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Evaluation of Toxicity of the Methanolic Root Extract of *Persicaria hydropiper* (L.) Delarbre Used for Fertility Regulation during Early Gestational Period in Female Albino Mice

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ABSTRACT

Ethnopharmacological relevance: Persicaria hydropiper is a medicinal herb that also holds significant culinary value in Southeast Asia. Among the Mishing tribe of Assam, the roots of this plant are traditionally used for female contraception.

Aim of the study: The present study aimed to evaluate the potential toxicological effects of the methanolic root extract of *P. hydropiper*, which was administered to female mice to prevent conception.

Materials and Methods: The assessment included acute toxicity tests, behavioural studies, biochemical analyses (SGOT and SGPT levels), histological examinations of the liver and kidney and genotoxicity tests using micronucleus assays.

Results: No notable signs of acute or behavioural toxicity were observed in extract-treated mice. In fact, treated groups exhibited central nervous system stimulating and anxiolytic effects at a dose of 1000 mg/kg body weight. A significant increase in locomotor activity was recorded on Day 1, Day 5, Day 6 and in ovariectomized (OVX) mice (81.7±0.40, 80.8±0.72, 82.0±0.26 and 80.3±0.15 cases/5 min, respectively), along with higher head-dip counts on Day 1, Day 4, Day 5 and Day 6 (34.5±0.43, 35.1±0.28, 35.0±0.26 and 33.6±1.45, respectively). Histological sections of liver and kidney tissues revealed nearly normal structures. Genotoxicity evaluations showed no significant variation in micronucleus frequency. However, a marked increase in SGOT was detected in ovariectomized females (123.65±2.86 IU/l), while SGPT levels rose in the Day 1, 2, 3 and 6 treated groups (52.52±1.81, 52.01±1.95, 54.84±2.00 and 49.37±1.78, respectively), suggesting mild hepatotoxic effects.

Conclusion: Studies on the toxicological aspects of any traditional medicine are necessary for the proper formulation of a drug. Most of the parameters selected for toxicity detection showed no significant alteration between the control and treated mice groups. Only mild hepatotoxicity was observed in the extract-treated group of female albino mice. Moreover, some improvements in terms of exploratory behaviour could be observed in the treated groups. Thus, the extract may be considered low or non-toxic at this present dose. Further studies are necessary to investigate toxicological impacts on other tissues and to identify the potentially toxic compounds within the extract, which could aid in the development of antifertility agents derived from this plant.

Keywords: Antifertility, Gestation, Methanolic Root Extract, *Persicaria hydropiper*, Toxicity, Traditional Medicine

1. Introduction

Traditional medicine encompasses a diverse range of health practices, knowledge, beliefs and approaches that involve the use of plant, animal and mineral-based remedies, along with spiritual therapies and manual techniques, for the diagnosis, prevention and treatment of illnesses. These practices are broadly classified into three categories: codified medical systems such as Ayurveda, Siddha and Unani; orally transmitted, knowledge-based folk medicines and other allied health practices (Telles et al., 2014). The use of various herbs to treat numerous ailments is a widespread and longstanding tradition (Sarma et al., 2019). Interest in traditional medicine is steadily increasing due to its accessibility, affordability, minimal technological requirements, adaptability and relatively fewer side effects compared to conventional modern therapies (Bodekar et al., 2005).

Since ancient times, plants have served both nutritional and medicinal purposes for human societies (Mensah et al., 2019). In many developing countries, limited access to modern healthcare services compels people to rely on traditional medicine to manage a wide range of health issues. Natural medicines are often presumed to be completely safe and free from adverse effects. However, this assumption is misleading (Efferth et a., 2011). A significant number of plants used in traditional medicine have been found to be toxic because of their bioactive compounds (Mensah et al., 2019). Herbal remedies can potentially cause nephrotoxicity, hepatotoxicity, cardiotoxicity, neurotoxicity and dermatotoxicity (Fatima and Nayeem, 2016). Given that many populations heavily depend on herbal medicine, it is essential to conduct thorough investigations to identify effective and safe herbal products while distinguishing beneficial remedies from harmful ones (Efferth et a., 2011).

Persicaria hydropiper Linn., an annual herb from the family Polygonaceae, is widely valued not only as a spice and flavoring agent for various traditional dishes but also for its broad medicinal applications (Huq et al., 2014). Among these, its antifertility properties have been documented (Hazarika et al., 2006a). The plant's extract is traditionally used to address menstrual irregularities (Blatter et al., 1998) and decoctions made from the whole plant are administered to manage excessive menstrual bleeding (Chevallier, 1996). In Bangladesh, women of the Garo tribe use the leaf juice to alleviate menstrual pain (Rahmatullah et al., 2009). Notably, in Assam, a north-eastern state of India, the Mishing tribal women customarily consume the powdered dry root of *P. hydropiper* to prevent unwanted pregnancy. Prolonged, continuous consumption of this powder for over a year reportedly results in permanent sterility (Hazarika et al., 2006a).

Despite its numerous medicinal benefits, several studies have reported the toxic effects associated with various extracts of this plant (Raihan et al., 2012; Kuroiwa et al., 2006). Evidence of the cytotoxic properties of its extracts has also been documented (Lajter et al., 2012; Ayaz et al., 2019; Sharif et al., 2014). These findings raise concerns about the safety of using this plant as medicine, especially among traditional communities where awareness of its potential toxicity may be limited. Considering these possible harmful effects, the use of this plant for contraception — whether before or after conception — could pose risks to both reproductive and general health. In light of this, the present study was designed to evaluate the toxicological effects of the methanolic root extract of *P. hydropiper* in both pregnant and ovariectomized mice, with particular focus on assessing its potential for acute toxicity, genotoxicity, hepatotoxicity, nephrotoxicity and neurotoxicity.

2. Materials and Methods

2.1. Collection of Plant

The roots of *P. hydropiper* were collected from paddy fields in Gohpur (26.87900° N, 93.60580° E) and Nagaon (26.34640° N, 92.68400° E) during the months of February to May 2018. The plant samples were authenticated at the Department of Botany, Gauhati University, Guwahati, Assam, and a voucher specimen was deposited there (Accession No. GUBH20373).

2.2. Preparation of Root Extract

The collected roots were thoroughly washed, shade-dried and then chopped into small pieces. These pieces were ground into a fine powder using a mixer-grinder, passing through a 60-mesh sieve. The powdered roots were soaked in methanol for 72 hours at room temperature (25±2ºC) and then filtered. The obtained filtrate was concentrated under reduced pressure at 27±2ºC and stored at -20ºC until further use.

2.3. Experimental Animals

For this study, adult cyclic female Swiss albino mice weighing 30±5 g were procured from the College of Veterinary Sciences, Khanapara, Guwahati, Assam. The animals were housed under standard laboratory conditions with natural light and ambient temperature. They were provided a routine diet consisting of Bengal gram along with water available *ad libitum*. All procedures for animal care and experimentation adhered to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and the study protocol received approval from the Institutional Animal Ethical Committee (IAEC) (Approval No.- IAEC/2021-22/03).

2.4. Experimental Design

Adult female mice over 60 days of age were selected for the study and monitored for their estrous cycles following the protocol described by Montes and Luque (1988). Only females exhibiting regular cycles were chosen and allowed to mate during the proestrous stage. Pregnancy was confirmed by the presence of a vaginal plug the following morning. The

pregnant females were then divided into five groups and administered either distilled water (control group) or varying doses of *P. hydropiper* methanolic root extract — 250 mg/kg body weight (Group 1), 500 mg/kg (Group 2), 750 mg/kg (Group 3) and 1000 mg/kg (Group 4). Treatments were given daily for six consecutive days. Following the treatment period, the animals were sacrificed and their uteri were examined to count implantation sites. The extent of pregnancy suppression was determined based on the number of implantation sites. The dose that resulted in complete (100%) suppression of pregnancy (1000 mg/kg body weight) was selected as the threshold dose for subsequent treatments.

A total of 12 groups comprising normal cyclic female mice were established — six designated as treated groups (At, Bt, Ct, Dt, Et and Ft) and six as control groups (Ac, Bc, Cc, Dc, Ec and Fc). The treated groups received the threshold dose of the methanolic root extract in the morning (between 9:00–10:00 AM) following confirmed positive mating. Additionally, two groups of ovariectomized females were included: one treated with the vehicle (OVX control, Oc) and the other with the extract (OVX treated, Ot). Ovariectomy procedures were carried out following the method of Hogan et al. (1986), as previously described by Hazarika et al. (2006a).

All extract-treated and control animals were regularly and individually observed for changes in body weight, behaviour and signs of toxicity. Mice from treated groups At, Bt, Ct, Dt, Et and Ft were sacrificed on Day 1, Day 2, Day 3, Day 4, Day 5 and Day 6 of positive mating, respectively. Corresponding control groups (Ac, Bc, Cc, Dc, Ec and Fc) were sacrificed on the same respective days. The ovariectomized groups were sacrificed on the sixth day of treatment.

Prior to sacrifice, animals were anesthetized with intraperitoneal pentobarbital and blood was collected via cardiac puncture. Following sacrifice, the uterus, liver and kidneys

were carefully dissected using sharp scissors and fixed in 10% formalin for histological examination. All procedures for animal handling and experimentation were conducted in accordance with the guidelines of CPCSEA. The experimental protocols received approval from the Institutional Animal Ethical Committee (IAEC) (Approval No. IAEC/2021-22/03).

2.5. Behavioural Studies

2.5.1. General Signs and Behavioural Observations

The animals in groups At, Bt, Ct, Dt, Et and Ft received a daily oral dose of 1000 mg/kg body weight of the extract for 1, 2, 3, 4, 5 and 6 days, respectively, following confirmed positive mating. Each animal was observed for six hours after treatment daily, continuing until the day of sacrifice. The corresponding control groups (Ac, Bc, Cc, Dc, Ec and Fc) were administered the vehicle and observed under the same conditions. Similarly, ovariectomized mice in the control (Oc) and treated (Ot) groups were given vehicle and extract treatments, respectively, for six consecutive days and monitored in the same way. Body weight of all animals was recorded daily until the day of sacrifice.

2.5.2. Open Field Test

The open field test was performed according to the method described by Kadri et al. (2018) to assess the general locomotor activity of both control and treated animals. Each animal was placed in the centre of an open field apparatus divided into six equal squares. The number of squares crossed and the duration of immobility were recorded for each subject.

2.5.3. Novel Object Recognition Test

This test was conducted using a polypropylene cage. On the day before the experiments, mice were allowed to freely explore the empty cage for 2 minutes. During the first trial, two

identical objects were placed at opposite positions inside the cage and the time spent exploring each object was recorded. Exploration was defined as the animal directing its nose towards an object within 2 cm and/or physically touching it with the nose. The second trial was conducted 24 hours after the first. This procedure was repeated daily up to the 6th day of pregnancy. In each successive trial, one of the identical objects was replaced with a novel object. The time spent exploring the familiar (F) and novel (N) objects was recorded separately. The discrimination index (DI) was calculated using the formula: (N–F)/(N+F). To avoid olfactory bias, the objects and apparatus were wiped with hydrogen peroxide after each trial. All trials were performed two hours after administering the extract each day.

2.5.4. Modified Hole Board Test

The modified hole board test was conducted following the method of Laskar et al. (2021). In brief, a grey wooden box was prepared at the institute, featuring 20 equidistant holes, each 3 cm in diameter, on the floor surface. The platform was elevated 28 cm above the ground using a stand. Each animal was placed in the apparatus facing the nearest wall and allowed to explore freely for 5 minutes. The total number of head dips was recorded, considering a head dip valid when the animal's eyes disappeared into the hole.

2.6. Estimation of SGOT and SGPT Levels

Blood samples were collected via cardiac puncture and left to clot. The clotted blood was then centrifuged at 2000 rpm for 5 minutes, after which the sera were separated and stored at –20°C until analysis. The quantitative in vitro estimation of SGOT and SGPT was carried out following the protocol recommended by the International Federation of Clinical Chemistry (IFCC, 1980).

2.7. Histological Study of Liver and Kidney

The liver and kidneys collected from sacrificed animals were fixed in 10% formalin. Histological processing was performed according to the method described by Culling (1972). Serial tissue sections of 5 μm thickness were prepared and stained with Delafield's hematoxylin and eosin. Stained slides were mounted using DPX and examined microscopically. Alternate sections were photomicrographed for documentation.

2.8. Genotoxicity Study

The genotoxic potential of the extract was assessed using a micronucleus assay. Peripheral blood was collected from the tail vein of each animal and three smears were prepared on clean, degreased slides. The smears were fixed with absolute methanol for 10 minutes, stained with 10% Giemsa for 5 minutes and mounted for microscopic observation. For each group, a total of 2000 erythrocytes were counted and the number of micronucleated erythrocytes was recorded.

2.9. Statistical Analysis

All data were expressed as mean values±standard error of mean (SEM). Statistical comparisons between control (vehicle-treated) and extract-treated groups were performed using SPSS software (version 16.0). Student's t-test was applied to determine significant differences, with a double-tailed paired probability (P) value of less than 0.05 considered statistically significant.

3. Results

3.1. Determination of Threshold Dose

Four different doses of the methanolic root extract of *P. hydropiper* were prepared by dissolving in water: 250 mg/kg, 500 mg/kg, 750 mg/kg and 1000 mg/kg body weight. Among

these, the dose of 1000 mg/kg body weight was found to be 100% effective in preventing pregnancy in female mice with confirmed positive mating (Fig. 2). Therefore, this dose was selected as the threshold for further studies on the antifertility property of the *P. hydropiper* crude root extract.

3.2. Changes in Body Weight

The body weight of all experimental animals, initially around 30 g, was recorded daily until the day of sacrifice. No significant changes in body weight were observed between the extract-treated and control groups throughout the study period (Fig. 3).

3.3. Assessment of Behavioural Toxicity

3.3.1. General Signs and Behaviour

All animal groups treated with 1000 mg/kg body weight of the extract exhibited no signs of toxicity based on behavioural observations. The treated mice displayed normal responses, including touch response, pain reflex, torch response, righting reflex and gripping ability. Additionally, no noticeable alterations were recorded in parameters such as urination, salivation, skin colour, food intake or water consumption (Table 2).

3.3.2. Open Field Test

The open field test was conducted for 5 minutes, 2 hours after the administration of the methanolic root extract. The same procedure was applied to the control groups. A significant increase in locomotor activity was recorded in the extract-treated groups on Day 1 (81.7 ± 0.40 cases/5 min), Day 5 (80.8 ± 0.72 cases/5 min), Day 6 (82.0 ± 0.26 cases/5 min) and in the OVX-treated group (80.3 ± 0.15 cases/5 min) when compared with their respective control groups (78.6 ± 1.01, 78.3 ± 0.75, 77.9 ± 0.59, and 75.9 ± 0.94 cases/5 min, respectively) (Fig. 4).

3.3.3. Novel Object Recognition Test

Observations for both control and treated groups were carried out over two consecutive days: one day prior to sacrifice (session 1) and on the day of sacrifice (session 2). No notable differences were observed in the time spent exploring objects between the control and treated groups. Similarly, no significant variations in novel object recognition behaviour or discrimination index were recorded between the groups (Fig. 5).

3.3.4. Modified Hole Board Test

In this test, the total number of head dips performed by the animals was recorded. A significant increase in head dip counts was observed in the extract-treated groups on Day 1 (34.5 ± 0.43), Day 4 (35.1 ± 0.28), Day 5 (35.0 ± 0.26) and Day 6 (33.6 ± 1.45), as compared to their respective control groups (Fig. 6).

3.4. Serum Biochemical Parameters

3.4.1. Determination of SGOT Level

Serum SGOT levels were evaluated in pregnant control mice from Day 1 to Day 6 of pregnancy, in extract-treated mice from Day 1 to Day 6 after confirmed positive mating and in OVX females following 6 days of treatment with either vehicle or methanolic root extract. In the control groups, SGOT levels ranged from 100.61 ± 2.48 IU/l to 127.46 ± 2.20 IU/l, whereas in the extract-treated groups, levels ranged from 121.06 ± 1.12 IU/l to 131.28 ± 2.74 IU/l. A significant increase in SGOT levels was noted in the OVX-treated group when compared to its control (Fig. 7).

3.4.2. Determination of SGPT Level

Serum SGPT levels were also assessed in both control and extract-treated groups. In control animals, SGPT values ranged from 36.14 ± 2.24 IU/l to 52.44 ± 2.38 IU/l, while in the extract-treated groups, levels ranged from 41.82 ± 1.52 IU/l to 55.42 ± 2.50 IU/l. Although all values remained within normal physiological ranges, a significant increase in SGPT levels was observed on Day 1 and Day 2 in the treated groups compared to their respective controls (Fig. 8).

3.5. Histological Study

3.5.1. Liver

The liver tissues of both control and extract-treated mice exhibited well-defined lobules with centrally located central veins. In both groups, the portal areas contained the hepatic artery, portal vein, and bile duct. Hepatocytes were arranged in radiating cords from the central vein extending towards the portal areas. These hepatocyte cords were separated by sinusoidal capillaries in both control and treated livers (Fig. 9). The overall histoarchitecture of the *P. hydropiper* methanolic root extract-treated livers appeared comparable to that of the control group, with no notable histopathological alterations (Fig. 9).

3.5.2. Kidney

Histological sections of both control and extract-treated kidneys revealed well-differentiated cortex and medullary regions. The cortex contained numerous renal corpuscles and renal tubules. Each renal corpuscle consisted of a glomerulus surrounded by Bowman's capsule. These structures were clearly identifiable in both control and treated kidneys, with no discernible differences in histoarchitecture between the two groups (Fig. 10).

3.6. Genotoxicity Study

The genotoxic potential of the methanolic root extract of *P. hydropiper* was assessed using the micronucleus assay in 2000 peripheral blood erythrocytes from both control and extract-treated groups. No significant increase in the frequency of micronucleated erythrocytes was observed in any of the treated groups when compared to their respective controls, indicating the absence of genotoxic effects (Fig. 11).

4. Discussion

The concept of contraception involves the prevention of conception and, more broadly, the prevention of pregnancy. In contemporary society, a variety of contraceptive methods are employed, including oral contraceptive pills, intrauterine devices (such as Copper-T), tubectomy and barrier methods like condoms (Pradhan et al., 2012). Alongside these modern techniques, the use of medicinal plants for fertility regulation has been an age-old practice among various communities across the globe (Das et al., 2017). Ethnobotanical and ethnomedicinal studies conducted in India and other countries have documented the use of numerous plant species over centuries, either as dietary adjuncts or in medicinal formulations, for reproductive control (Priya et al., 2012). Several plants, including *Ferula assa-foetida*, *Abrus precatorius*, *Acacia catechu*, *Borassus flabellifer*, and *Euphorbia caducifolia*, have been traditionally employed for such purposes (Gediya et al., 2011; Jain et al., 2012). These plants exert their antifertility effects through diverse mechanisms, such as anti-implantation, abortifacient, ecbolic, estrogenic and spermicidal actions. However, despite their contraceptive potential, many of these plants are also associated with varying degrees of toxicity (Priya et al., 2012).

P. hydropiper is a medicinal plant previously reported to possess antifertility and estrogenic properties (Huq et al., 2014). It demonstrates notable anti-ovulatory and anti-implantation effects, thereby contributing to fertility regulation (Kamboj and Dhawan, 1982).

Importantly, the effectiveness of plant-based contraceptives is often dose-dependent. In the present study, among the four tested doses of the methanolic root extract of *P. hydropiper* (250 mg/kg, 500 mg/kg, 750 mg/kg, and 1000 mg/kg body weight), only the highest dose of 1000 mg/kg body weight achieved 100% pregnancy prevention in female mice. This observation aligns with the findings reported by Hazarika (2006).

In this investigation, pregnant mice were treated with the methanolic root extract of *P. hydropiper* at a dose of 1000 mg/kg body weight up to the peri-implantation period (Day 6 of pregnancy). Notably, no mortality or signs of acute behavioural toxicity were observed at this dose. Parameters such as alertness, touch response, torch response, pain response, righting reflex, gripping reflex, urination, salivation, food intake and general physical appearance remained unaffected. Furthermore, no toxicity-associated behaviours like restlessness, tremors or convulsions were detected. This safety profile is comparable to observations made with other medicinal plants, such as *Pericampylus glaucus*, which was similarly considered safe due to an LD_{50} value exceeding 1000 mg/kg body weight (Kifayatullah et al., 2015). Additionally, a previous study demonstrated no mortality in mice even when treated with *P. hydropiper* extract at doses up to 4000 mg/kg body weight (Akhter et al., 2013), further supporting the relative safety of this plant extract at the dose used in the present study.

An alteration in body weight is often associated with the toxicity profile of any chemical or pharmacological agent (Kifayatullah et al., 2015). A decrease in body weight, typically resulting from reduced caloric intake due to appetite suppression following treatment, is considered a sign of potential toxicity (Madingou et al., 2016). In the present investigation, no significant change in body weight was observed in the extract-treated mice compared to the control groups. This suggests the probable absence of overt systemic toxicity resulting from the administration of the *P. hydropiper* methanolic root extract at the tested dose.

The treatment was also found to slightly increase locomotor activity in the open field test. As a reduction in locomotor activity is typically associated with central nervous system (CNS) depression, an increase in activity indicates the absence of such depressive effects (Devi et al., 2013). Additionally, the open field test is widely used as a measure of anxiety-related behaviour in rodents (Anchan et al., 2014). Animals experiencing heightened anxiety typically display thigmotaxis — a tendency to remain close to the walls of the test arena (Kraeuter et al., 2019)— which was not observed in the present study. The mild anxiolytic effect inferred from the increased locomotor activity might be attributed to the modulation of estrogen-sensitive pathways, such as the activation of GPR-30 receptors in the limbic system (Anchan et al., 2014). Furthermore, locomotor activity in this test also serves as an indicator of general well-being and stress response, as animals in poor health tend to exhibit reduced movement within the experimental environment (Kraeuter et al., 2019). The maintained or increased movement in treated groups, therefore, suggests good overall health following extract administration.

Similarly, the modified hole board test assesses exploratory and locomotor behaviour in rodents (Laskar et al., 2021). A decrease in head-dipping behaviour is interpreted as a sign of heightened anxiety, whereas an increase indicates anxiolytic-like activity and reduced anxiety levels (Saitoh et al., 2006). In the present study, a significant increase in head-dip counts was noted in the treated groups on Day 1, Day 4, Day 5 and Day 6. This reinforces the suggestion of an anxiolytic effect exerted by the extract. Such an effect could plausibly be mediated through the modulation of GABAergic neurotransmission, as has been suggested in other studies involving plant-based anxiolytics (Mahmud et al., 2017). Phytoconstituents like alkaloids, flavonoids and saponins are known to possess anxiolytic properties through the activation of GABA-A receptors and facilitation of chloride ion channel opening, thereby exerting CNS calming effects (Zaman et al., 2015). Previous phytochemical analyses have

confirmed the presence of such bioactive compounds in *P. hydropiper* extracts (Hazarika, 2006) and their involvement in the observed behavioural effects in this study is therefore likely.

In the Novel Object Recognition (NOR) test, an increase in the discrimination index (DI) reflects an enhancement in recognition memory [40]. This form of object memory is regulated by several factors, including ovarian steroid hormones such as estradiol (E_2) and progesterone (P_4) (Tuscher et al., 2015). In the present investigation, however, no significant changes were observed in the DI values of extract-treated groups compared to controls, suggesting that the extract did not exert a noticeable effect on memory performance within the short duration of the study. Interestingly, *β*-sitosterol, a phytoconstituent previously isolated from *P. hydropiper*, has demonstrated memory-enhancing effects in mice in other experimental models (Ayaz et al., 2017; Ye et al., 2020). Therefore, it is plausible that prolonged or chronic administration of the extract might reveal cognitive-enhancing properties not detectable under the current experimental conditions.

Exposure of liver tissue to toxic substances over a prolonged period can lead to hepatocyte injury, progressing to chronic hepatitis, fibrosis, portal hypertension and ultimately, liver failure (Das et al., 2017). In the present study, liver tissues from mice treated with the methanolic root extract of *P. hydropiper* up to day 6 of pregnancy maintained near-normal histoarchitecture. As the primary detoxifying organ, the liver acts as a frontline defence against toxins that cross the intestinal barrier. Kupffer cells, the resident macrophages of the liver, play a pivotal role in initiating inflammatory responses by recruiting neutrophils, which subsequently release pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukins (IL-1 and IL-6), chemokines and reactive oxygen species (ROS). These factors prime and activate neutrophils within the hepatic sinusoids and post-sinusoidal venules, potentially leading to hepatocellular damage and necrosis (Ramaiah et al., 2007a; Ramaiah et

al., 2007b). Infiltration of neutrophils is therefore a hallmark of liver injury and a critical contributor to hepatic inflammation (Xu et al., 2014).

Notably, no such pathological features—including neutrophilic infiltration, hepatocyte degeneration, or architectural disruption—were observed in the liver tissues of extract-treated mice in the current investigation. However, biochemical alterations in liver enzyme levels are regarded as sensitive indicators of subclinical hepatic injury (Rajanna et al., 1984). Among these, serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) are routinely evaluated to assess liver function and detect hepatocellular damage (Doolittle et al., 1987). In this study, a significant increase in SGOT levels was detected in the ovariectomized (OVX)-treated group, while a significant elevation in SGPT was noted on Day 1, Day 2, Day 3 and Day 6 in the extract-treated pregnant mice when compared to controls. A comparable, albeit mild, elevation in SGPT has also been reported in both male and female mice exposed to 4000 ppm water-paper leaf extract (Kuroiwa et al., 2006).

These findings indicate a very minimal, possibly transient, hepatic perturbation at the administered dose of 1000 mg/kg body weight of the methanolic root extract. Elevations in SGOT and SGPT generally suggest hepatocellular membrane damage, increased permeability or inflammation (Kifayatullah et al., 2015). While mild hepatotoxicity has been documented with several other medicinal plants traditionally used as contraceptives or abortifacients (Das et al., 2017), it is important to note that, according to widely accepted histopathological grading systems such as the Knodell and METAVIR scoring systems, no evidence of higher-order hepatic damage or fibrosis was detected in the present study within the peri-implantation treatment window (Knodell et al., 1981; Bedossa and Poynard, 1996). A few previous studies also documented a slight increase in SGOT and SGPT levels in pregnancy (Mutua et al., 2018).

Regarding renal toxicity, the kidneys play a critical role in maintaining physiological homeostasis and are particularly vulnerable to xenobiotic-induced injury. Nephrotoxic agents are often absorbed by both apical and basolateral transport systems in the renal proximal tubules, leading to cell damage, functional impairment and in severe cases, the formation of tubular crystals due to the concentrating ability of the kidneys (Vervaet et al., 2017). Structural injury and functional alterations typically manifest as histopathological changes, including tubular degeneration, interstitial inflammation or glomerular disruption (Andjelkovic et al., 2019). In the present study, the histological examination of kidney sections from extract-treated mice revealed no notable differences compared to the control group. The treated kidneys displayed normal histoarchitecture, including intact renal corpuscles, proximal and distal tubules, and preserved cortical and medullary regions. These findings suggest an absence of nephrotoxic effects at the tested dose and duration. Similar observations have been made by Kuroiwa et al. (2006), where treatment with 4000 ppm *P. hydropiper* leaf extract did not induce any detectable histopathological alterations in renal tissues.

Genotoxicity assessment is essential for traditionally used medicinal plants, as any damage or alteration to the genetic material can potentially lead to carcinogenesis or other genetic disorders (Mohmed et al., 2018). Among the established methods for evaluating genotoxicity, the peripheral blood micronucleus assay is widely regarded as a reliable and convenient technique. This assay detects the formation of micronuclei — which are acentric chromosomal fragments or whole chromosomes that fail to incorporate into daughter nuclei during cell division — serving as biomarkers of chromosomal damage and genomic instability (Aguilera-Rodríguez et al., 2021).

Previous studies have demonstrated genotoxic effects of certain medicinal plants and pharmaceutical contraceptives. For example, *Azadirachta indica* has been reported to induce

genotoxicity in male germ cells (Awasthy, 2001). Additionally, several contraceptive formulations such as Anovlar 21 and synthetic progestins have exhibited genotoxic potential in earlier experimental studies (Shyama et al., 1991; Siddique and Afzal, 2008; Hassan et al., 2018). These findings underscore the importance of thoroughly evaluating the genetic safety of medicinal plants with contraceptive properties. In the present investigation, no significant differences were observed in the frequency of micronuclei in peripheral blood cells between the extract-treated and control groups. This suggests that the methanolic root extract of *P. hydropiper*, at the administered dose of 1000 mg/kg body weight, does not exert genotoxic effects within the peri-implantation exposure period. Accordingly, the extract may be considered non-genotoxic at this dosage under the experimental conditions of the study.

5. Conclusion

Toxicological evaluation of traditional medicinal plants is essential to ensure their safety before potential therapeutic application. In light of this, the present study was undertaken to assess the possible toxic effects of the methanolic root extract of *P. hydropiper*, a plant traditionally used for fertility regulation by certain tribal communities. The findings revealed that most of the evaluated toxicity parameters remained unaltered in the extract-treated groups compared to the controls.

A slight but statistically significant elevation in serum SGOT and SGPT levels was observed in the treated mice, indicating mild hepatotoxicity. However, no histopathological damage to liver tissue was evident at the administered dose. Similarly, no signs of nephrotoxicity were detected, as reflected by the normal histology of kidney tissues in the treated groups. Additionally, some enhancement in exploratory and locomotor behaviours was noted, suggesting a possible anxiolytic effect of the extract without central nervous system depression.

Taken together, the extract may be considered low-toxic or non-toxic at the tested dose of 1000 mg/kg body weight per day, at least within the peri-implantation exposure period. Nonetheless, a comprehensive phytochemical analysis is warranted to identify the specific bioactive constituents responsible for the observed mild hepatotoxicity. Further in-depth toxicological studies, including chronic toxicity, reproductive toxicity, and genotoxicity assessments, are recommended to support the development of a safer, standardized herbal contraceptive formulation.

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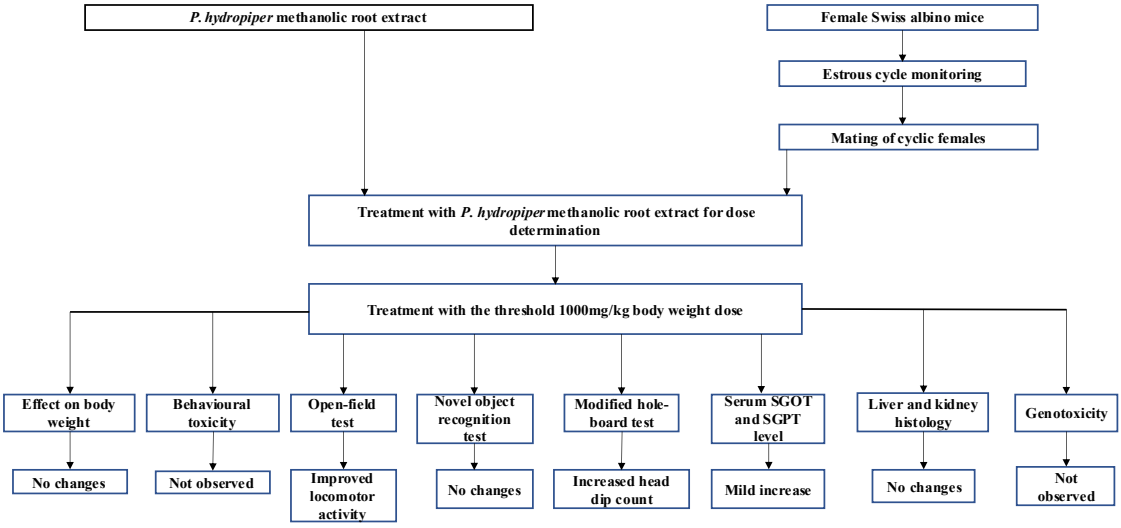

Fig. 1 Overview of the experimental design and brief results

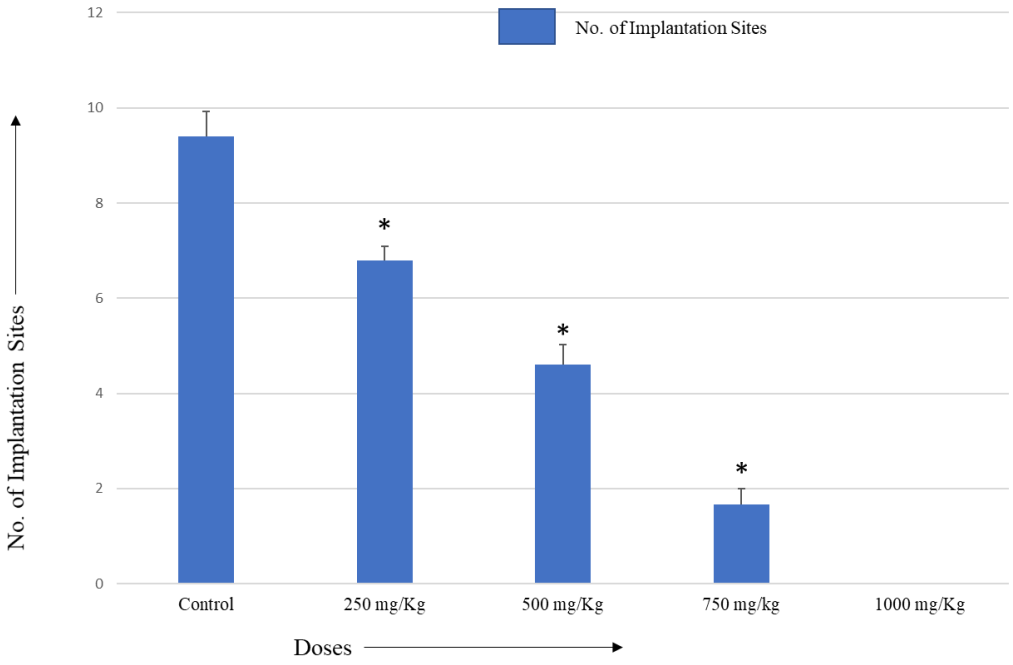

Fig. 2 Number of implantation sites in mice treated with water (control) and varying doses of methanolic root extract of *P. hydropiper* (n = 10). Data are expressed as mean ± SEM (n = 10). Statistical analysis was conducted using Student's *t*-test to compare treatment groups with the control. The asterisk (*) denotes statistically significant differences at the 95% confidence level ($p < 0.05$).

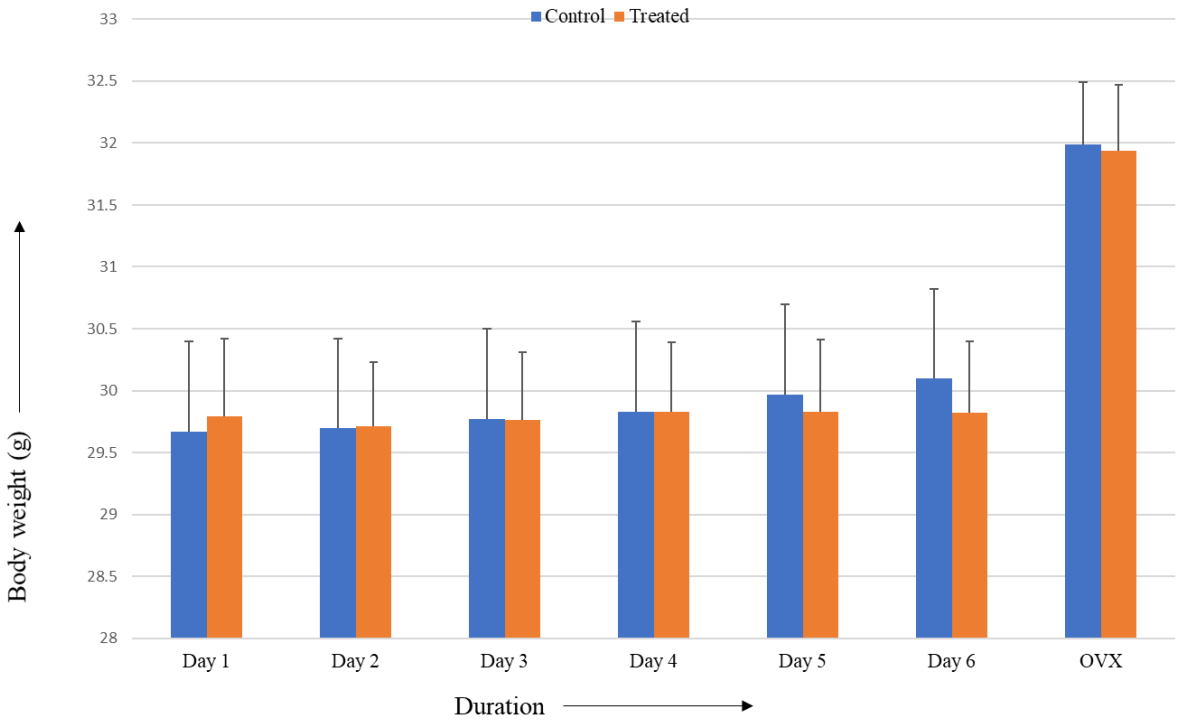

Fig. 3 Effect of methanolic root extract of *P. hydropiper* on body weight in control and CRE-treated groups of pregnant and ovariectomized mice that were administered a dose of 1000 mg/kg body weight. Data are presented as mean ± SEM (n = 10). Statistical comparisons between respective control and treated groups were performed using Student's *t*-test. No statistically significant difference was observed in the CRE-treated groups compared to their respective controls ($p < 0.05$).

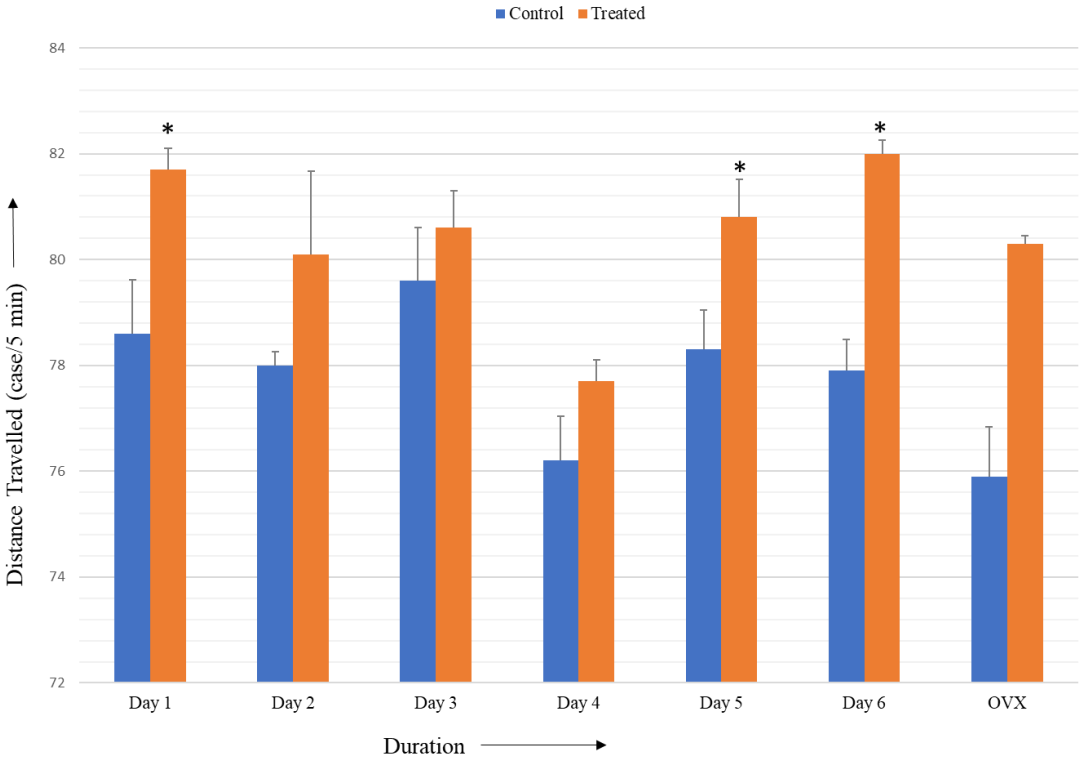

Fig. 4 Effect of methanolic root extract of *P. hydropiper* on total distance travelled (Case/5 min) in the Open Field Test in control and CRE-treated groups of pregnant and ovariectomized mice administered a dose of 1000 mg/kg body weight. Data are expressed as mean ± SEM (n = 10). Statistical comparisons between respective control and treated groups were conducted using Student's *t*-test. The asterisk (*) denotes statistically significant differences at the 95% confidence level ($p < 0.05$).

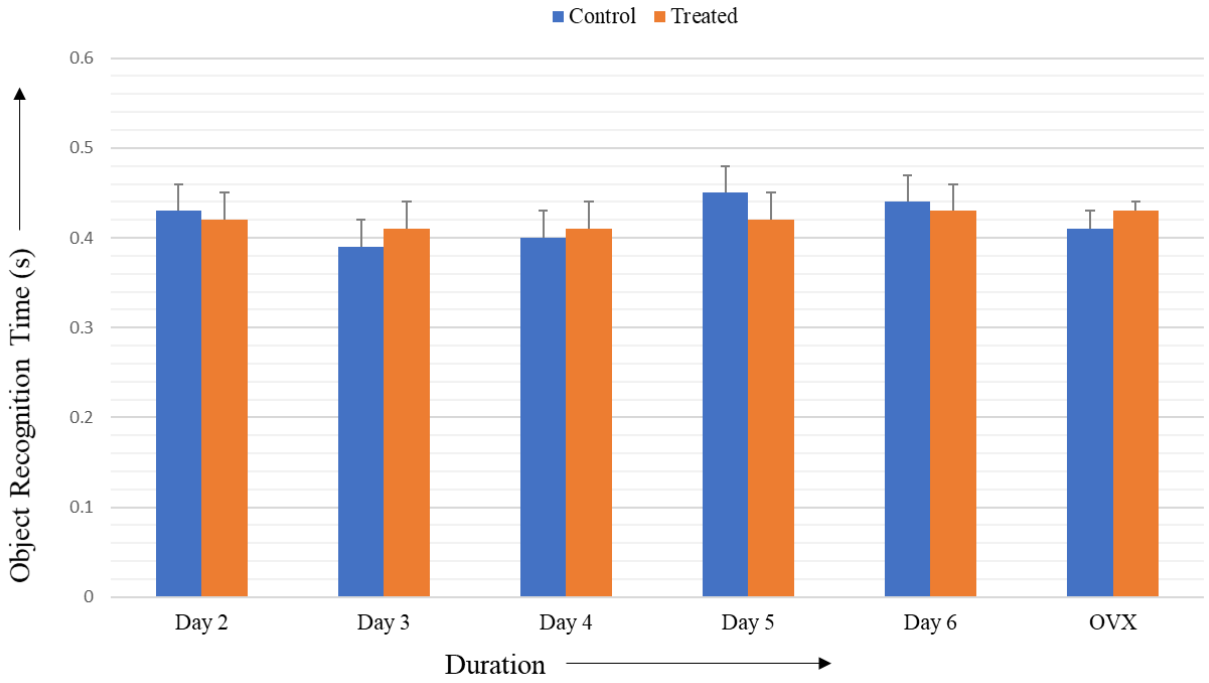

Fig. 5 Effect of methanolic root extract of *P. hydropiper* on object recognition time in the Novel Object Recognition Test in control and CRE-treated groups of pregnant and ovariectomized mice administered a dose of 1000 mg/kg body weight. Data are presented as mean ± SEM (n = 10). Statistical comparisons between respective control and treated groups were performed using Student's *t*-test. No statistically significant difference was observed in the CRE-treated groups compared to their respective controls ($p < 0.05$).

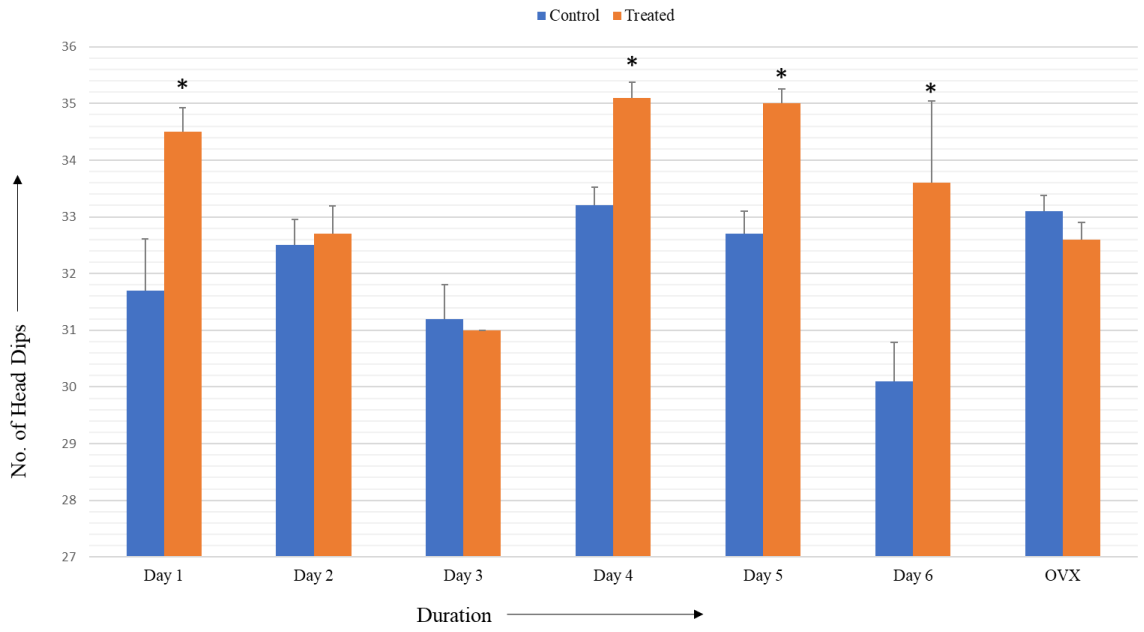

Fig. 6 Effect of methanolic root extract of *P. hydropiper* on exploratory behaviour, measured by head dips, in the Modified Hole Board Test in control and CRE-treated groups of pregnant and ovariectomized mice administered a dose of 1000 mg/kg body weight. Data are presented as mean ± SEM (n = 10). Statistical comparisons between respective control and treated groups were performed using Student's *t*-test. A statistically significant difference was observed in the Day 1, Day 4, Day 5 and Day 6 CRE-treated groups compared to their respective controls ($p < 0.05$).

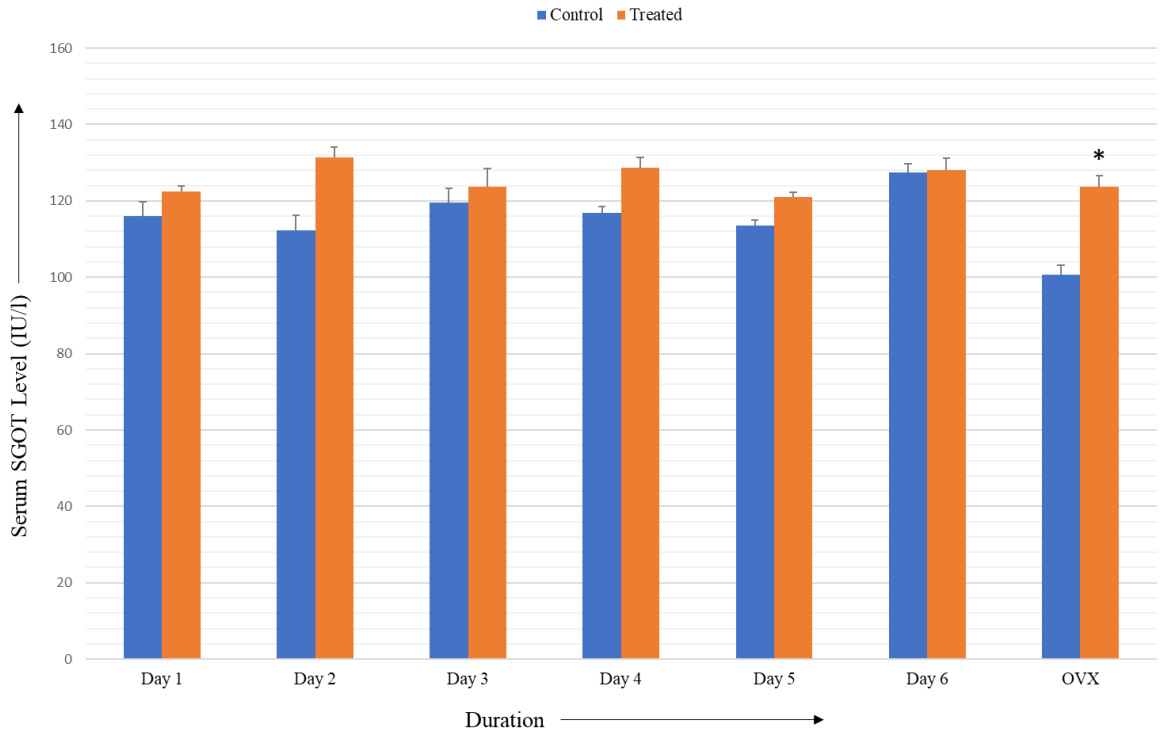

Fig. 7 Comparison of serum SGOT levels between control and CRE-treated groups of pregnant and ovariectomized mice administered a dose of 1000 mg/kg body weight. Data are presented as mean ± SEM (n = 10). Statistical comparisons between respective control and treated groups

were conducted using Student's *t*-test. A statistically significant difference was observed in the OVX CRE-treated group compared to its respective control ($p < 0.05$).

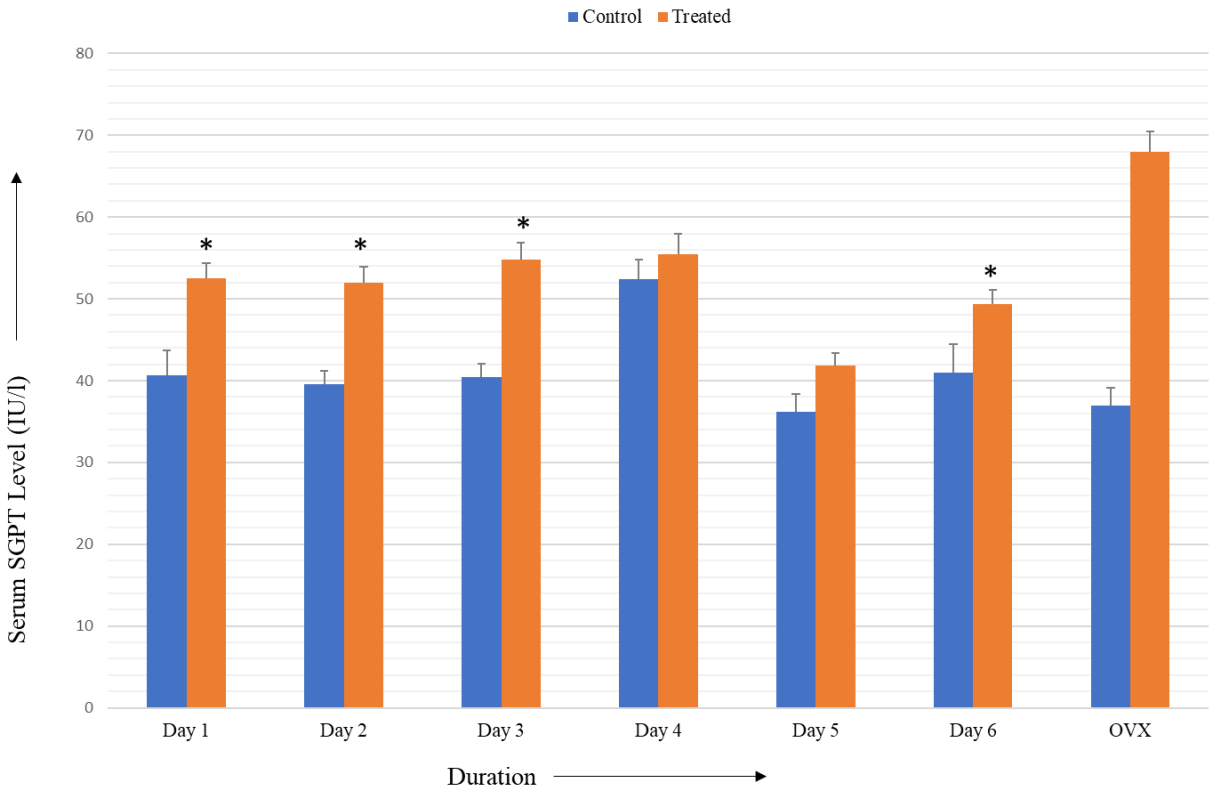

Fig. 8 Comparison of serum SGPT levels between control and CRE-treated groups of pregnant and ovariectomized mice administered a dose of 1000 mg/kg body weight. Data are presented as mean ± SEM (n = 10). Statistical comparisons between respective control and treated groups were performed using Student's *t*-test. A statistically significant difference was observed in the Day 1, Day 2, Day 3 and Day 6 CRE-treated groups compared to their respective controls ($p < 0.05$).

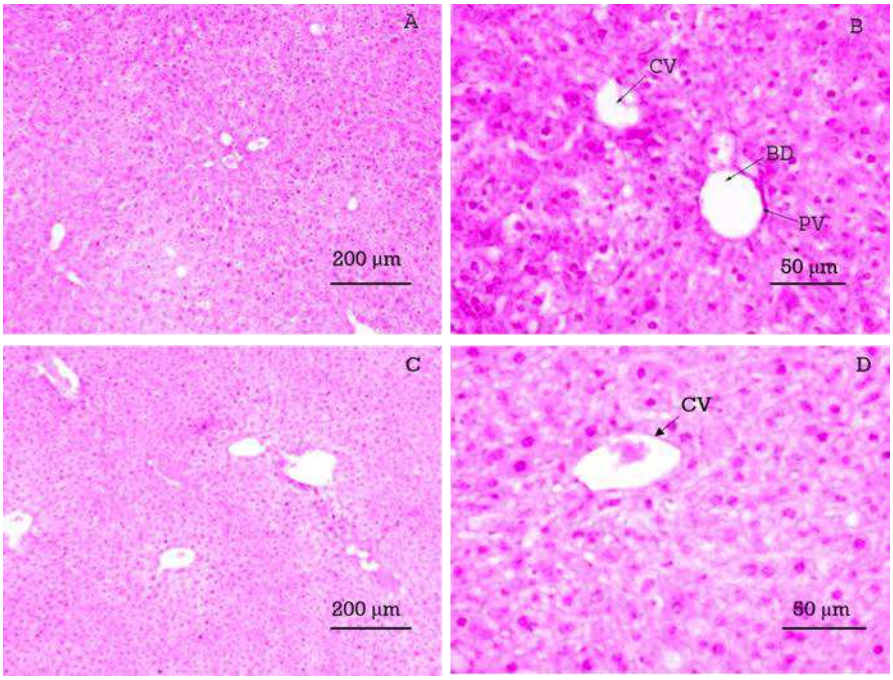

Fig. 9 L.S. of the liver of control mouse (Day 6 pregnancy) (A×10x, B×40x) and CRE-treated mouse (treated up to Day 6) (C×10x, D×40x). CV= Central vein, PV= Portal vein, BD= Bile duct

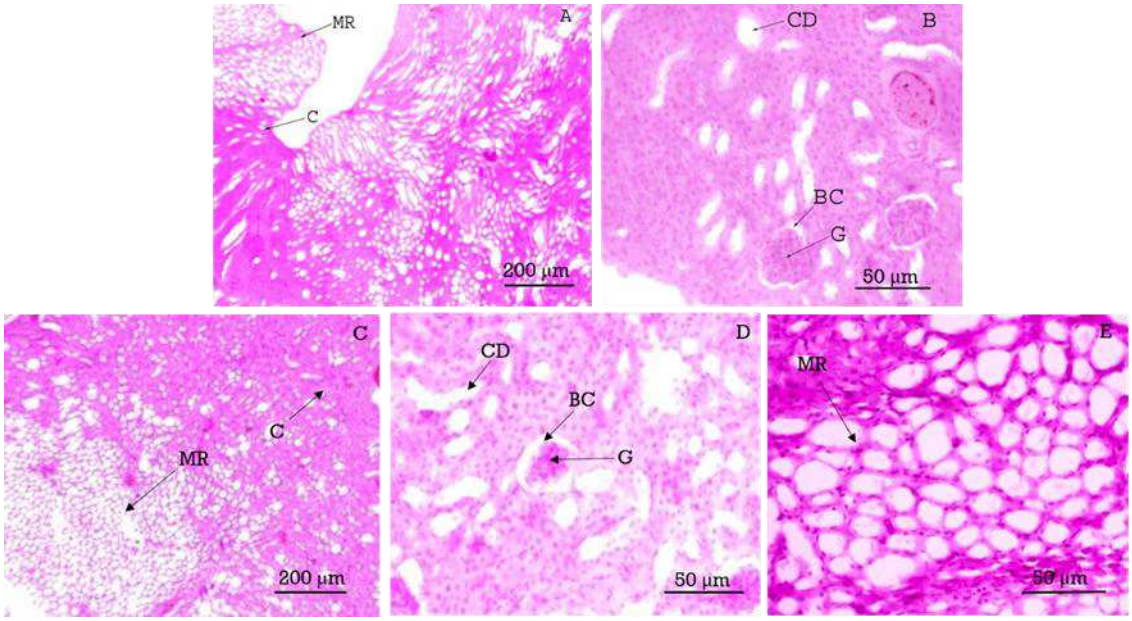

Fig. 10 L.S. of kidney of control mice (Day 6 pregnancy) (A×10x, B×40x) and CRE-treated mice (treatment up to Day 6) (C×10x, D, E×40x). MR= Medullary Ray, C= Cortex, G= Glomerulus, BC= Bowman's capsule, CD= Collecting duct

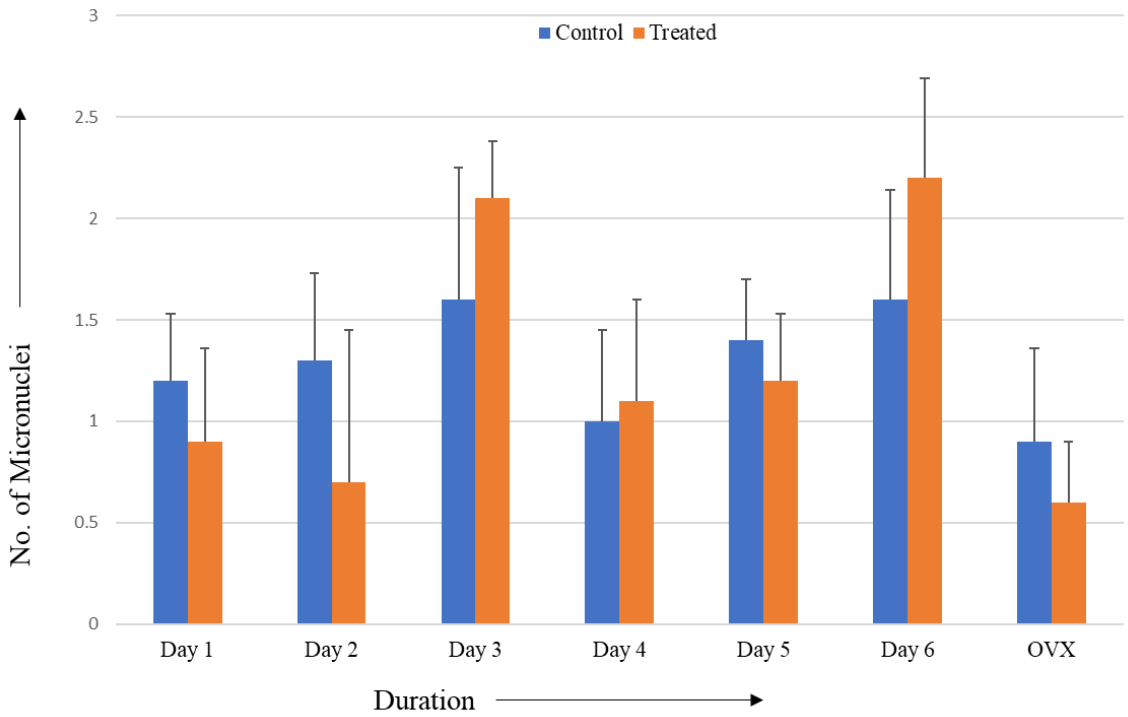

Fig. 11 Effect of *P. hydropiper* methanolic root extract on total micronucleus in peripheral blood of control and treated female albino mice administered a dose of 1000 mg/kg body weight. Data are presented as mean ± SEM (n = 10). Statistical comparisons between respective control and treated groups were performed using Student's *t*-test. No statistically significant difference was observed in the OVX CRE-treated group compared to its respective control ($p < 0.05$).

Table 1 Determination of the threshold dose of methanolic root extract of *P. hydropiper* required for suppression of pregnancy. Data are presented as mean ± SEM. An asterisk (*) indicates statistically significant differences at the 95% confidence level ($p < 0.05$).

Treatment	**Dose (mg/kg body weight)**	**No. of Mated Female**	**No. of Pregnant Females**	**Percentage of Pregnancy**	**No. of Implantation sites**	**Percentage of Suppression**
Control	Water	10	10	100%	9.40±0.52	0%
CRE	250	10	10	100%	6.80±0.29*	27.66%
CRE	500	10	6	60%	4.60±0.42*	51.06%
CRE	750	10	3	30%	1.67±0.33*	82.23%
CRE	1000	10	0	0%	0.00±0.00	100%

Table 2 Body responses exhibited by the control and *P. hydropiper* methanolic root extract treated mice groups

Body Response	**Control Groups (n=10)**	**Treated Groups (n=10)**
Alertness	Normal	Normal
Grooming	Not observed	Not observed
Restlessness	Not observed	Not observed
Touch Response	Normal	Normal
Torch Response	Normal	Normal
Pain Response	Normal	Normal
Tremors	Not observed	Not observed
Convulsion	Not observed	Not observed
Righting Reflex	Normal	Normal
Gripping	Normal	Normal
Pinna Reflex	Observed	Observed
Corneal Reflex	Observed	Observed
Writhing	Not observed	Not observed
Pupils	Normal	Normal
Urination	Normal	Normal
Salivation	Normal	Normal
Skin Colour	Normal	Normal
Lacrimation	Normal	Normal
Food Intake	Normal	Normal
Water Intake	Normal	Normal
Mortality	None	None
Diarrhoea	Not observed	Not observed
Coma	Not observed	Not observed