UNIVERSITI PUTRA MALAYSIA

MATERNAL TOXICITY AND TERATOGENIC EFFECTS OF \textit{Jatropha curcas} L. OIL IN PREGNANT \textit{Sprague dawley} RATS

YON THANNIA BINTI SAMAT

FPSK(m) 2013 34
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MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2013
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YON THANNIA BINTI SAMAT

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment on the Requirements for the Degree of Master of Science

October 2012
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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YON THANNIA BINTI SAMAT

October 2012

Chair: Associate Professor Sabrina Sukardi, PhD

Faculty: Medicine and Health Sciences

*Jatropha curcas* (L.) (*Euphorbiaceae*), is a large shrub, attaining 3-4 m in height, common in Brazil and also found in India and Africa in semi-wild conditions. Laboratory studies on the effects of *Jatropha curcas* on pregnancy is limited and the number of experimental animals used in most studies were insufficient for any firm conclusions to be drawn. Research however has shown that it has pregnancy terminating effects in rats and mice and was used widely in some African countries for contraceptive intentions. Feeding studies also showed that the whole seeds were highly toxic. Curcin, a toxic protein isolated from the seeds, inhibits protein synthesis in *in vitro* studies even though it is less toxic than ricin and abrin. The oil of *Jatropha* contains irritant phorbol esters which causes purgative and skin irritant effects and possess tumour-promoting (co-carcinogenic) properties. In this study, pregnant Sprague-Dawley rats were administered *Jatropha* crude oil (JCO) orally at doses of 0.175, 0.35 or 0.7 g/ml during embryogenesis (Gestation day (GD) 1-7) for early gestation group and during organogenesis on GD 8-14 for late gestation group to
examine any toxic effects. On day 21st of pregnancy, the rats were anesthetized with chloroform. Ovaries and uteri were removed by Caesarean section. Heart, ovaries, placenta, liver, intestines, stomach, kidneys, and lungs of dams were collected and weighed. In addition, number of fetuses were recorded and examined for obvious external malformations before subjected to fetal staining to assess teratogenic effects. Placentas were stored in 10% buffered formalin for subsequent histopathology examination to observe effects of JCO on placental morphology. Results were reported as means ± S.E.M. Data were analyzed with SPSS. Two-way ANOVA followed by Duncan post hoc test were used to determine the degree of significance for the various mean variables obtained and p < 0.05 was considered significant. Body weight of rats exposed to JCO at doses 0.35 and 0.7 g/ml examined were significantly lower (p < 0.05) than those of controls who only received corn oil. No fetuses were observed with external malformations in this study but for skeletal malformations, variations or abnormalities observed in fetuses from treated groups include dumbbell shape vertebrae, split vertebrae, wavy ribs, poorly ossified sternum and xiphisternum and hypoplastic sternum. Placentas of rats exposed to JCO showed histological changes in maternal-fetal interface, trophoblastic giant cell layers, and labyrinth layer with an increase in abnormal trophoblastic giant cells which has atypical shape with pyknotic and irregular nuclei. The results of this study indicate that JCO causes acute toxicity to the dams, induce teratogenic effects on fetuses and stimulate deleterious effects on placenta.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN TOKSIK TERHADAP IBU DAN TERATOGENIK OLEH MINYAK ASLI \textit{Jatropha curcas} DALAM TIKUS \textit{Sprague dawley} BUNTING

Oleh

\textsc{Yon Thannia Binti Samat}

Oktober 2012

Pengerusi: Profesor Madya Sabrina Sukardi, PhD

Fakulti: Perubatan dan Sains Kesihatan

\textit{Jatropha curcas} (L.) (Euphorbiaceae), adalah sejenis pokok tidak berkayu dengan ketinggian 3-4 m, yang berasal dari Brazil dan juga India dan Africa dalam keadaan separa liar. Ujikaji makmal akan kesan \textit{Jatropha curcas} semasa kebuntingan terhad dan bilangan haiwan kajian yang digunakan dalam ujikaji kebanyakan juga tidak mencukupi untuk membuat sebarang kesimpulan yang kuhuk. Penyelidikan telah menunjukkan bahawa ia mempunyai kesan keguguran pada tikus dan mencit dan juga digunakan dengan meluas di negara tertentu seperti Afrika untuk tujuan mencegah kehamilan. Kajian pemakanan telah menunjukkan bahawa keseluruhan biji adalah sangat toksik. Curcin, sejenis protein bersifat toksik dari biji, boleh merencat sintesis protein dalam kajian \textit{in vitro} walaupun ianya kurang toksik daripada ricin dan abrin. Minyak \textit{Jatropha} mengandungi phorbol ester yang mengakibatkan cirit birit serta kegatalan kulit dan juga mengandungi bahan-bahan penyebab tumor. Di dalam kajian ini, tikus \textit{Sprague-Dawley} yang bunting diberi minyak mentah \textit{Jatropha} melalui mulut pada dos 0.175, 0.35 dan 0.7 g/ml ketika pembentukan embrio iaitu pada hari ke 1-7 kebuntingan bagi kumpulan awal.
kebuntingan dan ketika pembentukan organ pada hari ke 8-14 kebuntingan bagi kumpulan akhir kebuntingan untuk mengkaji kesan toksiknya dalam tikus bunting. Pada hari kebuntingan yang ke 21, ibu tikus dikorbankan dengan overdos klorofom. Rahim serta ovari dikeluarkan secara kaedah potongan "Caesarean". Jantung, ovari, uri, hati, usus, perut, buah pinggang dan peparu ibu tikus dikeluarkan dan ditimbang. Bilangan janin dicatat dan kesemua janin diperhatikan untuk kecacatan luaran sebelum menjalani proses pewarnaan janin bagi mengkaji kesan teratogenik. Uri disimpan di dalam 10% cecair formalin bagi menjalani pemeriksaan histopatologi untuk mengkaji kesan minyak mentah *Jatropha* terhadap histologi uri. Keputusan dicatat dalam bentuk nilai min ± SEM. Data dianalisis menggunakan ANOVA dua hala diikuti dengan ujian Duncan post hoc dan nilai p<0.05 dianggap signifikan. Berat badan tikus yang terdedah kepada minyak mentah *Jatropha* dengan dos 0.35 dan 0.7 g/ml didapat lebih rendah secara signifikan (p < 0.05) berbanding berat badan tikus kawalan yang hanya menerima minyak jagung. Tiada janin yang mengalami kecacatan luaran dalam kajian ini tetapi untuk kecacatan tulang, variasi atau ketidak normalan yang dapat dilihat pada janin daripada kumpulan rawatan termasuklah vertebra berbentuk “dumbbell”, vertebra terpisah, rangka berombak, sternum, dan xiphisternum yang kurang osifikasi serta sternum hipoplastik. Uri tikus dari kumpulan rawatan menunjukkan perubahan histologi pada interfasa ibu- janin, lapisan sel gergasi trophoblastik dan lapisan labirin dengan peningkatan dalam sel gergasi trophoblastik mempunyai bentuk atipikal dan nukleus yang piknotik dan berbentuk tidak sekata. Keputusan kajian ini menunjukkan bahawa minyak mentah *Jatropha* mengakibatkan toksisiti akut kepada ibu tikus, menyebabkan kesan teratogenik terhadap janin dan mengakibatkan kesan kerosakan pada uri.
ACKNOWLEDGEMENTS

In the name of Allah, more gracious, most merciful. Praise and thanks must be given first to Him who has provided me with patience, health, courage and knowledge to complete this study. Peace and blessing of Allah be upon last Prophet Muhammad S.A.W.

I would like to express my sincere gratitude to Associate Professor Dr. Sabrina Bt. Sukardi, my supervisor for her guidance, assistance, wise advice and kind understanding all throughout my studies here and during the evaluation, finding and completion of my thesis to the end.

Next, I would like to also express my appreciation and thanks to laboratory assistants, Miss Farhatani (Science Officer), Miss Noridah (MLT) and Encik Shahidan (Supporting Staff) for the lab instruments that I used in this project and also not forgetting Encik Ramli who help me to get an animal supplier for my project and Dr. Khairulnizam for his wise advice.

Finally, to my beloved parents, a big appreciation for their kind support and understanding in completion of this project. Not forgetting also my husband Mohd Zuhri, my friends; Joan Blin, Muhammad Hussaini, Siti ‘Aishah, Nur Haziyah and also to my beloved lab mates who has been a great help throughout the completion of the project.
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Sabrina Sukardi, PhD**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Noordin bin Mohamed Mustapha, PhD**  
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**BUJANG BIN KIM HUAT, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia  

Date:
DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

YON THANNIA BINTI SAMAT
Date: 11 October 2012
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