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Anti implantation effects of *Jatropha curcas* crude oil when fed to pregnant Sprague dawley rats during the early gestation period

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Abstract
Jatropha curcas is a species of flowering plants in the spurge family, Euphorbiaceae. The seeds contain 27-40% oil consisting of curcin and tetramethylpyrazine which are toxic and cause abortion, fetotoxicity and teratogenic effects if consume during pregnancy. Twenty four *Sprague dawley* rats at early gestation weighing about 200-300g were randomized into four groups: Positive control group was orally administered with retinyl palmitate, negative control group with corn oil (vehicle), treatment group with 0.7 ml/kg body weight of the oil and the normal group was given distilled water. Females were paired overnight with male rats and presence of sperm in the vaginal smears indicated positive mating and considered pregnant day 1. Rats were dosed orally once daily from day 1 till day 7 of gestation. Maternal body weights were also recorded daily. Rats were euthanized by diethyl ether inhalation on day 8 and the uteri removed and stained with 1% ammonium sulphide. The number of implantation sites was counted and data obtained were analysed using SPSS. There is a significant reduction in number of implantation sites i.e., treatment groups, positive and negative control and normal group are 0.83±0.401, 4.5±0.428, 8.67±0.333, 9.17±0.307 respectively. There is also a significant difference between treatment and control group in maternal body weights. In addition, a high percentage (90%) of anti-implantation activity was recorded in treatment groups. The small number of implantation sites and high percentage of anti-implantation activity suggests that *Jatropha curcas* crude oil have anti-fertility effects when fed to pregnant rats during early gestation.
Keywords: *Jatropha curcas*; anti implantation; early pregnancy.

1.0. Introduction

The increasing use of herbal medicines has resulted in concern about efficacy and safety of these products. Public especially pregnant women should be made aware of the adverse effects of herbal products [1]. *Jatropha curcas* is a species of flowering plant in the family of Euphorbiaceae [2]. Around the world, this plant is known by nearly 200 different names, which indicated its significance and various possibilities of its uses [2]. In Malaysia, this plant is known as pokok jarak pagar. It is potentially used as an abortifacient, for contraception, purgative, anti inflammatory, anti helminthic, anti tumor agent, treat skin diseases and jaundice [3]. Analysis of its seeds shows variety of chemical composition such as protein, fat, carbohydrate and fibre. The seed content is about 35-40% of oil [4]. The seed is a source of highly poisonous toxalbumin known as curcin which have been found as the lead compound leading to abortion, have fetotoxicity and teratogenicity effects (5, 6). The contraceptive effect has been confirmed with laboratory rats [7]. A study conducted in 1963 demonstrated a 100% anti fertility effect when female rats were fed with the fruit and seed separately in a quantity of 3.3% of a stock diet. The diets were given for a total period of 25 days consecutively of which 10 were immediately preceding mating. The males and the control females were fed only the stock diet [7]. A previous study given methanol, petroleum ether and dichloromethane extract of *Jatropha curcas* indicate the abortifacient properties of the fruit. The effect could be accomplished even when the extracts were given from day 6^{th} to 8^{th} of pregnancy. Loss of body weight during the dosing period, ranging from slight to severe was seen in treated animals [8]. However, despite the abortifacient claims of *Jatropha curcas* in folklore medicine in African countries, laboratory studies on the effect of *Jatropha curcas* on pregnancy appear to be limited to Mameesh *et al*. (1963) and Goonasekera *et al*. (1995). Furthermore, there is not a single study that shows how *Jatropha curcas* affects early pregnancy. Therefore, the present study was undertaken to investigate the anti-implantation effects of feeding *Jatropha curcas* crude oil to pregnant *Sprague dawley* rats.

2.0. Methods

2.1. Production of *Jatropha curcas* Crude Oil

Jatropha curcas crude oil was purchase commercially from Agolink Sdn. Bhd, Malaysia. Several steps were taken to produce crude oil from the seeds. The shell of Jatropha kernel was first removed by using a shell remover. Then, the kernel and seeds were separated with a seed separator. The seeds were then left to dry for 3 to 5 days. After that, oil from the dried seeds was expelled by using oil expeller through screw type method where the seeds were pressed to release oil. The oil was left to settle down and a clear yellowish *Jatropha* crude oil was produced.

2.2. Preparation of Retiny Palmitate

Retinyl palmitate is purchased commercially from Fisher Scientific, Malaysia. It is dissolved in corn oil before feeding to the pregnant rats. According to Material safety data sheet Vitamin A USP MSDS for the Oral LD_{50} for retinyl palmitate in rats is 7910mg/kg. The optimum dose for retinyl palmitate to give effect is 90 mg/kg per day. For this experiment, we prepare 30,000USP/kg after converting the unit according to the international unit system.

2.3. Laboratory Animals

Twenty four pregnant *Sprague dawley* rats weighing 200-300g was obtained from the Laboratory Animal Unit, Faculty of Medicine and Health Sciences, University Putra Malaysia with ethics approval from the Animal Ethics Committee, UPM (ACUC). The animals were fed on standard pellet diet and water. The cages were cleaned every week. and animals were maintained under standard environmentally controlled room temperature and light.

2.4. Determination of Anti Implantation Activity

The female rats were mated overnight with the male rats at a ratio of 1:1 in special cages with a tray at the bottom of the cage. The presence of a vaginal plug in the pan or presence of spermatozoa in the vaginal smear was considered Day 1 of pregnancy. The female rat was transferred into different cages with free access to food and water. Then, they were randomized into control and test groups. Group A (positive control) was orally administered with retinyl palmitate, Group B (vehicle control) was orally administered with corn oil. Group C (treatment) was orally administered with 0.7 ml/kg body weight of *Jatropha curcas* crude oil and Group D (negative control) was orally administered with distilled water only. The treatment commenced on Day 1 until Day 7 where the pregnant rats were given the exact dosage depending on their weights. The animals were orally gavage daily for 7 days. The dose was determined according to the body weight. The maternal weight was recorded during the entire pregnancy (total weight gain) and during the treatment period. During pregnancy, the females were observed everyday for survival, changes in appearance, behaviour, signs of vaginal bleeding, food and water consumption. On day 8 of pregnancy, the rats were sacrificed using excess diethyl ether inhalation and their uteri removed by Caesarean section. The uteri of the pregnant rats were collected and kept in phosphate buffered saline in a Petri dish to prevent drying [9].

2.5. Uterus Examination

Implantation sites were visualized by staining the uterus with 1% ammonium sulphide for 20 min. The uteri were stained blue and implantation sites (if available) were identified by the unstained bands along the uterus and [10].

2.6. Anti-implantation Activity Calculation

The anti implantation activity for each sample was calculated using the following formula [11].

$$\text{Anti implantation activity} = \frac{\text{No of implants in control} - \text{No of implants in test group}}{\text{No of implants in control group}} \times 100$$

2.7. Statistical Analysis

The results were expressed as means ±S.E.M. Means were analyzed using One-Way ANOVA and Dunnett test to compare the mean differences between experimental groups. Values of P less than 0.05 were considered statistically significant.

3.0. Results and Discussion

3.1. Effect of *Jatropha curcas* crude oil on number of implantation sites

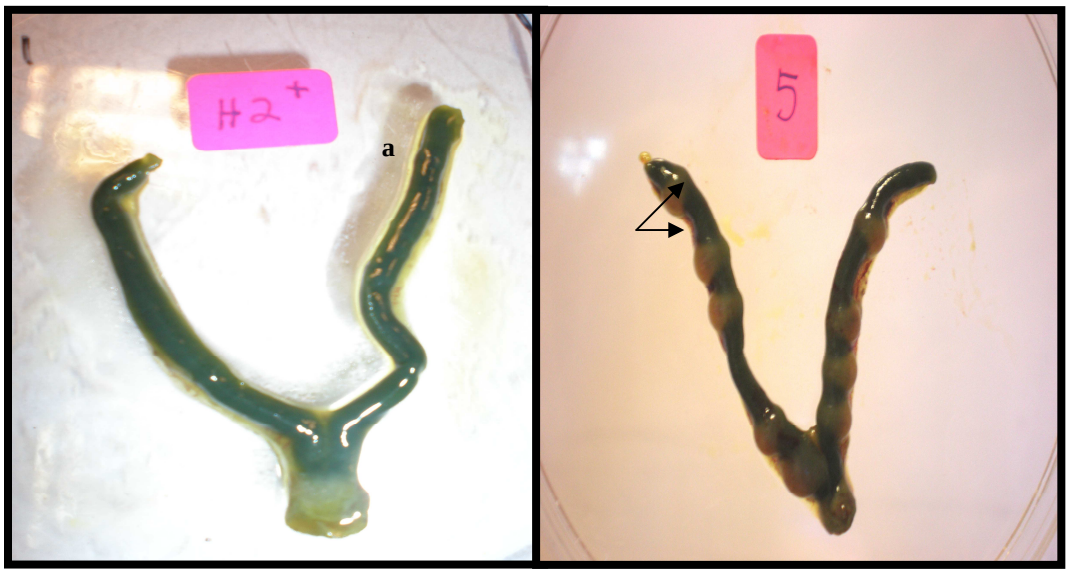
Number of implantation sites in group C was significantly reduced compared to group D (0.6±0.267 vs. 8.6±0.267). Mean ±SEM for number of implantation sites for all groups was shown in Table 1. The number of implantation sites in Group D and Group C was shown in Figure 1. The anti-implantation effects of *Jatropha curcas* crude oil may be due to natural toxic substances in the seeds i.e., curcin. Purified proteins from this fraction have been shown to inhibit protein synthesis in vitro in a way similar to ricin from castor (4). The seed also showed pregnancy terminating effects in rodents, but it is unclear whether this is due to a specific action or result of general toxicity (8).

Table 1. Means±SEM of number of implantation sites for all groups

Group	Means±SEM
Group A/Positive control/(Retinyl palmitate)	4±0.365*
Group B/Vehicle control (corn oil)	8.6±0.267
Group C/Treated group *(Jatropha curcas crude oil)*	0.6±0.267*
Group D/Negative control (distilled water)	9.17± 0.307
Total means	4.4±0.299

* Significance comparable with negative control at $p<0.05$

Figure 1. Implantation sites in uterus from A) Group C and B) Group D



Uterus from Jatropha curcas group Uterus from negative control group.
Note : implantation sites (a)

3.2. Effects of *Jatropha curcas* on anti-implantation activity

Table 2 summarizes the number or rats with implantation and the total number of implants in each group. It shows the percentage anti implantation activity exhibited by each groups in comparison to the corresponding controls. It was noted that some of the rats in group C did not show any implantation. As shown in Table 2, anti implantation activity of *Jatropha curcas* was 90%. The anti-implantation activity indicates the presence of one or more active ingredients in the seed of *Jatropha curcas*. It was previously reported that the anti implantation effect of such agents could be due to their estrogenic nature [12]. It has been reported that administration of low concentrations of compounds with estrogenic activity to many species during early pregnancy resulted in rapid passage of ova through oviducts and expulsion of ova from uterus [13]. The reduction number of implants observed in group C in the present study might be due to such properties of the extract. However, this is not conclusive since a decrease in implantation could be due to a non-conducive uterine environment rather than rapid transport. The exact mechanism of anti implantation in this study requires further investigation.

Table 2. The percentage of anti-implantation activity for each group

Groups	n	No of rats showing implantation	Total no of implants	Anti-implantation activity (%)
Group A	6	6	27	47
Group B	6	6	51	0
Group C	6	3	5	90
Group D	6	6	55	0

3.3. Effects of *Jatropha curcas* on maternal body weight

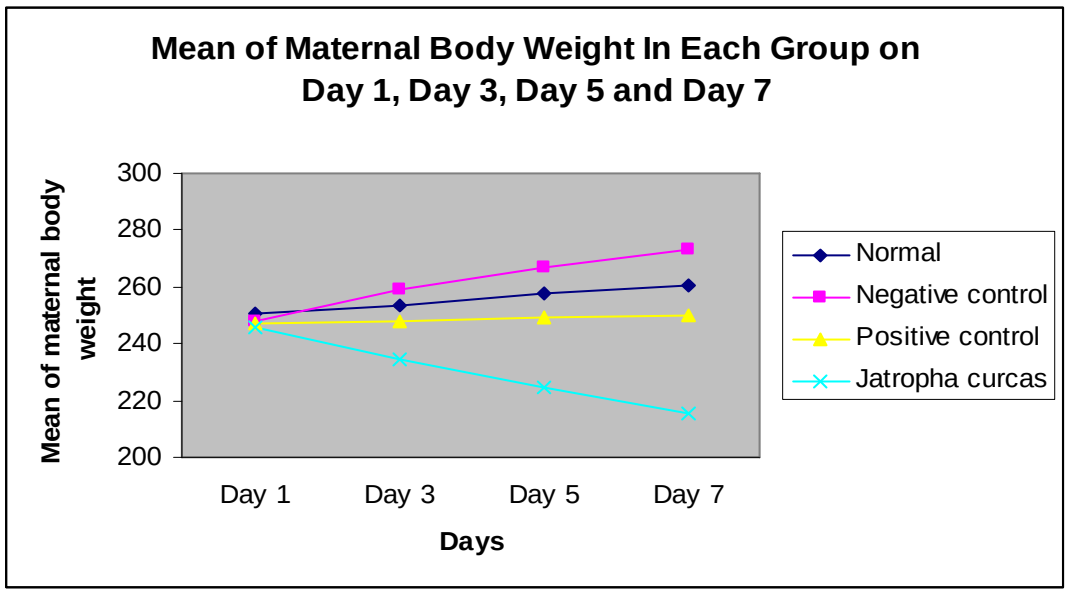
Body weight of rats in group C were significantly reduced compared with group D (Table 3). The means for maternal body weight on Day 1, Day 3, Day 5 and Day 7 for all groups were illustrated in Figure 2. As compared to positive control, maternal body weight is significantly reduced in group C. This suggests that *Jatropha curcas* crude oil has toxic effects on pregnant *Sprague dawley* rats as weight loss is considered to be a good indication of toxicity. This is in agreement with the results obtained from a study by Goonasekera *et al*. (1995), which showed loss in body weight in treated animals dosed with all extracts of *Jatropha curcas* seed.

Table 3. Means of maternal weight for each groups in early gestation period on Day 1, Day 3, Day 5 and Day 7

Groups/Days	Means±SEM			
	Day 1	Day 3	Day 5	Day 7
Group A	247.5±3.294	248.17±3.198	249.17±3.156	250±3.235
Group B	247.67±7.223	258.83±4.143	266.6 ±4.096	273.5±5.252
Group C	246±10.933	234.17±13.169	224.33±14.823*	215.17±16.051*
Group D	250.5±9.76	253.5±8.819	257.5±7.274	260.33±6.883
Total Means	247.9±7.8	248.67±7.33	249.4±7.33	249.75±7.86

* Significance comparable with negative control at $p<0.05$

Figure 2 : Changes in the mean of body weight of dams in each groups on day 1, day 3, day 5 and day 7



4.0. Conclusions

From the results obtained, it can be concluded that *Jatropha curcas* has anti implantation effects on pregnancy as the number of implantation sites are lower compared to negative control. In addition, *Jatropha curcas* showed a high percentage of anti- implantation activity thus supporting the evidence that the crude oil of seeds of *Jatropha curcas* can cause abortion. It can cause also acute toxicity in dams by reducing their weights during the dosing period. This study thus justifies the folkloric claim that *Jatropha curcas* crude oil has anti implantation effects when consumed during early pregnancy.

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