ORIGINAL ARTICLE

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Can the red clover combat the effect of the furan as an endocrine Disruptor on the ovary of albino rat?

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SUMMARY

Furan is an endocrine-disruptive chemical formed as a result of foods heat treatment as coffee, jarred and canned foods. It induces harmful effects on organisms. This study was intended to evaluate the effect of furan exposure on ovary, as a new rat polycystic ovary model (PCO), and the possible anti-inflammatory, anti-apoptotic and estrogenic effects of red clover (RC) dry extracts on it. Sixty adult female rats were divided into five groups (Control, RC, Furan, Protected and Treated groups). At the end of the experiment, Ovarian tissues were taken for histological (Hx & E and Picro – Sirius red stains), immunohistochemical (Bcl2, Er-β & COX-2 immunoexpression), statistical & morphometrical studies. The ovarian sections of Furan group showed significant decrease in the number of the follicles and corpora lutea, follicular degeneration as well as many cysts and significant increased deposition of collagen fibers between the theca cells extending to the medulla. Bcl-2 and Er-β immunoexpression were significantly decreased. However, the surface area percentage of COX-2 immunostain was significantly increased as compared to the control. The ovarian sections of the protected and treated groups with RC showed improvement in the histological and immunohistochemical alterations of furan induced ovarian injury. However, the treated group is significantly different from the protected group. It could be concluded that RC is more effective as a protected than treated agent against ovarian injury induced by furan.

Key words: Furan – Red Clover – Polycystic Ovary – Bcl2 – Er-β – COX-2

INTRODUCTION

Endocrine disruptive chemicals (EDCs) can simulate hormones (Vo et al., 2012). Such chemicals interfere with the normal function, regulation, and mechanisms of the hormone (Kim et al., 2012). EDCs executed their actions through nuclear hormone receptors, including estrogen, progesterone, androgen, thyroid, and retinoid receptors (Diamanti-Kandarakis et al., 2009). Thus, evaluation of EDCs is important.

EDCs are chemicals used in industry, agriculture, and pharmaceutical purposes or even heavy metals (Kabir et al., 2015; Monneret, 2007). Furan (C4H4O) is an EDC used in industry, produced in heat treatment of foods during processing, and preservation techniques (Cooke et al., 2014). Also found in cigarette smoke, and engine exhausts (Terrell et al., 2014). Therefore, determination of its effects as a new rat polycystic ovary model (PCO) is important due to its widespread use.

PCOS is a complex endocrine and metabolic disorder commonly affecting 5%-10% of women

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during reproductive age (Chiofalo et al., 2017; Asemi et al., 2015). It is manifested by infertility, polycystic ovaries, oligovulation, menstrual irregularity, hyperandrogenism, hirsutism, increased anxiety, and depression (Kubota, 2013; Goodarzi et al., 2011) and has many long-term complications as diabetes, obesity, insulin resistance, cardiovascular disease, and cancer (Qu et al., 2009; Laganà and Pizzo, 2015).

The etiology, and pathology of PCOS is unclear and its management requires varied approach. So, in the present study, we describe a new rat PCO model by furan that exhibits ovarian characteristics of the syndrome inducing histological changes and immunohistochemical changes in Er-β immunostain. We also demonstrate its inflammatory and apoptotic changes in the ovary by studying immunohistochemical expression of COX-2 and Bcl2. As Song and Tan (2019) stated that inflammatory stimuli triggers COX-2 and Bcl2 that leads to pathogenesis of the ovary.

Increasing use of herbal medications have led us to explore an alternative to conventional hormone replacement therapy (HRT), that prevents postmenopausal women pathologies but with a high breast and endometrial cancer incidence (Colditz et al., 1995). Such alternative should offer the hope of improved safety and greater compliance, also if it enhances Bcl-2, and inhibit COX-2 expressions, it has important role for delaying or decreasing cell apoptosis according to Song and Tan (2019), who related the pathogenesis of the ovary to inflammation where COX-2 and Bcl2 are triggered to it. Also, Bcl-2 considered to regulate apoptotic signaling in the ovary (Hsu and Hsueh, 2000; Hussein, 2005; Slot et al., 2006).

One of the natural estrogenic alternatives is red clover (RC) plant (Trifolium pratense L.) which is a dietary supplement that grows wild in America, Europe, Australia, Asia, and Northern Africa and is safe in the improvement of postmenopausal symptoms, maintenance of cardiovascular health with obvious effects on the breast, endometrium, and neural structures (Kawakita et al., 2009). The isoflavones (a major class of phytoestrogen) formononetin, genistein, daidzein, and biochanin A, are present in RC as malonates and glycosides (Wu et al., 2004). Isoflavones are the estrogenic compounds isolated from RC (Vishali et al., 2011; Liu et al., 2001), also known for their potential antioxidant properties (Tsao, 2010).

So we investigated the potential estrogenic effect of RC, a natural estrogenic alternative, as well as its anti-inflammatory and anti-apoptotic effects on the ovary of female rats exposed to furan.

MATERIALS AND METHODS

Chemicals

Furan, RC dry extracts, solvents and all markers were purchased from local distributer (Sigma chemical) Cairo, Egypt. The experimental dose of furan powder is (40 mg/kg) dissolved in 1 ml/kg corn oil (Hamadeh et al., 2004).

The experimental dose of Red clover powdered extract is (500 mg/kg day) dissolved in a concentration of 1.3 ml 0.1% carboxymethylcellulose (CMC) solution (Vishali et al., 2011; Burdette et al., 2002).

Animals and Husbandry

Adult female rats were purchased from and the experiment was done in the animal house of the Medical Research Institute, Alexandria University, Egypt. The study was conducted according to the guidelines of the animal care review board of the Faculty of Medicine, Menoufia University, Egypt and adhering to the guide for care and use of laboratory animals, and the study was approved by the ethics committee. Their average weight was 180-200 grams. The rats (5 rats in cage) were kept in a room with a controlled temperature (20-25°C) and humidity (45%-55%) under 12h/12h light/dark cycles. Water and food were given ad libitum. Prior to experiments, rats were left 7 days for acclimatization. Then, vaginal smear analysis was done daily for 10 days. A cotton-tipped sterile swab was rotated two or three times in the vaginal wall, then removed and plumbed out on a clean glass slide, stained with H&E. Detection of cornified cells, nucleated epithelial cells and leukocytes was done under the light microscope to screen sixty rats with normal 4–6 days estrous cycle (Marcondes et al., 2002; Salvetti et al., 2004). Estrous cycle ≥15 days was defined as cycle cessation (Xu et al., 2017).

Sixty adult female rats with regular estrous cycle started the experiment during the proestrus stage, and were allocated into five experimental groups:

Group I (Control G): 12 rats.

Subgroup IA (Negative control): 4 rats received only standard diet and ordinary water for successive 35 days.

Subgroup IB (Positive control): 4 rats received 1.3 ml 0.1% CMC orally for the last 21 days of the experiment.

Subgroup IC (Positive control): 4 rats received 1 ml/kg corn oil orally for the last 14 days of the experiment.

Group II (Red Clover G): 12 rats received RC (500 mg/kg/day) orally for the last 21 days of the experiment.

Group III (Furan G): 12 rats received furan (40 mg/kg) orally for the last 14 days of the experiment.

Group IV (Protected G): 12 rats received RC (500 mg/kg/day) orally for 21 days, after that received furan (40 mg/kg) orally for the last 14 days of the experiment.

Group V (Treated G): 12 rats received furan (40 mg/kg) orally for 14 days, after that received RC (500 mg/kg/day) orally for the last 21 days of the experiment.

at the end of the experiment, All rats were anaesthetized using ether inhalation and decapitated. Ovarian tissues were taken for histological (H&E and Picro-Sirius red stains), immunohistochemical studies of Bcl2, Er-β & COX-2 immunoexpression, morphometrical and statistical studies.

Histological examination

For histological studies, ovarian tissues were fixed with 10% formalin, then were embedded in paraffin, cut into 4 µm thick sections, stained with: hematoxylin and eosin stain and Picro – Sirius red stain and photographed by an Olympus C-5050 digital camera; Olympus Optical Co., Tokyo, Japan mounted on an Olympus BX51 microscope; Olympus Optical Co., Tokyo, Japan.

Immunohistochemistry

Ovarian tissues were fixed in 4% neutral paraformaldehyde for 2h. then, were dehydrated in gradient alcohol solutions, embedded in paraffin, and cut into 4–5 µm sections.

B-cell lymphoma 2 (Bcl2) immunoexpression

Staining was done using commercialized IHC kits according to the manufacturers' instructions. Sections were seen with 3,3′-diaminobenzidine (DAB), hematoxylin used as a counterstain, and photographed at 40× (Xu et al., 2017).

Estrogen Receptor β (Er-β) immunoexpression

Sections were rinsed 20 min in 0.001 M sodium citrate buffer in a microwave oven for antigen recovery. Endogenous peroxidase was narrated 20 min with 3% (v/v) hydrogen peroxide then, the slides were rinsed in 50 mM PBS plus 200 mM of sodium chloride and then, incubated 15 min with background sniper, for detection of estrogen receptor β (ER-β) (Caneguim et al., 2013).

Cyclooxygenase-2 (COX-2) immunoexpression

Sections were rinsed in 0.05 M citrate buffer to reveal antigens in a microwave oven. After 10 minutes inactivation of endogenous peroxidase in 3% hydrogen peroxide. Samples washed for five minutes in PBS, and dehydrated non fat milk (50 mg/ml). Afterthat, sections were washed with 0.05% PBST ween-20 (PBS-T) 3 times with overnight incubation with rabbit and goat polyclonal antibody for COX-2 (1:500 dilution, Abcam, UK). Then, were incubated with anti-rabbit and anti-goat secondary antibody for 90 minutes. Finally, sections were incubated with immunoreactivity complexes detected by 3, 3'-diaminobenzidine tetrahydrocholoride (Immunohistochemistry Accessory Kit, Bethyl Laboratories Inc., USA). Counterstain was done by Mayer's hematoxylin and mounted on crystal. Negative controls were involved by incubating sections with antibody dilution buffer rather than the primary antibody (Karimzadeh et al., 2013).

Morphometrical studies

Morphological analysis was assessed by using image J software (Maryland, USA). Ten non-overlapped fields (x 100) for each specimen from five different rats/group were examined histologically for the number of follicles, the number of corpus luteum and the area percentage of Picro-Sirius red stain and immunohistochemically for the number of positive cells stained with Bcl2, area percentage of Er-β and area percentage of COX-2. This was done in the department of anatomy, Faculty of Medicine, Menoufia University, Egypt.

Statistical analysis

Evaluation of collected data was done by IBM SPSS, Chicago, IL version 20.0. The data were Quantitative, expressed as mean and standard deviation and Mann Whitney U test was used to test the difference between each two independent groups. A P value (<0.05) was defined as a statistical significance.

RESULTS

Both group I (control G.) and group II (RC G.) showed non-significant difference regarding all the results, so they were represented as control group.

Haematoxyline & eosin stain

The ovarian stroma of the control adult female rats showed two regions. The outer one was the cortex, which contained follicles and corpora lutea separated by fibroblast like interstitial cells (Fig. 1). The inner region was the medulla, which was composed of loose vascular connective tissue with polygonal interstitial cells and large blood vessels (Fig. 1). The ovarian sections were surrounded by the tunica albuginea (Fig. 3). There were different stages of follicular differentiation: the primordial follicle with an oocyte surrounded with single layer of follicular cells; the follicles grew with increasing the size of oocyte together with multiplication of the layers of follicular cells forming the secondary follicle (Fig. 3). The mature graffian follicle appeared to contain an antrum (a cavity containing fluid) pushing the large oocyte to the eccentric aspect of the follicle; both the antrum and the oocyte were surrounded by cubical cells named as the granulosa cells (Fig. 4). Also, there were pale cells arranged in two layers: theca interna and theca externa surrounding the granulosa cells (Fig. 4). The ovarian section of adult female rats given furan (Furan group) showed significant decrease (##p value < 0.001) in the number of the follicles and corpora lutea as compared to the control group (Fig. 2); there was also follicular degeneration as well as many cysts (Figs. 1, 3). The degenerated follicles showed separation from the stroma, shrinkage of its oocyte, disorganization and desquamation of

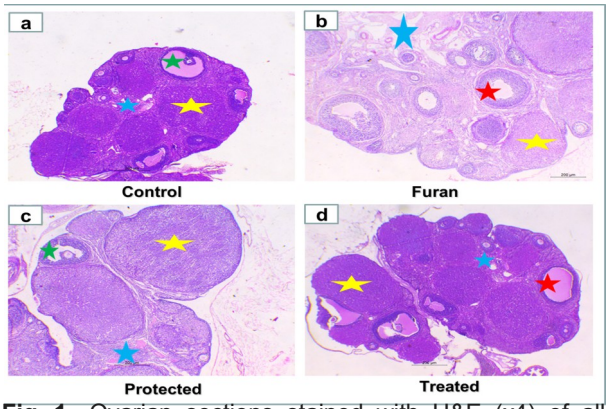

Fig 1. Ovarian sections stained with H&E (x4) of all experimental groups. **a&c** (control & protected groups): The ovarian sections of the animals of control and protected groups showing the mature graffian follicle (green star) together with the corpora lutea (yellow star) in the cortex, moreover, the medulla (blue star) reveals the normal blood vessels together with the interstitial cells. **b&d** (Furan & treated groups): The ovarian sections of furan and treated groups showing follicular cyst (red star) and corpora lutea in the cortex (yellow star) with cystic changes in the medulla (blue star).

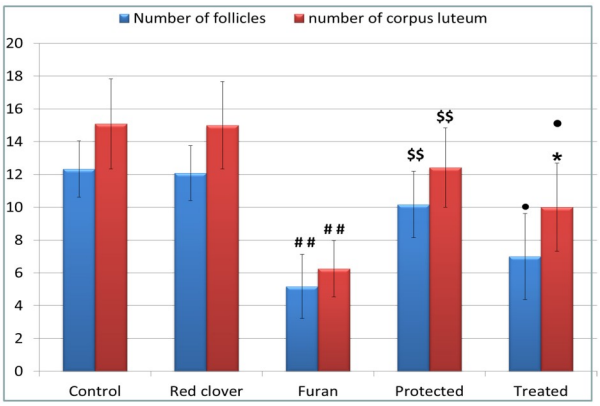

Fig 2. ##P & $$P values of significant decrease and increase (< 0.001) as compared to control and furan groups respectively, regarding the number of follicles and corpus luteum. *p value of significant increase (< 0.05) in the number of corpus luteum as compared to furan group. •p value of significant difference (<0.05) between the protected and treated groups regarding the number of follicles and corpus luteum.

granlosa cells, which showed pyknosis of their nuclei (Fig. 4). On protection of the adult female rats with RC (Protected group), their ovarian sections showed significant increase ($$p value < 0.001) in the number of the follicles and corpora lutea as compared to the furan group (Fig. 2), and also appeared with more or less normal preserved architecture regarding the graffian follicles (figs. 1&3), except for the vacuolations of follicular and theca cells (Fig. 4). On treatment of the adult female rats with red clover (Treated group), the ovarian sections showed non-significant increase in the number of follicles and significant increase (*p value < 0.05) in the number of corpora lutea as compared to the furan group (Fig. 2), with partial improvement of the architecture of the ovarian follicles together with the medulla (Fig. 4). But also the cysts and the degenerated follicles appeared (figs. 1&3). However, the treated group is significantly different from the protected group (•p value < 0.05) regarding the number of follicles and corpus luteum (Fig. 2).

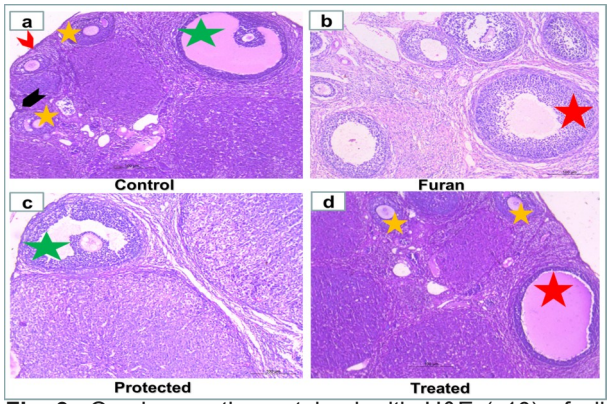

Fig 3. Ovarian sections stained with H&E (x10) of all experimental groups. The ovarian sections showing the covering tunica albuginea (red arrow head) in **(a,** control group**)**, the cortex contains primordial follicle (black arrow head) in **(a,** control group**)**, secondary follicle (orange star) in **(a&d,** control & treated groups**)**, mature graffian follicle (green star) in **(a&c,** control & protected groups**)** and follicular cyst (red star) in **(b&d,** furan & treated groups**)**.

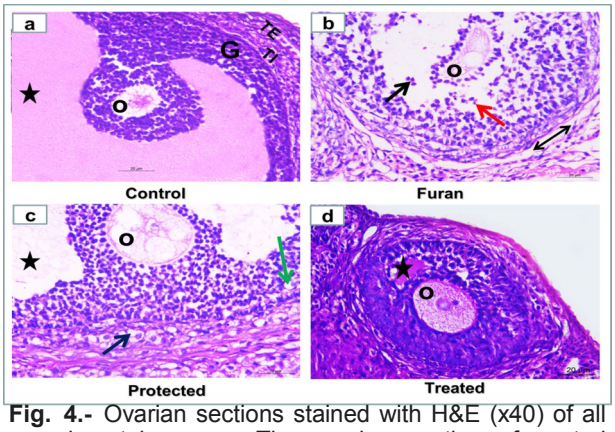

Fig. 4.- Ovarian sections stained with H&E (x40) of all experimental groups. The ovarian section of control group **(a)** showed the mature graffian follicle containing an antrum filled with fluid (black star) that is surrounded by granulosa cells (G), theca interna layer (TI) and theca externa (TE) with normal oocyte (o) seen also in the protected and treated groups **(c&d)**. There are vacuolations in the granulosa (green arrow) and theca interna cells (blue arrow) in the mature graafian follicle of the protected group **(c)**. The ovarian section of furan group **(b)** showed a degenerated oocyte (o), disorganized (red arrow), desquamated (black arrow) granulosa cells and separated theca cells (double headed arrow) from the stroma. The ovarian section of treated group (**d)** showed secondary ovarian follicle with little fluid (black star) between the follicular cells.

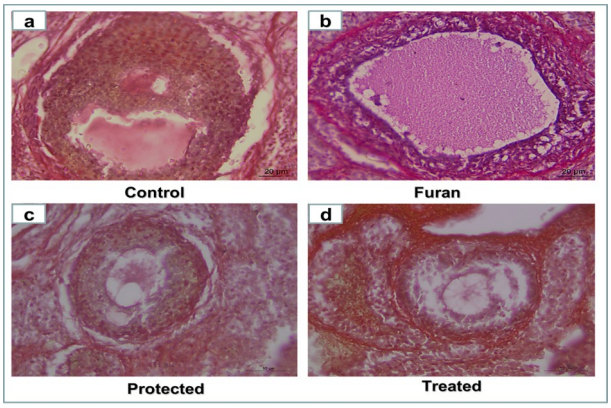

Fig 5. Representative Picro-Sirius red stained ovarian sections (x40) of all experimental groups showing a significant increase (##p value) in the area percentage of Picro - Sirius red staining of the ovarian section of furan group **(b)** as compared to control group **(a)**. Significant decrease ($$p &**p value) in the area percentage of Picro - Sirius red staining of the ovarian sections of protected and treated groups **(c &d)** as compared to furan group **(b)**.

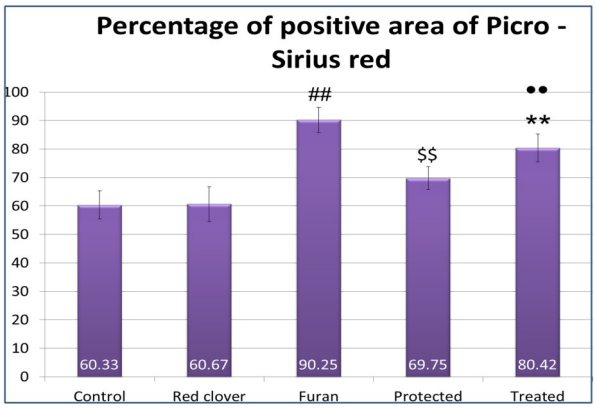

Fig 6. ##P value of significant increase < 0.001 as compared to control group. $$P & **P values of significant decrease as compared to furan group, ••p value of significant difference < 0.001 between treated and protected groups regarding the percentage of positively stained area by Picro-Sirius red.

Picro-Sirius red stain

The ovarian section of adult female rats given furan (Furan group) showed significant increase (##p value < 0.001) in the percentage of area stained with Picro-Sirius red as compared to the control group (Figs. 5, 6), indicating the increased deposition of collagen fibers between the theca cells extending to the medulla. The ovarian sections of adult female rats that were protected and treated with RC (protected and treated groups) showed significant decrease ($$p & **p values < 0.001) in the percentage of the area stained with Picro-Sirius red as compared to the furan group (Figs. 5, 6). However, the treated group is significantly different from the protected group (••p value < 0.001) (Fig. 6).

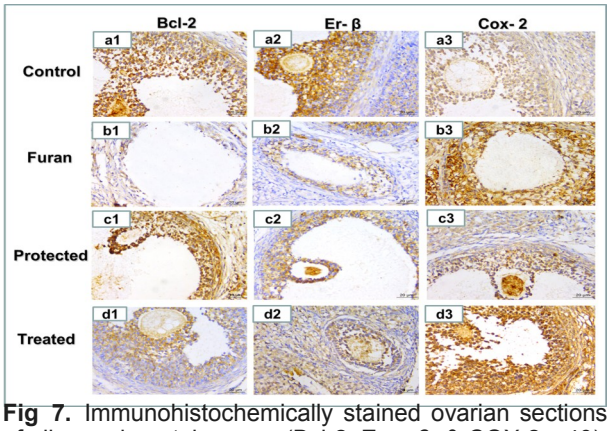

Fig 7. Immunohistochemically stained ovarian sections of all experimental groups (Bcl-2, Er- β, & COX-2, x40): The ovarian sections of control group **[a1** (Bcl-2), **a2** (Er-β), **a3** (COX-2)**]** showing strong expression for Bcl-2 and Er-β, and minimal expression for COX-2. The ovarian sections of furan group **[b1** (Bcl-2), **b2** (Er-β)**, b3** (COX-2)**]** showing significant downregulation of Bcl-2 & Er-β and upregulation of COX-2 expression as compared to control group. The ovarian sections of protected group **[c1** (Bcl-2), **c2** (Er-β)**, c3** (COX-2)**]** showing significant upregulation of Bcl-2 & Er-β and downregulation of COX-2 expression as compared to furan group. The ovarian sections of treated group **[d1** (Bcl-2), **d2** (Er -β), **d3** (COX-2)**]** showing significant upregulation of Bcl -2 & Er-β and downregulation of COX-2 expression as compared to furan group.

Immunohistochemical stains

The ovarian sections of control rats showed strong expression of the immunohistochemical stains (Bcl-2 & Er-β) in the granulosa cells and to a lesser extent in the theca cells (Fig. 7). The ovarian sections of the rats of furan group showed significant decrease (##p values < 0.001) in the number of Bcl-2 immunopositive cells and surface area percentage of Er-β immunostain respectively as compared to the control group (Fig. 8), while the ovarian sections of rats protected and treated by red clover showed significant increase ($$p & **p values <0.001) in the number of Bcl-2 immunopositive cells and surface area percentage of Er-β immunostain respectively as compared to the furan group (Fig. 8). However, the treated group is significantly different from the protected group (•p value < 0.05) (Fig. 8).

The ovarian sections of control rats showed minimal expression of the COX-2 immunohistochemical stain in the granulosa cells and to lesser extent in the theca cells (Fig. 7). The ovarian sections of the rats of furan group showed significant increase (##p values < 0.001) in the surface area percentage of COX-2 immunostain as compared to the control group (Fig. 8), while the ovarian sections of rats protected and treated by RC showed significant decrease ($$p & **p values < 0.001) in the surface area percentage of COX-2 immunostain as compared to the furan group (Fig. 8). However, the treated group is significantly different from the protected group (••p value < 0.001) (Fig. 8).

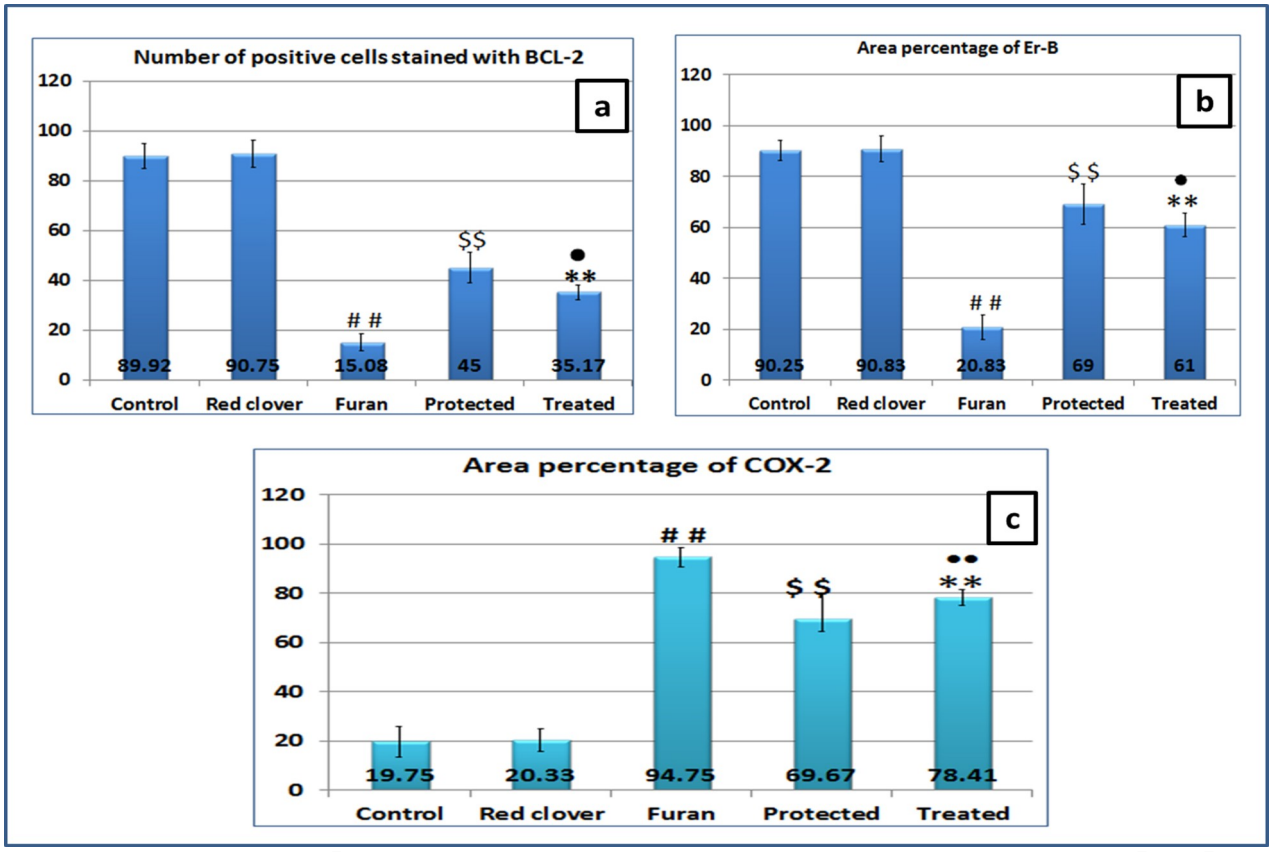

Fig. 8.- (a&b, Bcl-2 & Er-β**)** ##P value of significant decrease (< 0.001) as compared to control group. $$P & **P values of significant increase as compared to furan group, •p value of significant difference (<0.05) between treated and protected groups regarding the expression of Bcl2 & Er-β. **(c,** COX-2**)** ##P value of significant increase (< 0.001) as compared to control group. $$P & **P values of significant decrease as compared to furan group, ••p value of significant difference (<0.001) between treated and protected groups regarding the expression of Bcl2.

DISCUSSION

In this study, two main points of research was performed. Firstly, the effects of furan administration on ovarian morphology, Bcl2, Er-β & COX-2 immunoexpression, as a new rat PCO model. Secondly, the anti-inflammatory, anti-apoptotic and estrogenic effects of RC dry extracts on it.

Furan is a natural compound present in a wide range of foods (Seok et al., 2015). Formed mainly from food thermal processing as baking, cooking, roasting, sterilization, and pasteurization (Crews and Castle, 2007). Also, it is used in various foods subjected to heat treatment such as canned and jarred foods (US Food and Drug Administration, 2004).

PCOS is is a slight inflammatory (Kelly et al., 2001) and endocrine disease, which has multifactorial pathogenesis and heterogeneous clinical manifestations as hyperandrogenemia, hirsutism, chronic anovulation and vascular disorder (Conway et al., 2014).

Researches on the effect of furan toxicity in animals were limited. Therefore, the comprehensive purpose of our study was to investigate the first in vivo assessment of the effects of furan on ovary of female rats as a new rat PCO model inducing changes in the ovarian histology, Er-β immunoexpression as well as ovarian immunohistochemical markers of inflammation (COX-2) and anti-apoptosis (Bcl2) expression.

From the present study, data ascertained that furan induced ovarian morphology of PCO rat model that exhibit significant decrease (##p value < 0.001) in the number of the follicles and corpora lutea; there was also follicular degeneration, as well as many cysts. The degenerated follicles showed separation from the stroma, shrinkage of its oocyte, disorganization and desquamation of granulosa cells, which showed pyknosis of their nuclei. Selmanoglu et al. (2012) classified furan as a potential human carcinogen and induce histopathological kidney and liver damage of male rats. Also El-Akabawy and El-Sherif (2016) stated the oxidative changes in the adult rat testis of furan. Moreover, Uçar and Pandir (2017) demonstrated furan-induced histopathological alterations in the ovarian tissue after daily treatment, together with a marked reduction in CAT, SOD, GST, and GPx activities and a marked MDA elevation indicating its oxidative stress injury.

Also, in the current study, there is increased deposition of collagen fibers between the theca cells extending to the medulla in the Picro-Sirius stained

sections of the furan group as compared to the control group. As reported by the previous studies of Zhang et al. (2013), that demonstrated ovarian thickening of theca cells, an elevated level of collagen deposition and explained the rise of fibrosis involved in the pathology of PCO by the chronic inflammation. Also, Xiong et al. (2011) confirmed that the ovaries of PCOS patients demonstrated constant chronic inflammation with a larger number of immersed inflammatory cells.

Immunohistochemically, the furan group showed significant decrease (##p values < 0.001) in the number of Bcl-2 immunopositive cells and significant increase (##p values < 0.001) in the surface area percentage of COX-2 immunostain, confirming its inflammatory effect. Upregulation of COX-2 in both inflammation and cancer, confirming its ovarian inflammatory and apoptotic effects (Desai et al., 2018). Also, Arif et al. (2015) reported two folds upregulation of COX-2 in PCO when compared to superovulated ovaries in rats during ovulation. In contrast, Lee et al. (2017) showed downregulation of COX-2 mRNA in the woman granulosa cells with PCOS and Sander et al. (2011) found PCOS follicles with no change in COX-2 mRNA levels. Moreover, Bcl-2 family is evidenced as an ovarian regulator of apoptotic signaling (Hsu and Hsueh, 2000; Hussein, 2005; Slot et al., 2006). Their function can be either antiapoptotic (e.g. Bcl-2, Bcl-xL, and Bcl-W) or proapoptotic (Bax, Bim, Bad, and Bcl-xS) and can prevent cell apoptosis by inhibiting the release of Bcl-2 protein (apoptosis inducing factors) found in endoplasm omentum and mitochondrial membranes (Gürsoy et al., 2008; Johnson and Bridgham, 2002). Also Yang et al. (1997) reported that Bcl-2 could inhibit or delay apoptosis in ovarian cell.

Reactive oxygen species (ROS) can be generated from peripheral blood mononuclear cells in PCOS secondary to hyperandrogenism and hyperglycemia that induce inflammation, resulting in oxidative stress that activates nuclear factor κB (NFκB), and implicated in the expression of COX-2 and induction of IL-6 (Gonzalez et al., 2006; Porta et al., 2009). Furthermore, coincidence of inflammatory mediators with androgens can induce PCOS. Also the ovarian hyperandrogenism state seen in PCOS can result in dysgenesis (Song and Tan, 2019).

The ovarian sections of the rats of the furan group showed significant decrease (##p values < 0.001) in the surface area percentage of Er-β immunostain, in accordance with Jakimiuk et al. (2002), who stated that abnormal expression of estrogen receptors (ERs) could contribute to poor follicular development and ovulatory failure in PCOS as a defining characteristic of dominant follicles is high estradiol concentrations.

In this study, RC improved the ovarian morphology regarding the number of the follicles, corpora lutea and the graffian follicles, whereas the ovarian sections of the protected group showed significant increase ($$p value < 0.001) in the number of the follicles and corpora lutea as compared to the furan group with more or less normal preserved architecture regarding the graffian follicles. Treated group showed non-significant increase in the number of follicles and significant increase (*p value < 0.05) in the number of corpora lutea as compared to the furan group, with partial improvement of the architecture of the ovarian follicles. But also the cysts and the degenerated follicles appeared. Both protected & treated groups showed significant decrease ($$p &**p values < 0,001) in the percentage of area stained with Picro – Sirius red as compared to the furan group; this might be attributed to the anti-inflammatory capability of RC. Moreover, Bcl-2 and Er-β immunoexpression were significantly increased and the surface area percentage of COX-2 immunostain was significantly decreased in the protected and treated groups compared to the furan group, confirming its anti-inflammatory, anti-apoptotic and estrogenic capability. However, the treated group is significantly different from the protected group histologically and immunohistochemically.

These results are in harmony with Selloum et al. (2003), who attributed the therapeutic effects of many traditional herbal medicine to the flavonoids, and Saviranta et al. (2010), who confirmed that RC is rich in isoflavonoids, a Phenolic compound with multiple protective functions from oxidative stress induced damage in the plant and human cells. Moreover, the anti-inflammatory effect of flavonoids is assumed to result mainly from the inhibition of some key enzymes involved in inflammation and/or cell signaling pathways such as cyclooxygenase and lipoxygenase (You et al., 1999), protein kinase C and phosphoinositide 3-kinase (Walker et al., 2000; Selloum et al., 2001). Thus, inhibiting such enzymes could be valuable for treating inflammatory conditions. Also, Vishali et al. (2011) confirmed that RC estrogenic activity is largely due to isoflavones.

Isoflavones (a major class of phytoestrogens) bind to estrogen receptors; they have a higher affinity to bind to estrogen receptor β (ER-β) than to estrogen receptor α (ER-α) (Kuiper et al., 1997). And exhibit definite estrogenic effects at most in tissues expressing ER-β as bone or cardiovascular system (Enmark et al., 1997; Onoe et al., 1997).

CONCLUSION

Findings of the present study reinforce the significant role of RC extract in restoration of ovarian morphology, Bcl2, Er-β & COX-2 immunoexpression in furan-induced PCO, which have confirmed its anti-inflammatory and anti-apoptotic activities besides estrogenic effects. Such improvement is more prominent during protection than treatment.

So, this study proposes more research in this

area, because the results were hopeful and pave the way to important applications of RC, which is orally safe and inexpensive for treatment of PCOS.

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