Original Article

The Modulatory Effects of Pentoxifylline in Biochemical Changes Induced By 17α-Ethinyl Estradiol in the Rat Model

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ABSTRACT

Background: Ethinylestradiol (EE) has induced cholestasis and hepatotoxicity in animal studies through reducing bile acid uptake by hepatocytes and induce of oxidative stress. Pentoxifylline (PTX) is a drug that by inhibition of release or transcription of proinflammatory cytokine cause prevents oxidative stress of liver cell and reduction of damage. We aimed to evaluate the effects of pentoxifylline on liver injury induced by Ethinylestradiol in rats.

Methods: Twenty-four female Wistar rats (300±20 gr) were divided into four groups, equally. Duration of treatment was 5 consecutive days for each group. The control group Simultaneously received orally and subcutaneously normal saline. PTX group Simultaneously received Pentoxifylline orally and normal saline subcutaneously, EE Group Simultaneously received EE subcutaneously and normal saline orally. In the EE+PTX group, rats Simultaneously received EE subcutaneously and PTX orally. Rats were anesthetized and blood and tissue samples were collected for measurement of hematological and biochemical parameters.

Results: The EE administration increased the serum levels of ALP and MDA significantly. The EE administration also decreased albumin and GPX levels were significant. These aberrations were improved by PTX treatment in EE + PTX group. Most of hematological parameters were not significant by the EE. The plasma level of TNF- in the PTX+ES treated group showed a significant decrease in comparison to that in the ethinyl estradiol group.

Conclusion: PTX has partial capacity to protect against liver changes induced by Ethinyl Estradiol.

Keywords: Biochemical Parameters, Hepatotoxicity, Oxidative Stress, Pentoxifylline, 17α-Ethinylestradiol.

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INTRODUCTION

Estrogens are broadly used in animal and human practice. In small animal practice, indications of estrogens are treatment of hypogonadal obesity and hormonal urinary incontinence in bitches, anal adenoma, excess libido and prostatic hyperplasia in male dogs. In large animal, practice suggested for treatment of postpartum cow to decrease uterine infection, for treatment of metritis in cattle, for estrus synchronization and parturition induction in horse. Furthermore, in human, estrogen is widely used for postmenopausal symptoms, oral contraceptives, and carcinoma prostate. There are always increasing concerns about estrogen exposure directly as pharmaceutical formulation and indirectly as estrogen residues with food origin that can cause cytotoxicity and cancer of many organs, such as hepatotoxicity and hepatocellular carcinoma [1-3].

Ethinyl estradiol, a semisynthetic 17α-ethynylestradiol (17α-EE), is the highly potent estrogens.

17α-EE as tumor promotor encourages liver DNA synthesis and has also been shown to cause genotoxic damage through activation of reactive oxygen species (ROS). 17α-EE has also been noticed to cause intrahepatic cholestasis in experimental animals through 17α-EE glucuronide metabolite or ROS signaling pathway in part, or accumulation of bile salts in hepatocytes that can cause to increase oxidative stress and disruptions of plasma membrane and finally can lead to hepatotoxicity and fibrosis.

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The exact mechanism of the cytotoxicity by EE is unclear. However, cholestatic hepatotoxicity is often associated with hepatic inflammation that may be due to activation of inflammation pathway through inflammatory mediators such as proinflammatory cytokine (TNF-α and IL-6), and induction of oxidative stress which can lead to necrosis [3-5].

Pentoxifylline (PTX) is phosphodiesterase inhibitor that use as vasodilator and anti-inflammatory drug. PTX's effects were shown on the cytokine network and antifibrogenic actions. The effects of PTX on the cytokine network reduce phagocytic activity, superoxide anion production, and lysosomal enzyme. Moreover, it suppresses the TNF-α gene transcription and ultimately inhibits TNF-α release [6, 7].

We aimed to survey anti-inflammatory and antioxidative Pentoxifylline effects as the targets for prevention and treatment on possible damage caused by 17α-EE in female rat model.

MATERIAL AND METHODS

Chemicals

17α-ethinylestradiol was obtained from Aburaihan pharmaceutical Co. (Tehran, Iran). The ALT/AST kit was purchased from Ziestchem (Tehran, Iran). Pentoxifylline was ordered from Merck (Germany). The TNF- kit purchased from Bender med system company (1030 Vienna, Austria)

Animals and Treatment

Overall, 24 female Wistar rats (300±20) were purchased from the laboratory animal research center of Shahmirzad (Semnan University, Iran). They were housed in a room under controlled temperature (22±2 °C), lighting (12-h light/dark cycle) and humidity (40%-50%) conditions, and were treated with a standard pellet diet. All the animals were fed ad libitum with normal rat chow and free access to water. All the study was performed in approval with Laboratory Animal Research Center for Semnan University (LARC) and conduct in accordance with the guidelines of Center for the use and care of experimental animals. The animals were divided randomly into four equal groups:

Control Group: rats simultaneously received normal saline orally and subcutaneously (SC) for 5 d (with similar volume of EE for SC and PTX for oral).

Pentoxifylline Group (PTX): rats simultaneously received PTX orally at dosage 100 mg/kg and normal saline subcutaneously for 5 d.

Ethinyl Estradiol (EE) Group: rats simultaneously received E.E at dosage of 5 mg /kg /day and normal saline orally for 5 d.

EE+ PTX Group: rats simultaneously received E.E at dosage of 5 mg /kg /day and PTX orally for 5 d.

Biochemical and Hematological Assay

The animals were anesthetized by ether on the day 6 and blood samples were collected by cardiocentesis technique and immediately divided to two samples: one sample was for hematological assay in anticoagulant tube containing EDTA and another sample was for biochemical assay and serum were separated by centrifugation at 4000 rpm for 10 min and maintained at -20 ºC until assay time.

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), cholesterol, total proteins, and albumin in serum were calorimetrically measured according to available kit procedures. Lipid peroxidation levels were determined in homogenized liver tissue, according to the thiobarbituric acid (TBA) method of Esterbauer and Cheeseman (8). Liver GSH was estimated by Kuo and Hook standard method (9). Plasma TNF-α level was measured by TNF-α ELISA kit.

Statistical analysis

All results presented as mean±SE. The comparisons among groups were done by way ANOVA test and followed by Duncan test in SPSS ver. 21 (Chicago, IL, USA). Significant differences among groups were considered as $P<0.05$.

RESULTS

Biochemical Findings

There was difference significant in an increase of ALP activity and a decrease of Albumin levels in E.E. group in comparison with other groups (Fig1, 2). Despite increase of ALT, AST and GGT activity and decrease of cholesterol and total proteins values in E.E. group compared with other groups,there were no differences significantly among groups. Moreover, the plasma level of TNF- in the PTX+ES treated group showed a significant decrease in comparison to that in the ethinyl estradiol group (Fig3).

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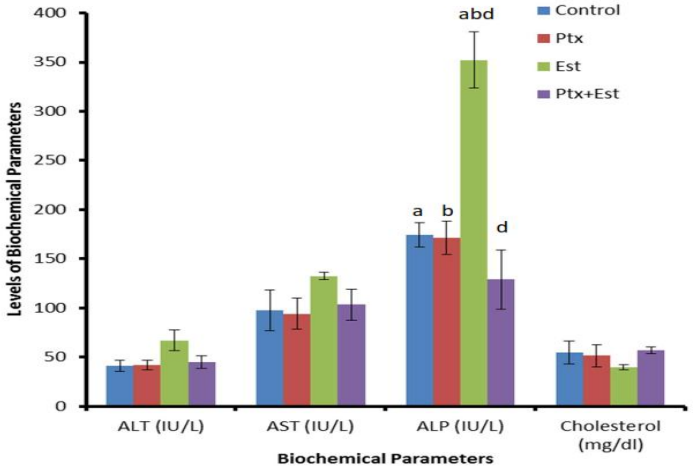

Figure 1. Comparison of biochemical parameters of ALT, AST, ALP and cholesterol in studied groups. [a] significant difference between control group and EE group, $P<0.05$. [b] significant difference between PTX group and Est group, $P<0.05$ and [d] significant difference between EE group and PTX+EE group, $P<0.05$.

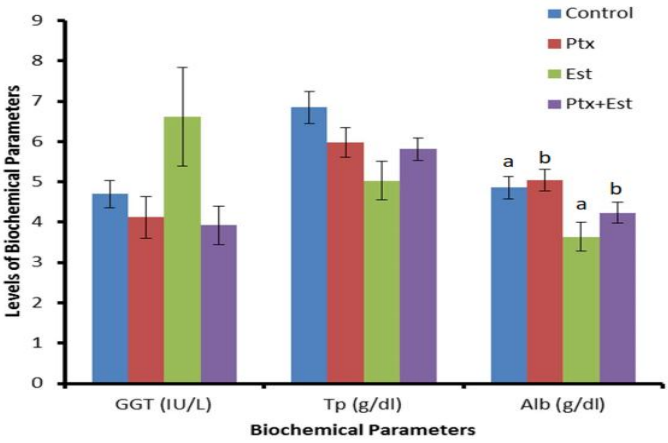

Figure 2. Comparison of biochemical parameters of ALT, AST, ALP and cholesterol in studied groups. [a] significant difference between control group and Est group, $P<0.05$. [b] significant difference between PTX group and EE group, $P<0.05$.

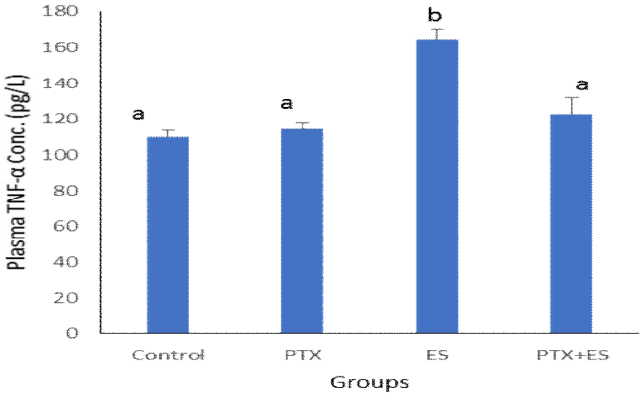

Figure 3. Comparison of biochemical parameters of TNF-α in studied groups. [a] significant difference between control group and EE group, $P<0.05$. [b] significant difference between PTX group and Est group, $P<0.05$.

Oxidative Stress Assays

EE produces a significant increase in MDA contents of liver tissue homogenate compared to control group ($P < 0.05$); while it produces a significant decrease in the level of GSH in rat liver homogenate compared to control group ($P < 0.05$). Oral administration of 100mg/kg pentoxifylline to rats for 5 d, showed a significant difference ($P < 0.05$) on either the contents of lipid peroxidation product (MDA) or on the level of reduced glutathione (GSH) in rat liver homogenate compared to control group.

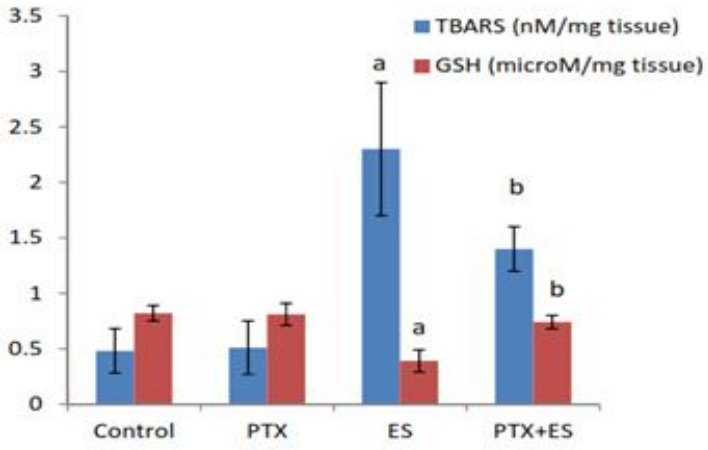

Figure 4. Comparison of lipid peroxidation (MDA) and GSH in studied groups. [a] significant difference between control group and EE group, $P<0.05$. [b] significant difference between PTX group and EE + PTX group, $P<0.05$.

Hematological Findings

Administration of EE at a dose of 5 mg/kg as SC for a 5-d period, compared with other group did not show a significant effect ($P< 0.05$) on Hgb, PCV, RBCs and WBCs values. (Figs. 3, 4)

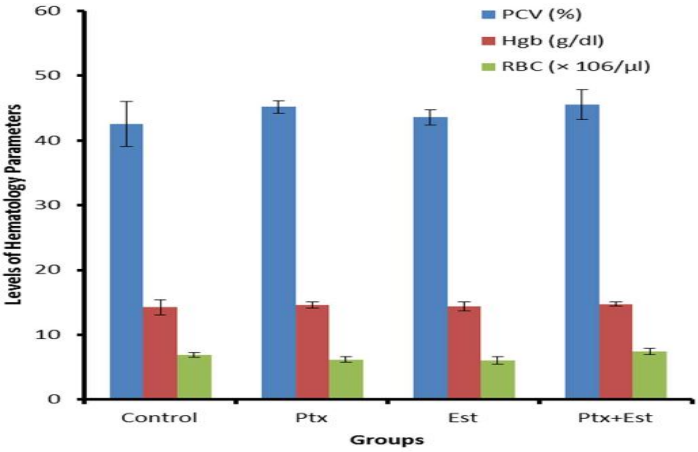

Figure 5. Comparison of hematological parameters PCV, Hgb, and RBC in studied groups. No difference significant has been shown among groups ($P< 0.05$).

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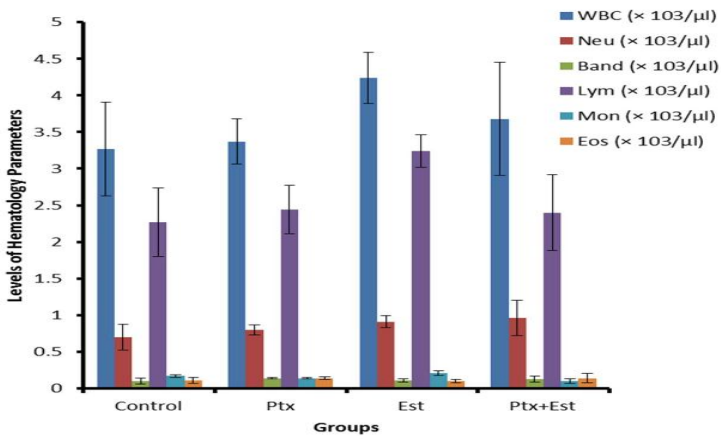

Figure 6. Comparison of cell blood count in studied groups. No difference significant has been shown among groups ($P< 0.05$).

DISCUSSION

In the present study, the effects of PTX as antioxidative agent were evaluated following 17α-Ethinyl Estradiol-induced changes in liver rat. EE-treated group showed a significant decrease in serum albumin, total protein, and liver GSH and increase in alkaline phosphatase and liver MDA compared to control group. In addition, difference significant was showed in levels of liver GSH, MDA, and activity of ALP between PTX group and PTX+EE group.

Although, there is not a significant difference among groups in values of alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase and cholesterol, increase in values of ALT, AST, and GGT, above the upper limit of reference and decrease in levels of serum cholesterol down the lower limit of reference were shown.

Cholestasis was induced by estradiol-17β-(β-D-glucuronide) for 5 consecutive days at the dose of 5 mg/kg body wt. subcutaneously. The EE-induced biochemical changes were shown in ALP, ALT, AST and bilirubin levels that significantly difference was statistically observed in ALP level from the control group. Silymarin as protective agent works through restoration of the impaired bile salt output [1]. Preventive effect of omega-3 fatty acids was surveyed on ethinyl estradiol-induced hepatosteatosis in female Wistar rat [10]. There was significant difference among group in AST and ALT levels. Increase of serum ALP activity and decrease of serum cholesterol level were biochemical markers of cholestasis.

Concerning the effects of significant elevation of hepatic MDA and a significant decrease of hepatic GSH contents in exposed rat with 17α-EE, can deduce that 17α-EE may induce oxidative stress process in hepatocytes and disrupt biological process. These results are in agreement with that showed damages of genotoxic and aberration of chromosomal are as a result of reactive oxygen species (ROS) and also, 17α-EE induce cytotoxicity in isolated rat liver by oxidative stress [4, 11]. Another study reported ethinyl estradiol damage liver through induction of oxidative stress. A significant increase in serum ALP, ALT and liver MDA and decrease significantly of serum cholesterol was in agreement with our study findings [12].

Significant decrease in serum albumin and total protein may suggest in correlation with oxidative stress that can cause disruption of hepatocyte protein structure and cells could not synthesis and release protein for circulation. Liver weight was measured and liver weight in ethinyl estradiol group was less than that in control group [1, 12]. In fact, serum albumin and total protein can be representative of liver weight that in our study was agreed with other studies.

Estrogens as pharmaceutical formulation have a lot of indications in animal and human practice. However, because of continuous use of such drugs and consumption of animal products containing these substances could cause adverse effects in some organs specifically in liver. The mechanism by which estrogens induces such effects is not still obvious, although some mechanism has been documented [1, 4, 13]. However, the liver, due to the site of anatomical to the blood supply and gastrointestinal system and also its ability to metabolize Ethinyl estradiol is the target organ [14].

Likely, light cholestasis produced by 5 d of ethinyl estradiol administration cause the initial toxic injury. In fact, toxic bile acids accumulation in hepatocytes may make to increase oxidative stress or disrupt the plasma membrane by their detergent activity or induce inflammatory response such as proinflammatory cytokines. In response to focal damage, likely, tissue-fixed macrophages, along with adjacent endothelial cells and epithelial cells are activated and secrete inflammatory products, subsequently. The latter ultimately results in the recruitment and activation of neutrophils and monocytes into the damaged site and the release of reactive oxygen species and the nitrogen-centered radical, nitric oxide, producing cell damage. Overproduction of these productions leads to more activation of macrophage and neutrophils in the site of damage and because of generalized liver damage that in the long-term lead to liver cirrhosis and fibrosis [14-16].

Another proposal mechanism in hepatocyte damage by EE is cholesterol esterification and oxidation products of cholesterol metabolism that

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cause increase of plasma membrane rigidity and generation of free radicals, respectively [4, 13].

In this study, concerning the antioxidative and antifibrotic, anti-inflammatory effects and of Pentoxifylline, specifically in inhibition of TNF-α as proinflammatory cytokines [7, 17-20], we used PTX. Our study in PTX+EE group in comparison with EE group showed significant difference in MDA, GSH, ALP and albumin parameters. Bile acids may induce inflammatory response or oxidative stress in liver cells and make focal tissue damage and PTX prevented these damages through inhibition of release of proinflammatory cytokines and stop of lipid peroxidation [21, 22]. Moreover, PTX reverses the rigidifying effect of EE on hepatocyte lipid membrane and prevent apoptotic genes expression and focal damages of liver [1, 12].

CONCLUSION

The possible modulatory effect of PTX against biochemical and oxidative stress changes was induced by 17α-Ethinyl Estradiol. Hepatoprotective effects of PTX against liver oxidative damages induced by EE have not been reported until now.

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