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[E. U. Eric, T. O. Alade, E. F. Eruvwahwe. "Gordon Dry Gin \(Moringa Citrus Blend\) Induce Adenomatous Hyperplasia in Female Sprague Dawley Rats", Journal of Advances in Medicine and Medical Research, 2019](#) ✕

[Journal of the Medical Sciences \(Berkala Ilmu Kedokteran\) Volume 53, Number 4, 2021; 329-335 http://dx.doi.org/10.19106/JMedSci005304202102 Effect of kelor \(Moringa oleifera Lam\) ethanolic leaves extract on uterus of pregnant mice and its teratogenicity](#) Desy

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1Department of Anatomy and Histology, Faculty of Medicine, Universitas Diponegoro, Semarang, 2Department of Medical Biology and Biochemistry, Faculty of Medicine, Universitas Diponegoro, Semarang, 3Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia ABSTRACT Submitted: 2019-11-28  
[Moringa oleifera Lam \(Moringaceae\) is a source of additional dietary. It](#)  
Accepted : 2021-05-18 contains 9 essential amino acids, calcium, iron, potassium, magnesium, zinc and vitamins A, C, E and B which have a major role in the immune system. Previous study reported that M. oleifera Lam can increase the hemoglobin of breastfeeding mothers. In contrast, M. oleifera Lam is well known as abortifacient plant, therefore it is not recommended to use during pregnancy. The aim of this study was to evaluate the effect of M. [oleifera Lam ethanolic leaves extract](#) M. oleifera Lam [on uterus of pregnant mice and its](#) teratogenic effect. Twenty pregnant female Balb/c mice were used in this experimental study. Mice were randomized divided into four groups with five mice in each group. Group C as control, mice were not given anything. Group T1-T3 were given M. oleifera Lam ethanolic leaves extract during pregnancy age 7-18 days with doses of 10, 20 and 30 mg/kg BW, respectively. Phytochemical analysis was performed quantitatively and qualitatively. On day 19, a caesarean section was performed to take the uterus, count the number of living foetus, dead foetus, disability, and other morphological abnormalities. No significant difference between all groups was observed. The abortifacient effect was not also observed in the uterus. Qualitative phytochemical analysis results showed that alkaloids, flavonoids, phenols, steroids, and tannins are identified in this extract. Quantitative steroids analysis results showed that the extract contained steroids of 1057.6 ppm. In conclusion, the M. oleifera Lam ethanolic leaves extract at dose of 30 mg/kg BW does not show any effect on the uterus of pregnant mice. Further study concerning the effects on other organs and hormones is recommended. ABSTRAK Kelor atau Moringa oleifera Lam (Moringaceae) merupakan sumber makanan tambahan. Kelor mengandung 9 asam amino esensial, kalsium, besi, kalium, magnesium, seng dan vitamin A, C, E dan B yang memiliki peran penting dalam system imun. Penelitian sebelumnya melaporkan kelor dapat meningkatkan hemoglobin pada ibu hamil. Namun demikian M. oleifera Lam juga dikenal sebagai tanaman aborsi yang tidak dianjurkan diberikan selama kehamilan. Penelitian ini bertujuan untuk mengkaji efek ekstrak etanol daun kelor (M. oleifera Lam) pada uterus mencit bunting. Dua puluh mencit Balb/c betina digunakan dalam penelitian ini. Mencit dibagi secara random menjadi empat kelompok dengan masing-masing kelompok lima ekor. Kelompok C sebagai kontrol tidak diberi perlakuan apapun. Kelompok T1-T3 diberi ekstrak etanol daun kelor (M. oleifera Lam) selama umur kebuntingan 7-18 hari dengan dosis berturut-turut 10, 20 dan 30 mg/kg BB. Analisis fitokimia dilakukan secara kualitatif dan kuantitatif. Pada hari ke 19, dilakukan bedah sesar untuk pengambilan rahim, penghitungan jumlah janin hidup, janin mati, cacat, dan kelainan morfologi lainnya. Tidak dijumpai perbedaan pada seluruh kelompok yang diamati. Efek abortifasien tidak terlihat pada rahim. Keywords: Hasil analisis fitokimia kualitatif menunjukkan adanya kandungan alkaloid, Moringa oleifera flavonoid, fenol, steroid, dan tanin dalam ekstrak uji. Hasil analisis steroid uterus secara kuantitatif menunjukkan ekstrak uji mengandung steroid 1057,6 ppm. teratogenic Simpulan, ekstrak etanol daun kelor (M. oleifera Lam) pada dosis 30 mg/kg extract BB tidak menunjukkan efek yang merugikan pada uterus mencit. Penelitian phytochemical lanjut disarankan untuk mengkaji efeknya pada organ lain. \*corresponding author: desy.armalina@gmail.com INTRODUCTION Moringa oleifera Lam is known by such regional names as benzolive, drumstick tree, kelor, marango, mlonge, mulangay, nébéday, saijhan, and sajna.1 Every part of this tree is edible, from the leaves, trunks, stems, all the way down to its root.1

Moringa oleifera Lam has various pharmacological activities such as analgesic, anti-inflammatory, diuretic, antihypertensive, antioxidant, and antitumor. In addition, M. oleifera Lam also has several active compounds, some of which are interesting because of their medicinal value. [Every part of M. oleifera Lam has been reported to](#) have positive [properties](#) which make a significant contribution to its diversity and significance as a medicinal plant.<sup>2</sup> In tropical countries, M. oleifera Lam trees have been used to combat malnutrition, especially among infants and nursing mothers. Moringa oleifera Lam leaves contain complete protein (9 essential amino acids), calcium, iron, potassium, magnesium, zinc, and vitamins A, C, E, and B which have a major role in the immune system.<sup>1</sup> It was reported that M. oleifera Lam leaves dry powder contains 25 times more iron than spinach, in which iron is one of the therapeutic agents for anemia.<sup>3</sup> Previous studies reported that M. oleifera Lam was used as a source of additional dietary to address malnutrition. Administration of M. oleifera Lam leaves powder to breastfeeding mothers with babies aged 3-4 months for 3 months can significantly increase hemoglobin levels similar with iron/folic acid administration as control group.<sup>4</sup> Moreover, consumption of M. oleifera Lam leaves powder can improve the nutritional status of children and prevent some micronutrient deficiencies like iron and vitamin A.<sup>5</sup> Although M. oleifera Lam is useful for the treatment of malnutrition, in contrast, M. oleifera Lam is well known as abortifacient plant, therefore it is not recommended to use during pregnancy.<sup>6</sup> A study in rat reported that M. oleifera Lam ethanolic bark extract possesses abortifacient activity that potential to be developed as contraceptive plant. This extract can induce post-coital antifertility effect and fetal resorption in pregnant rats.<sup>7</sup> Another study reported that M. oleifera Lam ethanolic leaves extract at dose 250 and 500 mg/kg BW produced antifertility by inhibiting the implantation in female rats.<sup>8</sup> The study was conducted to evaluate the effect of M. [oleifera Lam ethanolic leaves extract](#) M. oleifera Lam [on uterus of pregnant mice and its](#) teratogenic effect. MATERIALS AND METHODS Animal and design It was a laboratory experimental study conducted in the Animal Laboratory and the Department of Histology, Faculty of Medicine, Universitas Diponegoro, Semarang. Twenty female mice of Balb /C strain with 8-10 weeks of age, 25 to 35 g of weight, healthy, and without anatomical abnormalities were used in this study. Every three female mice were caged together with one male for 12 h, from 18:00 to 6:00 am. Day 0 of pregnancy was determined by the presence of a vaginal plug.<sup>9</sup> All pregnant mice were divided into four groups. Each group consisted of five mice that were randomly chosen and then acclimatized for seven days. Group C as control, mice were not given anything. Group T1-T3 were given M. oleifera Lam ethanolic leaves extract during pregnancy age 7-18 days with doses of 10, 20 and 30 mg/kg BW, respectively. Histopathological examination A cesarean section was performed to observe the teratogenic effects of mice's fetuses and the termination of pregnant mice for examination of microscopic preparations on the uterus on the 19th day. The uterus of mice was processed micro-technically and stained using hematoxylin and eosin (HE). Each preparation was read at five fields of view and analyzed using a microscope to evaluate the histopathological index. The histology observation were performed from appearance in endometrium and myometrium, find out the damage in epithelium, uterine glands, and inflammatory cell infiltration. For teratogenic effects, an observation was conducted on the fetus including the number of living fetuses, dead fetuses, fetuses with disabilities, or other morphological abnormalities.<sup>10</sup> The study was approved by the Research Ethics Committee of Faculty of Medicine, Universitas Diponegoro/Dr. Kariadi General Hospital, Semarang (No.05/EC/H/ FK-RSDK/2017). Phytochemical analysis Moringa leaves extract preparation A total of 100 g of fresh M. oleifera Lam leaves were macerated using 70% ethanol, with a ratio of sample: solvent was 1:3. The mixture obtained was [then evaporated at a temperature of 50 - 60°C](#)

until a thick extract was formed. Phytochemical screening of these extracts showed the presence of alkaloid, phenolic, saponin, steroid, and flavonoid compounds. Alkaloid identification Moringa extract of 0.5 g was dissolved in 2 N sulfuric acid, then Meyer reagent and Wagner reagent were added. The identification was made on the precipitation formed. Steroid identification A total of 0.5 g of the sample was dissolved in 2 mL of chloroform. The dissolved sample was then given 10 drops of acetic anhydride and 3 drops of concentrated sulfuric acid. The identification was made of the color change that occurs. Flavonoids identification A total of 0.5 g of the sample were given 0.1 mg of magnesium powder. As much as 0.4 mL of amyl alcohol and 4 mL of alcohol are added. The identification was made of the color change that occurs. Saponin identification A sample Moringa extract of 0.5 g was dissolved with 2 N hydrochloric acid. The solution was then heated in a water bath for 30 minutes. The identification was carried out on the formation of foam Phenolic compound identification A sample of 0.5 g was added with 10 mL of 70% ethanol and left for 30 minutes. 1 mL of the mixture was given 5 drops of FeCl<sub>3</sub> 5%. The identification was carried out of the color change that occurs. Statistical analysis The quantitative data was presented as mean ± standard deviation (SD) processed with the SPSS computer program. Data distribution was tested by different tests with Kruskal-Wallis and followed by Mann Whitney Test due to not normal distribution was observed. A p value < 0.05 was considered as significant. RESULTS Maternal and fetus observation Observation during termination was obtained that the fetus in the control and treatment group showed an average of 8-9 fetuses from one parent mice. No abortion or absorption in this study was observed. TABLE 1. Descriptive analysis of uterus damage index Groups Mean ± SD Control Group 1 treatment Group 2 treatment Group 3 treatment 0 ± 0 0 ± 0 0.4 ± 0.55 0.6 ± 0.55 Histological appearance in endometrium and myometrium found no damage in epithelium, uterine glands, and no inflammatory cell infiltration (FIGURE 1). There was found no significant difference between all groups (p = 0.077). Phytochemical analysis Phytochemical analysis results showed that there were alkaloids, flavonoids, phenols, steroids, and tannins in moringa leaf extract (TABLE 2). Control Group Treatment 1 Group Treatment 2 Group Treatment 3 Group FIGURE 1. Histopathological uterus result. Blue arrow: the endometrium, Red arrow: myometrium. 400x, HE TABLE 2. Qualitative analysis results on *M. oleifera* Lam extract Qualitative analysis Results Remarks Flavonoid Saponin Steroid Tannin Alkaloid Phenolic Brownish green to yellowish-green No foam is formed Brownish green becomes purplish-green Brownish green to blackish-blue Chocolate precipitation is formed Brownish green to blackish + blue + - + + + Phytochemical analysis of *M. oleifera* Lam leaves showed the presence of steroid compounds. This compound has a basic frame in the form of rings, with carbon and hydrogen atoms, or carbon, hydrogen, and oxygen, which are not aromatic. Steroid compounds have certain physiological effects, such as a regulator of growth in plants. In pharmaceutical fields, the steroid is used as an active material for making drugs. Quantitative testing of steroids showed that *M. oleifera* Lam leaves extract contained steroids of 1057.6 ppm. DISCUSSION The abortifacient effect was observed in the uterus where it causes endometrial epithelium damage of the uterine gland as well as making the endometrial milieu unfavorable for the implantation of the fertilized ovum.<sup>11</sup> According to the TABLE 1 and FIGURE 1 above, there was no significant difference in uterus damage compared to the control group. The in vitro administration of cold and hot *M. oleifera* Lam aqueous extracts significantly produced uterine contractility with varying intensities (89.7%).<sup>12</sup> The previous study reported that 100% abortifacient activity was observed when administered orally in aqueous and ethanolic extract of *M. oleifera* Lam at dose of 175 and 100 mg/ kg BW, respectively.<sup>13,14</sup> Another study reported that the *M. oleifera* Lam ethanolic extract has shown an abortifacient effect by

decreasing [in the number of litters from animals treated with the extract](#).<sup>12</sup> In vivo study of *M. oleifera* Lam leaves administration before and after mating, respectively produced 100% and 80% inhibition of conception.<sup>12</sup> The dosage that we used in the research was from the daily consumption of *M. oleifera* Lam leaves in the community 10-30 mg/ kg BW. This dose was lower than the previous study although some research found that the 25 mg/kg BW resulted in 26.26 % abortion.<sup>7</sup> Despite the numerous positive health benefits of *M. oleifera* Lam reported, the adverse effects of this plant were also identified.<sup>15</sup> *Moringa oleifera* Lam contains active compounds, such as alkaloids and other phytotoxins which have nerve-paralyzing properties and other adverse effects in high dose.<sup>16</sup> Some other active compounds isolated from *M. oleifera* Lam were moringine, moringinine, estrogen, pectinesterase, and phenols including tannin.<sup>2</sup> There are unverified reports that *M. oleifera* Lam stems, roots and flowers potentially contain harmful phytochemical ingredients, especially during pregnancy, which may help uterus contraction, leading to miscarriages in pregnant women. It is thought to prevent implantation in women, therefore it should be avoided by those trying to conceive as it serves as an abortifacient agent.<sup>17</sup> Finally, the toxicity *M. oleifera* Lam aqueous leaves extract was investigated in human peripheral blood mononuclear cells only at dose 20 mg/kg BW, whereas the genotoxicity on blood rat's cells was verified at dose 3000 mg/kg BW. The acute toxicity *M. oleifera* Lam leaves showed an oral LD<sub>50</sub> of 3000 mg/kg BW.<sup>18</sup> Though, all cited side effects were confirmed with doses that far exceed the totals used in food intake.<sup>19,20</sup> [Moringa oleifera Lam stems, leaves, flowers, pods and seeds have been reported to have bioactive secondary metabolites such as flavonoids \(quercetin, kaempferol and vanillin etc.\), phenolic acids \(ellagic acid, gallic acid, ferulic acid, chlorogenic acid and other phenolic acid in trace amount\), glycosides, and glucosinolates which have nutritional and several pharmaceutical](#) qualities.<sup>21</sup> Nevertheless, the existence and the quantity [of these metabolites vary with a geographic location, soil type and climate condition](#). Subject matter [and the presence of these metabolites also](#) rely on [the solvents used for the extraction](#).<sup>22</sup> The phytochemical analyses from the previous study of *M. oleifera* Lam leaves reported the same composition with this research showed: flavonoids ++, alkaloids ++, saponins +, tannins +, proteins ++, carbohydrate +, reducing sugars +, steroids ++ and terpenoids +.<sup>18</sup> ACKNOWLEDGMENT This study was supported by the Research Grant of PHK-PKPD, Universitas Diponegoro, Semarang. REFERENCES 1. Dhakar RC, MauryaSD, Pooniya BK, Bairwa N, Gupta MS. *Moringa*: The herbal gold to combat malnutrition. *Chronicles Young Sci* 2011; 2(3):119-25. <https://doi.org/10.4103/2229-5186.90887> 2. Padayachee B, Baijnath H. An updated comprehensive review of the medicinal, phytochemical and pharmacological properties of *Moringa oleifera*. *South African J Bot* 2020; 129:304-16. <https://doi.org/10.1016/j.sajb.2019.08.021> 3. Mun'im A, Puteri MU, Sari SP, Azizahwati. Antianemia effect of standardized extract of *Moringa oleifera* lamk. leaves on aniline induced rats. *Pharmacogn J* 2016; 8(3):255-8. <https://doi.org/10.5530/pj.2016.3.14> 4. 5. Zakaria Z, Hadju V, As S, Bahar B. The effect of moringa leaf extract in breastfeeding mothers against anemia status and breast milk iron content. *Int J Sci Basic Appl Res* 2015; 24(1):321-9. Zongo U, Savadogo B, Zoungrana SL, Sanou D, Savadogo A, Dicko MH, et al. Effect of moringa leaves powder consumption on young children nutritional and serum retinol status in Burkina Faso rural area. *Int J Nutr Food Sci* 2018; 7(4):148-54. [h t t p s : / / d o i . o r g / 1 0 . 1 1 6 4 8 / j . i j n f s . 2 0 1 8 0 7 0 4 . 1 6](https://doi.org/10.11648/j.ijnfs.20180704.16) 6. Balamurugan S, Vijayakumar S, PrabhuS, Yobesh JEM. Traditional plants used for the treatment of gynaecological disorders in Vedaranyam taluk, South India - An ethnomedicinal survey. *J Tradit Chinese Med Sci* 2018; 8(2):308-23. [h t t p s : / / d o i . o r g / 1 0 . 1 0 1 6 / j . j t c m e . 2 0 1 7 . 0 6 . 0 0 9](https://doi.org/10.1016/j.jtcme.2017.06.009) 7. Onyewuchi CB, Aprioku JS, Siminialayi IM. Evaluation of the effect of ethanol bark extract of *Moringa oleifera* on reproductive biology of non-pregnant Wistar

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