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ROLE OF ALLOPURINOL IN INDUCED ENDOMETRIAL HYPERPLASIA IN RATS

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Background and aim: Allopurinol is traditionally recommended for treatment of gout. The current study was planned to discover the influence and mechanism of allopurinol on endometrial hyperplasia (EH).

Experimental design: 24 female Wistar rats were classified into 3 groups. Control group: Rats were received carboxymethyl cellulose 1% orally. Endometrial hyperplasia group: Rats administered estradiol valerate (EV). Therapeutic group: Rats treated with allopurinol.

Results: In the EH group, there was significantly elevated serum uric acid and dyslipidemia. Also, significant elevation in NO and MDA tissue levels and significant reduction in tissue level of CAT, SOD, and Nrf2. Furthermore, tissue IL10 was significantly decreased while IL6, TNF-α, and NF-κB were significantly increased. Uterine PI3K, AKT, and mTOR genes were up-regulated. Histologically, there was increased endometrial thickness, stratification, numerous invaginations, and papillary formation of surface epithelium. Surface and glandular epithelium of endometrium revealed loss of BAX expression. All of the previously mentioned biochemical and histological alterations caused by estradiol-induced endometrial hyperplasia were reversed by allopurinol.

Conclusion: Therefore, these results revealed that allopurinol could suppress endometrial hyperplasia by targeting PI3K/AKT/mTOR and BAX signaling. In addition to its antioxidant and anti-inflammatory properties.

Keywords: Endometrial hyperplasia, BAX, apoptosis, xanthine oxidase, uric acid.

INTRODUCTION

Endometrial hyperplasia (EH) is the most common risk factor for endometrial cancer in females. Uterine inflammation has been directly linked to the pathophysiology of EH and has the potential to cause aberrant cell division and decreased apoptosis[1]. In the endometrium, wild cell proliferation, aberration, and carcinogenesis are caused by dysregulation of cellular apoptosis[2]. Given this situation, regulating apoptosis of EH cells would be a promising target for developing effective anti-EH agents. The growth of the endometrial stroma may be modulated by reactive oxygen species. Increased oxidative stress and antioxidant depletion are two pathologic conditions that may lead to endometrial stromal cell proliferation[3]. Metabolic causes play a significant role in the advance of endometrial malignant hyperplasia; the main risk factors are hyperuricemia, hyperlipidemia, and BMI> 25[4].

It has long been controversial to discuss how oxidative stress contributes to disease. Instead of using scavenging to get rid of ROS

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that have already formed, research has focused on ways to decrease their formation. The two main enzyme systems that produce ROS are xanthine oxidase and NADPH oxidase.The balance of ROS in the endometrium is preserved with both enzymatic and non-enzymatic anti-oxidative systems[5]. Based on the evidence, it appears that the ROS and its scavenging system contribute significantly to the function of the human endometrium.

Even though allopurinol has been used extensively for a long time, research on the drug is still ongoing in an effort to improve patient outcomes[6]. Allopurinol is an antioxidative drug that predominantly used to reduce uric acid levels. Its antioxidative potential has been demonstrated in earlier researches[7]. In addition to treating hyperuricemia, allopurinol has a major impact on the management of other illnesses. According to research, patients with gout receiving allopurinol can have a reduced incidence of manic symptoms, chronic kidney disease, prostate cancer, and cardiovascular disease and mortality[8-11]. Allopurinol also possesses anti-inflammatory and analgesic properties[12].

We hypothesized that oxidative stress and inflammation which induce cell proliferation and decrease apoptosis are key mechanisms that cause the imbalance of PI3K/AKT/mTOR and BAX in endometrial hyperplasia. Thus, the purpose of this study was to look into how allopurinol can attenuate estradiol valerate induced endometrial hyperplasia in female rats.

MATERIALS AND METHODS

Chemicals and drugs

Biodiagnostic Company for pharmaceuticals and chemical industries, Egypt provides carboxymethyl cellulose 1% (CMC), catalase (CAT), superoxide dismutase (SOD), nitric oxide (NO), malondialdehyde (MDA), uric acid (UA), high-density lipoprotein (HDL), total cholesterol and triglycerides. Low-density lipoprotein (LDL) was measured according to the Friedewald formula: LDL = Total cholesterol - (HDL + (TG/5)). Estradiol valerate purchased from Bayer Weimar GmbH and Co. KG (Weimar, Germany) in Cyclo-Progynova tablets (2 mg/tablet), while the supplier of allopurinol was AK Scientific, Inc. (USA).

Animals

Adult female Wistar rats weighing 190 ±20 grams have been used. Animals were got from the animal house, Faculty of Medicine, Sohag University, Egypt. Rats were preserved in the animal house with room temperature being preserved at 23±2°C and 50±10% humidity. Animals were permitted to have free access to food and water. Rats kept under normal light/dark cycle. The European Union Guidelines for the Care and Use of Laboratory Animals (European Union Directive 2010/63/EU) were firmly tracked during the study, which was directed with the approval of the Institutional Animal Care and Use Committee of Faculty of Medicine, Sohag University, Egypt (authorization number 5-5-2022-01).

Experimental design

Totally 24 female Wistar rats were allocated into 3 groups after one week of acclimatization; eight rats each and distributed randomly.

Control group: Rats were taken carboxymethyl cellulose 1% (CMC) orally as a vehicle.

Endometrial hyperplasia group: Rats were treated EV (2 mg/kg) orally daily for10 days[13].

Allopurinol + EV therapeutic group: Rats were treated daily with EV (2 mg/kg/ orally) for 10 days and then with allopurinol for another 10 days[14].

Sample collection

After the experiment was completed all animals were weighed, the rats of all groups were euthanized and given isoflurane anesthesia. Uterus carefully removed and cleaned with cold saline and was divided into 3 parts after weighing. Part of uterus was preserved in formalin 10% for histological and immunohistochemical investigation. The part used for gene expression study was quickly kept at -80°C after freezing in liquid nitrogen. The remaining part was homogenized in sodium phosphate buffer (pH 7.4) and centrifuged. The supernatant was separated for biochemical assay.

Biochemical analysis

According to Montgomery, Dymock[15] and Ohkawa *et al.*[16], NO and MDA levels were assayed respectively. Colorimetric technique analysis was used for both of them. The absorbance change was detected at 540 nm for NO and at 534 nm for MDA. The level of NO was reported as μmol/mg tissue and MDA as nmol/mg tissue.

Superoxide dismutase and CAT were measured according to Nishikimi *et al.*[17] and Aebi[18] respectively. At 550 nm, a change in tissue SOD absorbance was observed, and it was reported as U/mg tissue. Regarding CAT, a change in absorbance was observed at 510 nm and reported as U/mg tissue.

Nuclear factor erythroid 2-related factor 2 (Nrf2) was supplied by Cusabio Technology LLC (USA). It was measured by ELISA in accordance with manufacturer's instructions and expressed in ng/mg tissue.

An ELISA-specific kit was used to measure the tissue levels of interleukin 6 (IL6), IL10, tumor necrosis factor alpha (TNFα), and nuclear factor-κB (NF-κB). Kits for IL-10 and NF-κB were supplied by Cusabio, USA. Wuhan EIAab Science Co. Ltd., China, Elabscience., USA provided TNF-α and IL-6 kits respectively and expressed as pg/ mg tissue.

Quantitative real-time PCR analysis

Total RNA was isolated from uterus tissue samples utilizing TRI reagent (Bioshop Co, Canada). GoTaq 1-Step RT-qPCR kit from Promega Institution, USA was used to create the template cDNA. Applied Biosystems 7500 apparatus (USA), was used to perform the amplification. The initial denaturation step of PCR amplification process took place at 95°C for 10 minutes. Then, denaturation is carried out 40 cycles for 10 seconds at 95°C. 30 seconds of annealing at 60°C, followed by extension for 30 seconds at 72°C. The relative level of gene expression for each sample was measured using the $^{2-\Delta\Delta}$CT comparative method[19], which was standardized to the housekeeping gene β-actin. The nucleotide sequences of the primers were as follow (Table 1):

Table 1: Sequences for the studied genes' primers.

Gene	Primer sequence: 5′–3′
PI3K	F: CTC TCC TGT GCT GGC TAC TGT R: GCT CTC GGT TGA TTC CAA ACT
AKT	F: ATC CCC TCA ACA ACT TCT CAG T R: CTT CCG TCC ACT CTT CTC TTT C
mTOR	F: TGC CTT CAC AGA TAC CCA GTA C R: AGG TAG ACC TTA AAC TCG GAC C
β-actin	F: GGC ACC ACA CCT TCT ACA ATG R: GGG GTG TTG AAG GTC TCA AAC

PI3K: Phosphatidylinositol-3-kinase, AKT: Protein kinase B, mTOR: Mammalian target of rapamycin. F: Forward, R: Reverse.

Histopathological and Immunohistochemical studies

Histopathological and immunohisto-chemical analyses were performed on the formalin-preserved uterine specimen.

Histopathological evaluation

Hematoxylene and Eosin (H&E) stain was used for routine histological processing to evaluate the normal histological structure. Five micron (μm) tissue sections were prepared and examined under a light microscope (Olympus Corp., Tokyo, Japan).

Immunohistochemical study

Immunohistochemistry of BAX carried out using Avidin-Biotin immunoperoxidase complex technique (ABC). The chosen paraffin-embedded tissues were deparaffinized and rehydrated through a graded alcohol series. Few micron tissue sections were incubated in hydrogen peroxide to block endogenous peroxidase activity. Sections were incubated by the primary antibody (BAX, a mouse monoclonal antibody, Santa Cruz Biotechnology, Inc., sc-20067). After that, sections were incubated with the pre-diluted biotinylated secondary monoclonal antibody (mouse IgG1 binding protein (m-IgG1 BP), Santa Cruz Biotechnology, Inc., sc-525408) for 45 minutes after being washed by phosphate-buffered saline. Diaminobenzidine (DAB) kit was used for staining tissue sections before being counter-stained with Mayer's hematoxylin. Lastly, the sections were

dehydrated, cleared, and finally mounted by DPX then a slide cover was put. The immunostaining interpretation was done using light microscopy.

Statistical analysis

Version 26 of the SPSS software was used for all statistical analyses. One-way analysis of variance (ANOVA) was used to statistically analyze the data, and the results were expressed as mean ± SE. To assess the variations between groups, the Tukey post hoc test was used. When $p< 0.05$, the difference was deemed significant.

RESULTS

Effect of allopurinol on uterine weight, body weight and uterine somatic index

Table 2 showed that treatment of female rats with EV produced a significant rise in uterine weight, body weight and the uterine somatic index compared to the control group. Interestingly treatment with allopurinol showed significant drop in uterine weight, body weight and the uterine somatic index in comparison with endometrial hyperplasia group.

Effect of allopurinol on serum uric acid

The findings indicated that there was significant increase in serum uric acid in endometrial hyperplasia group compared to control group. After treatment with allopurinol there was significant drop in serum uric acid compared to endometrial hyperplasia group (Fig. 1).

Effect of allopurinol on lipid profile

Table 3 showed that there was significant elevation in total serum cholesterol, triglycerides and low-density lipoprotein and significant decline in high-density lipoprotein in endometrial hyperplasia group confronted to control group. However, treatment with allopurinol elevate high-density lipoprotein and reduce total serum cholesterol, triglycerides and low-density lipoprotein significantly faced to endometrial hyperplasia group.

Effect of allopurinol on inflammatory biomarkers

The results of this research revealed that the tissue level of TNFα, IL6 and NF-κB, were substantially greater in EH group than those of the control group, while the EH group's tissue level of IL10 was significantly reduced. In contrast to the EH group, allopurinol administration dramatically reduced the levels of TNFα, IL6 and NF-κB, and increased the levels of tissue IL-10 (Table 4).

Effect of allopurinol on oxidant and antioxidants

Present data showed that NO and MDA level were significantly higher in endometrial hyperplasia group faced to control group. While administration of allopurinol lower NO and MDA faced to endometrial hyperplasia group. In endometrial hyperplasia group, there were significant reduction in Nrf2, CAT and SOD in comparison with control group. Allopurinol therapeutic group showed significant increase in Nrf2, CAT and SOD opposed to endometrial hyperplasia group (Table 5).

Table 2: Effect of allopurinol on uterine weight and uterine somatic index of estradiol valerate induced endometrial hyperplasia.

Group	Uterine weight (gram)	Body weight (gram)	Uterine somatic index
Control group (-ve control)	(1.293 ± 0.049)	(195.25 ± 2.287)	(0.662 ± 0.018)
Estradiol valerate group (+ve control)	$(1.599 \pm 0.034)^{*}$	$(215.25 \pm 2.056)^{*}$	$(0.743 \pm 0.009)^{*}$
Allopurinol therapeutic group	$(1.30775 \pm 0.053)^{\#}$	$(205.75 \pm 2.175)^{\#}$	$(0.635 \pm 0.023)^{\#}$

Data were exhibited as mean ± SE (n= 8), $*p< 0.05$ faced to control group. $^{\#}p< 0.05$ faced to endometrial hyperplasia group.

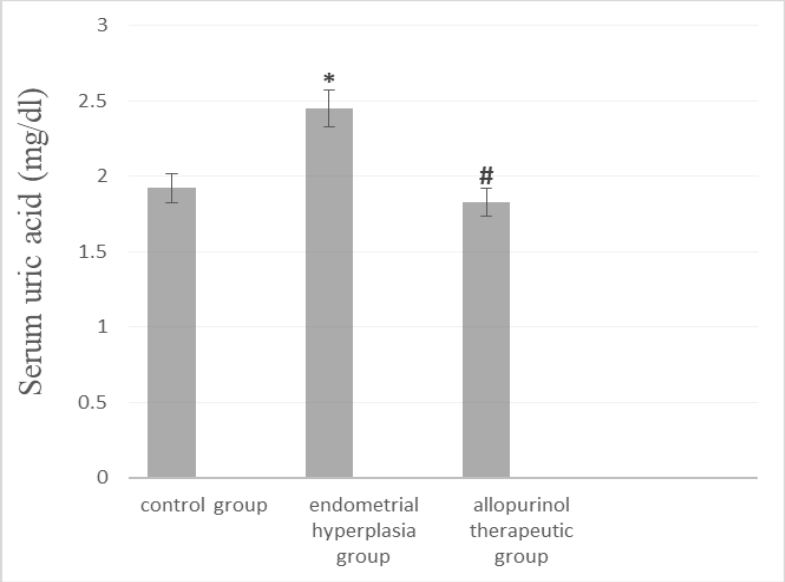

Fig. 1: Effect of allopurinol on serum uric acid in estradiol valerate induced endometrial hyperplasia. Records were exhibited as mean ± SE (n= 8), $^{*}p< 0.05$ faced to control group. $^{\#}p< 0.05$ faced to endometrial hyperplasia group.

Table 3: Effect of allopurinol on lipid profile in estradiol valerate induced endometrial hyperplasia.

Group	Total serum cholesterol (mg//dl)	Triglycerides (mg/dl)	High-density lipoprotein (mg/dl)	Low-density lipoprotein (mg/dl)
Control group (-ve control)	(102.08 ± 4.89)	(110.46 ± 10.88)	(48.21 ± 1.56)	(28.31 ± 2.65)
Estradiol valerate group (+ve control)	(217.49 ± 6.46)*	(261.42 ± 3.97)*	(30.49 ± 0.44)*	(134.54 ± 5.8)*
Allopurinol therapeutic group	(125.28 ± 6.09)#	(133.48 ± 4.78)#	(47.89 ± 1.16)#	(47.37 ± 3.37)#

Data were exhibited as mean ± SE (n= 8), $^{*}p< 0.05$ faced to control group. $^{\#}p< 0.05$ faced to endometrial hyperplasia group.

Table 4: Effect of allopurinol on inflammatory biomarkers in estradiol valerate induced endometrial hyperplasia.

Groups	TNFα pg/mg tissue	IL6 pg/mg tissue	NF-κB pg/mg tissue	IL10 pg/mg tissue
Control group	139.03 ± 3.12	14.08 ± 0.96	39.13 ± 2.99	16.99 ± 0.71
Endometrial hyperplasia group	409.97 ± 28.92*	38..99 ± 1.95*	63.54 ± 3.14*	6.98 ± 0.21*
Allopurinol therapeutic group	201.23 ± 12.08#	21.99 ± 1.28#	41.69 ± 3.11#	12.99 ± 0.64#

Data were exhibited as mean ± SE (n= 8) TNFα: Tumor necrosis factor α, IL6: Interleukin 6, NF-κB: Nuclear factor-κB, IL10: Interleukin 10.
$^{*}p< 0.05$ faced to control group. $^{\#}p< 0.05$ faced to endometrial hyperplasia group.

Table 5: Effect of allopurinol on oxidants and antioxidants in estradiol valerate induced endometrial hyperplasia.

Groups	NO (µmol/ mg tissue)	MDA (nmol/ mg tissue)	Nrf2 (ng/ mg tissue)	CAT (U/mg tissue)	SOD (U/mg tissue)
Control group	13.13 ± 1.10	46.01 ± 4.09	59.99 ± 3.07	538.98 ± 24.38	391.07 ± 14.98
Endometrial hyperplasia group	31.07 ± 1.19*	101.98 ± 7.41*	30.75 ± 2.57*	229.07 ± 21.79*	179.03 ± 8.21*
Allopurinol therapeutic group	17.99 ± 1.11#	59.62 ± 4.33#	54.52 ± 3.39#	521.00 ± 18.95#	339.98 ± 26.75#

Data were exhibited as mean ± SE (n= 8) NO: Nitric oxide, MDA: Malondialdehyde, Nrf2: Nuclear factor erythroid 2-related factor 2, CAT: Catalase, SOD: Superoxide dismutase.
*p< 0.05 faced to control group. #p< 0.05 faced to endometrial hyperplasia group.

Effect of allopurinol on gene expression

Figure 2 illustrates the up regulation of PI3K, AKT, and mTOR gene expression in the endometrial hyperplasia group relative to the control group, and the clear down regulation of the aforementioned genes in the allopurinol therapeutic group relative to endometrial hyperplasia group.

Gross, histological and immuno-histochemical studies

Gross picture showed uterus was within average size in control group. In EH group there was symmetrical diffuse enlargement and increased uterine size, after treatment with allopurinol there was marked reduction in uterine size and near to normal and average size (Fig. 3).

Sections of the control group stained with hematoxylin and eosin revealed the typical histologic appearance of the endometrial tissue. Surface epithelium of simple type; single layer of low columnar (cuboidal) epithelium, which invaginated with little branching and coiling into tubular gland within lamina propria. The lamina propria showed the endometrial glands, blood vessels, connective tissue, and a few inflammatory cells (neutrophils/ lymphocytes). The background is formed of stromal cells, blood vessels, connective tissues and few inflammatory cells (Fig. 4 (A&B)). BAX staining revealed diffuse strong nuclear expression of all surface epithelium, tubular glands, and stromal cells (red arrow) (Fig. 4 (C&D)).

In endometrial hyperplasia group H & E stained sections showed epithelial and stromal hyperplastic changes, epithelial changes involved both surface and glandular epithelium denotes stratification (more than one layer), and papillary infolding, while the glands increased in number. There is increased thickness of the surface epithelial layer due to proliferation and hyperplasia of the stromal cells (Fig. 5 (A&B)). BAX staining showed loss of nuclear expression of both surface and glandular epithelium (early changes). However, non-spindle membranous staining was noted. The stromal cells act as valid internal positive control and showed diffuse strong nuclear staining of spindle cells (Fig. 5 (C&D)).

In allopurinol therapeutic group, H & E staining revealed the surface epithelium is columnar type with focal stratification, absent papillary infolding, and less indulation. There is a decrease in the number of glandular elements with less cellularity of stromal cells (Fig. 6 (A&B)). BAX staining showed nuclear staining of both surface glandular epithelium and stromal cells with focal cytoplasmic/membranous expression (Fig. 6 (C&D)).

Figures 7 and 8 showing histopathological comparison and summary of different groups.

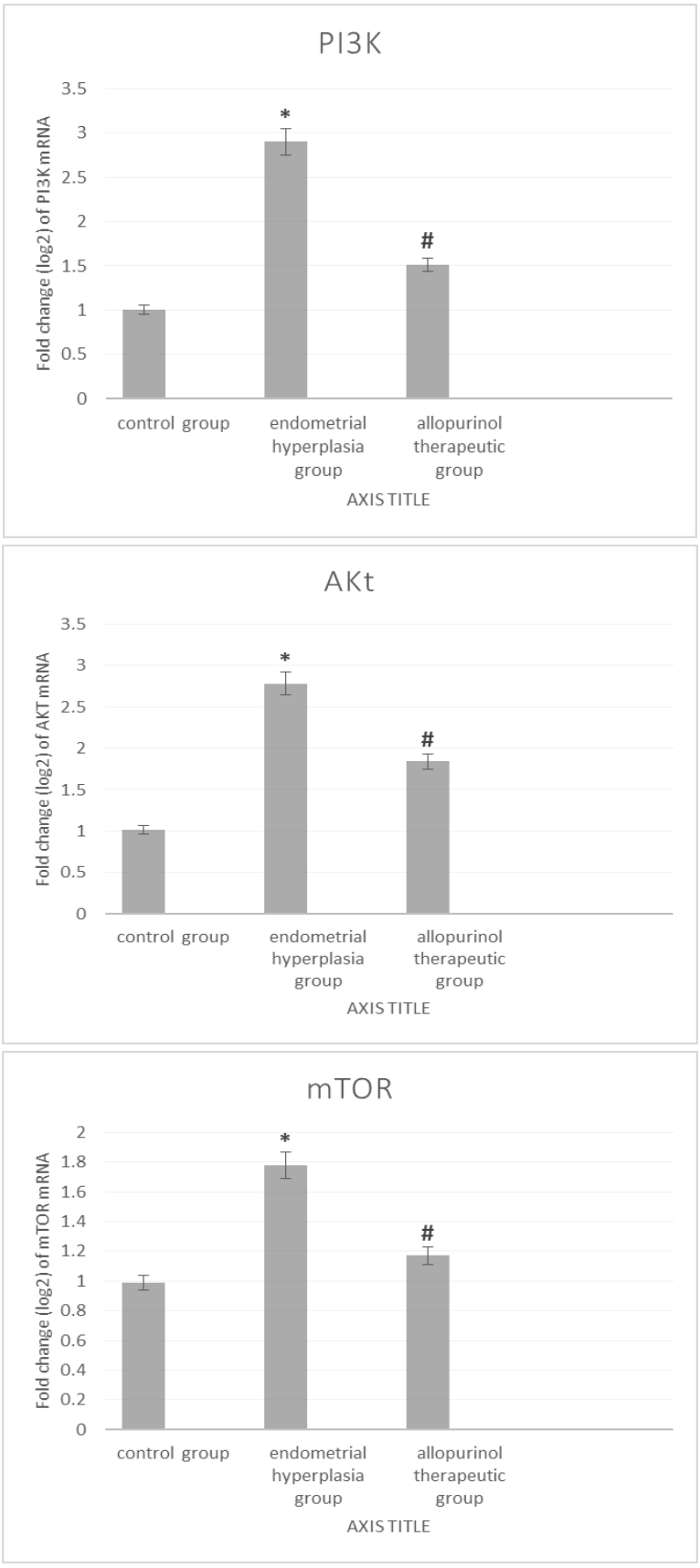

Fig. 2: Effect of allopurinol on PI3K, AKT and mTOR genes expression in estradiol valerate induced endometrial hyperplasia.

Data were exhibited as mean ± SE (n= 8). PI3K: Phosphatidylinositol-3-kinase, AKT: Protein kinase B, mTOR: Mammalian target of rapamycin. $^{*}p< 0.05$ faced to control group. $^{\#}p< 0.05$ faced to endometrial hyperplasia group.

Fig. 3: Gross study showing control group (Cont): Uterus was within average size, estradiol valerate group (EV): Symmetrical diffuse enlargement and increased uterine size, allopurinol therapeutic (Allo-t): Marked reduction in uterine size and near to normal and average size.

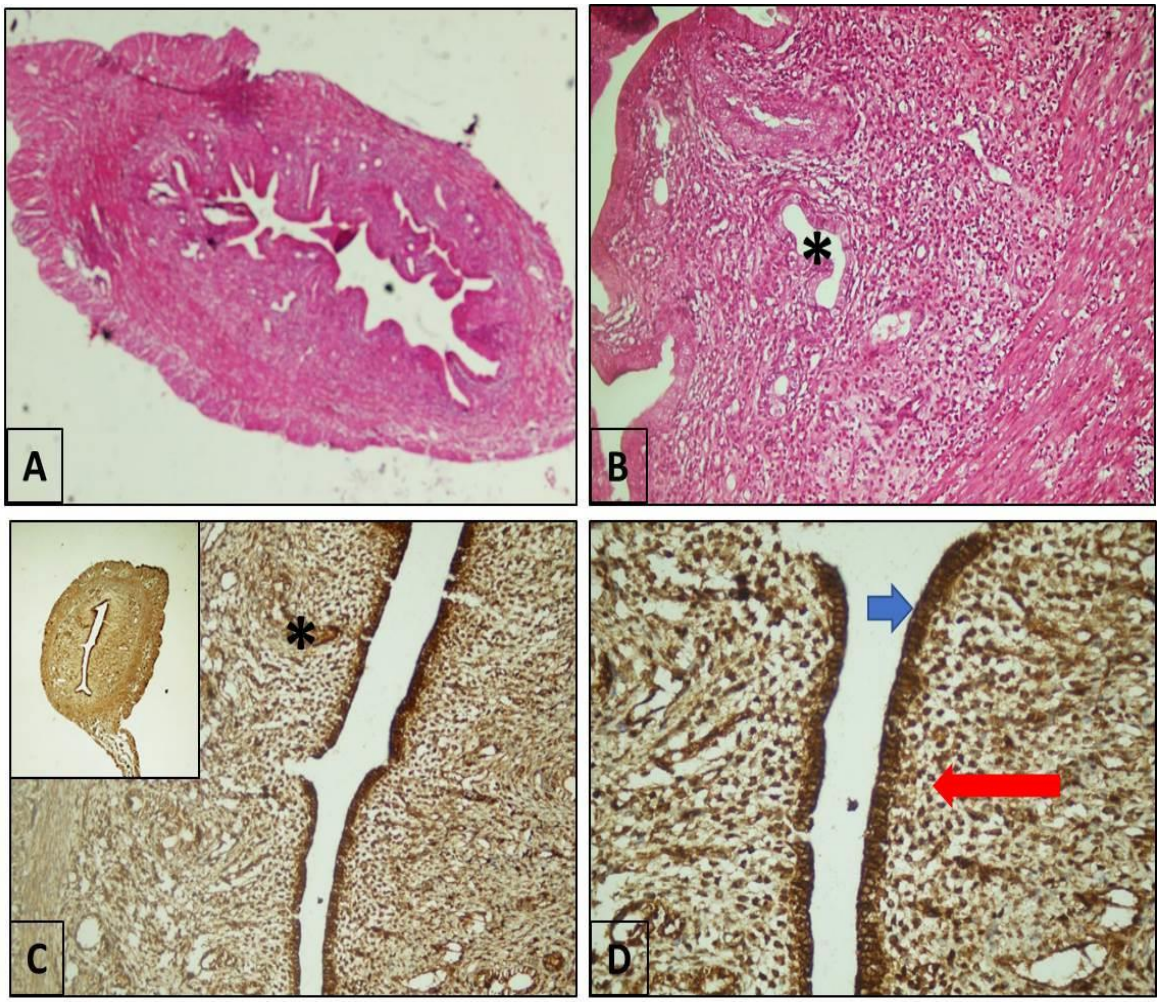

Fig. 4: Control group: (A&B) photomicrograph of H&E stained sections showing normal thickness of the endometrium with normal surface and glandular epithelium, (H&E Original; x40, x200). C&D) BAX stained sections revealed diffuse strong nuclear expression of all surface epithelium (blue arrow), tubular glands (astrix) and stromal cell (red arrow). (Original; insight x40, x200, x400).

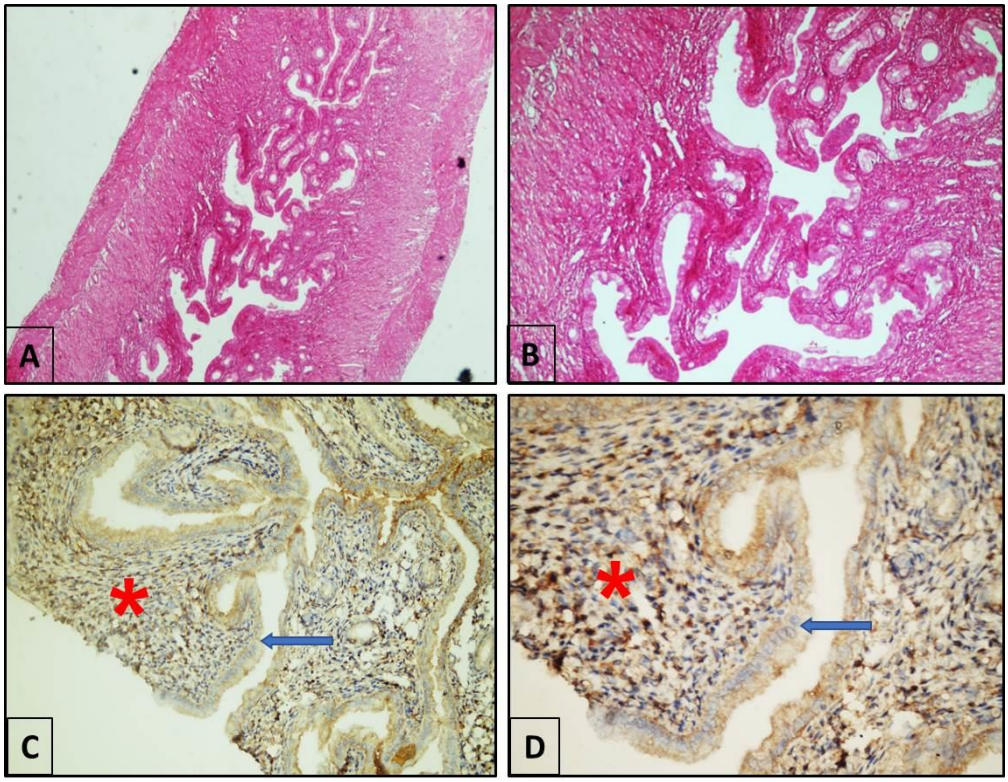

Fig. 5: Endometrial hyperplasia group: A&B) Diseased animals showed hyperplastic changes of both surface and glandular epithelium with no atypical changes, original (x40, x100). C&D) BAX stained sections of the same animal revealed loss of nuclear staining of surface epithelium (blue nuclei; blue arrows) and glandular epithelium, the stromal spindle cells (red astrix) showed diffuse nuclear expression, original (x 200,400).

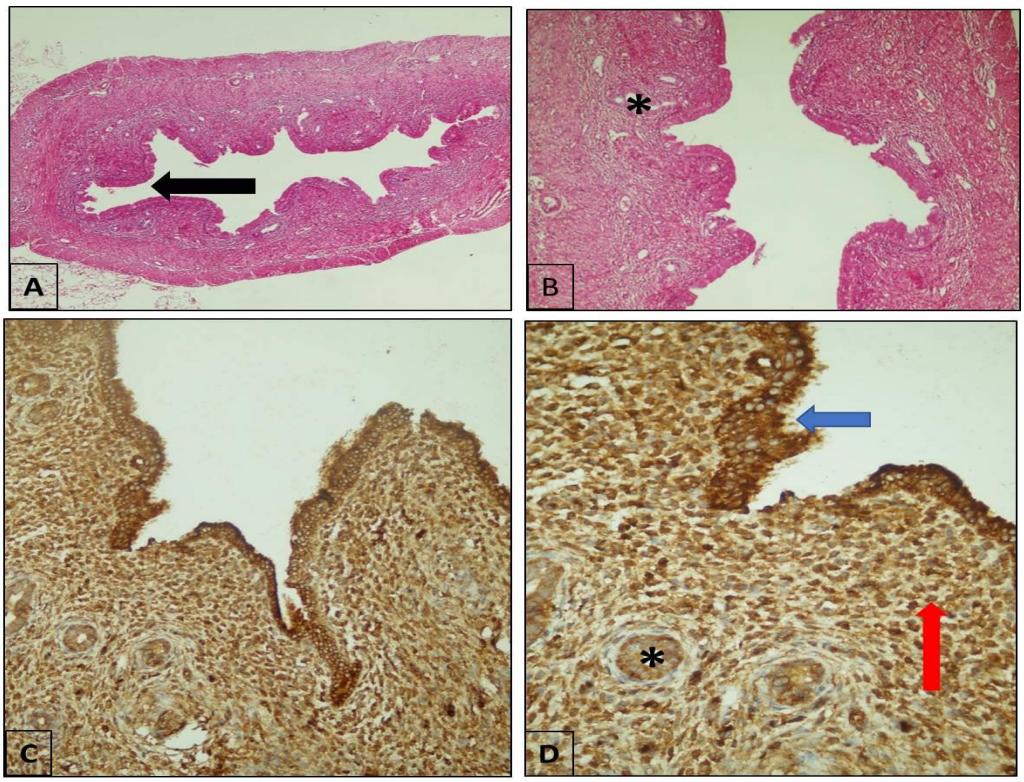

Fig. 6: In allopurinol therapeutic group H&E stained section showing marked improvement of surface epithelium invagination (black arrow), (A&B Original; x40,x100). C&D) BAX stained sections revealed retained nuclear /cytoplasmic expression of surface epithelium with focal stratifications (blue arrow), tubular glands (astrix), and stromal cells (red arrow). (Original; x200, x400).

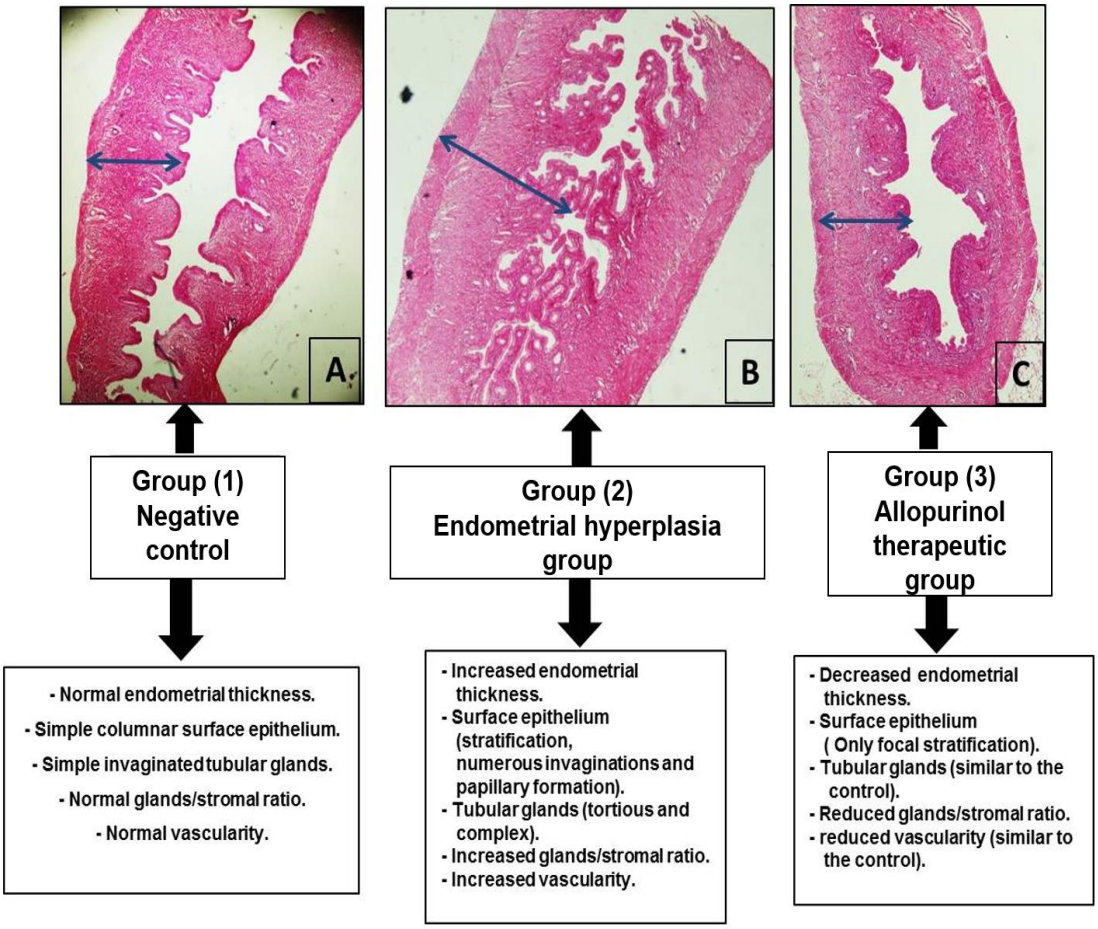

Fig. 7: Comparison between the experimental groups (H&E; original x40).

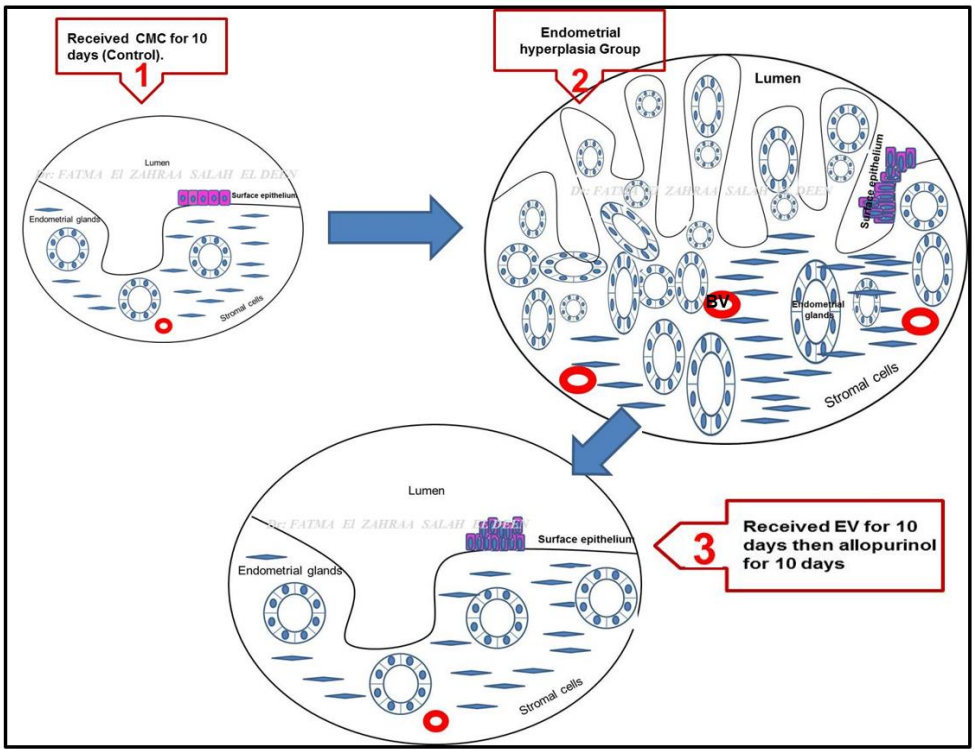

Fig. 8: Diagram summarizes the histopathological features and details for each group.

DISCUSSION

Our aim in this work was to deliver a novel pharmacological approach to ameliorate EH by investigating the therapeutic effect of allopurinol against EH and elucidating the underlying mechanisms.

The present results demonstrated elevation in uterine weight, body weight and the uterine somatic index in endometrial hyperplasia group when compared with control group. These findings are consistent with Abdelzaher and his coauthors[13]. Interestingly allopurinol dramatically reduced uterine weight, body weight and the uterine somatic index. İt was found a significant increase in serum level of uric acid, total cholesterol, triglycerides, LDL and significant decrease in HDL in endometrial hyperplasia group in contrast to control group. These finding are in harmony with Zhang and his coauthors, which reported that blood uric acid and hyperlipidemia were linked with the development of endometrial carcinoma[4]. According to Kitson *et al.*[20] women with endometrial cancer are more likely than women without the disease to experience metabolic abnormalities. However, in other researchs, hyperuricemia in particular has a stronger correlation with BMI and triglyceride level[21&22]. In the existing study, in addition to treating hyperuricemia allopurinol improves lipid profile. These finding are in agreement with Heimbach and his coworkers, who reported even though allopurinol is used to lower uric acid levels in gout patients, controlling elevated cholesterol levels, a significant comorbid condition in gout patients[23].

Pro-inflammatory agents are released by the endometrium in response to unopposed estrogen-mediated signaling, which exacerbates inflammation[24]. This is in harmony with the current results, which showed increased uterine tissue level of TNFα, IL6 and NF-κB and low level of IL10 in EV group in comparison with control group. In addition, in present results there is a disproportion between the oxidants and antioxidants parameters in EV group compared with control group. A vital factor contributing to the hyperplastic and cancerous changes in the endometrium is the imbalance between the oxidative and antioxidant pathways. This imbalance activates pro-inflammatory cytokines like TNFα, which initiates and sustains an abnormal inflammatory state. Nuclear factor-kappa B transcription factor is then activated and translocated to the nucleus, up regulating anti-apoptotic genes and causing excessive cell division, proliferation, hyperplasia, and dysplasia[25&26]. Allopurinol ameliorates these inflammatory changes and increase uterine tissue level of IL10 and decrease uterine tissue level of TNFα, IL6 and NF-κB. These results are coincident with Schlesinger and Brunetti, which reported the anti-inflammatory and analgesic effect of allopurinol[12]. Allopurinol inhibits the inflammatory signaling pathway through inhibition of NF-κB and pro-inflammatory cytokines TNFα and this mediates its anti-inflammatory effect.

The present results exhibited significant increase in the uterine tissue level of NO and MDA and significant decrease in Nrf2, CAT and SOD in EV group compared to control group. This results in oxidative stress which in agreement with Yıldırım *et al.*[5]. According to Prasad *et al.*[27] an elevation in estrogen level may result in oxidative stress injury by raising the concentration of reactive oxygen species, which in turn promotes cellular proliferation. The role of oxidative stress in disease has always been a contentious issue. Lipid peroxides are created when free radicals attack plasma lipoproteins, and MDA is the product of this acid hydrolysis. Thus, it serves as an indirect indicator of oxidized low-density lipoprotein[28]. This is in coordination with the current results. The present results have shown that antioxidant allopurinol efficiently preserves inner redox homeostasis and restoring the Nrf2, CAT and SOD. This is in matching with Luo *et al.*[29] which reported that allopurinol inhibited oxidative stress and triggered Nrf2, SOD. Moreover, Rodríguez-Rovira and his coauthors, which described that allopurinol acts as a potent antioxidant by scavenging ROS and combating XOR activity[30].

In normal cells, the PI3K/AKT/mTOR pathway is necessary for controlling physiological processes[31]. In many human diseases, the PI3K/AKT/mTOR signaling

pathway is highly activated[32]. The PI3K/AKT/mTOR pathway controls angiogenesis, growth, metabolism, survival, and proliferation in cells. Hyperactivation of the PI3K/AKT/mTOR pathway results in uncontrolled cell growth and survival[33]. This over activity rendering its inhibition a matter of considerable therapeutic interest. The current investigation revealed a significant increase in the expression of PI3K, AKT and mTOR genes in EH tissue samples when compared to control samples. The current finding is in coherence with Sivalingam *et al.*[34] which stated that there is increase in the expression of PI3K/AKT/mTOR in women with endometrial hyperplasia or endometrial cancer. In addition, Felip *et al.*[35] described the increased expression of PI3K/AKT/mTOR in endometrial cancer. Xanthine oxidase enzyme is crucial for oxidative stress generation. It stimulates mitochondrial ROS through the PI3K/AKT/mTOR signaling pathway. Activation of the PI3K/AKT/mTOR pathway triggers a signaling cascade that alters inflammatory responses, cellular metabolism, autophagy and apoptosis[36]. In harmony with the current results, allopurinol has strong antioxidant effects on blockade of aortic aneurysm through decreasing oxidative stress of the aorta by inhibiting XOR activity[30]. Based on above-mentioned data, it was postulated that allopurinol inhibits XOR and its subsequent stimulation of PI3K/AKT/mTOR pathway.

Greater estrogen level results in suppression of apoptotic signaling pathways that plays a role in the development of endometrial hyperplasia and endometrial cancer[37]. The BAX protein enhances the apoptotic vulnerability of cells in various organs[38]. We demonstrated that in EV group BAX staining showed loss of nuclear expression of both surface and glandular epithelium indicating decreased apoptosis. This is in accord with Granese and his coauthors, which stated that BAX expression increased after treatment of endometrial hyperplasia with Genistein in women[39]. In addition, in harmony with Alamoudi and his coworkers which reported that BAX expression significantly decreased in endometrial hyperplasia group in female rats[40]. To clarify the biological sensitivity of apoptotic proteins to allopurinol in endometrial hyperplasia, we assessed the expression of BAX in hyperplastic endometrium subjected to allopurinol treatment. The current results showed retained nuclear /cytoplasmic expression of surface epithelium with focal stratifications in BAX stained sections. In addition, results revealed decreased vascularity and reduced glands /stromal ratio and decreased vascularity similar to control. This elucidates the substantial effect of allopurinol on reducing endometrial thickness, as apoptosis is an essential mechanism for preserving tissue homeostasis and removing unhealthy cells[41].

This research clarifies the complex role of allopurinol in alleviating the pathophysiology of EH caused by estradiol valerate. Allopurinol treatment reduced endometrial thickness and retaining of apoptosis marker expression. Additionally, this attenuation coincides with a decrease inflammatory mediators and oxidative stress.The mitigation of inflammation and oxidative stress directly aids in the normalization of proliferation, as oxidative stress can stimulate signaling pathways that enhance cell proliferation and survival, thus aggravating EH[42&43]. According to Zhang and his coauthors uncontrolled cell proliferation of the endometrium caused by dysregulation of cellular apoptosis[2]. Moreover, allopurinol inhibits subsequent stimulation of PI3K/AKT/mTOR pathway. These interconnected therapeutic effects underscore the potential role of allopurinol as an innovative treatment approach for EH.

Conclusion

Allopurinol offered a treatment against estradiol valerate-induced hyperplasia via anti-inflammatory, antioxidant, apoptosis and down regulation of PI3K/AKT/mTOR pathway.

Conflict of interest

This work, without opposing goals.

Funding

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دورالألوبيورينول في فرط تنسج بطانة الرحم المستحث في الجرذان

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الخلفية والهدف: يوصى تقليديا باستخدام ألوبيورينول لعلاج النقرس. تم التخطيط للدراسة الحالية لاكتشاف تأثير وآلية عمل الألوبيورينول على فرط تنسج بطانة الرحم (EH).

التصميم التجريبي: تم تقسيم ٢٤ من أنثى الجرذان إلى ٣ مجموعات. المجموعة الضابطة: تم تلقي االجرذان كربوكسي ميثيل السليلوز ١٪ عن طريق الفم. مجموعة فرط تنسج بطانة الرحم: أعطيت الجزذان استراديول فاليرات (EV). المجموعة العلاجية: االجرذان المعالجة بالألوبيورينول.

النتائج: في مجموعة EH ، كان هناك ارتفاع ملحوظ في حمض اليوريك وعسر شحميات الدم. أيضا ارتفاع كبير في مستويات NO و MDA وانخفاض كبير في مستوى CAT و SOD و Nrf2 في الأنسجة. علاوة على ذلك، انخفض IL10 في الأنسجة بشكل ملحوظ بينما كان هناك زيادة في IL6 و TNF-α و NF-κB بشكل كبير. تم تنظيم جينات الرحم PI3K و AKT و mTOR. من الناحية النسيجية، كان هناك زيادة في سمك بطانة الرحم، والتقسيم الطبقي، والعديد من الخروقات، والتكوين الحليمي للظهارة السطحية. كشفت الظهارة السطحية والغدية لبطانة الرحم عن فقدان تعبير BAX. باستخدام الوبيورينول تم عكس جميع التغيرات البيوكيميائية والنسيجية المذكورة سابقا الناتجة عن تضخم بطانة الرحم الناجم عن الاستراديول.

الخلاصة: كشفت هذه النتائج أن الوبيورينول يمكن أن يثبط فرط تنسج بطانة الرحم من خلال استهداف إشارات mTOR / AKT / PI3K وBAX. بالإضافة إلى خصائصه المضادة للأكسدة والمضادة للالتهابات.