Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.6, No.18, 2016

A comparative Between the Effects of *Glycyrrhiza glabra* Roots Extract and Pioglitazone on Induced Poly Cystic Ovary Syndrome in Rats.

*Ali A. Ali and **Huda F. Hasan
*Student, MSc. Dept. of Veterinary Physiology and Pharmacology, College of veterinary Medicine, University of Baghdad.
** Asisst.Prof. Dept. of Veterinary Physiology and Pharmacology, College of veterinary Medicine, University of Baghdad.

Abstract
Study was designed to make comparison between effect of pioglitazone and *Glycyrrhiza glabra* extract for controlling poly cystic ovaries induced in the rats. (0.5 mg/kg) of Letrazole for 21 days was given to induce poly cystic ovaries. Thirty five rats were alienated into five groups: Group one (T1) left as control, group two (T2) was induced and handled with distilled water, group three (T3) was induced and handled with (300 mg /kg) of *Glycyrrhiza glabra* extract, group four (T4) was induced and treated with (20 mg/kg) body weight of piogiltazone, group five (T5) was induced and handled with (10mg/kg.) of piogiltazone and 150mg/kg of *Glycyrrhiza glabra* extract. The oral administration of letrazole to rats caused prolong the length of hours in proestrus, metaestrus and diestrus phases, in addition to prolong in days of estrous cycle and gradual significant decrease in hours of estrus phase until to disappear in cycle four. The length estrous cycle, prostrus, metaestrus and diestrus phases in T3, T4 and T5 revealed a statistical reduce when compared with T2 and no significant variation when compared with T1 in cycle five. The length of estrus phase hours showed a significant increase in T3, T4 and T5 as compared with T2 and no significant difference as compared with the T1 in cycle five. Study concluded the *Glycyrrhiza glabra* had the ability in treating poly cystic ovary and lead to augment positive consequence of piogiltazone.
Keywords: Estrous cycle, *Glycyrrhiza glabra*, Letrazole, Pioglitazone

1. Introduction

The reproductive system is essential to keep a species alive and improve breeding and to maintain the fertility and to lessen the adverse effect of medications, herbal plants are excellent substitute to chemical medications, solitary of the main cause for this is little side effect compared to medications, because their scavenging free radicals properties they decrease medications toxicity , furthermore, the herbal plants have capable role in management of numerous diseases which effect on efficacy of reproductive system like Poly-cystic ovary with least adverse effects, furthermore, to improve immunity of the body and also standardize menstrual cycle without changeable in hormonal level [1] . *Glycyrrhiza glabra* was established by its capability on decrease serum testosterone, *Glycyrrhiza glabra* flavonoids had effect on decrease the fat accumulation, blood glucose level and hyperglycemia that may be produced by poly cystic ovaries, via potentiating the peroxisome proliferating activating receptors gamma (PPAR-gamma) [2]. *Glycyrrhiza glabra* had anti-inflammatory and anti-allergic effects when treatment of bronchial asthma, allergic, dramatis and others many diseases [3]. Poly cystic Ovaries is solitary of the mainly ordinary endocrine abnormalities, it is label with, menstrual irregularities, un ovulation, infertility, testosterone hormone increase, hirsutism, insulin resistance, obesity, endometrial cancer, cardiovascular disease, and Type-II diabetes, polycystic ovaries treated with medications such as Metformin, Clomiphene, Spironolactone , Rosiglitazone and Pioglitazone, these drugs have many side effects on body such as congestive heart failure and osteoporosis so that most studies headed to use herbals plant for treating diseases and decrease the side effect of drugs . [4]. Pioglitazone is documented to amplify insulin action and receptors of insulin mechanisms effect through stimulating peroxisome proliferator activated receptor-gamma (PPAR-g) [5]. The study was aimed to make comparison between pioglitazone and *Glycyrrhiza glabra* roots extract effect on managing estrous cycle abnormalities which resulting from PCOS induced in rats.

2. Materials and Methods

2.1 Plant extraction: *Glycyrrhiza glabra* roots were cleaned from dirt by tab water and desiccated by oven at 25^0C, then the roots powdered by electric grinder then the fine powder was sieved. The roots powdered extracted

by using 70% ethanolic alcohol, this was completed by soxhlet apparatus, the process of extraction was made for 24h at temperature 50 C° , the rotary evaporator was used to concentrate the extract at 50 C° [6].

2.2 Animals of Experiment: Thirty five adult female rats in weights˷ 260 gram were catched from animals house of university of Karbala. Then animals were reserved underneath appropriate environmental condition of 22-25°C and the moisture between (50;50) in dark, 45% to 55% in light; The animals have been kept in cages plastic with measurement 25×30×50cm and the cages were altered every week, foodstuff was given as pellets.

2.3 Poly cystic ovary syndrome induction: Female rats were administered letrozole (0.5mg/kg) B.W to induce poly cystic ovary, letrazole was dissolved in 1% carboxy methyl cellulose (CMC) and given daily for 21 days**,** the length of phases of estrous cycle were determined along the periods of letrazole administration [7].

2.4 Design of Experiment: The two normal estrous cycles of thirty five female rats have been determined by examination the animal vaginal smear for two weeks before treatment and before induction the disease. Female rats (35 in numbers) were alienated into five groups, and 30 days was the periods of treatment as following: The rats of group one (T1) were left without any treatment, while animals of group two (T2) were induced and handled with distilled water given orally , the animals of group three (T3) were induced and handled with *Glycyrrhiza glabra* roots extract (300mg/kg) given orally, whereas the animals in group four (T4) were induced and handled with piogiltazon (20mg/kg) given orally and daily. Finally the animals of group five (T4) were induced and handled with piogiltazone (10mg./kg) and *Glycyrrhiza glabra* extract (150mg/kg).

2.5 Estrous cycle detection and the periods length of estrous cycle with its phases determination: Vaginal smears were taken from treated letrazole, *Glycyrrhiza glabra* ,piogiltazone , *Glycyrrhiza glabra* with piogiltazone, distilled water and control female rats groups, that examine was daily each 12 hrs, the swab was taken by inserting plastic micro pipette contains 0.2 ml normal saline into the vagina and the sample put on microscope slide, the smear on the slide was dried in room temperature and stained by metylene blue, then put the slide under microscope to classify it into one of four phases of estrous according to the cell types and shape, the phases of estrous cycle were determined by observing the vaginal smear two times through the day, as explained by Cooper *et al.* [8] and five cycles in each groups were examined, the duration of the estrous cycle and also the number of days that spent at the stage of the cycle was estimated as the following:

The estrous cycle phases length within hours = total hours of each phase cycle **/** total number of animals

The estrous cycle phases length in days = the phase estrus cycle length in hours/ 24 hrs.

2.6 Statistical analysis: SAS [9] is the Statistical Analytic System- has been used to product the altered factors in parameters of experiment. Least significant difference LSD test at (P<0.05) was used to significant evaluate between average in this experiment.

3. Results

3.1 Estrous cycle detection: The results of two estrous cycle detection of thirty five female rats before starting the experiment were showed in (figure 1), the proestrus phase, estrus phase, diestrus phase and metaestrus in cycle one showed no significant different (P<0.05) with proestrus phase, estrus phase, diestrus phase and metaestrus phase in cycle two respectively

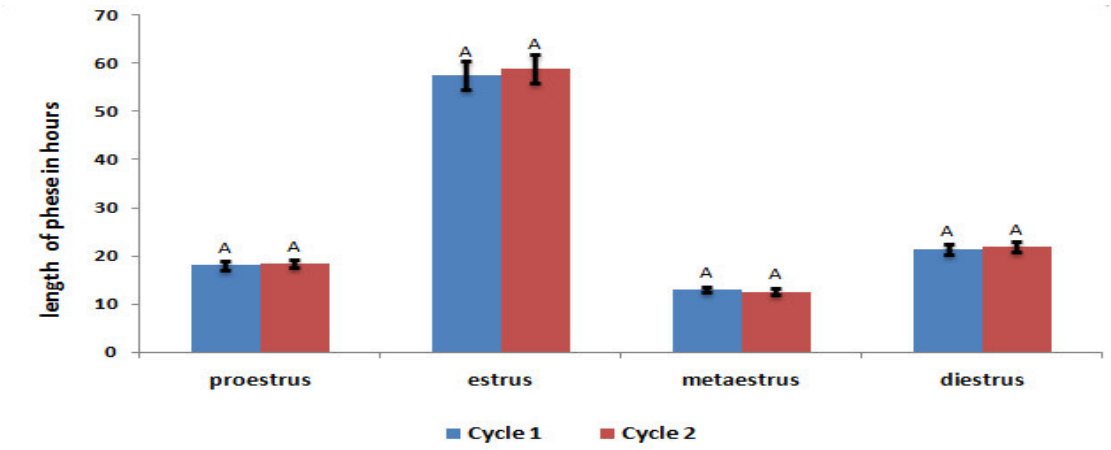

Figure (1): The phases of two estrous cycles of thirty five rats before beginning the experiments.

In addition, the results of Photomicrographs in all phases showed in (figure 4-2), the proestrus phase under light microscope showed predominant of nucleated epithelia cells and some corneum cells appeared, while the estrus phase distinguished by corneum squamous epithelia cells, which located in clusters. In metaestrus phase, there

was a mixed of cell types with a predominant of leucocytes and a little nucleated epithelial and corneum squamous epithelia cells, while in diestrus phase showed consistent of leukocytes.

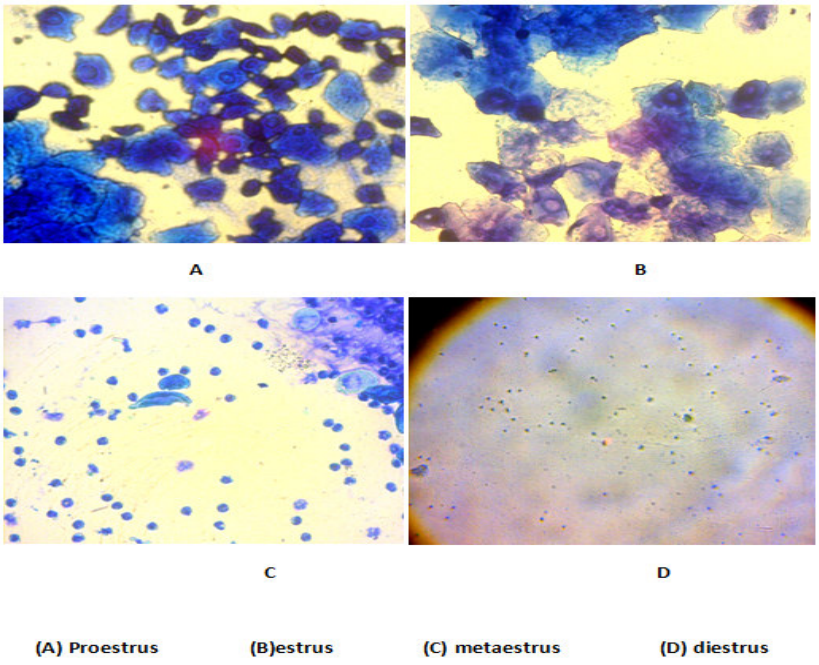

Figure (2): The photomicrograph of stained vaginal secretions from rats at (A) proestrus, mainly consisting of nucleated epithelia cells; (B) estrus, distinguished by presence a cornifing cells with nucleus; (C) metestrus, Consists of the three kind of cells, leukocytes, nucleated epithelia cells and cornified ,(D) in diestrus, presenting the major of leucocytes.

3.2 Effect of letrazole on estrous phases length in hours and estrous cycle length in days along periods of 21 days. The results of the effect of letrazole on proesrus phase after oral treatment rats are shown in figure (3). The proestrus of rats in letrazole treated group was 15.20 hr., in cycle one and started gradually significant increase ($P<0.05$) until reached to 37.44 hr. in cycle four. In addition, the proestrous phase of rats in letrazole treated group showed a significant rise in value ($P< 0.05$) in cycles 2, 3 and 4 in mean value (30.03 ± 2.82, 33.89 ± 2.72 and 37.44 ± 2.22) respectively, as compared with negative control group.

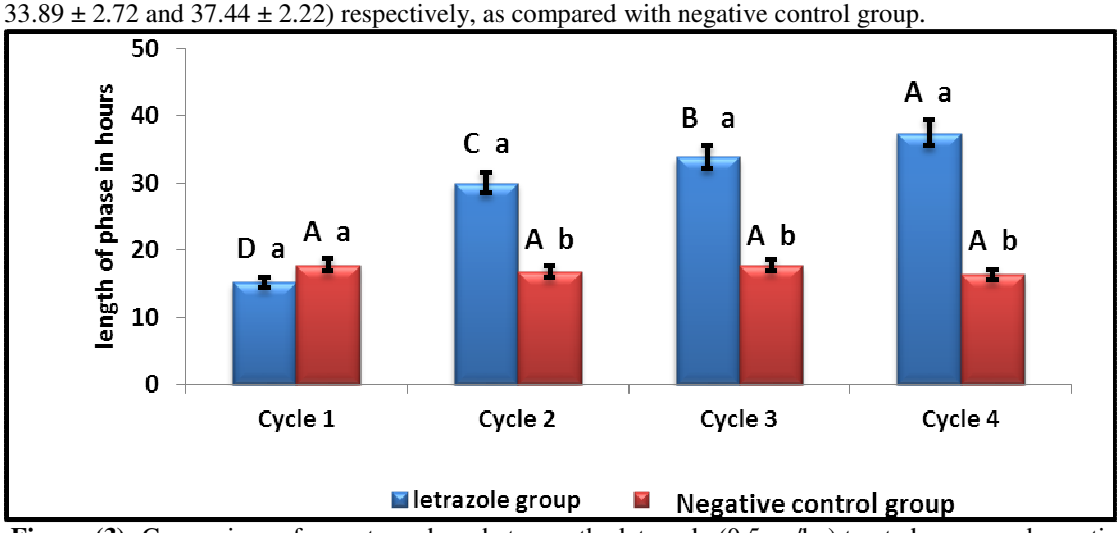

Figure (3): Comparison of proestrus phase between the letrazole (0.5mg/kg) treated group and negative control group. *dissimilar capital letters indicated significant variation ($P<0.05$), between groups. *dissimilar small letters indicated significant variation ($P<0.05$) within groups.

The results of estrus phase length in group adminstered letrazole appeared gradual important decrease ($P<0.05$) between cycles until disappeared in cycle four, the estrus phase of rats in letrazole treated group appeared a significant reduce ($P<0.05$) in the cycle 2 and 3 in mean value (20.50 ± 3.36, 6.00 ± 1.84) respectively, as

compared with cycle 2 and 3 of negative control group (57.34±1.75,58.18±2.53) respectively, as illustrated in figure (4).

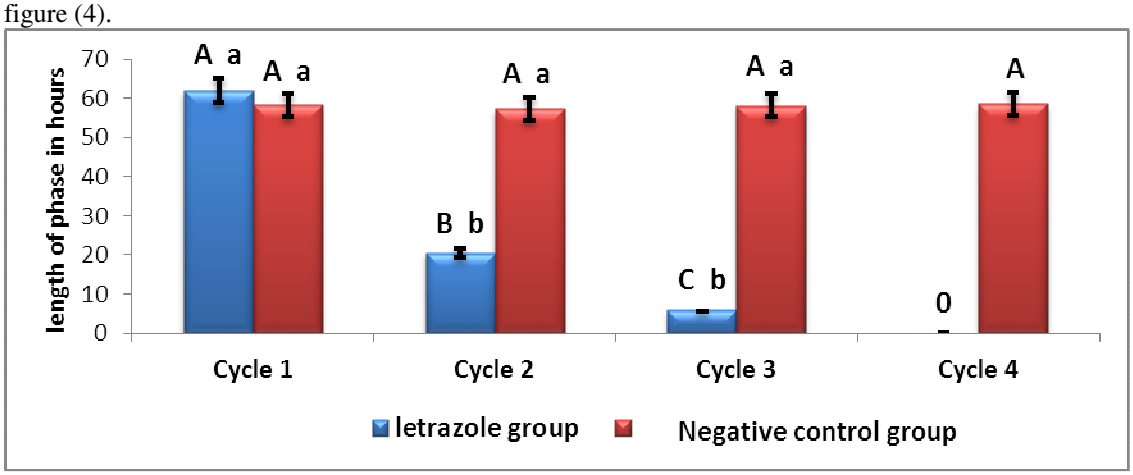

Figure (4): Comparison of estrus phase between the letrazole (0.5mg/kg) treated group and negative control treated group in hours.*dissimilar capital letters indicated significant variation (P<0.05), between groups. *dissimilar small letters indicated significant variation (P<0.05) within groups.

The length of metaestrus phase in rats of letrazole treated group was 16.01 hr. in cycle one and started gradually significant increase (P<0.05) until reached to 50.81 hr. in cycle four. In addition, the metaestrus phase in animals of letrazole handled group appeared a significant increase (P<0.05) in cycles 2, 3 and 4 in mean value (27.20±2.37, 33.20±2.78 and 50.81±3.50) respectively, when compared with negative standard group, as in figure (5).

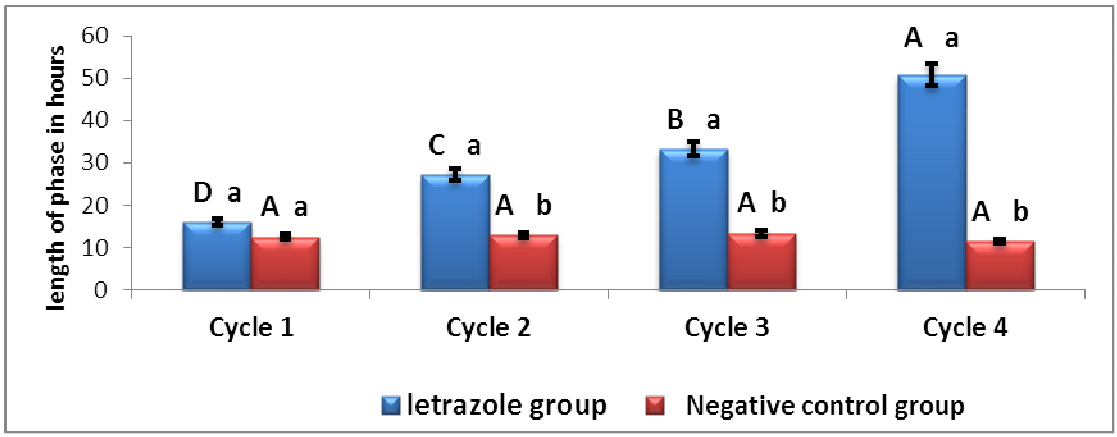

Figure (5): Comparison of metaestrus phase between the letrazole (0.5mg/kg) treated group and negative control group in hours.* dissimilar capital letters indicated significant variation (P<0.05), between groups. *dissimilar small letters indicated significant variation (P<0.05) within groups.

The length of diestrus phase in rats of letrazole treated group was 22.99 hr., in cycle one and started gradually significant increase (P<0.05) until reached to 62.50 hr., in cycle four, furthermore, the metaestrus phase in animals of letrazole handled group appeared important increase (P<0.05) in the cycles 2, 3 and 4 in mean value (40.37 ±3.10, 55.01 ±3.638 and 21.42 ±1.35) respectively, when compared with the negative standard group, as in figure (6).

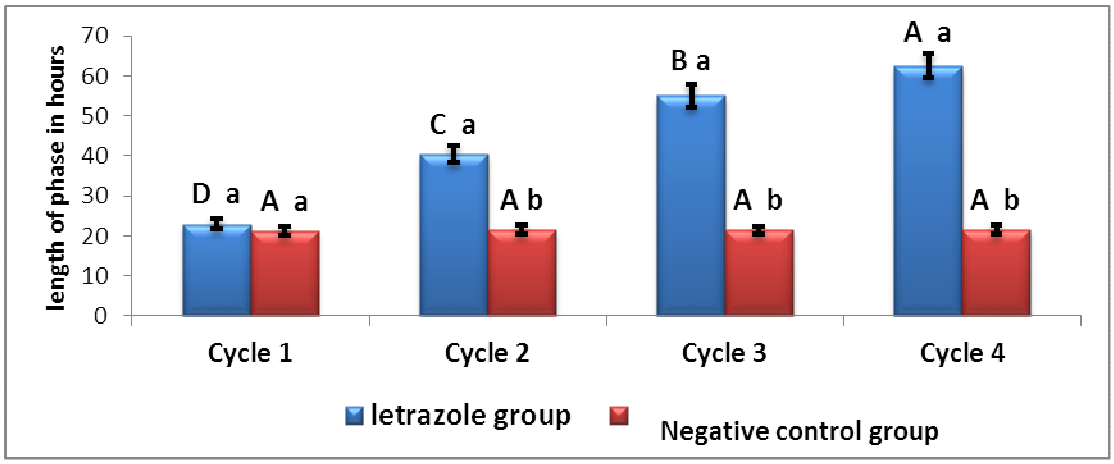

Figure (6): Comparison of diestrus phase between the letrazole (0.5 mg/kg) treated group and negative control group in hours.* dissimilar capital letters indicated significant variation (P<0.05), between groups. *dissimilar small letters indicated significant variation (P<0.05) within groups.

The length of estrous cycles in days is shown in figure (7). The estrous cycle in letrazole treated group was 4.88 days and started gradually significant increase (P<0.05) until reached to 6.20 days in cycle 4, in addition the days of estrous cycle in cycles three and four appeared important increase (P<0.05) in comparison with cycles 3 and 4 of control group.

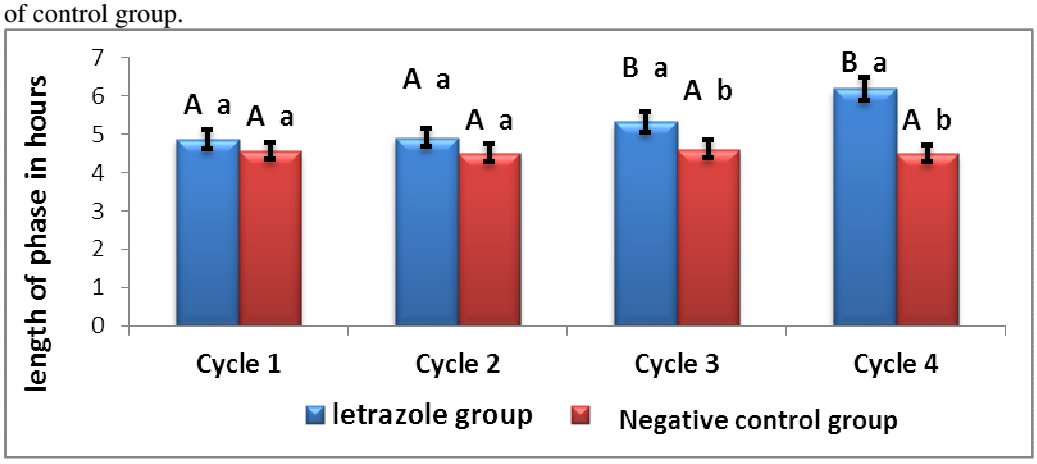

Figure (7): Comparison of estrous cycle between the letrazole (0.5mg/kg) treated group and negative control group in days.*dissimilar capital letters indicate significant variation (P<0.05), between groups. *dissimilar small letters indicate significant variation (P<0.05) within groups.

3.3 Effect of the treatments on estrous phases length in hours and estrous cycle length in days: The outcomes of the current research showed that oral administration of 300mg/kg *Glycyrhazia globra* extract, 20mg/kg of piogiltazone, 150mg/kg of *Glycyrhazia globra* extract and 10mg/kg of piogiltazone to rats resulted in a gradual important depletion (P<0.05) in hours of proestrus phase until reached to mean value (16.55 ±230, 18.98 ±3.09 and 13.84 ±2.24) respectively in cycle five. In addition, the proestrus phase in all cycles of *Glycyrhazia globra* extract, piogiltazone, *Glycyrhazia globra* extract and piogiltazone groups revealed a significant depletion (P<0.05) as compared with distilled water handled group. Furthermore, in cycle five proesrtus phase of *Glycyrhazia globra* extract and piogiltazone handled group revealed a significant depletion (P<0.05) in comparison with control group, whereas the *Glycyrhazia globra* extract group and piogiltazone group indicated no significant difference in comparison with standard group, as in figure (8).

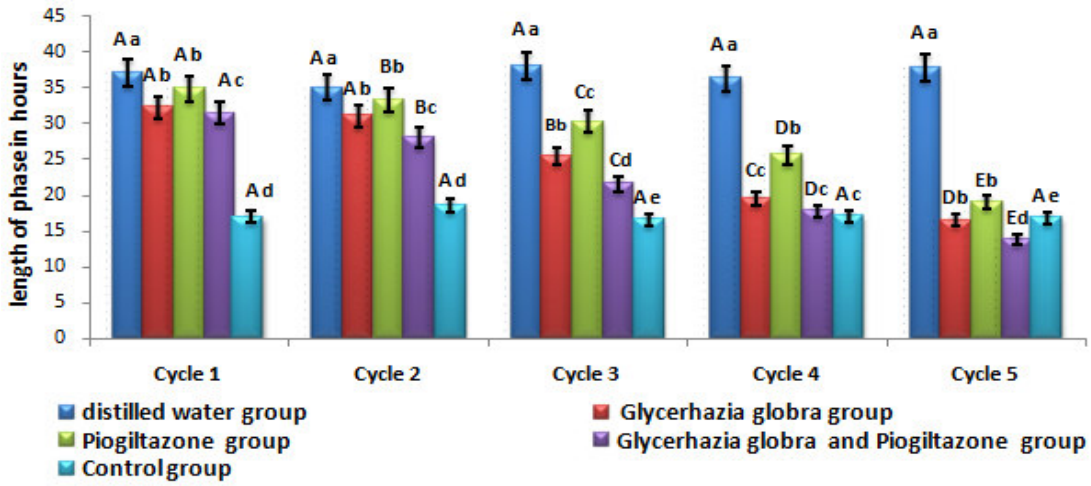

Figure (8): Illustrated the effect of *Glycyrhazia globra* extract 300mg/kg, piogiltazone 20mg/kg, *Glycyrhazia globra* extract 150mg/kg with piogiltazone 10mg/kg and distilled water on proestrus phase in hrs.*dissimilar capital letters indicated significant variation (P<0.05), between groups. *dissimilar small letters indicated significant variation (P<0.05) within groups.

The hours of estrus phase of distilled water treated group disappeared in all cycles, while in *Glycyrhazia globra* extract, piogiltazone, *Glycyrhazia globra* extract and piogiltazone groups were gradually and significantly increased (P<0.05) until reached to mean value (57.70 ±3.11, 55.90 ±3.11 and 59.03 ±3.05) respectively in cycle five. In cycle five the hours of estrus phase in *Glycyrhazia globra* extract, piogiltazone, *Glycyrhazia globra* extract and piogiltazone handled groups revealed no statically difference when compared with standard treated group as in figure (9).

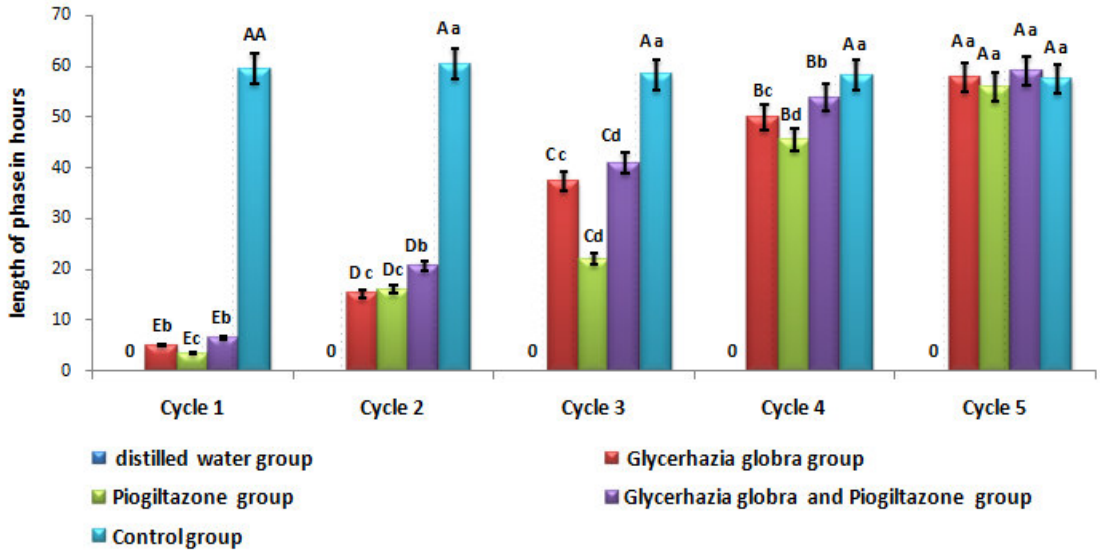

Figure (9): Illustrated the effect of *Glycyrhazia globra* extract 300mg/kg, piogiltazone 20mg/kg, *Glycyrhazia globra* extract 150mg/kg with piogiltazone 10mg/kg and distilled water on estrus phase in hrs.*dissimilar capital letters indicated significant variation (P<0.05), between groups. *dissimilar small letters indicated significant variation (P<0.05) within groups.

In the current study, the hours of metaestrus phase in distilled water handled group revealed no statistically difference (P <0.05) between cycles of the same column of distilled water treated group while *Glycyrhazia globra* extract, piogiltazone, *Glycyrhazia globra* extract and piogiltazone groups were gradually and significantly decrease (P<0.05) until reached to mean value (13.30 ±3.38, 14.40±2.40 and 12.31 ±1.85) respectively in cycle five. Furthermore, the hours of metaestrus phase in distilled water treated group indicated to statistical increase (P<0.05) in all cycles in comparison with control treated group, while the *Glycyrhazia globra* extract, piogiltazone, *Glycyrhazia globra* extract and piogiltazone groups revealed a statically decrease (P<0.05)

in all cycles in comparison with distilled water treated group and without significant variation (P<0.05) in comparison with control group in cycle five, as in figure (10).

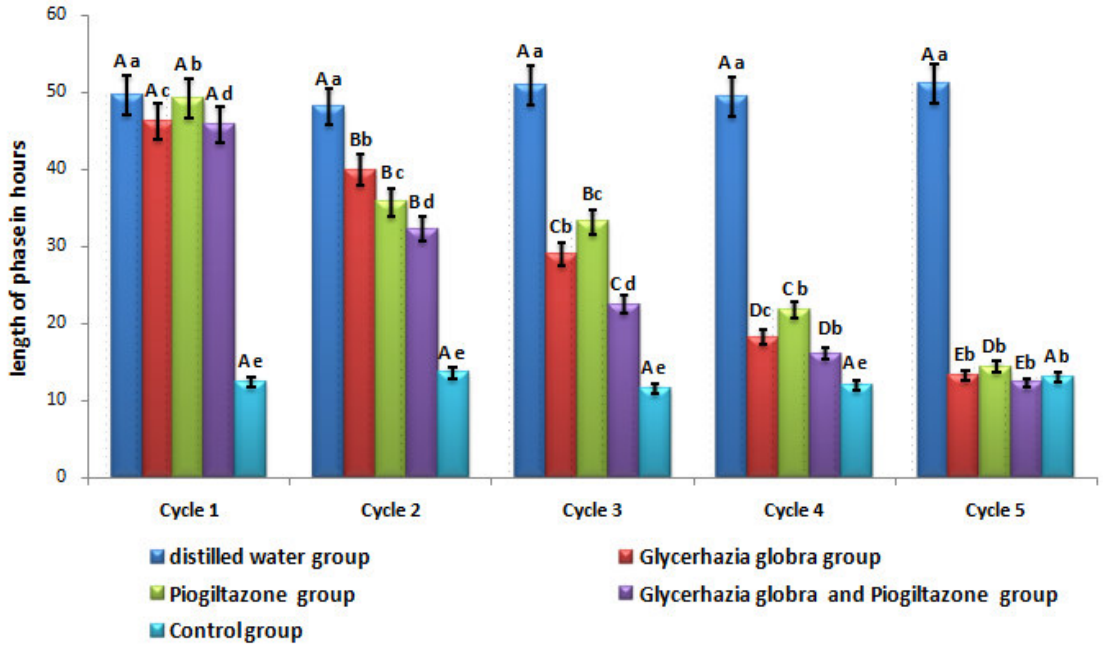

Figure (10): Illustrated the effect *Glycyrhazia globra* extract 300mg/kg, piogiltazone 20mg/kg, *Glycyrhazia globra* extract 150mg/kg with piogiltazone 10mg/kg and distilled water on metaestrus phase in hrs. *dissimilar capital letters indicated significant variation (P<0.05), between groups. *dissimilar small letters indicated significant variation (P<0.05) within groups.

The mean values of diestrus phase hours in different groups were clarified in figure (11). Diestrus phase in *Glycyrhazia globra* extract, piogiltazone, *Glycyrhazia globra* extract and piogiltazone groups were gradually and significantly decrease (P<0.05) until reached to average value(23.01±1.9, 22.95±1.94 and 20.53± 2.16) respectively in cycle five, in addition to no significant difference value (P<0.05) compared with standard group (20.50±2.59) in cycle five. On other hand, treatment of rats with distilled water caused a significant elevation (P< 0.05) in hours of diestrus phase in all cycles as compared with control group.

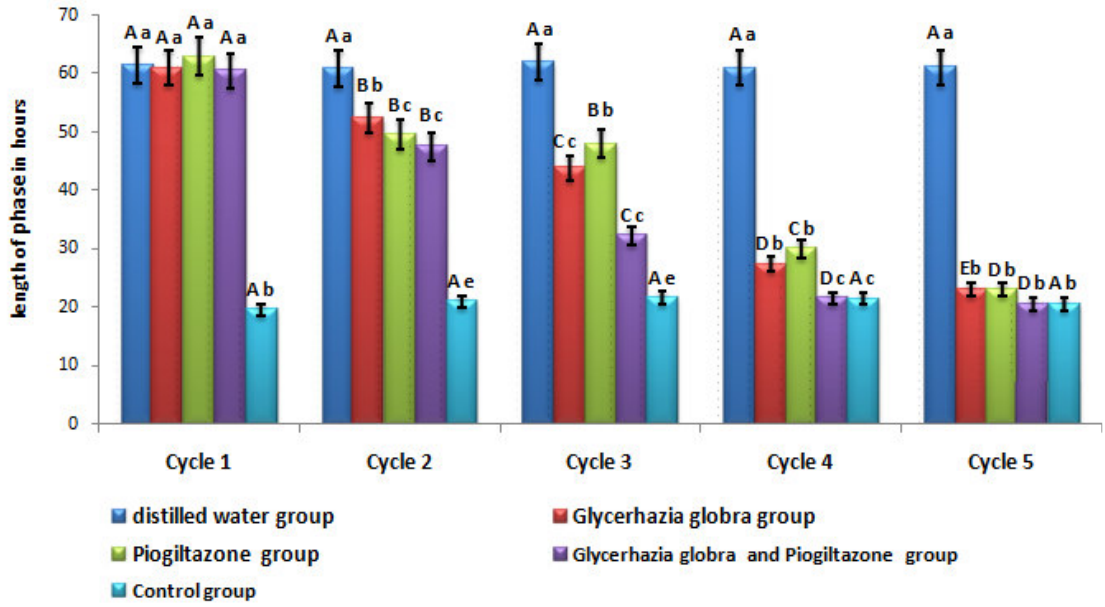

Figure (11): Illustrated the effect *Glycyrhazia globra* extract 300mg/kg, piogiltazone 20mg/kg, *Glycyrhazia globra* extract 150mg/kg with piogiltazone 10mg/kg and distilled water on Diestrus phase in hrs. *dissimilar capital letters indicated significant variation (P<0.05), between groups. *dissimilar small letters indicated significant variation (P<0.05) within groups.

The days of estrous cycles was illustrated in figure (12), the orally administration of *Glycyrhazia globra* extract to animals caused a significant depletion (P<0.05) in days of estrous cycle four and cycle five as compared with other cycles (one, two and three), while in piogiltazone and *Glycyrhazia globra* extract with piogiltazone treated groups caused significant depletion (P<0.05) in days of estrous cycles two, three, four and five when compared with cycle one. The days of estrous in *Glycyrhazia globra* extract, piogiltazone, *Glycyrhazia globra* extract and piogiltazone groups revealed significant depletion (P<0.05) in all cycles in comparison with distilled water treated group, furthermore, in cycles four and five consequences there were no significant difference (P<0.05) in mean value when compared with baseline group. The distilled water handled group revealed a significant raise (P<0.05) in the days of estrous cycle when compared with control group in the all cycles.

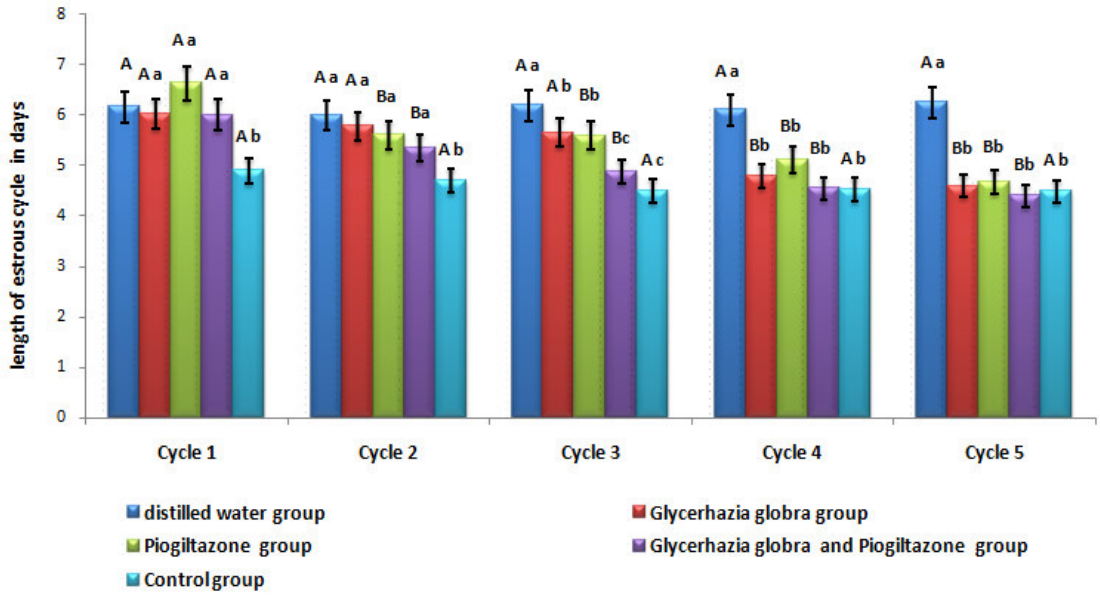

Figure (12): Illustrated the effect of, *Glycyrhazia globra* extract 300mg/kg, piogiltazone 20mg/kg, *Glycyrhazia globra* extract 150mg/kg with piogiltazone 10mg/kg and distilled water on estrous cycle in days. *dissimilar capital letters indicate significant variation (P<0.05), between groups. *dissimilar small letters indicate significant variation (P<0.05) within groups.

4. Discussion

4.1 Estrous cycle detection: Before starting the experiment the estrous cycles was detected to ensure from the fertility and regularity the estrous cycle of all female rats, and the results of photomicrographs in all phases agreed with results reported by Lohmiller and Swing, [10], they mentioned the characterization of each stage from stained smears obtained from the vaginal epithelium, including during the estrous cycle enucleated acidophilic cells are observed, during metestrus, leukocytes are also observed and shown in the insert, in the diestrus, numerous leukocytes can be seen during this phase, in proestrus phase large basophilic cell (LBC), with pre-acidophilic cells (PAC) nucleated acidophilic cells (NAC) was observed, while the estrus phase characterized by presence cornified nucleated cell.

4.2 Estrous phases length in hours and estrous cycle length in days : In this study, the oral administration of letrazole (0.5mg/kg) to induce poly cyctic ovary in rats caused irregularity in all phases of estrous cycle, such as prolonged the hours of proestrous, metaestrus and diestrus in addition decreased and disappeared the hours of estrus and increased the days of estrous cycle , this result may be attributed to Letrozole is considered from aromatase enzymes inhibitors, act by blocking step of aromatization, the levels of testosterone enhance and the estrogens decrease. This altitude of androgen levels stamped on the hypothalamus result in a non-cyclic discharge of gonadotrophins and abnormality in all estrous phases, the same result mentioned by Kafali, *et al*.,[7], in addition to testosterone is needed for usual folliculogenesis, mostly before time of follicular growth , and an intemperance of intra ovarian androgens lead to atresia follicles formation furthermore, the testosterone allowed the stimulation of the primary follicles by way of phosphatidylinositol-3-kinases-Akt- Foxo-3a and also depleted the growth differentiation factors 9 appearance in oocyte, leading to capture of preantral follicles growth, which can be clarified estrous cycle disturbances in PCOS, this outcomes supported by consequences

reported by Manuel, *et al.,* [11]. The estrous cycles measurements, altered to the ordinary sequences following handled rats with *Glycyrrhiza glabra* roots extract is due to the extract has the capability to manipulate on metabolism of androgen [12], several studies established that the *Glycerrhezia globra* closed effect of 17-20-lyase, 3-hydroxysteroid dehydrogenase, 17- hydroxysteroid dehydro-genase and activated the aromatase activity [13]. Estrogens androgens occupied in the metabolism and creation by these enzymes, in addition, the influence of the glycyrrhizic acid which establish in herbal product participated in ruling the hormones and activation the ovulation, *Glycyrrhiza glabra* roots extract in vitro produced endorsement the formation of estradiol and activate activity of aromatase by glycyrrhizic acid, therfore the estrous cycles after adminstered rats with *Glycyrrhiza glabra* roots extract it can be returned to the regular sequences, this result also agreed with Takeuchi , *et al.,*[14]. Moreover, *Glycerrhezia globra* had active component, glycyrrhizic acid, which is dependable for the pharmacological characteristic and also recommended to treatment female's sterility [15]. The oral administration of piogiltazone for 30 days caused normalize the phases of estrous cycle and this result may be belong to the Pioglitazone had effects on reproductive hormonal during PCOS both straightly taking place in the ovary or not directly via lessening the insulin resistance, on the way to support a straight outcomes on the ovary, in addition to pioglitazone inhibited the activation of the phospha-tidyl-Inositol 3-kinase in ovaries, at several pathways and may be bang the creation of ovarian androgens lead to normalize the menstrual cycles , restore the ordinary feedback influence of luteal steroids by its action on standardization the levels of LH serum and ovulation, for this reasons it may be normalized the phases of estrous cycle, remodel reproductive irregularity, renovate ovulation and decreased androgen arising symptoms in the animals with poly cystic ovary and increased averages of pregnancy, this result supported by [16]. The *Glycyrrhiza glabra* roots extract with piogiltazone treated group showed improvement in regularity of estrous cycle when compared with other groups this is possibly result from synergism effect between roots extract of *Glycyrrhiza glabra* and piogiltazone, on other hand the extract have the similar mechanisms of piogiltazone in ruling of hormones, furthermore the *Glycyrrhiza glabra* roots extract have great amount of active compenents which perhaps activated and also enhanced the beneficial effect of piogiltazone, in addition to maybe decreased adverse systemic effect of piogiltazone which considered as stress factors, this result agreement with results reported via [17].

5. Conclusion

This study was concluded the *Glycyrrhiza glabra* roots extract had the ability in treating poly cystic ovary and lead to augment positive consequence of piogiltazone when given together.

References

1. Kooti, W., Ghasemiboroon, M., Asadi-Samani, M., Ahangarpoor, A., Abadi, A. & Noori, M.(2014), "The effects of hydro-alcoholic extract of celery on lipid profile of rats fed a high fat diet" . *Adv Environ Biol.* 8(9): 325-30.
2. Decio, A.(2004), "Licorice reduces serum testosterone in healthy women". *Steroids J.* 69 (12): 763-66.
3. Armanini, D., Mattarello, M., Fiore, C., Bonanni, G., Scaroni, C., Sartorato, P., & Palermo, M. (2004), "Licorice reduces serum testosterone in healthy women". *Eur J Obstet Gynecol Reprod Biol* . 69(12):763–766.
4. Miyazaki, Y., Archana, M., Masafumi, M., Srikanth, M., Jean, H., Kenneth, C., Lawrence, J., Mandarino, M. & Ralph, A. (2002), "Effect of Pioglitazone on Abdominal Fat Distribution and Insulin Sensitivity in Type 2 Diabetic Patients" , *Journal of Clinical Endocrinology & Metabolism*. 87 (6): 4-9.
5. Chalasani, N.P., Sanyal, A.J. and Kowdley, K.V. (2009), "Pioglitazone versus vitamin E versus placebo for the treatment of non-diabetic patients with non-alcoholic steatohepatitis". *Contemp Clin Trials*. 30(1):88-96.
6. Vibha, J.B., Choudhary, K., Singh, M., Rathore, M.S.,& Shekhawat, N.S. (2009), "A Study on Pharmacokinetics and Therapeutic Efficacy of *Glycyrrhiza glabra*". *A Miracle Medicinal Herb Botany Research International.* 2(3) : 157-163 .
7. Kafali, H., Iriadam, M., Ozardali, I. & Demir, N. (2004), "Letrozole-induced polycystic ovaries in the rat: a new model for cystic ovarian disease". *Arch Med Res* . 35:103-108.
8. Cooper, M.D. & Kenneth, H. (1993), "Advanced Nutritional Therapies". Inc. Publishers. 87-94. ISBN 0-7852-7302-6.
9. SAS. (2012)."Statistical Analysis System User's Guide. Statistical" . Version 9. 1th ed, SAS. Inst. Inc. Cary. N. C. USA.
10. Lohmiller, J. & Swing, S.P. (2006), "Reproduction and Breeding". *Elsevier Academic Press*. 5: 147-164 .
11. Manuel, M., Miao, S., Julia, A.B., Fernand L, Henrik S, Malin L, Antoni J, & Elisabet, S. (2013), "Continuous Administration of a P450 Aromatase Inhibitor Induces Polycystic Ovary Syndrome with Metabolic and Endocrine Phenotype in Female Rats at Adult Age". *Endocrinology J*. 154(1):434–445.

12. Armanini, D., Bonanni, G. & Palermo, M. (1999), "Reduction of serum testosterone in men by licorice". *Engl J Med*. 341:1158.
13. Sakamoto, K., & Wakabayashi, K. (1988), "Inhibitory effect of glycyrrhetinic acid on testosterone production in rat gonads". *Endocrinol J*. 35: 333-342 .
14. Takeuchi, T., Nishii, O., Okamura, T. & Yaginuma, T. (1991), "Effect of paeoni- florin glycyrrhizin and glycyrrhetinic acid on ovarian androgen production" . *Am J Chinese Med*. 19:73–8 .
15. Armanini, D., Fiore, C., Mattarello, M., Bielenberg, J. & Palermo, M. (2002), "History of the endocrine effects of licorice". *Exp Clin Endocrinol Diabetes*. 110:257–61.
16. Glintborg, D., Hermann, A.P., Andersen, M., Hagen, C., Beck-Nielsen, H., Veldhuis, J.D. & Henriksen, J.E. (2006), "Effect of pioglitazone on glucose metabolism and luteinizing hormone secretion in women with polycystic ovary syndrome". *Fertil Steril*. 86:385–397.
17. Susan, A.J., Anthony, A., Caroline, A. S. & Alan, B. (2014), "Herbal medicine for the management of polycystic ovary syndrome (PCOS) and associated oligo/ amenorrhoea and hyperandrogenism". *BMC Complementary and Alternative Medicine.* 14:511-33.