



Effect of kelor (*Moringa oleifera* Lam) ethanolic leaves extract on uterus of pregnant mice and its teratogenicity

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ABSTRACT

Submitted: 2019-11-28

Accepted : 2021-05-18

Moringa oleifera Lam (Moringaceae) is a source of additional dietary. It 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. Hasil analisis fitokimia kualitatif menunjukkan adanya kandungan alkaloid, flavonoid, fenol, steroid, dan tanin dalam ekstrak uji. Hasil analisis steroid secara kuantitatif menunjukkan ekstrak uji mengandung steroid 1057,6 ppm. Simpulan, ekstrak etanol daun kelor (*M. oleifera* Lam) pada dosis 30 mg/kg BB tidak menunjukkan efek yang merugikan pada uterus mencit. Penelitian lanjut disarankan untuk mengkaji efeknya pada organ lain.

Keywords:

Moringa oleifera
uterus
teratogenic
extract
phytochemical

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.²

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.¹ 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.³ 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.⁴ 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.⁵

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.⁶

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.⁷ 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.⁸ 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.⁹ 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.¹⁰ 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 FeCl₃ 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	0 ± 0
Group 1 treatment	0 ± 0
Group 2 treatment	0.4 ± 0.55
Group 3 treatment	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).

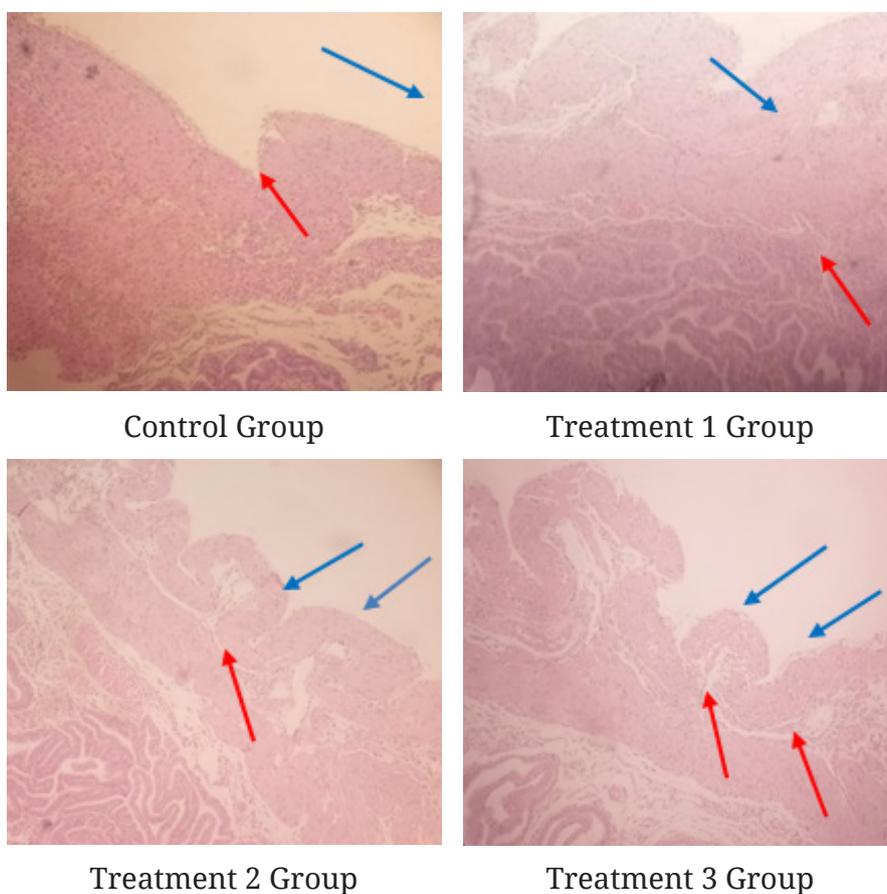


FIGURE 1. Histopathological uterus result. Blue arrow: the endometrium, Red arrow: myometrium. 400x, HE

TABLE 2. Qualitative analysis results on *M. oleifera* Lam extract

Qualitative analysis	Results	Remarks
Flavonoid	Brownish green to yellowish-green	+
Saponin	No foam is formed	-
Steroid	Brownish green becomes purplish-green	+
Tannin	Brownish green to blackish-blue	+
Alkaloid	Chocolate precipitation is formed	+
Phenolic	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.¹¹ 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%).¹² 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.^{13,14} 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.¹² *In vivo* study of *M. oleifera* Lam leaves administration before and after mating, respectively produced 100% and 80% inhibition of conception.¹² 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.⁷

Despite the numerous positive health benefits of *M. oleifera* Lam reported, the adverse effects of this plant were also identified.¹⁵ *Moringa oleifera* Lam contains active compounds, such as alkaloids and other phytotoxins which have nerve-paralyzing properties and other adverse effects in high dose.¹⁶ Some other active compounds isolated from *M. oleifera* Lam were moringine, moringinine, estrogen, pectinesterase, and phenols including tannin.² 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.¹⁷

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 LD₅₀ of 3000 mg/kg BW.¹⁸ Though, all cited side effects were confirmed with doses that far exceed the totals used in food intake.^{19,20}

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.²¹ 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.²² 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 +.¹⁸

ACKNOWLEDGMENT

This study was supported by the Research Grant of PHK-PKPD, Universitas Diponegoro, Semarang.

REFERENCES

1. Dhakar RC, Maurya SD, 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. 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.
5. 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. <https://doi.org/10.11648/j.ijnfs.20180704.16>
6. Balamurugan S, Vijayakumar S, Prabhu S, 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. <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 albino rats. *J Complement Altern Med Res* 2018; 5(2):1-7.
8. Agrawal SS, Vishal D, Sumeet G, Shekhar C, Ashish N, Parul D, *et al.* Antifertility activity of ethanol leaf extract of *Moringa oleifera* Lam. in female Wistar rats. *Indian J Pharm Sci* 2018; 80(3):565-70.
9. Fonseca BM, Correia da Silva G, Teixeira NA. The rat as an animal model for fetoplacental development: A reappraisal of the post-implantation period. *Reprod. Biol* 2012; 12(2):97-118. <https://doi.org/10.1016/s1642->

- 431x(12)60080-1
10. Klaassen C. Toxicology. The Basic Science of Poisons. Vol. 7th edition, McGraw-Hill. 2008. <http://www.loc.gov/catdir/enhancements/fy0726/2007015656-b.html>
 11. Obediah GA, Paago G. Effects of ethanolic extract of *Moringa oleifera* seeds and leaves on the reproductive system of female albino rats. SOJ Biochem. 2018; 4(1):1-8. <https://doi.org/10.15226/2376-4589/4/1/00131>
 12. Attah AF, Moody JO, Sonibare MA, Salahdeen HH, Akindele OO, Nnamani PO, et al. Aqueous extract of *Moringa oleifera* leaf used in Nigerian ethnomedicine alters conception and some pregnancy outcomes in Wistar rat. South African J Bot 2020; 129:255-62.
 13. Sethi N, Nath D, Shukla SC, Dyal R. Abortifacient activity of a medical plant *Moringa oleifera* in rats. Ancient Sci Life 1988; 7(3-4):172-4.
 14. Zade V, Dabhadkar DK. Abortifacient efficacy of *Moringa oleifera* stem bark on female albino rats. World J Pharm Res 2014; 3(3):4666-79.
 15. Annongu AA, Karim O, Toye A, Sola-ojo F, Kayode R, Badmos A, et al. Geo-assessment of chemical composition and nutritional evaluation of *moringa oleifera* seeds in nutrition of broilers. J Agric Sci 2014; 6(4). <https://doi.org/10.5539/jas.v6n4p119>
 16. Maizuwo AI, Hassan AS, Momoh H, Muhammad JA. Phytochemical constituents, biological activities, therapeutic potentials and nutritional values of *Moringa oleifera* (Zogale): a review. J Drug Des Med Chem 2017; 3(4):60-6. <https://doi.org/10.11648/j.jddmc.20170304.12>
 17. Dutta AK (2017) *Moringa oleifera*: a review on its importance and medicinal applications in recent age. World J Pharm Sci 2017; 6:1829-43. <https://doi.org/10.1007/s13596-020-00468-0>
 18. Ufelle SA, Onyekwelu KC, Achukwu PU, Ezeh CO, Ghasi S. Haematological effects of leaf extract of *moringa oleifera* lam in normal and myelo-suppressed wistar rats. Afr J Biomed Res 2018; 21:87-90.
 19. Asare AG, Gyan B, Bugyei K, Adjei S, Mahama R, Addo P, et al. Toxicity potentials of the nutraceutical *Moringa oleifera* at supra-supplementation levels. J Ethnopharmacol 2012; 139(1):265-72. <http://dx.doi.org/10.1016/j.jep.2011.11.009>
 20. Meireles D, Gomes J, Lopes L, Hinzmann M, Machado J. A review of properties, nutritional and pharmaceutical applications of *Moringa oleifera*: integrative approach on conventional and traditional Asian medicine. Adv Tradit Med 2020; 20(4):495-515. <https://doi.org/10.1007/s13596-020-00468-0>
 21. Mbikay M. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: a review. Front Pharmacol 2012; 3:24. <https://doi.org/10.3389/fphar.2012.00024>
 22. Singh AK, Rana HK, Tshabalala T, Kumar R, Gupta A, Ndhlala AR, et al. Phytochemical, nutraceutical and pharmacological attributes of a functional crop *Moringa oleifera* Lam: an overview. South African J Bot 2020; 129:209-20. <https://doi.org/10.1016/j.sajb.2019.06.017>