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ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 11, Issue, 01, pp.10708-10711, January, 2020

RESEARCH ARTICLE

EFFECTS OF FEEDING JATROPHA CURCAS CRUDE SEED OIL ON PLACENTAL HISTOLOGY DURING THE EARLY AND LATE GESTATION PERIOD IN FEMALE SPRAGUE DAWLEY RATS

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ARTICLE INFO

Article History:
Received 25th October, 2019
Received in revised form
29th November, 2019
Accepted 17th December, 2019
Published online 31st January, 2020

Key words:
Jatropha Curcas Seed Oil, Gestation Period, Placenta, Toxicity, Sprague-Dawley Rats, Contraception, Curcin, Histopathology, Trophoblastic Giant Cells, Labyrinth Layer.

ABSTRACT

Jatropha curcas (L.) (*Euphorbiaceae*), is a large shrub, common in Brazil, India and Africa and thrive well in semi-wild conditions. This plant has potential therapeutic properties in man and animals. The seeds contain 30% oil which produce high-quality biodiesel. Feeding studies showed that the seeds contain curcin, a toxin which inhibits protein synthesis in *in vitro* studies. The oil also has pregnancy terminating effects in rats and mice and is used for contraceptive purposes in some African countries. A study was conducted to determine toxicity effects of Jatropha seed oil (JCO) on the placenta when fed to female Sprague-Dawley rats during early gestation (day 1-7) and late gestation (day 8-14). On day 21st of pregnancy, rats were sacrificed and uteri removed. Placentas were processed for histopathology. The data obtained was analyzed with SPSS using Friedman Test and Wilcoxon test and a p value < 0.05 is considered to indicate a significant difference. Treated group showed histological changes in maternal-fetal interface, trophoblastic giant cell layers and labyrinth layer with an increase in abnormal trophoblastic giant cells having atypical shape with pyknotic irregular nuclei. The results indicate that JCO stimulate deleterious effects on the placenta which were more pronounced during late gestation.

Citation: ***Vetorya A/P Palavandran, Yon Thannia Samat, Herni Talib and Sabrina Sukardi.*** **2020.** "Effects of feeding jatropha curcas crude seed oil on placental histology during the early and late gestation period in female sprague dawley rats.", *Asian Journal of Science and Technology*, 11, (01), 10708-10711.

INTRODUCTION

Jatropha curcas or Purge nut from the Euphorbiaceae family is locally known as Pokok Jarak Pagar in Malaysia. A drought resistant shrub or tree, it thrives well in semi dry conditions. This plant originated in Mexico and Central America. It is widely used in traditional medicine. The oil from the seeds are processed for biofuel. The major fatty acids in Jatropha seed oil were oleic acid, linoleic acid , palmitic acid and stearic acid while the most prominent triglycerides were OLL and OOL (Akbar *et al*., 2009). The seeds contain 27–40% oil (Achten *et al*., 2007) (average: 34.4%) that can be processed to produce a high-quality biodiesel fuel, usable in a standard diesel engine. Edible (non-toxic) provenances can be used for animal feed and food (King *et al*., 2009; Moniruzzaman *et al*., 2015). Jatropha curcas plant can be use as a purgative, anti-inflammatory, antitumor, antihelminthic, diuretic agent, to treat gout, paralysis, skin diseases, rheumatic conditions, fever and jaundice andd as animal food (reviewed by Thomas *et al*., 2008; Devappa *et al*., 2010). Detoxification of Jatropha leaves must be carried out before it can be use for animal feed (Gomes *et al*., 2018).

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As early as the 1960s, the plant shoots were consumed for contraceptive purposes in some African countries (Watt and Breyer, 1962). Women take them as a source of anti-fertility drugs (Farnsworth *et al*., 1975; Chaudry, 1986). Various methanolic extracts of the seeds have contraceptive and abortive action in rats and mice (Goonasekara,1995), anti implantation effects in rats (Alwi and Sukardi, 2013) and skeletal malformation effects to rat fetuses from dams fed during early and late gestation (Mutalib *et al*., 2014). Curcin(seeds) are highly irritant and remains in the seed after the oil has been expressed. It contains the toxin having ribosome inactivating protein (RIP) activity (Barbieri *et al*., 1993). Curcin has been purified and characterized (Ji *et al*., 2010) and causes posioning if accidentally eaten (Singh *et al*., 2011). Phorbol esters which are tetracyclic diterpenoids have tumor promoting activity (Goel *et al*., 2007) and its discovery in seed oil raised awareness of the danger of public use of the seed oil and seed cake in Thailand and the need for cancer preventions (Fujiiki *et al*., 2017). Pregnant rat is an important animal model in reproductive testing (De Rijt, 2002). The rat placenta comprised of two structures:which are the choriovitelline placenta during the early stage of gestation and the chorioallantoic placenta during mid gestation. The junctional zone (at the maternal surface) is involve in endocrine and invasive function, the labyrinth zone

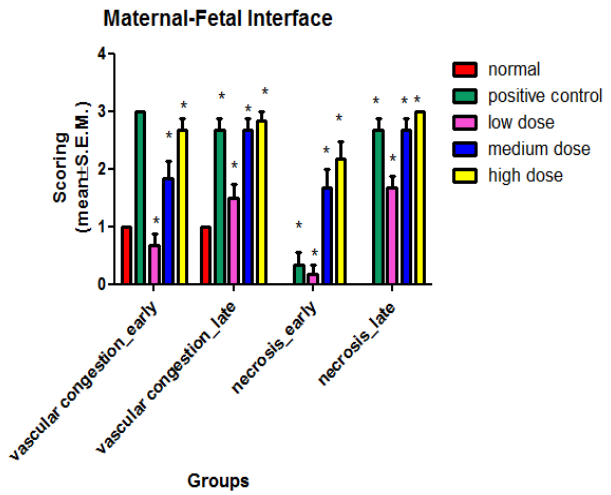

Figure 1. Effects on maternal-fetal interface in different groups during early and late treatment

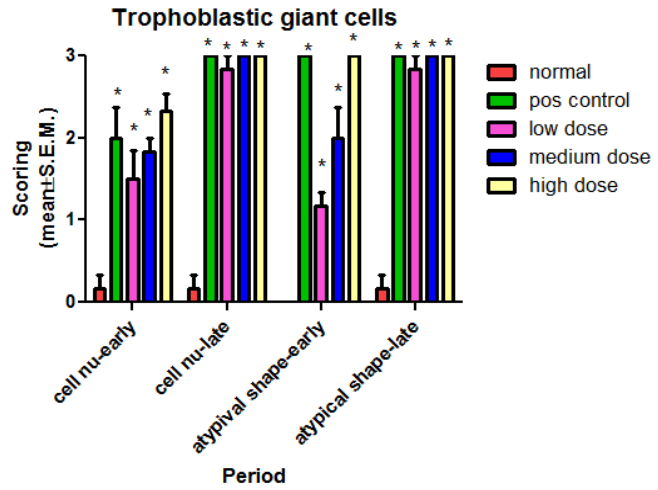

Figure 2. Effects on trophoblastic giant cells in different groups during early and late treatment

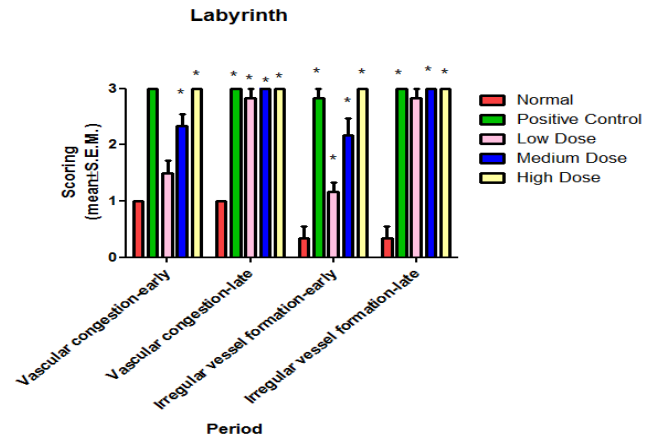

Figure 3. Effects on labyrinth in different groups during early and late treatment

(at the fetal surface) will form the transport barrier and both zones comprised of trophoblast cells which regulates transport of nutrients and wastes across the placenta. The interface between the maternal and fetal circulatory system is important for fetal development and growth of the fetus.

Bi-directional transfer of nutrients and oxygen between the maternal and fetal circulations occurs in rats (Raso *et al*., 2008). The similiarity between human and rat placenta is that both have the same origin (chorioallantoic membrane) and the hemochorion is where trophoblast cells are directly bathed by maternal blood (Knipp *et al*., 1999). The difference is human placenta is the villous type while rat placenta is the labyrinthine type (De Rijk, 2002). In addition, human pllacenta has only one layer of cells separating the mother and the fetus blood (hemomonochorial) while the rat has more than one layer (hemotrichorial). Placental circulation is also established quite early in women and much later in rodents. This study was conducted to observe the effects of feeding Jatropha curcas crude seed oil on placental histology in pregnant Sprague- Dawley rats dudirng the early and late gestation period.

METHODOLOGY

Animals: Sexually mature female Sprague dawley rats were used. The animals were adapted to laboratory surroundings and allowed to undergo three estrus cycles. Monogamous mating procedure was carried out and females were left overnight with proven male. Evidence of a vaginal plug and sperm in the vaginal smear will be designated as day one gestation. Oil was given through oral gavage daily for day 1-7 (early gestation) and days 8 - 15 (late gestation). Five groups (Low, Med and High Dose, Negative control (corn oil) and positive control (Retinyl palmitate). Dose of JCO was given according to Poon *et al*., (2013). Negative control is corn oil which is normally use at 10 ml/kg as a vehicle for test agent in teratogenic studies. Corn oil has been found to cause nephrotoxicity in pregnant and lactating dams thus providing a confounding factor (Sato *et al*., 2000). Therefore corn oil is given in one group of animals to confirm that effects observed may be due to the corn oil itself. Retinoids in retinyl palmitate was used as the positive control in this study as it represents one of the better studied classes of teratogens. Excess vitamin A and its metabolites, particularly retinoic acid can be teratogenic (Sommer, 2008).

Jatropha curcas crude seed oil: The oil was purchase commercially from Agolink Sdn. Bhd in Kluang, Johor, Malaysia. There are several steps that had to be taken to produce crude oil. Firstly the shell of Jatropha kernel was first removed by using shell remover. Then, the kernel and the seed will be separated by using seed separator. The seeds will then be left to dry for 3 to 5 days. After that, the oil from the dried seeds will be expelled by using oil expeller through screwed type method where seeds of Jatropha will be pressed until oil comes out. The oil will then be left to settle down until a clear yellowish Jatropha crude oil was produced.

Histopathology of placenta: On day 21 of gestation, all animals were sacrificed and the placentas collected and stored under -20˚C. Placentas were processed according to standard tissue processing procedures, stained with Haemotoxylin and Eosin and then viewed under light microscope to observed changes in placental histology.

Statistical Analysis: The data obtained were analyzed with SPSS (Statistical Package for Social Sciences) using Friedman Test and Wilcoxon test to determine the degree of significance

for the various mean variables of scores between control and treated groups. P value < 0.05 is considered to indicate a significant difference.

RESULTS AND DISCUSSION

The placenta showed no apparent inflammation and distortion in decidual layer in normal and low dose group respectivelyin both gestation groups. However, there is inflammation and distortion in the decidual layer in both medium and high dose group. The negative control group also showed normal trophoblastic giant cells with normal nuclei. However nuclei of trophoblastic giant cells in the positive conttol group was pynotic and irregular shaped. Trophoblastic cells and spongiotrophoblasts were also decreased in number in the medium and high dose group as well as the positive control group. Congestion in the labyrinth area is evident in low, medium and high dose groups which is similar to positive control group. These results are clearly shown in Figure 1: Effects on maternal-fetal interface in different groups during early and late treatment, Figure 2: Effects on trophoblastic giant cells in different groups during early and late treatment and Figure 3: Effects on labyrinth in different groups during early and late treatment. For all groups, each bar represents the mean value of scores obtained while the letters in superscript denotes significance at level of $P<0.05$ as compared to normal group.

The gestation period of day 1 to 7 of gestation (early group) is duing the implantation or embryogenesis stage while gestation period day 8 to 15 of gestation (late group) is the critical period of organogenesis which is equivalent to 3-6 weeks after fertilization in human. During the organogenesis, fetus is highly susceptible to exogenous toxic stimuli (Roberts and Lain,2002). The late treatment group shows more pathological changes in placental histology as compared to the early treatment group. In the maternal-fetal interphase, necrosis is irreversible injury to cells as a result of encounters with noxious stimuli invariably leading to cell death. Such noxious stimuli include infectious agents (bacteria, viruses, fungi, parasites), oxygen deprivation or hypoxia, and extreme environmental conditions such as heat, radiation, or exposure to ultraviolet irradiation (Adigum and Bhimji, 2018). Decidual necrosis causes a deficiency in the maternal vascular circulation leading to decreased placental circulation. The toxic substance in Jatropha oil might cause decidual cells to degenerate and die. Trpophoblastic giant cells (TGC) secrete paracrine and endocrine effectors to control placental growth and development reactions. TGC are precursor cells of more differentiated trophoblasts. Current study shows an increase in abnormal TGC i.e., atypical shape and pyknotic and irregular nuclei. J.curcas crude see oil might cause cells of spongiotrophoblasts to degenerate & die therefore stimulating trophoblasts to phagocytose and removed damaged cells. The labyrinth is responsible for fetomaternal nutrient exchange where placental vasculature development is needed to support fetal growth.Histological alterations to the labyrinth will decrease placental blood flow (Ishimura *et al*., 2002). Deficient vasculature in the labyrinth maybe due to placental angiogenesis during fetoplacental development. Decreased and irregular vessel formation maybe caused by reduced functional capacity of the trophoblastic barrier. mThese pathological changes may be due to toxic principles in the oils, probably irritant poisons as the seeds contain high amounts of phytotoxin similar to ricin of Ricinus communis. Curcin and phorbol derivatives are highly irritant compounds (Hass, *et al*., 2002). This compounds transcend the placenta and cause deleterious effects to normal cell architecture and impaired placental function.

Conclusion

Jatropha curcas crude oil causes deleterious effects to the placenta when fed to pregnant female Sprague dawley rats during early and late gestation. The relevant alterations are possible compensatory adjustment to maintain adequate metabolic exchange in the structure of the placenta. Therefore this may decrease the functional role of the placenta and contribute to adverse reproductive outcomes.

Acknowledgements: The authors thank UPM for the RUGS grant.

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