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The Abortificient Effects of Hydroalcoholic Extract of Lawsonia Inermis on BALB/c Mice

Ramin Esteki[1], Sepideh Miraj[2]

[1] General Practitioner, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2] M.D., Gynecologist, Fellowship of Infertility, Assistant Professor, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran

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Abstract

Introduction: According to the traditional beliefs of certain cultures, *Lawsonia inermis* has been reported to cause the abortion or termination of an undesirable pregnancy. The present study was undertaken with the goal of studying the effect of *Lawsonia inermis* extract on abortion in pregnant BALB/c mice in 2013 in Shahrekord, Iran.

Methods: This research study used an experimental methodology and was conducted in 2013 in Shahrekord, Iran. Forty female BALB/c mice (30-40 gm, 8-12 weeks old) were randomly assigned to 4 groups. One male mouse was included for each two female mice (1:2) and they were maintained in a protective cage habitat. Pregnancy of the mice was confirmed by means of a vaginal smear. The doses of 1 mg/kg and 10 mg/kg of the hydroalcoholic extract of *Lawsonia inermis* were injected intraperitoneally into pregnant mice beginning on the first day and continuing through the seventh day of pregnancy. The control group did not receive any treatment, but was left completely unadministered. On the eighteenth day of pregnancy, the uterine tubes of mice were removed. The subsequent embryonic absorption is considered to be an abortion. The data were analyzed using SPSS software version 22 using Fisher's exact test and the Kruskal-Wallis H tests.

Results: Abortions were observed more often in the experimental groups ($p< 0.01$).The mean of the serum estrogen level was significantly higher in the case control groups ($p< 0.01$) and the mean of progesterone level was also significantly lower in the experimental groups ($p< 0.01$).

Conclusion: The use of *Lawsonia inermis* during pregnancy may cause abortion and therefore it should be considered as contraindication or use with caution.

Keywords: Lawsonia inermis extract, BALB/c mice, Abortion, Serum estrogen, Progesterone

1. Introduction

The term abortion is generally used to describe any termination of pregnancy, whether it might be either spontaneous or deliberate, when it occurs before the fetus is developed enough to survive outside of the womb. Conventionally, abortion is referred to as the termination of a pregnancy before the twentieth week of pregnancy or when the fetus is less than 500 grams (1). Over 80% of abortions occur during the first 12 weeks of pregnancy and chromosomal abnormalities are responsible for at least half of such abortions. After the first quarter of gestation, both the abortion rate and the incidence of chromosomal abnormalities tend to be reduced. The literature suggests that the diagnosed rates of abortion are predicted to rise from 12% for women under 20 years to 26% for women over 40. The apparent mechanisms responsible for these abortion have not yet been ascertained, but in the first trimester of pregnancy, the death of the embryo or fetus nearly always occurs prior to spontaneous abortion. Abortion may be defined as including a threatened abortion (2), inevitable abortion, complete abortion, incomplete abortion, missed abortion (3, 4), and recurrent abortion (5). Throughout human history, individuals have tried to use many natural materials to facilitate abortion. Natural abortion methods can be used to reduce the risk of uterine rupture or a hole forming and additional benefits are that it can make it possible for patients to avoid having an

Corresponding author:
Assistant Professor Dr. Sepideh Miraj. Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran. Tel: +98.9126509952, Fax: +98.32225456, Email: miraj.sepide@gmail.com
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invasive surgery. The use of medicinal plants in Iran and other countries has been popular for a long time among non-health professionals and especially so in recent years, the use of medicinal plants to induce abortion has greatly increased (6, 7). Various studies have shown that women tend to use herbal remedies to treat problems such as dysmenorrhea, to relieve symptoms of menopause, menstrual disorders, mood disorders, the prevention of osteoporosis as well as problems that occur during pregnancy. Most pregnant women believe that natural treatments are not dangerous and do not have any side effects for either the mother or the fetus and due to such questionable beliefs may attempt to self-medicate with herbal medicines or other such preparations (8). *Lawsonia inermis* is a single-species genus of the Lythraceae family; its leaves, stems, bark, roots, flowers, and seeds have been used in various forms of traditional medicine. It has garnered greater levels of attention from scholars from many countries because of the variety of compounds that can be derived from Lawsonia as well as some of them that may have significant physiological properties. The plant is reported to contain quinones, phenyl propanoids, flavonoids, terpenoids, phenolic compounds, and fatty acids. Modern pharmacological studies have demonstrated that the plant performs as an antimicrobial, antioxidant, and demonstrates some anticancer and antiparasitic activity (9, 10). Henna leaf powder has been found to have a skin cancer chemo-preventive activity (11). Historically, henna was applied topically to treat joint pain, ulcers treatment of liver and digestive disorders, in the reduction of tissue loss in cases of leprosy, diabetic foot disorders, and ulcers (10). It has also been used to treat diabetes mellitus associated dyslipidemia (12). It has also traditionally been used to dye skin, hair, and fingernails (13, 14). It was reported to have anti-inflammatory effect (15). Alongside its therapeutic effects, it has lots of side effects. When involved in the process of dying hair and eyelashes with henna, one should be careful because there is a risk of damaging the eye (16). Henna can cause skin irritation as well as contact dermatitis (Red henna appears to be generally safe, with rare instances of contact allergy and Type 1 hypersensitivity reactions, but henna can cause contact allergies due to its ingredient PPD (17, 18). Henna leaves have been found to cause infertility in mice. Its leaves have narcotic effect and high levels of it can cause headaches as well as intoxication (19). *L. inermis* may have some properties of teratogenicity and should be used cautiously during pregnancy (20). It can also cause hemolytic anemia in glucose-6-phosphate dehydrogenase deficient (G6PD deficient) patients (15, 21).

Overall, henna coloring and its therapeutic properties are due to the existence of a substance generally called Lawsone (2-hydroxy-1,4-naphthoquinone) (13,16, 22), but this substance does not act alone, and especially, its coloring properties depend on other substances. Lawsone has been found to exhibit beneficial biological properties such as being an antioxidant, anti-inflammatory, antitubercular, and having antimalarial effects. Lawsone is able to undergo redox cycling and chelation of trace metal ions (14). Lawsonia has very low poisoning effects and causes heart rate to slow down with increasing effectiveness as the scope of its contact increases. Its antidiarrheal effect is due to muscle tonus reduction and mass movement (peristalsis) of the colon. Its anti-inflammatory, analgesic, and anti-fever effects has been reported when taking 500 mg per of Lawsonia per kilogram of body weight (19). The antibacterial and antifungal effects of henna have also been also attributed to Lawsonia (13). In a study conducted by Aguwa in 1987, methanolic henna root extract was investigated to determine its abortificient activity. This extract has shown to be most abortificient effect in mice, rats, and guinea pigs and that its effect was dose-dependent. However, no mechanism of action of the isolated tissue was observed. The results of the study showed that the methanolic henna root extract had a strong toxic effect on the fetus as well as the mother and induced an abortion (23). In a study conducted in 1999 by Fatahi Bafghi et al., the effectiveness of the henna leaf extract on Leishmaniasis in BALB/c mice were examined. This study's results showed that the use of henna extract reduced the rate of weight loss, but it was not very remarkable. The extract also significantly reduced the expansion as well as the increase in average diameter of the lesions, so that the mice's lesions scarified in the control group as compared to the mice receiving the henna extract was much larger, although all mice eventually died (24). In a study conducted in 2008 by Behdani and his colleagues, the antibacterial activity of aqueous and ethanol henna extract were examined against Staphylococcus aureus and Pseudomonas aeruginosa. The results indicated a wide range of activity of aqueous and ethanol henna extract against bacteria. The findings of this study suggested the use of henna in the treatment of skin and wound infections. It also seems that this natural substance can be a promising agent in the prevention and treatment of skin diseases (25). In a 2007 study by Malek, the efficacy of methanol henna extract was investigated for the treatment of contact dermatitis in mice and it was demonstrated that it produced after 7 days of henna extract administration that there was zero percent lymphocyte infiltration (26). The side effects of some other plants on pregnancy were evaluated in some studies. For example, effects of water extract of Crocus sativus were evaluated on growth of BALB/c mice on days 6 to 12 of pregnancy. It was shown that the consumption of this extract during this period can trigger side effects on the fetus (27). In a study conducted by Gagandeep et al., it showed that henna causes permanent infertility in mice (28). It should be noted that no similar study on the

abortificient effects of *Lawsonia inermis* leaves was found. Thus, the aim of this study was to investigate the abortificient efficacy of *Lawsonia inermis* in BALB/cmice.

2. Material and Methods

2.1. Study design

This experimental study was conducted at a cellular and molecular research center, Shahrekord University of Medical Sciences, Shahrekord, Iran in 2013.

2.2. Subjects

Firstly, 45 BALB/c mice ranging in age between 8 and 12 weeks were selected from among groups where the completely identical environmental conditions had been maintained. To begin, 5 mice were sacrificed to determine their serum estrogen and progesterone and the remaining 40 mice were divided into four groups.

2.3. Inclusion criteria

Female BALB/c mice aged 8 to 12 weeks, who were kept in the same light and with proper sanitary conditions (12 hours of light and 12 hours of darkness) and without any food restrictions were included in this study.

2.4. Interventions

To begin with, 5 mice were sacrificed to determine the serum estrogen and progesterone and the remaining mice were divided in groups of 10. Two of these female mice were fertilized by a male mouse. The other mice were fertilized in the same manner. The time of vaginal plug observation was considered as the zero day of pregnancy (26). However, since this method was not foolproof, an eosin smear 3% was prepared from the vaginal secretions of mice and the existence of sperm was considered as fertilized. Another way that fertilization was determined as well as the certainty of its having occurred was the weight gain of female mice after fertilization. As soon as of the fourth day of pregnancy and there afterward, 3 to 10 % will be added to the mouse's body weight, weight gain was regarded as fertility. Then, the mice were randomly divided into 4 groups. A non-intervention control group, a group injected with distilled water, and the other two groups, in which each was injected with different doses of the plant extract intraperitoneally over different days of gestation (29). Firstly, blood was collected using a syringe from the heart of mice in order to use its serum to measure the serum estrogen and progesterone by kit. Then, the mice underwent a cesarean section. The caesarean cut was in a reverse Y-shaped form. The uterus is in a Y shape, which two branches of Y are the mouse uterus that at the two ends it leads to the ovaries and the long branch is the urethra (infertile womb). After the cesarean, the uterine tubes were opened and fetal absorption in the tubes was considered to be an abortion (33, 34).

2.5. Outcomes

The primary outcome of our analysis was determining the frequencies of abortion and the secondary outcome of our analysis was the serum estrogen and progesterone measurement.

2.6. Sampling and randomization

In each group, ten mice and in sum 40 mice formed the research sample (25).Two groups were selected as the control groups and two groups were selected as the experimental case groups. One of the control groups formed the non-injected group and the other control group was only injected with distilled water. Both case groups were intraperitoneally injected with henna extract prepared at concentrations of 1 and 10 mg/kg over days 0-7. Beginning on days 18-19, blood sampling of the mice was performed to measure their levels of serum estrogen and progesterone. Then, the mice underwent a caesarean section and observations were made with a tubal embryo absorption being considered as an abortion. First, shoots of the henna plant were collected from around the city of Yazd, Iran and a botanist from the Agricultural Organization Research Center verified and confirmed the identity of the henna plant samples. The gathered plant material was dried for a week at normal temperatures in the shade, and then the extraction process was performed by maceration. The process by which this was performed was as follows, 100 grams of dried and ground herbs were mixed with 500 cc of ethanol with an 80% concentration. The extraction procedure was carried out while shaking and repeated stirring the mixture at room temperature and eventually establishing an equilibrium concentration between the plant substances in solvent and plant tissue, this was when the extraction procedure was terminated (30). Then 400 ml of extract was passed through micron measurement of filter paper and then moved to the rotary devices in a vacuum during which the ethanol was evaporated. Then, the solution was kept for two days at the temperature 40° C in order to obtain 8gm of dried powder extract (13, 31, 32). It should be noted that the natural ethanol used in this method was evaporated so to ensure that the alcohol content would

have no bearing on the results of the study. Then, the rats were randomly divided into four groups, consisting of two control groups without intervention and by injecting 0.3 cc of distilled water and the other two groups, each received different doses of 1 and 10 mg per kg of the refined extract that was injected over the course of 7 consecutive days. This amount of extract was added to 0.3 cc of distilled water and was injected. Given that the gestational period of BALB/c mice is known to last 21 days, a double dose of henna leaves was intraperitoneally injected into the mice once a day for 7 days, starting from day 0 to day 7.This injection was performed in such a way that the subcutaneous injection is slowly inserted until the researcher feels or hears the sound of the puncturing of peritoneal wall, then injection was started. On the day 18-19 of gestation, the mice were anesthetized by ether in accordance with humane and ethical considerations.

2.7. Statistical methods

The collected data were entered and were analyzed using Statistical Package for the Social Sciences (IBM© SPSS©) Version 22 (IBM© Corp., Armonk, NY, USA) by means of the Fisher Exact Test, the Kruskal-Wallis H Test, and the Dunn's Post Hoc Test.

2.8. Research ethics

The mice were first anesthetized under standard conditions and then sacrificed so that fetal absorption in uterine could be investigated and so that they would suffer less stress.

3. Results

In the study, entitled "The Effect of hydro-alcoholic henna leaf extract (Lawsonia Inermis) on embryos of BALB/c mice", the following results were obtained. A total of 40 mice were randomly divided into 4 groups of 10 individuals. The first group formed the control group and the second group received only injections of distilled water, the third group received injections of a 1 mg/kg dose of henna leaf extract, and the fourth group received a 10 mg / kg dose of henna leaf extract. In the study, the abortificient effects of the henna leaf extract were observed in the two groups. The Fisher's Exact Test showed a significant difference between the groups in terms of affecting abortion ($p = 0.003$). The frequencies of abortion between the groups were summarized in Table 1. The serum estrogen and progesterone of the mice after the eighteenth day of measurement as well as the results of these measurements are summarized in Table 2.

Table 1. Frequency Distribution of abortion in groups under study

Group	Abortion (%)	
	Have	Do not have
Control	0	100
Distilled water	0	100
Extract 1 mg/kg	50	50
Extract 10 mg/kg	50	50

Table 2. Serum estrogen and progesterone mean in the groups under study

Variable	Group	Mean ± SD	Minimum	Maximum	p-value
Serum estrogen	Control	27.81±7.51	20.3	46.9	< 0.001
	Distilled water	27.67±3.92	22.4	36.4	
	Extract 1 mg/kg	51.01±1.72	47.3	53.2	
	Extract 10 mg/kg	51.63±0.95	50	53	
Progesterone	Control	29.24±3.94	20.3	36.2	0.003
	Distilled water	27.94±2.34	22	30.5	
	Extract 1 Mg/kg	24.76±2.9	20.5	28.9	
	Extract 10 Mg/kg	23.6±5.35	11	29	

It demonstrated a significant difference between the groups in terms of the serum estrogen and progesterone. It showed that the serum estrogen of groups receiving 1 mg per kg and 10 mg per kg were not different, but these groups were, in fact, found to be different from control groups. On the contrary, there was also not found to be any difference in the serum estrogen of the control groups. Regarding the progesterone levels, the control groups and the group that received distilled water showed no difference from each other. Furthermore, the distilled water group also showed no difference from the other groups, but the control group showed a significant difference in relation to the

case groups. In addition, the case groups were not significantly different from each other in terms of the progesterone levels. In the case groups, the mean of the serum estrogen was greater and the mean of the progesterone level was less. Regarding the serum estrogen, there was found to be a significant difference between the control groups. Considering the progesterone levels, there was found to be a significant difference between the control and the case groups. The results indicated that there was a significant difference between the groups in terms of the incidence of abortion ($p = 0.003$). The results demonstrated that there were higher rates of abortion seen in the case groups. Based on the studies undertaken, there was a significant difference between the groups with regard to the serum estrogen and progesterone, in such a way that the serum estrogen in the control and case groups showed differences ($p< 0.001$). Also, in the case of progesterone, the control group showed a significant difference in relation to the case groups ($p = 0.003$). The mean of the serum estrogen in the case groups was greater and the mean of progesterone found to be less and given that the high level of progesterone and low level of serum estrogen is needed to continue pregnancy, the low average of progesterone and the high level of serum estrogen is another possible reason for the abortion and may be a property of the henna leaf extract.

4. Discussion

Although henna was used as a medicinal herb for thousands of years in traditional folk medicine for therapeutic purposes, but in relation to its effects during pregnancy, except in a few limited reports, there is not a verifiable, empirical study supporting its use as an abortifacient. The study aimed to determine the effects of henna leaves extract on abortion as well as its effects on the serum estrogen and progesterone rates in late pregnancy. The results showed that in the case groups (consumers of henna leaf extract) that 50% of the embryos were absorbed, while such fetal absorption was not observed in the control group. The teratogenicity effect of this plant was investigated by Jafarzade et al. and in their study, the antioxidant activity and the possible side effects of L. inermis hydroalcoholic extract on development of congenital abnormalities in BALB/c mice was assessed and it was shown that it has potential teratogenic effect that is in accordance with our results (20, 36). In a study by Aguwa, methanolic extract of the henna root has shown to be highly abortificient in mice, rats, and guinea pigs. As well, in this study the genetic compound of henna in both watery and alcoholic extracts were compared and the results showed that the rate of epigenetic is more in hydroalcolic extract (37). Apigenin was known in the past as a drug, and therefore, flowers containing large amounts of apigenin were used to treat insomnia, seizures, shortness of breath, and decrease in nerve pain. Apigenin is a serum estrogen flavonoid that exists in aromatic vegetables like henna (38). Gradplatto et al. examined apigenin metabolism in rats after a single dose. In their study, it was observed that apigenin is metabolized slowly and its absorption and elimination were also done slowly. Thus, the accumulation of flavonoids in the body was likely to occur (39). As we know from the literature, henna leaf contains 1-hydroxy naphtokinon, multiple phenolic glycosides, coumarin, flavonoids and cineol and given that the apigenin flavonoids are serum estrogenic and flavonoids are one of the henna's chemical components; thus, it can be argued that the likely presence of this serum estrogenic compound in the henna extract can be one of the possible causes of abortion. It may be concluded that due to the presence of this flavonoid, henna (*Lawsonia inermis*) leaf may cause changes in the level of performance of the hypothalamus-pituitary-gonadal axis. The combination of 2-hydroxy-1 and 4-naphtokinon existing in henna extract may hemolysis due to its antioxidant properties. It metabolizes hepatically and changes in the liver into a toxic metabolism and then, the metabolites cross the placenta and may cause abortion (40). In previous studies conducted on different plants, different results were obtained. Studies carried out on Peganum harmala have shown that the consumption of this plant causes an interruption in fetal growth and it is an abortificient plant (35). In a review study, various medicinal plants have been reviewed for their antifertility effects such as Polygonum hydropiper Linn, Citrus limonum, Piper nigrum Linn, Juniperiscommunis, Achyanthesaspera, Azadirachtaindica, Tinosporacordifolia, and Barleriaprionitis and it was concluded that many of these medicinal plants act through an antizygotic mechanism (41). During a recent study that was conducted on henna extract, it became clear that this plant is able to cause abortion in mice. But, its abortificient effects and its effect on the hormones of serum estrogen and progesterone in our study were not found to be dose-dependent. On the other hand, it was clear that this plant demonstrated a significant difference between the case and control groups regarding its serum estrogen and progesterone levels. However, its abortificient effects need to be studied further. Nevertheless, it is recommended that the plant be used with caution during pregnancy. Furthermore, in a study concerning (G6PD)-deficient male newborns with acute hemolysis a few days after applying henna dye over the body were investigated, the laboratory analyses revealed significant anemia, reticulocytosis, and indirect hyperbilirubinaemia among newborns. The clinical findings support the harmful effects of henna on G6PD-deficient red blood cells (42).

5. Conclusions

The goal of the research study was to investigate the potential effects of *Lawsonia inermis* extract on gestation of mice embryos in pregnant mice. The results indicated that the use of *Lawsonia inermis* during pregnancy may cause abortion, and therefore, the researchers of this study highly suggest that Lawsonia should be used with great caution or else considered to be counter indicated for pregnant individuals. Furthermore, it is recommended that additional studies should be conducted to determine if any other side effects or counter indications should be considered when Lawsonia compounds are used by pregnant women.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

Both authors contributed to this project and article equally. Both authors read and approved the final manuscript.

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