Prenatal developmental toxicity study of herbal tea of *Moringa stenopetala* and *Mentha spicata* leaves formulation in Wistar rats

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ABSTRACT

Background: *Moringa stenopetala* and *Mentha spicata* leaves have long been used to treat diabetes, hypertension, asthma, and other ailments. Herbal tea of *M. stenopetala* and *Mentha spicata* leaves formulation showed better antidiabetic and antihypertensive activities. This study investigated the prenatal developmental toxicity potential of the herbal tea of *M. stenopetala* and *M. spicata* leaves blend in rats.

Methods: Wistar pregnant rats were randomly distributed into four groups (n = 8). Group I (control) dams received distilled water. Group II-IV dams were treated with 559.36, 1118.72, and 2237.44 mg/kg of herbal tea of *M. stenopetala* and *Mentha spicata* leaves formulations, respectively, during days 5–19 of gestation. Maternal mortality, clinical signs, body weight changes, and food consumption were recorded. On gestation day 20, cesarean sections were performed, and maternal parameters of systemic toxicity (e.g., body weight, serum biochemistry, organ weight, and macro-pathology) as well as reproductive toxicity (e.g., number of corpora lutea, implantations, resorptions (early/late), pre/postimplantation losses, number of fetuses (live/dead), and fetal body weights, length, and their sex ratio) were evaluated. Fetuses were further examined for external, soft tissue, and skeletal alterations.

Results: No herbal tea-related maternal deaths or overt toxic symptoms were observed. The measured maternal systemic and reproductive toxicity parameters showed no herbal tea-associated significant alterations at any dosage levels. Moreover, there were no overt toxic effects of the herbal tea on the fetal external, visceral, or skeletal prenatal growth and development.

Conclusion: The study findings demonstrated that the herbal tea of *M. stenopetala* and *M. spicata* leaves blend could be relatively safe/low toxic to pregnant rats and developing fetuses. The no-observed-adverse-effect level (NOAEL) of herbal tea for maternal toxicity, fetotoxicity, and teratogenicity in rats is estimated to be > 2237.44 mg/kg/day.

Abbreviations: CRL, Crown Rump Length; EPHI, Ethiopian Public Health Institute; OECD, Organization for Economic Corporation and Development; TMMRD, Traditional and Modern Medicine Research Directorate.

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1. Introduction

Traditional African medicine is the oldest of all therapeutic systems in Africa. It has remained the most affordable and easily accessible treatment option for the majority of Africa’s population [1,2]. In Ethiopia, about 80% of the population relies on traditional medicine for primary health care, and over 95% of traditional medicinal preparations are of plant origin. Ethiopia is home to more than 732 species of medicinal plants, of which around 12–19% are endemic to the country [3,4]. In many areas of Ethiopia, Moringa stenopetala and Mentha spicata have long been used for their medicinal and nutritional benefits [5,6].

Moringa stenopetala is a medicinal plant of the Moringaceae family. It is native to Southern Ethiopia, Northern Kenya, and Eastern Somalia. In Southern Ethiopia, a large number of populations use the leaves of M. stenopetala as a source of food (i.e., eaten as vegetables) and herbal medicine. The leaves are prepared as a tea to treat diabetes, hypertension, malaria, asthma, stomach problems, and retained placenta [5–9]. The leaves are reservoirs of several beneficial secondary metabolites, viz., alkaloids, flavonoids, glycosides, glycoproteins, saponins, and phenolic compounds [10–12]. Several pharmacological studies showed that M. stenopetala leaves exhibit a wide range of biological activities, including antidiabetic, anti hypertensive, anti hypolipidemic, and anti inflammatory effects [13–15].

Mentha spicata is a medicinal and aromatic herb of the Lamiaceae family. It is native to temperate regions of Europe and western and central Asia, but these days it is widely distributed and grown in several regions of the world [16,17]. Since ancient times, the aerial parts of M. spicata have been used as a flavoring agent in numerous food products and as an herbal medicine in folk remedies. In particular, the leaves of this plant are prepared and administered as tea infusion or decoction to treat several diseases, including diabetes, hypertension, and various digestive, skin, and respiratory disorders [6,16–19]. Several pharmacological studies have confirmed that the leaves of M. spicata have antidiabetic, diuretic, antioxidant, antibacterial, anti inflammatory, analgesic, and antipyretic properties [20–22]. Phytochemical analyses of the leaves revealed the presence of several phytochemical bioactive compounds, including alkaloids, flavonoids, phenolic acids, carvone, carvacrol, and terpenes [20,23,24].

Nowadays, herbal formulations have gained widespread acceptance because it has been proven that herbal blends containing two or more distinct herbs generate better therapeutic results than individual herbs [25,26]. Such polyherbal materials are commonly prepared and used for the treatment of a variety of chronic diseases, including kidney failure [27], hypertension [28], and diabetes mellitus [29]. The herbal formulation prepared from the blended leaves of M. stenopetala (a primary/main therapeutic herb) and M. spicata (a secondary/supportive herb) exhibited better diuretic and antihyperglycemic activities than the primary herb (M. stenopetala) and the other herbal formulations, including the blended leaves of M. stenopetala and Cymbopogon citratus as well as the primary herb and Thymus serratulaceus blends. Moreover, the herbal formulation of M. stenopetala and M. spicata has demonstrated better physicochemical properties (e.g., optimal total phenolic and flavonoid contents, ash value, and moisture content, as well as better particle size, angle of repose, bulk and tapped density), sensory acceptability (e.g., good taste and flavor), and microbial quality properties than the other herbal preparations [30].

Furthermore, the safety and toxicity potentials of herbal formulations for M. stenopetala and M. spicata leaves were evaluated in previous studies using rats. The Acute Toxic Class Method procedure found the acute oral LD50 of herbal tea in rats to be > 5593.6 mg/kg body weight [30]. The repeated dose 90-day (subchronic) oral toxicity study conducted on male and female rats revealed no herbal tea-induced major toxic events at doses of 559.36, 1118.72, and 2237.44 mg/kg body weight [31]. During the chronic oral toxicity study, both male and female rats treated with different concentrations of herbal tea (up to a high dose of 2237.44 mg/kg/day) showed no in-life adverse events (e.g., death, overt toxic symptoms, loss of body weight, or altered food intake) throughout the 360-day treatment period. At the end of this long-term experiment, macro-pathological and histological examinations of the major organs (e.g., liver, kidneys, heart, pancreas, and spleen) revealed no significant pathologic alterations in the test groups compared with the control group findings [32]. However, the prenatal developmental safety and toxicity potential of herbal formulations of M. stenopetala and M. spicata leaf blends have not been studied yet. The prenatal developmental toxicity study will provide information regarding the adverse effects of prenatal exposure on the pregnant test animals (e.g., maternal systemic and reproductive toxicity effects) and on the developing fetuses (e.g., death, altered growth, or structural abnormalities in the fetus) [33].

Pregnancy is a period when herbs and herbal preparations are usually contraindicated [34]. Many adverse effects have been reported with the use and/or administration of herbs and herbal blends during pregnancy, including urogenital malformation, trachea-esophageal fistula, spina bifida, and imperforate anus in humans [35,36], maternal systemic and reproductive adverse effects, fetal death, retarded growth, and teratogenicity effects in rats [37–39]. In light of this information, assessing the prenatal developmental toxicity of the herbal formulation under study is necessary to ensure its safety before human use. The present study investigated the potential adverse effects of herbal tea of M. stenopetala and M. spicata leaf blend on pregnant rats and developing fetuses following maternal oral exposure during gestation days 5–19. The herbal tea formulation was tested according to the Organization for Economic Cooperation and Development (OECD) guidelines for testing chemicals to establish any dosage-related response and no-observed-adverse-effect level (NOAEL).

2. Materials and methods

2.1. Plant materials collection, formulation and extraction

The leaves of Moringa stenopetala were collected from an experimental site at Arba-Minch University, Southern Region, Ethiopia (6°01’59” N and 37°32’59” E). The Mentha spicata leaves were harvested from the Wondo Genet Medicinal and Aromatic Plants Research Center’s botanical garden in the Sidama Region, Ethiopia (7°1’N and 38°35’E). The leaves of M. spicata (Batch No. 019) and M. stenopetala (Batch No. 029) were authenticated, and voucher specimens were deposited in the herbarium of the Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia.

The leaves of both herbs were cleaned from extraneous materials, shade dried, cut into small pieces by hand, coarsely crushed using an electric mill, and sieved to a mesh size of 212 μm to obtain the proper size of leaf granules. The individual plant leaf granules were packed in aluminum foil and kept in a container with a lid, sealed, and stored in a cool, dry place until used. The coarse powders of M. stenopetala and M. spicata were weighed individually and blended in a ratio of 85% and 15%, respectively, using an electric blender to prepare a homogeneous herbal formulation. The total weight of animals at each dosage level was used to determine the amount of the herbal (leaves) formulation to be measured.

The measured herbal formulation was held on filter paper in a funnel-placed glass beaker and a cup (100 ml) of boiled distilled water (94.5 °C) was poured over it. Finally, the herbal tea infusion was cooled at room temperature and then orally given to the test animals. The tea infusions were prepared shortly prior to administration [15,30]. The different doses (559.36, 1118.72, and 2237.44 mg/kg body weight) selected for the present study were chosen based on previous studies [15,30–32].

2.2. Experimental animals and ethical considerations

Healthy young adult nulliparous female and fertility-proven male
rats (Rattus norvegicus) were collected from the Animal Breeding Unit of EPHI. Throughout the study period, rats were accommodated in the experimental animal room of the Traditional and Modern Medicine Research Directorate (TMMRD) of EPHI, which was kept at a temperature of 22–30 °C with a relative humidity of 50–60% and with a 12-hour light-dark cycle. The rats were kept in clean plastic cages (with wood shavings as bedding material) and supplied with conventional laboratory (pelleted) diets with unlimited drinking water. Before testing, all rats were acclimated to laboratory conditions for seven days.

The European Union guidelines Directive 2010/63/EU were used for the housing, care, and use of the experimental rats [40]. For euthanasia, the American Veterinary Medical Association (AVMA) guidelines were utilized [41]. At the end of the experiment, the carcasses of the sacrificed rats and unused fetal specimens were disposed of humanely based on the laboratory standards of the EPHI. The experimental protocols were approved by the Institutional Review Board of the College of Health Sciences, Addis Ababa University (no. AAUMF-010/18/ANAT), in compliance with the Organization for Economic Cooperation and Development (OECD) guideline test no. 414, for testing chemicals on prenatal developmental toxicity studies [35].

2.3. Experimental design

For mating, two female rats cohabitated with a single male rat per cage. Following mating, a vaginal smear was taken and examined for the presence of spermatozoa to confirm pregnancy. On the day on which a vaginal plug (sperm) was observed (the first positive finding) in the vaginal smear was recorded as day 0 of gestation (GD 0).

Thirty-two pregnant rats were randomly distributed into four groups (n = 8) and then randomly assigned to a control group and three test groups. The control group (G I) received distilled water. The test groups (G II, III, and IV) received the herbal tea of M. stenopetala and M. spicata leaves blend (at doses of 559.36, 1118.72, and 2237.44 mg/kg body weight, respectively) from day 5 to 19 of gestation. The herbal tea and vehicle (distilled water) were administered orally by intragastric tube at a dosing volume of 2 ml/100 g body weight [33]. The maternal and fetal toxicity parameters in all study groups were investigated. For reporting animal research, the ARRIVE guidelines were applied [42].

2.3.1. Maternal in-life observations and measurements

All pregnant rats were observed twice daily for mortality, moribundity, and all signs of toxicity, including abortion, premature delivery, vaginal bleeding, convulsions, and pertinent behavioral changes. The body weight of each animal was measured on day 0, on day 5 (the first day of dosing), and thereafter, at three-day intervals during the dosing period, and finally, on day 20 (the day of scheduled humane killing). Food consumption was measured (n = 2/cage) on days 0, 5, and thenceforward at a three-day interval during the treatment period.

2.3.2. Maternal biochemical evaluation

On day 20 of gestation, the pregnant rats were anesthetized by pentobarbital injection (150 mg/kg body weight), and then about 3–5cc of blood from each dam was collected by cardiac puncture and placed in a plane test tube. The blood sample was allowed to clot for one hour at room temperature, centrifuged for ten minutes using an electrical centrifuge at 3000 rpm, and then the serum was analyzed using an Automated Clinical Chemistry Analyzer (Huma Star 80, Germany) for assessing the values of alanine transaminase (ALT), alkaline phosphate (ALP), aspartate transaminase (AST), triglyceride (TG), triiodothyronine (T3), thyroxine (T4), thyroid stimulating hormone (TSH), creatinine, urea, uric acid, total cholesterol, and total protein.

2.3.3. Maternal post-mortem examination

Following blood sample collection, the dams were humanely sacrificed and inspected for macro-pathological abnormalities. The liver, kidney, ovary, placenta, and gravid uterus were removed, cleaned from any adherent tissues, and weighed.

The number of corpora lutea in both ovaries of each dam was counted under a dissecting microscope (XTL3101). The gravid uterus of each dam was dissected longitudinally, and then the chorician and amniotic sacs were opened to expose the fetuses. The corresponding placentae were cleaned from adherent tissues, inspected grossly, and weighed. The total number of live and dead fetuses, early and late resorptions, and total implantation sites were recorded. The live fetuses were weighed and sexed, and their crown-rump lengths were also measured. The fetuses were then examined for external craniofacial, trunk, genitalia, tail, and limb developmental abnormalities. Right after the external examination, the fetuses were examined for skeletal and visceral anomalies [33].

2.3.4. Fetal skeletal examination

For skeletal examination, approximately half of the fetuses per litter were fixed in 95% ethanol, macerated in 2% potassium hydroxide solution, bleached in pure acetone, and stained with 0.001% aqueous Alizarin red solution. Then, the stained specimens were cleared by an increasing concentration of glycerin-based solutions (20%, 50%, and 80%) and were preserved in 100% glycerin until examined. The specimens were examined by the experimenter blind to the control and treated group fetuses. The skull, hyoid, sternum, ribs, vertebrae, and limb (fore and hind) bones were examined for treatment-related malformations [43–46].

2.3.5. Fetal visceral evaluation

Nearly half of the fetuses were fixed in Bouin’s solution and serially sectioned for visceral examination. The updated Wilson technique [47] was used to accomplish serial sectioning in the head, neck, thoracic, abdominal, and pelvic areas. Soft tissue (visceral) anomalies were investigated in the head (for hydrocephaly, anophthalmia, and cleft palate), neck (for athymia and thyroid agenesis), thoracic (for tracheoesophageal fistula, atrial septal defect, and apulmonism), abdominal (for diaphragmatic hernia, asplenia, and agastria), and pelvic (for acystia, and anorchia) [48,49].

2.4. Statistical Analysis

The data gathered in this research were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 24 software (SPSS Inc., Chicago, IL, USA). The maternal body weight, food consumption, organ weight, serum biochemicals, fetal body weight, and length were analyzed using a one-way analysis of variance (ANOVA), followed by a Dunnett’s test to compare the differences between the test groups and the control group. The frequencies or percentages of malformations were analyzed through the Chi-square test followed by Fisher’s exact test. The results are expressed as M ± SD (mean ± standard deviation). P-value < 0.05 was considered statistically significant.

3. Results

3.1. Effects of the herbal tea on maternal in-life parameters

There were no herbal tea-related maternal deaths during the treatment period. All dams in the control and test groups survived to schedule terminal sacrifice. Throughout the gestation period, maternal clinical examinations did not reveal test item-related overt clinical signs in any of the treated group dams.

The body weight and food consumption data of the dams are shown in Table 1. Mean body weight between each weighting interval and overall mean weight gain for the treated groups were statistically comparable to those of the control group. All the test groups exhibited lower corrected mean body weight. The medium and higher dose groups had lower net body weight gain, while the low dose group dams had higher net body weight gain when compared with the control group.
The observed differences were minor and statistically insignificant for all treated groups (P > 0.05). Throughout gestation, food consumption showed a gradual increase for all study group dams (Table 1). However, no significant differences were observed in food consumption between the control and treated groups as a function of time (P > 0.05).

### 3.2. Effects of the herbal tea on maternal biochemicals and gross necropsy

Table 2 shows the results of biochemicals and weights of organs in pregnant rats on GD 20. In the test groups, serum ALT and AST increased, while AST, creatinine, and urea levels decreased non-significantly in a dose-dependent manner when compared with the control group. All the other parameters (i.e., T3, T4, TG, TSH, uric acid, total cholesterol, and total protein) showed non-significant and dose-dependent changes in comparison with the control (P > 0.05).

| Parameters | Experimental groups | P-value | Control | 559.36 | 1118.72 | 2237.44 |
|------------|---------------------|---------|---------|--------|---------|---------|
| Biochemicals | ALT (IU/L)          | –       | 65.80   | 66.47  | 67.90   | 70.34   |
|             | AST (IU/L)          | –       | 13.05   | 10.27  | 6.44    | 4.65    |
|             | T3 (µg/ml)          | –       | 0.66    | 0.89   | 0.93    | 0.12    |
|             | T4 (µg/dL)          | –       | 0.19    | 0.24   | 0.34    | 0.05    |
|             | Creatinine (mg/dL)  | –       | 0.35    | 0.34   | 0.33    | 0.32    |
|             | Urea (mg/dL)        | –       | 0.05    | 0.03   | 0.03    | 0.04    |
|             | Uric acid (mg/L)    | –       | 1.70    | 5.37   | 2.70    | 0.82    |
|             | Total cholesterol (mg/dL) | – | 79.05 | 81.01 | 79.84 | 81.84 |
|             | Total protein (mg/dL) | – | 4.47 | 4.42 | 4.45 | 4.46 |
|             | Liver (g)           | –       | 0.22    | 0.19   | 0.23    | 0.29    |
|             | Kidney (g)          | –       | 10.98   | 10.41  | 10.69   | 10.95   |
|             | Ovary (g)           | –       | 0.11    | 0.10   | 0.11    | 0.11    |
|             | Placenta (g)        | –       | 0.02    | 0.01   | 0.01    | 0.02    |
|             | Thyroid gland (mg)  | –       | 0.08    | 0.07   | 0.08    | 0.07    |
|             | Gravid uterus (g)   | –       | 55.75   | 55.38  | 55.63   | 55.63   |

Values are presented as mean ± standard deviation (M ± SD); one-way ANOVA followed by a Dennett’s test (n = 8).

Table 2 shows the results of biochemicals and weights of organs in pregnant rats on GD 20. In the test groups, serum ALT and AST increased, while AST, creatinine, and urea levels decreased non-significantly in a dose-dependent manner when compared with the control group. All the other parameters (i.e., T3, T4, TG, TSH, uric acid, total cholesterol, and total protein) showed non-significant and dose-dependent changes in comparison with the control (P > 0.05). No herbal tea-induced macro-pathological changes or lesions on the external morphology, body cavities, and major viscera (e.g., liver, kidney, thyroid gland, ovary, gravid uterus, and placenta) of any of the test group dams. Moreover, the absolute weights of the liver, kidney, thyroid gland, ovary, gravid uterus, and placenta in the treated group dams were statistically comparable with the values in the control group dams (P > 0.05).

### 3.3. Effects of the herbal tea on maternal reproductive parameters

Table 3 summarizes the cesarean section data. There was no abortion or premature delivery in any of the study groups. The number of litters with viable fetuses in the test groups was comparable to that of the control group. The average number of corpora lutea, implantations, late resorption, and total resorptions decreased insignificantly in a non-dose-dependent manner as compared with the control group.

The mean number of early resorptions and dead fetuses (Fig. 1a & b) as well as pre-and post-implantation losses were slightly higher in dams at 2237.44 mg/kg when compared with the control group dams. In addition, the indices of pre-and post-implantation losses (%) increased as well as pre-and post-implantation losses were slightly higher in dams in a dose-dependent manner as compared with the control group. The mean body weight and crown-rump length (CRL) of viable (male/female) fetuses showed a slight increase in the low and medium dose groups, while they exhibited an insignificant decrease in the high dosage group as compared with their values in the control group. There was no significant difference in the sex ratio (female/male) between the herbal tea-treated and vehicle-treated groups.
The external morphological examinations on the craniofacial, thoracic, abdominal, limbs, vertebral column, external genital, and tail revealed no herbal tea-related external morphological anomalies or malformations in any of the treated group fetuses (Table 4 and Fig. 2a & b). The soft tissue examinations were carried out in the viscera of the head, neck, thoracic, abdominal, and pelvic regions. No remarkable visceral anomalies or malformations were observed in the test group fetuses when compared with the viscera of the control group fetuses (Table 4 and Fig. 2c-g).

Furthermore, no herbal tea-associated skeletal malformations were seen on the skull, hyoid, vertebrae, ribs, pectoral girdle, pelvic girdle, or fore-and hind-limbs of any test group. The caudal vertebrae, sternabrae, metacarpus, metatarsus, fore-paw, and hind-paw phalanges demonstrated delayed or reduced ossifications in all study groups, including the control group. There was no significant difference in the number of fetuses (litters) with delayed ossification between the control and test groups.

Moreover, the incidence (percentage) of retarded ossification in the test group was statistically comparable with the control group, as summarized in Table 4, and as shown in Fig. 3.

4. Discussion

In Ethiopia, Moringa stenopetala and Mentha spicata have been used for many years for their potential medicinal and nutritional benefits. The leaves of these individual plants are widely utilized in the traditional system of medicine to treat various ailments, including diabetes, hypertension, stomach problems, and respiratory disorders [5,6]. Herbal formulations made from the leaves of M. stenopetala and M. spicata showed better antidiabetic and antihypertensive effects in rodent models. These herbs’ leaf formulations were non- or low-toxic to rodents after short and long-term oral administration [30–32]. Nevertheless, the prenatal developmental toxicity potential of herbal (leaves) formulation of these two medicinal plants has not been investigated yet. Therefore, the present study investigated the prenatal developmental toxicity of the herbal tea of M. stenopetala and M. spicata leaves formulations (at doses of 559.36, 1118.72, and 2237.44 mg/kg) in Wistar albino rats.

Death, clinical signs, decreased food or water consumption, loss of body weight, decreased uterus-corrected body weight, decreased absolute organ weight, and macroscopic and histopathological lesions are all indicators of maternal toxicity to a test substance [50,51]. In the present study, oral administration of herbal tea during gestation did not result in mortality or signs of morbidity (abortion, premature delivery, vaginal bleeding, prolonged diarrhoea, convulsions) in the pregnant rats treated up to the maximum dose of 2237.44 mg/kg body weight. Throughout gestation, body weight and food consumption exhibited progressive changes for all study group dams. The body weight and food consumption measurements between each weighting interval revealed no significant variations between the test and control groups. Furthermore, the terminal (overall) and net body weight gains, as well as the corrected body weight findings, showed no herbal tea-related significant changes when compared with the vehicle (control) group. These results indicate that the administration of herbal tea could not induce in-life adverse effects in pregnant rats. The present maternal in-life findings are in agreement with earlier toxicity studies of extracts of Mentha piperita leaves [50], Salacia lehmbachi root [52], and Achyranthes aspera leaves [39], which asserted that the prenatal oral administration of different doses of the extracts did not produce in-life adverse effects (i.e., mortality, morbidity, loss of body weight, or loss of food intake) in pregnant rats.

In the current study, no macro-pathological lesions were observed in the external and internal viscera of dams treated with different doses of herbal tea infusions. The mean absolute weights of the gravid uterus, placenta, ovary, thyroid gland, liver, and kidney in the treated groups were statistically comparable with the control group values. In addition, maternal serum biochemistry analyses did not reveal significant alterations in the biochemical parameters of the treated group dams.

Table 3

Caesarean section data by dosage group.

| Parameters                          | Control          | 559.36 mg/kg | 1118.72 mg/kg | 2237.4 mg/kg | p value |
|-------------------------------------|------------------|--------------|---------------|--------------|---------|
| Pregnant rats (n)                   | 8                | 8            | 8             | 8            |         |
| Premature delivery (n)              | 0                | 0            | 0             | 0            |         |
| Abortion (n)                        | 0                | 0            | 0             | 0            |         |
| Litter size/group (n)               | 8                | 8            | 8             | 8            |         |
| Live fetuses/group (n)              | 79               | 78           | 74            | 72           |         |
| Male & female live group (n)        | 46 & 33          | 43 & 35      | 45 & 39       | 40 & 32      |         |
| Sex ratio (male:female)             | 1.07 ± 1.08      | 1.07 ± 1.08  | 1.07 ± 1.08   | 1.07 ± 1.08  |         |
| No. of corpora                       | 12.38 ± 12.13    | 11.88 ± 12.00| 10.38 ± 10.50 | 5.18         |         |
| lutea                               | 1.69 ± 1.04      | 1.25 ± 1.20  |               |              |         |
| No. of implantations                | 1.83 ± 0.76      | 1.06 ± 1.41  |               |              |         |
| No. of early resorptions             | 0.88 ± 0.75      | 0.88 ± 1.00  | 0.88 ± 0.85   |              |         |
| No. of late resorptions              | 0.50 ± 0.50      | 0.25 ± 0.28  | 0.38 ± 0.25   |              |         |
| No. of total resorptions             | 1.38 ± 1.25      | 1.26 ± 1.25  | 1.25 ± 0.98   |              |         |
| No. of late implantations loss       | 0.52 ± 0.46      | 0.71 ± 1.04  |              |              |         |
| No. of pre-implantation loss         | 1.13 ± 1.13      | 1.50 ± 1.50  | 1.50 ± 0.52   |              |         |
| No. of post-implantation loss        | 0.35 ± 0.64      | 0.76 ± 1.00  |              |              |         |
| Post-implantation loss (%)           | 9.35 ± 9.23      | 12.37 ± 12.57| 15.19 ± 6.39  | 0.639        |         |
| No. of dead fetus                   | 0.00 ± 0.00      | 0.25 ± 0.40  |              |              |         |
| No. of viable fetuses               | 9.88 ± 9.75      | 9.13 ± 8.88  | 0.25 ± 0.25   |              |         |
| Male live fetuses                   | 1.55 ± 0.89      | 0.64 ± 1.25  |              |              |         |
| Female live fetuses                 | 5.75 ± 5.38      | 5.50 ± 0.73  |              |              |         |
| Male live fetuses (females)         | 0.89 ± 1.69      | 1.31 ± 1.41  |              |              |         |
| No. of dead fetuses                 | 4.13 ± 4.38      | 3.36 ± 0.88  | 0.729         |              |         |
| Weight of male fetuses (g)          | 1.25 ± 1.41      | 1.51 ± 1.36  |              |              |         |
| Weight of female fetuses (g)        | 4.79 ± 4.99      | 5.26 ± 4.69  | 0.682         |              |         |
| Weight of female fetuses (cm)       | 0.74 ± 0.85      | 1.15 ± 1.15  |              |              |         |
| Weight of female fetuses (cm)       | 4.36 ± 4.61      | 4.59 ± 3.92  | 0.507         |              |         |
| CRL of male (g)                     | 1.41 ± 0.83      | 0.36 ± 0.17  |              |              |         |
| CRL of female (cm)                  | 0.42 ± 0.26      | 0.15 ± 0.46  |              |              |         |
| CRL of male fetuses                 | 5.75 ± 5.96      | 6.00 ± 0.54  | 0.737         |              |         |
| CRL of female (cm)                  | 0.58 ± 0.31      | 0.11 ± 0.25  |              |              |         |
| CRL of male + female (cm)           | 5.90 ± 6.03      | 6.17 ± 0.74  | 0.163         |              |         |

Values are presented as mean ± standard deviation (M ± SD); one-way ANOVA followed by a Dennett’s post hoc test (n = 8).

a CRL = Crown Rump Length.

received groups.

The average number of male fetuses in the test groups was slightly lower than in the control group. The mean number of female fetuses displayed a slight increase in the low dose group, while it presented a minor decrease in the medium and high dose groups in comparison with the vehicle group. Furthermore, the mean body weight and CRL of male fetuses, as well as female fetuses in the test groups, were statistically comparable with the values of the respective control groups, as summarized in Table 3.

3.4. Effects of the herbal tea on fetal growth and development

The external morphological examinations on the craniofacial,
These maternal systemic toxicity findings indicate that the prenatal oral administration of herbal tea of *M. stenopetala* and *M. spicata* leaves may not induce significant effects on the serum biochemistry and gross necropsy of pregnant rats at the studied dose levels. Similar results were observed with previous prenatal developmental toxicity studies of extracts of *Orthosiphon stamineus* leaves [53], *Parkia platycapha* leaves [54], and *Salacia lehmbachii* root [52], which revealed that oral administration of the extracts at different doses during the days 5–19 of gestation produced no significant systemic toxicity effects in rats.

The number of corpora lutea, implantation sites, early/late resorptions, live/dead fetuses, sex ratio, pre/post-implantation loss, and gravid uterus weight are viewed collectively as maternal reproductive...
Fig. 2. Photographs showing the fetal external and visceral developmental findings (Bouin’s fixation). (A) Normal external anatomy of the fetuses from control group. (B) A normal external anatomy of the fetuses from high dose group. (C) Normal palate at high dose group (white arrow). (D & E) Sections through the brain displaying normal ventricles, cerebrum, and eyeballs from high dose group. (F) Section through the neck viewing thyroid (Thy), trachea (Tr) and esophagus (Eso). (G) Section through the chest demonstrating normal pericardium (Pr), blood vessels (Bv) and Lungs (L) from high dose group. (H & I) Section of through the abdomen showing normal digestive and genitourinary viscera at high dose group.
parameters and are used for evaluating the toxic effects of a test compound on pregnancy [51,55]. In this prenatal toxicity study, the maternal reproductive indices (corpora lutea, implantation sites, early/late/total resorptions, pre-and post-implantation loss, sex ratio, live and dead fetuses) showed no statistically significant differences between the control and treated groups. Furthermore, all study groups’ maternal reproductive data were within the historical control reference ranges for rats [56,57]. These maternal reproductive findings were consistent with earlier prenatal developmental toxicity studies conducted with *Bulbine natalensis* stem extract in rats [58], *Mentha piperita* leaf extract in mice [50], *Salacia lehmbackii* root extract in rats [52], and *Ginkgo biloba* herbal extract in rats [59].

Significant changes in the fetal toxicity indices (body weight, crown-rump length (CRL), and placental weight) are indicative of a test compound’s potential toxicity [60]. In this study, results of the fetal body weight, CRL, and placenta weight in the test groups were comparable with those of the control group fetuses, suggesting that the herbal tea of *M. stenopetala* and *M. spicata* leaves blend may not retard prenatal

Fig. 3. Photographs showing different ossification centers of rat fetuses at high dosage group. (a) Supra-occipital (So), external occipital (Eo), first cervical vertebrae (CV), ribs (Rs), lumbar vertebrae (Lv), sacral vertebrae (Sv) and caudal vertebrae (Cv). (b) Vertebral column with thoracic vertebrae (Tv) and lumbar vertebrae (Lv). (c) Hyoid (Hy) bone and sternum (St) with six sternebrae. (d) Forelimb phalanges (Flp), metacarpus (Mc), hindlimb phalanges (Hlp), and metatarsal (Mt) bones. (e) Hindlimb phalanges (Hlp) and metatarsal (Mt) bones. (f) Forelimb phalanges (Flp), metacarpus (Mc), radius (Ra), humerus (Hu), and scapula (Sc) (Alizarin red stained).
growth of the exposed fetuses at the studied dose levels.

Furthermore, the herbal tea exposed group fetuses did not exhibit test article-induced soft tissue and external morphological anomalies in comparison with the vehicle-received group fetuses. In the skeletal examination, a few fetuses in all study groups, including the control group, displayed delayed/reduced ossification centers in their caudal vertebrae, sternum, metacarpus, metatarsus, and fore- and hind paw phalanges.

These skeletal findings could not indicate disrupted development due to treatment with herbal tea by *M. stenopetala* and *M. spicata* leaves blend, as they were observed both in the control and test group fetuses, and they did not show dose-dependent changes in the frequency of occurrence. The changes in the caudal vertebrae, sternum, metacarpus, metatarsus, and fore- and hind paw phalanges may indicate a delayed schedule of events and could be interpreted as a transient delay, as these bones show ossifications mostly late in gestation or shortly after birth [43, 46, 61].

5. Conclusion

The study findings demonstrated that prenatal oral administration of the herbal tea of *M. stenopetala* and *M. spicata* leaves blend produced no significant toxic effects on maternal systemic and reproductive parameters as well as on the fetal developmental toxicity indices at the tested dose levels. Based on the study results, the herbal tea formulation could be safe or low toxic to pregnant rats and developing fetuses, in doses up to 2234.44 mg/kg. The no-observed-adverse-effect level (NOAEL) for prenatal developmental toxicity of the herbal tea of *M. stenopetala* and *M. spicata* leaves blend is 2237.44 mg/kg/day body weight. The results of this study could provide relevant toxicological information on the prenatal developmental toxicity potential of the herbal tea of *M. stenopetala* and *M. spicata* leaf formulations. The analysis reported in this research may help to predict safe exposure levels for humans, thereby ensuring public safety. The data obtained from this study could also be used as baseline data for future preclinical and clinical investigations.

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CRediT authorship contribution statement

Abdu Hassen Musa: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, and Writing-original draft. Asfaw Debella: Conceptualization, Methodology, Resources, Supervision, Validation, Writing-review and editing. Girmai Gebru, Eyasu Makonnen, Mesfin Asefa: Supervision, Methodology, Resources, Validation, Writing-review and editing. Samuel Woldekidan, Abiy Abebe, Boki Lengiso and Chala Basheha: Investigation, Methodology, Validation, Writing-review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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