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RESEARCH ARTICLE

EFFECT OF *GLYCYRRHIZA GLABRA* ROOTS IN COMBATING POLY CYSTIC OVARIAN SYNDROME (PCOS) IN LETROZOLE INDUCED INFLAMMATION IN RATS

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ABSTRACT

The polycystic ovary syndrome (PCOS) is a hyper androgenic disorder associated with chronic oligo-anovulation and polycystic ovarian morphology.*Glycyrrhiza glabra* Linn. Commonly known as Licorice is one of the most widely used herb from the ancient medical history of Ayurveda, both as a medicine and as a flavouring agent. Letrozole induction was reported to cause an inflammatory condition which may contribute to insulin resistance, hyperlipidemia leading to metabolic syndrome. The aim of the present study was to assess the effect of *Glycyrrhiza glabra* root extract in the treatment of letrozole induced PCOS in female wistar rats. Administration of letrozole led to an increase in the levels of CRP, TBARS and Liver marker enzymes in rats treated with letrozole. Glycyrrhiza *glabra* root extract treatment significantly decreased thelevels which were found to be elevated in PCOS rats induced by letrozole. The effect of *Glycyrrhiza glabra* was found to be comparable with clomiphene citrate, which is being used as a major medicine in the treatment of PCOS. The findings of the present study suggested that the *Glycyrrhiza glabra* root extract could be used as an adjunct therapy for the management of PCOS.

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INTRODUCTION

Body has defence mechanisms against oxidants in the form of both enzymatic and non-enzymatic antioxidants. Oxidants have both physiological and pathological role in female reproduction. Oxidative stress causes damage to the ovaries and ovulation as well as in implantation procedures. Polycystic ovarian syndrome is the most frequent endocrinological disorder that affects women of childbearing age, leading to metabolic alterations, such as hyperandrogenism, obesity, menstrual irregularities, insulin resistance and polycystic ovaries. Several genetic and environmental factors have been correlated with manifestations of this syndrome (Reis *et al.,* 2017). Furthermore, increased oxidative stress and inflammatory cytokines in the blood and histological samples of women with PCOS directed investigators to suspect that inflammatory responses and oxidative stress might play pivotal roles in the pathogenesis of PCOS (Showell *et al.,* 2013). In PCOS patients, the elevated oxidative stress causes disturbance in antioxidants balance leading to harmful effects of reactive oxygen species including endometriosis, infertility, abortion, birth defects, preeclampsia, injury to ovarian epithelium's DNA, excessive apoptosis and alteration in cell signalling process (Agrawal *et al*., 2005).PCOS is associated with increased systemic inflammation. This is evidenced by elevated CRP and TNF-α (Kelly *et al*., 2001). Many studies have indicated the implication of secondary metabolites from medicinal plants on the regulation of reproductive functions (Jha *et al*., 2010).Unfortunately, there is minimal evidence that complementary and alternative therapy is safe and efficacious. Therefore, new treatment strategies including complementary and alternative therapy need to be evaluated to alleviate PCOS, regulate hormones, and improve quality of life in PCOS patients. Licorice (*Glycyrrhiza glabra* L., / Fabaceae) is a well-known medicinal herb and it is the oldesest and widely used herbs from the ancient medical history of Ayurveda, both as a medicine and also as a flavouring to disguise the unpleasant flavour of other medications. In the traditional system of medicine, the roots and rhizomes of G. glabra have been employed clinically for centuries for their anti-inflammatory, antiulcer, expectorant, antimicrobial and anxiolytic activities (Asl and Hosseinzadeh, 2008). Liquorice has been shown to have great antioxidant, free radical scavenging (Mambro and Fonseca, 2005) and anticonvulsant activities (Nassiri-Asl *et al*., 2007) It has been shown to decrease circulating levels of testosterone in men (Rafi *et al*.,2002)

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This study aimed to investigate the possible effects of *Glycyrrhiza glabra* root extract in in letrozole induced PCOS rats using biochemical and inflammatory parameters.

MATERIALS AND METHODS

Collection and Extraction of Plant Materials

Glycyrrhiza glabra plants were collected from botanical garden at Tamil Nadu Agricultural University, Coimbatore. The plant roots were shade-dried at room temperature and then ground into powder. The sample was extracted into solvents of increasing polarity using a soxhlet apparatus. About 20 g of each powdered sample was extracted with 250 mL of methanol. The extracts were filtered and concentrated in rotary evaporator to obtain crude extracts. They were stored at -20°C until use.

Experimental animals

The study protocol was approved by Institutional Animal ethical committee (AUW.IAEC.2015.BC:01). The experimental study was carried out using 30 adult female Wistar rats body weight, 150-170g, were obtained from the Laboratory Animal Department of Kovai Medical Centre and Hospital. Animals were housed in polypropylene cages lined with husk beddings. The cages were maintained clean and hygienic Animals were kept in the standard conditions of light and temperature controlled room with a 12-12h dark-light cycle, temperature 25±2ºC and humidity 45±50% and were randomly divided into six groups. The rats were fed with commercial pellet rat feed and water *ad libitum* and were also weighed weekly throughout the duration of the experiment.

Grouping of animals

The rats were divided into six groups with 5 rats in each. Group I rats were kept as the control group received 1% carboxy methyl cellulose. Group II rats were received oral dose of letrozole (1mg /kg b.w) dissolved in 1% CMC once daily for 28 days. Group III rats received letrozole (1mg /kg b.w) along with standard drug (clomiphene citrate 1 mg/kg.b.w) for 28 days. Group IV rats received letrozole for 14 days and then supplemented with *Glycyrrhiza glabra* root extract (100mg/kg. b.w). for 28 days. Group V rats given orally letrozole at a dose of 1 mg/kg b.w in combination with *Glycyrrhiza glabra* root extract (100 mg/ Kg.b.w) once daily for 28 days. Group VI rats supplemented with *Glycyrrhiza glabra* extract alone for 28 days.

Blood sampling

At 24 h post the last dose of treatment and after 18 h fasting period, the rats were weighed and blood samples were collected by retino orbital puncture into different eppendorf tubes. The blood samples were centrifuged at 3000 r/min for 15 min and plasma and was separated for biochemical assay. The plasma was stored in a freezer at -20 °C till further analysis

Biochemical analysis

TBARS was estimated by the method of Bishayee and Balasubramaniam (1971), C–Reactive protein (CRP) was estimated by kit method (Ridker et al 2000), Aspartate amino transferase (AST) by Reitman and Frankel (1957) and Alanine amino transferase (ALT) by Kings Method, (1965).

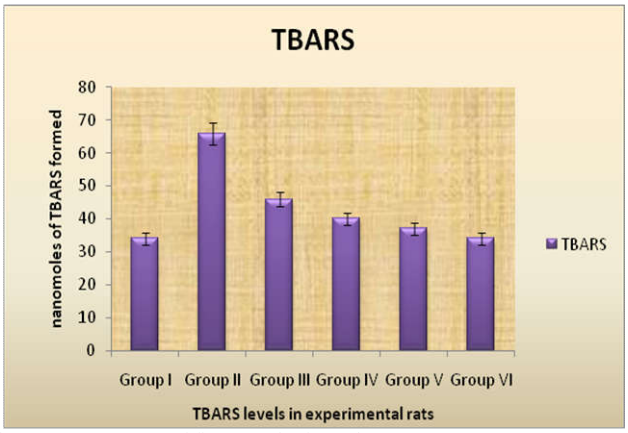
Statistical analysis

Values are expressed as mean ±SD. Statistical significance was determined by one-way analysis of variance. The values with $p< 0.05$ were considered to be significantly different.

RESULTS AND DISCUSSION

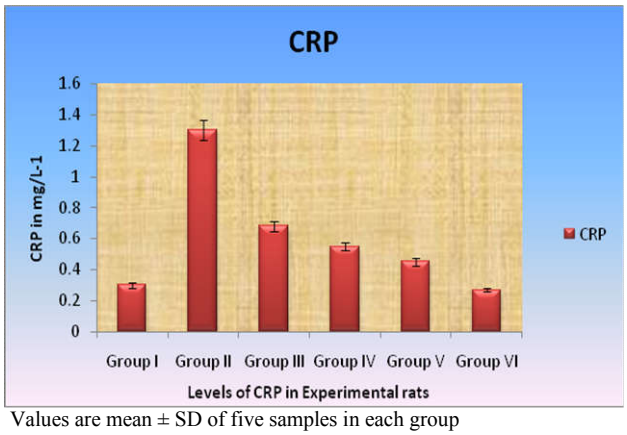
Effect of *Glycyrrhiza glabra* on plasma TBARS levels

Plasma TBARS concentration was detected significantly higher in letrozole induced PCOS rats when compared with normal control. In contrast, treatment with plant extract decrease the elevation of TBARS significantly and retained than clomiphene citrate treated and it close to that of normal control as shown in Figure 1.



Values are mean ± SD of five samples in each group
Significant at 5% level ($p<0.05$)

Figure 1. Effects of Glycyrrhiza glabra on TBARS levels in Experimental rats



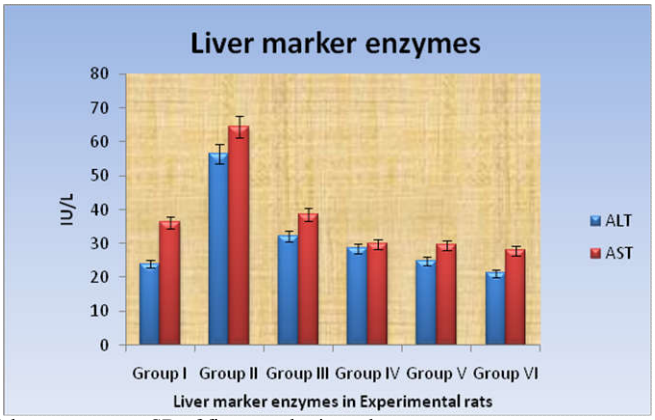
Values are mean ± SD of five samples in each group
Significant at 5% level ($p<0.05$)

Figure 2. Effects of Glycyrrhiza glabra root extract on CRP levels

Serum CRP concentration was significantly higher in letrozole induced PCOS rats as compared with normal control. However, treatment with Glycyrrhiza glabra significantly reduced CRP concentration in rats treated with letrozole.

Effects of *Glycyrrhiza glabra* on the levels of liver marker enzymes

The levels of ALT and AST concentration were significantly higher in letrozole induced PCOS rats when compared with normal control.



Values are mean ± SD of five samples in each group
Significant at 5% level ($p<0.05$)

Figure 3. Effects of *Glycyrrhiza glabra* on the levels of liver marker enzymes

In contrast, treatment of *Glycyrrhiza glabra* decrease the elevation of liver marker enzymes significantly and retained it close to that of normal control as shown in figure 3. The present data showed that *Glycyrrhiza glabra* significantly decrease the selected inflammatory biomarkers and oxidative stress biomarker, and liver marker enzymes in letrozole-induced PCOS rats. Letrozole, a non-steroidal aromatase inhibitor, was used to induce PCOS in the rats. It was found that cystogenesis in the letrozole-induced poly cystic ovaries was associated with several alterations in biochemical factors. Letrozole reduces conversion of androgens to estrogens in the ovary and results in a condition termed hyperandrogenemia. Hyperandrogenism as a key regulator in the pathogenesis of a majority of PCOS cases developed cystogenesis by impairing maturation of developing follicles in the ovaries. (Goodarzi *et al*., 2011).

Experimental models and clinical studies data suggest that hyperandrogenism is progenitor of chronic lowgrade inflammation that in turn directly stimulates excess ovarian androgen production (Gonzàlez, 2012). In recent decades, more studies have shown that oxidative stress and low grade inflammation are the two main pathways in the pathogenesis of PCOS (Rezvanfar *et al*., 2016). Elevated TBARS levels in PCOS rats suggest enhanced lipid peroxidation leading to tissue damage and inability of antioxidant defense mechanisms to prevent free radical attack.*Glycyrrhiza glabra* has been demonstrated to reduce testosterone level (Saris and Wardle 2010). Isoflavones are a sub group of phytoestrogens, with structure similar to 17-B-estradiol and capable of binding to estrogen receptors. *Glycyrrhiza glabra* (licorice) contains not only triterpene saponins (glycyrrhizin), flavonoids polysaccharides but also various isoflavonoids, glabrone and genesitin. This phytoestrogens may be responsible for the management of PCOS (Vijayalakshmi and abhilasha, 2013).

Oxidative stress is considered as an important pathological feature of PCOS and women with PCOS have decreased total antioxidant status (Jungbauer and Medjakovic 2014). Treatment with Glycyrrhiza glabra root extract significantly reduced LPO, letrozole-induced PCOS model of rats. The antioxidant potencies of isoflavones are structurally related and closely associated with the presence of hydroxyl groups. Genistein is the most powerful antioxidant and isoflavones of Glycyrrhiza glabra roots and it is well documented in previous studies.

The present study demonstrated that *Glycyrrhiza glabra* treatment decrease serum CRP compared to the PCOS control. C-reactive protein is an inflammatory marker, which is a member of the group of acute phase proteins and the level of CRP increases in response to inflammation. (Rhodes *et al*., 2011). According to the results of Shapna (2010) *Glycyrrhiza glabra* has the most powerful antioxidant and radical scavenging activity which may be the reason to alleviate and prevent chronic diseases.

ALT, a sensitive indicator of liver cell injury, is a cytosolic enzyme and is thought to be a more specific indicator of liver damage than AST (Kalpan 2002). In our study a significant increase in the ALT and AST levels may be due to letrozole induction (Mehmet *et al* 2010). Glycyrrhizin present in the roots of *Glycyrrhiza glabra* may significantly inhibits membrane lipid peroxidation might be responsible for the activity. 18β-glycyrrhetic acid (an aglycone of glycyrrhizin acid) shows hepatoprotective activity by inhibiting both free radical generation and lipid peroxidation (Jeong et al 2002).

Conclusion

The current results suggest that application of *Glycyrrhiza glabra* root extract with dual antioxidant and anti-inflammatory effects might be effective in the management of PCOS. So that it can be used as an adjunct therapy sideways to currently used drugs in PCOS management.

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