187 (0.999)	7.29 ± 0.01
4	Total Tannins (mg/g Tannic acid equivalent)	Spectrophotometric analysis y = 0.0003x - 0.0043 (0.991)	19.86 ± 0.27
5	Total Alkaloids (mg/g Atropine equivalent)	Spectrophotometric analysis y = 0.0005x + 0.0015	18.67 ± 0.27

SD = Standard deviation



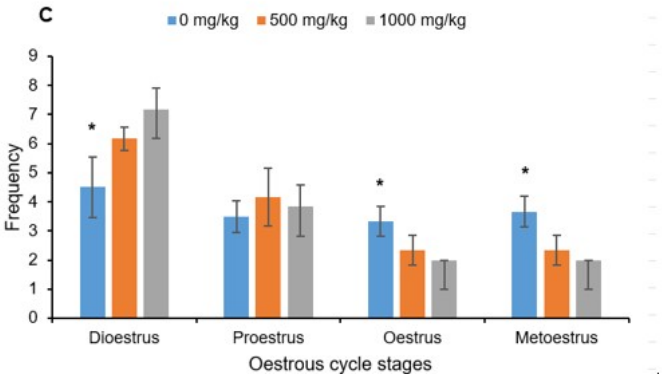


Fig. 1: Effect of AIHM extract on the Wistar rat's oestrous cycle during a 15-day treatment period. A: Oestrous stage at day-1 and day-15 of treatment. B: Number of oestrous cycles and average duration of each cycle. C: Frequency of each oestrous stage. * Significantly different at $p < 0.05$

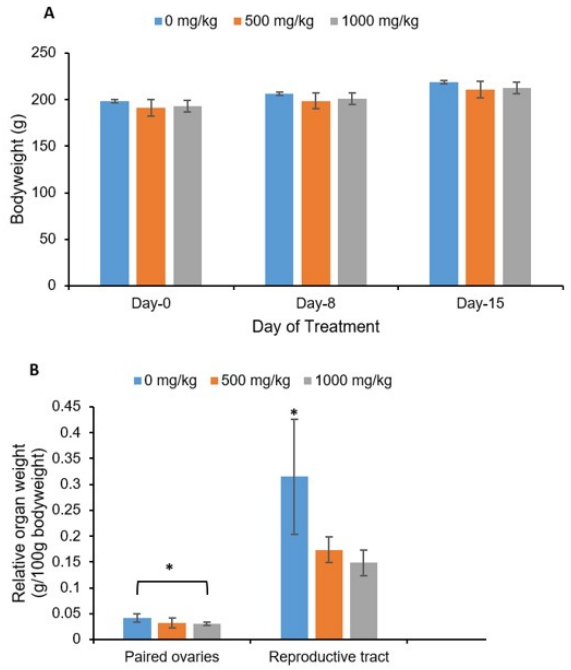


Fig. 2: Effect of AIHM extract on the morphometry of the Wistar rat's reproductive organs
* Significantly different at $p < 0.05$

In Fig. 1, AIHL treatment resulted in a lengthened oestrous cycle and prolonged vaginal dioestrus, indicating elevated progesterone levels, consistent with Auta and Hassan (2016). Lower relative ovarian and reproductive tract weights in AIHL-treated groups (Fig. 2) suggest progesterone influence. Despite this, AIHL did not alter serum progesterone or lipid levels, indicating that its active compounds may act as non-hormonal ligands on progesterone receptors.

CONCLUSION

AIHM extract exhibited progesterone-like activity by extending dioestrus and the overall oestrous cycle while decreasing ovarian and reproductive tract weights. Further research is needed to identify the active compounds and their mechanisms of action.

REFERENCES

Auta, T. and Hassan, A. T. (2016). Alteration in oestrous cycle and implantation in Mus musculus administered aqueous wood ash extract of *Azadirachta indica* (neem). *Asian Pacific Journal of Reproduction, 5*(3): 188-192. https://doi.org/10.1016/j.apjr.2016.03.003

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology, 54*: 275 - 287

Marcondes, F.K., Bianchi, F.J., Tanno, A.P. (2002). Determination of the estrous cycle phases of rats: some helpful considerations. *Brazillian Journal of Biology, 62*: 609-614. https://dx.doi.org/10.1590/S1519-69842002000400008

S-Prateek V., Neralla, M., Baskar V., & Satheesh, T. (2023). Comparative extraction and bioactive potential of the leaf extracts of *Azadirachta indica* for combatting postoperative head and neck infections: an in vitro study. *Cureus, 15*(12), e51303. https://doi.org/10.7759/cureus.51303

Tepongning, R.N., Mbah, J.N, Avoulou, F.L., Jerme, M.M., Ndanga, E.-K.K., Fekam, F.B. (2018). Hydroethanolic extracts of *Erigeron floribundus* and *Azadirachta indica* reduced *Plasmodium berghei* parasitemia in Balb/c mice. *Evidence Based Complementary Alternative Medicine, 2018*(1): ID156710. https://doi.org/10.1155/2018/5156710