Original Article

Effectiveness of Histopathological Changes of Induced Thin Layer Endometrium by Pentoxifylline and Pentoxifylline-Loaded Poly Lactic-co-Glycolic Acid on Female Rats

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ABSTRACT

Pentoxifylline (PTXF) is a vasoactive agent that plays a significant role in the treatment of thin-layer endometrium cases. The PTXF, also identified as oxpentifylline, is a member of xanthine derivatives and a competitive nonselective phosphodiesterase inhibitor leading to the elevation of intracellular cAMP, inhibition of tumor necrosis factor and leukotriene synthesis, activation of protein kinase A, and reduction of inflammation and innate immunity. Moreover, it is used as an agent to relieve muscle pain in people with peripheral artery disease (vascular irregularities). It is also an acceptable choice for the treatment of radiation-induced fibrosis. Therefore, the present study aimed to determine the advantageous impact of PTXF and PTXF-loaded poly lactic-co-glycolic acid (PLGA) on female rats after being exposed to ethanol to create a thin layer of the endometrium. For this purpose, 50 female rats were selected and divided into five groups (G1: negative normal control, G2: positive control, G3: PLGA only, G4: preference PTXF, and G5: PLGA-PTXF groups) for a 20-day treatment period. In this study, the histopathological section revealed a perfect improvement in the tissues of the uterine horn of female rats that induced endometria and were treated with PLGA-PTXF. In this group of rats, clear healing was achieved and there was an increase in the thickness of endometrium and myometrium, compared to the ordinary PTXF-treated group which had the lowest recovery characteristics. However, the positive control group underwent a significant decrease in terms of endometrium and myometrium thickness as well as vascular and glandular density. This study showed that the PTXF-loaded PLGA had the capacity to heal the thin layer of the endometrium by improving the levels of histopathological changes, especially regarding the thickness of the endometrium and myometrium more than the ordinary PTXF.

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Keywords: Endometrium female rats, Myometrium thickness, Nanoparticles, Pentoxifylline-loaded poly lactic-co-glycolic acid, Vascular and glandular density

1. Introduction

Thin endometrium is necessary for a fruitful pregnancy; the threshold thickness for uterine endometrium is less than the endometrial thickness which is referred to as successful planting (1). For the endometrium to be considered thin, it must be thinner than the threshold thickness; hence, it is described as the minimal expanse between the echogenic interfaces of myometrium and endometrium, Asherman syndrome, and other etc. (2).

In the implantation failure due to thin endometrial, there is an increased number of inflammatory cytokines, such as IFN-γ, while the metabolism and anti-oxidative stress are down-regulated by numerous genes in the thin endometrium (3). In this regard, several studies have explored the thin endometrium and it has been found that the control endometrium obviously had various mRNA expression profiles, proposing that irregular gene expression is included in implantation malfunction in a thin endometrium (4). Therefore, animals with thin endometrium have been subjected to a variety of therapeutic approaches to increase endometrial thickness and its associated receptivity (5).

These methods include hormonal organization of human chorionic gonadotropin and estradiol, gonadotropin-releasing hormone agonist, platelet-rich plasma application, electrical stimulation, regenerative medicine, tamoxifen, endometrial receptivity array, and PTXF intrauterine infusion of growth factors, such as granulocyte colony-stimulating factor (6,7). Despite the wide range of treatments, the majority of options only slightly alter the endometrial thickness and are not currently allowed (8,9). The therapy regimens of thin-layer endometrium have been challenging; therefore, further explorations are essential for the amplification and ideal management of patients with a thin endometrium (10).

Angiogenesis is considered to play a key role in various female reproductive developments and be involved in the formation of a corpus luteum, growth of dominant follicles, and endometrial pattern. Endometrial angiogenesis is vital in endometrium renewal after menstruation and supply of vascularised endometrium for a successful pregnancy (11). The PTXF is the vasoactive agent that plays a significant role in the treatment of the thin layer endometrium case (12). Pentoxifylline (PTXF), which is recognized as an oxpentoxifylline, is a member of xanthine derivatives and a competitive nonselective phosphodiesterase inhibitor leading to elevation of intracellular cAMP, inhibition of tumor necrosis factor (TNF) and leukotriene synthesis, activation of protein kinase A (PKA), and reduction of inflammation and innate immunity. Moreover, it can be used as an agent to relieve muscle pain in people with peripheral artery disease (vascular irregularities) and is an acceptable choice for the treatment of radiation-induced fibrosis (13–15).

2. Materials and Methods

2.1. Experimental Design

In this study, 50 female rats were divided into five equal groups, including G1: control negative and G2: control positive groups with induced thin endometrial that were treated with distilled water. The other groups, G3, G4, and G5, were treated with 1.1 mg/kg of poly lactic-co-glycolic acid (PLGA), 34 mg/kg of ordinary PTXF, and 29.64 mg/kg of PTXF-loaded PLGA, respectively, for 20 days after they were subjected to the injection of ethanol 95% in the uterine horn (0.5 ml) for about 5 min.

2.2. Doses of Pentoxifylline and Pentoxifylline-Loaded Nanoparticle

The PTXF was administered orally at doses of 15, 20, 25, 30, and 35 mg/kg/day with concentrations of solutions of 7.5, 10, 12.5, 15, and 17.5 mg⁄ml to rats in G1, G2, G3, G4, and G5, respectively. Furthermore, 0.2 ml/100 g b.w. of each stock solution of PTXF was administered orally to the rats by stomach tube.

2.3. Preparation of Poly Lactic-Co-Glycolic Acid-Pentoxifylline Nanoparticles

Nanoparticles were prepared by the nanoprecipitation method (16) as illustrated in figures 1 and 2 (17,18).

2.4. Induction of Thin Layer Endometrium

In total, 55 adult female rats were used in the experiment of effective dose detection for ordinary PTXF and PTXF-loaded PLGA. Moreover, 50 female adult rats were utilized in experiment two, in which the surgical techniques were performed according to the recorded method (figure 3-5) (19). Regarding specimen collection, all rats were sacrificed in the estrus phase after recovery of two normal estrus cycles. The phases of the estrous cycle were determined by observing the vaginal smear as described (20).

2.5. Histological Study

During the treatment period, the animals were anesthetized by a ketamine (80 mg/kg) and xylazine (12 mg/kg) cocktail at a dose of 0.1 ml/100 g b.w. that was injected intraperitoneally for the secession of tissue for the histopathological assessment of uterine tissues. The samples were put in a 10% formalin solution and then prepared following the steps proposed by previous research (21,22).

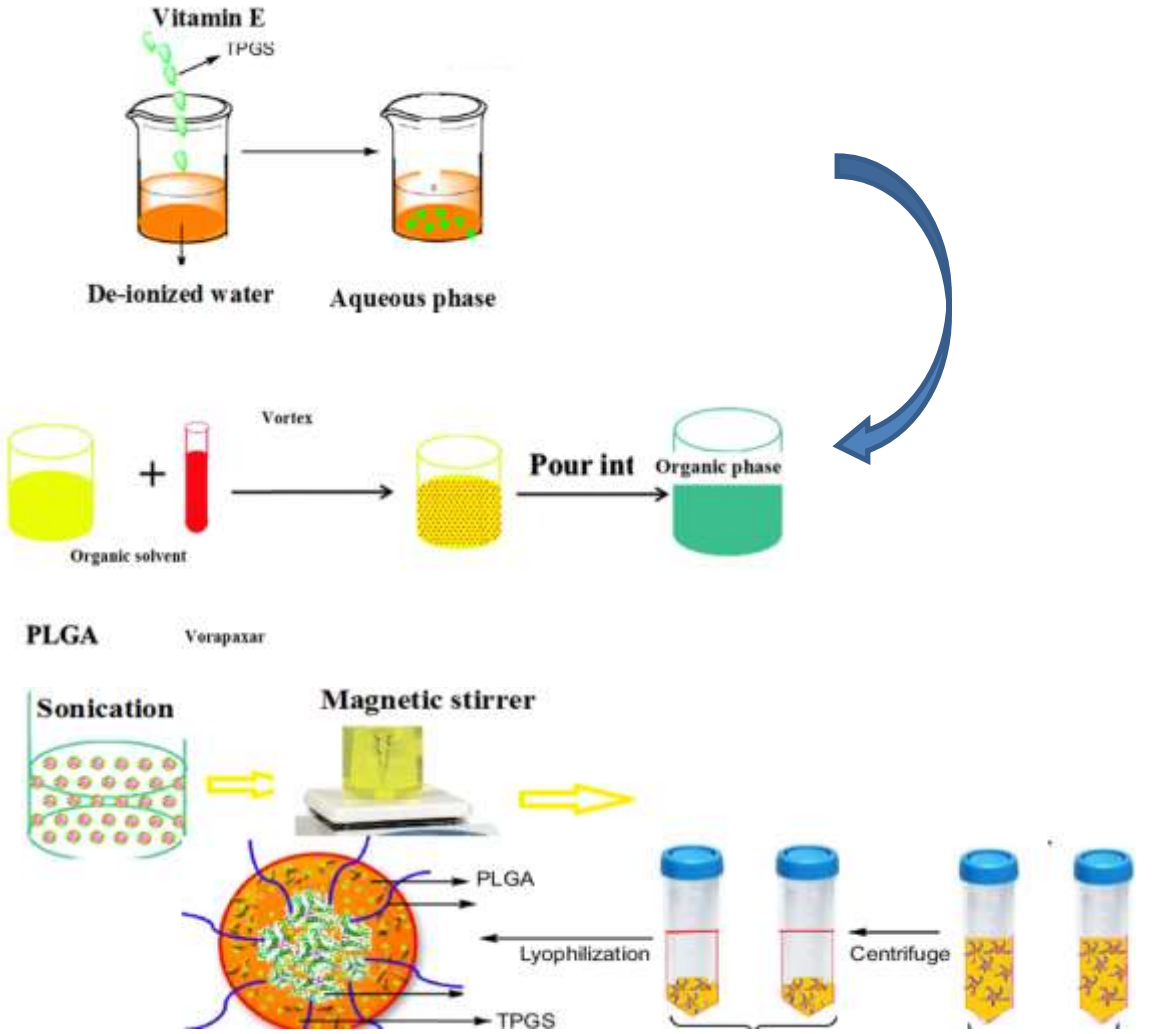

Figure 1. nanoprecipitation method for preparing PLGA –Pentoxifylline polymer nanoparticles (Jasim *et al*. (2019) (18)

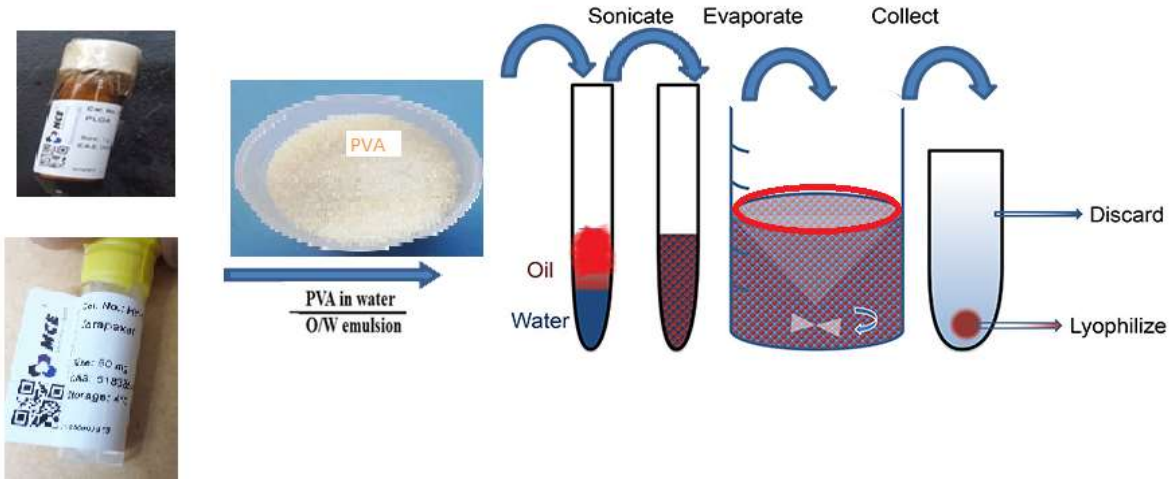

Figure 2. Polyvinyl alcohol O/ W emulsion for preparing PLGA - Pentoxifylline polymer nanoparticles (Jasim *et al*. (2019) (18)

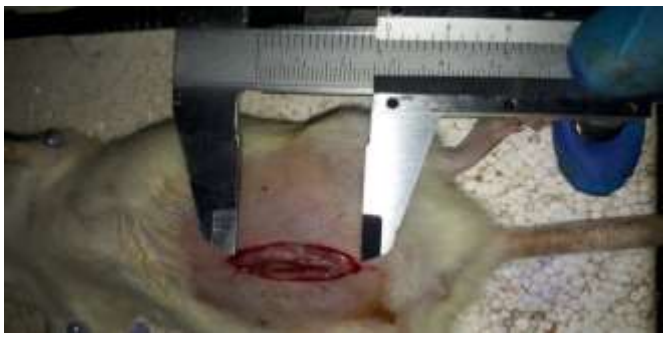

Figure 3. An incision of approximately 2.5 cm in rats was made into the lesser abdomen through the skin and primary layers

Figure 4. Injection 95% ethanol into the horn of the uterus with a 16-gauge needle of a 1 mL syringe

3. Results

3.1. Histopathological Changes of The Uterus

The section of the endometrial layer of rats with induced thin endometrium after two estrus cycles showed severe vascular degeneration and infiltration of inflammatory cells in the endometrium mucous as illustrated in figure 6. The histopathological section of the endometrial layer of rats with induced thin endometrium after 20 days of treatment with distilled water (positive control group) showed severe vascular degeneration in the endometrium mucosa. Moreover, myometrium was infiltrated by mixed acute and chronic inflammatory cells as shown in figure 6. However, the histopathological section of the endometrial thin layer of rats that were treated with PTXF-loaded PLGA showed mild vacuolation, mild edema, and proliferative endometrial glands as shown in figure 7.

Figure 5. The abdomen of the rat was closed by silk non-absorbable suture 80 cm. curved cutting 20 mm

Nevertheless, the histopathological section of rats with an endometrial thin layer, that were treated with ordinary PTXF, revealed secretory endometrial glands with slightly loose stroma as shown in figure 8, compared to rats in the negative control group as illustrated in figure 9. However, the histopathological

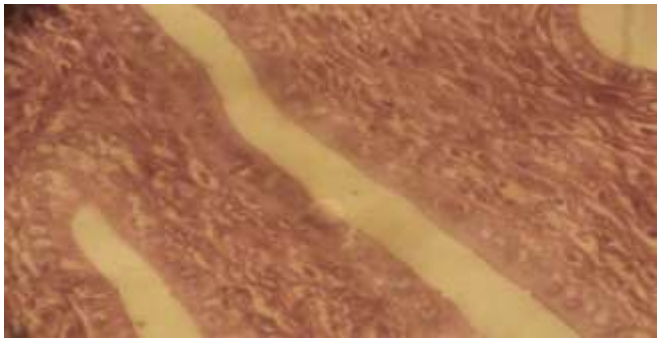

Figure 6. Histopathological section of endometrial layer of rats induced endometrium after two estrus cycle, showed sever vascular degeneration and infiltration of inflammatory cells in the endometrium mucosa. H&E 400x.

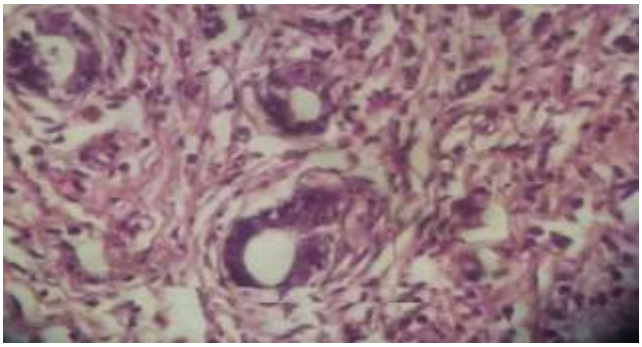

Figure 7. Histopathological section of endometrial thin layer rats treated with Pentoxifylline loaded PLGA, showed mild vasculation , mild edema and proliferative endometrial glands. H&E 400 x.

section of the endometrial thin layer of rats treated with only PLGA revealed prominent infiltration by eosinophils as depicted in figure 10.

The group of rats subjected to ethanol to induce thin layer endometrium underwent a decrease in the thickness of endometrium and myometrium as well as inflammatory changes, such as acute and chronic inflammation with edema and congestion. This result can be attributed to ethanol. Reactive oxygen species (ROS) creation, caused a slight reduction in cell viability, an elevation in the cellular NADH/NAD1 ratio, acute and chronic ethanol exposure, exacerbated mitochondrial ROS production, and therefore, cell death (23,24).

The pathological conditions concerned with ethanol-induced ROS formation have been described and include oxidative protein modification, antioxidant depletion, and mitochondrial dysfunction. In addition, ethanol may cause an uprising in inflammatory substances in the uterine horn in the female rats which leads to the establishment of acute and chronic inflammation. These inflammatory cells may undergo activation (one of the pattern recognition receptors recognizes pathogen-associated molecular patterns or damaged-related molecular patterns) which leads to the release of inflammatory mediators and the cytokines that cause outward symptoms of inflammation. The mediator molecules alter the blood vessels to allow leukocytes, mostly neutrophils and macrophages, to migrate into the tissue by

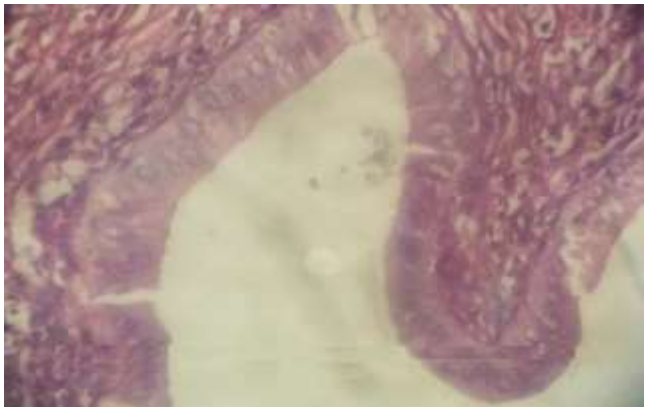

Figure 6. Histopathological section of endometrial layer of rats induced endometrium after 20 days treatment with distilled water (Control positive treated group), showed sever vascular degeneration in the endometrium mucosa and myometrium is infiltrated by mixed acute and chronic inflammatory cells. H&E 400 x.

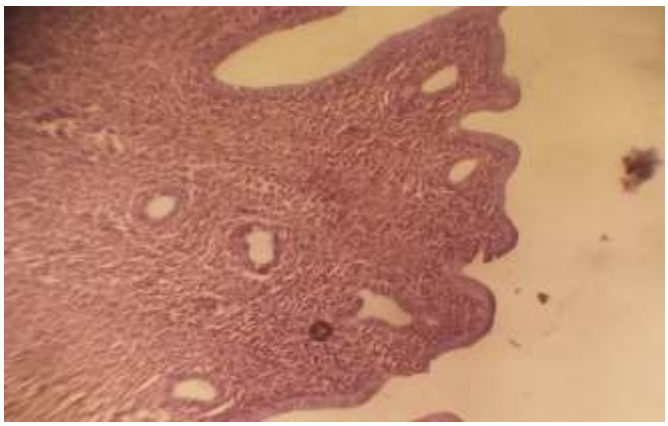

Figure 8. Histopathological section of endometrial thin layer rats treated with ordinary Pentoxifylline , secretory endometrial glands with slightly loose stroma. H&E 100 x.

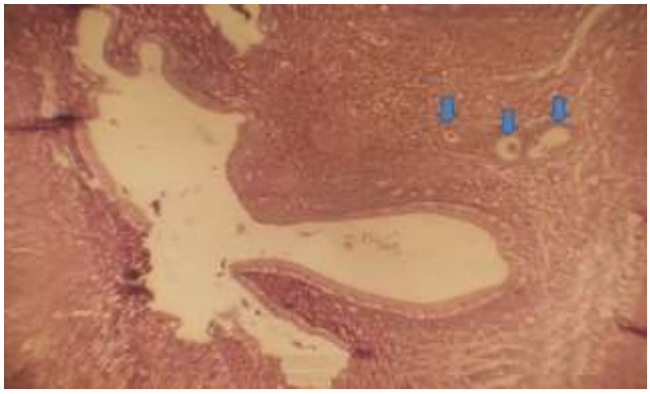

Figure 9. Histological section of endometrial layer rats (control negative). H&E 100x.

extravasation outside of the blood vessels (25,26).

The neutrophils may travel along a chemotactic gradient created by the local cells to reach the site of injury and enhance the release of interleukin 6 (IL-6) and TNF α levels. These results were in line with those of previous studies (27, 28) that explained the presence of proinflammatory substances, such as cytokines and tumor necrosis factors, as proinflammatory cytokines and discharged by macrophages due to secretion of ROS as a series of acute inflammatory reaction, in addition to that ROS had been involved in the activation of many intracellular signaling pathways, such as mitogen-activated protein kinases (29,30). Therefore a competitive nonselective phosphodiesterase inhibitor caused the decrease of TNF α, IL-1, and IL-6.

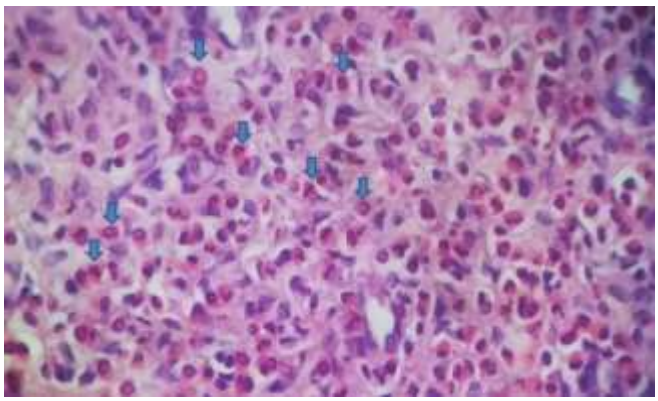

Figure 10. Histopathological section of endometrial thin layer rats treated with Empty PLGA, prominent infiltration by eosinophils (arrows). H&E 400x.

Moreover, the increase of glutathione peroxidase and superoxide dismutase may be due to the antioxidant effectiveness of treatment with PTXF (31,32).

Improvement in the thickness of the endometrium and myometrium as well as the histopathological changes in the uterus of the rats treated with ordinary PTXF may be due to its mechanism of action through the inhibition of phosphodiesterase enzyme. Moreover, antagonist adenosine receptors (A1 and A3) caused an increase in cAMP by elevation of the adenyl cyclase activity in the uterine horn. Other factors that caused the retrieval of the thickness of endometrium and myometrium may be attributed to the effectiveness of PTXF on the activity of sexual glands, especially the ovarian gland, that led to the secretion of estradiol where the function of the latter is to produce sexual features in female rats in addition to increasing the thickness of endometrium and myometrium (33).

4. Discussion

The results showed an improvement in the thickness of the endometrium and myometrium as well as the histopathological changes when treated with PLGA-PTXF. This result can be attributed to the adverse effects of the latter that may have affected the other pathophysiological levels of female reproductive organs in rats by decreasing the dose, whereas in PLGA- PTXF the part of principle properties is formed as nanoparticle to increase its activity, drug delivery, penetrations and arrived to the target organ speedily and augmented the antioxidants effectiveness with minimal adverse effects that led successfully to remodeling of damaged tissues (34,35). Therefore, the loaded and formation by nanoparticle techniques led to good results with minimum side effects, in contrast to the ordinary PTXF treatment group that apparently required high doses with the highest concentrations and long duration of action.

Based on the results of this study, PLGA can be characterized and it can be concluded that the dimethyl sulfoxide-related organic phase alone gives

the shape, particle size with a small diameter, negatively charged, in addition to suitable drug loading and encapsulation efficiency. Therefore, the concentration (0.03%) of vitamin E-TPGS is the optimal concentration utilized as a stabilizer and coating of PLGA. However, PLGA-PTXF regimen treatment with different concentrations had the ability to recover female rats that were subjected to ethanol which led to a thin layer of endometrium in the uterine horn. Therefore, treatment via PLGA-PTXF regimen resulted in clear histopathological development as is evident in the uterine horn tissues of female rats.

5. Recommendations

It is recommended to use PLGA-loaded drugs via a non-participation method for drug delivery in the treatment of problems of the respiratory system. Moreover, it is advised to employ the PLGA- PTXF regiment in treatment of the reproductive system disorders in male rats. In addition, it should be noted that the pharmacokinetic efficacy of PTXF-loaded PLGA needs further studies.

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Authors' Contribution

Study concept and design:A.A.H;R.S.S; H.H.F;J.A.M
Acquisition of data:
A.A.H;R.S.S; H.H.F;J.A.M
Analysis and interpretation of data:A.A.H;R.S.S; H.H.F;J.A.M
Drafting of the manuscript:
A.A.H;R.S.S; H.H.F;J.A.M
Critical revision of the manuscript for important intellectual content:
A.A.H;R.S.S; H.H.F;J.A.M
Statistical analysis:
A.A.H;R.S.S; H.H.F;J.A.M

Ethics

Not applicable.

Conflict of Interest

No conflict of interest.

References

1. Guyton AC, Hall JE. Chapter 81 Female physiology before pregnancy and female hormones. Textbook of medical physiology 11th ed WB Saunder Co. 2006;1018ff.
2. Baker JM, Chase DM, Herbst-Kralovetz MM. Uterine microbiota: residents, tourists, or invaders? Frontiers in immunology. 2018;9:208.
3. Maekawa R, Taketani T, Mihara Y, Sato S, Okada M, Tamura I, et al. Thin endometrium transcriptome analysis reveals a potential mechanism of implantation failure. Reproductive Medicine and Biology. 2017;16(2):206–27.
4. Beyer AM, De Lange WJ, Halabi CM, Modrick ML, Keen HL, Faraci FM, et al. Endothelium-specific interference with peroxisome proliferator activated receptor gamma causes cerebral vascular dysfunction in response to a high-fat diet. Circulation research. 2008;103(6):654–61.
5. Chien L-W, Au H-K, Chen P-L, Xiao J, Tzeng C-R. Assessment of uterine receptivity by the endometrial-subendometrial blood flow distribution pattern in women undergoing in vitro fertilization-embryo transfer. Fertility and sterility. 2002;78(2):245–51.
6. Blanquicett C, Kang B-Y, Ritzenthaler JD, Jones DP, Hart CM. Oxidative stress modulates PPARγ in vascular endothelial cells. Free radical biology and medicine. 2010;48(12):1618–25.
7. Koohi MK, Shahroozian E, Ghazi-Khansari M, Daraei B, Javaheri A, Moghadam-Jafari A, et al. The pretreatment effects of pentoxifylline on aflatoxin B1-induced oxidative damage in perfused rat liver. International Journal of Veterinary Research. 2011;5(1):43–72.
8. De Geyter C, Schmitter M, De Geyter M, Nieschlag E, Holzgreve W, Schneider HPG. Prospective evaluation of the ultrasound appearance of the endometrium in a cohort of 1,186 infertile women. Fertility and sterility. 2000;73(1):106–13.
9. Khani A, Khorasgani EM. Investigating the Effect of Hydroalcoholic Extract of Eryngos on Plasma Concentration of Blood Glucose, Blood Cells and Pancreatic Tissue in Diabetic Rats. Iranian Journal of Veterinary Medicine. 2021;15(4).

10. Dreisler E, Poulsen LG, Antonsen SL, Ceausu I, Depypere H, Erel CT, et al. EMAS clinical guide: assessment of the endometrium in peri and postmenopausal women. Maturitas. 2013;75(2):181–90.
11. Gałczyński K, Jóźwik M, Lewkowicz D, Semczuk-Sikora A, Semczuk A. Ovarian endometrioma–a possible finding in adolescent girls and young women: a mini-review. Journal of ovarian research. 2019;12(1):1–8.
12. Eftekhar M, Tabibnejad N, Tabatabaie AA. The thin endometrium in assisted reproductive technology: An ongoing challenge. Middle East Fertility Society Journal. 2018;23(1):1–7.
13. Flint H, Cotter MA, Cameron NE. Pentoxifylline effects on nerve conduction velocity and blood flow in diabetic rats. International journal of experimental diabetes research. 2000;1(1):49–58.
14. Essayan DM. Cyclic nucleotide phosphodiesterases. Journal of Allergy and Clinical Immunology. 2001;108(5):671–80.
15. Deree J, Martins JO, Melbostad H, Loomis WH, Coimbra R. Insights into the regulation of TNF-a production in human mononuclear cells: the effects of non-specific phosphodiesterase inhibition. Clinics. 2008;63:321–8.
16. Al-Rubaee SH, Al-Azawi TS, Taha AA. Duodenal Histomorphological Changes in Broilers Administered poly d, l-lactic-coglycolic acid (PLGA) Nanoparticles Encapsulated with Peptide. The Iraqi Journal of Veterinary Medicine. 2020;44(1):80–8.
17. McCall RL, Sirianni RW. PLGA nanoparticles formed by single-or double-emulsion with vitamin E-TPGS. JoVE (Journal of Visualized Experiments). 2013;(82):e51015.
18. Jasim AM, Hasan HF, Awady MJ. Preparation of Vorapaxar loaded with Vitamin E TPGS and PVA emulsified PLGA nanoparticles In vitro studies. Research Journal of Pharmacy and Technology. 2019;12(9):4503–10.
19. Zhao J, Zhang Q, Li Y. The effect of endometrial thickness and pattern measured by ultrasonography on pregnancy outcomes during IVF-ET cycles. Reproductive Biology and Endocrinology. 2012; 10(1):1–6.
20. Zarrow MX. Experimental endocrinology: a sourcebook of basic techniques. Elsevier; 2012.
21. Kittel B, Ruehl-Fehlert C, Morawietz G, Klapwijk J, Elwell MR, Lenz B, et al. Revised guides for organ sampling and trimming in rats and mice–Part 2: a joint publication of the RITA) and NACAD) groups. Experimental and Toxicologic Pathology. 2004;55(6):413–31.
22. Griffin S, Masood MI, Nasim MJ, Sarfraz M, Ebokaiwe AP, Schäfer K-H, et al. Natural nanoparticles: a particular matter inspired by nature. Antioxidants. 2017;7(1):3.
23. Nordmann R, Ribière C, Rouach H. Implication of free radical mechanisms in ethanol-induced cellular injury. Free Radical Biology and Medicine. 1992;12(3):219–40.
24. Rouach H, Fataccioli V, Gentil M, French SW, Morimoto M, Nordmann R. Effect of chronic ethanol feeding on lipid peroxidation and protein oxidation in relation to liver pathology. Hepatology. 1997; 25(2):351–5.
25. Pollice PF, Rosier RN, Looney RJ, Puzas JE, Schwarz EM, O'Keefe RJ. Oral pentoxifylline inhibits release of tumor necrosis factor-alpha from human peripheral blood monocytes: a potential treatment for aseptic loosening of total joint components. JBJS. 2001;83(7):1057–61.
26. Motamedi S, Asghari A, Jahandideh A, Abedi G, Mortazavi P. Effects of Echinacea Purpurae Extract on Testicular Ischemia/Reperfusion (I/R) Injury in Rat. Iran J Vet Med. 2019;13:303–13.
27. Olanow CW. A radical hypothesis for neurode-generation. Trends in neurosciences. 1993;16(11):439–44.
28. Wee Yong V. Inflammation in neurological disorders: a help or a hindrance? The Neuroscientist. 2010; 16(4):408–20.
29. Liu R, Li J, Song J, Sun J, Li Y, Zhou S, et al. Pinocembrin protects human brain microvascular endothelial cells against fibrillar amyloid-β1− 40 injury by suppressing the MAPK/NF-κ B inflammatory pathways. BioMed Research International. 2014;2014.
30. Onwuama KT, Nzalak JO, Dzenda T, Hambolu JO, Salami SO. Onset and Stages of Osteogenesis in the Rabbit (Oryctolagus cuniculus) using Diaphonisation. Iranian Journal of Veterinary Medicine. 2022;16(3).
31. Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. Japi. 2004;52(794804):4.
32. Hayyan M, Hashim MA, AlNashef IM. Superoxide ion: generation and chemical implications. Chemical reviews. 2016;116(5):3029–85.

33. Westwood FR. The female rat reproductive cycle: a practical histological guide to staging. Toxicologic pathology. 2008;36(3):375–84.
34. Atabek ME, Kurtoglu S, Selver B, Baykara M. Effectiveness of pentoxifylline on the cross-sectional area of intima media thickness and functions of the common carotid artery in adolescents with type 1 diabetes. 2011;
35. Ghotbitabar Z, Asghari A, Hassanpour S, Jahandideh A. Effects of Quebracho Tannin Extract on Testicular Ischemia-/Reperfusion. Iranian Journal of Veterinary Medicine. 2022;16(4).