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Embryotoxicity Test of the Anti-anemia Drug from *Alternanthera sessilis*

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Alternanthera sessilis **is a plant native to wetlands that is commonly known in the Philippines as "bunga-bunga" (Tagalog). Currently,** *A. sessilis* **is available as a natural product that can combat anemia in the Philippines. Anemia, together with diabetes, is one of the complications experienced during pregnancy. This study aimed to identify the safety of** *A. sessilis* **as a supplement for pregnant mothers by testing its embryotoxic and possible teratogenic effects in pregnant mice. The experimental design consisted of supplementation of 7.3 mg/mL (low) and 73 mg/mL (high)** *A. sessilis* **to pregnant dams compared with 10,000 IU/kg of isotretinoin (positive control), soybean oil (vehicle control), and distilled water (negative control group). Reproductive parameters, histological parameters, and gene expression pattern changes were observed in the embryos of pregnant dams. Dams and embryos in the isotretinoin group had reduced reproductive performance and induced teratogenicity. Administration of a low dose (7.3 mg/mL) of** *A. sessilis* **capsule showed no significant changes in the reproductive performance of dams and embryo development (E11.5). Although not significant, a higher dose (73 mg/mL) of** *A. sessilis* **resulted in a slight reduction in reproductive performance and embryo growth when compared with the negative control group. According to the immunohistochemistry results, the high-dose group of** *A. sessilis* **had a significant increase in the expression of BCL-2 anti-apoptotic protein in the embryonic liver (E11.5) when compared with the negative control group and the isotretinoin group. The findings in this study suggest that the use of** *A. sessilis* **as a supplement at a low dose can be beneficial during pregnancy.**

Keywords: *Alternanthera sessilis,* BCL-2, immunohistochemistry, isotretinoin

INTRODUCTION

A variety of complications may arise during pregnancy – including anemia, gestational diabetes, and hypertension – which will require pharmacological therapy. The use of herbal medicinal products, which are typically marketed as dietary supplements, is rapidly growing. A common reason why pregnant women use herbal medicinal plants is the belief that natural products are safer than conventional medicine. However, there is a lack of understanding about the potential risks to both the mother and the fetus. *Gingko biloba*, for example, is a herbal supplement with antioxidant and free radical scavenging bioactive properties. Gingko biloba has been shown to cause malformations in the gross morphology of embryos *in vivo*. As a result, *Gingko biloba* may have a teratogenic effect when given to pregnant mothers (Zehra *et al.* 2010). Teratogenic substances negatively impact normal development. Teratogens employ different mechanisms of action through multiple signaling pathways. Understanding the processes of teratogenicity provides opportunities for

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therapeutic approaches that may protect pregnant mothers from teratogenic chemicals (Ipulan-Colet 2019).

To find an alternative medicine to alleviate pregnancy complications, it is necessary to investigate the potential teratogenic effects of plants with proven medicinal use. *Alternanthera sessilis* is another natural product with numerous bioactive properties. It is a plant native to wetlands and is commonly known as "bunga-bunga" in the Philippines (Tagalog). Several studies have examined and supported the claims associated with its traditional use. It has anti-microbial, antioxidant (Kota *et al.* 2017), anti-diabetic, anti-inflammatory, wound-healing, nootropic, and hepatoprotective properties (Shehzad *et al.* 2018). Previous research has indicated that it improves hemoglobin and serum ferritin levels in iron deficiency anemia (Arollado and Osi 2010). However, no studies have examined the possible teratogenic effects of *A. sessilis.*

Currently, *A. sessilis* is available as a natural product that can combat anemia in the Philippines (Arollado and Osi 2010). Anemia, together with diabetes, is one of the complications experienced during pregnancy (Arollado and Osi 2010; Tan and Kim 2013). Based on previous studies, *A. sessilis* may have therapeutic effects on anemia and diabetes. The purpose of this research is to determine the safety of *A. sessilis* as an alternative treatment for pregnant women by evaluating the embryotoxic effects on pregnant mice. Gestational parameters, histological parameters, and gene expression pattern changes were observed in the embryos of pregnant dams treated with *A. sessilis* for 3 d at the mid-gestation stage.

Animal Handling and Housing

The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of the Philippines Diliman with IACUC approval reference number AP-2020-10-R. Male and female ICR mice, 3–6 mo of age, weighing 28–35 g, were obtained from the Research Institute for Tropical Medicine. Mice were transferred to polycarbonate cages with stainless steel tops (315 x 232 x 250 mm) and maintained for 1 wk. Female mice were maintained in holding cages with a maximum of five females per cage. Male breeding mice are housed separately. Food and water were given *ad libitum* at room temperature (23–26 °C), humidity (40–60%), and 12 h light/dark in the animal house of the Institute of Biology. After 1 wk of acclimation, the female mice were examined for signs of estrus. The reddish and swollen vulva is an indicator of the beginning of estrus (Ajayi and Akhigbe 2020). Female mice that were confirmed to be in estrus were subjected to mating, in which the female mice were transferred to a male breeder cage between 05:00–08:00 PM with a ratio of one male to two female ratio. The next day, at 09:00 AM, the vaginal plug was checked, which indicated the success of the mating procedure. The appearance of the plug was regarded as Day 0.5 of pregnancy. Pregnant mice were transferred and caged separately and allocated into five groups.

Treatment with *A. Sessilis*

Alternanthera sessilis was in capsule form, non-heme iron (anti-anemic). The capsules contain 450 mg of the active ingredient, which has been ground, dispersed, and completely dissolved in distilled water at room temperature. Healthy pregnant mice were divided into five groups (N = 5). The treatment groups include a positive control treated with teratogenic dose (10,000 IU/kg) of vitamin A (isotretinoin, ACNETREX 10 mg, Thailand), a negative control group treated with distilled water, a vehicle control group treated with soybean oil (Jolly Puregoodness, Soya Oil, Philippines), and *A. sessilis*-treated groups that received increasing doses of the plant extract (7.3 and 73 mg/mL, respectively).

The dosage was determined using the animal-estimated dose equation below (Nair and Jacob 2016):

$$\text{Animal estimated dose} = \text{Human dose} \div \frac{\text{Human km}}{\text{Animal km}} \quad (1)$$

One capsule contains 450 mg of *A. sessilis*, based on the calculation of animal estimated dose, a low dose (7.3 mg/mL) corresponds to 45 mg of the capsule, and a high dose (73 mg/mL) corresponds to 450 mg of the capsule. The pregnant mice were treated for 3 d (E8.5, E9.5, and E.10.5) *via* oral gavage, and embryos were harvested at E11.5.

Embryo Collection and Fetal Assessment

Pregnant mice were sacrificed through cervical dislocation at E11.5. The embryos were harvested to determine the weight and litter number, as well as to check for morphological defects. The fetal assessment was done to investigate the effect of the treatments on the embryonic development of mice. The number of live and dead fetuses, as well as the presence of resorptions, was recorded. External malformations of the head (shape), forelimbs, hind limbs, and digits of the fetus were observed (Herrera and San Diego 2009). Nikon Imaging Software was used to assess the crown-to-rump length, anterior-posterior diameter (APD), brain region area (forebrain, midbrain, and hindbrain), hind limb, and forelimb of embryos.

Histological and Immunostaining Analysis

Mice embryos were collected from each treatment group and were preserved in 4% PFA (paraformaldehyde 95%, powder, Sigma-Aldrich, USA) in PBS (PBS tablets, Biomatik Corporation USA) for at least 3 d. Tissue

processing of the embryos was performed at the Hi-Precision Diagnostic Center. The tissue was processed using xylene and paraffin and embedded in a sagittal position. Each paraffin block was sectioned at 0.5 μm, and slides were stained with H&E for histological assessment (Cardiff *et al.* 2014). For the qualitative, quantitative, and semi-qualitative assessment of histological slides, three randomly selected representative embryos per treatment replicate were used for histological and histomolecular analysis. Each organ tissue (neural, cardiac, and liver) was assessed with a total of three fields of view per sample (Herrera and San Diego 2009). Histological slides for the whole embryo were observed and imaged using Moticam ProS5 Lite 3.0 software. Specific structures from the embryo such as the neuroepithelium of the telencephalon, heart, and liver were analyzed for both quantitative and qualitative analysis. The qualitative analysis was based on the study of Chen *et al.* (2017), Savolainen *et al.* (2009), and Crawford *et al.* (2010), which described the organ development of mice at different stages of gestation. For quantitative analysis, the average cell count (cell count/area of structure in a field of view) was determined using ImageJ analysis software.

Immunohistochemical staining was performed at the HP Diagnostic Center using the B-cell lymphoma 2 (BCL-2) primary antibody. Immunohistochemical analysis followed a semiquantitative scoring where the staining intensity was scored as 1 (weak), 2 (medium), and 3 (strong). The quick score (Q-score) was used to determine the intensity and proportion of biomarkers of interest (Charafe-Jauffret *et al.* 2004; Hollenbach *et al.* 2021). The scoring was performed by two independent investigators.

Statistical Analysis

The obtained results were expressed in mean ± SEM. The normality of the data was analyzed using the Shapiro-Wilk test, and the homogeneity of equal variances was analyzed using Levene's test. One-way analysis of variance (ANOVA) was used for statistical comparisons, followed by Tukey's pairwise *post hoc* comparison test. Statistical analysis was performed using R-4.2.1 for Windows. Significance among means was determined at p-value ≤ 0.05.

RESULTS

The result of reproductive performance is presented in Figure 1A. The gestation index is expressed as the percentage of live implants out of the total number of implants. The gestation index was significantly lower ($p = 0.007$) in the isotretinoin (10,000 IU/kg) treated group than that of the distilled water control and *A. sessilis* low dose group (7.3 mg/mL). The gestational indices of *A. sessilis* high dose (73 mg/mL) and soybean control groups were slightly lower than that of the negative control, but these differences were not statistically significant.

Embryonic weight was significantly reduced ($p = 0.04$) in the isotretinoin-treated group (Figure 2A). In contrast, average embryonic weight was not significantly different between negative control and low-dose treated groups. However, there was a minimal reduction in the average weight of embryos both in the high dose and soybean oil group, albeit not statistically significant.

Morphometric measurements are presented in Figure 3A, there is a significant reduction ($p = 0.02$) in the crown-rump length of embryos in the isotretinoin group than that of the distilled water and low dose group. There is also a minimal reduction in the crown-rump length of embryos under the high dose and soybean oil group but is not significantly different from the negative control group. Furthermore, only the isotretinoin-treated group was significantly reduced ($p = 0.001$) in terms of brain region area. There were no significant differences in the mean APD, hindlimb length, and forelimb length among all groups.

Embryos were evaluated qualitatively for head, heart, and liver abnormalities based on histological appearance (Figure 4). When compared with the positive and negative control, there were no morphological deformities in the brain, heart, or liver in the low- and high-dose groups of *A. sessilis.*

Cell count data were derived from the number of visible cells divided by the area of the tissue seen in the field of view (Figure 5A). The isotretinoin group exhibited a significant increase ($p < 0.001$) in the cell count of the neuroepithelium of the ventral telencephalon compared to the other treatment groups. Also, a significant reduction in Cell count was observed in the heart trabeculae ($p = 0.028$) and liver ($p = 0.021$) of the isotretinoin-treated group. Moreover, there were no significant differences in the cell count of the neuroepithelium of the ventral telencephalon, heart trabeculae, and liver of embryos treated with low and high doses of *A. sessilis* when compared to the negative control group.

The expression of BCL-2 was assessed semi-quantitatively based on the Q-score. Although not statistically significant, an increase was observed in the expression of BCL-2 in the neuroepithelium of the telencephalon, mesenchyme cells surrounding the vertebrae, and liver in both low and high doses of *A. sessilis*. However, only the liver cells exhibited a significant increase in the expression of BCL-2 ($p = 0.004$). As expected, the expression of BCL-2 in the positive control group ($p < 0.05$) decreased except for the heart trabeculae, compared with the negative control.

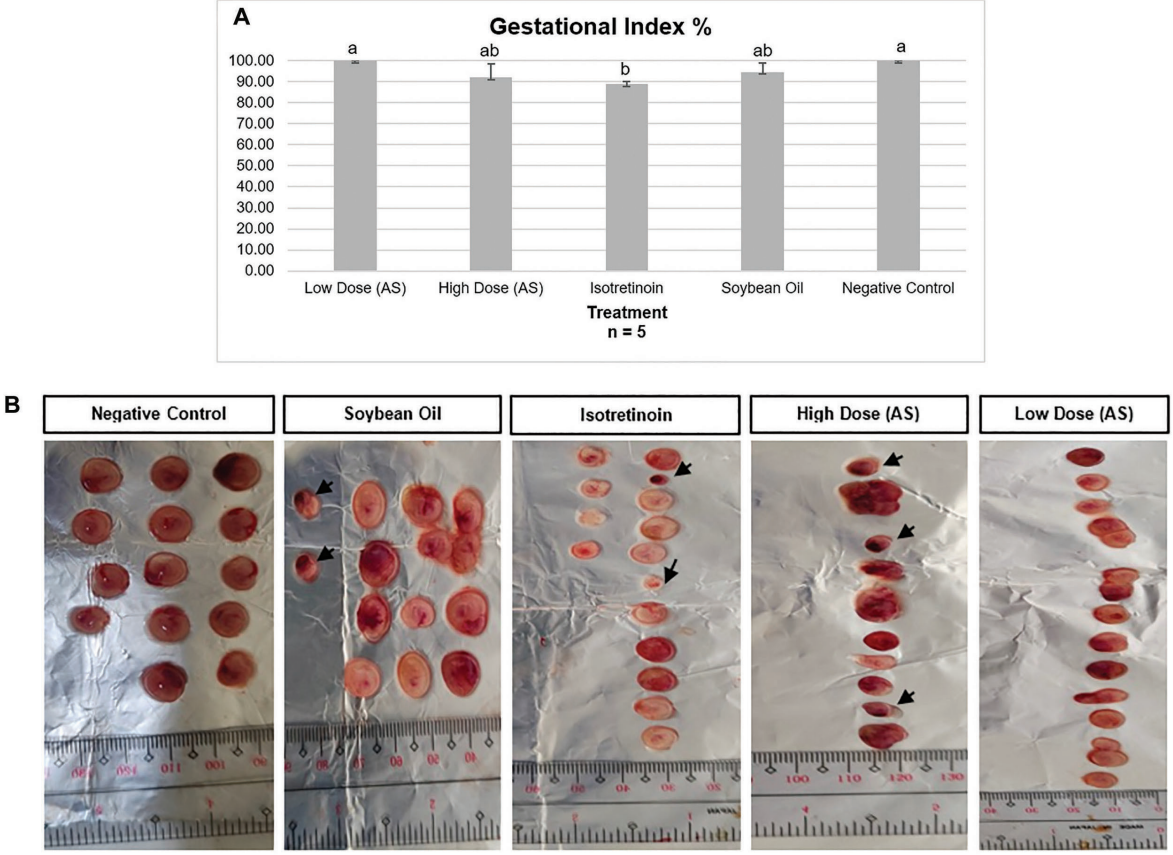
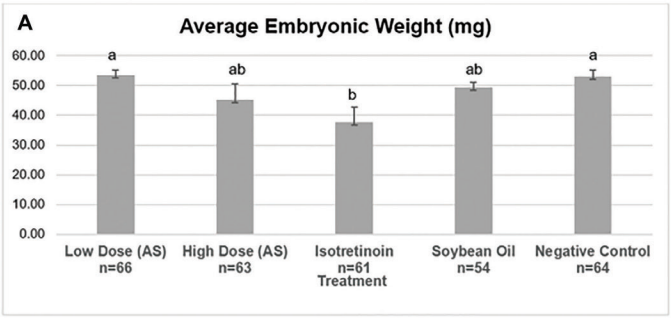


Figure 1. [A] Reproductive performance of dams per treatment group; [B] representative photos of harvested embryos. Arrows represent embryo resorption.



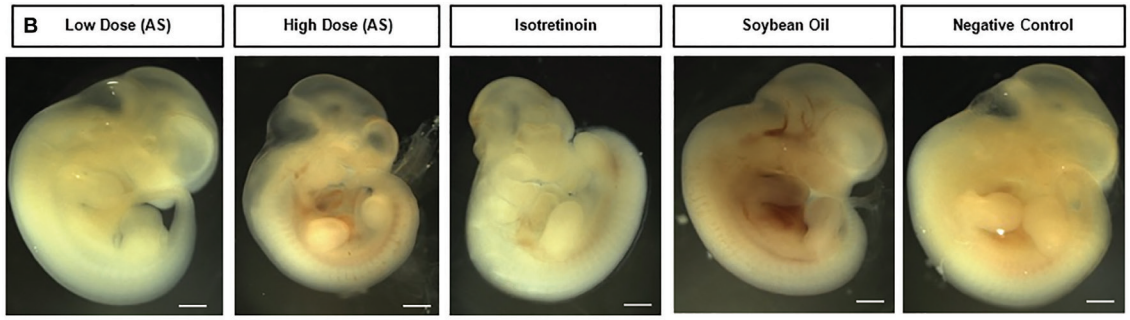


Figure 2. [A] Average weight of harvested embryos among treatment groups; [B] representative photomicrographs (15X) of embryos per treatment group. Scale bar: 0.483 mm. *Values are means of N = 5 replicates per treatment, where n = the number of collected embryos per treatment.

A

	Treatment groups				
	Low dose (AS) n = 66	High dose (AS) n = 63	Isotretinoin (PC) n = 61	Soybean oil (VC) n = 54	Distilled water (NC) n = 64
Crown-rump length, mm	5.56[a]	4.84[ab]	4.63[b]	5.23[ab]	5.40[a]
Brain region area, mm^2	0.79[a]	0.77[a]	0.63[b]	0.81[a]	0.82[a]
Anteroposterior diameter (APD), mm	0.44	0.45	0.44	0.45	0.45
Hindlimb length, mm	0.23	0.21	0.21	024	0.24
Forelimb length, mm	0.22	0.23	0.22	0.23	0.22

[1]Values are means of N = 5 replicates per treatment, where n = the number of collected embryos per treatment
[2]Treatments were [1] low dose *A. sessilis* at 7.3 mg/ mL, [2] high dose *A. sessilis* at 73 mg/mL, [3] positive control at 10,000 IU (PC, isotretinoin, ACNETREX 10 mg, Thailand), [4] soybean oil as vehicle control (VC, Jolly Puregoodness, Soya Oil, Philippines), and [5] distilled water as negative control (NC).
[a–b] Means within a row lacking a common superscript letter have significant differences following ANOVA ($p < 0.05$); Tukey's was used as *post hoc* method for comparison of means

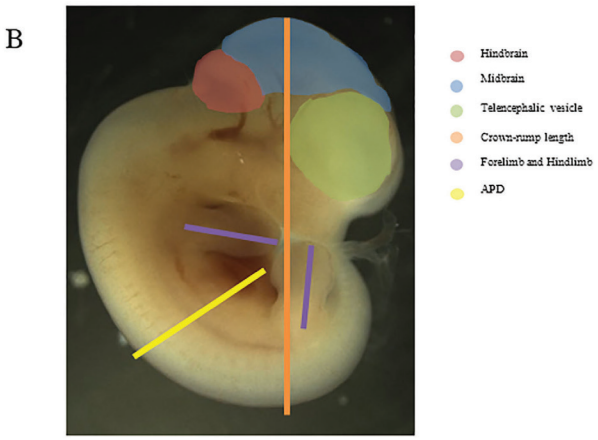
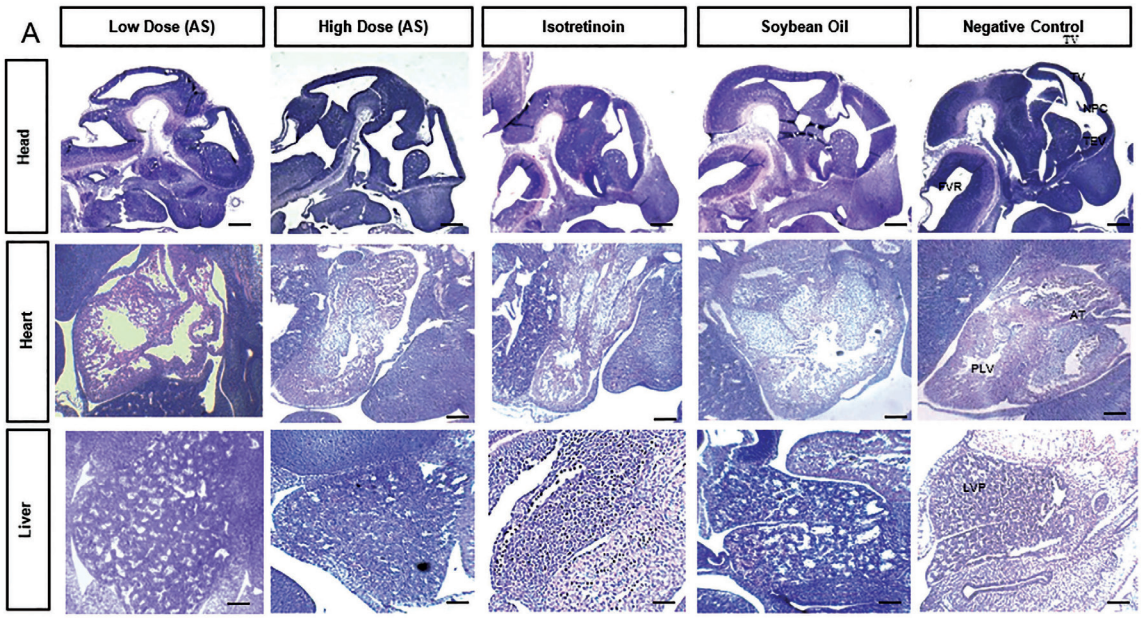


Figure 3. [A] Morphometric measurements of embryos among treatment groups; [B] photo representation of an embryo at E11.5 depicting the known measurement for the following structures (Molnár and Price 2016).



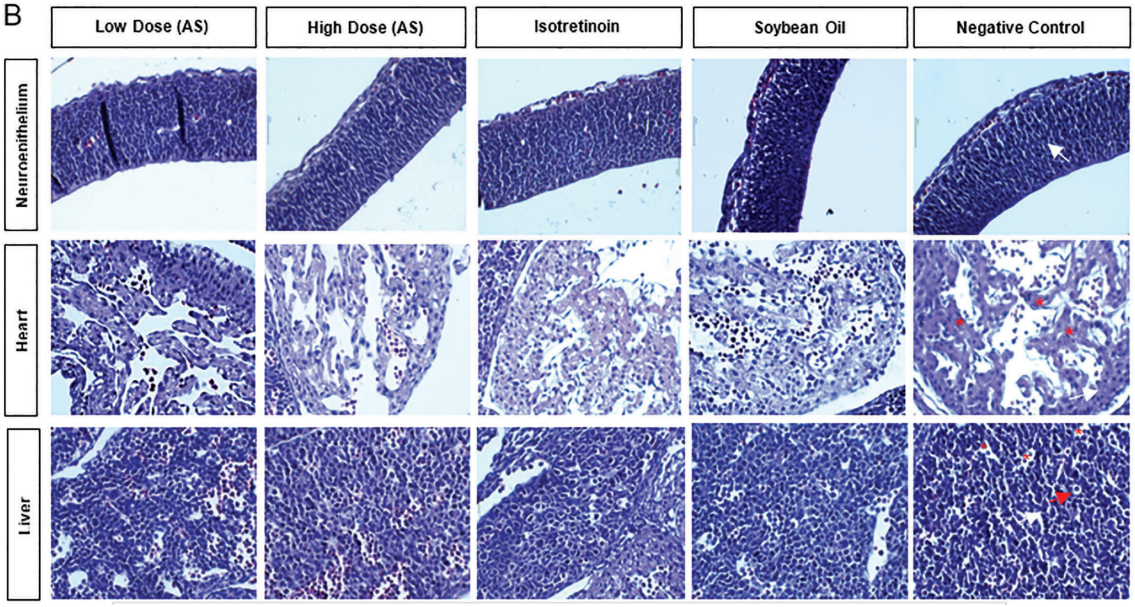


Figure 4. Representative photomicrographs (100X) in a sagittal section of whole E11.5 embryos with H&E stain. Head: [TV] third ventricle; [NPC] neopallial cortex; [TEV] telencephalic vesicle; [PG] pituitary gland; [FVR] fourth ventricle roof (Chen *et al.* 2017). Heart: [PLV] primitive left ventricle; [AT] atrium (Savolainen *et al.* 2009). Liver: [LVP] liver parenchyma. Scale bar: 100 µm. *Values are means of N = 3 randomly selected embryos per treatment (n = 3), where n is the number of fields of view observed per sample.

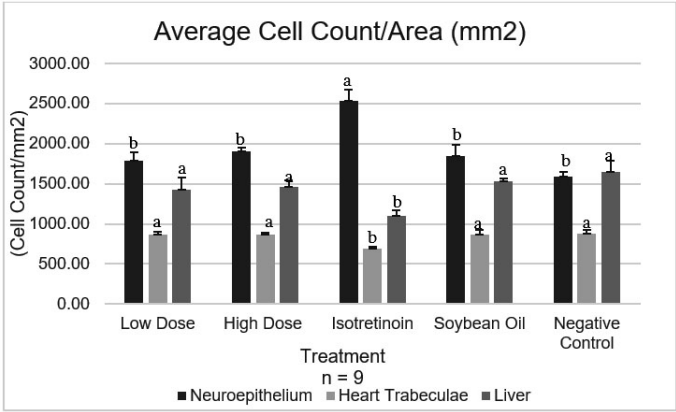


Figure 5. [A] Average of cell count in neuroepithelium, heart trabeculae, and liver among treatment groups. [B] Representative photomicrographs (400X) sagittal sections of neuroepithelium, heart, and liver of E11.5 embryos with H&E stain. Neuroepithelium: [white arrow] ependymal layer. Heart: [asterisk] heart trabeculae; [white arrow] ventricular wall. Liver: [asterisk] hepatic sinusoids; [red arrow] hepatic cords; [white arrow] endothelial cells (Crawford *et al.* 2010) Scale bar: 100 µm. *Values are means of N = 3 randomly selected embryos per treatment (n = 3), where n is the number of fields of view observed per sample.

DISCUSSION

A. sessilis has been shown to have anti-anemic and anti-diabetic properties, which will be beneficial if used as a supplement during pregnancy. This study administered *A. sessilis* to pregnant mice to investigate its potential teratogenicity. Treatments were administered during the gestational period from E8.5–E10.5, and embryos were collected at E11.5. This is because the initial stage of organ development occurs during this period (Chen *et al.* 2017; Savolainen *et al.* 2009; Crawford *et al.* 2010). At E8.5, the brain and heart develop *in utero*, with the heart being the most prominent organ. Srinivasan *et al.* 1998; Turnbull 1999). The neural tube separates into three distinct regions – namely, the forebrain, midbrain, and hindbrain – at E9.5 (Turnbull 1999; Chen *et al.* 2017). In E10.5, both the forelimb and hindlimb buds become apparent (Kaufman 1995). In addition, the liver primordium may be seen lying posterior to the heart, where it expands for the first time at E10.5 (Srinivasan *et al.* 1998; Crawford *et al.* 2010). The

A

	Treatment groups				
	Low dose (AS)	High dose (AS)	Isotretinoin (PC)	Soybean oil (VC)	Distilled water (NC)
Brain (neuroepithelium of ventral telencephalon)					
Percentage of positive cell count, %	70.00[a]	85.56[a]	36.67[b]	50.00[ab]	81.00[a]
Q-score	178.89[a]	↑208.89[a]	46.67[b]	100.00[ab]	172.22[a]
Neural crest cells derivatives (mesenchyme cells)					
Percentage of positive cell count, %	75.56[a]	83.33[a]	33.33[b]	70.00[a]	83.33[a]
Q-score	156.67[a]	↑178.89[a]	53.33[b]	140.00[a]	182.22[a]
Heart (heart trabeculae)					
Percentage of positive cell count, %	57.78[a]	83.33[a]	58.89[a]	48.89[a]	60.00[a]
Q-score	145.56[a]	↑216.67[a]	98.89[a]	97.78[a]	150.00[a]
Liver					
Percentage of positive cell count, %	81.11[a]	77.78[a]	20.00[b]	63.33[a]	76.67[a]
Q-score	↑215.56[ab]	↑221.11[a]	26.67[c]	126.67[b]	153.33[ab]

[1]Values are means of three replicates per treatment (n = 3), where n is the number of fields of view observed per treatment
[2]Treatments were: [1] low dose *A. sessilis* at 7.3 mg/mL, [2] high dose *A. sessilis* at 73 mg/mL, [3] positive control at 10,000 IU (PC, isotretinoin ACNETREX 10 m, Thailand), [4] soybean oil as vehicle control (VC Jolly Puregoodness, Soya Oil, Philippines, and [5] distilled water as negative control (NC)
[3]Immunoreactivity score (Q-score) = proportion of biomarker of interest (%) x score intensity (SI)
[a–c] Means within a row lacking a common superscript letter have significant differences following ANOVA ($p < 0.05$); Tukey's was used as *post hoc* method for comparison of means

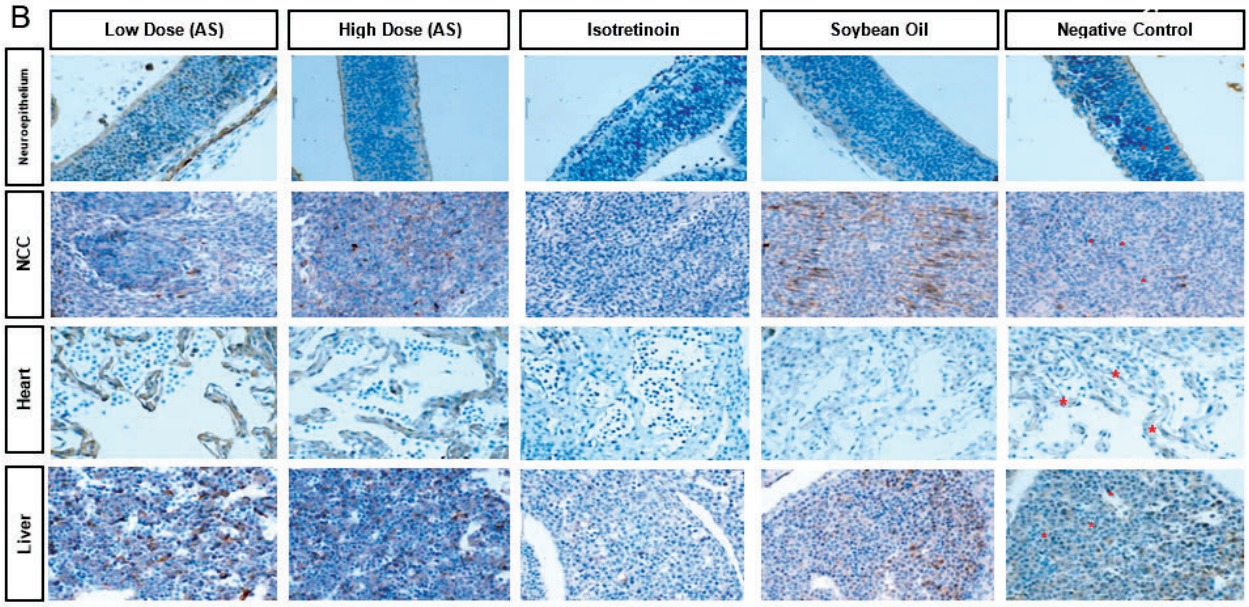


Figure 6. [A] Percentage of positive cell count and Q-score among treatment groups. [B] Representative photomicrographs (400X) of gene expression of BCL-2 in sagittal sections of neuroepithelium, heart, and liver. Neuroepithelium: [asterisk] ependymal cells. Neural crest cell derivatives: [asterisk] mesenchyme cells. Heart: [asterisk] heart trabeculae. Liver: [asterisk] hepatocytes; [white arrow] hepatic sinusoids (Crawford *et al.* 2010). Scale bar = 100 µm. *Values are means of N = 3 randomly selected embryos per treatment (n = 3), where n is the number of fields of view observed per sample.

purpose of the study was to see how *A. sessilis* affected the early development of organs in the embryo.

This study used a teratogenic dose of 10,000 IU/kg to induce embryotoxicity. The introduction of isotretinoin activates all-trans retinoic acid or ATRA-induced p53 overexpression, which promotes pro-apoptotic signaling into the tissues (Melnik 2018). Activation of apoptosis in the mesenchymal cells resulted in the teratogenic effects of the isotretinoin. In this study, it was shown that dams and embryos in the isotretinoin group in this study have reduced reproductive performance and showed embryotoxic phenotypes.

Based on the morphological data, there were minimal reductions in the gestational index and embryonic weight in the high-dose and soybean oil groups. These results suggest that *A. sessilis* may not negatively affect embryo implantation and maintenance. However, administration of *A. sessilis* at a high dose may result in reduced reproductive performance of treated dams. The growth of the fetus during intrauterine life is reflected in the weight at birth. In addition, the minimal reduction in the total length of embryos in the high-dose group can be related to their embryonic weight, which suggests that a higher dose of *A. sessilis* may have a negative effect on embryo growth. There are no available published articles on the teratogenic effect of *A. sessilis* in the literature. However, other studies that evaluated the teratogenic potential of other herbal medicinal plants found the same results, with fetal weight decreasing during administration (Golalipour *et al.* 2011). The herbal plant medicine studied had no reported teratogenicity, but Golalipour *et al.* (2011) suggested that several factors can affect embryo growth and maintenance. The availability of nutrients and the placenta's ability to provide these nutrients to the fetus in suitable amounts are two factors that greatly influence fetal growth. It may also be connected to the volume and rate of placental growth, both of which may have an impact on fetal growth and development (Golalipour *et al.* 2011). There were also minimal reductions in the weight of embryos from the soybean oil group (vehicle control). Dams fed with a high-fat diet (HFD) have lower fetal weights. HFD exposure results in maternal hypertension and placental malfunction, both of which are linked to the potential restriction of fetal growth (Yamaguchi *et al.* 2023).

The qualitative histological appearance was assessed based on the specific developmental processes that occur during E11.5 and confirmed by quantitative analysis using cell count. Based on the literature, proliferation in the neuroepithelium of the ventral telencephalon is increased between E10–E12. For E11.5, the ependymal layer is mostly found in the neuroepithelium. This layer has a high nucleus-to-cytoplasm ratio and a high mitotic figure (Chen *et al.* 2017). As stated in E11.5, the histological appearance of the neuroepithelium in embryos treated with both low and high doses follows normal development, as confirmed by its cell count in the neuroepithelium. The data result suggests that there are no negative effects of *A. sessilis* on the development of the brain, at least during the development of the neuroepithelium at E11.5. Among the treatments, the isotretinoin group had a significantly higher ependymal layer cell count. The increase in cell count can be linked to ATRA, which activates pro-apoptotic signaling and adversely affects cranial neural crest gene expression (Melnik 2017).

During heart development, progressive septation of the common outflow tract, atria, and ventricles is one of the primary heart developmental processes at E11.5. Between E11.5–E14, the most active proliferation and significant growth in ventricular wall thickness occurred. Significant ventricular trabeculations begin at E11. Trabeculation primarily increases myocardial oxygenation in the absence of coronary circulation (Savolainen *et al.* 2009). The degree of trabeculation of the primitive heart in the *A. sessilis*-treated groups was comparable with that in the negative control group. (Figure 4B). These data suggest that embryos in the *A. sessilis* group have no negative effects on trabecular formation, indicating that the primitive development of the heart did not have any complications during its development. This is because a decrease in trabecular formation often results in embryonic lethality because of a decrease in oxygen exchange in myocardial tissue, which can also lead to cardiomyopathy and heart failure (Wu 2018). This type of malformation was observed in the isotretinoin group, where the degree of trabeculation and its cell count were reduced. Other studies have reported that cell proliferation in heart tissue was also inhibited upon isotretinoin treatment (Huang *et al.* 2001; Melnik 2017; Wiens *et al.* 1992).

During normal development of the liver at E11.5, the tissue consists of hepatic cords with endothelial-lined sinusoids. The hepatoblasts present in the cord are undifferentiated and have large basophilic nuclei. Cells are densely packed and vary in size and form at the developmental stage of E11.5. Furthermore, immature erythropoietic cells are found in primitive hepatic cords and nucleated red blood cells (RBCs) are present in the sinusoids at this stage of development (Crawford *et al.* 2010). There were no observed malformations in the development of the liver tissue of the embryo from the low- and high-dose groups of *A. sessilis* compared with the negative control. The hepatic cords and endothelial sinusoids are apparent and the hepatocytes are compact, which was confirmed by the cell count of the liver tissue. The presence of nucleated RBCs within the sinusoids was also present in the embryos in both the low- and high-dose groups of *A. sessilis*. The liver tissue of embryos in the isotretinoin group is loosely

packed and the variation in cell size and shape of the liver is absent, which was confirmed by the reduction in the cell count in the liver tissue of the embryo. Moreover, sinusoids and RBCs were fewer in the isotretinoin group (Figure 5B). Vitamin A is known to inhibit embryonic liver development by increasing apoptosis and suppressing cell division (Aslan *et al.* 2022). Overall, these data suggest that at a histological level, qualitative and quantitative assessment of embryos in low- and high-dose groups of *A.sesillis* did not exhibit any forms of malformations in the development of the head, heart, and liver. The recorded data also indicate that *A. sessilis* has no negative effect during the early stage of organ development in mouse embryos.

The expression of the target protein BCL-2 was used in this study to assess the regulation of apoptosis in *A. sessilis-treated* groups. Apoptosis activation is signaled in the mitochondria. The BCL-2 protein family, in particular, regulates the balance of apoptotic activity. There are two types of BCL-2 families: anti-apoptotic [BCL-2, BCL-x(L), and MCL1] and pro-apoptotic [BIM, BAD, BAX, and BAK proteins]. IGF1, which mediates antiapoptotic stimuli, activates the signaling of the following BCL-2 proteins (Hilmi *et al.* 2008). During normal development of embryos, the expression of BCL-2 anti-apoptotic protein is upregulated during early neural development (neural plate and neural plate formation), as well as brain and spinal cord formation (Opferman and Kothari 2018). Another study reported the decreased expression of pro-apoptotic protein, Bax, in the foregut diverticulum and increased expression of BCL-2 during 10.5 and 11.5 d of mice gestation. Moreover, the expression of BCL-2/BAX tends to increase during the developmental process of the primitive heart area (Sun *et al.* 2002). According to the literature, the expression of BCL-2 is expected to be high in the neural, cardiac, and liver tissues of the developing embryos at E11.5, as demonstrated in the results of the present study (Figure 6B). As previously reported, there was an increase in the expression of BCL-2 in both the low- and high-dose groups of *A. sessilis*, which suggests its antiapoptotic effect specific in the liver tissue. *A. sessilis* is known to have anti-inflammatory, antioxidant, and hepatoprotective biological functions because of its high concentration of polyphenol compounds (Kanagarasu *et al.* 2017). Ellagic acid (EA), a scavenging radical compound, is a major bioactive polyphenolic constituent found in *A. sessilis* (Mondal *et al.* 2015). EA is known to have anti-apoptotic and anti-inflammatory effects, and a study has reported that EA administration downregulates Bax protein and upregulates BCL-2 in the liver and brain in a dose-dependent manner (Chen *et al.* 2018). The presence of EA in *A. sessilis* may explain the increase in the expression of BCL-2 in the liver of *A. sessilis*-treated groups.

CONCLUSION

In conclusion, this study reports the effect of the plant extract *A. sessilis* on the reproductive performance of dams and the development of its embryos. The low dosage of *A. sessilis* does not affect the reproductive performance and development of embryos. However, *A. sessilis* given at a higher dose may result in reduced embryo maintenance and growth. Based on the findings in this study, care should be taken if *A. sessilis* will be used during pregnancy since exposure to high doses could lead to possible detrimental effects during early embryonic development. However, intake of *A. sessilis* at a low dose showed possible anti-apoptotic effects, which should be further investigated. The increased expression of BCL-2 in the *A. sessilis*-treated group supports *A. sessilis's* potential anti-apoptotic, anti-inflammatory, and anti-oxidative effects. This study shows that using *A. sessilis* as a supplement during pregnancy, especially at low doses, does not confer embryotoxic effects.

RECOMMENDATIONS

The study discovered that a low dose of *A. sessilis* had no negative impact on the early development of organ structures in the embryo. To ascertain the safety of *A. sessilis* throughout the gestation period, it is also necessary to screen for teratogenicity at other stages of development.

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