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Original Research Article

Therapeutic Modulation of Multisystemic Complications in Letrozole-Induced PCOS by Camellia sinensis L. Leaves

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Abstract: Polycystic ovarian syndrome is metabolic and reproductive disorder which is growing especially in Asian countries. Compounds from Camellia sinensis leaves can be used against PCOS with improved hepatic, renal and pancreatic profile. In current study, Letrozole(1.0 mg/kgb.w./day) induced adult healthy female Sprague Dawley rats (150-200gm) had been treated with metformin (50 mg/kg b.w.) and polarity based C. sinensis leaves extracts (50 mg/kg b. w.) for followed by hormonal and metabolite profiling by standard kit method and histological analysis of ovary, uterus, hepatic, renal and pancreatic tissues at 40X. Statistical analysis of results ($p<0.0001$) showed that LH, testosterone, HOMA-IR, ALT,AST,ALP and bilirubin in PCOS model decreased in rats treated with dist. water extract (1.2± 0.4 mlU/ml, 1.2±0.4 ng/dl, 0.054 ±0.03,50.0±4.35 IU/L,208.3 ±17.4 IU/L,192.6±17.4 IU/L,0.1 ±1.6 mg/dl) as compared to metformin (0.9± 0.4 mlU/ml, 2.09±0.078 ng/dl, 0.24 ±0.03, 33.3.6 ±14.0 IU/L,181.3 ±32.0 IU/L,115.3 ± 30.8 IU/L,0.8±0.1 mg/dl) respectively. Increase in FSH and progesterone level in animals treated with n-hexane extract was statistically significant (6.03±0.32 mlU/ml and 9.72±2.3 pg/dl) when compared to positive control group (2.13±0.99 mlU/ml and 9.05±1.27 pg/dl) respectively while this extract reduced estradiol level significantly in rats treated with n-hexane extract and metformin (48± 5.2pg/ml). Concentration of urea creatinine and uric acid has been decreased significantly by ethyl acetate extract (36.6 ±10.1mg/dl,0.56±0.05mg/dl,3.9±0.04mg/dl) as compared to metformin(37.33 ±4.04 mg/dl,0.63 ±0 mg/dl,3.4±1.04) respectively. Histopathological observation endorsed that PCOS induced rats showed typical peal string cystic follicle like appearance and ovarian parenchyma under ovarian stoma with mild inflammation of liver and interstitial nephritis in kidney, whereas pancreatic tissues were normal, and this repair was more visible in rats treated with dist. water extract. Active compound behind this study can be a useful tool for pharmaceutical companies.

Keywords: Polycystic ovary syndrome, Camellia sinensis, polarity-based extracts, hepatic, renal, pancreatic, biochemical and hormonal profile, histopathology.

INTRODUCTION

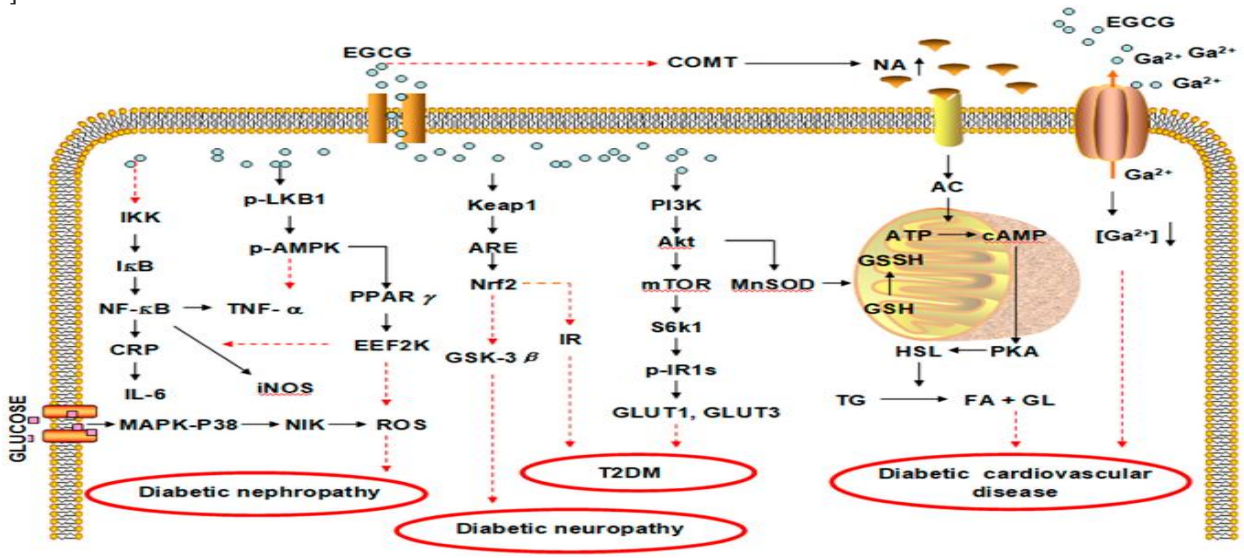
PCOS is one of the most frequent disorders among reproductive-age women worldwide. It is considered as a metabolic, reproductive and endocrinal disorder with multifaceted clinical presentations [1]. According to an estimation all over the world about 4–10% of women of reproductive age do suffer from PCOS with highest prevalence in Asian countries [2].

Exact etiology of PCOS still remains indefinable but hyperandrogenemia and hyperinsulinemia are considered as the main reason. The increased insulin resistance (IR) in ovarian tissue causes impaired metabolic signaling at one side and on other led to intact mitogenic and steroidogenic activity in ovarian tissue that favors hyperandrogenemia

[3]. High levels of androgens may lead back to IR by increasing levels of free fatty acids and modifying muscle tissue, perpetuating the IR-hyperinsulinemia-hyperandrogenemia cycle and symptoms like hirsutism, acne, central adiposity, reproductive dysfunction such as infertility, menstrual irregularity, miscarriage, and pregnancy complications.[4,5,6].PCOS also led to metabolic complications e.g. more chances of gestational diabetes (GDM), impaired glucose tolerance, and increase chance of suffering from type 2 diabetes. This all ultimately result in increased risk of cerebrovascular and cardiovascular disease, anxiety and depression of the patients [7]. These above complications and risks ultimately reduced not only the quality of life of patient [8] but also cause economic burden to individuals and governments [9].

As primary cause of PCOS is increased insulin resistance. Insulin resistance can be improved by the use of different drugs like thiazolidinediones and metformin with lifestyle modification. As thiazolidine dionesare related with many side effects so metformin is usually considered as safest to decrease insulin resistance in PCOS patients .Through the Adenosine monophosphate (AMP)-activated protein kinase (AMPK) pathway metformin decreases the hepatic glucose production and decrease peripheral insulin resistance. But the use of metformin is related to many side effects like diarrhea, nausea and abdominal cramps on one hand and psychological financial burdens of patients on other hand due to persistent disease [10].

So, it is necessary to investigate alternative therapy agents which may cure PCOS. This alternative therapy may include plant extracts like *C. sinensis,* which is commonly known as green tea. It is fermented tea, used all over the world and contains many compounds like polyphenols, such as catechins, flavonols, theaflavins, and the arubigins, alkaloids, vitamins and minerals. These compounds have role in reducing insulin resistance by following pathways [11].



The molecular mechanisms of green tea compound ,EGCG against diabetes mellitus and its complications. EGCG has shown effects against T2DM by improving IR, against diabetic cardiovascular disease by decreasing TG and [Ga2+], against diabetic nephropathy by decreasing ROS and against diabetic neuropathy by increasing Nrf2. The arrow means the direction of actions, and the black full lines indicate upregulation and red dotted lines refer to downregulation or inhibition. CRP, C-reactive protein; MAPK p38-NIK, NF-κB inducing kinase; LKB1, kelch-like ECH-associated protein-1; EEF2K, eukaryotic elongation factor-2 kinase; ARE, antioxidant-responsive element; GSK-3β, glycogen synthase kinase-3β; IR, insulin resistance; MnSOD, Mn superoxide dismutase; NA, noradrenalin; s6k1, ribosomal protein S6 kinase 1; AC, adenylatecyclase; HSL, hormone-sensitive lipase; TG, triglyceride; FA, fatty acid; GL, glycerinum; GSH, glutathione; GSSH, oxidized glutathione; mTOR, the target of rapamycin; EGCG, epigallocatechingallate; IKK, IκB kinase; NF-κB, nuclear factor-κB; iNOS, inducible nitric oxide synthase; TNF-α, tumor necrosis factor-α; Nrf2, nuclear factor-erythrocyte-associated factor 2; PI3K, phosphatidylinositol 3-hydroxykinase; Akt, protein kinase B; AMPK, adenylic acid-activated protein kinase; T2DM, type 2 diabetes mellitus; GLUT, glucose transporter type; PKA, protein kinase A; ATP, adenosine triphosphate; cAMP, cyclic Adenosine monophosphate; COMT, catechol-O-methyltransferase, an enzyme responsible for the degradation of noradrenalin. settings

Figure 1: *C.sinensis*compound improving hyperglycemia and related symptoms;, against diabetic cardiovascular disease by decreasing TG and [Ga^{2+}], against diabetic nephropathy by decreasing ROS and against diabetic neuropathy by increasing Nrf2. The arrow means the direction of actions, and the black full lines indicate upregulation and red

dotted lines refer to downregulation or inhibition. CRP, C-reactive protein; MAPK p38-NIK, NF-κB inducing kinase; LKB1, kelch-like ECH-associated protein-1; EEF2K, eukaryotic elongation factor-2 kinase; ARE, antioxidant-responsive element; GSK-3β, glycogen synthase kinase-3β; IR, insulin resistance; MnSOD, Mn superoxide dismutase; NA, noradrenalin; s6k1, ribosomal protein S6 kinase 1; AC, adenylatecyclase; HSL, hormone-sensitive lipase; TG, triglyceride; FA, fatty acid; GL, glycerinum; GSH, glutathione; GSSH, oxidized glutathione; mTOR, the target of rapamycin; EGCG, epigallocatechingallate; IKK, IκB kinase; NF-κB, nuclear factor-κB; iNOS, inducible nitric oxide synthase; TNF-α, tumor necrosis factor-α; Nrf2, nuclear factor-erythrocyte-associated factor 2; PI3K, phosphatidylinositol 3-hydroxykinase; Akt, protein kinase B; AMPK, adenylic acid-activated protein kinase; T2DM, type 2 diabetes mellitus; GLUT, glucose transporter type; PKA, protein kinase A; ATP, adenosine triphosphate; cAMP, cyclic Adenosine monophosphate; COMT, catechol-O-methyltransferase, an enzyme responsible for the degradation of noradrenalin.

Most of these compounds are anti-oxidants and have documented role in lowering of blood sugar, insulin resistance[11], [figure 1],body weight, cancer, cardiovascular disease [12,13].

Primary Pathogenesis of polycystic ovary syndrome includes hyperinsulinemia. In this respect, this study was conducted to assess the effects of green tea extracts with metformin to cure PCOS with hepatic, renal, and pancreatic effects.[14].

2. MATERIAL AND METHOD

2.1 Study Design:

It is an experimental study, conducted at Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore from September 2021 to Feburary2022.

2.2 Collection and Preparation of Plant Extracts

C. sinensis leaves were obtained from Shinkiari region of Pakistan, identified by expert taxonomist at Government College University, Lahore with botanical number of GC.Herb.Bot.3779.Than fresh leaves were shade dried at room temperature grind, followed by grinding of dried leaves into powdered form (80 mesh) by mechanical means, added n-hexane(1:10 ratio) and kept for shaking for 24 hours in shaker incubator (K-J-201BD), followed by the centrifugation for 15 minutes at 5000 rpm (SIGMA 203,43191) and filtration through Whatman filter paper 1.0. Filtrate had been shade dried at room temperature while next solvent (ethyl acetate, methanol and dist. water respectively) had been added in residue with repetition of previous procedure. Dried filtrate has been re- dissolved in 15 % DMSO to prepare stock solution (1.0 mg/ ml) [15].

2.3 Animal Selection:

Adult female Sprague Dawley rats (150-200gm) were housed in standard stainless-steel cages at controlled room temperature and 60-70% relative humidity, fed a standard laboratory diet, and given free access to water. Sick and ill rats were removed from the experiment.

Animals were divided into the following seven groups.

- **Group 1:** Vehicle (received water only).
- **Group 2:** Negative Control group. PCO induction with 1mgLetrozole/ kg b.w. of rat for 21 days [16].
- **Group 3:** Positive control group. PCO Induction metformin (50 mg/kg b.w. of rat) for 21 days [17]
- **Group 4:** Experimental group. PCO Induction+ n-hexane extract (50 mg/kg b.w. of rat) [18]
- **Group 5:** Experimental group. PCO induction+ ethyl acetate extract (50 mg/kg b.w. ofrat) [18]
- **Group 6:**Experimental group. PCO induction+ methanolic extract of(50 mg/kg b.w. ofrat) [18]
- **Group 7:**Experimental group. PCO induction+ distilled water extract (50 mg/kg b.w. ofrat) [18]

2.4 Collection and Analysis of Vaginal Smears

The PCOS-induced rat has a protracted diestrous phase. Throughout the study period, animals were weighed every week while rats were held from back to collect vaginal smear with wet swab and by inserting pre-filled (with 0.9% normal saline) tip of narrow plastic pipette into the rat's vagina, but not deeply to avoid puncturing, followed by observation of smear under compound microscope[19] by using clean grease-free microscope slides and afterwards stained with methylene blue or crystal violet stain to identify different stages of esterous cycle.[19].

2.5 Hormonal and biochemical analysis:

Once extract was induced, after one week rat were dissected by open chest method to collect blood in gel tubes via the left ventricle puncture by using a 19-21 gauge needle[20].Serum Follicle stimulating hormone (FSH), Luteinizing hormone(LH), testosterone, estrogen, insulin and progesterone has been quantified in rat blood by ELISA method[20]while serum bilirubin, glucose, ALT, AST, urea, creatinine and uric acid were measured by using kit method through automated chemistry analyzer [21].While HOMA-IRwas

calculated by following formula

HOMA-IR=[glucose] (mmol/l) × [insulin] (µU/ml)/ 22.5, formulation [22].

2.6 Histopathological analysis:

Ovaries, Uterus, Pancreas, Liver and Kidneys were preserved with 10% neutral formalin. The specimens were dehydrated in descending ethanol grades, cleaned with xylene, and embedded in paraffin wax. Sections of 4-5µm thickness were cut, stained with haematoxylin and eosin, and inspected under a microscope at 40X to identify any cellular alterations.

2.7 Statistical analysis:

The data was analyzedby using Two-Way ANOVA by considering P<0.05 level of significance through GarphPad prism 8.0 while SEM has been calculated by using mathematical calculation on MS Excel 19.

3. RESULT:

3.1. Identification of PCOS in vaginal smear of rats

Stained vaginal smears of rats in control group showed regular proestrous, estrous diestrous and metestrous phases. In proestrous phase figure 2 (i) well-formed round nucleated epithelial cells in clustersare seen. Estrous phase is characterized by cornified squamous cells found in clusters. The PCOS induced rats showed persistent diestrous phase (Figure 2).

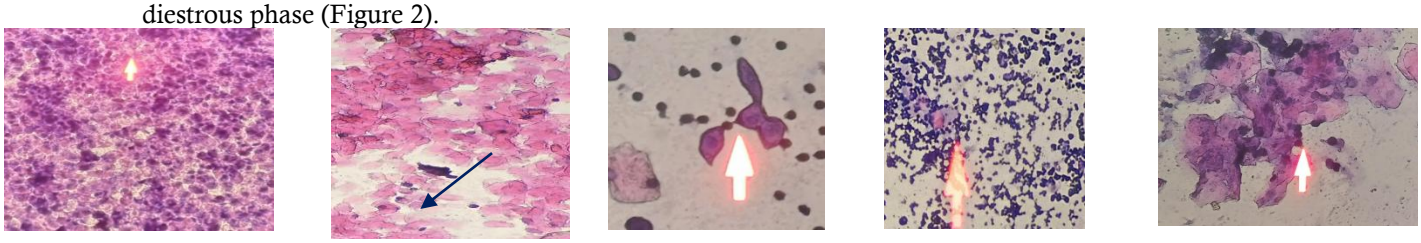
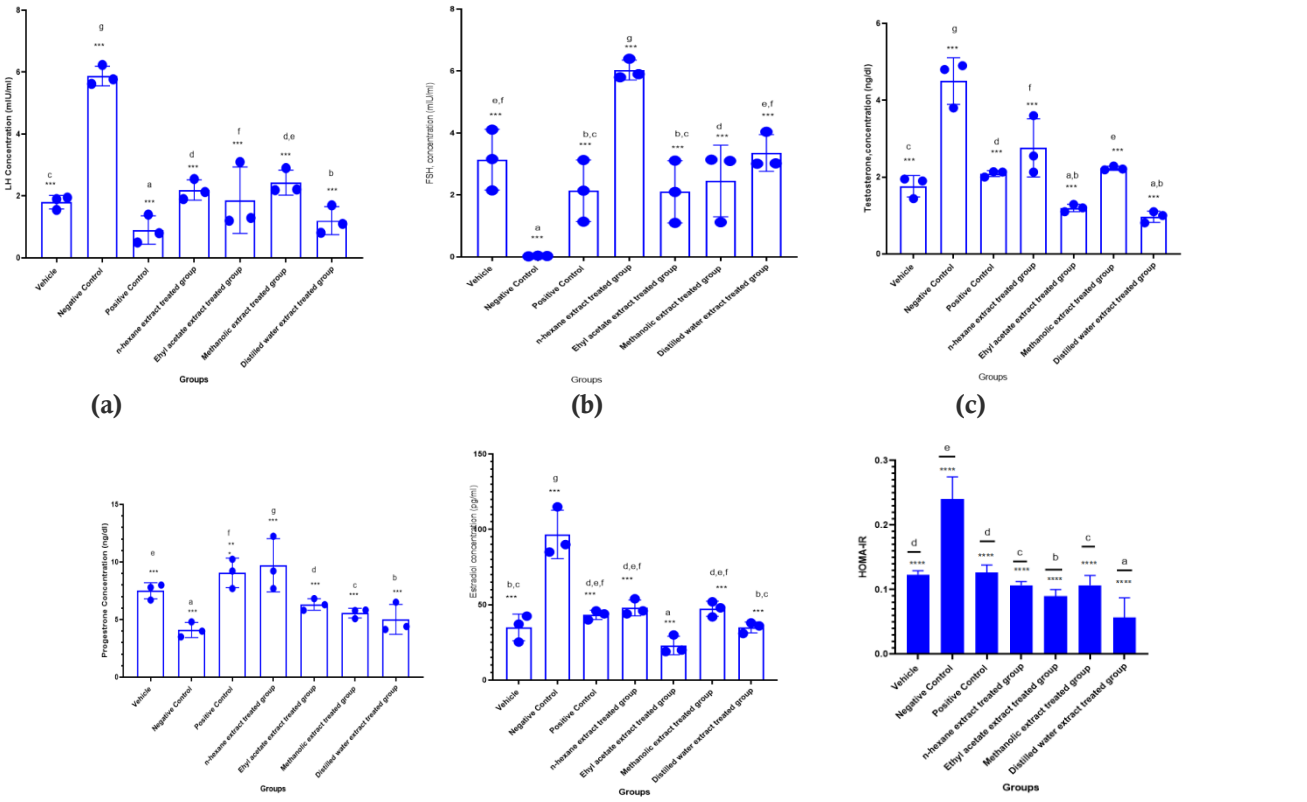


Figure 2 (i) Proestrous (ii) Estrous (iii)Diestrous(iv) Metestrous phases of vaginalsmears in vehicle group (v) Diestrous phase in PCOS

3.2 Hormonal profile of rats

Statistical analysis revealed that the negative control group had considerably higher LH levels (5.87±0.31mlU/ml) compared to the vehicle group (1.8±0.21mlU/ml).The increased LH level in the PCOS model dropped considerably across all experimental groups (p<0.0001), with the greatest reduction observed in animals treated with distilled water extracts (1.2± 0.4mlU/ml), followed by n-hexane (2.0± 0.47mlU/ml), ethyl acetate (1.17± 0.41mlU/ml) and methanolic extracts (1.2± 0.12mlU/ml) and these results are in accordance to the results of positive control group (0.9± 0.4mlU/ml) [Figure3 (a)]. The study found that the negative control group had lower FSH levels (0.03±0.01mlU/ml) than the vehicle group (3.14±0.98mlU/ml). As compared to positive control group (2.13±0.99mlU/ml), most significant increase in FSH level has been shown by rats treated with n-hexane extract(6.03±0.32mlU/ml), followed by rats treated with methanolic(2.45±1.15mlU/ml),ethyl acetate (2.1±1.0mlU/ml) and distilled water extracts(0.02±0.01mlU/ml) (p<0.0001). [Figure 3 (b)]. Statistical analysis revealed considerably higher levels of testosterone in the negative control group (6.15±1.2ng/dl) as compared to vehicle(2.0±0.29ng/dl), while in all experimental groups, testosterone level decreased, which was most significant in animalstreated with distilled water extract(1.2±0.4ng/dl) at (p<0.0001)followed by rats treated with ethyl acetate (1.8±1.07ng/dl), n-hexane (2.7.±0.7ng/dl) and methanolic extracts (2.23±0.05ng/dl) with comparison to positive control group (2.09±0.078ng/dl) [Figure 3 (c)].Statistical findings clearly showed that in negative control group, significant decrease in progesterone level(4.1±0.6pg/dl) as compared to vehicle (7.5±0.5pg/dl). Restoration of progesterone levels was more evidentin rats treated with n-hexane extract(9.72±2.3pg/dl) as compared to positive control(9.05±1.27pg/dl) at p<0.0001, followed by rats treated with ethyl acetate, methanolic and distilled water extract (6.31±0.5pg/dl, 5.5±0.4pg/dl and 5.05±1.2 pg/dl respectively) [Figure 3 (d)]. Statistical findings suggested that level of estradiol substantially decreased in negative control group(28.3± 20.12pg/ml) when compared to vehicle (96.6 ± 16.07pg/ml) (p<0.0001). This decreased level restored in positive control group (48± 5.2pg/ml) and equally in animas treated with n-hexane extract (48± 5.2pg/ml). Significant reduction in estradiol level had been observed in rats treated with ethyl acetate extract (23±6.08pg/ml), followed by rats treated with methanolic and distilled water extracts (38± 16.3pg/ml and 39± 9.8pg/ml respectively) [Figure 3 (e)].)]. Statistical analysis revealed a significant increase in HOMA-IR in the negative control group (0.24 ±0.03) compared to the vehicle (0.12 ±0.05) and positive control group (0.12 ±0.01), while rats treated with distilled water extracts had a substantial decline in HOMA-IR (0.054 ±0.03) followed by rats treated with n-hexane, ethyl acetate and methanolic extracts (0.106 ±0.005, 0.09 ±0.01 and 0.1066 ±0.015 respectively) with p<0.0001 and alpha0.05 [Figure 3 (f)].



(a) **(b)** **(c)**

(d) **(e)** **(f)**

Figure 3 Concentration of hormones in rats (a) LH, (b) FSH, (c) Testosterone, (d) progesterone, (e) estradiol (f) HOMA-IR

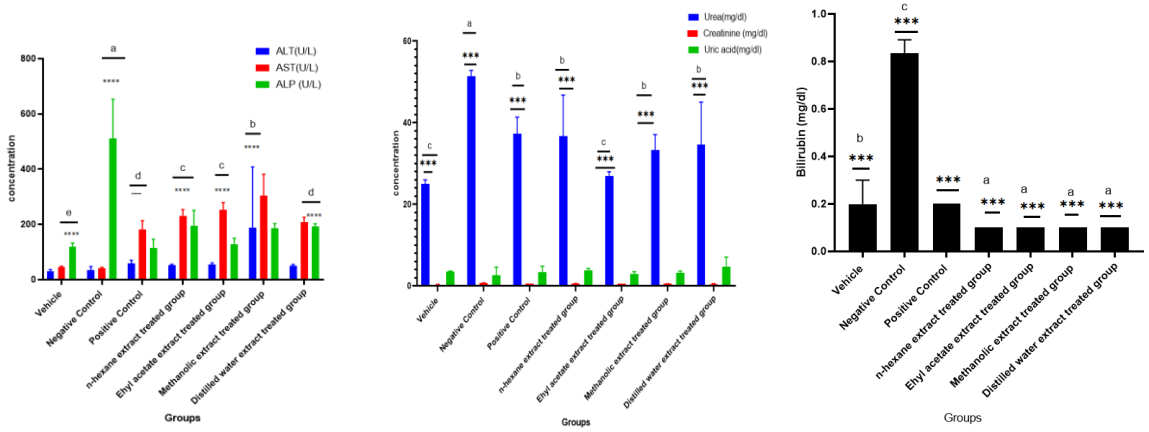
a – g = Comparison of animal groups from most significant results to less significant. ***= P<0.001, **= P<0.01, *= P<0.05.

3.3 Enzymatic and Biochemical profile of all animals

Statistically analyzed data showed that ALT levels has been significantly increased in positive controlled group (33.3.6 ±14.0 IU/L) when compared with normal vehicle group(28.6 ±7.09 IU/L) .In all experimental groups ALT levels were found significantly increased as compare to positive control (58.3 ±11.8 IU/L).Methanolic extracts of green tea has elevated ALT levels to maximum (188.3 ±219.3IU/L). Lowest disturbance in normal ALT levels were seen in animal group who has given distilled water extracts of green tea (50.0 ±4.35 IU/L) .Statistically evaluation of ALT levels in group with n-hexane extracts of green tea were (51 ±4 U/L) and with ethyl acetate extracts is (53.3 ±6.3 IU/L).After statistically analysis of serum AST level it was found that all experimental groups given green tea extracts has caused elevation in enzyme level when compared with normal vehicle (44.3 ±4.04 IU/L)[Figure 4 (a)].In experimental groups, AST became elevated. Experimental group with n-hexane (231.3 ±23.5IU/L),ethyl acetate extract group (253.3 ±25.6IU/L,methanolic extract (303.3 ±78.0IU/L) and in distilled water extract group (208.3 ±0.03 IU/L) respectively While ALT in positive control is (181.3 ±32.0 IU/L.This result shows that experimental group with distilled water extracts has disturbed AST least (208.3 ±17.4 IU/L)[Figure 4 (a)].Statistically analyzed data showed that ALP is increased in positive control group (510.3±12.6IU/L) as compared to normal vehicle (119.6 ± 142.9 IU/L).In positive control group ALP was found significantly decreased (115.3 ± 30.8 IU/L).data analysis of experimental groups shows elevated levels with maximum effect in n- hexane extract group(195 ±55.4IU/L) and minimum effect in group who has given methanolic extract (186 ±16.64IU/L)[Figure 4 (a)].Bilirubin data analysis showed its slight elevation in negative control group (0.8±0.1 mg/dl) when compared with normal (0.2 ±0.05 IU/L).This level became normal when given all green tea extracts.In all experimental groups bilirubin remain (0.1 ±1.6 mg/dl).In positive control group bilirubin level was found (0.2 ± 3.3mg/dl)[Figure 4 (c)].

Statistical analysis of blood urea demonstrate that it significantly increased in negative control group (51.3 ±1.5 mg/dl) as compared to normal vehicle group (25.1 ±1mg/dl). Wheras, in positive control group blood urea mean level was found(37.33 ±4.04 mg/dl).In experimental group with ethyl acetate, urea level disturbed least (27.5 ±1mg/dl).In experimental group with n-hexane urea levels were found maximum increased (37.3 ±10.1mg/dl) while in methanolicand dis tilled water extract given experimental groups, urea remains (33.36 ±3.7mg/dl) and (34.6 ±10.4 mg/dl) respectively[Figure 4 (b)].. Creatinine levels increased significantly in the

negative control group (0.72 ±0.1 mg/dl) compared to the normal vehicle group (0.31 ±0.05 mg/dl), according to statistical analysis. In the positive control group, creatinine levels averaged 0.63 ± 0 mg/dl. In the experimental group using distilled water, creatinine levels were found to be least altered.(0.5±0.1 mg/dl).In experimental group with, ethyl acetate creatinine levels were found maximum increased (0.65 ±0 mg/dl) while in methanolic and n hexane extract given experimental group creatinine remains (0.53±0.05 mg/dl) and (0.56±0.05 mg/dl) respectively. After statistically analysis of uric acid it was analyzed that it significantly decreased in negative control group (2.7±1.93 mg/dl) when it compared to normal vehicle group (3.5 ±0.15 mg/dl).In Positive control group uric acid mean level was found(3.4 ±1.44 mg/dl).In experimental group with ethyl acetate ,uric acid levels found least disturbed (2.9±0.58 mg/dl).In experimental group with distilled water extracts uric acid were found maximum increased (4.8 ±2.27mg/dl) while inn-hexane and methanolic extract given experimental groupsuric acid remains (3.9±0.4 mg/dl) and (3.3±0.4 mg/dl) respectively[Figure 4 (b)].



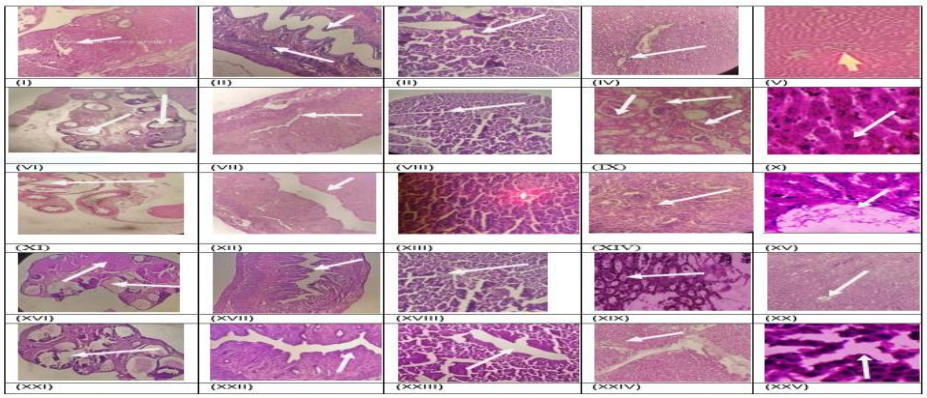
(a) (b) (c)

Figure 4Concentration of (a) Enzymes (b and c) Biochemical parameters of all animals

a – g = Comparison of animal groups' results, from most significant to least significant.***= P<0.001, **= P<0.01, *= P<0.05.

3.4. Histological profile of all animal groups

In the negative control group, the histological characteristics of the ovary exhibited multiple sub capsular cystic follicles like pearls of strings, while endometrium and myometrium of its uterus were unremarkable, pancreas was normal, kidney had mild tubular interstitial nephritis and intra tubular casts, and liver had mild peripheral inflammation [Figure 05 (VI-X)]. Compared to the vehicle, which exhibited normal ovarian parenchyma with corpus leuteum, unremarkable uterine, kidney with normal glomeruli, tubules, and interstitium, and liver with normal hepatic lobule, no steatosis, hepatocystitis and ballooning or inflammation [Figure 05 (I-V)].In rats treated with distilled water extract, ovarian cortex showed corpus leuteum while in rats treated with methanolic extract, graffian folliclewas also observed. All other organs including uterus, pancreas and liver were unremarkable in all experimental group except in rats treated with methanolic extract which showed congestion of blood vessels in blood vessel of kidneys [Figure 05 (XVI- XXXV)]. These histological results were much better than Positive control group in which ovary had relatively few cystic follicles and corpus leuteum while itsuterus,pancreas and kidney were unremarkable and liver hadmild peripheral inflammation [Figure 5 (XI-XV)].



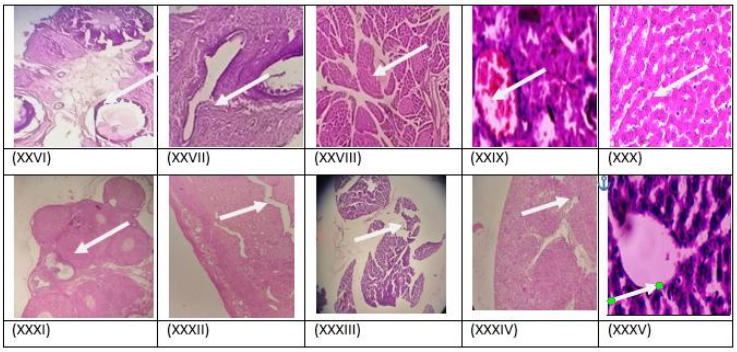


Figure 05 Histopathological features (40X) of Vehicle (I-V), Negative control group (VI-X), Positive control group (XI-XV), Experimental groups: ethyl acetate extract induced (XVI-XX), n-hexane extract induced (XXI-XXV),Methanolic extract induced (XXVI-XXX), distilled water extract induced (XXX-XXXV)

DISCUSSION

Letrozole belongs to the third generation of non-steroidal aromatase inhibitors, which blocks the conversion of testosterone into estradiol [23, 24 Rising levels of testosterone promote hyperandrogenemia and resemble the PCOS image by increasing LH and serum testosterone while reducing FSH, estrogen, and progesterone . At present there is no definite cure of PCOS, but currently metformin is used all over the world to treat metabolic and endocrinal disorders related to this disease . Metformin improves PCOS by enhancing insulin sensitivity in the hepatocytes and peripheral tissues. Metformin therapy lowered serum LH and testosterone levels while restoring FSH, estradiol, and progesterone levels [25].Like metformin, C.sinensiscompounds has the ability to enhance glucose metabolism and insulin sensitivity. These compoundsdecreases the hepatic gluconeogenesis by regulatingthe related genes and protein-tyrosine phosphorylation. They also decrease the absorption of carbohydrates from intestinal cells [26].In current study, hormonal profile of PCOS induced rats were improved by all C.sinensisextracts as compared to metformin.In a study comparing old and new methods to treat metabolic aberrations on PCOS also suggests effectiveness of metformin and C.sinensis[27].From all C. sinensis extracts, distilled water and n-hexane extracts showed maximum improvement in hormonal profile of PCOS induced animals, becausedistilled water dissolved many substances and non toxic in nature while n-hexane is well absorbed and metabolized in body[28].. FSH levels increased significantly by n-hexane extract followed by distilled water extract. Insulin resistance was also improved in rats treated with distilled water extract. Same pattern of hormonal increase and decrease is reported in a study which shows that Water-soluble extracts of green tea and catechin demonstrate similar benefits on gonadotropins, β-estradiol, progesterone, testosterone and ovarian follicle in polycystic ovarian syndrome rat model[29].Serum testosterone levels reduction was maximum with methanolic extracts, while Serum estradiol decreasedwith n-hexane extract. Progesterone decreased in PCO induced rat model and become elevated in rats treated with metformin and n-hexane extract of C.sinensis. Decreased levels of serum testosterone was reported in study showing green tea's effect on the metabolic and hormonal features of polycystic ovarian syndrome in overweight and obese women suffering from the condition.[30].Liver function test was done to find any liver injury. Commonly done liver function test are serum aminotransferases, alkaline phosphatase and bilirubin. Serum transaminases elevation shows the liver injury and functional disturbance of liver cell membranes, raised ALP shows disparity in the transport of metabolites while enzymes and serum bilirubin elevation shows hepatic cell function disturbance .All liver function test were found elevated when letrozolewas given to induce PCO model. These enzymes and bilirubin became decreased when metformin was given. Green tea extracts also decreased the enzyme markers of liver function test and also decreased the bilirubin.This result are same as done in study in which green tea extracts effects on liver function test was tested on females [31]. All green tea extracts decreased the bilirubin most as compare to metformin but increased the liver enzymes when compared to positive control.

To assess renal function tests, urea, uric acid, and creatinine are usually considered. In current study all these renal function markers were found elevated in PCO induced rat model but they all became normal when animals were given control drug or green tea extracts . Promising nephron protective role was also explained in a study in which green tea compound effects were observed in various kidney disorders [32]. Histopathological evidence of methanolic extracts showed blood vessels congestion which is may be due to solvent or compounds. There is no

evidence about such injury was found in previous literature.

Histopathology findings of current study are also in support of hormonal profile and biochemical changes in control and experimental groups. These morphological changes showed typical pearl string like cystic appearance in ovarian parenchyma of PCOS model. These cysts are reduced with dissolution of ovarian cyst and even appearance of graffian follicle by metformin and plant extracts.Hydro alcohol C.sinensis extracts found improvement in ovarian morphology previously [33].These findings are in accordance to already done workwhich showed that ethyl acetate and n-hexane extracts of C.sinensishavecaffeine, epicatechin,epicatechin 3-gallate, epigallocatechin, quercitine, theobromine, theophyllinewhile distilled water and methanolic extracts haveL-theanine and current findings of biochemical, hormonal and histopathological studies are attributed to these compounds[34].

CONCLUSION

Current study results suggest that the utilization of the green tea extracts, especially n-hexane and distilled water can be beneficial to cure polycystic ovarian disease by decreasing and improving symptoms of PCO as compared to metformin. This alternative therapy can be considered but more study work is required guaranteeing protected results.

Conflict of interest:

All authors declare no conflict of interest.

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