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RESEARCH ARTICLE
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QUERCETIN AS THERAPEUTIC SOURCE FOR OVARIAN, UTERINE, AND HEPATO-RENAL PROFILE OF SPRAGUE DAWLEY RATS WITH LETROZOLE-INDUCED POLYCYSTIC OVARIAN SYNDROME

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ABSTRACT: Females of reproductive age are affected by Polycystic ovary syndrome (PCOS) all over the world.Traditional methods of treatment like metformin don,t provide cure but just symptomatic relieve and with many side effects. Current study compared the results in terms of improving the hormonal, biochemical, enzymatic, and histopathological profile along with nephro-renal protective activity in PCOS induced rat models, after treatment with quercetin, (50 mg/kg) in Letrozole (1.0 mg/Kg b. w./day) induced PCOS female *Sprague Dawley* rats (150-200gm) by keeping metformin (50 mg/Kg b.w.) as positive control. Statistically analyzed results ($p<0.0001$) showed that LH, homeostatic model assessment insulin resistance (HOMA-IR), AST, bilirubin and uric acid decreased in PCOS rats after treatment with quercetin, (0.8±0.05ng/dL, 0.07±0.05, 55.6 ±4.0 IU/L, 0.2 ±3.3 mg/dL and 2.3±0.7 mg/dL) as compared to control (2.09 ± 0.08 ng/dL, 0.12 ± 0.01, 181.3 ± 32.0 IU/L, 0.2 ± 0.1 mg/dL, 4.7 ± 0.15 mg/dL) respectively. Restoration in progesterone and estrogen were found significant in experimental groups (13.4±2.3 pg/dL, 53.5± 1.5pg/m\L) as compared to control (9.05±1.2pg/dL, 48± 5.2pg/mL) respectively with restoration of normal ovarian stroma from typical pearl string cystic appearance and normal hepato-renal tissues in histology. So it is concluded that quercetin, can be helpful to cure or mange PCOS .

KEY WORDS: Polycystic ovary syndrome,Quercetin, Metformin

INTRODUCTION

PCOS is worldwide known as a complex metabolic and endocrinal. It effects the reproductive health of females in all over the world with prevalence of about 10% (Maria et al. 2022). Asian countries have shown highest prevalence of this disease. (Bulsara et al 2021). PCOS is heterogeneous syndrome with unclear etiology and wide clinical spectrum.majority of research describes the interactions among various genetic, epigenetic, environmental factors that cause this disease. Along with etiological factors hyperandrogenemia and hyperinsulinemia are found to be the main pathological factors of PCOS, and these both are interrelated (Glueck et al.2019). In PCOS, increased insulin levels causeimpaired metabolic signaling to cells but simultaneously increasessteroidogenesis activity in ovary and decreases hepatic serum hepatic binding globulin (SHBG) synthesis which favors hyperandrogenemia. The PCOS provokes excessive ovarian/adrenal

androgen secretion and. more insulin signaling in (Witchelet al. 2019).The hyperinsulinemia-hyperandrogenemia cycle led to more growing follicles in women with PCOS and cause premature growth arrest of antral follicle. All gives classical ovarian phenotype with string of pearl like appearance on ultrasound (Azziz et al.2019).

PCOS being a complex disease is always challenging to treat. Due to associated risks like obesity, cardiovascular diseases, infertility, and diabetes mellitus the impact of PCOS on life of patient and governments is huge. (Joham et al. 2022). Although many pharmacological treatments are available now that give symptomatic relief to PCOS patients like clomiphene citrate, metformin, oral contraceptive e.g., cyproterone acetate etc (Gholamali.et al. 2012)but the usage of all these is usually associated with many side effects (Jianget al. 2022). Most common medicine used to cure symptoms of PCOS is metformin. This drug is known to lowers blood glucose by increasing peripheral glucose intake and inhibition of hepatic gluconeogenesis so improve peripheral insulin resistanceso it also effects in improving obesity, hirsutism, triglycerides, and high-density lipoprotein cholesterol levels in patients with diabetes and PCOS (Garzia,et al. 2022). At molecular level, this drug inhibits the mitochondrial respiratory chain in the liver, leading to activation of AMPK, pathway enhancing insulin sensitivity (via effects on fat metabolism) and lowers cAMP, thus reducing the expression of gluconeogenic enzymes. Metformin also has AMPK-independent effects on the liver that may include inhibition of fructose-1, 6-bisphosphatase by AMP (Al Khalifah et al. 2016). Although metformin has many beneficial effects, but it is also related to someserious side effects like lactic acidosis, hypoglycemia, high fever, dehydration and increase incidence of myocardial infarction(Hamid and Mahmoud;2014). To investigate and discover new domains of management or treatment of a PCOS many plant-based drugs can be good alternatives as they contain natural compounds so can be potential therapeutic substitutes of metformin in curing disease. Plants contain flavonols and phytoestrogen and are useful to cure PCOS because they have potential to decrease hyperinsulinemia and hyperandrogenemia(Rosenfield et al. 2016).Quercetin is flavanol, found in citrus fruit, berries,onion, and tea plants. It has many antioxidant properties (Anand et al 2017), decreases blood glucose and liver glucose content, regenerate beta pancreatic cells and efficiently increase insulin release from them (Ay et al 2017). This research project gives an opportunity to further study the effects of quercetin,on PCOS being a potential therapeutic agent to reduce hyperinsulinemia.

MATERIALS AND METHODS

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. Animals were fed with a standard laboratory diet and gave free access to water.Sick and ailing rats were excluded from the study. Animals were divided into the followingfour groups with three replications each as follows;

1. Vehicle received water only
2. Negative control group has PCO induction with 1mg Letrozole/ kg b.w. of rat for 21 days (Alkalby, et al. , 2017)
3. Positive control group has PCO Induction+metformin (50 mg/kg b.w. of rat) for 21 days (Fatima,et al., 2021)
4. Experimental group was PCO Inducted+ Quercetin (50-mg/Kg) of quercetin,administered as intra peritoneal (Oza,et al. 2019)

Collection and analysis of vaginal smears and blood

PCOS induced rats have persistent diestrous phase. Throughout the study period, animals were weighed every week, while rats had been holed 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).
After one week of extract induction, rat was dissected by open chest method to collect blood in vacutainer tube systems and placed vertical upright position in racks in transportation boxes and stored in the dark, at 4 °C (Elmlinger et al. 2011).

Histopathological analysis
Ovaries, uterus, pancreas, liver and kidneys were preserved in 10% neutral formalin (MERCK) and 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 hematoxylin (BIOFAR) and eosin and examined at 40 µm under microscope (model number XSZ-107BN ,made in USA).

RESULTS

Physical Appearance of Vaginal Smears
Stained vaginal smears of rats in the control group showed regular proestrous, estrous, diestrous and metestrous phases. In proestrous phase [Figure 1 (I)] well-formed round nucleated epithelial cells in clusters were seen. Estrous phase [Figure 1 (ii)] was characterized by cornified squamous cells found in clusters. The diestrous phase [Figure 1(iii)] was characterized by prominent leukocytes with few epithelial and cornified cells. The metestrous [Figure 1 (iv)] has shown large number of leucocytes and a small number of large, non-granular and anucleated cornified epithelial cells. The PCOS induced rats showed persistent diestrous phase with prominent leukocytes [Figure 1(v)].

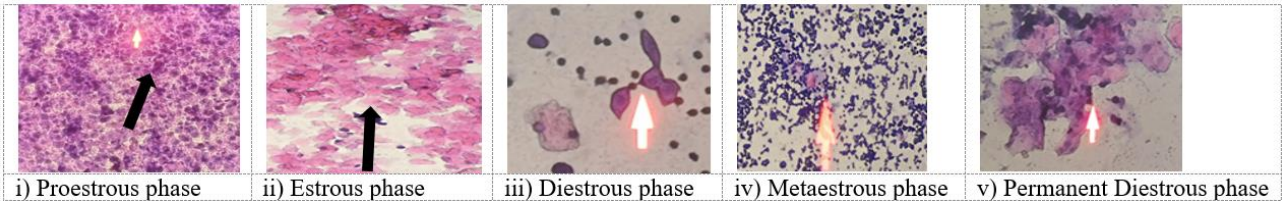
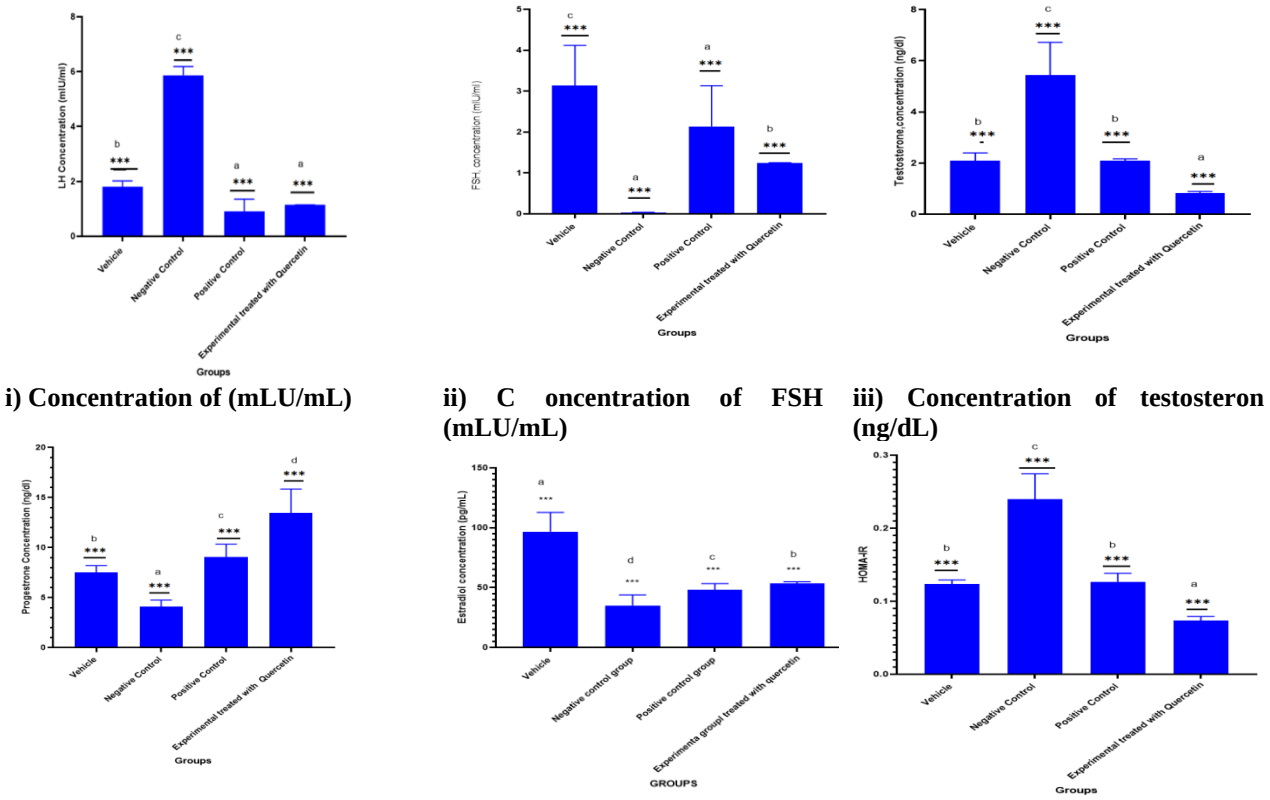


Figure 1 Phases obtained at vaginal smears

Hormonal Profile of Animals
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 become decreased significantly in experimental groups with quercetin, and reached to value of normal vehicle group (1.1± 0.0mLU/mL)but maximum reduction of LH level has been done in animals treated with positive control drug metformin (0.8± 0.5mLU/mL) (Figure 2(i)).
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). FSH level in positive control group was found (2.5±1mLU/mL).Experimental group quercetinhas increased the FSH level to (1.24±0.01mLU/mL) ($p<0.0001$) {Figure 2 (ii)}.
Statistically analyzed results showed significant elevation in testosterone levels in negative control group (6.15±1.2 ng/dL) as compared to vehicle (2.0±0.29 ng/dL). In experimental group, testosterone level become decreased maximally (0.8±0.05 ng/dL) at ($p<0.0001$) when compared to positive control group (2.09±0.08 ng/dL) {Figure 2 (iii)}.
Statistically analyzed results showed that in negative control group, there was significant decrease in progesterone level (4.1±0.6 pg/dL) as compared to vehicle (7.5±0.5pg/dL). Restoration of progesterone levels was more evident in rats treated with quercetin, (13.4±2.3 pg/dL) as compared to positive control (9.05±1.2 pg/dL) at $p<0.0001$ {Figure 3.2 (iv)}.
Statistically analyzed results showed that level of estradiol significantly decreased in negative control group (28.3± 20.12 pg/mL) as compared to vehicle (96.6 ± 16.07 pg/mL) ($p<0.0001$). This

decreased level restored in experimental group (53.5± 1.5 pg/mL) maximally in comparison of control drug metformin induced group (48± 5.2 pg/mL {Figure 2 (v)}.
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). Animals treated with quercetin,have lowest HOMA-IR mean value (0.07±0.05) {Figure2 vi)}.



i) Concentration of (mLU/mL) **ii) C oncentration of FSH (mLU/mL)** **iii) Concentration of testosteron (ng/dL)**

iv) Concentration of progestrone (ng/dL) **v) Estradiol concentration (pg/mL)** **vi) HOMA-IR**

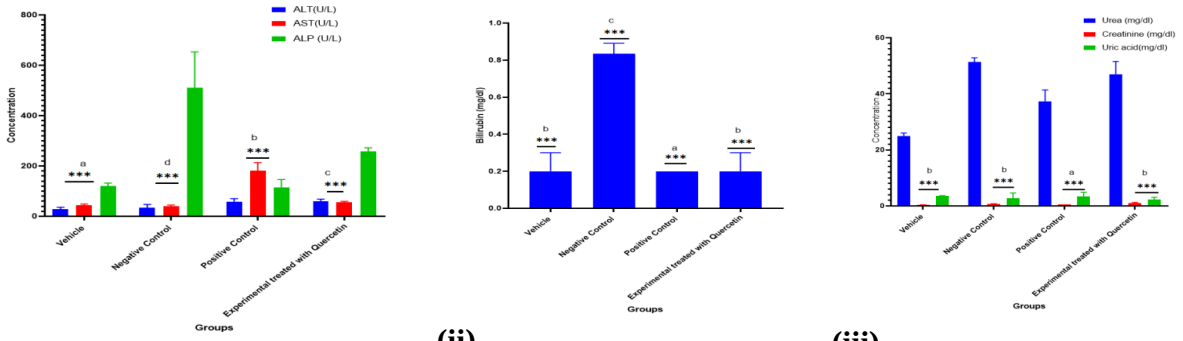
Figure 2 Hormonal profile of animals.

(i) LH concentration **(ii)** FSH concentration **(iii)** Testosterone concentration **(iv)** Progestrone concentration **(v)** Estradiol concentration **(vi)** HOMA-IR. (a – d) = Comparison of animal groups from most significant results to less significant. ***= P<0.001, **= P<0.01, *= P<0.05

Enzymatic and Biochemical Profile of animals

Statistically analyzed data showed that ALT levels has been significantly increased in positive controlled group (33.3.6 ±14.0 IU/L) as compared to vehicle (28.6 ±7.09 IU/L). In experimental group, ALT levels slightly decreased (56.5±11.8 IU/L) as compare to positive control (58.3 ±11.8 IU/L). Serum AST level decreased in negative control group (40.3±4.0IU/L), as compared to vehicle (44.3 ±3.7IU/L), while in experimental group, it decreased (55.6 ±4.0 IU/L) as compared to positive control (181.3 ±32.0 IU/L). 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 experimental group (257.3 ±15.7U/L) {Figure 3 (i)}.
Analysis of bilirubin showed its slight elevation in negative control group (0.8±0.1 mg/dl) as compared to vehicle (0.2 ±0.05 IU/L). This level became normal when rats were treated with control drug (metfromin) and quercetin, .In both groups bilirubin remain same (0.2 ±3.3 mg/dL). In positive control group bilirubin level was found 0.2 ± 0.1mg/dL) {Figure 3 (ii)}. Statistical analysis showed that blood urea significantly increased in negative control group (51.3 ±1.5 mg/dL) as compared to vehicle (25.1±1 mg/dL). In Positive control group, blood urea mean level was found 41.3±1.5

mg/dL which was less than in animals treated with quercetin, urea level (47.3 ±4.58mg/dL). After statistically analysis of creatinine, it was analyzed that it was significantly increased in negative control group (0.72 ±0.1 mg/dL) as compared to vehicle (0.31±0.05 mg/dL). In positive control group, creatinine mean level was 0.8±0.1 mg/dL while in rats treated with quercetin, it was slightly increased (1.03±0.25 mg/dL). Statistically analysis of uric acid showed that it was significantly decreased in negative control group (2.7±1.93 mg/dL) as compared to vehicle (3.5 ±0.15 mg/dL) and positive control group (4.7 ±0.15 mg/dL) while it was almost equal to experimental group animals (2.3±0.7 mg/dL) {Figure 3 (iii)}.



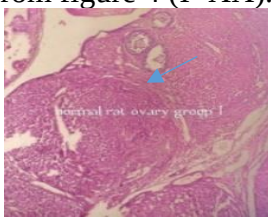
(i) **(ii)** **(iii)**

Figure 3 (i) Enzymatic profile (ii) Concentration of serum Bilirubin (iii) Urea ,Creatinine and Uric acid concentration

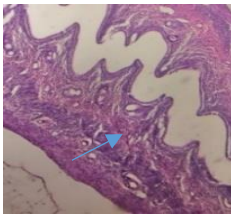
(a – d) = Comparison of animal groups from most significant results to less significant. ***= P<0.001, **= P<0.01, *= P<0.05

Hisological Profile of animals

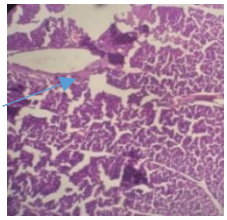
Histological analysis revealed multiple changes in ovarian, uterine, hepto-renal and pancreatic tissues which were ranging from normal to toxic levels in different groups of animals and is evident from figure 4 (I- XX).



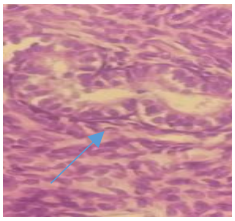
(I) Ovarian parenchyma shows corpus leuteum



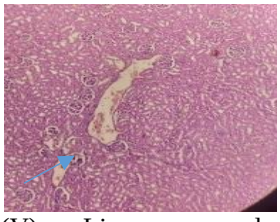
(II) Uterus endometrium and myometrium are un remarkable



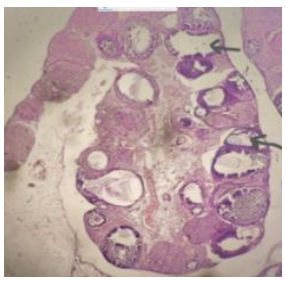
(III) Pancreas. Showing islets of Langerhans



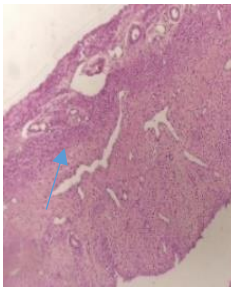
(IV) Kidney unremarkable with normal glomeruli tubules and interstitium



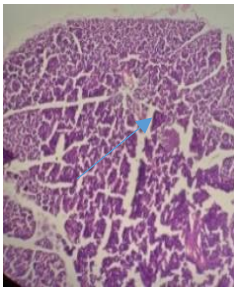
(V) Liver normal hepatic lobule, no steatosis, hepatocystitis,balloonin g or inflammation



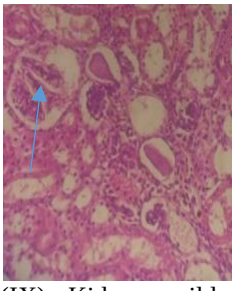
(VI) Ovary Multiple sub capsular cystic follicles are seen like pearl of strings



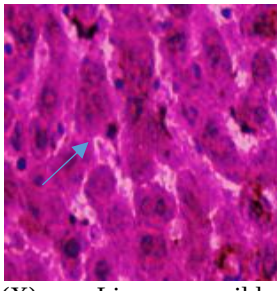
(VII) Uterus endometrium and myometrium are unremarkable



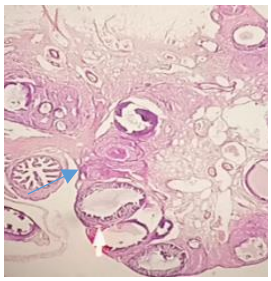
(VIII) Pancreas unremarkable



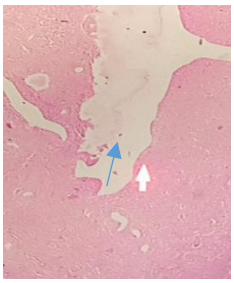
(IX) Kidney mild tubular interstitial nephritis and intra tubular casts are seen



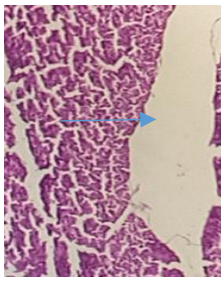
(X) Liver mild peripheral inflammation



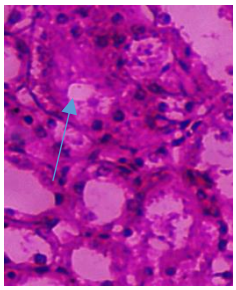
(XI) In ovary relatively few cystic follicles are seen and corpus leuteum is also seen



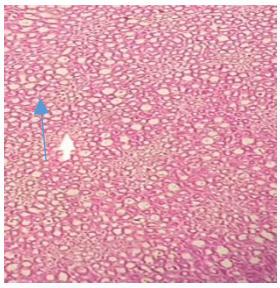
(XII) Uterus endometrium and myometrium are unremarkable



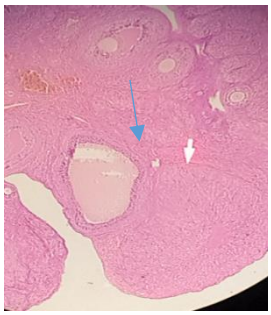
(XIII) Un remarkable Pancreas showing islets of Langerhans



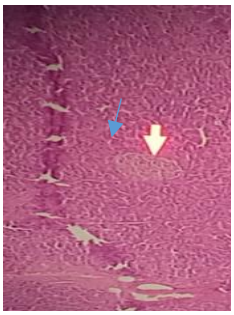
(XIV) Kidney unremarkable glomeruli, tubules and interstitium



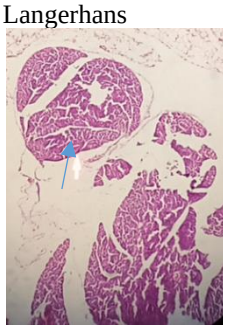
(XV) Liver shows mild peripheral inflammation



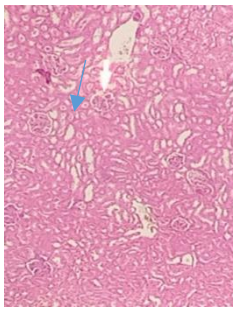
(XVI) In ovary 3 to 4 residual cystic follicles are seen with corpus leuteum



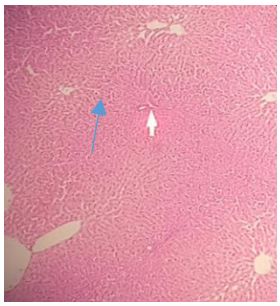
(XVII) Uterus proliferative endometrium and myometrium is unremarkable



(XVIIII) Un remarkable Pancreas showing islets of Langerhans



(XVIX) Kidney unremarkable glomeruli, tubules and interstitium



(XX) Liver shows normal triads and central vein

Figure 4 Histopathological features of animals at (40 µm)

Vehicle (I-V), Negative control group (VI-X), Positive control group (XI-XV), Experimental group (XVI-XX)

DISCUSSION

Being acomplicated and multifaceted disease it is difficult to cure and manage PCOS. Currently many drugs including metformin is used to treat the PCOS patients. Metformin is a biguanide and known to lowers blood glucose by increasing peripheral glucose intake and inhibition of hepatic gluconeogenesis. It alsoreduce the PCOS related hyperinsulinemia by increasing the insulin sensitivity at hepatocytes and peripheral tissues and it also reduce hyperandrogenemia,which arethe main culprits of thisdisease. Research proved that the treatment with metformin decrease the serum LH, and testosterone and restore FSH, estradiol and progesterone levels (Jiang S et al. 2022).

In current study PCOS model in female Sprague Dawley rats was induced by us of Letrozole. Letrozole isa third generation of non-steroidal aromatase inhibitorsand it act by preventing conversion of testosterone in estradiol (Arentz et al.2017). This increased level of testosterone level mimics the PCOS picture by increasing the LH, and serum testosterone while decreasing FSH, estradiol and progesterone. Incurrent study all leterozole induced animals exhibited PCOS characters as explained in previous studies (Maharjan et al. 2010).Current study compare the results of metformin and quercetin, in terms of improving the hormonal, biochemical, enzymatic, and histopathological profile improvement along with nephro renal protective activity in PCOS induced rat models. Quercetin,is a natural polyphenols it is known to decrease blood glucose and liver glucose content and due to antioxidant abilities it regenerate beta pancreatic cells and efficiently increase insulin release from them.It was reported that estrous cyclicity of PCOS induced rat model became improved after quercetin,administration. In a few recent clinical trials, it was proved that natural polyphenols like quercetin, is helpful in reducing insulin resistance and reduce androgens (Wang et al.2017).In our study we found that after administration of quercetin, a significant decrease in LH, testosterone level as compared to metformin.This positive finding of quercetin is in

accordance of (Banaszewska et al. 2016) but no current study has been found that compared the effects of quercetin,with metformin on PCOS. After giving quercetin,, it was also observed that there is increased of serum FSH, progesterone and estradiol levels as compare to metformin .No comparative study between quercetin, and metformin found to our knowledge but same promising results of quercetin,alone is reported by (Jahan S et al2018).More over in our study improvement of insulin resistance by quercetin, is another metabolic finding when calculated by HOMA-IR in comparison to metformin .This improvement is due to significant decrease in blood glucose level and serum insulin concentration. This finding is also in same, reported in (Shah et al.2016). Besides hormonal profile, biochemical investigations of blood were also done to rule out any hepto- renal protective role of quercetin,Serum transaminases alanine transaminase (ALT), Aspartate transaminase (AST) and alkaline phosphatase (ALP) elevation shows the liver injury and functional disturbance of liver cell membranes, while raised bilirubin shows derangement in the transport of metabolites, which is the main cause of functional disturbance of hepatic cells.Letrozole causes mild liver injury and interstitial nephritis as described by (Gharia et al2017) and (Puri, et al 2020). In current study there was restoration of hepatic functions with quercetin, was observed in comparison of metformin .The anti oxidative role of quercetin, on liver is reported by many studies (Zhao et al 20121)most research articles reported nephroprotective role of quercetin,like (Yang et al2019) but current study found a slightly increased of renal enzymes as compare to metformin Histological improvement in PCOS induced rat model withquercetin, is another parameter of our study. Improvement in cystic appearance is clearly seen in our quercetin,giving rat model while uterus and pancreas remain normal. This histo morphological improvement is also reported by (Neisy et al. 2019)

CONCLUSION

It is concluded that the quercetin, as compare to metformin had shown more promising results to cure PCOS by regulating hormonal, biochemical and ovarian architecture in PCOS models,.In this context this study can be helpful for pharmaceutical companies to find further cure PCOS.

Patent

Not applicable

Authors Contribution

Dr Asma Ahmed supervised the whole work, designed the project, and gave final approval to this manuscript. Ghazala Waris performed whole experiment in lab, Search the literature for this manuscript, provided English editing services, and wrote first draft of this manuscript. Rehana Badar collected the literature and provided technical support and English editing services for this manuscript, Provided English editing services. Hasan Akbar Khan, Provided English editing services and his support for statistical analysis of the data. Samra Hafeez helped in preparation of plant extracts and provided technical support.

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Current research work has been approved by ethical approval committee of institute of molecular biology and biotechnology, The university of Lahore under approval No(Approval No: USM/Animal Ethics approval/2009/[45] [140])

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Data Availability Statement:
Not applicable

Conflict of Interest:
All authors declare no conflict of interest.

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Sample Availability:
Not applicable

Abbreviations:
PCOS Polycystic ovary syndrome

Limitation of Study:
The current data can be used for the further in vivo trial of active compound (S) through chromatographic techniques.

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