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ORIGINAL ARTICLE

BIOCHEMICAL AND HISTOLOGICAL CHANGES IN REPRODUCTIVE ORGANS OF EXPERIMENTAL RATS AFTER DIENOGEST THERAPY

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Summary

The precise method of action of dienogest on the production and development of endometriosis lesions is unknown, and its controversial effect on endometrial thickness has been under investigation. In the following study the Dienogest's effects on the target animal's histology of female reproductive organs, including the tissues from the Fallopian tubes, ovaries, and uterus, as well as the impact of drug administration on the liver's enzymes and the drug's effects on triglycerides, body weight, and HbA1c, have all been studied. The findings of the following experiment indicated that there was no significant elevation of liver enzymes. The little to no elevation of the liver enzymes indicated that the drug did not induce stress on the hepatic cells and according to the subsequent experiment it is safe for clinical use. Moreover, after 10, 20, and 30 days of blood administration, the level of blood TG significantly decreased, and after 30 days of intake, the level of blood sugar significantly decreased. However, there were no appreciable changes after 10 and 20 days. After 30 days of treatment, the rats' weight also showed a very minor drop. In addition to it, the results of histological changes in the tissue in the following study represented that there were evident changes in the tissues which comprised of decline in blood circulation, fibrosis in tissues, and degeneration of follicles.

Key words: Dienogest; contraceptive; progesterone; liver; metabolic effects

Introduction

It has been indicated in multiple trials that Dienogest is a synthetic oral progestogen with a different pharmacological profile that is approved for the treatment of the disease of endometriosis at a dose of 2 mg/day in adults (1). It has a significant progestational effect and a mild anti-gonadotropic effect, but little to no androgenic, glucocorticoid, or mineralocorticoid action (2). Dienogest has little affinity for the progesterone receptor (10% of progesterone) and at a dose of 2 mg/day, it lowers estradiol levels only slightly. It has good oral bioavailability and a long half-life which is responsible for making it suitable and appropriate for daily administration through the oral route (3). The following findings have led to multiple trials of the drug through which Dienogest has been approved as a monotherapy for the treatment of endometriosis in multiple states like Japan, Australia, Europe, and Singapore based on this trial evidence (4).

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The search for targeted therapy with few side effects has long been underway, but no superior drug is available. Recently, selective progesterone receptor modulators (SPRMs) and next-generation progestins have been suggested or postulated for the treatment of endometriosis (5). For example, eugenolastase acetate (Esmya), an SPRM, has long been used to treat uterine fibroids. Esmya can reduce the size of fibroids by preventing proliferation and stimulating apoptosis. Although its effectiveness in treating endometriosis has not been thoroughly studied, it may also be effective. Dydrogesterone, a progestin produced from 9-, 10-progesterone, a component of Duphaston, has been shown to be effective in treating endometriosis pain without affecting pregnancy. There is insufficient evidence for its use in the treatment of endometriosis. Another progestin, dienogest, produced by 19-nortestosterone, has been used to treat symptoms of endometriosis (6, 7). By causing metaplasia and preventing implantation and angiogenesis of endometrial lesions, it prevents the development of these lesions. The precise method of action of dienogest on the production and development of endometriosis lesions is unknown, and its controversial effect on endometrial thickness has been noted (8). Even so, the possibility of recovery of endometriosis after discontinuation of Esmya, Duphaston and Dienogest has not been studied. The following experiment-based study aims to evaluate the effects of Dienogest on the liver, endometrium tissues, and thyroid during different treatment time periods, as well as on uterine tissue structure.

Materials and Methods

Test drug: In this trial, desogestrel was used as a test drug. It is an oral progestin used as monotherapy, as a preferred method of contraception, or in combination with ethinylestradiol for the treatment of endometriosis. It is said to have the chemical formula C20H25NO2 and a molecular weight of about 311.4 g/mol. Its half-life is about 7.5 hours and its bioavailability is 90%. It is metabolized in the liver and eventually excreted through the urine.

Animal: The target samples selected for use in subsequent experimental studies were after obtaining permission, 8-week-old female Sprague-Dawley rats were purchased from the market. These animals were housed at an ideal room temperature of 23 to 28 degrees Celsius with a relative humidity of approximately 51% and maintained in a diurnal balance with 12-hour light and 12-hour dark cycle (14, 15). During the test period, the rats were provided with sterilized solid food appropriate to their needs and unrestricted access to water. Before any of the rats were used in the experiment, vaginal smears were performed to determine whether they were in the oestrous cycle. At the end of each 10 days following dienogest therapy, 10 animals were bled to death while being given sodium pentobarbital (50 mg/kg) for anaesthesia. The animals used in this study were cared for in accordance with the policies of Mochida Pharmaceuticals Ltd. and protocols were followed in the handling of these animals.

Experimental design: A total of 40 rats were included in the study which was divided into 4 groups, a controlled group, and three dienogest groups of 10 days, 20 days, and 30 days respectively. The liver function and uterus histological analysis has been done separately.

Histological study: ovary, uterus, and Fallopian tubes were collected from the sacrificed animal and fixed in formalin overnight. The tissue slices were then prepared, stained with eosin-hematoxylin and examined under the microscope for pathological changes (Figures 1, 2, 3).

Biochemical measures: Liver function has been analyzed by evaluating the levels of AST and ALT in 10, 20, and 30 days respectively. In addition to it, HbA1c, TG, T4, and BS have also been analyzed. Biochemical parameters were measured using the following kits, for T4 Biomerux kit France (VIDAS instrument), TG & Glucose kit from Biolabo France, HbA1c kit from HUMAN USA, and Roche Reflotron Test Strips for GOT and GPT.

Statistical analysis: The results of each experiment were expressed as the mean and S.D. One-Way ANOVA followed by a posthoc test was used to assess the significance of differences between groups in multiple group comparisons. For comparisons between two groups, the Student's t-test was used. All values were statistically significant with ($p<0.05$).

Results

According to the histological study of the uterine tissues, ovarian tissues, and the tissues from the Fallopian tube, there were no abnormalities in the results of the control group. Normal and healthy structures of the tissues were

visible, and the ovary and its mature primary and secondary follicles - clearly supported by granulosa cells, antrum and effusion - were no exception. The submucosa, mucosa, muscularis and secretory ducts were among the features that were valued. However, in all three intervention groups, structural changes were evident, including follicular degeneration, slight restriction of blood circulation due to arterial congestion, and increased fibrosis already observed in the connective tissue. In addition to this, infiltration of inflammatory cells was seen.

After 10 days of drug treatment, the second group showed a slight mucosal thickening, infiltration of inflammatory cells in the submucosa, necrosis of secretory gland cells, and vascular occlusion. During 20 days of drug induction, there was an increased folding of mucosal cells, significant hyperplasia of the mucosal epithelial tissue, and infiltration of polymorphonuclear inflammatory cells - especially eosinophils - into the cells of the submucosa. Inflammation of the Fallopian tubes in the group receiving Denovo pregnancy for 30 days was characterized by necrosis of glandular epithelial cells, infiltration of polymorphonuclear inflammatory cells of the submucosa, especially eosinophils, obstruction of blood vessels and necrosis of glandular cells. Staining with hematoxylin and eosin (Figures 1, 2, 3).

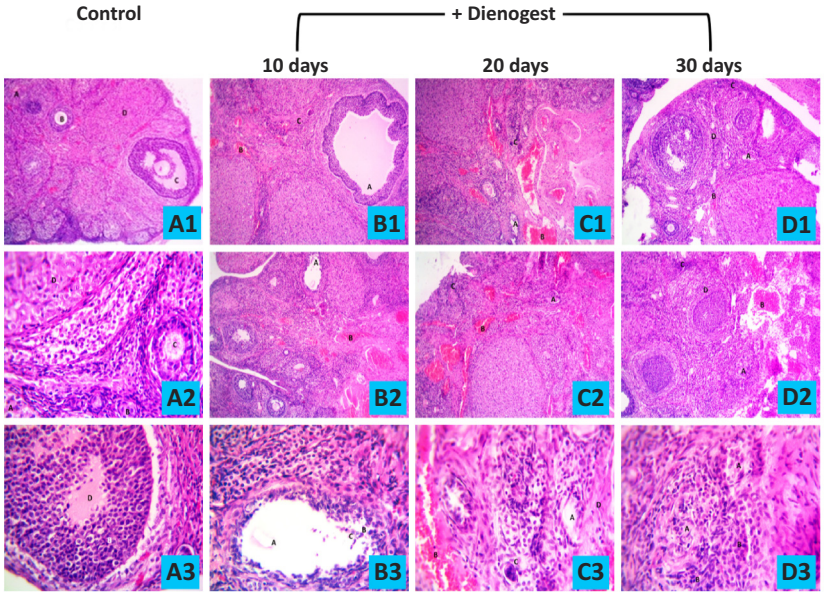

Figure 1. Histological changes associated with ovary following exposure to Dienogest over three timepoints; 10 days (B), 20 days (C), 30 days (D) compared to dienogest-free group (A). (A) Normal architecture of ovarian tissue representing by primordial, primary or secondary, mature follicle (characterized by granulosa cells, cumulous cells,antrum) , and corpus luteum. Atretic follicle (degeneration of follicles), congestion of blood vessels, and increased fibrous connective tissue between follicles, infiltration of inflammatory cells (B, C, D). Eosin-Hematoxylin stain. 100X (A1,B1, B2, C1, C2, D1, D2); 400X (A2, A3, B3, C3, D3).

In liver function analysis, levels of ALT=alanine aminotransferase and AST=aspartate aminotransferase were observed in all four groups. In the control group, the levels of AST and ALT were 210 U/L and 70 U/L, respectively, which were in the normal range. The second group that received the drug for 10 days was the dienogest group, which showed almost no significant increase in the levels of the enzymes. In the third group that received the drug for 20 days, AST increased from 200 U/L to 220 U/L, while the results of ALT were unchanged. In the last group, AST increased from 200 U/L to 300 U/L, while ALT remained generally unchanged (figure 4).

The analysis of drug impact on the TG indicated that there was a marked decrease in the level of blood TG after 10, 20, and 30 days of blood administration and the blood sugar levels fell after 30 days of intake, although in 10 and 20 days there were no significant changes. In addition to it, there was a slight decrease in the weight of the rats after 30 days of treatment. Weight loss associated with dienogest therapy leads to reduced weight with time. Thyroxine, blood sugar, and HbA1c were only slightly affected mainly during the first 10 days and returned to normal with continuous therapy thereafter (figure 5).

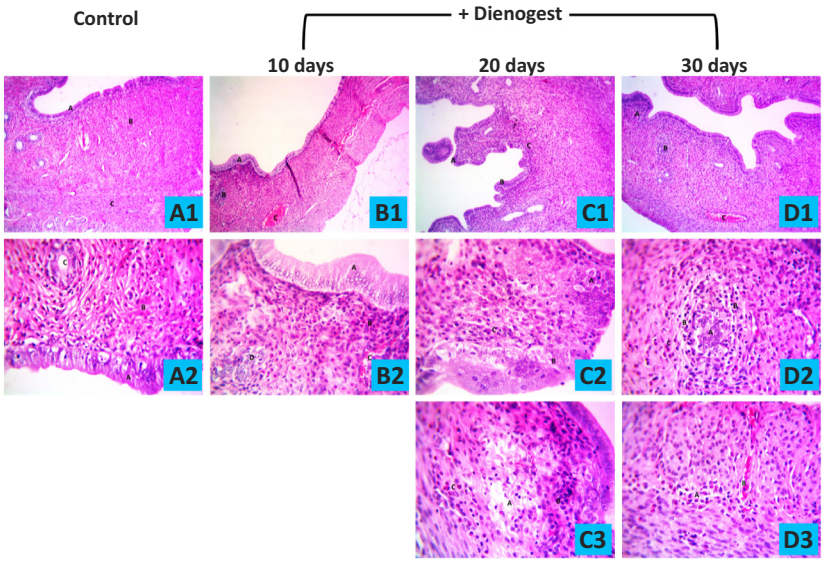

Figure 2. Histological changes associated with Fallopian tube following exposure to Dienogest over three timepoints; 10days (B), 20days (C), 30 days (D) compared to dienogest-free group (A). (A) Normal architecture representing by mucosa, submucosa, muscularis layer, and secretory ducts. (B) Thickening of mucosa, infiltration of inflammatory cells in submucosa, congestion of blood vessels, necrosis of glandular cells. (C) Increased folding of mucosa, hyperplasia of mucosal epithelium, and infiltration of polymorphnuclear inflammatory cells in submucosa specially eosinophils. (D) Salpingitis representing by necrosis of glandular epithelial cells and by infiltration of polymorphnuclear inflammatory cells in submucosa specially eosinophils , necrosis of glandular cells and congestion of blood vessels. Eosin-Hematoxylin stain. 100X (A1,B1, C1, D1); 400X (A2, B2, C2, C3, D2, D3).

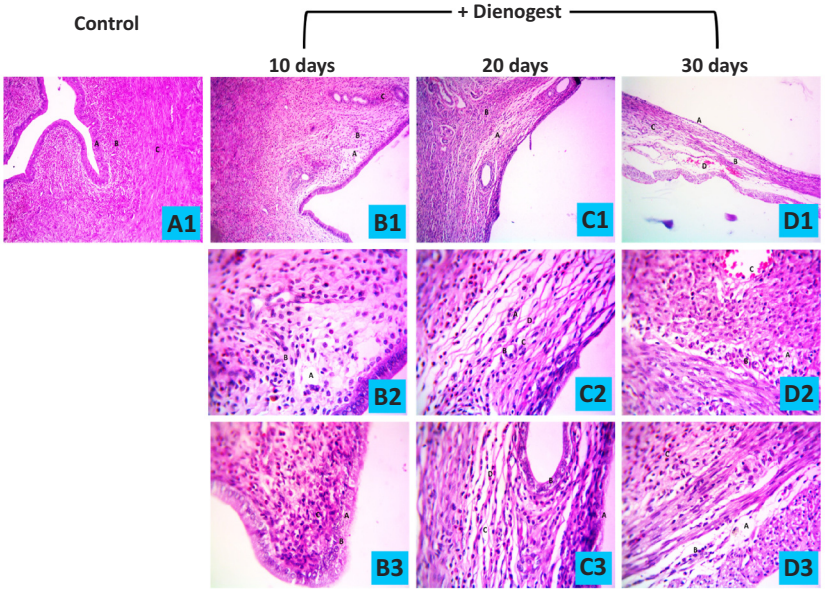

Figure 3. Histological changes associated with uterus following exposure to Dienogest over three timepoints; 10days (B), 20days (C), 30 days (D) compared to dienogest-free group (A). (A) Normal architecture representing by mucosa, submucosa, muscularis. thickening of mucosa, infiltration of inflammatory cells in submucosa, congestion of blood vessels, necrosis of glandular cells. (B) Degeneration and necrosis of mucosal epithelium and infiltration of polymorphnuclear inflammatory cells in surrounding with eosinophils. (C) edema between myometrium fibers and infiltration of polymorphnuclear inflammatory cells leading to myometritis. (D) myometritis representing by edema between myometrium fibers, infiltration of polymorphnuclear inflammatory cells in surrounding with esinophils alongside congestion of blood vessels. Eosin-Hematoxylin stain. 100X (A1, B1, C1, D1); 400X (B2, C2, D2, B3, C3, D3).

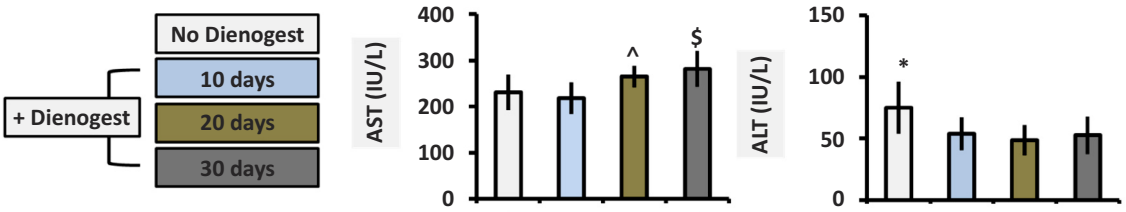

Figure 4. Liver enzyme changes associated with dienogest use. Data expressed as mean±SD. *#^$P<0.05 as compared to other groups. AST=Aspartate transaminase, ALT=Alanine transaminase.

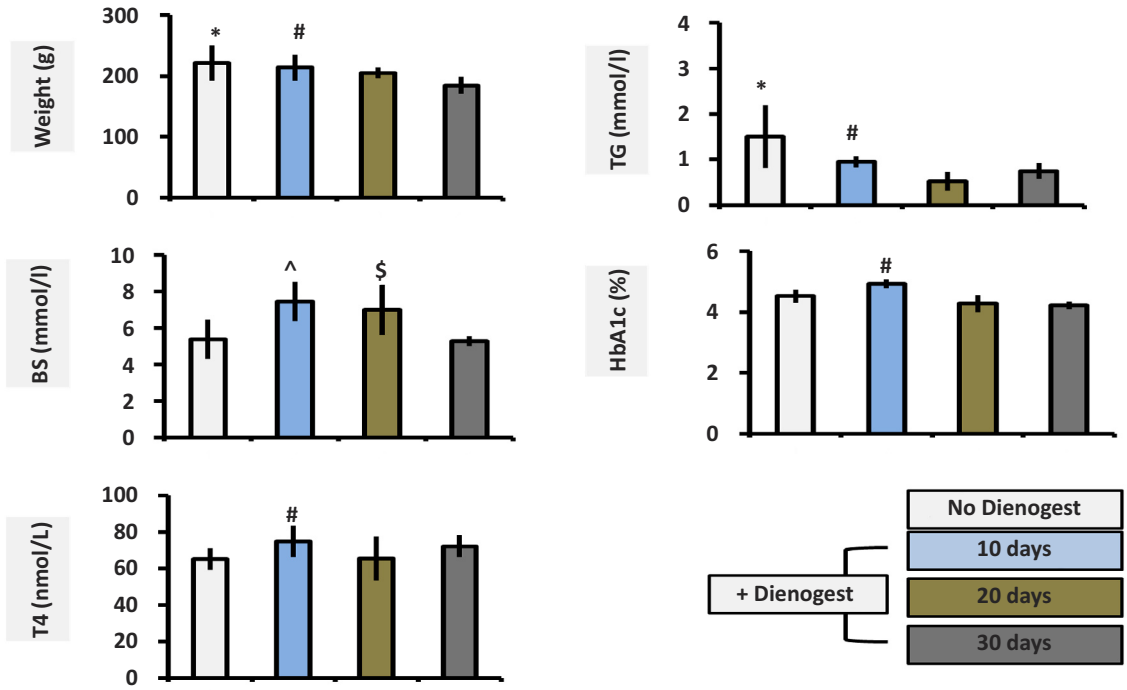

Figure 5. Glycemic and metabolic changes associated with dienogest use. Data expressed as mean±SD. *#^$P<0.05 as compared to other groups. TG=Triglycerides, BS= blood glucose, T4=Thyroxine, HbA1c=Glycated hemoglobin.

Discussion

In the detailed evaluation of the effects of the drug Dienogest on the target animal in different aspects, including the effects of Dienogest on the histology of female reproductive organs including the tissues from the Fallopian tubes, ovaries and uterus, the impact of the drug administration on the liver enzymes, and its effects on triglycerides, body weight, and HbA1c has been evaluated. The results of histological changes in the tissue in the following study represented that there were evident changes in the tissues which comprised of decline in blood circulation, fibrosis in tissues and degeneration of follicles. The following results have been seconded by the study which stated that dienogest administration resulted in an increase in uterine infiltrating natural killer (NK) cells in the glandular endometrium (16, 17). The response of NK cells to dienogest varied depending on the site of immune cell infiltration. In addition, the effects of dienogest on uterine NK cells in adenomyosis may be beneficial in terms of embryo implantation and fetal protection for pregnancies that occur after the end of treatment (18). It has been confirmed that statins modulated circulatory lymphocyte count (19), and Progesterone is structurally derived from cholesterol as a precursor molecule (20, 21). Therefore, they might share the same effects on immune cells infiltration into tissues.

From the analysis of the liver enzymes in the groups of the sample which were given the intervention, i.e. Dienogest for different periods, it was found that there was no significant elevation of liver enzymes. The little to no elevation of the liver enzymes indicated that the drug did not induce stress on the hepatic cells and according

to the subsequent experiment it is safe for clinical use. These findings of our study have been supported by multiple other studies like (22-24) a study based on the evaluation of the effects of Dienogest on liver enzymes indicated that no deviations from the normal range were observed during the treatment period which was one, three, and six months. There was a slight significant decrease in ASAT and ALAT and a slight significant increase in LDH and BILI ($p<0.05$), but these remained within normal laboratory values. Since no adverse metabolic side effects of dienogest have been observed to date, it can be considered an effective new alternative therapy for the treatment of endometriosis (24). Furthermore, another study mentioned that at the high dose, dienogest showed no adverse effects on lipid metabolism, liver enzymes, fasting insulin, or glucose. There were no reports of menopausal symptoms or adverse androgen-related effects (20).

Contrary to the following findings, it has been found in the conclusion of the research that in patients with liver diseases some adverse effects have been observed associated with the use of Dienogest, therefore it is not the best choice of drug for patients with liver problems (26). Another study analyzed the general adverse effects which have been associated with the use of Dienogest, these included gain in weight, mood swings, menstrual irregularities, and gastric discomfort (27). However, these findings have been rare and there is limited data which supports this. Although according to the following study the use of Dienogest is clinically safe, however more studies are required on human subjects to confirm the findings.

As per the results of this study the blood TG levels decreased significantly after 10, 20 and 30 days of blood administration, and blood glucose levels decreased significantly after 30 days of intake. However, no significant changes occurred after 10 and 20 days. The body weight of the rats also decreased only very slightly after 30 days of treatment.

Conclusion

The following experimental study aims to investigate the effects of Dienogest on the liver and uterine tissue structure during different treatment periods which were 10 days, 20 days, and 30 days after the treatment and these groups were compared with the controlled group which received the placebo. The specific mechanism of action of Dienogest on the formation and progression of endometriosis lesions is unknown, and a debatable effect on endometrial thickness has been identified. It was shown that there was no substantial increase in liver enzymes. The low to no rise of liver enzymes suggested that the medicine did not cause stress on the hepatic cells and that it is safe for clinical use, according to the subsequent experiment. Therefore, it can be concluded that Dienogest is safe and it does not impose any adverse effects on metabolism.

Conflict of interest

The authors declare no conflict of interest concerned in the present study.

Adherence to Ethical Standards

The study was approved by the Research Ethical Committee and Scientific Committee in the Department of Dental Basic Science of College of Dentistry / University of Mosul with approval number (UOM/COM/MREC/20-23(15) in 07.02.2021).

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