Ameliorative Effect of *Terminalia chebula* Retz. in Regulating the Hepato and Renal Physiological Impairments in Letrozole Induced PCOS Rat Model

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Abstract:

This study aims to investigate the phyto-therapeutic effect of Terminalia chebula Retz. in regulating the impact of letrozole-induced polycystic ovary syndrome (PCOS) on various aspects of reproductive and physiological health including the oxidative stress, of liver and kidney. In this study, the experimental animals were categorized into six groups: Group 1- Control; Group 2- Letrozole-induced PCOS group and untreated; Group 3- PCOS-induced and treated with Metformin; Group 4-6 PCOS-induced and treated with the Terminalia chebula Retz. fruit extract at different concentration (100, 200,400 mg/kg. bw (p.o)). Additionally, the estrus cycle regulation, weight of the experimental animals and their liver and kidney weight along with its histopathology, functional analysis and antioxidant levels were assessed in experimental rats. Letrozole-induced PCOS resulted in irregular estrus cyclicity and a protracted diestrus stage. Except LPx, tissue antioxidant levels of SOD, GSH, GPx, and Catalase were shown to be decreased in both the liver and kidney samples. The functional analysis of liver and kidney showed a impairments in the levels of SGOT, SGPT, ALP, bilirubin, blood sugar, urea, uric acid and creatinine as an indicator of liver and kidney damage. These tissue damages were been evidenced by the histological assessment and compared to the control group. These entire alterations in the organ weights, antioxidant and histomorphology that were observed during the successful induction of PCOS got restored to normal after being treated with the fruit extract of T. chebula in the experimental animals and implied its potentiality in managing the complications of PCOS.

Keywords: *Polycystic ovarian syndrome; Letrozole; Terminalia chebula Retz.; Oxidative stress; LFT; KFT.*

1. Introduction

Polycystic ovarian syndrome (PCOS) is a complex endocrine condition that is estimated to affect about 116 million women (3.4%) globally [1]. It is defined by a number of clinical characteristics, including hyperandrogenism, menstrual abnormalities, and presence of multiple cysts in the ovaries [2]. Women with PCOS have an unfavourable reproductive profile, which includes a higher risk of preeclampsia, gestational diabetes mellitus, and pregnancy-induced hypertension [3]. Alterations in the biochemical parameters are being assessed through various non-invasive functional analysis like kidney function test (KFT), liver function test (LFT), lipid profiles [4]. Insulin resistance is believed to cause alterations with glucose metabolism, which can therefore have an impact on blood glucose levels as well as the biochemical constituents of blood serum [5]. For instance, several researches have revealed that women with PCOS typically have higher levels of fasting glucose and insulin as a sign of insulin resistance such HOMA-IR (homeostatic model assessment of insulin resistance) [6]. On concerning these crucial roles of biochemical parameters in the pathophysiology of PCOS, it is being relied on the diagnosis, evaluation, and treatment of PCOS [7]. Since, the majority of current PCOS therapies largely entail hormone medication and surgical management, which can result in serious illnesses and side effects, it is critical to obtain a better understanding of the relationship between PCOS and biochemical parameters. This knowledge can lead to better diagnostic standards, personalised treatment strategies, and focused interventions for women who are dealing with this difficult issue. Exploring the possibility of phytotherapy as an alternative treatment for PCOS and its complications is a viable option for addressing these difficulties. *Terminalia chebula* Retz. a deciduous tree in the Combretaceae family, has been shown to have antioxidant, anti-cancer, anti-diabetic, and anti-hyperlipidemic properties [8]. The fruit of *T. chebula* has higher proportions of tanins such as chebulanic acid, ellagic acid, chebulagic acid, gallic acid corilagin, and chebulinic acid [9]. In this investigation, the ability of *T. chebula* to control the physiology and biochemical abnormalities of the liver and kidney in the treatment of PCOS was assessed.

2. Materials and methods

2.1. Chemicals and reagent

Letrozole (Cat. No. L6545-50MG) was obtained from Sigma-Aldrich Chemicals Private Ltd. Bangalore, India, and the Randox kit, Sugar (Cat. No. GL 364), Urea (Cat. No. UR 2795), Uric acid (Cat. No. UA 2796), Creatinine (Cat. No. CR 2789). ALP (Cat. No. AP 2779), SGPT (Cat. No. AS 1202), SGOT (Cat. No. AS 1202), Bilirubin total (Cat. No. BR 2784).

2.2 Plant sample collection and extract preparation

The fresh fruits *of T. chebula* were collected from Top Sengattupatti, Thuraiyur (Tk), Tiruchirappalli (Dt), Tamilnadu - 621011. The plant specimen was authentified and certified (Certified No. BSI/SRC/5/23/2022/Tech/678) by the Botanical Survey of India (Southern Regional Centre), Coimbatore, Tamil Nadu, India. The collected fruits were cleaned shade-dried to ground as a fine powder with an electronic mixer. Soxhlet extraction was done with 50 g of powdered sample and 500 mL ethanol to get the ethanolic fruit extract of *T.chebula*.

Then the extract was filtered and kept in a rotating evaporator at 75 °C under reduced pressure for vapourization.

2.3. Animals

Matured female albino Wistar rats (*Rattus norvegicus*) weighing 150 – 180 g, used in this study were purchased from College of Veterinary and Animal Sciences, Mannuthy, Kerala. At a regulated environment (25 °C), the animals were maintained in cages made of polypropylene providing a standard laboratory feed (Sai Durga Feeds and Foods, Chennai) and limited water with a 12h light: 12h dark cycle. By following the regulations of CPCSEA's protocol, ethical approval from the institution's animal ethics committee (Protocol number: IAEC/BDU/P 25/2018/Dt.07.08.2018) was brought for the use of animals in this study.

2.4. Assessment of estrous cyclicity & vaginal cytology

Estrus cycle in the rat consists of four different phases namely, Proestrus, Estrus, Metestrus, and Diestrus. Vaginal smears taken between 6:30 and 8:00 AM in the experimental period were microscopically observed for the presence of distinct cell types on respect to the stages of estrus cycle [10]. Rats with regular cycles (lasting 4-5 days) were further included in the study.

2.5. PCOS Induction and treatment

For this experiment, 30 female Albino Wistar rats were used, and they were equally categorized as six experimental groups (5 animals/group). Animals without any treatment and given optimum condition as the Group I - control (Con), and the animals treated with the Letrozole (L) dissolved in 0.5% CMC for 21 days at a dosage of 1 mg/kg body weight as the Group II - Negative control (Letrozole-induced PCOS group), animals in Group III were given 20 mg/kg.bw (p.o)/day of metformin (L+MET). Animals in Group IV, V, and VI received 100, 200 and 400 mg/kg.bw (p.o) of an ethanolic fruit extract of *T. chebula* for 28 days after PCOS Induction and represented as L + TC, L+ TC 2, L+ TC 3. The successful induction of PCOS in the experimental animals was confirmed by assessing the estrus cycle irregularity.

2.6. Physiological assessment

The animals in each group were weighed weekly throughout the course of the experiment for monitoring their body weight. In addition, after the experiment was completed successfully, the livers and kidneys of the experimental animals were procured, weighed, and preserved for future studies.

2.7. Blood collection and tissue processing

After experimental period, the animals were sacrificed through Co_2 inhalation and the blood samples were collected via cardiac puncture using a syringe fitted with #22 needle. The collected blood samples were preserved in EDTA tubes and clot activator tubes for the haematological analysis and active serum separation. Then the serum were separated and stored at −20 °C. In addition, liver and kidney samples were procured from the animals of each experimental groups, cleaned free of fat and the weight were recorded. The organs were flash-frozen using liquid nitrogen and stored at −80 °C until further analysis.

2.8. Liver function test

ALP (Cat. No. AP 2779), SGPT (Cat. No. AS 1202), SGOT (Cat. No. AS 1202), Bilirubin total (Cat. No. BR 2784).

2.9. Blood sugar level and Kidney function test

Sugar (Cat. No. GL 364), Urea (Cat. No. UR 2795), Uric acid (Cat. No. UA2796), Creatinine (Cat. No. CR 2789).

2.10. Antioxidant evaluation

The antioxidant level of liver and kidney were analyzed using the standard protocols as follows: Superoxide dismutase (SOD) [11] , reduced glutathione (GSH) [12] , Glutathione peroxidase (GPX) [13] , Catalase (CAT) [14] , Lipid peroxidation (MDA) [15] .

2.11. Histology

After the experimental period, liver and kidney were harvested and weighed, then the organs were fixed in formalin (10%) and dehydrated using xylene and ethyl-alcohol at various concentrations. Using a rotary microtome (Leica, USA), tissue sections of a thickness of 5 nm were taken from ovarian and uterine samples, mounted on a glass slide, and dried. Deparaffinized, rehydrated, and also stained using H&E (Himedia, USA), then by using a light microscope (Magnus, India), the histological sections were examined to determine their pathology.

3. Results

3.1. Vaginal cytology

The Letrozole-induced PCOS group demonstrated altered cyclic phases and a decreased number of cycles (21%) in the cellular pattern during the course of the experiment in the extensive investigation of vaginal cytology (Figure 1). Following letrozole therapy, the animals receiving metformin experienced regular estrus cyclicity (44%) with its four distinct stages. Surprisingly, estrus cyclicity in PCOS rats that received 200 (58%), and 400 (54%) mg/kg/bw of *T. chebula* fruit extract had largely restored to normal. meanwhile, only 25% of estrus cyclicity was restored in the other treatment group after receiving 100 mg/kg/bw (p.o).

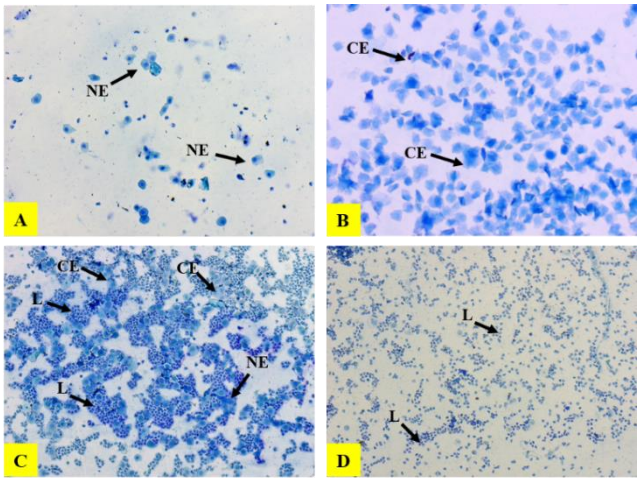

Figure 1. Vaginal cytology

A-Proestrus, **B**-Estrus, **C**-Metestrus, **D**-Diestrus.

3.2. Physiological assessment

The animals in the PCOS group had a remarkable weight gain that eventually led to increased BMI and obesity than in the other experimental groups which were being treated with metformin and *T. chebula* fruit extract; On this treatment, the body weight of the experimental animals were brought normalized with a restoration of normal physiology. The letrozole induced obesity in the PCOS animals caused a notable increase in the weight of the metabolically active organs like liver and kidney (Figure 2-a & 2-b). These increased weights are then restored to normal when they are treated with *T.chebula* fruit extract at 200 and 400mg/kg bw (p.o) concentrations of PCOS.

The increase in the weight of liver could probably be due to increased adiposity around the hepatocytes and the campaign of lymphatic cells in the damaged liver. The fat accumulation in the liver of PCOS animals can ultimately accelerate the fatty liver formation that in term lead to impairment of the function of liver.

Being the primary excretory organ of the body kidney tends to filter all the toxicants that could probably give rise to metabolic toxification. The continuous exposure to the aromatase inhibitor (letrozole) in the PCOS rat during induction resulted in the inflammation and increased weight of the kidney.

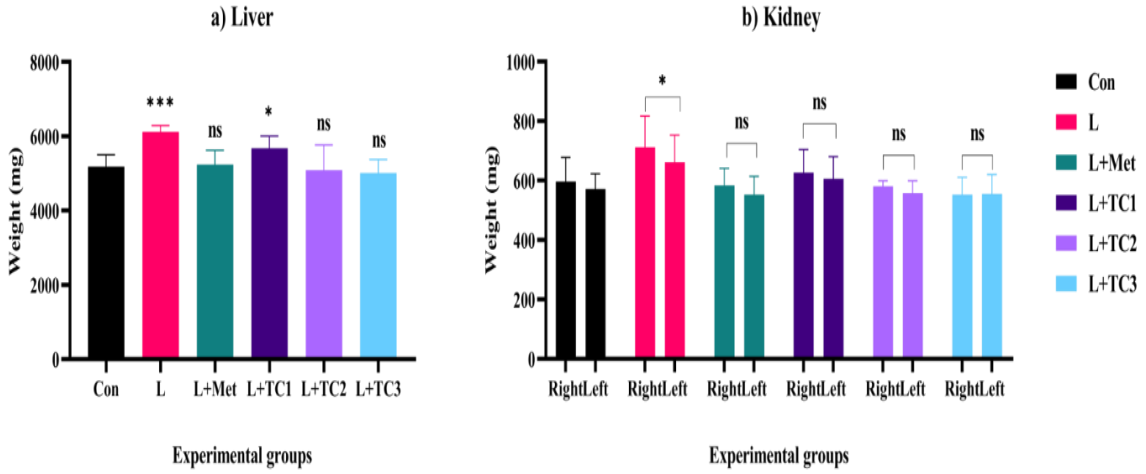

Figure 2. Physiological assessment

The bar graphs representing the physiological parameters; **a)** Liver weight; **b)** Kidney weight of the experimental groups such as Control (Con), Letrozole induced PCOS group (L), metformin treated group (L+Met), and groups treated with *T. chebula* fruit extract at different concentrations of 100, 200 and 400 mg/kg.bw (p.o) (L+TC1, L+TC2 and L+TC3). The data were analysed and presented as mean ± SEM, n = 5, *p < 0.05; **p < 0.01; ***p < 0.001; ns – nonsignificant.

3.3. Liver Function Test

3.3.1. ALP Level

The serum concentration of alkaline phosphatase (ALP) in the PCOS affected animals was much higher than the control group animals. On the context of the groups which were treated with metformin and *T.chebula* had slightly reduced levels of ALP which is almost comparable to the control group (Figure 3-a).

3.3.2. SGPT Level

In this test, a significant elevation in the levels of SGPT was found in the PCOS group as compared to the control. Moreover, treatment with metformin and *T. chebula* at a concentration of 200 and 400 mg/kg resulted in lowered SGPT level as in the control (Figure 3-b). illustrates the impact of *T. chebula* treatment at different doses on the SGPT level of PCOS rats.

3.3.3. SGOT Level

The level of Serum glutamic oxaloacetic transaminase (SGOT), also known as aspartate aminotransferase (AST), was much higher in the PCOS group than in the experimental groups. However, treatment with metformin and *T. chebula* at 200 and 400 mg/kg (p.o) concentrations substantially decreased SGOT levels in comparison to the control groups. Figure 3-c depicts the effect of *T. chebula* therapy at various doses on the SGOT level of PCOS rats.

3.3.4 Bilirubin

The serum bilirubin level in the PCOS affected animals were lowered. On the other hand, there were no measurable alterations in the experimental animals that are being treated with the *T. chebula* fruit extract (Figure 3-d).

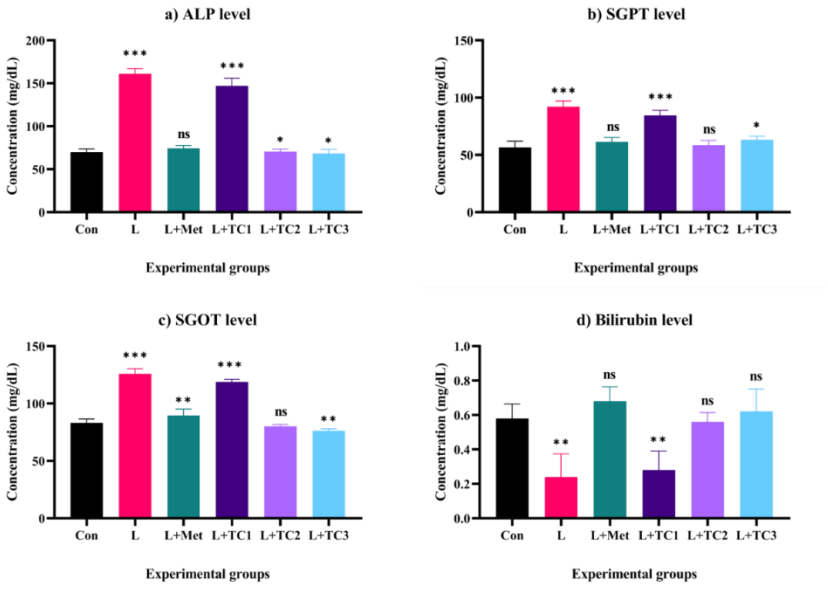

Figure 3. Liver Function Test

a) ALP, **b**) SGPT, **c**) SGOT, **d**) Bilirubin level of the experimental groups such as Control (Con), Letrozole induced PCOS group (L), metformin treated group (L+Met), and groups treated with *T. chebula* fruit extract at different concentrations of 100, 200 and 400 mg/kg.bw (p.o) (L+TC1, L+TC2 and L+TC3). The data were analysed and presented as mean ± SEM, n = 5, *p < 0.05; **p < 0.01; ***p < 0.001; ns – nonsignificant.

3.4. Blood sugar level and Kidney Function Test

3.4.1. Blood sugar level

The letrozole induced PCOS animals had higher levels of serum glucose concentration that could be a sign of aromatase induced diabetic condition. On the other hand, other experimental groups which include treatment with metformin and *T. chebula* fruit extract showed reduced blood sugar level that was appropriately comparable with the control group (Figure 4-a).

3.4.2. Creatinine level

The induction of PCOS in animal models caused a significant increase in creatinine levels. In contrast, the animals administered with 200 and 400 mg/kg bw (p.o) of *T. chebula* had a lower creatinine levels (Figure 4-b). Statistical results of serum creatinine levels in all treatment groups showed no significant differences ($p > 0.05$).

3.4.3. Urea level

The results of the measurement of serum urea levels of the experimental groups are presented in Figure 4-c. Based on this results, it can be stated that the letrozole induced PCOS rat and the experimental animals administered with 100 mg/kg.bw of *T. chebula* had increased serum urea levels and were significantly different ($p < 0.05$) compared to the other experimental groups.

3.4.4. Uric acid level

The results of uric acid measurements in PCOS animal models show that acid levels in the PCOS induction group and the group treated with *T. chebula* at a dose of 100 mg/kg.bw (p.o) are significantly higher levels of uric acid ($p < 0.05$) from the control group (Figure 4-d).

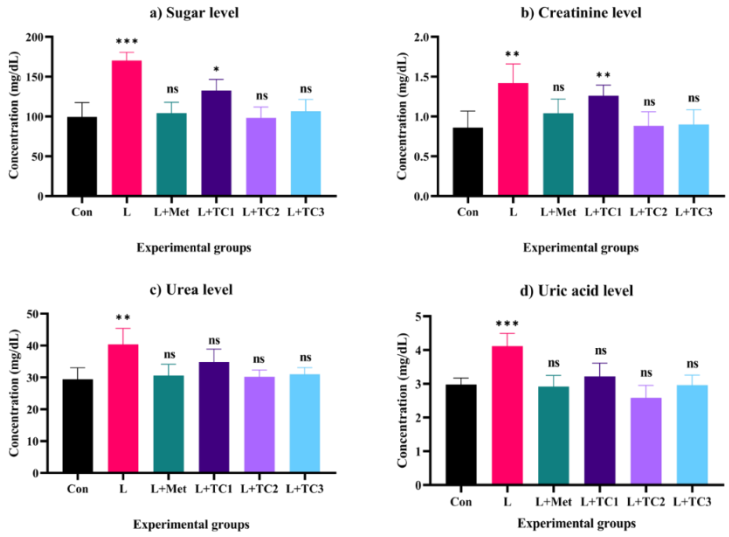

Figure 4. Blood sugar level and Kidney Function Test

a) Sugar, **b**) Creatinine, **c**) Urea, **d**) Uric acid, level of the experimental groups such as Control (Con), Letrozole induced PCOS group (L), metformin treated group (L+Met), and groups treated with *T. chebula* fruit extract at different concentrations of 100, 200 and 400 mg/kg.bw (p.o) (L+TC1, L+TC2 and L+TC3). The data were analysed and presented as mean ± SEM, n = 5, *p < 0.05; **p < 0.01; ***p < 0.001; ns – nonsignificant.

3.5. Antioxidant (Liver & Kidney)

3.5.1. Superoxide dismutase

When compared to the control group, the SOD activity was considerably lower in the untreated PCOS animals. However, it was shown to be marginally higher in the liver and kidney tissues in the control and groups treated with 200 and 400 mg/kg.bw (p.o) (Table 1&2).

3.5.2. Reduced glutathione

The liver and kidney tissues of the experimental animals belonging to the PCOS group showed very slight decrease in the GSH level compared to other experimental groups, which is generally indicative of less substantial alterations in the neutralization of GSH free radical (Table 1&2).

3.5.3. Glutathionine peroxidase

The levels of GSH and GPx were more closely correlated in the experimental groups. The levels of GPx have been found to be lower in the PCOS groups than the control and other experimental groups that had been administered with the *T. chebula* fruit extract as the GSH levels measured in the PCOS group slightly declined which had an impact on the levels of GPx (Table 1&2).

3.5.4. Catalase

The rats in the Letrozole-induced PCOS group showed considerably less catalase activity in their liver and kidney tissues compared to the control group and the treatment groups. This raises the possibility that the antioxidant defence mechanism in the Letrozole-induced PCOS animals may be impaired (Table 1&2).

3.5.5. Lipid peroxidation

The levels of LPO in the liver and kidney tissues were significantly higher in the PCOS group in contrast to the control group which displayed an increased MDA activity, this confirms the concept that PCOS is associated with hyperlipidaemia (Tables 1&2). When the experimental animals were given medium and high dosages (200 & 400 mg/kg.bw (p.o)) of the fruit extract, the increase in lipid peroxidation free radical production was brought to normal depending on the concentration of the administered dosage.

Experimental Groups	SOD (U/mg protein)	GSH (U/mg protein)	GPx (U/mg protein)	CAT (U/mg protein)	LPx (µ mole/mg protein)
Con	6.7 ± 1.32	4.1 ± 0.36	111 ± 9.53	72 ± 9.16	3.2 ± 1.21
L	2.1 ± 0.9**	1.7 ± 0.52 **	63 ± 5**	44 ± 8.71*	12.3 ± 3.35*
L+Met	6.2 ± 1.75 ns	3.4 ± 0.36 ns	99 ± 10.81 ns	65 ± 6.24 ns	4.3 ± 1.55 ns
L+TC1	3.5 ± 0.45**	1.9 ± 0.30**	79 ± 8.54**	47 ± 7.54*	10.9 ± 2.59**
L+TC2	8 ± 1.2 ns	4.5 ± 1.21 ns	119 ±10.53 ns	79 ± 8.54 ns	3 ± 0.7 ns
L+TC3	7.1 ± 0.88 ns	3.9 ± 0.45 ns	106 ±14.5 ns	75 ± 6.24 ns	3.5 ± 1.32 ns

Table :1 Liver Antioxidant Assay

Table :1 Assessment of effect of *T. chebula* fruit extract on ROS enzyme levels in the liver tissues of experimental groups. SOD – Superoxide dismutase; GSH – Reduced glutathione; GPX – Glutathione peroxidase, Catalase, and LPX – Lipid peroxidase. Values are represented as mean ± SEM, number of animals per group = 3. Where, *P < 0.05 compared to control; ns-non-significant compared to control; LET-Letrozole-induced PCOS group and untreated; LET + Met - PCOS-induced and treated with Metformin; LET + TC1-PCOS-induced and treated with the *T. chebula* fruit extracts (100 mg/kg.bw (p.o)), LET + TC2 -(200 mg/kg.bw (p.o)), LET + TC3 (400 mg/kg.bw (p.o)).

Table :2 Kidney antioxidant assay

Table :2 Assessment of effect of *T. chebula* fruit extract on ROS enzyme levels in the kidney tissues of experimental groups. SOD – Superoxide dismutase; GSH – Reduced glutathione; GPX – Glutathione peroxidase, Catalase, and LPX – Lipid peroxidase. Values are represented as mean ± SEM, number of animals per group = 3. Where, *P < 0.05 compared to control; ns-non-significant compared to control; LET-Letrozole-induced PCOS group and untreated; LET + Met - PCOS-induced and treated with Metformin; LET + TC1-PCOS-induced and treated with the *T. chebula* fruit extracts (100 mg/kg.bw (p.o)), LET + TC2 -(200 mg/kg.bw (p.o)), LET + TC3 (400 mg/kg.bw (p.o)).

Experimental Groups	SOD (U/mg protein)	GSH (U/mg protein)	GPx (U/mg protein)	CAT (U/mg protein)	LPx(µ mole/mg protein)
Con	11.4 ± 2.50	5.6 ± 0.52	170 ± 9	32.4 ± 2.42	8 ± 0.8
L	4.7 ± 1.57 *	1.5 ± 0.50 ns	120 ± 9.53**	19.8 ± 3.93 ns	18.1 ± 0.65***
L+Met	9 ± 1.73 ns	4.8 ± 0.91 ns	167 ± 8.18 ns	30 ± 2 ns	9.1 ± 0.55 ns
L+TC1	6.4 ± 2.14 ns	2.1 ± 0.95 ns	129 ± 10.53*	23.1 ± 6.39 ns	16.5 ± 1.43**
L+TC2	12.2 ± 1.31 ns	6.7 ± 0.36 ns	182 ± 8.88 ns	36 ± 3.60 ns	7.3 ± 0.79 ns
L+TC3	10.4 ± 1.63 ns	6 ± 0.52ns	173 ± 5.29 ns	34 ± 2.64 ns	7.8 ± 0.52 ns

3.6. Histological assessment

3.6.1. Histopathology of Liver

Histological findings of the liver among experimental groups were displayed in Figure 5, the liver sections from the control group had a typical hepatic morphology. An enhanced inflammatory response, necrosis of hepatocytes as necrotic patches, localised fat cell accumulations and vacuole degradation are predominantly observed in the letrozole induced PCOS group in comparison to the control. Sections from the L+Met group displayed modest vacuolar degeneration and some necrotic hepatocytes. In some areas of this group, sinusoidal dilatations and congestion were also seen. But in the experimental groups treated with 200 and 400 mg/kg.bw (p.o) of *T. chebula* fruit extract certain improvements in degenerative alterations that almost showed the normal anatomical structure of the liver which evidenced the effect of *T. chebula* in the restoration of healthy hepatocytes.

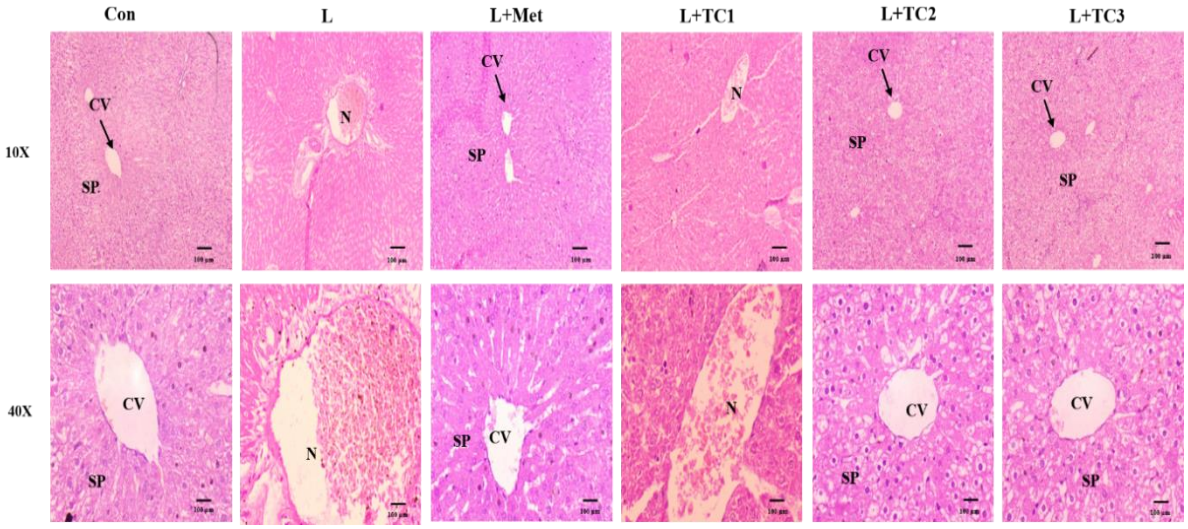

Figure 5. Histopathology of Liver

CV- Central Vein, **SP**- Sinusoidal Space, **N**- Necrosis.

3.6.2. Histopathology of kidney

After receiving letrozole for 21 days, female rats showed serious kidney injury, including the acute tubular necrosis, atrophy of renal glomerular capillaries (Destroyed Glomerulus) with enlarged Bowman's space, and loss of the proximal tubule brush border. The renal tissue of the control and the animals treated with metformin exhibited normal structure in the cortex and medulla regions. On the other hand, the treatment with 200 and 400 mg/kg.bw (p.o) dosages of *T. chebula* fruit extract restored the glomerulus, basement membrane, and capillaries to their normal by improving bowman's space. The anatomy of the kidney in the control and metformin treated groups was quite normal (Figure 6).

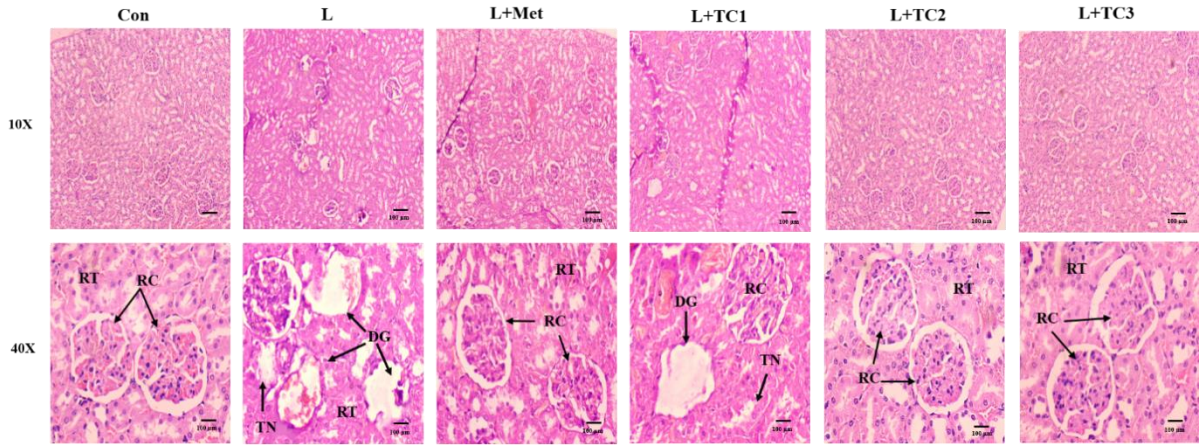

Figure 6. Histopathology of Kidney

RC- Renal Corpuscles, **RT**- Renal Tubules, **DG-** Destroyed Glomerulus, **TN**-Tubular Necrosis

3. Discussion

The increased weight of liver and kidney in the letrozole induced PCOS rats may be due to the excessive fat accumulation as a result of fat disposal and the inflammatory response to the metabolic toxicants like free radical production [16]. The liver contains the enzymes Alanine transaminase (SGPT), Aspartate transaminase (SGOT), Alkaline phosphatase (ALP), and bilirubin. These enzymes assist in the body's breakdown of amino acids, the conversion of proteins into energy for the liver cells, and the production of bilirubin [17]. The rate of release of these enzymes into the bloodstream will increase when the liver is damaged [18]. Despite the fact that letrozole-inducion in PCOS rats caused an increase in the levels of these enzymes it got returned to normal when rats were given higher dosages of *T.chebula* (200 and 400 mg/kg. bw (p.o)) and metformin. The serum glucose level is identified as the biomarker of insulin resistance in the PCOS animals that may lead to type II diabetic condition which cause elevated sugar levels [19]. Urea, Uric acid and creatinine are the predominantly removed by the kidneys after it is formed from the breakdown of proteins and nitrogen-containing substances. As a result, an increase in those levels in blood serum can indicate impairment in the kidney function, implying a probable failure of its ability to properly filter and eliminate waste products [20]. As the evidence for kidney's impairment, the letrozole induction in PCOS rats had higher levels of urea, uric acid and creatinine in the serum compared to the other experimental groups [21]. PCOS is regulated by oxidative stress, which occurs when antioxidants such as SOD, CAT, GSH, and GSH-Px are deficient, resulting in an excess of reactive oxygen species [22]. This can have an adverse effect on ovarian activities such as ovulation, folliculogenesis, and oocyte maturation [23]. CAT and GPx levels rise to neutralise H2O2 and preserve ovarian function. PCOS patients had lower GSH levels as well as higher reactive oxygen species [24]. Similarly, oxidative stress occurs in the liver and kidneys of PCOS rats, resulting in lower levels of endogenous antioxidants such as SOD, CAT, GSH, and GPx [25]. *T.chebula* treatment, on the other hand, recovers these antioxidant levels, indicating that it may have an antioxidative impact in PCOS. Superoxide (O2 -), a major oxygen radical, is converted by SOD into the dangerous chemical H2O2, which GSH-Px then converts into water [26]. GSH is a vital antioxidant that is

produced in the cytosol through two ATP-dependent mechanisms. Glutamyl cysteine synthetase first transforms glutamate and cysteine into glutamyl cysteine, which is then transformed into GSH and located in the endoplasmic reticulum, nucleus, and mitochondria [27]. High ROS and peroxide levels may have also contributed to GSH depletion [28]. In comparison to the control group, the GSH levels in the letrozole-induced PCOS condition were considerably reduced [29]. Based on these results, it seemed probable that the PCOS group's increased ROS generation which constituted the GSH depletion. GSH acts as the substrate for GSH-Px conversion hence the level of GSH-Px depends on the amount of GSH in the environment and so a decrease in GSH production will have a negative impact on GSH-Px activity [30]. Histological examination revealed that the liver tissue of the control group had a normal morphology. The rise in inflammatory cells was notable in the PCOS group with localised necrotic hepatocytes and moderate vacuolar degeneration, fat deposition and necrotic regions were also found in rats [31]; [32]; [33]. When an aromatase inhibitor letrozole is used for the induction of PCOS, there will be an increased the blood pressure and proximal sodium reabsorption. Thus, in PCOS models, hyperandrogenism and increased blood pressure, can be the factor which probably will mediate renal injury. This present study has also evidenced the renal injury in the form of acute tubular inflammations with necrosis and the occurrence of lymphatic cells. These conditions made an increase in the levels of urea, uric and creatinine in the serum of a PCOS rat models. However, those animals who received metformin and high dose of *T.chebula* treatment simultaneously showed a remarkable restoration of normal histomorphology of liver and kidney among experimental animals.

4. Conclusion

In conclusion, this study has evaluated physical and metabolic alterations like weight gain, increased organ weight of liver and kidney, along with the levels of oxidative stress related enzyme in letrozole-induced PCOS in rats. These changes could be result of inflammation, oxidative stress, and excessive fat storage in the liver and kidney which causes deterioration in their function. However, treatment with *T. chebula* showed encouraging outcomes in terms of enhancing liver and kidney function, regaining enzyme levels and activities, and lowering oxidative stress. With its ability to reduce weight gain, enzyme changes, and organ damage, *T. chebula* treatment has promise as a therapeutic approach for letrozole-induced PCOS. The dosage and duration of the medication need to be optimised, and more study is required to understand the underlying mechanisms. Overall, our research contributes to our understanding of PCOS and provides new therapeutic ideas by highlighting the negative effects of letrozole-induced PCOS and the potential of *T. chebula* therapy as a promising treatment option.

5. Declarations

Acknowledgment

Vignesh Kalimuthu acknowledges University Research Fellowship, TANSCHE, DST-TARE, and RUSA 2.0, Biological Sciences for the financial assistance and DST-FIST for the infrastructure provided at Department of Animal Science, Bharathidasan University.

Ethical statement

The use of animals in the study received proper approval from the institution's animal ethics committee, and the care of the animals was provided following the CPCSEA's protocol (Protocol No: IAEC/BDU/P 25/2018/Dt.07.08.2018).

Conflict of Interest

The authors declare that there is no conflict of interest.

Author contributions

The study's design and conceptualization were done by Vignesh Kalimuthu and Kadalmani Balamuthu. Swathi Chandran Manimegalai contributed to the gathering and evaluation of the data. Everyone who took part in the article's development offered suggestions and constructive feedback. The final manuscript benefited from the discussion and approval of all the contributors.

6. References

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