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THERAPEUTIC EFFECTS OF CAMELLIA SINENSIS (L.) ON REPRODUCTIVE, HEPATO-RENAL AND PANCREATIC PROFILE OF SPRAGUE DAWLEY RATS

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Abstract

Polycystic ovarian syndrome is metabolic and reproductive disorder which is growing especially in Asian countries. Compounds from *Camellia sinensis (L.) 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 (L.)* 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.Statistically analyzed results ($p<0.0001$) showed that LH, testosterone, HOMA-IR, ALT, AST, ALP and bilirubin decreased in rats treated with dist. water extract (1.2± 0.4 mIU/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 mIU/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 mIU/ml and 9.72±2.3 pg/dl) as compared to positive control group (2.13±0.99 mIU/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, while pancreatic tissues were normal and this restoration was more evident in rats treated with dist. water extract. Active compound behind this study can be a useful tool for pharmaceutical companies.

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Keywords: Polycystic Ovary Syndrome, *Camellia Sinensis (L.),* Polarity Based Extracts, Hepatic, Renal, Pancreatic, Biochemical and Hormonal Profile, Histopathology, Homa-Ir

1. INTRODUCTION

All over the world, PCOS is known as one of the most common disorder among the women of reproductive age. It is considered as a metabolic, reproductive and endocrinal disorder with multifaceted clinical presentations(Bulsara et al 2021).According to an estimation all over the world about 4–10% of women of reproductive age suffer from PCOS with highest prevalence inAsian countries (Jabeen et al 2020)

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 (Harada ,2022).High levels of androgens may lead back to IR by increasing levels of free fatty acids and modifying muscle tissue (Witchelet al 2019), perpetuating this IR-hyperinsulinemia- hyperandrogenemia cycle and symptoms like hirsutism, acne, central adiposity, reproductive dysfunction such as infertility, menstrual irregularity, miscarriage and pregnancy complications (Anjum et al 2020). 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 (Akre et al 2022), anxiety and depression of the patients(Rocha et al ,2019)]·These above complications and risk ultimately reduced not only the quality of life of patientbut also cause economic burden to individuals and governments((Joham et al 2022).·

As primary cause of PCOS is increased insulin resistance .Insulin resistance can be improved by the use of differentdrugs like thiazolidinediones andmetformin with life style modification. As thiazolidinedionesare 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 decrease the hepatic glucose production and decrease peripheral insulin resistance but the use of metformin is related with many side effects like diarrhea, nausea and abdominal cramps at one hand and psychological financial burdens of patients on other hand due to persistent disease (Shamim et al 2022)

So it is necessary to investigate alternative therapy agents which may cure PCOS. This alternative therapy may include use of plant extracts like *C.sinensis (L.)* which is commonly known as green tea, sabzqehwa ,sabz chai locally .It is fermented tea, used all over the world and contains many compounds like polyphenols, such as catechins, flavonols, theaflavins, and thearubigins, alkaloids, vitamins and minerals. These compounds have role in reducing insulin resistance by following pathways [Figure 1].

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Most of these compounds are anti-oxidants and have documented role in lowering of blood sugar, insulin resistance (Meng et al 2019), [figure 1], body weight, cancer, cardiovascular disease (Jager et al., 2022).

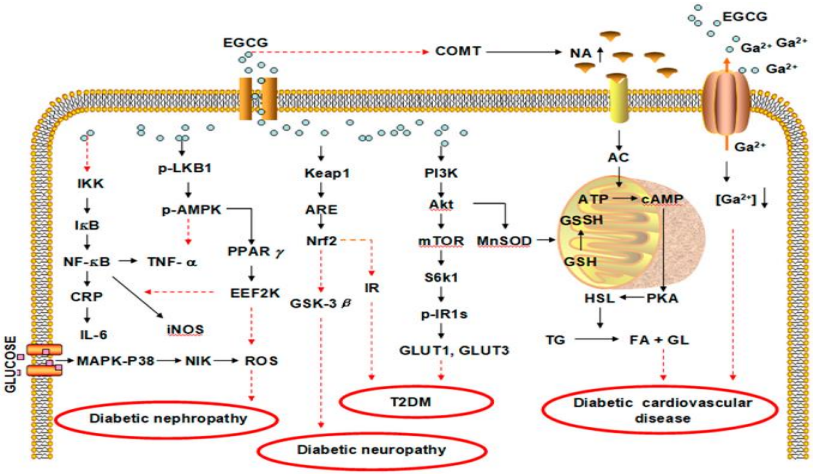

Figure 1: Compound of *C. sinensis* *(L.)* isimproving hyperglycemia and related symptoms

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 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 up regulation and red dotted lines refer to down regulation 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

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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.

Primary Pathogenesis of polycystic ovary syndrome includes hyperinsulinemia. So in this context this study was done to compare the effects of green tea extracts with metformin to cure PCOS with hepatic, renal and pancreatic effects (Maleki et al 2021) and (Khan et al 2021)

2. MATERIALS AND METHODS

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.

Collection and Preparation of Plant Extracts

C. sinensis (L.) 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) has 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) (Abubakar et al 2020).

Animal Selection

After ethical approval(Approval No: USM/Animal Ethics approval/2009/[45] [140])adult female *Sprague Dawley rats* (150-200gm) had been housed in standard stainless steel cages at controlled room temperature and 60-70 % relative humidity and fed with standard laboratory diet with free access to water. Sick and ailing rats were excluded from the study.

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Animals were divided in 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 (Xu, et al 2020).
- **Group 3:** Positive control group. PCO Induction+metformin (50 mg/kg b.w. of rat) for 21 days (Fatima et al 2021)
- **Group 4:** Experimental group. PCO Induction+ n-hexane extract (50 mg/kg b.w. of rat) (Akinmoladun, et al 2022)
- **Group 5:** Experimental group. PCO induction+ ethyl acetate extract (50 mg/kg b.w. ofrat)(Akinmoladun, et al 2022)
- **Group 6:** Experimental group. PCO induction+ methanolic extract of(50 mg/kg b.w. ofrat)(Akinmoladun, et al 2022)
- **Group 7:** Experimental group. PCO induction+ distilled water extract (50 mg/kg b.w. ofrat)(Akinmoladun, et al 2022)

Collection and Analysis of Vaginal Smears

PCOS induced rat have persistent diestrous phase. Throughout the study period, animals were weighed every week while rats had been held from back to collect vaginal smear with wet swab and by inserting pre- filled (with 0.9 % normal saline, (BIOFAR)tip of narrow plastic pipette (BIO HIT)into the rat's vagina, but not deeply to avoid puncturing, followed by observation of smear under compound microscope model number XSZ-107BN,made in USA) (Ajayi et al 2020) by using clean grease-free microscope slides and afterwards stained with methylene blue or crystal violet stain(BIOFAR) to identify different stages of estrous cycle (Ajayi et al 2020).

Hormonal and biochemical analysis

After one week of extract induction, 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 (Asinas et al 2021).Serum Follicle stimulating hormone (FSH), Luteinizing hormone(LH), testosterone, estrogen, insulin and progesterone has been quantified in rat blood by ELISA method (Asinas et al 2021)while serum bilirubin, glucose, ALT, AST, urea, creatinine and uric acid were measured by using kit method through automated chemistry analyzer [21]. While HOMA-IR was calculated by following formula

HOMA-IR= [glucose] (mmol/l) × [insulin] (µU/ml)/ 22.5, formulation (Asefaw et al 2020).

Histopathological analysis

Ovaries, Uterus, Pancreas, Liver and Kidney were preserved in 10% neutral formalin (MERCK). The specimens were dehydrated in descending grades of ethanol brought (MERCK), cleared in xyleneand embedded in paraffin wax purchased from BIOFAR chemicals. Sections of 4-5µm thickness had been cut and stained with haematoxylin (BIOFAR) and eosin and examined at 40X under compound microscope ,(model number XSZ-107BN ,made in USA) to observe any cellular changes.

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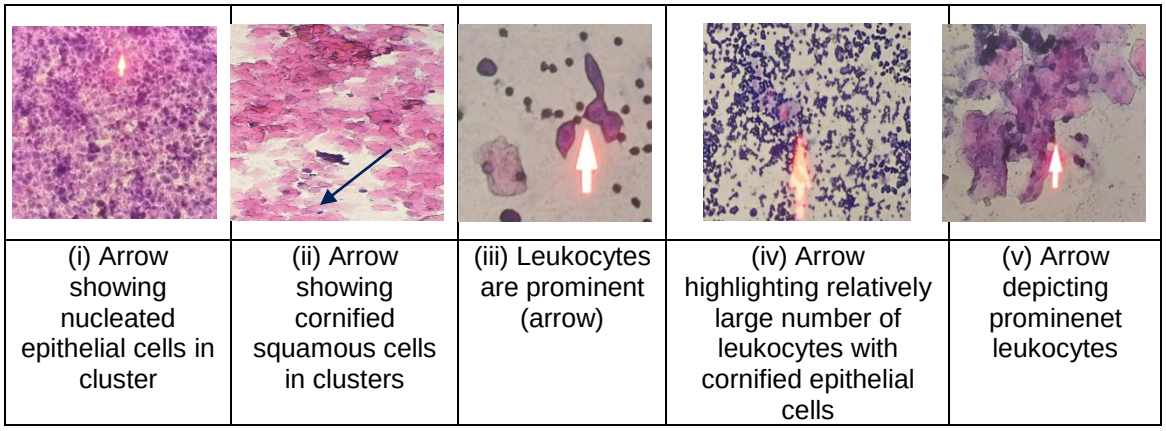
Statistical analysis

The data was analyzed by 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. RESULTS

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 clusters are seen. Estrous phase [Figure 2 (ii)] is characterized by cornified squamous cells found in clusters .The diestrous phase [Figure 2 (iii)] is characterized by prominent leukocytes with few epithelial and cornified cells. The metestrous [Figure 2 (iv)]is shown with large number of leucocytes and a small number of large, non-granular and anucleated cornified epithelial The PCOS induced rats showed persistent diestrous phase[Figure 2 (v)]



(i) Arrow showing nucleated epithelial cells in cluster	(ii) Arrow showing cornified squamous cells in clusters	(iii) Leukocytes are prominent (arrow)	(iv) Arrow highlighting relatively large number of leukocytes with cornified epithelial cells	(v) Arrow depicting prominenet leukocytes

Figure 2: (i) Proestrous (ii) Estrous (iii) Diestrous (iv) Metestrous phases of vaginal smears in vehicle (v) permanent Diestrous phase in PCOS induced rat

Hormonal profile of rats

Statistically analyzed results showed that negative control group has significantly increased LH level (5.87±0.31mlU/ml) as compared to vehicle (1.8±0.21mlU/ml).This elevated LH level in PCOS model decreased significantly in all experimental groups ($p<0.0001$) but maximum reduction has been done 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)]. It has been proven statistically that in negative control group, FSH level decreased (0.03±0.01mlU/ml) as compared to vehicle (3.14±0.98mlU/ml).

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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)].

Statistically analyzed results showed significant elevated levels of testosterone in 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 animals treated 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) as comparison to positive control group (2.09±0.078ng/dl) [Figure 3 (c)]. Statistically analyzed results showed that in negative control group, there was significant decreased in progesterone level (4.1±0.6pg/dl) as compared to vehicle (7.5±0.5pg/dl).

Restoration of progesterone levels was more evident in 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)]. Statistically analyzed results showed that level of estradiol significantly decreased in negative control group (28.3± 20.12pg/ml) as 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 animals 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)]. Statistically analyzed results showed significant increase in HOMA –IR in negative control group (0.24 ±0.03) as compared to vehicle (0.12 ±0.05) and positive control group (0.12 ±0.01) while rats treated with distilled water extracts had significant reduction 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)].

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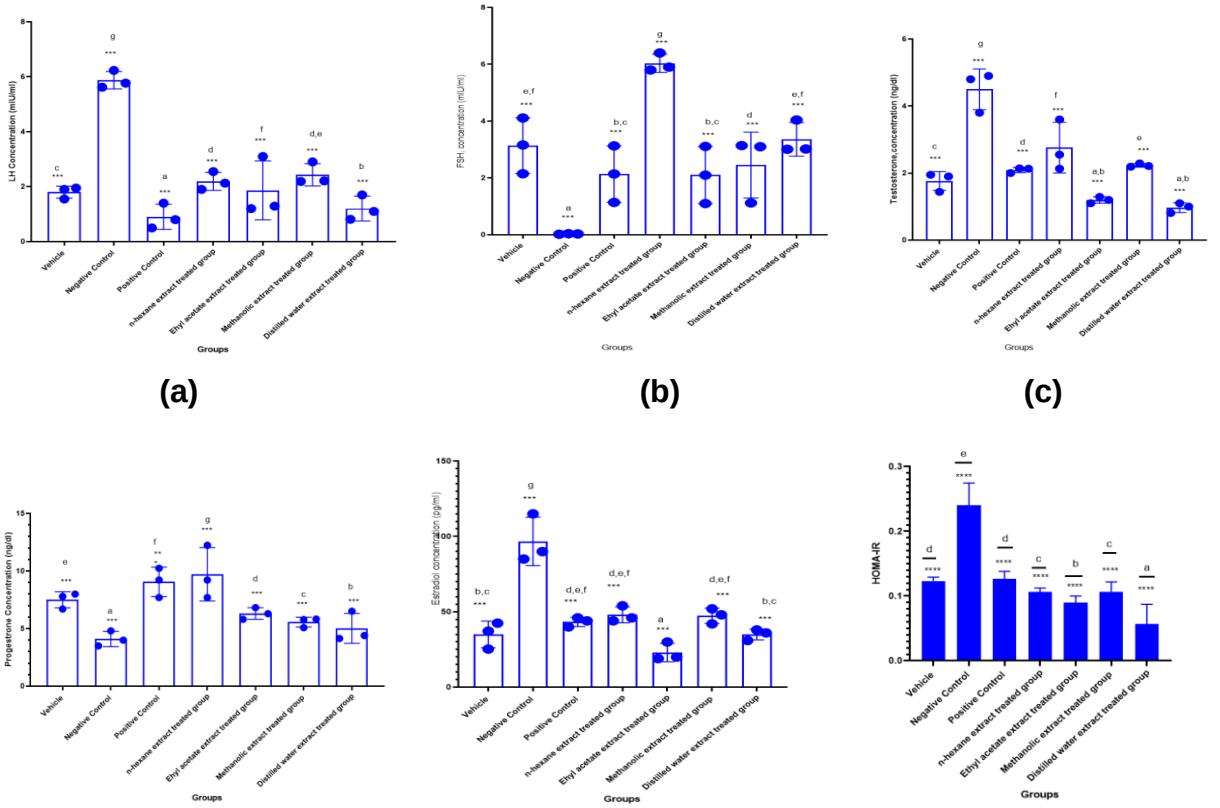
Figure 3: Concentration of hormones in rats (a) LH, (b) FSH, (c) Testosterone, (d) progesterone, (e) estradiol (f) HOMA-IR



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

LH= Luteinizing hormone, FSH= Follicle Stimulating Hormone, HOMA-IR= Homeostatic Model Assessment for Insulin Resistance

Enzymatic and Biochemical profile of all animals

Statistically analyzed data showed that ALT levels have been significantly increased in positive control group (33.3.6 ±14.0 IU/L) when compared with vehicle (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 animals treated distilled water extracts of green tea (50.0 ±4.35 IU/L). Statistically evaluation of ALT levels in rats treated with n-hexane and ethyl acetate extracts of green tea was 51 ±4 U/L and 53.3 ±6.3 IU/L. Serum AST level was elevated in all experimental groups as compared to vehicle (44.3 ±4.04 IU/L) [Figure 4 (a)]. ALT levels in rats treated with n-hexane, ethyl acetate, methanolic and distilled water extract was found to be

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(231.3 ± 23.5IU/L, 253.3 ± 25.6IU/L, 303.3 ± 78.0IU/L and 208.3 ±0.03 IU/L respectively) as compared to positive control group (181.3 ±32.0 IU/L). This result shows that experimental group with distilled water extracts has least disturbed AST level (208.3 ±17.4 IU/L) [Figure 4 (a)]. Statistically analyzed data showed that ALP increased in positive control group (510.3±12.6IU/L) as compared to vehicle (119.6 ± 142.9 IU/L) and positive control group, where it significantly decreased (115.3 ± 30.8 IU/L). Data analysis of experimental groups showed elevated ALP levels by n- hexane extract (195 ±55.4IU/L) and least in rats treated with methanolic extract (186 ±16.64IU/L) [Figure 4 (a)]. Slight elevation in bilirubin has been observed in negative control group (0.8±0.1 mg/dl) as compared to vehicle (0.2 ±0.05 IU/L). Bilirubin level became normal in rats treated with all green tea extracts as compared to positive control group (0.2 ± 3.3mg/dl) [Figure 4 (c)].

Statistical analysis of blood shows that urea significantly increased in negative control group (51.3 ±1.5 mg/dl) as compared to vehicle (25.1 ±1mg/dl). In Positive control group blood urea level was 37.33 ±4.04 mg/dl. In experimental group treated with ethyl acetate extract, disturbance in urea level was least (27.5 ±1mg/dl), while in rats treated with n-hexane extract, there was maximum increase in urea level (37.3 ±10.1mg/dl). In rats treated with methanolic and distilled water extracts, urea was found to be 33.36 ±3.7mg/dl and 34.6 ±10.4 mg/dl respectively [Figure 4 (b)].

Similarly creatinine level significantly increased in negative control group (0.72 ±0.1 mg/dl) as compared to vehicle (0.31 ±0.05 mg/dl) and positive control group (0.63 ±0 mg/dl). In experimental groups, rats treated with distilled water extract, creatinine level was least disturbed (0.5±0.1 mg/dl) as compared to rats treated with ethyl acetate extract in which there was maximum increase (0.65 ±0 mg/dl) in its concentration. Rats treated with methanolic and n hexane extracts had 0.53±0.05 mg/dl and (0.56±0.05 mg/dl respectively creatinine concentration. 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 vehicle (3.5 ±0.15 mg/dl).

In Positive control group its level was 3.4 ±1.44 mg/dl. In experimental groups, treated with ethyl acetate extract, uric acid levels was least disturbed (2.9±0.58 mg/dl) while in rats treated with distilled water extracts, maximum increase in uric acid (4.8 ±2.27mg/dl) has been observed while it was constant in animals treated with n-hexane and methanolic extracts (3.9±0.4 mg/dl and (3.3±0.4 mg/dl respectively) [Figure 4 (b)].

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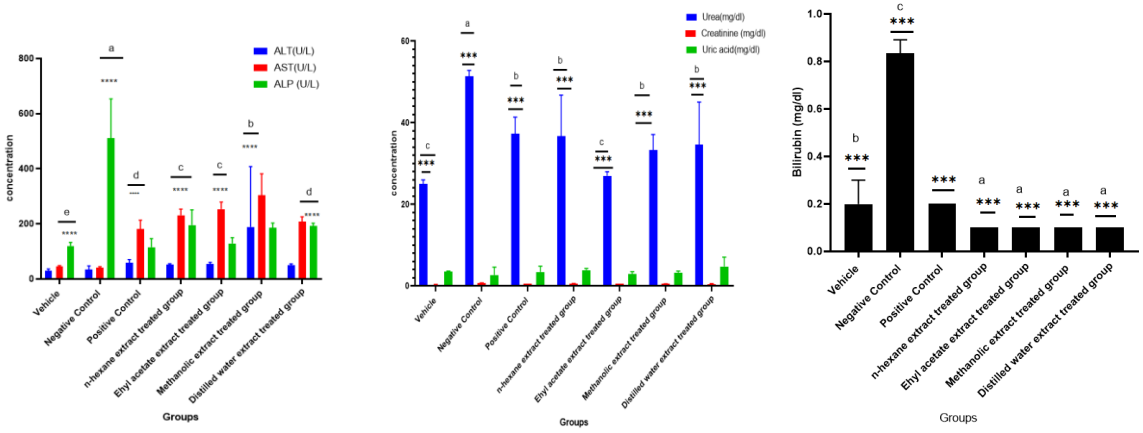
Figure 4: Concentration of (a) Enzymes (b) and (c) Biochemical parameters of all animals



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

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

Histological profile of all animal groups

In negative control group, histological features of ovary showed multiple sub capsular cystic follicles like pearl of strings while endometrium and myometrium of its uterus were un-remarkable, pancreas was normal, kidney had shown with mild tubular interstitial nephritis and intra tubular casts and liver had mild peripheral inflammation [Figure 05 (VI-X)] as compare to vehicle, which showed normal ovarian parenchyma with corpus leuteum, unremarkable uterus, kidney with normal glomeruli, tubules and interstitium and liver with normal hepatic lobule, with 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 follicle was 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 its uterus, pancreas and kidney were unremarkable and liver had mild peripheral inflammation [Figure 05 (XI-XV)].

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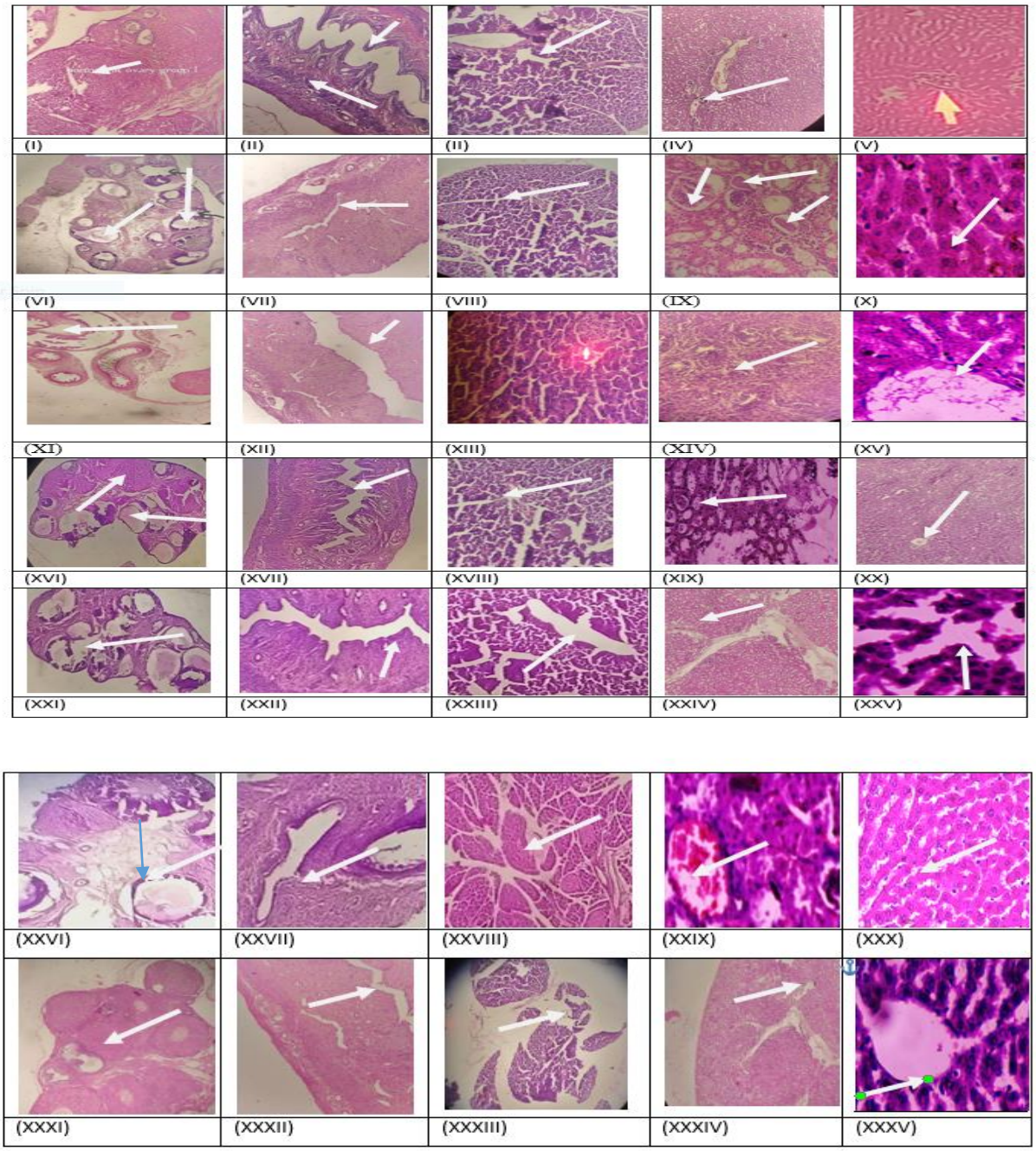

Figure 5: Histopathological features (40X) of Vehicle (I-V), Negative control group (VI-X), Positive control group (XI-XV), Experimental groups: ethyl acetate extract induced (XVI-XX), n-hexane extract induced (XXI-XXV), Methanolic extract induced (XXVI-XXX), distilled water extract induced (XXX-XXXV)

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4. DISCUSSION

Letrozole is third generation of non-steroidal aromatase inhibitors so it prevents conversion of testosterone in estradiol (Gu et al 2022) Increased level of testosterone leads to hyperandrogenemia and mimics the PCOS picture by increasing LH, and serum testosterone while decreasing FSH, estradiol 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 improve the PCOS by increasing insulin sensitivity at hepatocytes and peripheral tissues. The treatment with metformin decreased the serum LH, and testosterone and restore FSH, estradiol and progesterone levels (Jiang et al 2022).

Herbs are a safe and nutritive option to support ovarian function, endocrine feedback loops, thyroid function, blood sugar regulation and metabolism. Because PCOS is an endocrine disorder, herbs that balance hormone levels can be very helpful in improving most PCOS symptoms, including amenorrhea, infertility, acne, and excessive facial hair. Like metformin, *C. sinensis (L.)* compounds has the ability to enhance glucose metabolism and insulin sensitivity. These compounds decreases the hepatic gluconeogenesis by regulating the related genes and protein-tyrosine phosphorylation. They also decrease the absorption of carbohydrates from intestinal cells (Xu et al 2020).In current study, hormonal profile of PCOS induced rats were improved by all *C. sinensis (L.)* extracts 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 (L.)* (Bargiota et al 2021). From all *C. sinensis (L.)* extracts, distilled water and n-hexane extracts showed maximum improvement in hormonal profile of PCOS induced animals, because distilled water dissolved many substances and nontoxic in nature while n-hexane is well absorbed and metabolized in body (Abubakar et al 2020).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 aqueous extract of green tea and catech in have beneficial effects in similar pattern on gonadotropins, β-estradiol, progesterone, testosterone and ovarian follicle in polycystic ovarian syndrome rat model (Sadoughi et al, 2017) Serum testosterone levels reduction was maximum with methanolic extracts, while Serum estradiol decreased with n-hexane extract. Progesterone decreased in PCOS induced rat model and become elevated in rats treated with metformin and n-hexane extract of *C.sinensis (L.).*

Decreased of serum testosterone levels was reported in study showing effect of green tea on metabolic and hormonal aspect of polycystic ovarian syndrome in overweight and obese women suffering from polycystic ovarian syndrome (Tehrani et al, 2017).Serum transaminases elevation shows the liver injury and functional disturbance of liver cell membranes, raised ALP shows derangement in the transport of metabolites while

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enzymes and serum bilirubin elevation shows hepatic cell function disturbance. Enzymes and bilirubin decreased when metformin was given. Green tea extracts also decreased the enzyme markers of liver function and bilirubin. These result are same as done in study in which green tea extracts effects on liver functions was tested on females (Yu et al 2017). All green tea extracts decreased the bilirubin as compare to metformin but increased the liver enzymes when compared to positive control. In current study, urea, uric acid, and creatinine were found elevated in PCO induced rat model but they all became normal when animals were given control drug or green tea extracts. Promising nephrototective role was also explained in a study in which green tea compound effects were observed in various kidney disorders (Kanlaya et al 2019). Histopathological evidence of organs treated with methanolic extracts showed blood vessels congestion which was might be due to solvent or compounds. There was no evidence about such injury in previous literature. Histopathological 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 (L.)*extracts found improvement in ovarian morphology previously (Ishikawa et al 2022).These findings are in accordance to already done work which showed that ethyl acetate and n-hexane extracts of *C. sinensis (L.)* have caffeine, epicatechin, epicatechin 3-gallate, epigallocatechin, quercitine, theobromine, theophylline while distilled water and methanolic extracts haveL-theanine and current findings of biochemical, hormonal and histopathological studies are attributed to these compounds(KC et al 2020). All these features are attributed to phenolic compounds of green tea, such as catechins, especially (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG) and (-)-epicatechin (EC), which acted as natural antioxidants and constitute 6–16% of its dry leaves (Menget al2019)Catechins are superior to glutathione, vitamin C and flavonoids and neutralised free radicals and facilitate the detoxification of enzymes, such as catalase, glutathione peroxidase and glutathione reductase.

Numerous studies showed the various beneficial effects of green tea extract and its catechins on health, including high antioxidant (Tang et al 2019) osteoprotective (Huang,et al 2020), neuroprotective (Pervin et al 2019), anti-cancer , anti-hyperlipidaemia and anti-diabetic (Torello et al 2018) effects, and that green tea extract and its catechins improve fertility in humans and animals (Kongchian et al2020).Daily consumption of green tea has positive effects on the male and female reproductive systems. Effect of green tea on female reproductive system is demonstrated by a decrease in ovarian cancer risk among southern Chinese women who regularly drink green tea (Guan et al2020)This finding is further supported by the shrinkage of total fibroid volume and reduced fibroid-specific symptom severity in women with symptomatic fibroids after 4 months of supplementation with 800 mg of green tea extract (Dai et al, 2020 and Sayed et al. , 2019).A high content of EGCG inhibits the proliferation of leiomyoma tumour (fibroid

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tumour) and induce apoptosis (Khadilkar et al 2019).National Institutes of Health and Androgen Excess and PCOS Society consider hyperandrogenism one of the must-have criteria for diagnosing PCOS. Green tea or its derivatives inhibits increase in testosterone level in PCOS women/ PCOS-induced animals, however, no big difference has been found in testosterone, SHBG, free androgen, androstenedione, DHEA-S, FSH and LH levels between the green tea treatment group and placebo group in obese PCOS women. Moreover nearly half of women diagnosed with PCOS have insulin-resistant hyperinsulinism. Green tea or its derivatives normalise hyperinsulinism in PCOS. A clinical trial recorded a significant reduction in fasting insulin on overweight and obese PCOS women after green tea tablet treatment, but there was no change in fasting insulin, fasting glucose, 2 h post-load glucose and fasting G:I ratio in obese PCOS women who consumed capsules containing 2% freeze-dried tea powder for 3 months. The study on PCOS rat model found no difference in fasting insulin level, but significant reductions in fasting glucose and HOMA-calculated insulin resistance were recorded (Kamal et al 2021).

5. CONCLUSION

Current study results suggest that the use of the *C. sinensis (L.)* 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 consider but more study work is require to guarantee protected results.

6. Conflict of Interest:

All authors declare no conflict of interest.

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8. Author's Contribution GI: Performed experimental study and draft this manuscript, AA: Supervise the whole experimental study and review this article, RB: review present manuscript and proof read. AA: helped in lab experiment, S and AM: co-worker while lab work

References

1) Abubakar AR, Haque M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of pharmacy & bioallied sciences; 12 (1):1.

2) Ajayi, A. F., & Akhigbe, R. E. (2020). Staging of the estrous cycle and induction of estrus in experimental rodents: an update. Fertility research and practice; 6 (1), 1-15.

3) Akre, S., Sharma, K., Chakole, S., & Wanjari, M. B. (2022). Recent advances in the management of polycystic ovary syndrome: a review article. Cureus; 14 (8).

4) Akinmoladun, A. C., Falaiye, O. E., Ojo, O. B., Adeoti, A., Amoo, Z. A., & Olaleye, M. T. (2022). Effect of extraction technique, solvent polarity, and plant matrix on the antioxidant properties of

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 03 | 2023
DOI 10.17605/OSF.IO/7X3TV

Chrysophyllum albidum G. Don (African Star Apple). Bulletin of the National Research Centre; 46 (1), 1-9.

5) Anjum, F., Shahid, M., Jilani, M. I., Oranab, S., Farooq, S., Nazir, A., ... & Iqbal, M. (2020). Evaluation of antioxidant potential and cytotoxic behavior of different varieties of *Allium sativum*. Polish Journal of Environmental Studies; 29 (6), 44-47.

6) Asefaw, Tsegay, Mistre Wolde, Abebe Edao, Aster Tsegaye, Gebre Teklu, Feven Tesfay, and Gebreslassie Gebremariam. (2020). Assessment of liver and renal function tests among gasoline exposed gas station workers in Mekelle city, Tigray region, Northern Ethiopia. PLoS One; 15 (10), e0239716.

7) Asinas, J. M., Khaiwi, W., Miller, A., & Newland, P. (2021). An effective approach for endocrine dynamic function tests workflow and reporting using *Cerner millennium* (R). American Journal of Clinical Pathology; 156, S50-S50.

8) Bulsara, J., Patel, P., Soni, A., & Acharya, S. (2021). A review: Brief insight into Polycystic Ovarian syndrome. Endocrine and Metabolic Science; 3, 100085.

9) Bargiota, A., & Diamanti-Kandarakis, E. (2021). The effects of old, new and emerging medicines on metabolic aberrations in PCOS. Therapeutic advances in endocrinology and metabolism; 3 (1), 27-47.

10) Dai, W., Ruan, C., Zhang, Y., Wang, J., Han, J., Shao, Z., & Liang, J. (2020). Bioavailability enhancement of EGCG by structural modification and nano-delivery: A review. Journal of Functional Foods; 65, 103732.

11) Fatima H, Shahid M, Jamil A, Naveed M. (2021) Therapeutic potential of selected medicinal plants against carrageenan induced inflammation in rats. Dose-Response; 26, 19 (4):15593258211058028.

12) Gu, Y., Zhou, G., Zhou, F., Wu, Q., Ma, C., Zhang, Y. ... & Hua, K. (2022). Life modifications and PCOS: old story but new tales. Frontiers in Endocrinology; 488.

13) Guan, Q. H., Shi, W. J., Zhou, L. S., Tao, A. L., & Li, L. (2020). Effect of epigallocatechin-3-gallate on the status of DNA methylation of E-cadherin promoter region on endometriosis mouse. Journal of Obstetrics and Gynaecology Research; 46 (10), 2076- 2083.

14) Harada, M. (2022). Pathophysiology of polycystic ovary syndrome revisited: Current understanding and perspectives regarding future research. Reproductive Medicine and Biology; 21 (1), e12487.

15) Huang, H. T., Cheng, T. L., Lin, S. Y., Ho, C. J., Chyu, J. Y., Yang, R. S., ... & Shen, C. L. (2020). Osteoprotective roles of green tea catechins. Antioxidants; 9 (11), 1136.

16) Ishikawa, A., Matsuda, T., Gam, H., Kanno, M., Yamada, M., Ikegami, N., & Sakamaki-Sunaga, M. (2022). Effect of green tea extract ingestion on fat oxidation during exercise in the menstrual cycle: A Pilot Study. Nutrients; 14 (19), 3896.

17) Jager, T., Mokos, A., Prasianakis, N. I., & Leyer, S. (2022). First- page settings order article reprints open access article pore-level multiphase simulations of realistic distillation membranes for water desalination. Membranes.

18) Jiang, S., Tang, T., Sheng, Y., Li, R., & Xu, H. (2022). The effects of letrozole and metformin combined with targeted nursing care on ovarian function, LH, and FSH in infertile patients with polycystic ovary syndrome. Journal of Healthcare Engineering; 2022.

19) Joham, A. E., Norman, R. J., Stener-Victorin, E., Legro, R. S., Franks, S., Moran, L. J., ... & Teede, H. J. (2022). Polycystic ovary syndrome. The Lancet Diabetes & Endocrinology.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 03 | 2023
DOI 10.17605/OSF.IO/7X3TV

20) Jabeen, A., Yamini, V., Amberina, A. R., Eshwar, M. D., Vadakedath, S., Begum, G. S., & Kandi, V. (2022). Polycystic ovarian syndrome: prevalence, predisposing factors, and awareness among adolescent and young girls of South India. Cureus, 14 (8).

21) Kamal, D. A. M., Salamt, N., Zaid, S. S. M., & Mokhtar, M. H. (2021). Beneficial effects of green tea catechins on female reproductive disorders: A review. Molecules; 26 (9), 2675.

22) Kanlaya, R., & Thongboonkerd, V. (2019). Protective effects of epigallocatechin-3-gallate from green tea in various kidney diseases. Advances in Nutrition; 10 (1), 112-121.

23) KC, Y., Parajuli, A., Khatri, B. B., & Shiwakoti, L. D. (2020). Phytochemicals and quality of green and black teas from different clones of tea plant. Journal of Food Quality; 2020, 1-13.

24) Khadilkar, S. S. (2019). Can polycystic ovarian syndrome be cured? Unfolding the concept of secondary polycystic ovarian syndrome. The Journal of Obstetrics and Gynecology of India; 69, 297-302.

25) Kongchian, A., Keawboonlert, N., Boonrak, T., Lookyee, S., Buasri, K., Surongkul, N., & Tangpong, J. (2020). Anti-hyperlipidemia and anti-obesity properties of *Garcinia atroviridis* and *Camellia sinensis* extracts in high-fat diet mice. Walailak Journal of Science and Technology (WJST); 17 (10), 1126-1138.

26) Lewandowski, K. C., Skowrońska-Jóźwiak, E., Łukasiak, K., Gałuszko, K., Dukowicz, A., Cedro, M., & Lewiński, A. (2019). How much insulin resistance in polycystic ovary syndrome? Comparison of HOMA-IR and insulin resistance (Belfiore) index models. Archives of Medical Science; 15 (3), 613-618.

27) Maleki, V., Taheri, E., Varshosaz, P., Tabrizi, F. P. F., Moludi, J., Jafari-Vayghan, H., ... & Alizadeh, M. (2021). A comprehensive insight into effects of green tea extract in polycystic ovary syndrome: a systematic review. Reproductive Biology and Endocrinology; 19, 1-8.

28) Meng, J. M., Cao, S. Y., Wei, X. L., Gan, R. Y., Wang, Y. F., Cai, S. X., ... & Li, H. B. (2019). Effects and mechanisms of tea for the prevention and management of diabetes mellitus and diabetic complications: An updated review. Antioxidants; 8 (6), 170.

29) Padole, N., Chandankhede, H., Deshmukh, R., Chatakwar, P., Dandekar, S., & Baheti, J. (2022). A review: phytochemical investigation and medicinal applications of herb's. Asian Journal of Pharmaceutical Research and Development; 10 (6), 137-145.

30) Pervin, M., Unno, K., Takagaki, A., Isemura, M., & Nakamura, Y. (2019). Function of green tea catechins in the brain: Epigallocatechin gallate and its metabolites. International journal of molecular sciences; 20 (15), 3630

31) Rehman, A. U., Khan, S. G., Bokhari, T. H., Anjum, F., Akhter, N., Rasool, S., ... & Arshad, A. (2020). Synthesis, characterization, antibacterial, hemolytic and thrombolytic activity evaluation of 5-(3-chlorophenyl)-2-((N-(substituted)-2-acetamoyl) sulfanyl)-1, 3, 4-oxadiazole derivatives. Pakistan Journal of Pharmaceutical Sciences; 33 (2, Suppl), 871-876.

32) Rocha, A. L., Oliveira, F. R., Azevedo, R. C., Silva, V. A., Peres, T. M., Candido, A. L., ... & Reis, F. M. (2019). Recent advances in the understanding and management of polycystic ovary syndrome. F1000Research; 8.

33) Sayed, S., & Raheleh, R. (2019). Comparing the effect of aqueous extract of green tea and catechin on gonadotropins, beta-estradiol, Progesterone, testosterone and ovarian follicle in polycystic ovarian syndrome rat model. Journal of Advances in Medical and Biomedical Research; 27 (125):9-15

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 03 | 2023
DOI 10.17605/OSF.IO/7X3TV

34) Shamim, H., Jean, M., Umair, M., Muddaloor, P., Farinango, M., Ansary, A., ... & Mohammed, L. (2022). Role of metformin in the management of polycystic ovarian syndrome-associated acne: a systematic review. Cureus; 14 (8).

35) Tehrani, H. G., Allahdadian, M., Zarre, F., Ranjbar, H., & Allahdadian, F. (2017). Effect of green tea on metabolic and hormonal aspect of polycystic ovarian syndrome in overweight and obese women suffering from polycystic ovarian syndrome: A clinical trial. Journal of education and health promotion; 6.

36) Tang, G. Y., Zhao, C. N., Xu, X. Y., Gan, R. Y., Cao, S. Y., Liu, Q., ... & Li, H. B. (2019). Phytochemical composition and antioxidant capacity of 30 Chinese teas. Antioxidants; 8 (6), 180.

37) Torello, C. O., Shiraishi, R. N., Della Via, F. I., de Castro, T. C. L., Longhini, A. L., Santos, I., ... & Saad, S. T. O. (2018). Reactive oxygen species production triggers green tea-induced anti-leukaemic effects on acute promyelocytic leukaemia model. Cancer Letters; 414, 116-126.

38) Witchel, S. F., Oberfield, S. E., & Peña, A. S. (2019). Polycystic ovary syndrome: pathophysiology, presentation, and treatment with emphasis on adolescent girls. Journal of the Endocrine Society; 3 (8), 1545-1573.

39) Xu, R., Bai, Y., Yang, K., & Chen, G. (2020). Effects of green tea consumption on glycemic control: a systematic review and meta-analysis of randomized controlled trials. Nutrition & Metabolism; 17 (1), 1-13.

40) Xu, J., Dun, J., Yang, J., Zhang, J., Lin, Q., Huang, M., ... & Lin, Y. (2020). Letrozole rat model mimics human polycystic ovarian syndrome and changes in insulin signal pathways. Medical science monitor: international medical journal of experimental and clinical research; 26, e923073-1.

41) Yu, Z., Samavat, H., Dostal, A. M., Wang, R., Torkelson, C. J., Yang, C. S., ... & Yuan, J. M. (2017). Effect of green tea supplements on liver enzyme elevation: results from a randomized intervention study in the United States. Cancer Prevention Research; 10 (10), 571-579.