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

Ameliorating Role of Lactoferrin Against Letrozole Induced Toxicity on Liver of Female Rats

Asmaa N. Hamad a, Heba M. El Ghaweet b, Nermeen I. Ashry c & Sameh M. Shabana d.

Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt

**** Correspondence to:*** *asmaanasser273@gmail.com, 01125778896*

Received:10/12/2024
Accepted: 28/12/2024

Abstract: Background: Drug-induced liver damage is a rare but significant form of liver disease caused by various medications. Discontinuing the offending medication is usually the treatment. The present study aimed to investigate the effectiveness of Lactoferrin as an antiinflammatory protein in letrozole induced liver toxicity. Methods: 28 female Wistar albino rats, weighing 200 ± 20 g, were randomly categorized into 4 groups including: Group A: control group (0.4 ml of saline /rat/day), Group B: letrozole group (1 mg.kg.d, for 30 days), Group C: Lactoferrin group (300 mg of LF/kg.d, for 30 days). Group D: Letrozole plus Lactoferrin group; They were given letrozole a long with lactoferrin in the same previous doses. After 24 hours from the last dose, rats were sacrificed by cervical dislocation, their abdominal cavities were opened, liver was dissected, and histopathological changes were examined. Results:letrozole modelling resulted in a significant rise in body weight. Also, it induced hepatic steatosis and lipid accumulation in the liver. Lactoferrin treatment decreased liver congestion and hepatocellular damage. Conclusion: Lactoferrin had therapeutic effects on liver of rats treated by letrozole due to its anti inflamatory activities.

keywords: Lactoferrin; Letrozole; Aromatase enzyme; Hepatotoxicity.

Introduction

Drug-induced liver damage is a rare, significant, and difficult kind of liver disease. Potential cause when examining a patient with liver illness.[1] The enzyme letrozole aromatase helps postmenopausal women convert adrenal androgens to estrogens.[2] Letrozole causes PCOS by preventing testosterone from being converted to estrogen, which leads to circulating hyperandrogenism and hormonal imbalance.[3] Aromatase present in the brain, muscle, liver, kidney, adrenal glands, and subcutaneous fat, where it is involved in the low-level production of estrogens. For postmenopausal women, these tissues are the main source of estrogen. Letrozole is also recommended as a first line therapy for postmenopausal women with estrogen receptor-positive breast cancer.[1]

Liver had traditionally been letrozole-affected target organ. 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).[4] Prior research discovered that three months following the introduction of letrozole. Hepatic transaminases were significantly raised, and positive results for smooth muscle antibodies and ANA were seen. Liver tests returned to normal within three weeks of quitting letrozole, however letrozole-induced elevation of liver enzymes was still present. Thus, letrozole-induced liver injury might be caused by a toxic or immunogenic metabolite.[5]

The whey protein found in the milk of most mammals, presumably contains lactoferrin (LF). Colostrum has up to seven times the amount of LF of mature milk. Lactoferrin is produced by human body cells and is present in a variety of human organs and body cells. kidneys, lungs, gallbladder, pancreas, gut, gut, liver, prostate, saliva, tears, sperm, cerebrospinal fluid, urine, bronchial secretions, vaginal discharge, synovial fluid, blood plasma, umbilical cord blood, and immune system cells have all been shown to contain it. It is found wherever the body requires prompt and efficient defence against outside dangers.[6] LF had a direct cytoprotective impact on hepatocytes as well as a significant protective effect in mice that was independent of its capacity to bind iron. Treatment improved hepatic microcirculation abnormality and markedly increased liver sinusoidal endothelial cell dysfunction. LF seems to rely on hepatic resident macrophages, or Kupffer cells (KCs), for its protective function. It had a significant protective effect on mice and reduced hepatic microcirculatory dysfunction through the activation of hepatic macrophages.[7] Furthermore, LF promoted apoptosis, decreased oxidative stress, and improved liver function in rats with liver fibrosis, indicating that it may be a suitable treatment option for liver fibrosis patients.[8]

Our study designed to characterize the efficacy of lactoferrin in alleviation of hepatic toxicity caused by letrozole.

Material 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.22.10.4).

2.1 Chemicals:

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

2- Lactoferrin: The source of lactoferrin was Hygint Pharma,), was purchased from Amr Samir Pharmacy, Dakahlia, Egypt.

2.2 Experimental animals: In this experimental study, 28 female Wistar albino (Rattus norvegicus) rats, weighting 200 ± 20g (9-10 weeks), were procured from Egyptian Vaccine Company VACSERA, Giza, Egypt and housed in the animal house of Zoology Department, Faculty of Science, Mansoura University. The rats were separated into 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 groups:

The rats were randomly divided into four groups (7 rats for each group) as the following:

Group A: Control group received (0.4 ml of 0.9% NaCl /rat/day) orally for 30 days,

Group B: Lactoferrin group rats were received (300 mg/kg/day) orally for 30 days[9],

Group C: Letrozole group, the rats were received (1 mg/kg/day) of letrozole orally for 30 days[10],

Group D: Combined group (Letrozole + Lactoferrin), rats were received letrozole (1 mg/kg/day) along with Lactoferrin (300 mg/kg/day) orally for 30 days.

After 24 hours from the last dose, rats were sacrificed by cervical dislocation, their abdominal cavities were opened, livers were dissected, histopathological changes were examined and the body weights of rats in all experimental groups were recorded.

2.4 Measurements of body weights:

The absolute (g.) and relative (g/100g fresh tissue) body weight was measured in the study groups weekly.

2.5 Histological techniques: Liver from the different animal groups was separated and fixed immediately in 10% phosphate buffered formalin (pH 7.4), dehydrated in ascending series of ethyl alcohol, then liver was 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.[11]

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 $p < 0.05$.

Results:

Body weight

As shown in Fig.1 The body weight of the animals treated with letrozole was greater than that of untreated animals. The groups' food consumption and body weight increased significantly in comparison to the control group. Rats treated with letrozole and lactoferrin exhibited improved body weight but still varied from the control.

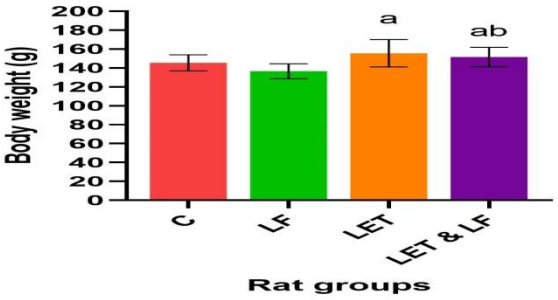

Figure (1): Histogram illustrates body weight (g) of the different studied groups.

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

a: significant as compared to control.

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

C: Control, LF: Lactoferrin, LET: Letrozole, LET & LF: Letrozole and Lactoferrin.

Hepatic histopathological investigation:

Control rats' livers met normal hepato-histological criteria; their hepatocytes (H) were organized in anastomosing cords, with normal portal veins (PV) and blood sinusoids (S) between. The hepatocytes were polyhedral cells that had some binucleated hepatocytes reported. They also had conspicuous nucleoli and central, spherical, vesicular nuclei with finely vacuolated cytoplasm. Kupffer cells (K) and flat endothelial cells surrounded the blood sinusoids (Figs. A&B). As the control group, livers treated with lactoferrin showed a normal histological appearance (Figs. C&D). Conversely, rats treated with letrozole, as shown in (Figs. E&F), showed histometric abnormalities in comparison to the control group; aberrant hepatocytes were seen in disordered hepatic cords with blood sinusoidal dilatation and blockage. Hepatocytes had deep acidophilic cytoplasm and black nuclei; some hepatocytes were seen to be pale, and some were binucleated. When the combined group's histological findings were compared to those of the control and letrozole groups, they showed improved hepatic tissue, normal hepatic cord organization with healthy hepatocytes and blood sinusoids between, and a normal hepatic central vein. There were found to be polyhedral hepatocytes with center rounded vesicular nuclei and acidophilic coarsely vacuolated cytoplasm. Normal sinusoids with mildly dilated endothelium and Kupffer cells lining them were seen. A thick-walled, normal hepatic central vein was seen (Figs. G&H).

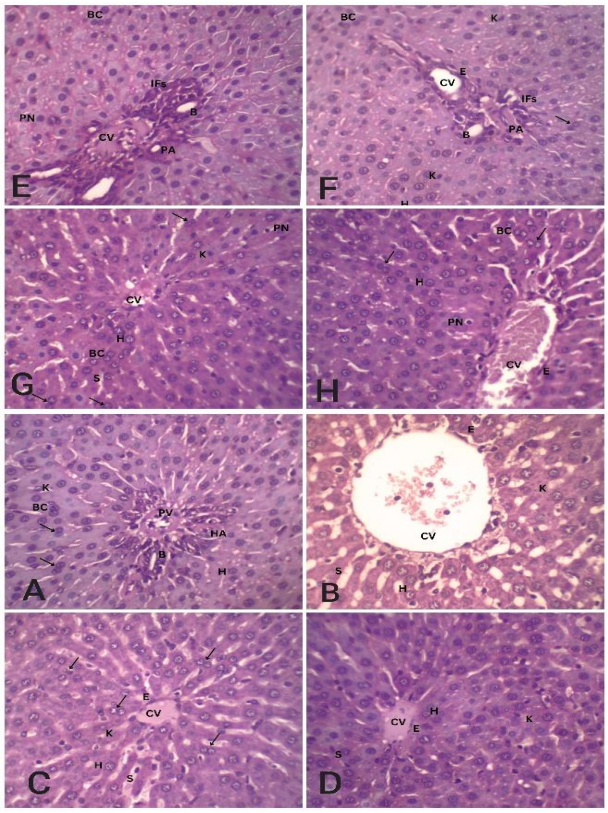

Figure (2) (A-H): photomicrographs of histological sections of liver of rats from control and different animal groups (H&E) (X. 100 µm). Figs. A&B: control group showing the presence of central vein with

endothelial cells, binucleated, with normal heterocytes, Kupffer cells and normal sinusoids. Figs. C&D: Lactoferrin supplemented group showing normal pattern like control group like central vein, binucleated and normal heterocytes. Figs. E&F: Letrozole group showing the presence of numerous pyknotic nuclei with interleukins fibrosis cell and bile duct, heterocytes. Figs. G&H: letrozole group supplemented with lactoferrin showing central vein with endothelial cells, with normal heterocytes, Kupffer cells, dilation sinusoids, binucleated, pyknotic nuclei, and irregular arrangement in raw of hepatocytes.

Abbreviations; Hepatocytes (H), Sinusoids (S), Portal Vein (PV), Bile Ducts(B), Kuffer cells (K), Endothelial cells (E), Binucleated (BC).

Immunohistochemistry of TGFP of liver

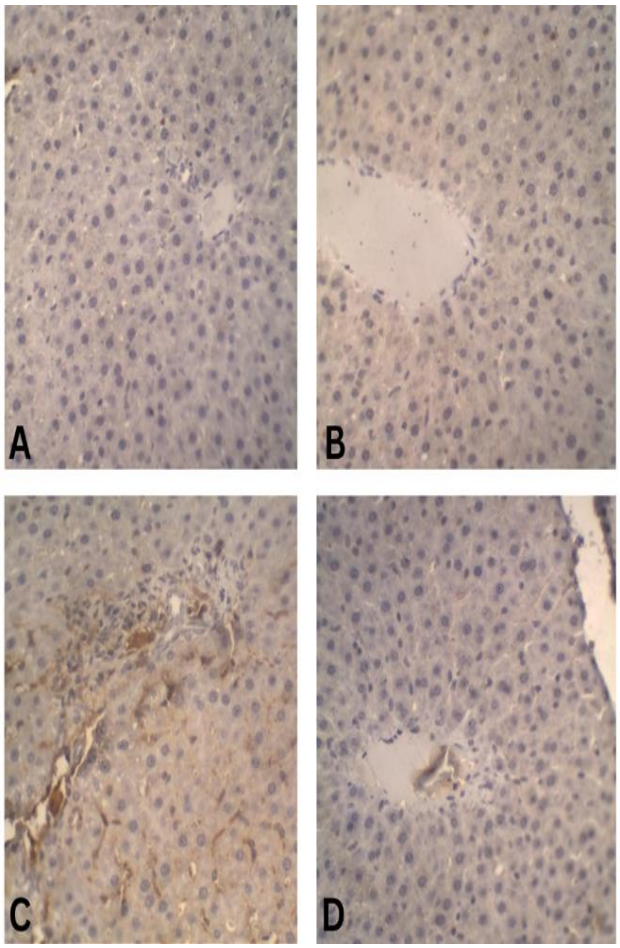

Figure (3): photomicrographs of paraffin embedded liver immunohistochemically stained with antibody of TGFP from control and different animal groups (X. 100 µm). Showing that, the immune- histochemical reaction appear brown and the counterstaining background is blue in color. Fig. A: Section of the liver from control rats showing weak immunoreaction brown nuclear staining, showing strong reaction of TGFB. Fig. B: Section of the liver from lactoferrin supplemented group showing weak positive nuclear staining, showing weak reaction of TGFB. Fig. C: Section of the liver from letrozole showing strong immunoreaction nuclear staining, showing strong reaction of TGFB. Fig. D: Letrozole group supplemented with lactoferrin group showing moderate brown nuclear immunostaining of staining.

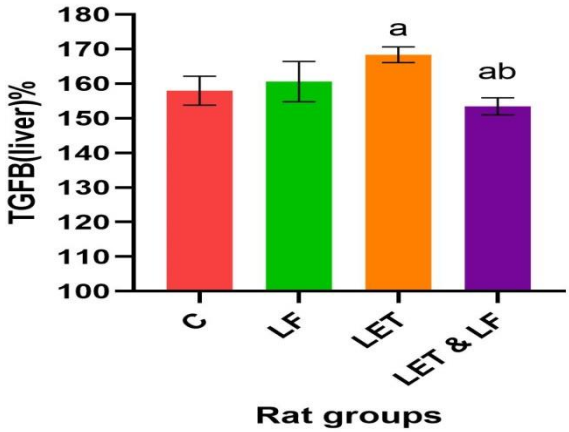

Figure (4): Histogram illustrating image analysis of TGFP in liver showing the percentages of immunohistochemical reaction of TGFB that is overexpressed in liver of rats treated with Letrozole and improved in the group treated with Letrozole supplemented with lactoferrin.

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

a: significant as compared to control.

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

C: Control, LF: Lactoferrin, LET: Letrozole, LET & LF: Letrozole and Lactoferrin.

Discussion

Letrozole is a complex medication used as an ovulation-induction treatment for women with various causes of infertility, endocrine therapy for menopausal breast cancer, and reversible aromatase inhibitor therapy[12],

has several phenotypic characteristics, such as hair loss, heat flashes, and sleeplessness[13], and endocrinopathies.

It prevents the synthesis of estrogen, which results in hyperandrogenism, hyper-

accumulation of testosterone, dysregulation of GnRH secretion, and subsequent hypersecretion of LH and downregulation of FSH. These endocrine dysfunctions promote polycystic ovaries and ovarian impairments with oligo-ovulation or anovulation.[14, 15]

Increased metabolic abnormalities were seen, including obesity, dyslipidemia, hyperinsulinemia, and insulin resistance. Letrozole was also reported to exacerbate tendinopathies, arthralgias, fractures, and osteoporosis.[16] The present results are consistent with the conclusions of (Kubatka, Sadlonova et al. 2008)[17], that letrozole resulted in weight gain. Letrozole administration resulted in a significant increase in body weight in the study (Maharjan, Nagar et al. 2010)[18], which was attributed to fat deposition. Letrozole medicine induces hepatic steatosis and lipid accumulation in the liver. These changes point to aberrant liver function even if their histological structures did not manifestly alter. It has been demonstrated that letrozole medication causes hepatic steatosis and lipid buildup in the liver.[19] The body weight of the animals treated with letrozole was greater than that of the untreated animals, so when comparing the groups of letrozole to the control group, there was a significant increase in food intake and body weight gain and these results were consistent with those of (Kubatka, Sadlonova et al. 2008).[17] TGF-β signaling, shedding light on the characteristics of the inflammatory milieu. One cytokine linked to inflammation is transforming growth factor-β (TGF-β), which has two functions in the development and suppression of tumors. Using TGF-β signaling to provide details about the inflammatory milieu's properties. The bulk of mediators and inflammatory cells are in the tumor microenvironment, where they play a critical role in regulating the growth, division, and metastasis of cancer cells.[20] TGF-β1 levels were higher in letrozole groups than in control groups. The kind of tissue and the cell's epigenetic background affect TGF-β function.[21] The dual role of TGF-β in tumorigenesis is a significant characteristic. In the early stages, it suppresses tumor growth, but in later stages, it encourages tumor cell spread.[21, 22] Shown that TGF-β stimulates the development of hepatocarcinogenesis.[23] Shown that increased TGF-β activity was linked to the hepatitis B virus's ongoing presence in liver tissues. Transforming growth factor (TGF)-β1 is dysregulated in women with PCOS.[24] A multitude of cells generate the multifunctional cytokine transforming growth factor (TGF)-β1, which may contribute to the pathogenesis of the condition.[25] Tissue and organ fibrosis can be successfully improved by blocking the TGF-β1 pathway. Research has indicated that TGF-β was the primary cause of organ fibrosis. In many tissues, TGF-β overexpression leads to significant fibrosis. The TGF-β signaling system plays a crucial role in the development and genesis of fibrosis. When compared to the control groups, the letrozole group's TGF-β1 levels were clearly higher.[3] In other study, group that was given letrozole. PCOS rats exhibited hyperglycemia along with changed lipid and hormonal profiles. There was clear evidence of interstitial fibrosis and strong TGF-β positivity.[26]

Conclusion: Lactoferrin had therapeutic effects on liver of rats treated by letrozole due to its antiinflamatory activities.

Reference:

1. Diabetes, N.I.o., Digestive, and K. Diseases, (2012) LiverTox: clinical and research information on drug-induced liver injury.: National Institute of Diabetes and Digestive and Kidney Diseases.
2. Dellapasqua, S., and M. Colleoni, (2010*)* Letrozole. Expert Opinion on Drug Metabolism & Toxicology,. **6**(2): p. 251-259.
3. Zhou, Y., H. Lan, Z. Dong, W. Li, B. Qian, Z. Zeng, W. He, J.-L.J.O.M. Song, and C. Longevity, (2022) Rhamnocitrin attenuates ovarian fibrosis in rats with letrozole-induced experimental polycystic ovary syndrome.. **2022**.
4. Gheena, S., D.J.H. Ezhilarasan, and e. toxicology, (2019) Syringic acid triggers reactive oxygen species–mediated cytotoxicity in HepG2 cells.. **38**(**6**): p. 694-702.

5. Gharia, B., K. Seegobin, S. Maharaj, N. Marji, A. Deutch, and L. Zuberi, (2017) Letrozole-induced hepatitis with autoimmune features: a rare adverse drug reaction with review of the relevant literature. Oxford Medical Case Reports,. **2017**(11).
6. Kowalczyk, P., K. Kaczyńska, P. Kleczkowska, I. Bukowska-Ośko, K. Kramkowski, and D. Sulejczak, (2022) The Lactoferrin Phenomenon—A Miracle Molecule.. **27**(9): p. 2941.
7. Yin, H., L. Cheng, M. Holt, N. Hail Jr, R. MacLaren, and C.J.H. (2010) Ju, Lactoferrin protects against acetaminophen-induced liver injury in mice.. **51**(3): p. 1007-1016.
8. Hessin, A., R. Hegazy, A. Hassan, N. Yassin, and S. Kenawy, (2015) Lactoferrin Enhanced Apoptosis and Protected Against Thioacetamide-Induced Liver Fibrosis in Rats. Open Access Maced J Med Sci,. **3**(**2**): p. 195-201.
9. Farid, A.S., M.A. El Shemy, E. Nafie, A.M. Hegazy, E.Y.J.D. Abdelhiee, and c. toxicology, (2021) Anti-inflammatory, anti-oxidant and hepatoprotective effects of lactoferrin in rats.. **44**(**3**): p. 286-293.
10. Naser, M., H. Sheida, N. Alireza, and H.J.J.o.T.C.M. Abdolkarim, (2022) Protective efficacy of dark chocolate in letrozole-induced ovary toxicity model rats: hormonal, biochemical, and histopathological investigation.. **42**(5): p. 741.
11. Weesner, F.M., (1964*)* General zoological microtechniques.: Williams & Wilkins Company.
12. Haynes, B., M. Dowsett, W. Miller, J. Dixon, A.J.T.J.o.s.b. (2003) Bhatnagar, and m. biology, The pharmacology of letrozole.. **87**(1): p. 35-45.
13. Bae, K., H.-S. Yoo, G. Lamoury, F. Boyle, D.S. Rosenthal, and B.J.I.C.T. Oh, (2015) Acupuncture for aromatase inhibitor–induced arthralgia: a systematic review.. **14**(**6**): p. 496-502.
14. Pachiappan, S., K. Ramalingam, A.J.R.J.o.P. (2023) Balasubramanian, and Technology, Evaluation of Gymnema sylvestre R. Br. against Letrozole induced polycystic ovarian syndrome in rats.. **16**(1): p. 385-390.
15. Younas, A., L. Hussain, A. Shabbir, M. Asif, M. Hussain, F.J.E.-b.C. Manzoor, and A. Medicine, (2022) Effects of fagonia indica on letrozole-induced polycystic ovarian syndrome (PCOS) in young adult female rats.. **2022**.
16. Gaillard, S., and V.J.B.C.R. Stearns, (2011) Aromatase inhibitor-associated bone and musculoskeletal effects: new evidence defining etiology and strategies for management.. **13**(2): p. 205.
17. Kubatka, P., V. Sadlonova, K. Kajo, G. Nosalova, and Z.J.N. Fetisovova, (2008*)* Preventive effects of letrozole in the model of premenopausal mammary carcinogenesis.. **55**(1): p. 42.
18. Maharjan, R., P.S. Nagar, L.J.J.o.A. Nampoothiri, and i. medicine, (2010*)* Effect of Aloe barbadensis Mill. formulation on Letrozole induced polycystic ovarian syndrome rat model.. **1**(4): p. 273.
19. Chaiyamoon, A., S. Bunsueb, and S.J.I.J.o.M. Iamsaard, (2020) Changes of Tyrosine Phosphorylation in Liver and Kidney of Polycystic Ovarian Rats Induced by Letrozole.. **38**(4).
20. Guo, L., Y. Zhang, L. Zhang, F. Huang, J. Li, and S.J.T.B. Wang, *MicroRNAs, TGF-β signaling, and the inflammatory microenvironment in cancer.* 2016. **37**: p. 115-125.
21. Roberts, A.B., and L.M.J.P.o.t.N.A.o.S. Wakefield, (2003) The two faces of transforming growth factor β in carcinogenesis.. **100**(15): p. 8621-8623.
22. Akhurst, R.J., and R.J.T.i.c.b. Derynck, (2001) TGF-β signaling in cancer–a double-edged sword.. **11**(11): p. S44-S51.
23. Wang, B., S.-H. Hsu, S. Majumder, H. Kutay, W. Huang, S.T. Jacob, and K.J.O. Ghoshal, (2010) TGFβ-mediated upregulation of hepatic miR-181b promotes hepatocarcinogenesis by targeting TIMP3.. **29**(**12**): p. 1787-1797.
24. Yang, P., Q.-J. Li, Y. Feng, Y. Zhang, G.J. Markowitz, S. Ning, Y. Deng, J. Zhao, S. Jiang, and Y.J.C.c. Yuan, (2012*)* TGF-β-miR-34a-CCL22 signaling-

induced Treg cell recruitment promotes venous metastases of HBV-positive hepatocellular carcinoma.. **22**(**3**): p. 291-303.
25. Rashad, N.M., A.I. Amin, A.E. Ali, and M.H.J.M.E.F.S.J. Soliman, (2018) Influence of obesity on soluble endoglin and transforming growth factor β1 in women with polycystic ovary syndrome.. **23**(4): p. 418-424.
26. Morsi, A.A., E.A. Mersal, A.R.H. Farrag, A.M. Abdelmoneim, A.M. Abdelmenem, and M.S.J.A.H.e.C. Salim, (2022) Histomorphological changes in a rat model of polycystic ovary syndrome and the contribution of Stevia leaf extract in modulating the ovarian fibrosis, VEGF, and TGF-β immunoexpressions: Comparison with metformin.. **55**(1): p. 9-23.