MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Therapeutic Role of Auraptene Against Letrozole-Induced Estrogen Disturbance in Female Rats

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Received:6/3/2024
Accepted: 20/3/2024

Abstract: Background: Estrogen insufficiency impedes organs functions and induces cytotoxicity, as estrogen is considered the pivotal regulator for normal development and function of multi-organs. The present study was designed to investigate the effectiveness of Auraptene, the monoterpene coumarin derivative compound with anti-oxidant, anti-inflammatory, multi-protective effects in improvement of estrogen insufficiency-induced cytotoxicity in sensitive organs like liver and cerebellum. Methods: 20 female Wistar albino rats, weighing 110±130g, were randomly categorized into 4 groups including: Group A: control group (0.5 ml of 0.9% NaCl /rat/day), Group B: AUR group (0.15 mg/kg for 2 weeks). Group C: letrozole group (0.04 mg/kg, for 2 weeks), Group D: Letrozole plus AUR group; letrozole (0.04 mg/kg) along with AUR (0.15 mg/kg) for 2 weeks. After 24 hours from the last dose rats were sacrificed by cervical dislocation, their skulls and abdominal cavities were opened, cerebellum and liver were dissected, and histopathological changes were examined. Results: Auraptene treatment evidently amended letrozole-cytotoxicity, letrozole treatment incited hepatic-microstructure alterations including distortion of portal area, sinusoidal dilation along with hepatocellular necrosis and vascular degeneration. Likewise, cerebellar sections revealed cerebral cortex obstruction, Purkinje cells shrinkage with a reduction in their numbers and empty areas in between them, as well as neural vacuolation and cells clumping in granular layer. Conclusion: These findings confirm the potential effects of auraptene in attenuate Letrozole-induced estrogen insufficiency cytotoxicity in female rats**.**

keywords: Estrogen; Aromatase enzyme; Letrozole; Auraptene; Cytotoxicity.

1. Introduction

Estrogen is a pivotal hormone as it is nearly involved in most physiological processes including the development, growth, reproduction, and maintaining both gender's sexual organs as well as the secondary sexual characteristics. As well, estrogen is the hypothalamic functions, cognitive and the neuroendocrine functions regulator, it also had role in maintaining homeostasis, and making the liver less volatile to different chronic liver diseases.[1]

Nowadays, Letrozole which is the potent third-generation non-steroidal oral aromatase inhibitor is widely used as a first line therapy for metastatic breast cancer in postmenopausal women[2], as well as an ovulation induction in fertility clinics for PCOS women, or for women with unexplained infertility.[3] Letrozole is acting through blocking estrogen production without affecting the other steroidogenic pathways[4], it acts through non-covalently binding to the aromatase heme moiety, causing cytochrome P450 enzyme reversible inhibition, which in turn block the conversion of testosterone and androgens to estrogens by approximately 99.1%.[3,5] Estrogen deficiency was found to be associated with different endocrine, metabolic, and reproductive damage including endocrine disorders, hyperinsulinism, hyperlipidemia, obesity, and oxidative damage.[6]

Liver and cerebellum have traditionally been considered to be letrozole- affected target organs. Liver is well known to have an indispensable role in the glucose metabolism,

homeostasis, as well as detoxification of xenobiotics and many drugs, it is directly dealing with toxicity etiological factors, which affected the normal biochemical and physiological functions, causing chronic liver diseases (CLD), and leading to various pathological demonstrations like hepatic inflammation, cirrhosis, fibrosis, steatosis, portal hypertension, and hepatocellular carcinoma (HCC).[7] In literature estrogen deficiency in female rat model caused oxidative damage in liver and developed prominent fibrosis. The estradiol treatment reversed the effects of estrogen deficiency, reduced the lipid peroxidation, and enhanced the deposition of type I and III fibril forming collagens. Also, treatment menopausal patients with aromatase inhibitor like tamoxifen had been found to induce the profibrogenic cytokine as well as hepatic fibrosis.[8,9]

Estrogen has efficient role in the normal development and functions of cerebellum in both gender's animals. The cerebellar cortex has been found to be an estradiol source, as cerebellar Purkinje cells is considered as a vital site for neurosteroid hormone formation in many vertebrates, as well as for many several kinds of steroidogenic enzymes, such as cytochrome P450 which located in the Purkinje cells.[10] In rat models ERα expression was detected in the scattered Purkinje cells, and ERβ was detected in the granular cell layer as well as the cytoplasm of most Purkinje cells.[11,12] In adult rodents, estrogen has been found to improve synaptic efficacy and boost cerebellum-mediated behaviors.[13] Also estrogen is required for mGluR1a signaling regulation which is responsible for supporting the parallel fiber (PF)-Purkinje cell synaptic function, which improving cerebellar physiology.[14]

Herbal medicines have been widespread through the whole world. As they were found to be more potent on their biological activity than synthetic one, also they had low toxic effects than the synthetic medicines.[15] Plants especially citrus fruits are good nutrition sources and has vital importance effects on human health as they contain plenty of vitamins and macronutrients. Citrus products are rich in polyphenolic, coumarins, and flavonoid compounds, they have been found to be antioxidant-rich substances which consistently have been shown to have vital effects in the prevention, controlling and treatment of various diseases.[16]

Auraptene (AUR) which is scientifically known as 7-geranyloxycoumarin, is a bioactive monoterpene coumarin ether, auraptene is isolated from many citrus fruits like bael fruit (*Aegle marmelos*), Seville orange (*Citrus aurantium*), lemons, oranges, and grapefruits.[17] Auraptene has been used widely as an anti-cancer treatment for many cancer cell lines including breast, colon, ovarian and prostate cancer. Nowadays, AUR is used for prevention, treatment and control many diseases such as cystic fibrosis, nonalcoholic fatty liver, and hypertension, it has the ability to overcome oxidative damage, inflammation, apoptosis and improve cell growth. Due to the impressive potential effects, auraptene have; like anti-oxidant, anti-inflammatory, anti-genotoxic, anti-coagulant, anti-bacterial, anti-fungal, neuroprotective and hepatoprotective effects.[18,19] However, the therapeutic effects of auraptene are still need to be investigated more extensively. The aim of this study is to investigate auraptene's potential effects in improvement letrozole-induced estrogen deficiency-cytotoxicity in liver and cerebellum.

2. Materials and methods

All care and procedures of the study protocol were conducted in accordance with the guidelines of Egyptian Bioethics of the Mansoura University Animal Care and Use Committee (MU-ACUC), code number: MU-ACUC (SC.MS.22.11.10).

2.1. Chemicals

1. Letrozole (Femara®) is a product of Novartis (Basel, Switzerland), was purchased from Amr Samir Pharmacy, Dakahlia, Egypt.

2. Auraptene was purchased from Sigma Aldrich co (Sigma, St. Louis, MO, USA).

2.2. Experimental animals:

In this experimental study, 20 female Wistar albino rats weighing about 110±130g, were purchased from Helwan Breading Farm, Ministry of Health, Egypt and housed in the animal house of Zoology Department, Faculty of Science, Mansoura University. The rats were separated in ventilated metal cages, they were

maintained at (22 ± 3) °C and 50%-60% humidity,12 h light cycle and 12 h dark cycle, food and fresh water were provided to animals *ad libitum*, rats were allowed to be acclimatized to laboratory conditions for two weeks before the experiment.

2.3. Experimental design

The rats were randomly divided into four groups (5 rats for each group) as the following: Group A: control group received (0.5 ml of 0.9% NaCl /rat/day), Group B: AUR group rats were received (0.15 mg/kg) of auraptene orally for 14 days, Group C: Letrozole group, letrozole tablets (2.5 mg) dissolved in 0.9% NaCl, the rats were received (0.04 mg/kg) of letrozole orally for 14 days, letrozole dose was selected corresponding to the recommended daily human dose (2.5 mg) in a person weighing 60 kg[20], Group D: combined group (Letrozole + AUR), rats were received letrozole (0.04 mg/kg) along with AUR (0.15 mg/kg) orally for 14 days.

After 24 hours from the last dose, rats were sacrificed by cervical dislocation, their skulls and abdominal cavities were opened, cerebellum and liver were dissected and histopathological changes were examined.

2.4. Histopathological investigations

Liver and cerebellum from the different animal groups were separated and fixed immediately in 10% phosphate buffered formalin (pH 7.4), dehydrated in ascending series of ethyl alcohol, then they were cleared in xylene and mounted in molten paraplast at 58-62ºC. Five µm histological sections were cut, stained with Hematoxylin & eosin then histomorphological changes were assessed using Olympus® microscope.[21]

2.5. Morphological analysis

Five µm cerebellar-H&E stained sections were used for analyzing Purkinje cells numbers using the ImageJ imaging software.

2.6. Statistical analysis

Statistical analysis was performed using the GraphPad Prism v5.02.419 software, Data are presented as mean ± standard error (SE), Statistical significance and the difference among groups were evaluated by one-way post-hoc analysis of variance (ANOVA). Differences were considered significant at $p < 0.05$.

3. Results

3.1. Hepatic histopathological investigations

Liver depicted in **(Figs. 1&2),** A microscopic examination of control rats showed normal hepato-histological criteria, the hepatocytes (H) were arranged in anastomosing cords with the blood sinusoids (BS) in between and normal central vein (CV) **(Fig. 1A)**. The Hepatocytes were polyhedral cells with central rounded vesicular nuclei with prominent nucleoli and acidophilic finely vacuolated cytoplasm, some binucleated hepatocytes were recorded. The blood sinusoids were lined with flat endothelial cells and Kupffer cells (KC) **(Fig. 2A)**. AUR-supplemented livers revealed normal histological appearance as control group **(Figs. 1B & 2B).**

On the other hand, letrozole-treated rats depicted in **(Figs. 1C & 2C)** exhibited histo-hepatic lesions compared to control group, abnormal hepatocytes were detected in disorganized hepatic cords with obstruction and dilation of blood sinusoids. Acute hepatocytes necrosis with acute chronic inflammatory cellular infiltration were observed around the degenerated central vein (DCV). Hepatocytes revealed dark nuclei and deep acidophilic cytoplasm, some pale hepatocytes were observed **(Fig. 2C).** Meanwhile, histopathological observations for combined group compared with control and letrozole group revealed an improvement in hepatic tissue, normal organization of hepatic cords with healthy hepatocytes and blood sinusoids in between as well as normal hepatic portal vein **(Fig. 1D).** Polyhedral hepatocytes with acidophilic finely vacuolated cytoplasm and central rounded vesicular nuclei were detected, some hepatocytes were binucleated. There were normal sinusoids with endothelial cells and Kupffer cells lining with mild dilation. Normal hepatic central vein with thick wall was noticed **(Fig. 2D)**.

3.2. Cerebellar histopathological investigations

Histomorphological examination for cerebellum sections of control group **(Fig. 3A)** showed a normal cerebellar inner white matter (WM) covered by an outer cortex which is consisted of outer molecular layer (ML),

middle Purkinje layer (PL) and inner granular layer (GL), Control Purkinje cells exhibited central vesicular nuclei (N), also they exhibited numerous small closely packed granular cells (GC) with dark spherical nuclei. Basket cell (BC) as well as Perineural glial cells were seen around Purkinje cells **(Fig. 4A)**. Same control histological criteria were observed in auraptene-supplemented rats **(Figs. 3B & 4B).**

Conversely, letrozole treatment disturbed the cerebellar-microstructures as illustrated in **(Figs. 3C & 4C)**, letrozole disturbed cerebellar Purkinje cells arrangement, there was an abnormal irregularity and shrinkage of Purkinje cells with observed empty areas in between. Rats exhibited a significant reduction in Purkinje cells numbers ($p < 0.05$, one-way ANOVA) **(Fig. 5 & Table 1)** compared to control rats. The granular layer showed clumping of cells as well as neuronal vacuolation **(Fig. 4C)**. On the other hand, cerebellum of auraptene-letrozole treated group revealed normal histological criteria. In comparison with letrozole, AUR restored normal cerebellar cortex appearance, but there were a few distorted cells, with mild empty spaces in between Purkinje cells**,** and few clumping cells in granular layer **(Figs. 3D & 4D)**. Also, there was a significant increase in number of Purkinje cells ($p < 0.05$, one-way ANOVA) **(Fig. 5 & Table 1)**.

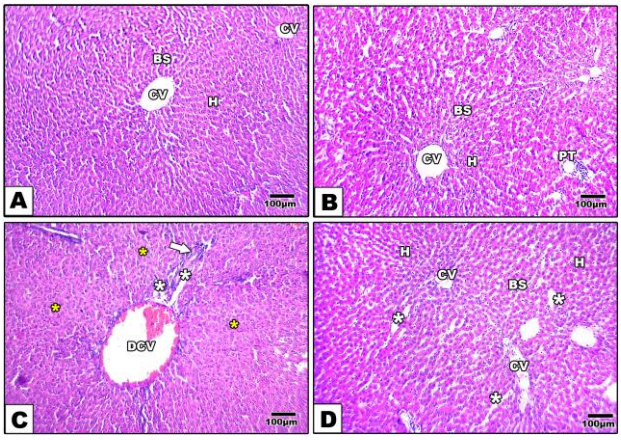

Plate 1: (Figs. A-D): photomicrographs of histological sections of liver of rats from control and different animal groups (H&E) (X. 100 µm). Abbreviations: H: hepatocytes, BS: blood sinusoids, CV: central vein, PT: portal tract, DCV: degenerated central vein. Hepatic section of control **(Fig. A)** & auraptene- treated rats **(Fig. B)** revealed normal hepatic histological appearance, hepatocytes are arranged in anastomosing plates with blood sinusoids in between and normal portal vein and normal portal tract. Letrozole-treated rats **(Fig. C)** showed abnormal hepatocytes **(yellow asterisks)** arranged in disorganized hepatic cords, obstruction and dilation of sinusoids **(asterisks)**, hepatocytes necrosis with inflammatory cellular infiltration **(thick arrow)** were observed around the degenerated central vein. Auraptene-letrozole treated group **(Fig. D**) revealed an improvement in hepatic architecture.

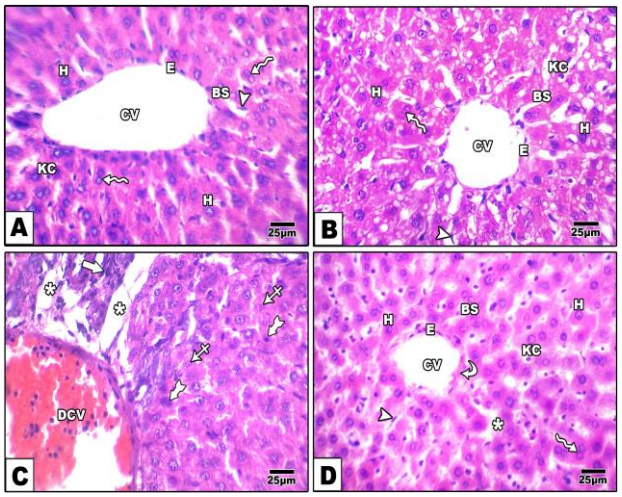

Plate 2: (Figs. A-D): photomicrographs of histological sections of liver of rats from control and different animal groups (H&E) (X. 25 µm). Abbreviations: H: hepatocytes, BS: blood sinusoids, CV: central vein, KC: Kupffer cells, E: endothelial cells, DCV: degenerated central vein. Hepatic section of control **(Fig. A) &** auraptene- treated rats **(Fig. B)** revealed normal hepatic histological appearance, polyhedral **hepatocytes with acidophilic finely vacuolated cytoplasm and central rounded** vesicular nuclei with prominent nucleoli, some hepatocytes are binucleated **(zigzag arrow),** sinusoids are lined with flat endothelial cells **(arrowhead)** and Kupffer cells, the hepatic central vein are lined with flat endothelial cells. Letrozole-treated rats **(Fig. C)** showed hepatocytes with dark nuclei and deep acidophilic cytoplasm **(tailed arrows)**, some pale hepatocytes **(crossed arrows)**, dilation of sinusoids **(asterisks),** degenerated central vein surrounded by necrotic hepatocytes with inflammatory cellular infiltration **(thick arrow).** Auraptene-letrozole treated group **(Fig. D**) revealed normal hepatic criteria with mild dilation sinusoids **(asterisk)**, central vein with thick wall **(curved arrow).**

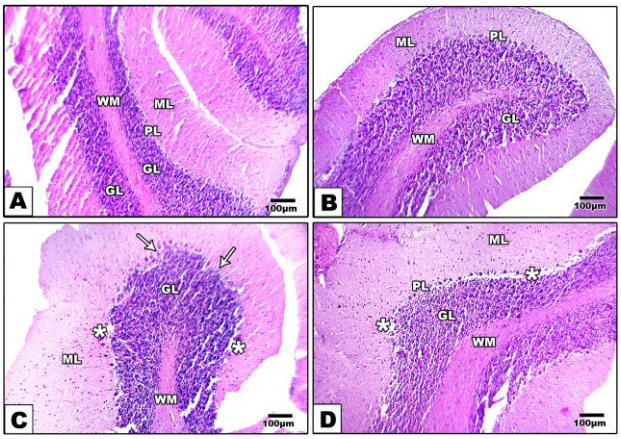

Plate 3: (Figs. A-D): photomicrographs of histological sections of cerebellum of rats from control and different animal groups (H&E) (X. 100 µm). Abbreviations: WM: white matter, ML: Molecular layer, PL: Purkinje cell layer, GL: granular layer. Cerebellar section of control rats **(Fig. A)** showed normal white matter & normal outer cortex; outer molecular layer, middle Purkinje layer and inner granular layer. Auraptene-treated rats **(Fig. B)** exhibited normal cerebellar histological microstructures as control group. Letrozole- treated rats **(Fig. C)** revealed shrunken, irregular Purkinje layer **(arrows)** with separated areas **(asterisks).** Auraptene-letrozole treated group **(Fig. D)** showed normal cerebellar histological appearance as control group with few separated areas in between Purkinje cells.

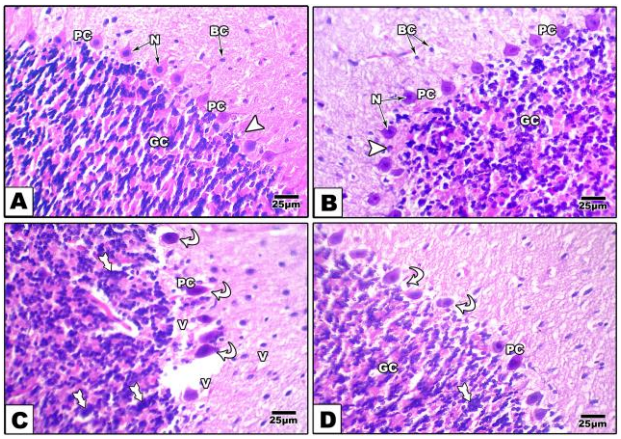

Plate 4: (Figs. A-D): photomicrographs of histological sections of cerebellum of rats from control and different animal groups (H&E) (X. 25 µm). Cerebellar section of control rats **(Fig. A)** showed Purkinje cells with central vesicular nuclei, numerous small closely packed granular cells with dark spherical nuclei. Basket cell and Perineural glial cells **(arrowhead)** around Purkinje cells. Auraptene-treated rats **(Fig. B)** showed normal cerebellar histological microstructures as control group. Letrozole- treated rats **(Fig. C)** revealed disturbed arrangement of Purkinje cells, shrinkage Purkinje cells with empty spaces in between **(curved arrows)**, clumping granular cells **(tailed arrows)** with neural vacuolation. Auraptene-letrozole treated group **(Fig. D)** revealed normal cerebellar histological appearance as control group, there was mild Purkinje cells distortion **(curved arrows)** and few granular cells clumping. BC: basket cell, PC: Purkinje cells, N: Purkinje cells nuclei, GC: granular cells, V: vacuolated neurons.

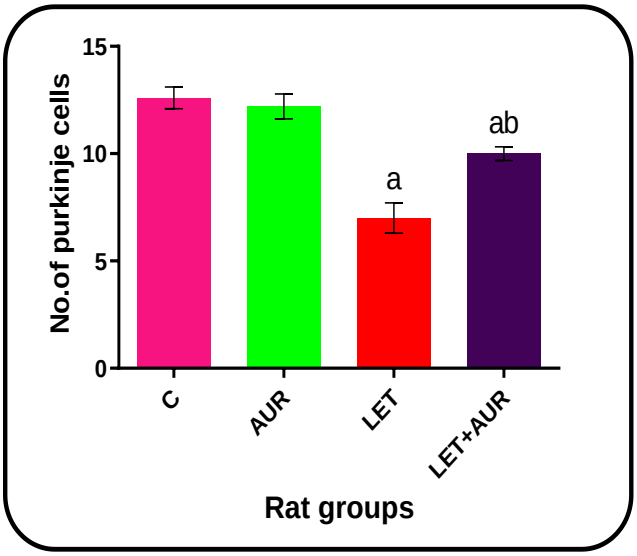

Figure (5): Number of Purkinje cells in control and different rat groups.

a, b significant changes at $p<0.05$.

a: significant as compared to control.

b: significant as AUR + LET group compared to LET group.

C: Control AUR: Auraptene LET: Letrozole.

Table 1: Number of Purkinje cells in control and different rat groups.

Parameter	Rat group	Mean+SE	% of change related to a control group	% of change related to LET group
No. of Purkinje cells	C	13 ±0.51		
	AUR	12 ±0.58	-7.69	
	LET	7 ±0.71[a]	-46.15	
	AUR +LET	10 ±0.32[ab]	-23.08	42.86

Results are presented as means ±SE and % of change.

(n=5 for each group).

a, b significant changes at $p<0.05$.

a: significant as compared to control.

b: significant as AUR + LET group compared to LET group.

(*): % of change related to control group.

(**): % of change related to LET group.

C: Control AUR: Auraptene LET: Letrozole.

4. Discussion

As a complex drug using as a menopause breast carcinoma- endocrine therapy as well as an ovulation-induction cure for women with different infertility reasons, letrozole as a reversible aromatase inhibitor treatment[3], has multi-phenotypic parameters like insomnia, hot flashes, hair thinning[22], and endocrinopathies. It inhibits estrogen synthesis, causing hyperandrogenism, testosterone hyper-accumulation, dysregulation GnRH secretion, which in turn leading to LH hypersecretion along with FSH downregulation, those endocrine dysfunctions are stimulating polycystic ovaries and ovarian impairments with oligo-ovulation or anovulation.[23,24] Increasing metabolic disturbances; such as hyperinsulinemia, insulin resistance, dyslipidemia and obesity were observed,[25,26] also letrozole was been found to stimulate arthralgias, tendinopathies, bone fractures and osteoporosis.[27]

In literature, estrogen receptors (ER) had been found in hepatocytes as well as Kupffer cells, and hepatic stellate cells. Low estrogen stimulates production of IL-6 and proinflammatory cytokines, ultimately increasing hepatic inflammation. In addition, it was stated that estrogen deficiency increases lipogenesis related genes expression and decreases lipolysis related genes expression, resulting in nonalcoholic-fatty liver diseases.[28,29] Indeed, estrogen can induce changes in gene expression and hepatic function, through estrogen-ER complex bind to specific DNA sequences, known as estrogen-response elements, which promote transcription of a various genes that regulate cell cycle, DNA replication, cellular differentiation, apoptosis, and angiogenesis, in addition to modulate hepatocellular function through extranuclear ER interacting with kinase signaling cascades.[30] Evidence from past literature indicates that letrozole disturbances hepatic function, as the hepatic parameters like ALP, AST, LDH, and bilirubin were found to be increased after treatment with letrozole[31], similar results observed in Chaiyamoon *et al.*[32] study, who indicated an increment in lipid profile and Tyrosine phosphorylation (TyrPho) protein expressions of hepatic 32 and 27 kDas. The present results revealed a sinusoidal dilation and obstruction with disorganization of hepatic cords, same results were in agreement with Azouz *et al.*[26] study. Furthermore, the present histological investigation revealed portal area distortion with acute inflammation and central vein degeneration, acute hepatocellular necrosis with pale or dark nuclei hepatocytes and dilated sinusoids were noticed. Similar hepatic-toxicity were reported by Koubaa-Ghorbel *et al.*[6]; Yu *et al.*[33], as results of oxidative damage induced by estrogen deprivation.

Aromatase inhibitors effects on nervous system are controversial and are increasing interest recently, Interestingly, Purkinje cells has been found to express multiple neurosteroidogenic enzymes; such as cytochrome P450[34], 3- hydroxysteroid dehydrogenase (3-HSD)[10] and 17-HSD[35]. Cerebellum has the ability for *in situ* testosterone aromatization to estrogen, as well as complete reserve of enzymatic steps for cholesterol synthesize into gonadal steroids.[36] There is evidence that a significant correlation exists between estrogen deficiency and neuroendocrine and cerebellar impairments. Estrogen can act *in situ* as a neuromodulator in addition to support basal cerebellar neurotransmission through regulation of mGluR1a signaling in Purkinje neurons.[14]

In past studies, cerebellum's estrogen is considered the key regulator of neural development and synaptic plasticity, it induces Purkinje cell dendritic growth, spine density, synaptogenesis, and excitability.[36] In addition to have a role in preservation of cerebellar gray matter as a function of aging.[37] The present histological investigation revealed cerebral cortex obstruction, reduction in Purkinje cells numbers with extensive Purkinje cell irregularity and shrinkage. As reported in Kara *et al.*[38] study, estrogen deficiency and hyperglycemia in ovariectomized rats caused

degeneration in Purkinje cells membrane and mitochondria, swollen organelles, formation and vacuolization of edema, with increasing Purkinje cells apoptotic activity.

Purkinje cells numbers in female rats were found to correlate directly with the concentration of plasma 17β-estradiol hormone, as well as plasma sex steroid hormones, which are very protective against immaturation and death of Purkinje cells.[39] The morphological changes in the present study were supported by many reports, estrogen induces dendritic growth and spinogenesis in Purkinje cells through acting directly on the Purkinje cells' ERβ-mediated mechanisms. Aromatase inhibitors bind to ERs, resulting in activating transcription through activating protein-1 response elements and blocking transcriptional activation of ER-β expression in the developing Purkinje cells *via* classical estrogen response element.[34] Letrozole as an aromatase inhibitor can alter glutamate-evoked excitation of Purkinje cells leading to neural-issues, as estrogen was found to be required for the activation of NMDA receptors in adult female rats for alterations in gene expression and hippocampal CA1 pyramidal cell dendrite spine density.[34]

Auraptene (AUR) , a rare nature bioactive monoterpene coumarin, is a research hot spot in recent decade, AUR has a long history as a medicine as well, it is well known for its anti-carcinogenic, anti-oxidant, anti-inflammatory, anti-coagulant, and anti-bacterial effects. Also, it is known to act as a cardioprotective, neuroprotective and hepatoprotective treatment for diseases associated with oxidative stress.[18] AUR has been recognized as an inhibitory tumorigenesis-cure in various cancer cell lines; like breast, ovarian, prostate, esophagus and colon cancers in rodent models.[17]

In the current study, it was determined that AUR administration along with letrozole, prevent the expecting hepatic damage which results from estrogen deprivation and oxidative damage due to treatment only with letrozole. AUR disturbed hepatocellular necrosis, liver exhibited normal portal area, as well as a normal organization of hepatic cords with healthy hepatocytes and mild dilated sinusoids. As a natural medicine, AUR shows hepatoprotective, antioxidant, and anti-inflammatory activities in chronic hepatic diseases in rat models, AUR reduces cystic fibrosis, hypertension, and nonalcoholic fatty liver.[40] Gao *et al.*[41] study examining AUR effectiveness in liver mice injury, AUR maintained bile acids homeostasis *via* regulation of FXR-target genes such as Bsep, Ntcp, Mrp2, Cyp7a1, and Cyp8b1, and downregulated TGF-β1 and α-SMA expression. Also, AUR was found to have anti-hyperlipidemia effects, AUR enhanced lipolysis in HepG2 hepatocytes *via* reduction white adipose tissue accumulation, which in turn overcome lipid profile abnormalities.[42]

The underlying mechanism for AUR effects may contribute to its improvement of oxidative damage, reduction of inflammatory cytokines and transcription of pro-apoptotic genes, resulting in reduction of hepatic damage. In past studies, AUR treatment in different doses for cholestatic liver diseases for 7 days *in vivo and in vitro* reduced mice cholestatic mortality percentage, also it reduced bile acid synthesis and uptake and increased their efflux into intestine, as accumulation of bile acids causing hepatocellular apoptosis and liver injury. Also, AUR induced liver regeneration-related gene promoted *via* suppression of liver inflammation- related gene and inflammatory markers such as IL-1β, IL-6, TNF-α, and NF-κB.[43,44]

In the current study, combined group exhibited normal healthy cerebellar histological criteria. AUR showed the ability to maintain normal cerebellar-cytoarchitecture. Estrogen deficiency- induced ROS and chronic inflammation, giving rise to normal cells apoptosis. The effects induced by AUR in cerebellum would have contributed to coumarins' anti-inflammation and neuroprotective activities, through AUR moiety geranyloxyl group which is responsible for auraptene integration with cells. Both geraniol and geranyl acetate group suppress ROS production and inflammation-induced cell toxicity.[45] Other studies demonstrated that coumarins have the ability to reduce pro-inflammatory mediators' levels and ROS , such as TNF-α, IL-1β, IL-8, and NO.[46] In rat models, coumarin has been found to decrease neuronal cells degeneration, and inhibit mitochondrial

dysfunction, also coumarin exerted neuroprotection effect in cerebral middle artery through reducing the water content and infract size of rat brains.[47] Coumarins are considered as a potential therapeutic resource for treatment and prevention some CNS diseases including traumatic brain injury, neural damage and chronic cerebral hypoperfusion-induced cognitive deficits and hippocampal neuronal damage, acute ischemic stroke, and transient focal cerebral ischemia.[48,49,50] Okuyama *et al.*[51] study proved the AUR-neuroprotective effects for CNS and peripheral tissues in cerebral ischemia, AUR suppressed neural death, hippocampal microglial activation as well as COX-2 expression in hippocampal astrocytes through suppression of the expression of protein in LPS-treated RAW264.7 cells and thereby inhibit neural apoptosis.[45]

5. Conclusion

The present study concluded that auraptene had the potential to lighten and ameliorate letrozole-induced cytotoxicity including oxidative damage, inflammation, apoptosis, as well as alteration histological criteria of vital organs. Auraptene can be potential therapeutic treatment for estrogen deficiency in females.

Abbreviations: AST: Aspartate aminotransferase; AUR: Auraptene; CLD: Chronic liver diseases; COX-2: Cyclooxygenase-2; ER: Estrogen receptors; ERα: Estrogen receptor-α; ERβ: Estrogen receptor-β; FSH: Follicle-Stimulating hormone; GnRH: Gonadotropin-releasing hormone; HCC: Hepatocellular carcinoma; IL-1β: Interleukin -1β; IL-6: Interleukin-6; LDH: Low-density lipoprotein; LH; Luteinizing hormone; NO: Nitric oxide; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; PF: Parallel fiber; PCOS: Polycystic ovarian syndrome; ROS: Reactive oxygen species; ALP: Total alkaline phosphatase, TNF-α: Tumor necrosis factor- α.

4. References

1 Weigel, N. L., & Rowan, B. G. (2001). Estrogen and progesterone action. Endocrinology. Philadelphia: WB Saunders. 2053-2060.

2 Slamon, D. J., Diéras, V., Rugo, H. S., Harbeck, N., Im, S. A., Gelmon, K. A., Lipatov, O. N., Walshe, J. M., Martin, M., Chavez-MacGregor, M., Bananis, E., Gauthier, E., Lu, D. R., Kim, S., & Finn, R. S. (2024). Overall Survival With Palbociclib Plus Letrozole in Advanced Breast Cancer. *Journal of Clinical Oncology*. **42(9),** 994-1000.

3 Haynes, B. P., Dowsett, M., Miller, W. R., Dixon, J. M., & Bhatnagar, A. S. (2003). The pharmacology of letrozole. *Journal of Steroid Biochemistry and Molecular Biology*. **87**, 35-45.

4 Swain, S. M. (2005). Aromatase inhibitors-a triumph of translational oncology. *The New England journal of medicine*. **353(26)**, 2807-2809.

5 Chumsri, S. (2015). Clinical utilities of aromatase inhibitors in breast cancer. *International journal of women's health*. **7,** 493-499.

6 Koubaa-Ghorbel, F., Chaâbane, M., Jdidi, H., Turki, M., Makni-Ayadi, F., & El Feki, A. (2020). Salvia officinalis mitigates uterus and liver damages induced by an estrogen deficiency in ovariectomized rats. *Journal of Food Biochemistry*. **45(5)**, e13542.

7 Gheena, S., & Ezhilarasan, D. (2019). Syringic acid triggers reactive oxygen species-mediated cytotoxicity in HepG2 cells. Human and Experimental Toxicology. **38(6)**, 694-702.

8 Yasuda, M., Shimizu, I., Shiba, M., & Ito, S. (1999). Suppressive effects of estradiol on dimethylnitrosamine-induced fibrosis of the liver in rats. Hepatology. **29**, 719-727.

9 Bentzen, S. M., Skoczylas, J. Z., Overgaard, M., & Overgaard, J. (1996). Radiotherapy-related lung fibrosis enhanced by tamoxifen. *Journal of the National Cancer Institute*. **88,** 918-922.

10 Ukena, K., Kohchi, C., & Tsutsui, K. (1999). Expression and activity of 3β-hydroxysteroid dehydrogenase/Δ5 -Δ4 -isomerase in the rat Purkinje neuron during neonatal life. Endocrinology. **140**, 805-813.

11 Pérez, S. E., Chen, E. Y., & Mufson, E. J. (2003). Distribution of estrogen receptor alpha and beta immunoreactive profiles in the postnatal rat brain. Brain research. Developmental brain research. **145**, 117-

139.

12 Price, R. H. Jr., & Handa, R. J. (2000). Expression of estrogen receptor-beta protein and mRNA in the cerebellum of the rat. Neuroscience Letters. **288**, 115-118.

13 Smith, S. S., Woodward, D. J., & Chapin, J. K. (1989). Sex steroids modulate motor-correlated increases in cerebellar discharge. Brain Research. **476(2)**, 307-316.

14 Hedges, V. L., Chen, G., Yu, L., Krentzel, A. A., Starrett, J. R., Zhu, J. N., Suntharalingam, P., Remage-Healey, L., Wang, J. J., Ebner, T. J., & Mermelstein, P. G. (2018). Local Estrogen Synthesis Regulates Parallel Fiber-Purkinje Cell Neurotransmission Within the Cerebellar Cortex. Endocrinology. **159(3)**, 1328-1338.

15 Ng, M. K., Abdulhadi-Noaman, Y., Cheah, Y. K., Yeap, S. K., & Alitheen, N. B. (2012) .Bioactivity studies and chemical constituents of Murraya paniculata (Linn) Jack. *International Food Research Journal*. **19(4)**, 1307-1312.

16 García-Salas, P., Gómez-Caravaca, A. M., Arráez-Román, D., Segura-Carretero, A., Guerra-Hernández, E., García-Villanova, B., & Fernández-Gutiérrez, A. (2013). Influence of technological processes on phenolic compounds, organic acids, furanic derivatives, and antioxidant activity of wholelemon powder. Food Chemistry. **141**, 869-878.

17 Genovese, S., & Epifano, F. (2011). Auraptene: a natural biologically active compound with multiple targets. Current Drug Targets. **12**, 381-386.

18 Bibak, B., Shakeri, F., Barreto, G. E., Keshavarzi, Z., Sathyapalan, T., & Sahebkar, A. (2019). A review of the pharmacological and therapeutic effects of auraptene. BioFactors. **45(6)**, 867-879.

19 Askari, V. R., Rahimi, V. B., Zargarani, R., Ghodsi, R., Boskabady, M., & Boskabady, M. H. (2021). Anti-oxidant and anti-inflammatory effects of auraptene on phytohemagglutinin (PHA)-induced inflammation in human lymphocytes. Pharmacological Reports. **73(1),** 154-162.

20 Tiboni, G. M., Marotta, F., Rossi, C., & Giampietro, F. (2008). Effects of the aromatase inhibitor letrozole on in utero development in rats. Human Reproduction. **23(8)**, 1719-1723.

21 Weesner, F. M. (1968). General Zoological Microtechniques. The Williams and Wilkins Company Baltimore. 230.

22 Bae, K., Yoo, H. S., Lamoury, G., Boyle, F., Rosenthal, D. S., & Oh, B. (2015). Acupuncture for aromatase inhibitor–induced arthralgia: a systematic review. Integrative Cancer Therapies. **14,** 496-502.

23 Pachiappan, S., Ramalingam, K., & Balasubramanian, A. (2023). Evaluation of Gymnema sylvestre R. Br. against Letrozole Induced Polycystic Ovarian Syndrome in rats. *Research Journal of Pharmacy and Technology*. **16(1)**, 385-390.

24 Younas, A., Hussain, L., Shabbir, A., Asif, M., Hussain, M., & Manzoor, F. (2022). Effects of Fagonia indica on Letrozole-Induced Polycystic Ovarian Syndrome (PCOS) in Young Adult Female Rats. Evidence-Based Complementary and Alternative Medicine. 2022, 1397060 .

25 Yu, J., Ding, C., Hua, Z., Jiang, X., & Wang, C. (2021). Protective effects of berberine in a rat model of polycystic ovary syndrome mediated *via* the PI3K/AKT pathway. *Journal of Obstetrics and Gynaecology Research*. **47(5)**, 1789-1803.

26 Azouz, A. A., Ali, S. E., Abd-Elsalam, R. M., Emam, S. R., Galal, M. K., Elmosalamy, S. H., Alsherbiny, M. A., Hassan, B. B., Li, C. G., & El Badawy, S. A. (2021). Modulation of steroidogenesis by Actaea racemosa and vitamin C combination, in letrozole induced polycystic ovarian syndrome rat model: promising activity without the risk of hepatic adverse effect. Chinese Medicine. **16(1)**, 36.

27 Gaillard, S., & Stearns, V. (2011). Aromatase inhibitor-associated bone and musculoskeletal effects: new evidence defining etiology and strategies for management. Breast Cancer Research. 13,

205.

28 Ohashi, T., Kato, M., Yamasaki, A., Kuwano, A., Suzuki, H., Kohjima, M., & Ogawa, Y. (2018). Effects of high fructose intake on liver injury progression in high fat diet induced fatty liver disease in ovariectomized female mice. Food and Chemical Toxicology. **118**, 190-197.

29 Cohen, J. C., Horton, J. D., & Hobbs, H. H. (2011). Human fatty liver disease: old questions and new insights. Science. **332(6037)**, 1519-23.

30 Zuo, Q., Park, N. H., Lee, J. K., Santaliz-Casiano, A., & Madak-Erdogan, Z. (2023). Navigating nonalcoholic fatty liver disease (NAFLD): Exploring the roles of estrogens, pharmacological and medical interventions, and life style. *Steroids.* 203, 109330.

31 Aydin, M., Oktar, S., Ozkan, O. V., Alçin, E., Oztürk, O. H., & Nacar, A. (2011). Letrozole induces hepatotoxicity without causing oxidative stress: the protective effect of melatonin. Gynecological Endocrinology. **27(4),** 209-215.

32 Chaiyamoon, A., Bunsueb, S., & Iamsaard, S. (2020). Changes of Tyrosine Phosphorylation in Liver and Kidney of Polycystic Ovarian Rats Induced by Letrozole. *International Journal of Morphology*. **38(4),** 919-923.

33 Yu, Y. M., Zhou, B. H., Yang, Y. L., Guo, C. X., Zhao, J., & Wang, H. W. (2022). Estrogen Deficiency Aggravates Fluoride-Induced Liver Damage and Lipid Metabolism Disorder in Rats. Biological Trace Element Research. **200(6),** 2767-2776.

34 Sakamoto, H., Mezaki, Y., Shikimi, H., Ukena, K., & Tsutsui, K. (2003). Dendritic growth and spine formation in response to estrogen in the developing Purkinje cell. Endocrinology. **144(10),** 4466-4477.

35 Dinkel, K., Rickert, M., Möller, G., Adamski, J., Meinck, H. M., & Richter, W. (2002). Stiff-man syndrome: identification of 17 beta-hydroxysteroid dehydrogenase type 4 as a novel 80-kDa antineuronal antigen. *Journal of Neuroimmunology*. **130(1-2),** 184-193.

36 Dean, S. L., Wright, C. L., Hoffman, J. F., Wang, M., Alger, B. E., & McCarthy, M. M. (2012). Prostaglandin E2 stimulates estradiol synthesis in the cerebellum postnatally with associated effects on Purkinje neuron dendritic arbor and electrophysiological properties. Endocrinology. **153(11),** 5415-5427. PMID: 23054057; PMCID: PMC3473195.

37 Ghidoni, R., Boccardi, M., Benussi, L., Testa, C., Villa, A., Pievani, M., Gigola, L., Sabattoli, F., Barbiero, L., Frisoni, G. B., & Binetti, G. (2006). Effects of estrogens on cognition and brain morphology: involvement of the cerebellum. Maturitas. **54(3),** 222-228.

38 Kara, A., Unal, D., Simsek, N., Yucel, A., Yucel, N., & Selli, J. (2014). Ultra-structural changes and apoptotic activity in cerebellum of post-menopausal-diabetic rats: a histochemical and ultra-structural study. Gynecological Endocrinology. **30(3)**, 226-231.

39 Janmaat, S., Akwa, Y., Doulazmi, M., Bakouche, J., Gautheron, V., Liere, P., Eychenne, B., Pianos, A., Luiten, P., Groothuis, T., Baulieu, E., Mariani, J., Sherrard, R. M., & Frédéric, F. (2011). Age-related Purkinje cell death is steroid dependent: RORα haplo-insufficiency impairs plasma and cerebellar steroids and Purkinje cell survival. *Age; Journal of the American Aging Association*. **33**, 565-578.

40 Sakata, K., Hara, A., Hirose, Y., Yamada, Y., Kuno, T., Katayama, M., Yoshida, K., Zheng, Q., Murakami, A., Ohigashi, H., Ikemoto, K., Koshimizu, K., Tanaka, T., & Mori, H. (2004). Dietary supplementation of the citrus antioxidant auraptene inhibits N,N-diethylnitrosamine-induced rat hepatocarcinogenesis. Oncology. **66(3),** 244-252.

41 Gao, X., Wang, C., Ning, C., Liu, K., Wang, X., Liu, Z., Sun, H., Ma, X., Sunab, P., & Meng, Q. (2018). Hepatoprotection of auraptene from peels of citrus fruits against thioacetamide-induced hepatic fibrosis in mice by activating farnesoid X receptor. Food & Function. **9(5).** 2684-2694.

42 Nagao, K., Yamano, N., Shirouchi, B., Inoue, N., Murakami, S., Sasaki, T., &

Yanagita, T. (2010). Effects of citrus auraptene (7-geranyloxycoumarin) on hepatic lipid metabolism *in vitro* and *in vivo*. *Journal of Agricultural and Food Chemistry*. **58(16),** 9028-9032.

43 Gao, X., Fu, T., Wang, C., Ning, C., Kong, Y., Liu, Z., Sun, H., Ma, X., Liu, K., & Meng, Q. (2017). Computational discovery and experimental verification of farnesoid X receptor agonist auraptene to protect against cholestatic liver injury. Biochemical pharmacology. **146**, 127-138.

44 Wang, J., Fu, T., Dong, R., Wang, C., Liu, K., Sun, H., Huo, X., Ma, X., Yanga, X., & Meng, Q. (2019). Hepatoprotection of auraptene from the peels of citrus fruits against 17α-ethinylestradiol-induced cholestasis in mice by activating farnesoid X receptor. Food & Function.**10**, 3839-3850.

45 Murakami, A., Nakamura, Y., Tanaka, T., Kawabata, K., Takahashi, D., Koshimizu, K., & Ohigashi, H. (2000). Suppression by citrus auraptene of phorbol ester-and endotoxin-induced inflammatory responses: role of attenuation of leukocyte activation. Carcinogenesis. **21(10),** 1843-1850.

46 He, W., Chen, W., Zhou, Y., Tian, Y., & Liao, F. (2013). Xanthotoxol exerts neuroprotective effects *via* suppression of the inflammatory response in a rat model of focal cerebral ischemia. Cellular and Molecular Neurobiology. **33(5),** 715-722.

47 Sun, M., Hu, J., Song, X., Wu, D., Kong, L., Sun, Y., Wang, D., Wang, Y., Chen, N., & Liu, G. (2013). Coumarin derivatives protect against ischemic brain injury in rats. *European Journal of Medicinal Chemistry*. **67**, 39-53.

48 Ji, H. J., Hu, J. F., Wang, Y. H., Chen, X. Y., Zhou, R., & Chen, N. H. (2010). Osthole improves chronic cerebral hypoperfusion induced cognitive deficits and neuronal damage in hippocampus. *European Journal of Pharmacology*. **636(1-3),** 96-101.

49 He, Y., Qu, S., Wang, J., He, X., Lin, W., Zhen, H., & Zhang, X. (2012). Neuroprotective effects of osthole pretreatment against traumatic brain injury in rats. Brain Research. **1433**, 127-136.

50 Mao, X., Yin, W., Liu, M., Ye, M., Liu, P., Liu, J., Lian, Q., Xu, S., & Pi, R. (2011). Osthole, a natural coumarin, improves neurobehavioral functions and reduces infarct volume and matrix metalloproteinase-9 activity after transient focal cerebral ischemia in rats. Brain Research. **1385**, 275-280.

51 Okuyama, S., Minami, S., Shimada, N., Makihata, N., Nakajima, M., & Furukawa, Y. (2013). Anti-inflammatory and neuroprotective effects of auraptene, a citrus coumarin, following cerebral global ischemia in mice. *European Journal of Pharmacology*. **699(1-3)**, 118-123.