Plumbagin Causes Abortive Effects When Fed to Pregnant Sprague Dawley Rats during the Late Gestation Period

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Abstract:  
There are many plants in folk medicine which are used as contraceptive or abortive agents during early pregnancy. Plumbago is a genus of 10-20 species of flowering plants in the family Plumbaginaceae, native to warm temperate to tropical regions of the world. Plumbagin is a natural product found in many plants. In this study, plumbagin was obtained from Plumbago rosea. The aim of this study was to determine the effects of plumbagin on pregnant Sprague dawley rats and to observed possible teratogenic effects to foetuses. Mated females were randomly assigned to different experimental groups and treated during mid pregnancy on gestation day (GD) 8-15. The treatment consisted of ingestion by gavage of 32.5 mg/kg per day of plumbagin. All the animals were observed for toxic effects, abortion, water and food consumption, and weighed daily to monitor toxicity throughout the experiment. The maternal weight gain was recorded during the entire pregnancy (total weight gain) and during the treatment period. On day 21 of pregnancy, females were sacrificed by chloroform poisoning, and their uteri removed by Caesarean section. Placenta were taken and weighed while the fetuses undergo fetal staining. The number of fetus was recorded and all fetuses were examined for obvious external malformations before subsequent processing. For skeletal examination, the numbers of skeletal elements were counted and any malformations or variations were recorded. Results were reported as means ± S.E.M. Data were analyzed by one-way ANOVA followed by Duncan test and P<0.05 was considered significant. The results obtained showed that plumbagin has significantly decrease the body weights of pregnant rats when compared to control and olive oil groups where the weight of control, olive oil and plumbagin are 270.71±4.412, 242.75±2.767 and 219.02±1.932 respectively. There was no significant difference in fetal skeletal anomalies between control, olive oil and plumbagin groups. There is only one out of six (1/6) dams with pups in plumbagin group when compared to control and olive oil group which is six out of six (6/6) and five out of seven (5/7) respectively. This preliminary experiment suggests that plumbagin showed significant abortifacient activity in late pregnant Sprague dawley rats and cause acute toxicity to the dams by reducing its body weight without any teratogenic effects.

Keywords: Plumbagin, sprague dawley rats, late gestation period, abortive effects

1. Introduction  
In folk medicine, plants were used as a contraceptive to avoid pregnancy and as abortive agents during the early stage of pregnancy. There are some medicinal plants that are dangerous on pregnancy (Review by Daniyal and Akram, 2015). Effects on the embryo development are varies depending on the period of maternal exposure to xenobiotics (Rogers and Kavlock, 2001). Plumbagin is a plant-derived naphthoquinone possessing a number of pharmacological activities such as antimicrobial activity (Nair et. al., 2016), antimalarial activity (Wiriyaporn et al., 2014), anticarcinogenic effects (Lee et. al., 2012), antifertility action, (Sandeep et. al., 2011) and anti-atherosclerosis effects (Machi and Shah, 2012).

Plumbagin is named after the plant genus Plumbago, from which it was originally isolated. Plumbagin can be discovered in roots of Plumbago spp. (Plumbago europea, P. rosea, P. zeylanica) (Panichayupakaranant et al., 2002). Plumbago rosea can be found in tropical regions of India. In many traditional medical procedures, the mature roots of Plumbago rosea were used to cure many different diseases (Komariah et al., 2003). According to Uma Devi et al., 1999, the roots of Plumbago zeylanica which has plumbagin could cause abortifacient, antifungal and antibacterial effects. The purpose of this study is to evaluate the possible toxic effect of plumbagin from Plumbago rosea on pregnant Sprague dawley rats during organogenesis by observing whether this compound interferes with the normal development of the embryo.
2. Materials and Methods

2.1. Preparation of Plumbagin

Plumbagin is purchased commercially from Fisher Scientific. It is dissolved in olive oil before feeding to the pregnant rats. According to Premakumari et al. (1977), the LD₅₀ for plumbagin in rats is 65mg/kg. For this experiment, we prepare 50% concentration of LD₅₀ which is 32.5 mg/kg.

2.2. Animals

Twenty-one sexually mature fertile female Sprague dawley rats weighing approximately 150-200g were obtained from Animal Unit of Faculty of Medicine and Health Sciences, University of Putra Malaysia with ethics approval from the Animal Ethics Committee of University of Putra Malaysia with the reference number 00222. The animals were housed in individual cages in a temperature and light controlled room (25 to 28°C, 12 h light/dark), with water and food ad libitum. Rats were given 7 days to adapt to the environment and to undergo 2 consecutive estrus cycles.

2.3. Experimental Design

Each female rat is mated with a male rat and kept overnight in their cages with a tray at the bottom of the cage. This is for the purpose of collecting vaginal plugs and for easy observation of appearance of the plug. In the morning, when the vaginal plug is detected, it will be considered as day 0 of pregnancy and the following day is day 1 post-coitum. The mated females were randomly assigned to different experimental groups and later treated during mid pregnancy on gestation day (GD) 8-15 during organogenesis to detect whether plumbagin has any teratogenic effects. The treatment consisted of ingestion by gavage of 32.5 mg/kg per day of plumbagin or olive oil (control groups), on GD 8-15. During pregnancy, the rats were closely observed for survival, changes in appearance, behaviour, and signs of vaginal bleeding, food and water consumption, and weighed daily to monitor toxicity throughout the experiment. The maternal weight gain was recorded during the entire pregnancy (total weight gain) and during the treatment period. On day 21 of pregnancy, the females were killed by chloroform inhalation and their uterine horns removed by Caesarean section. Placenta of dams were taken and weighed while the fetuses undergo fetal staining. The number of fetuses was recorded and all fetuses were examined for obvious external malformations before subsequent processing. For skeletal examination, the number of skeletal elements was counted and any malformations or variations were recorded.

3. Statistical Analysis

All data were expressed as means ± S.E.M. (standard error of means). Data on maternal organ weights, fetal weights and placenta weights were subjected to one-way analysis of variance (ANOVA), followed by the Duncan test to compare the mean differences between experimental groups while data on body weight, regression analysis was applied. P value less than 0.05 was selected as the level of statistical significance.

4. Results and Discussions

4.1. Effects of Plumbagin on Maternal Body Weight

The effect exerted by the oral administration by gavage of plumbagin at dose 32.5 mg/kg was shown in Figure 1.0. Body weight of pregnant rats showed a significant decrease (p < 0.05) when compared to the control and olive oil group where the mean weights ± S.E.M of control, olive oil and plumbagin are 270.71 ± 4.412, 242.75 ± 2.767 and 219.02 ± 1.932 respectively.

![Figure 1: Effects of Plumbagin on Maternal Body Weight, Data Shows the Mean Weight ± S.E.M in Different Treatment Groups, C – Control; OL – Olive Oil; PL – Plumbagin * P < 0.05. A and B Denote Significance](image-url)
4.2. Effects of Plumbagin on Fetus Numbers, Fetus and Placental Weights

The results for number of fetuses, fetus and placental weight in all groups are shown in Table 1.

|                              | Control | Olive Oil | Plumbagin |
|------------------------------|---------|-----------|-----------|
| Number of females            | 6       | 7         | 6         |
| Number of females with fetuses | 6      | 5         | 1         |
| No. of fetuses (mean ± S.E.M) | 10.67 ± 0.803 | 7.00 ± 2.082 | 1.33 ± 1.333 |
| Fetal weight (g) (mean ± S.E.M) | 3.76 ± 0.134 | 3.83 ± 0.126 | 3.7783 |
| Placental weight (g) (mean ± S.E.M) | 0.68 ± 0.060 | 0.69 ± 0.014 | 0.63 |

Table 1: Fetus Numbers, Fetus and Placenta Weights of Control Group, Olive Oil Group and Plumbagin Group

4.3. Examination for Skeletal Anomalies

The results for skeletal anomalies in all groups are presented in Table 3.

|                              | Control | Olive Oil | Plumbagin |
|------------------------------|---------|-----------|-----------|
| Total number of fetuses      | 64      | 49        | 8         |
| External malformations       |         |           |           |
| Affected litters             | 0/6     | 0/7       | 0/6       |
| Affected foetuses            | 0/64    | 0/49      | 0/8       |
| Skeletal malformations       |         |           |           |
| Affected litters             | 2/6     | 1/7       | 1/6       |
| Affected fetuses             | 2/21    | 1/11      | 2/4       |
| Vertebral anomalies          | 2/21    | 1/11      | 2/4       |
| Damaged fetuses              | 43      | 38        | 4         |

Table 2: External and Skeletal Malformations and/or Variations in Fetuses of Different Groups

The weight gain of mothers during pregnancy was altered by treatment with plumbagin and because weight loss is considered to be a good indication of toxicity, this suggests that plumbagin gives toxic effect to mothers. Another indication of toxicity is the alterations in placental weight in the treated groups. Decreased number of fetuses in mother in plumbagin groups suggests the lethal effect of plumbagin without any teratogenic effects. This is because the dose given may not be enough to cause teratogenic effects to fetuses. This preliminary experiment suggests that plumbagin showed significant abortifacient activity in late pregnant Sprague Dawley rats and cause acute toxicity to the dams by reducing its body weight without any teratogenic effects. To assess prenatal toxicity caused by chemical agents, factors that should be considered are the phase of pregnancy, the doses given and the difference in the sensitivity that mammals have when exposed to these agents. To start appropriate experimental protocols, these factors are very important. The selection of the dosage for this study was adapted from Premakumari et al. (1997) (LD50 65 mg/kg). Thus, to prepare the dosage of plumbagin for plumbagin group, 50% of LD50 is made which is 32.5 mg/kg. The plumbagin is dissolved in olive oil because it cannot be dissolved in distilled water or Tween 20. However, these serve a slight problem as olive oil also has antioxidant properties as plumbagin and showed similar effects in the experiment.

Maternal parameters, such as food and water consumption, body weight changes and clinical signs of toxicity were determined, permitting a clear assessment of maternal homeostasis integrity. The rats were treated with 32.5 mg/kg per day of plumbagin from day 8 to 15 of gestation which is during organogenesis. During the period of organogenesis, effects of chemicals or drugs may be visible as abortions, malformations or retarded development. Rogers et al. (2001) states that fetus can undergo toxic effects due to maternal toxicity. The weight gain of mothers during pregnancy was altered by treatment with plumbagin and because weight loss is considered to be a good indication of toxicity, this suggests that plumbagin has a toxic effect on the dams. Decreased number of fetuses in plumbagin groups suggests lethal effect of plumbagin without any teratogenic effects. This suggests that the dosage given to the dams may not be sufficient to cause teratogenic effects to the fetuses. According to Lemonica (1996), a comparison of the teratogenic effect of chemical substances between humans and animals showed that laboratory animals are generally more resistant than humans.

In this study, the results showed an abortifacient activity of plumbagin in pregnant rats. This is due to the vaginal bleeding observed in rats in plumbagin groups. The vaginal bleeding occurred during the treatment of plumbagin which is within day 8 to 15 of gestation. Initially, rats in plumbagin groups appeared pregnant (swollen belly) with the detection of vaginal plugs after being kept with male rats overnight. In addition, indication of positive pregnancy is by the increase in weight gain drastically by the rats each day before the treatment begins. When the treatment of plumbagin begins, there are behavioural changes of the rats such as inactivity and crouching in the corner of the cage, decreased food and water consumption and a reduction in weight gain were observed. The abdominal part of the rats became reduced in size thus this indicates that the rats have aborted. The aborted fetuses cannot be observed since rats are placentalphyia where they ate their aborted fetuses immediately once the fetuses come out from the vagina. Rats in plumbagin group gave the lowest number of babies when compared to other groups. This indicates abortifacient activities of plumbagin in pregnant rats. When the rats are dissected, the uterine horn of the rats with no babies from plumbagin group appeared dilated and hypervascularized. There are also raised areas on the uterine wall.
Preimplantationary loss was resulted when plumbagin treatment was given during the first seven days of gestation where uterine proteins of 13000, 19000, 26000 and 75000 Da molecular weights were abolished. (Review by Jain et al., 2014). Aborted rats that were administered Plumbago zeylanica root powder from day 6 to 17 of gestation were found out with absent of proteins having molecular weights 55000 and 65000 Da. Thus, these results suggest that to maintain pregnancy, proteins having molecular weights of 55000 and 65000 Da are required while proteins of 13000, 19000, 26000 and 75000 Da influence the implantations.

Plumbagin did not alter fetuses and placenta mass in doses up to 32.5 mg/kg in rats. The weight of fetuses and placenta are normal and were not significantly different in all groups. The size of fetuses from control, olive oil and plumbagin are similar and there were no fetuses with external malformation observed. For the skeletal anomalies, dumbbell-shaped vertebrae were observed in fetuses from control, olive oil and also in plumbagin groups. Dumbbell-shaped vertebral anomalies are common abnormalities which can be observed even in fetuses from normal groups (Hussein pers. com., 2008). Thus, skeletal examinations of the fetuses did not reveal any malformations, anomalies or variations that could be attributed to the treatment. Some toxic substances can affect the organs such as kidneys, liver, nervous system, heart and muscles of the body. Damage to organs "system-wide" outside the original point of contact is known as systemic effect. Thus, these organs may be damaged while operating this function. Recent studied by Tilak et al. (2002) states that plumbagin, isolated from Plumbago zeylanica show antioxidant effects in the type of membrane protective properties in mitochondria from three different rat tissues, liver, brain and heart. Research done by Boskou et al. (2005) said that olive oil has high amount of tocopherols, carotenoids, sterols and phenolic compounds which are natural antioxidants. O-dihydroxy-phenolics are very strong antioxidants found in extra-virgin olive oils. There is a possibility that both plumbagin and olive oil’s effects can be damaging to body organs especially on the liver and kidneys. However, the liver and kidneys were not examined in this study.

5. Conclusion
The results obtained suggest that plumbagin can cause acute toxicity in dams by reducing its appetite and causing generalized weight loss. No teratogenic effects were observed in fetuses from plumbagin treated group. Therefore, plumbagin only has abortifacient effects when fed to Sprague dawley rats during late pregnancy.

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