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Effect of Berberine hydrochloride on bone morphogenic protein 4 in hyperandrogenic polycystic ovarian syndrome female rats

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ABSTRACT
Berberine (BBR) is a plant alkaloid and a quaternary ammonium salt that can be seen in a variety of plants of Berberidaceae family. BBR has been used for thousands of years for its antimicrobial, antihypertensive, antitumor, lipid lowering and antihyperglycemic effect. Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age, with an incidence of 5-10% according to women's health center statistics. Hormonal abnormalities that are associated with PCOS may have a rule in its etiology. 80% of females with PCOS presented with symptoms of hyperandrogenemia, elevated serum levels of androgen together with decreased estrogen, may contribute to negative feedback on the release of FSH and LH hormones from the pituitary gland. Bone morphological protein -4 has a major rule in the biosynthesis of steroid hormones in the zona reticularis of the adrenal gland and in the gonads. Through its modulatory effect on cyp17A1. Cyp17A1, a 56 kDa enzyme located in endoplasmic reticulum considered to be the rate-limiting enzyme responsible for the conversion of cholesterol to androgen in the theca cells , this enzyme found to be overexpressed in women with PCOS.

Keywords: hydrochloride, morphogenic, protein, rats.

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INTRODUCTION
Berberine (BBR)
Berberine is a plant alkaloid (figure-1) that can be seen in a variety of plants of Berberidaceae family all over the word including Coptidis Chinensis Franch, Phellodendron chinense, Berberis vulgaris, Berberis aristata (Tree turmeric), Hydrastis Canadensis (goldenseal) and many other spices of plants (1,2,3).

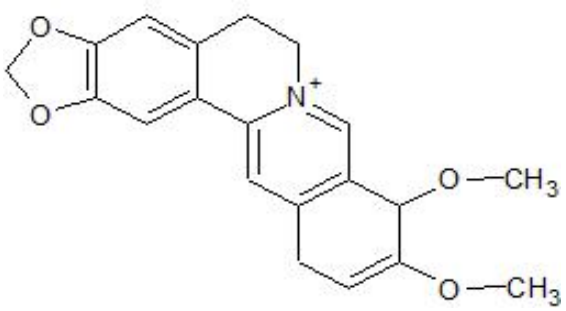

Fig.1: Structure of Berberine, Chem Sketch 2019

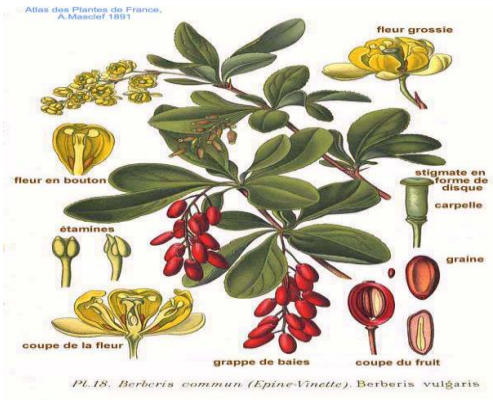

Fig.2:Berberis vulgaris (2).

In this study Barberry Berberis vulgaris was used (figure 2) for its availability and previous knowledge of its effect in different folk medicine. BBR has been used for thousands of years to reduce blood glucose and weight reduction which both can be useful in individuals with PCOS(4) (5) (6) .

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age, with an incidence of 5-10% according to women's health center statistics (7,8). PCOS is characterized by features including clinical hyperandrogenism (hirsutism) and biological hyperandrogenemia (elevated serum levels of androgen), ovulatory arrest (menstrual dysfunction), thickened ovarian Stroma, polycystic ovarian appearance on Ultrasonography and, infertility (8). PCOS is usually accompanied by metabolic disorders including diabetes mellitus (increased insulin resistance) and obesity (9).

Hyperandrogenemia in PCOS

80% of females with PCOS presented with symptoms of hyperandrogenemia ,hirsutism and acne (10) . This elevated levels of androgens contribute to a positive feedback on the ovaries , result in further production of ovarian androgen. This is due to an overexpression of enzymes involved in androgen biosynthesis in the theca cells . Cyp17A1, the rate-limiting enzyme responsible for the conversion of cholesterol to androgen in the theca cells found to be overexpressed in women with PCOS (11). The activation of p38 MAPks (mitogen-activated protein kinase) by a variety of environmental factors particularly p38a isoform will increase the activity of 17-20 lyase through phosphorylation also increases hyperandrogenemia (12).

Bone morphological protein -4 (BMP-4)

BMP-4 has a major rule in the biosynthesis of steroid hormones in the zona reticularis of the adrenal gland and in the gonads (13).BMPs are group of signaling proteins that involved in bone synthesis (14). It is now accepted that BMPs are involved in signaling far beyond bone synthesis . BMPs are a member of Transforming growth factor –β family (TGF- β) which includes activins , inhibins, anti-Müllerian hormone and many other proteins (15). There is about (15) subtypes of BMPs isolated and differentiated in human body (16). Most of BMPs play an important role in chondrogenesis and osteogenesis like, BMP2 and BMP12 (17). BMP4,6 and 7 are involved in sex hormone synthesis in theca cells (18). BMP4 is highly expressed in the adrenal gland cortex (zona glomerulosa , zona fasciculata and zona reticularis), and involved in steroidogenesis (19).Through its modulatory effect on cyp17A1, BMP4 can affect the androgen synthesis, and thus its expression can be important in PCOS. A study in 2015 shows that the level of BMP4 expression is directly correlated with the mRNA expression of the steroidogenic enzyme CYP17. In this study, (Rege et al. 2015) claimed that increased expression of BMP4 has a negative feedback effect on the expression of CYP17 enzyme in adrenal gland with no effect on other steroidogenic enzymes CYP11A1 and HSD3B2 .It was found that treatment of the cell line of adrenal cell with BMP4 resulted in decreased levels of DHEA and androstenedione parallel to an increase in pregnenolone, progesterone, 17_-hydroxyprogesterone, and 11-deoxycorticosterone (13) .In another study, It was found that treating the theca cells with a certain dose of BMP4 will result in a significant reduction in the production of Androstendion and progesterone even in the presence of LH stimulation (18). Normal BMP4 inhibit the synthesis of Androgen (testosterone) in the ovarian theca cells and promote the production of Estrogen . In Theca cells BMP4 binds to its receptors (BMPRI & BMPRII) this leads to phosphorylation of smad and p38 pathways , smad complex is then transferred to the nucleus to promote CYP19A1 synthesis and suppress the expression of CYP17A1 thus increase estrogen and decrease androgen synthesis . When there is an increase in serum levels of androgen in PCOS , this will result in suppression of ovarian production of BMP-4 by increase expression of androgen receptors (AR) . Decreased expression of BMP4 results in increase CYP17A1 and decreased CYP19A1 expression leading to further increase in androgen production (figure 3) (20).

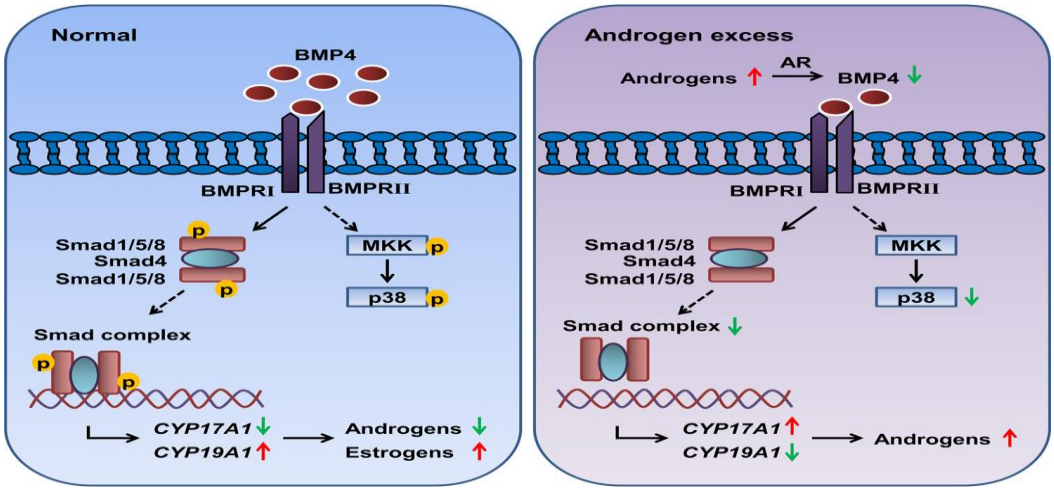

Fig.3: how BMP-4 modulate the androgen synthesis through its effect on CYP17A1 (20).

The aim of this study is to determine the effect of BBR on serum levels of BMP4 and is it possible that BBR by effecting BMP4 can reduce CYP17A1 and hence reducing hyperandrogenemia in PCOS?

MATERIALS AND METHOD

Chemicals and instroments that were used in this study all were of analytical grade . supplement BBR capsules 450 mg (Berberine hydrochloride 60% extract) was obtained from aSquared nutrition –USA, Chloroform was obtained from (SDFCL chemicals-India) , Sesame oil from (Spectrum chemicals) and Testosterone propionate from ORGANON OSS Holland. The hematoxyline and eiosen stains for histopathological study were obtained from (SyrBio-Switzerland) . The labretory instroments , Centrifuge (Hettich Universal – Germany) , Heated paraffin embedding Module Leica EG1150 H (Leica-biosystems – Germany) , Semi-automated rotary microtome Leica RM2245 (Leica-biosystems – Germany) and Microplate reader (MyBiosource-USA) were used .

The BMP4 and CYP17A1 sandwich ELISA 96 well kits were obtained from (MyBiosource-USA) .

Animals

Ethical approval

Animals used in this study were handled according to the ethical principles of laboratory animals protocols .

Animal mating

seven (200-250) gram body weight female Wistar rats were kept in dams for mating with males in controlled condition of (22-25 °C) degrees centigrade ,free access to food and water and dark environment to allow mating . Animals were kept in the animal house of collage of pharmacy / Mustansiriyah University. Pregnancies were confirmed by observation of vaginal plug . Pregnant females then separated from the males and each one placed in a single dam with free access to tab water and food with 12/12hours light / dark cycle for 28 days of pregnancy .

New born animals

Total number of 30 female offsprings were obtained . The animals were divided in to 3 groups (control (n=10) , PCOS (n=10) and BBR (n=10))

Induction of PCOS

At day 21 after birth animal received 1mg /100g testosterone propionate s,c injection at the dorsum of the neck for the following 28 days. A 250 mg/ml testosterone ampoule mixture of different salts (SustanonR 250mg/ml ,ORGANON OSS Holland , made by Holland) was diluted in 24ml exactly and carefully measured sesame oil (Spectrum Chemical 500 ml) obtaining a 10mg/ml concentration of testosterone .A 1ml insulin syringe then used to withdraw the final dilution so that each unit in the syringe (0.01 ml) will contain 0.1 mg testosterone propionate . The rats then received 1mg/100 g body weight for 28 days .For conformation of PCOS 5 rats were sacrificed . The ovaries were extracted and stained then examined under light microscope .

Berberine hydrochloride

BBR supplement (Berberine hydrochloride 450 mg/cap ,aSQUARED NEUTRITION ,USA) was obtained for oral administration by gavage for BBR treated group . Two capsules were emptied in a 50 ml beaker and carefully weighed for 900mg of Berberine . 9 ml on sesame oil was added to the beaker and thoroughly mixed to produce a light yellowish suspension .Each ml of the suspension contain 100mg of BBR .Using an insulin syringe (each 1 unit contain 1 mg BBR) the exact does for each rat was precisely calculated according to the body wt. 100 mg BBR/kg was chosen to be the dose to be given to the animals on daily bases for 28 days .

Body weight calculation

Animals body weight was measured on weakly basis from week 3 (PCOS induction) to week 7 (day of sacrifice) .

Blood sample collection

Blood was withdrawn from the animals in two different occasions ,first at the start of the induction of PCOS (retro orbital puncture) and then at the day of the sacrifice (cardiac puncture). For retro orbital puncture , The animal was anesthetized using chloroform (SDFCL chemicals-India) then a capillary tube was used to draw blood from the retro orbital sinus (21), then a jell tube was used to collect the blood. Blood sample then centrifuged at approx. 1000rpm for 20 min to separate the serum. For Cardiac puncture , the animal was anesthetized using chloroform (SDFCL chemicals-India). A 10 cc syringe was used to draw blood from the left ventricle . blood was transferred to a jell tube and allowed to clot for 2 hours then centrifuged at 1000rpm for 20 min. All serum sample were placed in Eppindrof tubes and stored at(-40 C) deep freeze to be used for measuring bl. Hormones and markers .

Measurement of BMP4 and CYP17A1 enzyme in serum

For measurement of BMP4 and CYP17A1 concentrations . ELISA technic was used . The 96 well that are provided with the ELISA kit are pre-coated previously with a monoclonal antibody specific to steroidgenic proteins (BMP4 and CYP17A1). A specific standard or the sample is then can be added to the wells so that the specific antigen can interact with the capture antibody inside the wells. Biotin antibody is used for the detection in which after the addition of Biotin antibody , it will react with captured antigen. Avidin-Horseradish Peroxidase (HRP) is then used to interact with biotin conjugated antibody in the wells. A coloring agent is then added to the wells (TMB substrate) which interact with (HRP) causing blue discoloration of the wells . a stop solution consisting of sulfuric acid is then added to end the color change . the samples then placed in micro plate reader to read their optical density on 450 nm .

histopathological studies

Ovaries were harvest as mentioned earlier and then placed immediately in 10% buffer formalin for 2 days.

Tissue preparation and fixation was carried out using Bancroft and Stevens histological technique (22) . Tissue was withdrawn from formalin buffer and cut in to half to facilitate the absorption of alcohol during fixation .ovaries then placed in specially designed cassette and thoroughly washed with distilled water. After fixation, dehydration and wax imbedding , the tissue was sectioned using semi-automated microtome (Leica-biosystems – Germany) at 5 micrometer thickness. The tissue is then stained using Hematoxylin and Eiosen stains then fixed on slides and examined using light microscope (Leica-biosystems – Germany) .

statistical analysis

The statistical analysis of this study was performed using one way and two way ANOVA in addition to Least significant differences (LSD) using SAS (Statistical Analysis System - version 9.1). for determination of the significance value a post hoc equations were performed with a confidence of 95% , $P < 0.05$. using SPSS software .

RESULTS

Serum Testosterone concentration

Table 1 : serum free testosterone in nmol/l for all treated groups pre and post treatment there is a significant decrease in serum testosterone concentration in BBR croup compared to PCOS group (P<0.05).

group	Pre treatment Free testosterone nmol/L	Free testosterone nmol/ L After treatment
control	1.3±0.24	1.3 ±0.24
100 mg /kg BBR	7.2±0.92	1.72 ±0.47
PCOS	8.1±1.01	7.6 ±1.55

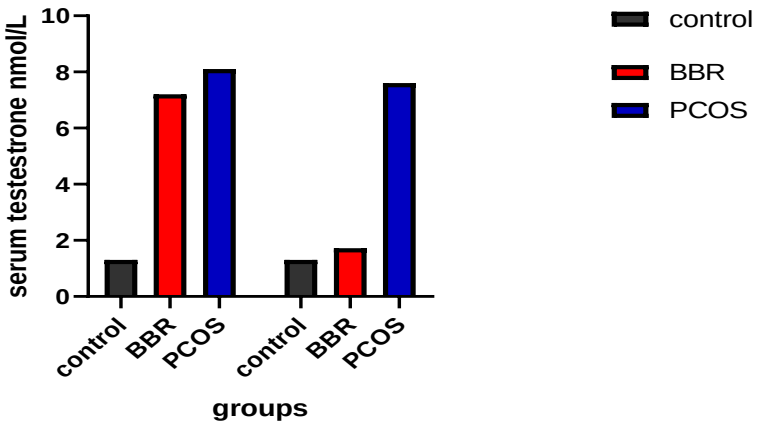

Fig.4 : serum free testosterone in Nano mole / liter , in pre and post treatment , black column represent control , red represent BBR , blue represent PCOS , and purple represent cyproterone

As seen in figure (4) there is a significant difference between the BBR treated group and PCOS group in pre and post treatment when compared to control group P value (< 0.05) . This indicates that testosterone levels was reduced in PCOS group after administration of 100 mg/kg BBR.

CYP17A1 concentration using sandwich ELISA

Cyp17A1 enzyme concentrations were plotted in a chart with (pre), representing pretreatment and (post), post treatment (figure 5) . compared to PCOS group (blue columns) , BBR treated group showed a significant decline in the enzyme activity($p<0.05$) .

Table 2: CYP17 A1 steroid genic enzyme concentration in all groups in pre and post treatment . Means with a different small letter in the same column significantly different (P<0.05). Means with a different capital letter in the same row significantly different (P<0.05).

CYP17A1	Pre	Post
Control	A3.14±0.52c	A3.06±0.72b
BBR	A8.86±0.49b	B2.85±0.27bc
PCOS	A10.07±1.06a	A9.91±1.03a
LSD	0.8896	

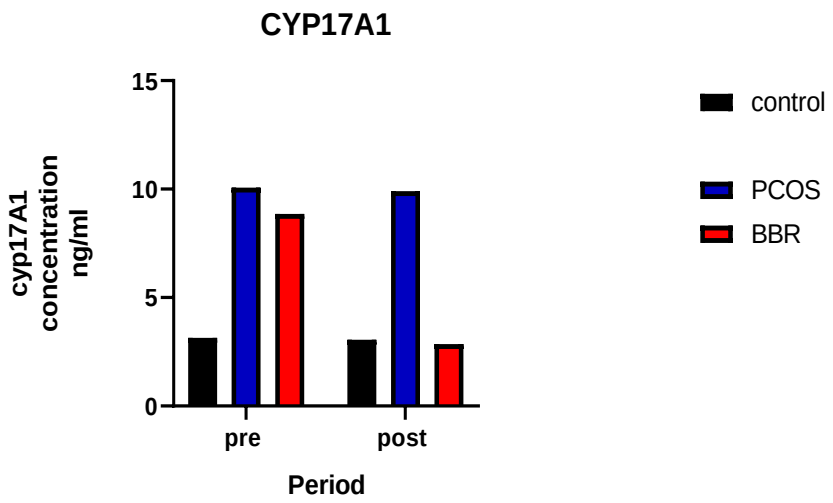

Fig.5: cyp17A1 enzyme concentration in ng/ml measured using sandwich ELISA technic , in all animals in pre and post treatment period

BMP4(bone morphogenic protein -4) serum concentration

Table 3: BMP4 mean concentration in pg/ml . Means with a different small letter in the same column significantly different (P<0.05). Means with a different capital letter in the same row significantly different (P<0.05).

BMP4	Pre	Post
Control	A88.30±5.84a	A89.86±8.49a
BBR	B18.23±4.08b	A96.21±12.59a
PECOS	A20.79±4.33b	A20.72±2.64c
LSD	11.189	

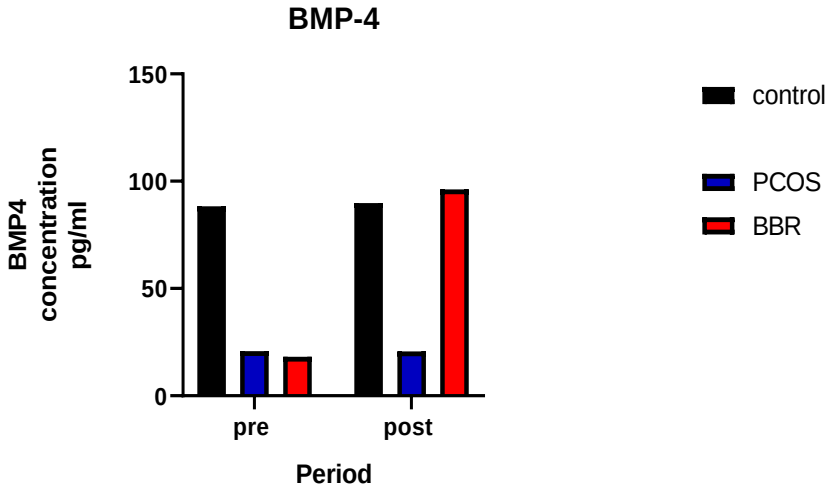

Fig.6: BMP-4 concentration in pg/ml measured using sandwich ELISA technic , in all animals . (pre) represent pretreatment , (post) represent post treatment

As shown in figure (6) BBR treated group showed a significant increase in BMP4 ($p < 0.05$) when compared to the PCOS group . BBR treated group showed an increase in BMP4 serum level from (18.24 pg/ml) in pretreatment (figure 6) to (96.21 pg/ml) in post-treatment compared to (88.3 pg/ml) in control and (20.73 pg/ml) in PCOS group.

Body weight changes

Average body weight. of BBR treated groups were compared to the other groups(control and PCOS).

Results revealed that the differences in the BW. of the control group was not significant through advanced period(P>0.05). The differences were significant (P≤ 0.05) in the BBR as the BW decreased significantly, whereas the PCOS group showed significant increase (P≤ 0.05) along with advanced period.

Concerning the differences among groups within each period, results obtained that the differences were not significant after one week whereas the differences were significant (P≤ 0.05) for other periods. After two weeks, the BW of all groups were significantly (P≤ 0.05) higher than control.

Table 4 : Average body weight comparison during the 4 week treatment period (28 days) . Means with a different small letter in the same column significantly different (P<0.05).Means with a different capital letter in the same row significantly different (P<0.05)

	Week1	Week2	Week3	Week4
Control	A193.60±5.72a	A188.80±4.32c	A189.00±2.54c	A189.60±4.16b
BBR	A199.92±7.45a	A205.68±3.84a	B185.04±5.95c	C177.40±5.59c
PCOS	C198.60±2.60a	C202.00±3.24a	B212.00±3.08a	A241.40±14.09a
	6.7883			

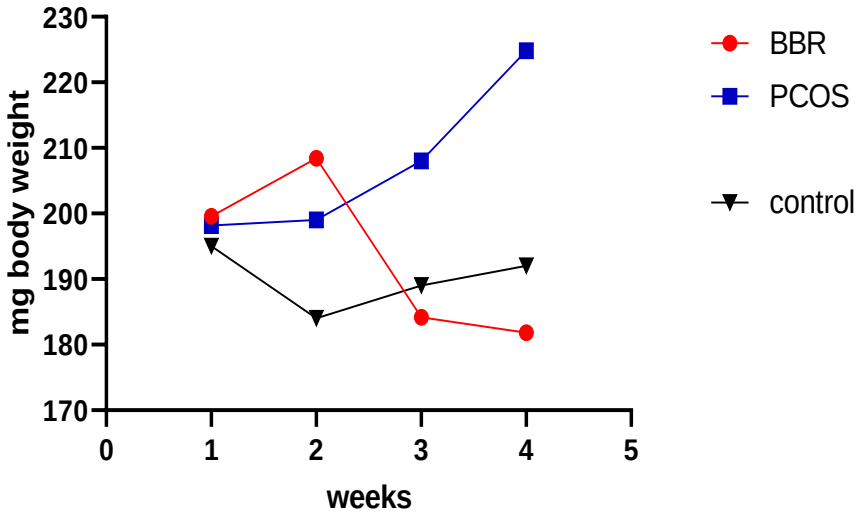

Fig.7 : Average body weight of BBR treated group (red line) compared to PCOS (blue line) , control (black line) .

Histopathology

The ovarian medulla is very small in control group, as seen in figure (8 d,e), much smaller than the PCOS group, high blood supply, and small ovarian volume. The most notable observation was the thickness of the ovarian cortex (stroma) (e), which is barely noticeable, which is the typical case in the female ovary. Ovarian follicles can be seen in various stage of development(8 a), (PF, SF) in addition to the presence of corpus luteum (CL) . Regarding PCOS group the ovarian medulla is more noticeable as seen in figure (9 b) at x100 ,starting from ovarian Hilum which attach the ovary to the tube and run down around the follicles . less blood supply can be seen compared to control group . the ovary is rather large in size compared to control group ,there is complete absence of corpus luteum and large number of primary immature follicles at the ovarian cortex . fewer number of secondary and mature follicles and a large number of small and large ovarian cysts . As can be seen in figure (9 c) x400 and in figure (10 d) x400 the cortex is very thick compared to control group.

In BBR group we can notice various changes that the ovaries gone through after the treatment of the PCOS disease using BBR. Figure (11 A&B) shows an over view of the rat ovaries at x40 on the light microscope ,here we can notice the absence of the large ovarian cysts (a characteristic feature of PCOS) and more fleshy texture of the ovarian tissue .the cortex is very thin ,with well diffused OM. In slide C&D on x100 we can see the SF,MF,CL and some blood vessels .in slides E&F on x400 we can notice the presence of few number of PF(around 3-5 in each ovary) and MF .

Microscopic examination of the control group

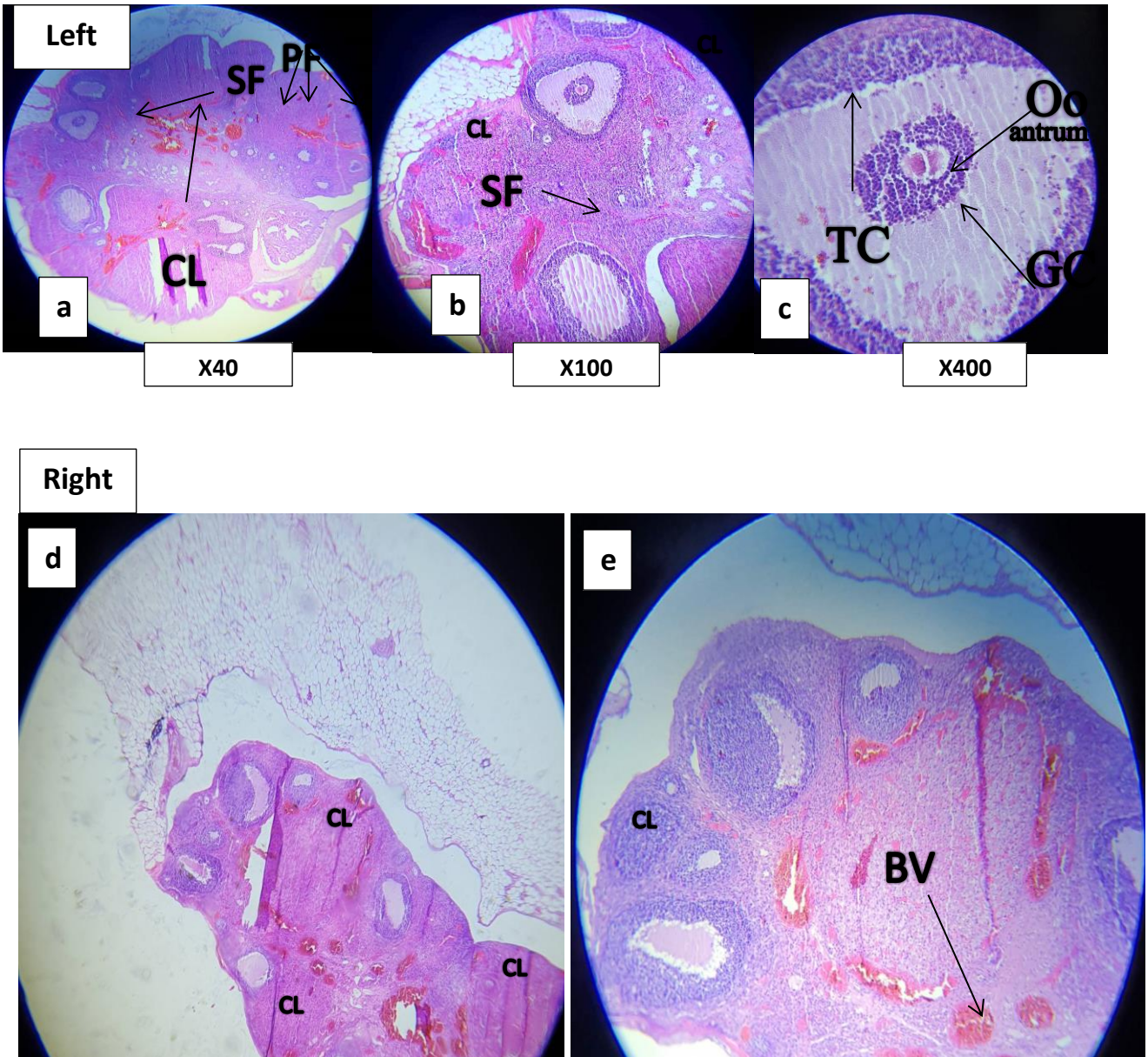

Fig.8: Normal left and right female rat ovaries shown on (x40,x100and x400) using light microscope and EH stain. SF(secondary follicle), PF(primary follicle) CL(corpus luteum),Oo (Oocyte), GC(granulosa cells),TC (thica cells)BV(blood vessels)

Microscopic examination of PCOS group

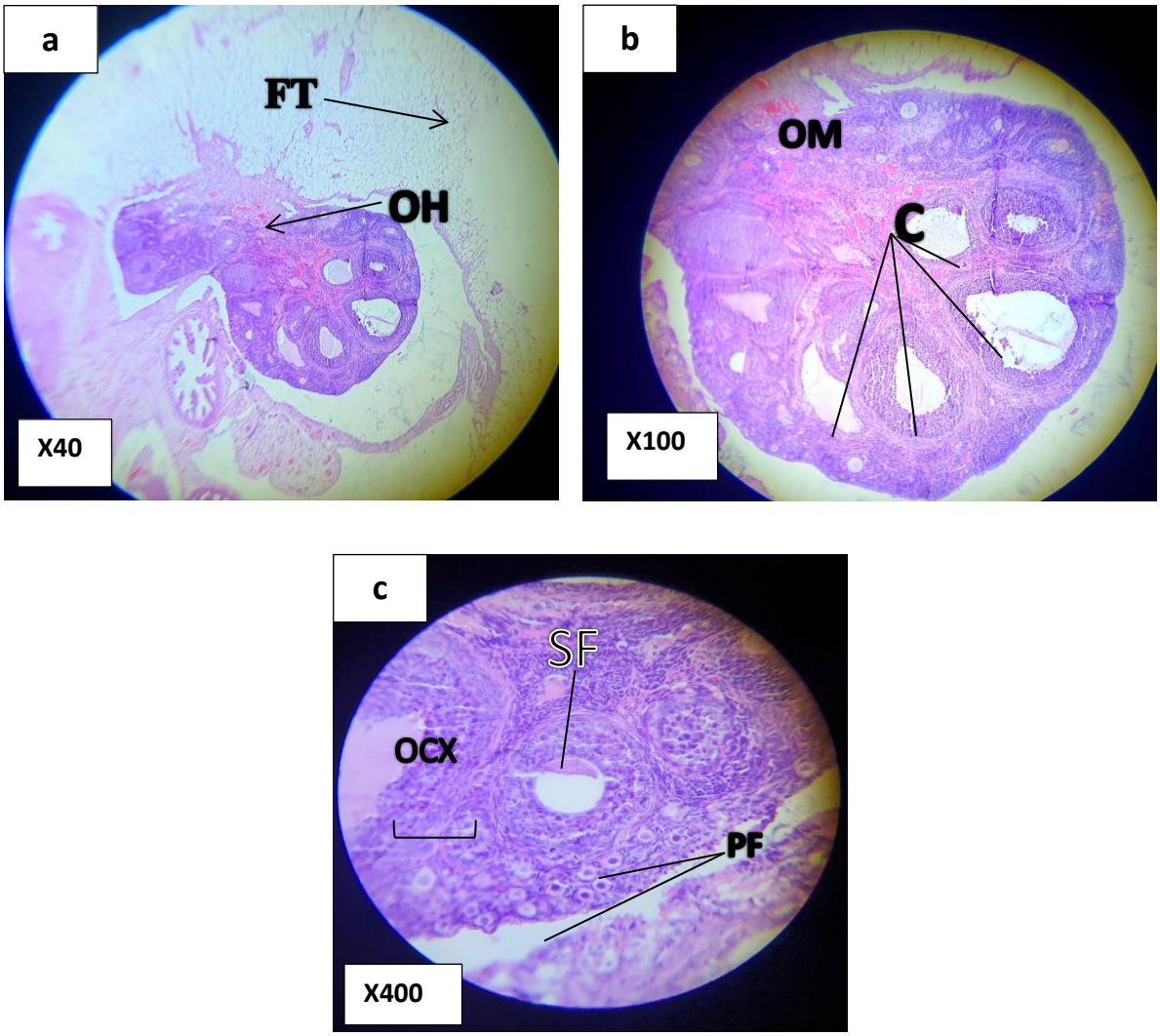

Fig.9 : Left rat polycystic ovary examined under light microscope (x40,x100 and x400) using E&H stain . FT(fat tissue),OH (ovarian hilum), OM (ovarian medulla) ,SF (secondary follicle) , PF(primary follicle) ,OCX (ovarian cortex). Note the size of fatty tissue around the ovary and the presence of multiple large empty cysts. Also the presence of multiple small primary follicles. The ovarian cortex is much thicker than normal ovary .

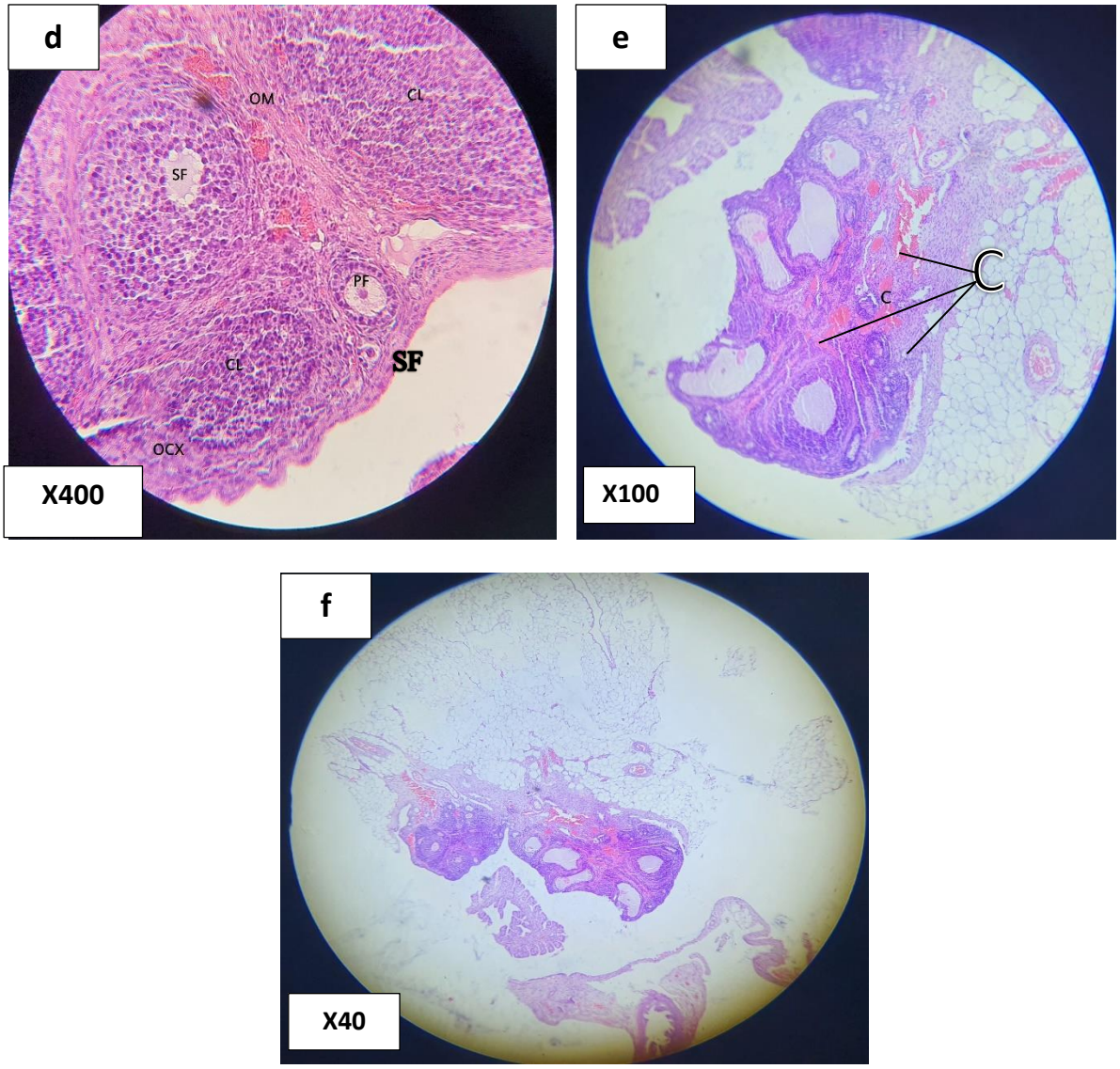

Fig.10: Right polycystic rat ovary . PF(primary follicle), OM(ovarian medulla),OCX(ovarian cortex), SF(secondary follicle), C(ovarian cyst) .

BBR treated group

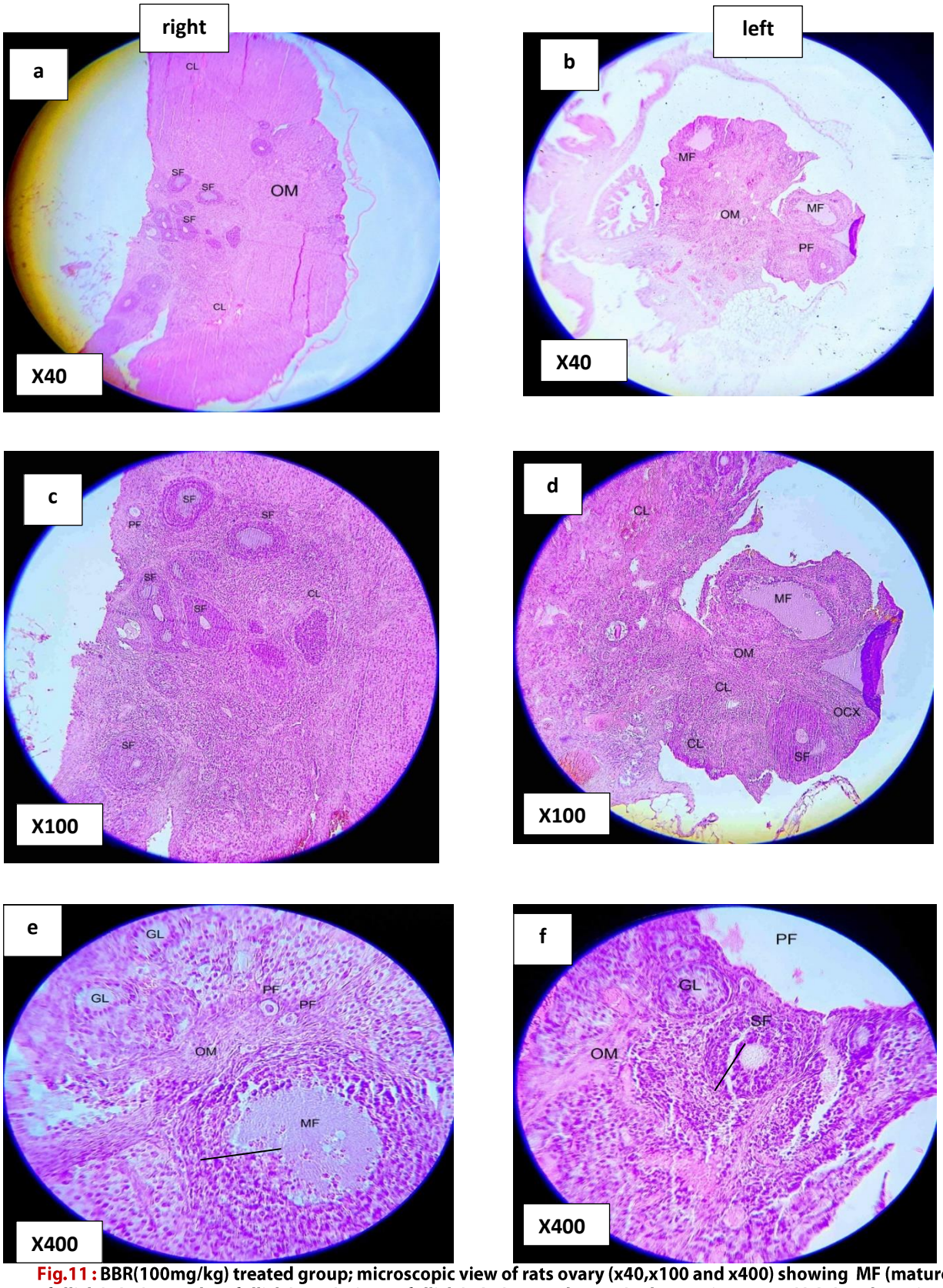

Fig.11 : BBR(100mg/kg) treated group; microscopic view of rats ovary (x40,x100 and x400) showing MF (mature follicle), SF (secondary follicle) , PF (Primary follicle), CL (corpus luteum) , the ovarian cortex(OCX) and ovarian medulla(OM) .note the small number of primary follicles and the absence of ovarian cysts

Ovarian features and difference between groups

Table 5: Number of PF,SF,CL,LS and SC in each group . Means with a different small letter in the same column significantly different (P<0.05) .

	P.FOLLICAL	S.FOLLICAL	CL	Lc	Sc
Control	6.00±1.58b	5.20±1.92a	6.60±1.67a	0.00±0.00b	2.00±1.58b
BBR	5.60±1.81b	4.40±1.14a	5.20±2.86a	0.20±0.44b	4.20±1.48a
PCOS	27.80±5.11a	6.60±2.07a	0.00±0.00b	6.20±1.30a	4.80±0.83a
LSD	3.9204	2.3796	2.5262	0.9943	1.7352

Primary follicles

Results revealed that the difference in mean primary follicles between PCOS group and other groups is highly significant (p<0.05) with a mean of 27.8 compared to 6.00 and 5.60 in control and BBR groups respectively as shown in figure (12) The study showed that there is comparable results between control and treatment group in which, these groups respond to treatment and the mean primary follicle number decreased significantly reaching numbers close to that of normal control group (p<0.05) compared to PCOS group.

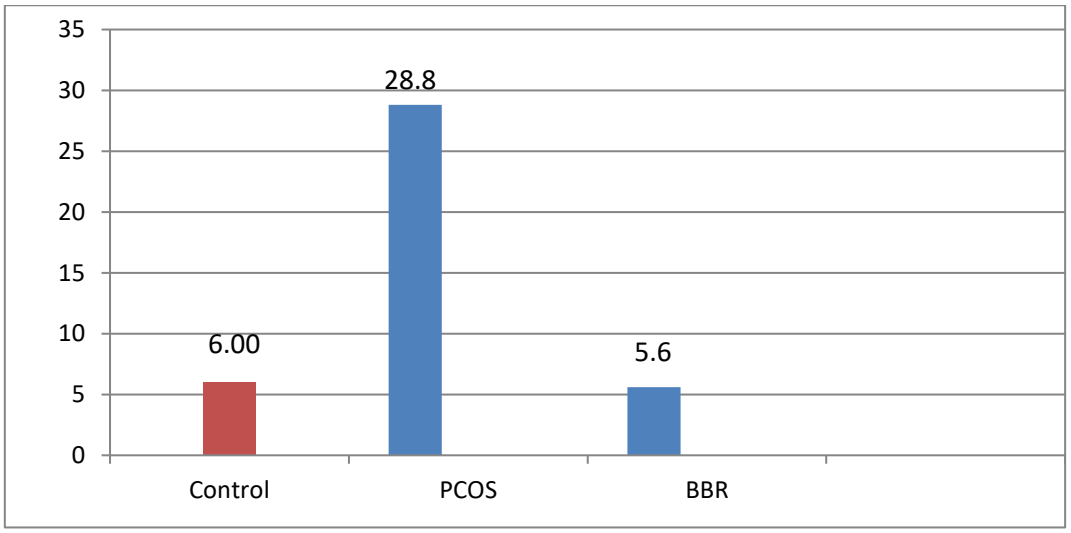

Fig.12: Number of primary follicles in all groups.

Secondary follicles

As shown in figure (13) there is no significant difference in the number of secondary follicles between the groups (p>0.05) .

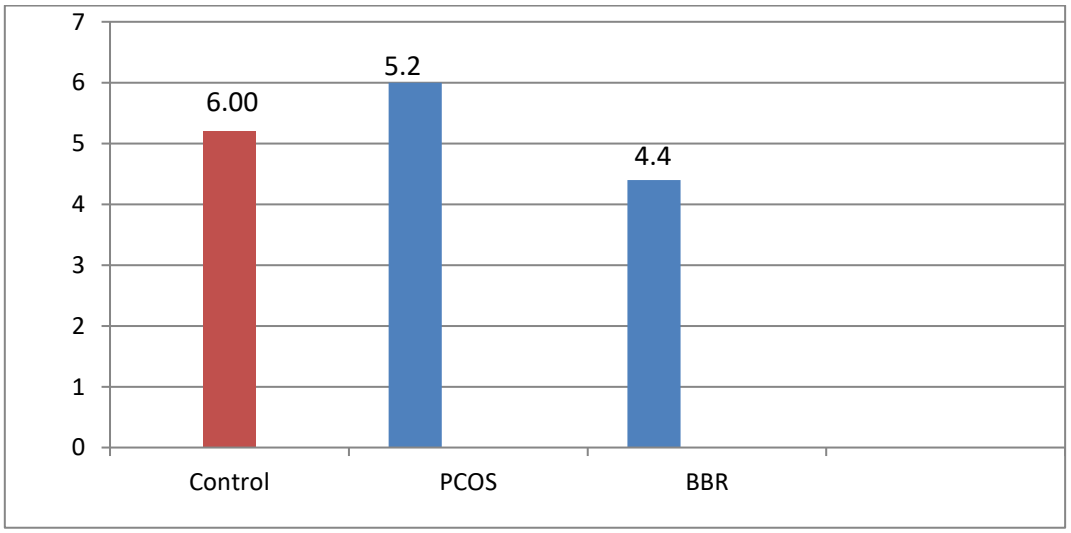

Fig.13: Number Of Secondary Follicles In All Groups

Corpus luteum

Results in figure(14) shows that there is a significant difference in the number of corpus luteum between the PCOS group and other groups ($p<0.05$) in which where is no CL in the PCOS group . When comparing the treatment group to the control group , we can observe that there is almost the same number of CL in BBR with no significant difference ($p>0.05$) between these groups .

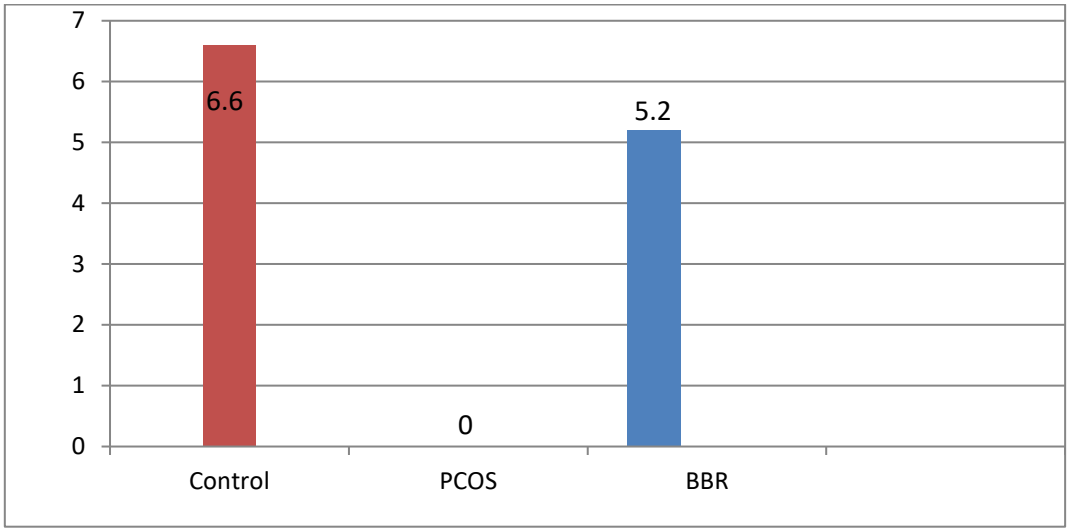

Fig.14: Number of Secondary Follicles in All Groups.

Large cysts

According to results showed in figure (15) there is a significant difference between the mean number of large cysts in PCOS group and the rest of groups ($p<0.05$) . In BBR group , the mean LC dropped from 6.20 in pretreatment to almost (0.00) after resaving treatment for 28 days ($p<0.05$) , when compared to control group .

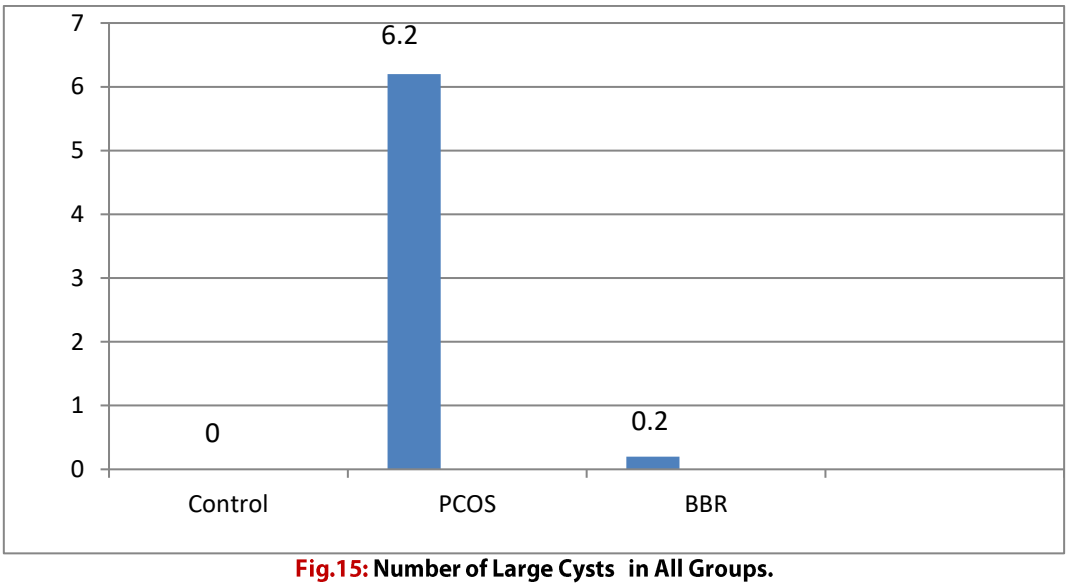

Fig.15: Number of Large Cysts in All Groups.

Small cysts

According to results showed in figure (16) there is no significant difference between the mean number of large cysts in PCOS group and BBR group ($p>0.05$).

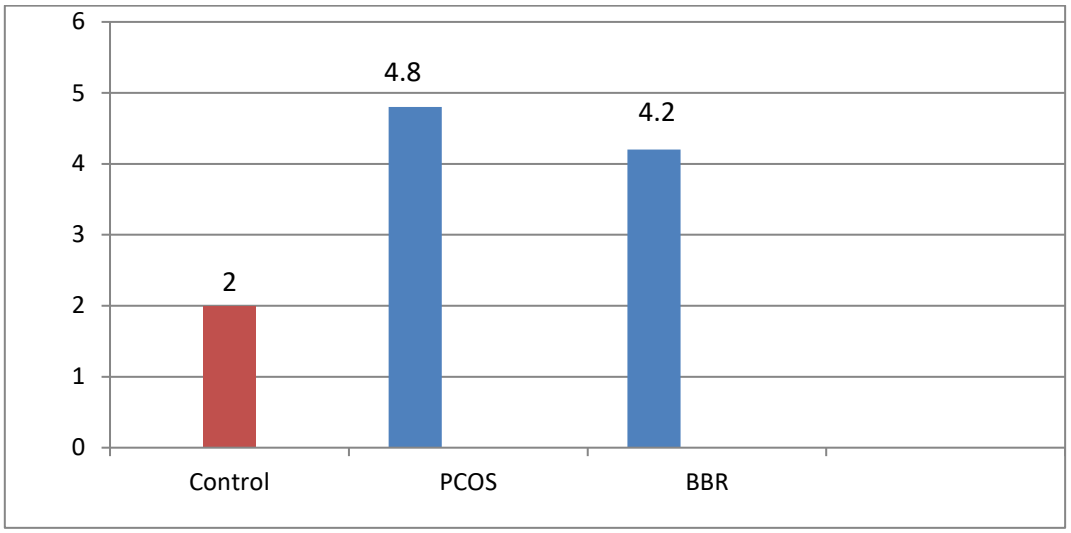

Fig.16: Number of Small Cysts in All Groups

DISCUSSION

Effect of BBR on bone morphogenic protein 4 (BMP-4)

In PCOS, females with hyperandrogenism, the signaling pathways of BMP-4 is impaired. Resulting in a lower serum concentration of this protein (20). In our study, we noticed that the serum concentration of BMP-4 was significantly reduced in PCOS model rats (20.79±4.33 pg/ml). Compared to (89.86±8.49 pg/ml) in the control group (figure3-8) that is a 76.86% decrease . After administration of 100 mg/kg, BBR to the animals BMP-4 serum concentrations reached (96.21±12.59 pg/ml), this significant elevation of serum BMP-4 was comparable to that of cyproterone acetate group (70.07±16.80 pg/dl). However, BBR caused a more considerable increase in serum BMP-4. These results indicate that the BBR effect on BMP-4 expression is an essential factor in reducing hyperandrogenism and decreasing the symptoms of PCOS. These findings agree with (Liu et al., 2017), who ruled out that hyperandrogenism results in decreased expression of BMP-4 . in other words, increasing BMP-4 concentration can be considered an approach in management of diseases like PCOS by effecting CYP17A1 and androgen synthesis in female ovaries (20) . In another study by (Glister et al., 2005), it was concluded that treating theca cells with BMP4 leads to significant suppression of Androgen synthesis (18). As a result, BBR, by its ability to increase BMP-4 levels and reducing CYP17A1 and cytochrome b5 reductase, acts on three critical steps in androgen synthesis in PCOS .

Effect of BBR on CYP 17A1 enzyme

The results of this study showed that BBR has a potent inhibitory effect on the CYP17A1 enzyme when compared to the control and PCOS groups. Serum CYP17A1 concentrations dropped by 67.833% (from 8.86±0.39 to 2.85±0.42) ng/ml compared to PCOS (10.07±1.) ng/ml (figure3-5). BBR was able to produce lower serum testosterone concentration by decreasing CYP17A1, a key enzyme in the synthesis of androgen (23) . These findings approve with the work done by (Tian et al., 2016), who claimed that Berberine was able to reduce CYP17A1 gene expression in prostate cancer through the inhibition of Aldo-keto reductase family one member C3 enzyme activity (24) . So, in other words, BBR, by inhibiting the key enzyme in androgen synthesis, can reduce hyperandrogenism in females with PCOS.

The effect of BBR on serum testosterone

All animals with PCOS had an elevated serum testosterone (8.5 ± 0.61) nmol/l when compared to the healthy animals (1.3 ± 0.24) nmol/l.(25,26) .

The animals were aggressive and had evidence of hair loss around their bodies with more muscular appearance of the limbs and torso, and they were acting almost like male rats(27,28).

While the healthy animals were friendly with thicker fur and smaller muscles.After the treatment of the animals with BBR for 28 days, from the first week, we noticed that there is some change in the animals' behavior (aggressiveness and libido) the animals start to grow fur again and the musclenaization starts to decrease (28). We compared the results from the BBR treated group with the control and PCOS groups. Results showed that the testosterone levels in BBR treated group decreased from (7.2±1.37) to (1.7±0.41) nmol/l compared to PCOS group (8.5 ± 0.61) nmol/l. This decline in serum testosterone was comparable to that's of control group (1.3 ± 0.24) nmol/l , indicating that BBR has a great impact on testosterone.

Effect of BBR on the animal's body weight

One of the most characteristic features of PCOS is increased body weight and overall obesity (29,30). lifestyle therapy, medication, and herbal preparations had been used over decades for decreasing the body weight in PCOS women. Insulin sensitizing agents (metformin) may be one of the most known medications that are used for weight reduction in PCOS. Nevertheless, Metformin has no FDA approval to be used as a weight-reducing agent, and its use remains off-label (30) .BBR is known for its ability to reduce weight by several pathways (31) In this study, the animal's weight was measured on a weekly bases throughout the treatment

(4weeks) (table 4). The change in the weight of control and PCOS groups was compared to that of BBR. The results of this study showed that there was a significant reduction in body weight in the BBR group compared to other groups (figure 7). There was an average loss of (-22.52 g) with (11.0553% decrease) in BBR group compared to (-4g) with (2.07254% decrease) in the control group. On the other hand PCOS group kept gaining weight with an average gain of (+42.4g) (21.7172% increase). Excellent ability of BBR to reduce weight in this study agrees with the work done by (Xu, J. H 2017) (6) and (Zhang et al.) who both proved that BBR reduces body weight by increasing the intestinal integrity and thus reducing Obesity (32) . This study also agree with the work done by (Zhang Z,2014) who ruled out that Berberine can reduce body weight by decreasing the

Effect of BBR on ovaries morphology

In PCOS, there are some histopathological changes that we should observe in order to determine if there was any improvement in the disease after treatment. This include number of large cysts, small cysts, corpus luteum, secondary follicles and primary follicles (33) the most important change will include the change in the number of primary follicles since in PCOS there is an increased number of small follicles in the ovarian cortex (34) there is also few number of large cysts which is the characteristic feature of PCOS. Hence, the name (polycystic). The presence of corpus luteum indicates the occurrence of ovulation and the response of the treatment (33).

Results from our study showed that after treatment with BBR, there was a significant decline in the number of PF when compared to the PCOS group, the number of PF decreased from (27.80±5.11) in pretreatment to (5.60±1.81) in BBR treated group. This indicates an 80% decrease (figure 12). Regarding the large cysts (LC) we found out after treatment with BBR for 28 days, there was a complete disappearance of large cysts in animal ovaries (figure 15) compared to the PCOS group (6.20±1.30). Considering corpus luteum (CL), BBR group had multiple CL inside the ovarian tissue this indicates the occurrence of ovulation (figure 8, 10). In PCOS due to hyperandrogenism the female ovary ceases the process of ovulation and only large ovarian cysts remains (9), as a result, there is no CL in a PCOS ovary.

CONCLUSION

According to the results of the current study, one can conclude that Berberine due to its ability to reduce androgen synthesis through lowering steroidogenic enzymes (CYP17A1) and increasing the synthesis of BMP-4 a key protein in the expression of CYP17A1 by reducing its expression at higher BMP-4 concentrations, is able to counteract the hyperandrogenism accompanying PCOS disease and decreasing testosterone concentrations.

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