Original Article

Nanonized progesterone formulation for improved oral bioavailability in healthy and pregnant rabbits

Gaurav K Jain[1], Jagriti Sharma[1], Nimesh Modi[2], Tejas Kothari[2], Nazeer Hasan[3], Nitin Sharma[4], Amirhossein Sahebkar[5], Prashant Kesharwani[3]*

[1]Center for Advanced Formulation Technology (CAFT), Delhi Pharmaceutical Science and Research University (DPSRU), Pushp Vihar, New Delhi-110017, India

[2]Corona Remedies Pvt. Ltd., Mondeal Business Park, "Corona House", Block C, S. G. Highway, Apex St, near Gurudwara, Thaltej, Ahmedabad, Gujarat-380059, India

[3]School of Pharmaceutical Education and Research, Jamia Hamdard, Mehrauli - Badarpur Rd, near Batra Hospital, Block D, Hamdard Nagar, New Delhi-110062, India

[4]Pharmacy, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly, Uttar Pradesh 243202, India

[5]Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

Email: prashantdops@gmail.com

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Abstract

The aim of this study is to evaluate and compare the oral bioavailability of nanonized progesterone (nano-PG) and micronized progesterone (micro-PG) sustained release tablet formulation in healthy and pregnant rabbits. High pressure compressed gas technology reduces the particle size from 1.72 ± 2.5 µm (micro-PG) to 800 ± 35 nm (nano-PG). DSC and XRD showed that both micro-PG and nano-PG were crystalline and exist as form I. Higher melting enthalpy of nano-PG indicated improved drug stability whereas XRD showed slight reduction in degree of crystallanity following nanonization. Nano-PG demonstrated 2-fold higher solubility in SDS aqueous solution and significantly higher permeability ($p < 0.05$) across porcine intestine compared to micro-PG. The pharmacokinetics of nano-PG and micro-PG was conducted in healthy and pregnant rabbits. The Cmax of nano-PG was higher in healthy and pregnant rabbits however the difference was significant in healthy rabbits only. The nano-PG demonstrated 30% and 18% higher bioavailability compared to micro-PG in healthy and pregnant rabbits, respectively. In conclusion, nanonization improves solubility, dissolution and bioavailability of PG in rabbits without affecting solid state characters.

Keywords: oral bioavailability; nanonized progesterone; sustained release tablet; solubility; drug delivery.

1. INTRODUCTION

Oral progesterone (PG), available as synthetic and natural PG, is used to treat various acute or chronic gynecological conditions including menorrhagia, amenorrhea, contraception, luteal support and to pregnant woman for maintenance of pregnancy [1], [2]. The use of synthetic PGs for luteal support and in pregnant woman is limited owing to their inherent androgenic activity [3]. The synthetic PGs results in reduced estrogenic benefits [4], adverse effects on the lipoprotein metabolism [5] and cardiovascular system [6] and teratogenicity [7]. Conversely, natural PG, devoid of androgenic activity, has no effect on lipoprotein metabolism or cardiovascular system or induce teratogenicity [7], [8]. In addition, several reports suggest that natural PG has a favorable effect on blood vessels [9]. The major problem with natural PG is its poor oral bioavailability due to its unfavorable physico-chemical properties [10], [11]. It is classified as Class II drug by Biopharmaceutical Classification System (BCS) with high lipophilicity (Log P = 3.9) and very low aqueous solubility (10 mg/mL) [10]. Further, it also undergoes hepatic metabolism when given by oral route [12]. Recently, lipidic delivery systems [13], microemulsions [14], self-emulsifying delivery systems [15] and surfactants [16] have been used to enhance the solubility of PG. Although, these technologies demonstrated improved solubility of PG but there are few major concerns: (a) reduced stability of PG in lipids and of lipids themselves and (b) final product is oral softgel, which is second choice compared to tablets and (c) reduced likelihood of sustained release of PG [13] – [16]. Presently, sustained release (SR) PG formulations are preferred due to reduce hepatic metabolism and improved patient compliance [17].

Considering these limitations, micronized PG (micro-PG) was developed, which offer improved stability and bioavailability [18], [19]. Micro-PG sustained release tablet is commercially available and is approved for variety of gynecological conditions and from the results of Postmenopausal Estrogen/Progestin Interventions (PEPI) trial it was evident that it was first choice formulation for estradiol-opposing therapy in postmenopausal women [20]. It is well documented that micronization decrease the size of PG particles, increases effective surface area and facilitates dissolution in GIT [21], [22]. Most likely, with enhanced drug dissolution, the bioaccessibility and bioavailability also increases [22]. Considering the relation between particle size and bioavailability, it is expected that reducing the particle size from micron to nano would further increase the bioaccessibility and bioavailability. Nanonization based bioaccessibility enhancement of drugs has been previously reported by our group [23] – [26]. The purpose of this research is to evaluate the bioavailability of micro-PG and nanonized PG (nano-PG) sustained release tablet (200 mg) using healthy and pregnant female rabbits. The nano-PG was processed via high pressure compressed gas (HPCG) proprietary technology (Corona remedies Pvt. Ltd, Ahmedabad, India) and formulated into sustained release tablet. The physico-chemical properties and permeability of nano-PG was also evaluated and compared with micro-PG.

2. MATERIALS AND METHODS

2.1. Materials and Formulations

Micro-PG was received as gift sample from La Chandra Pharmalab Pvt. Ltd (Vaghrol, India). Micro-PG was processed into nano-PG using HPCG proprietary technology. The grinding chamber was sterilized with special modulatory control to form the interface of the API with the jet stream of compressed air to ensure maximum uniformity of nanonized particles. The micro-PG and nano-PG was compressed into sustained release tablets at GMP facility of Corona remedies Pvt. Ltd (Ahmedabad, India). Briefly, 200 mg of micro-PG or nano-PG was blended with 100 mg HPMC and other excipients to form micro-PG tablet (200 mg PG) or nano-PG tablet (200 mg PG).

2.2. Characterization of nano-PG

The particle size of nano-PG was determined by dynamic light scattering (DLS) using particle size analyzer (Nano ZS, Malvern Instruments, Malvern UK) at 25□C. The diluted dispersion of particles in distilled water was analyzed at 90□ detection angle. The phase transition of the nano-PG was investigated using differential scanning calorimeter (Pyris 6, Perkin-Elmer, USA). Five milligram of sample was placed in aluminium pan and heat run was conducted from 50-400□C under an inert environment using nitrogen. Thermal analysis of the samples was performed using thermogravimetric analysis (TGA, DTG-60H, Shimadzu, USA) between temperature range 40-500°C under nitrogen and a heating rate of 10°C/min. The crystallinity of the nano-PG was studies using powder X-ray diffraction (PXRD, PANalytical, Xpert Pro Diffractometer JB Eindhoven, Netherlands). Cu-Kα was used as radiation source at a scanning speed of 0.05°/min. Micro-PG was used for comparison.

2.3. Solubility Studies of nano-PG

The solubility of micro-PG and nano-PG was determined in discriminating media containing aqueous media with different concentrations (0%, 1.25%, 2.5% and 5%) of sodium dodecyl sulphate (SDS). Excess amount of drug was added to 1 mL of media and allowed to equilibrate for 24 h on bath shaker. The insoluble drug was separated by filtration and the PG dissolved in media was determined spectrophotometrically at 242 nm.

2.4. Permeability Studies of nano-PG

The permeation of nano-PG was performed on Franz diffusion apparatus using porcine intestine. Previous reports suggest that porcine intestine could be an alternative for human intestinal permeability [27]. Porcine intestine (ileum) obtained from local slaughterhouse was sectioned and washed with Kreb's medium prior to use. A 3.0 cm2 segment was then mounted between receptor and donor compartment for permeability experiments. The receptor compartment was filled with 5% SDS aqueous solution and stirred with magnetic bead at 100 rpm. The temperature of the diffusion cell was maintained at 37□C by circulating water. Samples from the receptor compartment was collected at 30 min and 1 h and replaced with same volume of fresh medium. The concentration of PG in the samples was analyzed spectrophotometrically. The cumulative amount of drug permeated through intestine was calculated. Studies were done in triplicate and micro-PG was used for comparison.

2.5. Dissolution of nano-PG tablets

The dissolution of micro-PG and nano-PG sustained release tablets (200 mg) was conducted in 5.0 % SDS aqueous solution as dissolution medium using USP II dissolution apparatus. The temperature of the dissolution medium was maintained at 37±0.5□C by thermostatically controlled water bath. Five mL of sample was withdrawn at 30 min, 1 h, 2h, 4h, 6h, 8h, 12h and 24 h, filtered, suitably diluted and analyzed spectrophotometrically at 242 nm. After each sampling, 5 mL of fresh dissolution media was replaced to maintain the sink conditions.

2.6. Pharmacokinetics of nano-PG tablets in Rabbits

2.6.1 Animals

Bioavailability studies was conducted on healthy and pregnant female New Zealand rabbits weighing 2.25 ± 0.25 kg. Pregnancy was induced by mating sexually mature female rabbits with fertile males. The pregnancy was confirmed by abdominal palpation on day 15 considering day of mating as day 0. The 20-day pregnant animals were used for the pharmacokinetic study [28]. Total sixteen healthy and sixteen pregnant animals were used for the study. All the animals were housed in separate cages under standardized conditions of humidity (50±10%), temperature (23±2°C) and light (12 h light-dark). They had free access to standard diet and tap water. Animals were fasted 4 h pre-dose and continued till 6 h post-dose but had free access to water. All care and handling of rabbits adhered to the animal ethical guidelines with approval from Institutional Animal Ethics Committee of Shri Ram Murti Smarak Institute of Medical Sciences (Approval No. IAEC/SRMS/2022/1/12, Dated: 31-01-2022).

2.6.2 Study design

Pharmacokinetic study was conducted in healthy as well as pregnant female rabbits. Both healthy and pregnant rabbits were divided into 2 groups, each group consisted of 8 rabbits (n = 8). First group received oral micro-PG tablet while second group received equivalent nano-PG tablet. The tablets were administered intact orally to the animals through stomach tube. The tablets were placed deep into the throat and immediately 15 mL of water was given by a syringe to prevent sticking of tablet to animal throat and to facilitate swallowing [29]. The rabbit blood (0.5 mL) was collected through marginal ear vein following anesthesia of ears with a topical lidocaine cream. The blood was collected at predetermined time intervals using 1.0 mL syringe in serum separator tubes. The serum was separated and stored at -80°C for further analysis. The serum concentration of PG was determined by commercial kits using radio immunoassay as per supplier specifications.

2.7. Statistical Analysis

All the experiments were performed independently in triplicate. Statistical analysis was performed by GraphPad Prism Software 7.0c (La Jolla, CA). The pharmacokinetic data were calculated using Win Nolin. The statistical analysis of the difference between groups was tested by one-way ANOVA, followed by the Post hoc test for between-group comparisons. All the results are presented as mean ± standard deviation (S.D.) and $p < 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

As a drug delivery platform, nanonized drugs have shown to provide a number of advantages including enhanced solubility, permeability and bioavailability, improved stability and a formulation acceptable for oral route of administration [23]–[26]. The use of nanosizing for enhancing bioavailability of poorly water soluble drugs has been applied effectively over last few years, however, there are no reports in literature on nanonization of PG. Since micronization has shown to improve the bioavailability of PG [21], [22], therefore it was of interest to determine if nanonization could further impact the bioavailability. To ascertain this all the comparisons were made with micro-PG. The HPCG technology used in the present research for nanonization of PG has potential industrial feasibility and all the batches of nano-PG and nano-PG sustained release tablets were prepared in GMP facility. The use of sustained release tablets for micro-PG and nano-PG allows actual interpretation of the results since sustained release PG tablets are most widely used [17].

3.1. Characterization of nano-PG

The particle size and size distribution of nano-PG and micro-PG is shown in Fig. 1. The HPCG processing resulted in marked decrease in particle size and the mean size changed from 1.72 ± 2.5 µm (micro-PG) to 800 ± 35 nm (nano-PG). Low polydispersity index (0.15), suggested that the nano-PG particles were uniform. DSC was conducted to determine any thermal transition that occurred due to nanonization (Fig. 2). The absence of glass transition peak and presence of a sharp melting endotherm (130□C) in DSC thermogram of both micro-PG and nano-PG indicated that both the powders were crystalline and no phase change occurred during nanonization (Fig. 2). However, higher melting enthalpy of nano-PG (298.7 J/g) compared to micro-PG (129.2 J/g) indicated improved drug stability following nanonization. Such improvement in stability following nanonization was also reported earlier by our group when lumefantrine was milled using DYNO mill [23]. TGA thermogram (Fig. 3) showed that both nano-PG and micro-PG were thermally stable up to 250°C and thereafter degraded from 250°C to 350°C. From the thermogram (Fig. 3) it was evident that mass loss was higher for nano-PG (95.4% to 8.9%) compared to micro-PG (93.4% to 14.9%), however degradation started bit late for nano-PG. Such observations have been reported previously where nanosized particles with comparatively higher surface area showed increased thermal degradation [30]. Of note the results of our DSC and TGA findings were conflicting though the difference in degradation rates was very small. The XRD diffractogram of micro-PG and nano-PG are shown in Fig. 4. The XRD pattern demonstrated that both micro-PG and nano-PG were crystalline. However, decrease in peak intensities of 2-theta peaks at 5.2, 5.88 and 6.02 for nano-PG suggested that degree of crystallanity was lower for nano-PG (Fig. 4). Similar reduction in intensity of 2-theta peaks with decrease in crystallanity or with increase in amorphism were previously observed for several compounds [31], [32]. Additionally, XRD result was in agreement with DSC and support the presence of form I of progesterone [33]. From characterization studies, it appeared that HPCG milling had no effect on crystalline form however a slight reduction in degree of crystallanity was observed.

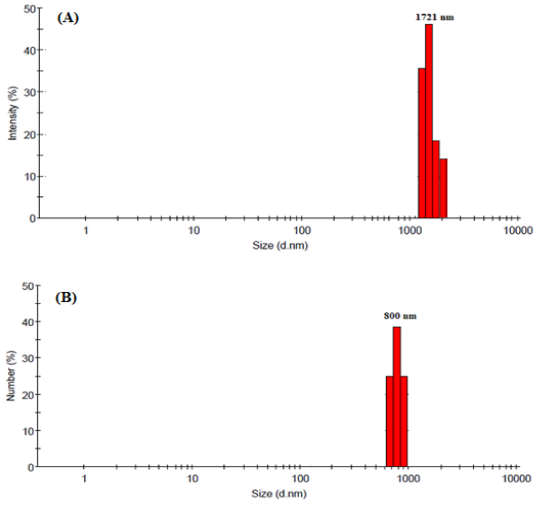

Fig. 1: Particle size and size distribution of (A) micro-PG and (B) nano-PG

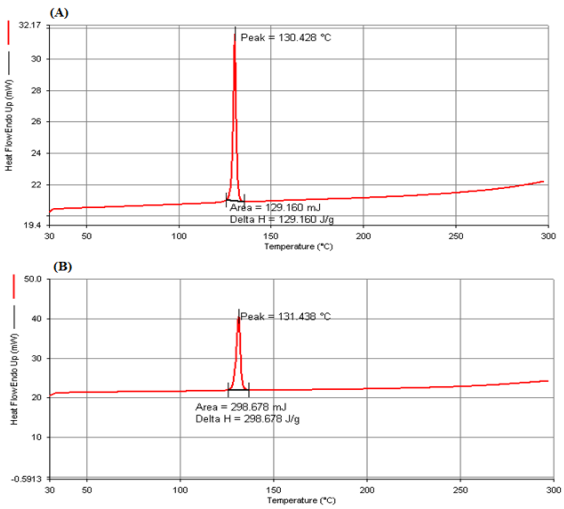

Fig. 2: DSC thermogram of (A) micro-PG and (B) nano-PG

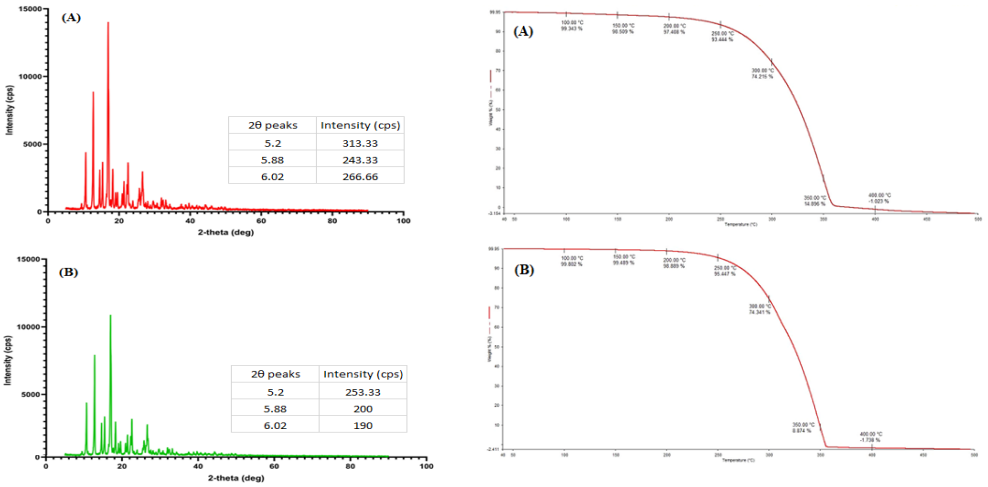

Fig. 3: TGA thermograms of (A) micro-PG and (B) nano-PG Fig. 4: XRD pattern of (A) micro-PG and (B) nano-PG

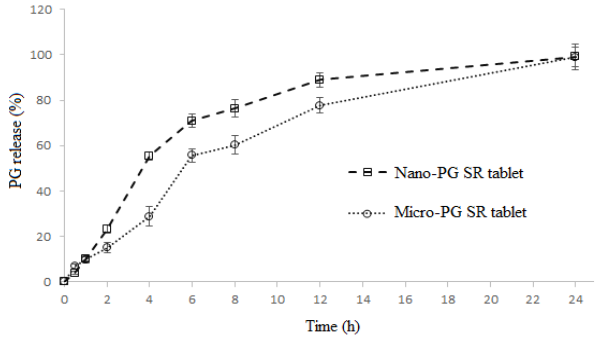

Fig. 5: Drug release profile of sustained release tablets of micro-PG and nano-PG

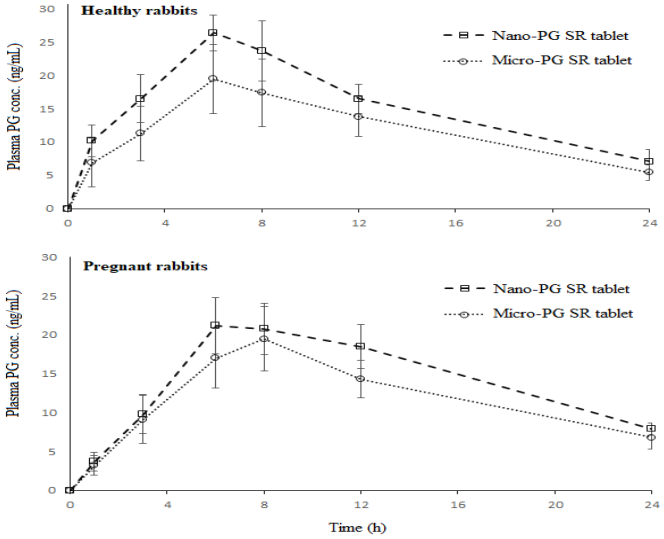

Fig. 6: Pharmacokinetic profile of sustained release tablets of micro-PG and nano-PG in healthy and pregnant rabbits

3.2. Solubility Studies of nano-PG

To compare and discriminate the solubility of nano-PG and micro-PG, aqueous media with various SDS concentrations was used since being highly insoluble in water it is difficult to analyze the enhancement of solubility, if any, in aqueous media. As anticipated, the aqueous solubility of nano-PG (27.8 ± 1.4 µg/mL) and micro-PG (30.0 ± 2.1 µg/mL) was comparable. Interestingly, in 1.25% SDS aqueous solution the solubility of nano-PG (172.0 ± 5.2 µg/mL) was nearly 2-fold higher than that of micro-PG (98.0 ± 6.9 µg/mL). Similar observations were obtained in 2.5% SDS aqueous solution and the solubility of nano-PG (540.0 ± 15.7 µg/mL) was 2-fold higher than micro-PG (290.0 ± 11.8 µg/mL). At 5% SDS aqueous solution, nano-PG (1280.0 ± 30.5 µg/mL) demonstrated 1.3-fold higher solubility than micro-PG (980.0 ± 45.9 µg/mL). Notably, the solubility of nano-PG was consistently higher than that of micro-PG. The improved solubility might be due to increased effective surface area due to nanonization. Similar observations following nanonization has been cited in literature [23] – [26].

3.3. Permeability Studies of nano-PG

The permeability of nano-PG and micro-PG was determined across porcine intestine. At 30 min, the permeability of micro-PG (48.0 ± 3.2 %) was similar to that of nano-PG (44.5 ± 5.6 %). Interestingly, at 1 h the permeation of nano-PG (89.2 ± 2.5 %) was significantly higher than micro-PG (77.4 ± 4.3 %). Thus, nanonization technology used in the present research not only improved the solubility but also increased the permeation of PG.

3.4. Dissolution Studies of nano-PG

Considering low aqueous solubility of PG [10] and based on results of solubility studies, 5% SDS aqueous solution was used as dissolution media. The use of discriminating media allows evaluation of difference in dissolution between micronized and nanonized PG [23]. The dissolution profiles of nano-PG and micro-PG sustained release tablet is shown in Fig. 5. Both the tablets showed sustained release profile over period of 24 h. Further, as expected, nano-PG tablet showed improved dissolution compared to micro-PG tablet. At 12 h, the drug release from nano-PG tablet was 88.9%, significantly higher ($p < 0.05$) than that released by micro-PG tablet (77.7%). According to the Noyes–Whitney equation, the decrease in particle size and increase in effective surface area leads to dissolution rate enhancement [23], [34]. Also, the decrease of particle size below 1 µm increases the saturation solubility [34]. Thus, nanonization of PG greatly increases the specific surface area and resulted in enhancement of solubility, permeability and dissolution significantly better compared to micro-PG. These promising in vitro results encouraged us to perform in vivo bioavailability studies in rabbits.

3.5. Pharmacokinetics of nano-PG tablets in rabbits

The pharmacokinetics of nano-PG and micro-PG was conducted in healthy and pregnant rabbits in order to ascertain changes due to pregnancy on bioavailability of PG. Based on the previously published report, a 20-day pregnant rabbits were used in the present study [28]. All the calculations were done considering initial PG concentration in the animal as zero. The plasma PG concentration vs time following oral administration of nano-PG and micro-PG sustained release tablet to healthy and pregnant rabbit is shown in Fig. 6 and the pharmacokinetic data is tabulated in Table 1. The t1/2 and MRT for both nano-PG and micro-PG tablet remained same in healthy and pregnant rabbits, however, the values were higher in pregnant rabbits. Similarly, the tmax for both the formulations (6 h) was same in healthy rabbits but in pregnant rabbits the tmax of micro-PG tablet was increased to 8 h from 6 h for nano-PG tablets. As expected, the Cmax of nano-PG tablet (26.4 ± 1.2 ng/mL) was significantly higher compared to micro-PG tablet (19.5 ± 3.7 ng/mL) in healthy rabbits. The Cmax for nano-PG tablet was also higher in pregnant rabbits but the difference was not significant ($p > 0.05$). Although, it is important to note that the tmax of micro-PG is different from nano-PG. In healthy rabbits, the nano-PG tablet (AUC, 464.5 ± 8.8 ng/ml/h) demonstrated 30% (1.3-fold) higher bioavailability compared to micro-PG tablet (AUC, 355.0 ± 14.1 ng/ml/h). However, in pregnant rabbits the increase in bioavailability following nanonization was 18%. The enhancement of bioavailability was expected because nano-PG has demonstrated increased solubility and permeability compared to micro-PG. Interestingly, nano-PG tablet demonstrated comparable bioavailability in healthy (464.5 ± 8.8 ng/ml/h) and pregnant (461.3 ± 7.4 ng/ml/h) rabbits but micro-PG tablet showed difference in bioavailability between healthy (355.0 ± 14.1 ng/ml/h) and pregnant (388.4 ± 12.5 ng/ml/h) rabbits. Also, to note that standard deviation for AUC of nano-PG is lower than that of micro-PG. Based on the result, it could be hypothesized that nano-PG could overcome variation in drug absorption, a problem associated with micro-PG [18]. However, more detailed studies are needed to support this hypothesis.

Table 1. Pharmacokinetic parameters following oral administration of sustained release tablet of nano-PG (200 mg) and micro-PG (200 mg) to healthy and pregnant female rabbits.

Pharmacokinetic Parameters	**Healthy Rabbits**		**Pregnant Rabbit**	
	Micro-PF (n=8)	Nano-PF (n=8)	Micro-PG (n=8)	Nano-PG (n=7)
$t_{1/2}$ (h)	9.3	9.4	10.7	10.4
T_{max} (h)	6	6	8	6
C_{max} (ng/ml)	19.5 ± 3.7	26.4 ± 1.2	19.5 ± 4.1	21.2 ± 1.6
$AUC_{0-\infty}$ (ng/ml/h)	355.0 ± 14.1	464.5 ± 8.8	388.4 ± 12.5	461.3 ± 7.4
$AUMC_{0-\infty}$ (ng/ml/h^2)	5630.2 ± 266.9	7325.8 ± 132.5	7273.2 ± 332.5	7853.8 ± 175.2
$MRT_{0-\infty}$ (h)	15.8 ± 1.6	15.8 ± 0.5	18.7 ± 1.9	18.2 ± 0.8

In conclusion, HPCG technology yield nanosized PG particles without affecting solid state characters of PG. Further, nanonization could be an effective approach to enhance solubility and bioavailability of oral PG thereby reducing its dose and related side effects.

Conflict of interest

All the authors report no conflicts of interest.

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