# Agaricus Subrufescens Ameliorates Ovarian Dysfunction and Regulates Altered Biochemical Parameters in Rats with Letrozole Induced Polycystic Ovarian Syndrome

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# Abstract

### Objective

To examine the impact of an ethonolic extract of *Agaricus subrufescens* on rat models of Polycystic Ovarian Syndrome (PCOS) produced by letrozole.

### Methods

Thirty female Wistar rats were split into five six-rat groups. Negative controls received 1 mL of 0.5% carboxy methylcellulose (CMC). Other groups received letrozole (1 mg/kg) for 21 days to induce PCOS. Positive control animals were slaughtered on day 22. The test and standard groups received treatment from the 22nd to the 36th day. The test group received *Agaricus subrufescens* ethanoic extract (200 mg/kg, 400 mg/kg, p.o.) and the standard group clomiphene citrate (1 mg/kg). Estimated blood glucose, total cholesterol, triglycerides, and hormonal changes like increased testosterone, oestrogen, and decreased progesterone with menstrual irregularity confirmed by vaginal smears and histopathological ovary changes in polycystic ovarian disease control.

### Results

*Agaricus subrufescens* reduced blood glucose, testosterone, anovulation, and menstrual irregularity. All therapies markedly corrected SGOT and SGPT levels. Letrozole-induced PCOS rats had elevated urea and creatinine. This study suggests that *Agaricus subrufescens* therapy protects renal function by lowering serum urea and creatinine. In letrozole-induced PCOS rats, suppressing hepatic synthesis, increasing ovarian follicle immaturity, and boosting androgen discharges increase liver and ovary weight. Endocrine organ weight decreased in all treatment groups. PCOS control histopathology indicated more cysts and theca lutein cells. PCOS control rats had more cysts than treatment groups.

### Conclusion

Oral letrozole produced polycystic ovarian disease in this research. It showed elevated blood glucose, total cholesterol, triglycerides, and hormonal changes like increased testosterone and oestrogen and decreased progesterone with menstrual irregularity confirmed by vaginal smears and histopathological changes in the ovary of polycystic ovarian disease control. Blood glucose, total cholesterol, and testosterone levels dropped in *Agaricus subrufescens* treatment groups.

# 1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endrocrine disorders, characterised by the accumulation of many cysts (fluid-filled sacs) on the ovaries, hyperandrogenism, ovulatory dysfunction, abdominal obesity, and other metabolic disturbances. PCOS causes infertility, monthly irregularity, hirsutism, and increased testosterone production [1, 2]. PCOS causes acne, acanthosis nigricans, insulin insensitivity, hyperandrogenism, and persistent anovulation [3–6]. Long-term effects include cancer, type

II diabetes, dyslipidemia, hypertension, and cardiovascular disease. Weight loss and inactivity can reverse PCOS's reproductive and metabolic effects. Lipid imbalance, oxidative stress, insulin resistance, and genetics may cause PCOS [7, 8]. It is caused by hypothalamic-pituitary axis, insulin, and ovarian dysfunction. Insulin resistance and obesity are linked [9]. Insulin resistance increases ca cell androgen production by luteinizing hormone and inhibits liver cell sex hormone binding globulin (SHBG) synthesis. It decreases follicular stimulating hormones in granulose cells, arresting follicular development [10].

Insulin-resistant and non-insulin-resistant PCOS are common. Insulin-resistant PCOS, or "Type 1 PCOS," causes most PCOS symptoms. Weight gain, ovulatory disturbances, facial hair, hair loss, and acne. Insulin-resistant PCOS causes hyperglycemia and excessive testosterone. Insulin resistance affects the hypothalamus pituitary ovarian axis, prompting ca cells to generate more androgen and decrease it in liver cells, causing hyperandrogenism, anovulation, and polycystic ovarian syndrome. Vitamin D, iodine, hormone-disrupting contaminants, thyroid disease, and adrenal stress cause non-insulin-resistant PCOS. Anti-diabetics cannot treat non-insulin-resistant PCOS or help women reduce hormonally-induced weight. Natural remedies. Iodine, vitamin D, magnesium, zinc, and testosterone-lowering herbs can be taken alongside dairy abstinence. Natural progesterone balances hormones and ovulates [11–15]. About 4–12% of US reproductive-age women have PCOS. 10% of women have PCOS. 6.5–8% European women have PCOS. 2.2–26%. Despite no evidence, experts believe 10% of Indian women have PCOS. PCOS is a genetically complicated endocrine disorder with an unknown cause and complex pathogenesis. PCOS symptoms may overlap with normal physiologic maturation in the two years after menarche, delaying clinical diagnosis. Slim women with familial PCOS may acquire weight. Hirsutism, acne, female pattern hair loss, acanthosis nigricans, seborrhea, striae, and acrochordons were 78%, 48%, 31%, 30%, 29%, 13%, and 9% [16–18]. Ovaries with multiple small follicles (cysts 4 to 9 mm in diameter) are polycystic. Tiny follicles cannot ovulation. Unbalanced oestrogen, progesterone, LH, and FSH. Adrenals and ovaries produce androgens. DHEA, DHEAS, testosterone, androstenedione are androgens. LH and insulin levels in PCOS women may increase androgens [19, 20] .

The main goal of this study is to assess the efficacy of *Agaricus subrufescens* on PCOS using blood glucose level estimation, detection of menstrual cycle abnormalities, biochemical estimation, hormonal analysis, and follicular cyst development in rats with letrozole-induced PCOS.

# 2. Materials & Methods

# 2.1 *Plant Profile*

Almond mushroom, mushroom of the sun, God's mushroom, mushroom of life, royal sun agaricus, jisongrong, himematsutake, and others are names for Agaricus subrufescens, a species of mushroom in the Agaricaceae family. Agaricus subrufescens is a tasty almond-scented food. In traditional and alternative medicine, the fungus is known as a medicinal mushroom with anti-cancer qualities. Agaricus mushrooms have not been adequately tested as a food, dietary supplement, or medication [21, 22].

## 2.1.1 *Plant collection, drying and authentication*

The plant was collected and dried on filter paper sheets in shade at room temperature. The plant dried in a month. Again dried and powdered. Nallamalla Forest in Andhra Pradesh yielded fresh Agaricus subrufescens. Dr. Ramadevi, Associate Professor of Dr. Y.S.R. Horticulture University, West Godavari, Andhra Pradesh, India, validated the plant.

## 2.1.2 *Extraction Procedure*

The dried and powdered material was extracted in ethanol using Soxhlet system for 18 hours. In a Soxhlet device, powdered material was dissolved in ethanol (1:3) and extracted. The round-bottom flask extract was evaporated to dryness to produce a yellowish-brown *Agaricus subrufescens* extract (EAS), which was stored in an airtight container. The crude EAS was stored at room temperature, phytochemically analysed to detect plant secondary metabolites [23].

## 2.1.3 *Stock solution preparation*

Using CMC as a diluting solvent, a 1 mg/ml solution was prepared.

## 2.1.4 *Test dose preparation*

200 mg/ml and 400 mg/ml solutions prepared from the aforementioned 1 mg/ml solution. Animals received doses in accordance with their body weight.

## 2.1.5 *Standard dose preparation*

CMC was used as a diluting solvent to prepare 1 mg/ml solution of clomifene citrate. Animals received doses in accordance with their body weight.

# 2.2 Phytochemical screening

Several qualitative tests were done to check the existence of phytochemical analysis in the ethanolic crude extract of *Agaricus Subrufescens* (EAS) [24, 25].

## 2.2.1 *Acute Toxicity Studies*

Acute toxicity studies followed organization for economic cooperation and development (OECD) draught standards 425 from CPCSEA. With only water, the animals fasted for 4 hours. Over 14 days, mice were given 1000 and 2000 mg/kg of *Agaricus subrufescens* ethanoic extract and examined for mortality and physical and behavioural abnormalities. The Institutional Animal Ethical Committee authorised the experiments [26].

## 2.2.2 *Animal groups design*

Group-I: Normal control (Received 2ml distilled water orally chow diet)

Group-II: Letrozole (LTZ) control; Scarified on day 22

Group-III: LTZ + clomiphene citrate (STD); Received 1.0mg/kg body weight dose of clomiphene citrate for 15days post letrozole induction.

Group-IV: LTZ + EAS (Treatment-1); Received 200mg/kg body weight dose of clomiphene citrate for 15days post letrozole induction.

Group-V: LTZ + EAS (Treatment-2); Received 400mg/kg body weight dose of Clomiphene citrate for 15days post letrozole induction

## 2.2.3 *Study Design*

Mahaveer Enterprises provided 180–220 g Wister albino female rats, were kept under 27 ± 2°C, 80 ± 10% humidity and a 12 hour light/dark cycle were maintained for all animals. They were fed a conventional rat diet and had unrestricted access to water. Three rats with husk bedding were in each case. The trial began after 7 days of acclimatisation. Letrozole at 1.0 mg/kg dissolved in 0.25% CMC (2 ml/kg body weight vehicle) was given to female rats daily for 21 days to develop polycystic ovary. On the 22nd day, PCOS control group mice were anaesthetized with diethyl ether and blood was taken from retro orbital puncturing to estimate blood glucose, oestrogen, progesterone, and total cholesterol. Uteruses, ovaries, kidneys, livers, and hearts were weighed after animals were slaughtered. The standard group animals received clomiphene citrate for 15 days, whereas the treatment group received varying doses of plant extract. On the 36th day, animals were anaesthetized with diethyl ether and retro orbital punctured to measure blood glucose, oestrogen, progesterone, and total cholesterol, then sacrificed to weigh the uterus, ovaries, kidneys, liver, and heart [6, 27, 28].

## 2.2.4 *Preparation of Vaginal smear*

The vaginal epithelium's response to ovarian hormones determines the vaginal smear method's ovarian function study value. The vaginal epithelium's reaction to ovarian hormones was measured by daily smears at 10am. Daily, at 10am smears were taken. Diethyl ether anaesthetized female animals were placed on their ventral surfaces. Wet a cotton ear swab with regular saline. I carefully spun the rat's vagina swab clockwise after inserting it to a specific depth. I cautiously took the vaginal swab and rolled it on a microscopic glass slide. Drying crystal violet or methylene blue discoloured the glass slide. Water carefully washed and dried the glass slide for one minute. Then 10X light microscopes examined cells [29].

## 2.2.5 *Estimation of Biochemical parameters*

The mice were anaesthetized with diethyl ether on the 22nd day (just the PCOS-induced group) and 36th day (all groups). The retroorbital plexus was used to take blood, and the serum was separated by centrifuging whole blood without anticoagulants at 3000 rpm for 10 min. Blood glucose, total cholesterol, triglycerides, SGOT, SGPT, urea, and creatinine were measured using normal laboratory methods [30, 31].

## 2.2.6 *Hormonal estimation, organs weight measurement, body weight monitoring*

Enzyme immunoassay kits measured serum testosterone, oestrogen, and progesterone [32]. After the study, all animals were sacrificed and their liver, kidney, heart, uterus, and ovary were taken, cross-examined, and weighed. A weighing balance was used to weigh the animals every morning until the experiment ended [4]. Every morning, feed intake was subtracted.

## 2.3 *Histopathology*

Ovaries were dissected shortly after scarification. They were then formalin-fixed for 16 hours. After fixation, samples were dehydrated by immersing them in a series of alcoholic solutions of increasing strength, usually 70%, 90%, and 100% alcohol, for 15 minutes each. After 20 minutes in xylene, samples were placed in a tissue embedding cassette and covered with molten pure paraffin wax. 20 minutes at 20°C. They were sectioned at least 2µm thick and viewed under a microscope.

# 3. Results & Discussion

Phytochemical screening of *Agaricus Subrufescens* ethanolic extract revealed the presence of alkaloids, carbohydrates, glycosides, tannins/ polyphenols, phytosterols, triterpinoids, flavonoids, saponins and proteins.

Table 1
Phytochemical investigation of *Agaricus Subrufescens*

| S. No. | Test | Presence |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Carbohydrates | + |
| 3 | Glycosides | + |
| 4 | Tannins or Phenolic Compounds | + |
| 5 | Phytosterols and Triterpennoids | + |
| 6 | Flavonoids | + |
| 7 | Saponins | + |
| 8 | Proteins | + |

One oral dose of letrozole at 1 mg/kg body weight in female rats caused polycystic ovarian syndrome. The animals experience irregular estrus cycles, anovulation, hormonal imbalance, aberrant follicular development, hyperlipidemia, and hyperglycemia. These findings indicated PCOS induction. Letrozole-induced PCOS management reduced weight. Compared to normal and PCOS control, treatment groups had significant weight changes. Natural healing did not affect weight compared to normal or PCOS control. Letrozole-controlled PCOS lowered feed consumption. All treatment groups had higher feed consumption than normal and PCOS controls. Natural recovery feed intake was similar to normal and PCOS control. PCOS diagnosis relied on hormonal testing. PCOS raises oestrogen. Letrozole-induced

PCOS rats had higher blood oestrogen and testosterone and lower progesterone levels than the PCOS control group. Letrozole enhances pituitary sensitivity to GnRH, increasing leutinizing hormone (LH) and insulin levels, which mostly worsen their steroidogenesis abnormalities. Hyperandrogenism results from androgen excess. *Agaricus subrufescens* normalised them.

All treatment groups had significantly lower oestrogen and testosterone levels than letrozole-induced PCOS rats. Anovulation occurs when progesterone levels drop, preventing ovulation. The PCOS control group had lower progesterone levels. Treatment groups outperformed PCOS and normal control groups. Letrazole-induced PCOS rats had high blood glucose. Letrozole enhances testosterone production, insulin resistance, and hyperinsulinemia, as established. Treatment groups reduced blood glucose more than letrazole-induced PCOS control, while the natural recovery group did not. Letrozole-induced PCOS rats had elevated total cholesterol and triglycerides. High levels may promote obesity and cardiovascular disease. *Agaricus subrufescens* reduced cholesterol and triglycerides in all treatment groups (Groups III, IV, and V) compared to letrazole-induced PCOS control. Smears were collected on all animals for a total of 36 days. Smears were taken each day at 10 am.

Table 2
Effect of various treatments on Body weights in Letrozole induced PCOS rats

| Group | Initial | After Induction | After Treatment |
|---|---|---|---|
| Normal Control | 181.2 ± 1.43 | 187.1 ± 0.40 | 196.6 ± 3.38 |
| LTZ | 180.2 ± 2.26 | 169.4 ± 3.20 | 173.1 ± 3.24 |
| LTZ + STD | 180.6 ± 0.378 | 173.8 ± 2.35 | 181.3 ± 2.34* |
| LTZ + EAS (200mg/kg) | 182.6 ± 3.21 | 178.2 ± 3.25 | 183.5 ± 4.32** |
| LTZ + EAS (400mg/kg) | 181.1 ± 3.41 | 171.5 ± 4.57 | 189.2 ± 4.21** |

The above Values are expressed as Mean ± SEM, n = 6.used one way ANOVA to calculate statistical significance of various groups at *P < 0.05,**P < 0.01 by using Dunnette multiple comparison test.

Table 3
Effect of various treatments on Feed intake in Letrozole induced PCOS rats

| Groups | Intake of feed during induction period (21days) | Feed intake during treatment period (15 days) |
|---|---|---|
| Normal Control | 25.28 ± 1.45 | 39.51 ± 1.15 |
| LTZ | 18.36 ± 1.12 | 29.56 ± 1.21 |
| LTZ + STD | 22.88 ± 1.13 | 37.09 ± 1.41 |
| LTZ + EAS (200mg/kg) | 28.32 ± 1.21 | 38.58 ± 1.33* |
| LTZ + EAS (400mg/kg) | 33.15 ± 1.12 | 42.12 ± 1.15* |

Values are expressed as Mean ± SEM, n = 6.used one way ANOVA to calculate statistical significance of various groups at,* P < 0.001 by using Dunnett multiple comparison test.

Table 4
Effect of various treatments on Hormones in Letrozole induced PCOS rats

| Groups | Estrogen (pg/ml) | Progesterone (ng/ml) | Testosterone (ng/ml) |
|---|---|---|---|
| Normal Control | 7.05 ± 0.25 | 31.24 ± 1.12 | 6.91 ± 0.61 |
| LTZ | 21.45 ± 0.43 | 20.1 ± 1.46 | 16.17 ± 1.02 |
| LTZ + STD | 10.92 ± 0.46** | 30.80 ± 1.42* | 11.32 ± 0.16** |
| LTZ + EAS (200mg/kg) | 12.19 ± 0.17** | 32.1 ± 0.94* | 12.12 ± 0.90** |
| LTZ + EAS (400mg/kg) | 11.15 ± 0.54** | 39.52 ± 1.25** | 10.26 ± 0.65** |

The above Values are expressed as Mean ± SEM, n = 6.used one way ANOVA to calculate statistical significance of various groups at,*P < 0.01,**P < 0.001 by using Dunnette multiple comparison test.

Table 5
Effect of various treatments on Blood Glucose in Letrozole induced PCOS rats

| Groups | Initial Blood Glucose | Blood Glucose after Induction | Blood Glucose after Treatment |
|---|---|---|---|
| Normal Control | 112.5 ± 2.51 | 120.2 ± 2.574 | 107.6 ± 3.66 |
| LTZ | 113.3 ± 2.12 | 196.6 ± 7.46 | 172.3 ± 4.24 |
| LTZ + STD | 108.7 ± 3.26 | 193.2 ± 1.24 | 138.6 ± 7.51*** |
| LTZ + EAS (200mg/kg) | 108.6 ± 3.24 | 198.5 ± 2.11 | 161.5 ± 4.45* |
| LTZ + EAS (400mg/kg) | 113.6 ± 2.13 | 188.8 ± 3.62 | 154.2 ± 2.41** |

Values are expressed as Mean ± SEM, n = 6.used one way ANOVA to calculate statistical significance of various groups at*P < 0.05,**P < 0.01,***P < 0.001 by using Dunnette multiple comparison test.

# 3.1 Biochemical Parameters

Table 6
Effect of various treatments on cholesterol and triglycerides in Letrozole induced PCOS rats

| Groups | Cholesterol (mg/dl) | Triglycerides (mg/dl) |
|---|---|---|
| Normal Control | 172.6 ± 2.27 | 149.6 ± 2.34 |
| LTZ | 229.4 ± 9.52 | 184.6 ± 4.31 |
| LTZ + STD | 189.5 ± 6.51* | 168.4 ± 1.32* |
| LTZ + EAS (200mg/kg) | 181.5 ± 2.48* | 161.2 ± 2.12** |
| LTZ + EAS (400mg/kg) | 170.2 ± 5.91** | 151.5 ± 1.09** |

Values are expressed as Mean ± SEM,n = 6.used one way ANOVA to calculate statistical significance of various groups at*P < 0.05,**P < 0.01,by using Dunnette multiple comparison test.

Table 7
Effect of various treatments on SGOT and SGPT levels in Letrozole induced PCOS rats

| Groups | SGPT (mg/dl) | SGOT (mg/dl) |
|---|---|---|
| Normal Control | 35.41 ± 1.12 | 32.10 ± 1.17 |
| LTZ | 57.25 ± 4.23 | 58.24 ± 3.54 |
| LTZ + STD | 37.12 ± 2.42* | 45.28 ± 2.38* |
| LTZ + EAS (200mg/kg) | 36.04 ± 1.52*** | 37.25 ± 1.24* |
| LTZ + EAS (400mg/kg) | 28.52 ± 1.34 *** | 32.24 ± 1.21 * |

Values are expressed as Mean ± SEM, n = 6 used. one way ANOVA to calculate statistical significance of various groups at*P < 0.05,**P < 0.01,***P < 0.001 by using Dunnette multiple comparison test.

Table 8
Effect of various treatments on Creatinine and Urea in Letrozole induced PCOS rats

| Groups | Creatinine (mg/dl) | Urea (mg/dl) |
|---|---|---|
| Normal Control | 0.98 ± 0.03 | 25.83 ± 1.13 |
| LTZ | 2.12 ± 0.344 | 5.17 ± 1.22 |
| LTZ + STD | 1.23 ± 0.06* | 31.24 ± 0.92** |
| LTZ + EAS (200mg/kg) | 1.23 ± 0.07* | 32.23 ± 1.61** |
| LTZ + EAS (400mg/kg) | 1.11 ± 0.08** | 33.44 ± 0.71* |

The above Values are expressed as Mean ± SEM,n = 6.used one way ANOVA to calculate statistical significance of various groups at*P < 0.05,**P < 0.01,***P < 0.001 by using Dunnette multiple comparison test.

Table 9
Effect of various treatments on weights of Ovaries & Uterus in Letrozole induced PCOS rats

| Groups | Ovaries | Uterus |
|---|---|---|
| Normal Control | 0.208 ± 0.001 | 0.592 ± 0.03 |
| LTZ | 0.256 ± 0.02 | 0.806 ± 0.04 |
| LTZ + STD | 0.151 ± 0.01* | 0.531 ± 0.03** |
| LTZ + EAS (200mg/kg) | 0.169 ± 0.01*** | 0.346 ± 0.02 |
| LTZ + EAS (400mg/kg) | 0.155 ± 0.01* | 0.282 ± 0.03*** |

Values are expressed as Mean ± SEM,n = 6.used one way ANOVA to calculate statistical significance of various groups at*P < 0.05,**P < 0.01,***P < 0.001 by using Dunnette multiple comparison test.

# 4. Conclusion

In this study, oral letrozole promoted polycystic ovarian disease. It showed elevated blood glucose, total cholesterol, and triglycerides, hormonal changes like increased testosterone and oestrogen and decreased progesterone, and menstrual irregularity confirmed by vaginal smears and histopathological changes in the ovary of polycystic ovarian disease control. *Agaricus subrufescens* treatment groups decreased blood glucose, total cholesterol, and testosterone. It may be attributed to phytoconstituents like glycosides, sugars, alkaloids, saponins, terpinoids, proteins, steroids, and phenolic substances. *Agaricus subrufescens* decreased blood glucose, testosterone production, and anovulation and monthly irregularity. For better polycystic ovarian disease treatment and management, active constituent isolation and full-scale clinical investigations are needed. In this study, PCOS control group SGOT and SGPT levels were considerably higher than normal control. It shows PCOS-related hepatic dysfunction. In all treatments, SGOT, SGPT, and ALP levels decreased. Renal impairment may be caused by elevated serum urea and creatinine in letrozole-induced PCOS rats. Atherogenesis begins with endothelial cell failure. This study suggests that *Agaricus subrufescens* reduces renal function impairment, as shown by a decrease in serum urea and creatinine. Inhibiting hepatic synthesis, increasing ovarian follicle immaturity, and raising androgen secretions in the letrozole-induced PCOS rat increase liver and ovary weight. All treatment groups show a weight reduction in these endocrine organs. It may help *Agaricus subrufescens*. Histopathological studies of PCOS control indicated more cysts and theca lutein cells than these therapy groups. Cyst numbers were much lower in treatment groups than in PCOS control animals.

# Abbreviations

PCOS : Polycystic Ovarian Syndrome, CMC : Carboxy methylcellulose, LTZ : Letrozole, SGOT : Serum Glutamic Oxaloacetic Transaminase, SGPT : Serum Glutamic Pyruvic Transaminase, *SHBG : Sex hormone binding globulin*, LH : Luteinizing Hormone, FSH : Follicle Stimulating Hormone, DHEA : Dehydroepiandrosterone, DHEAS : Dehydroepiandrosterone sulphate, EAS : *Agaricussubrufescens* extract, GnRH : Gonadotropin releasing hormone

# Declarations

I hereby declare that this submission is entirely my own work, in my own words, and that all sources used in researching it are fully acknowledged and all quotations properly identified.

### Ethics approval

The study was approved by IAEC (IAEC/SVCOP/007/2022-23) and conducted according to the CPCSEA guidelines.

### Consent to Publication

All the authors have read and agreed to the final copy of the finding as contained in the manuscript.

### Availability of data and materials

The datasets/information used for this study is available on reasonable request.

### Conflicting interest

All authors report that there was no conflict of interest in this work.

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### Author Contributions

Conceptualisation: S. P. N. B, P. B. B. R, K. S. K

Methodology and investigation: S. P. N. B, P. B. B. R, K. S. K, M. J, S. O

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Writing—review and editing: S. P. N. B, P. B. B. R, K. S. K, M. J, R. K, M. B, B. R, H. B, S. O

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# Figures

**Figure 1**

*Agaricus Subrufescens*

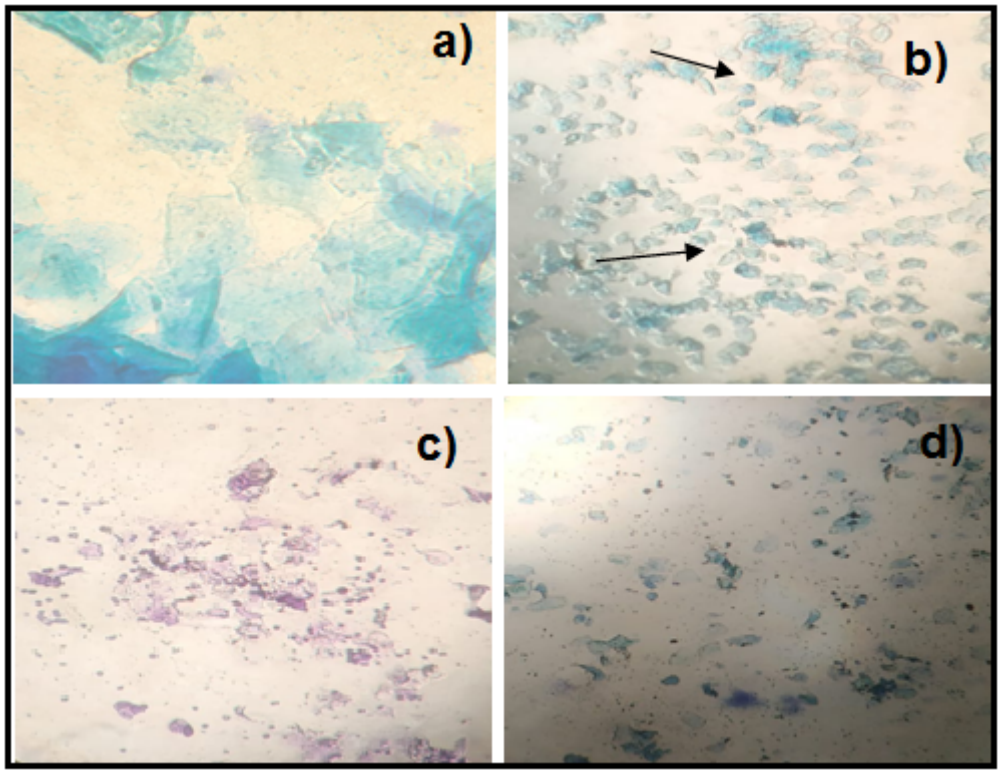



**Figure 2**

Group-I animal cells in the estrus cycle under a microscope;

a) Proestrous b) Estrus c) Metaestrus d) Diestrus

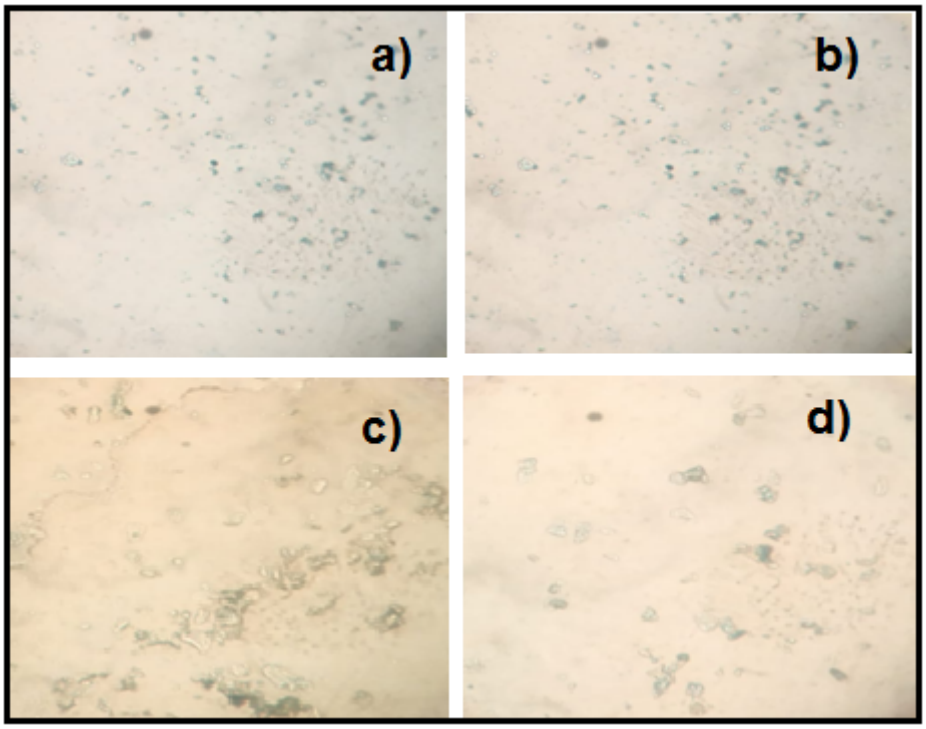



**Figure 3**

Letrozole induced animal cells in the estrus cycle under a microscope;

a) Proestrous b) Estrus c) Metaestrus d) Diestrus

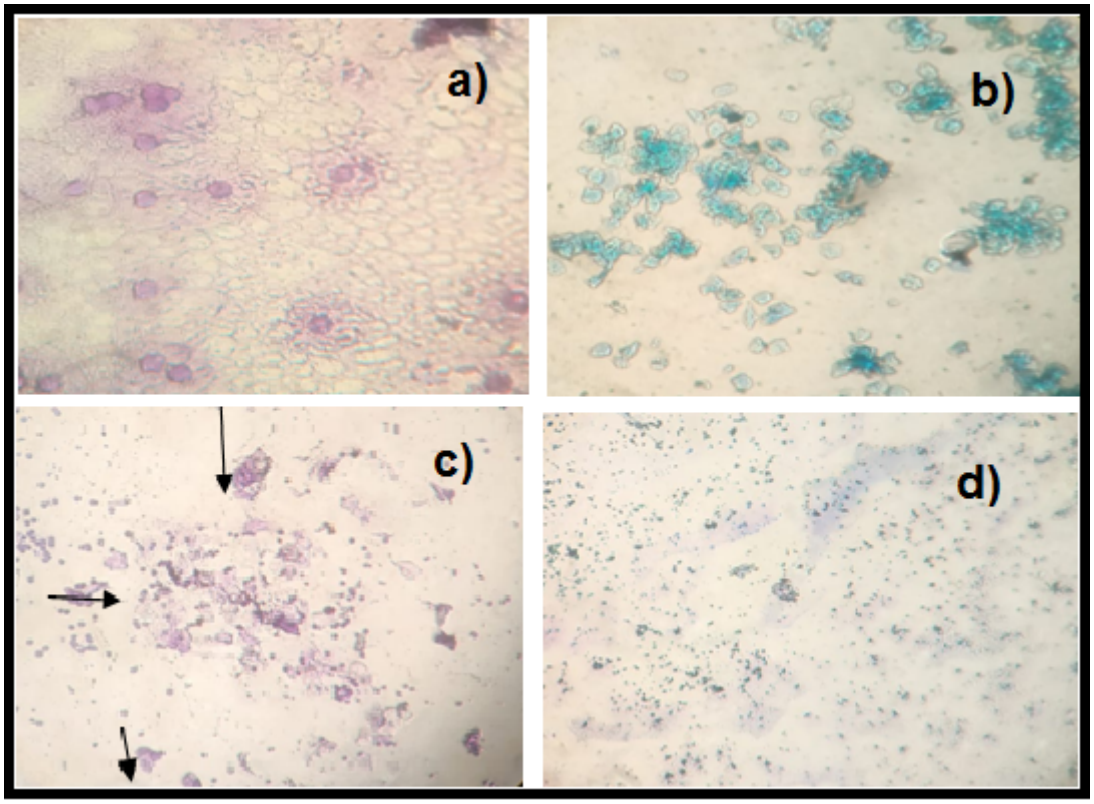
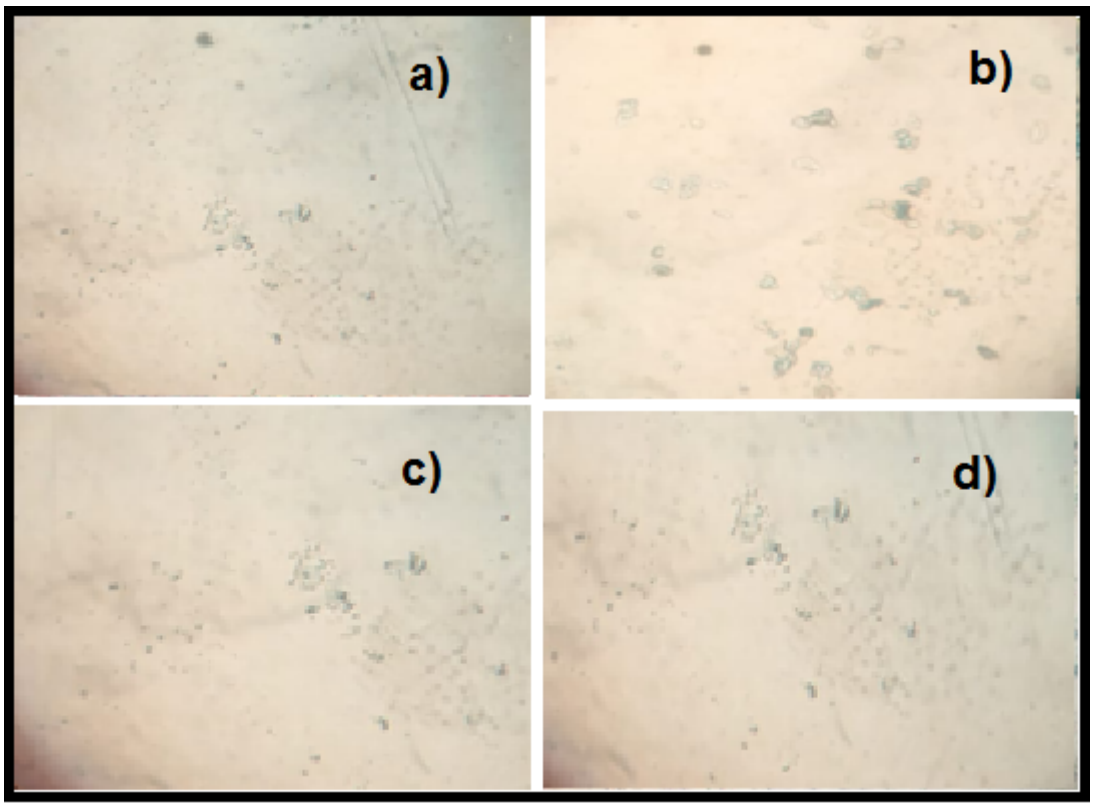



**Figure 4**

Treatment group animal cells in the estrus cycle under a microscope;

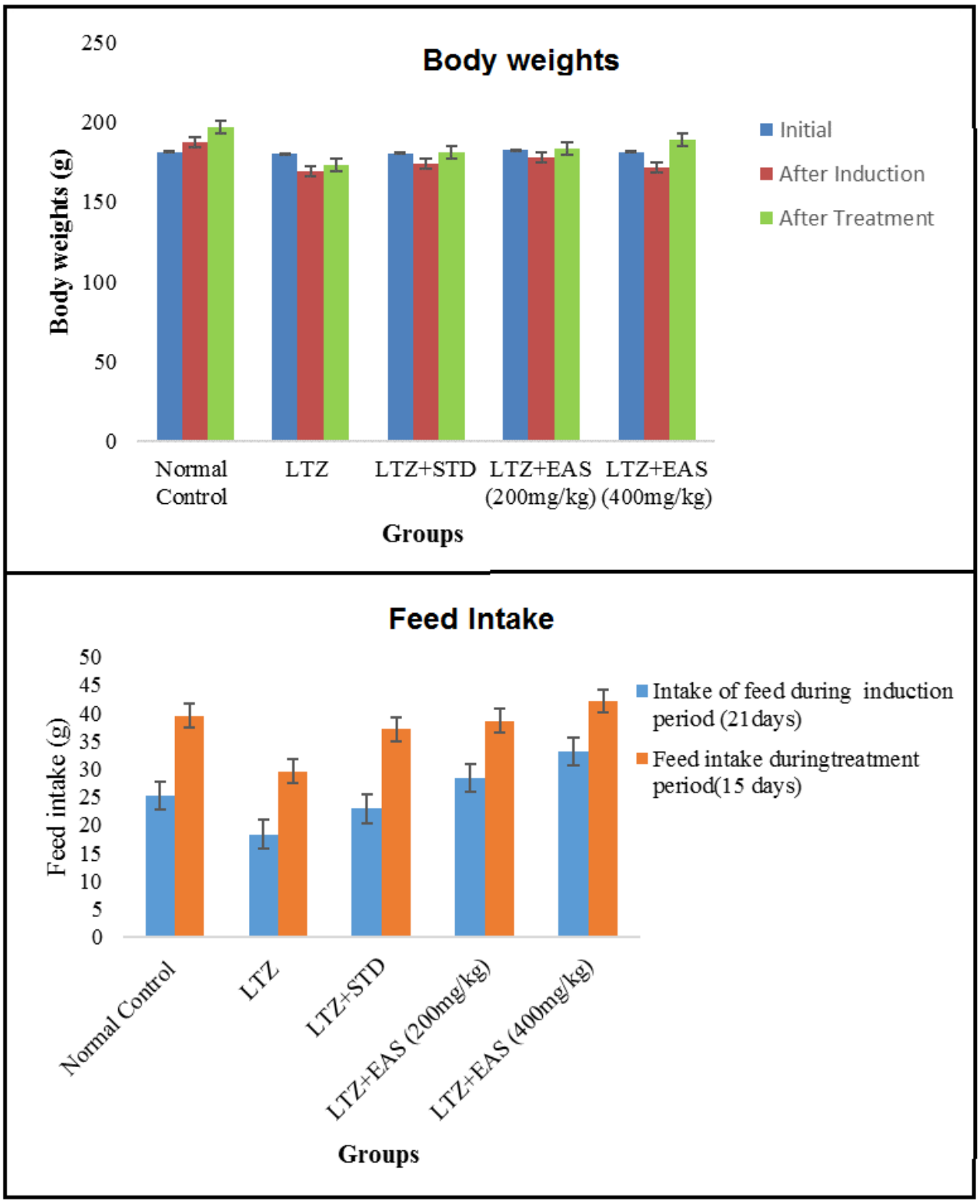
a) Proestrous b) Estrus c) Metaestrus d) Diestrus



## Figure 5

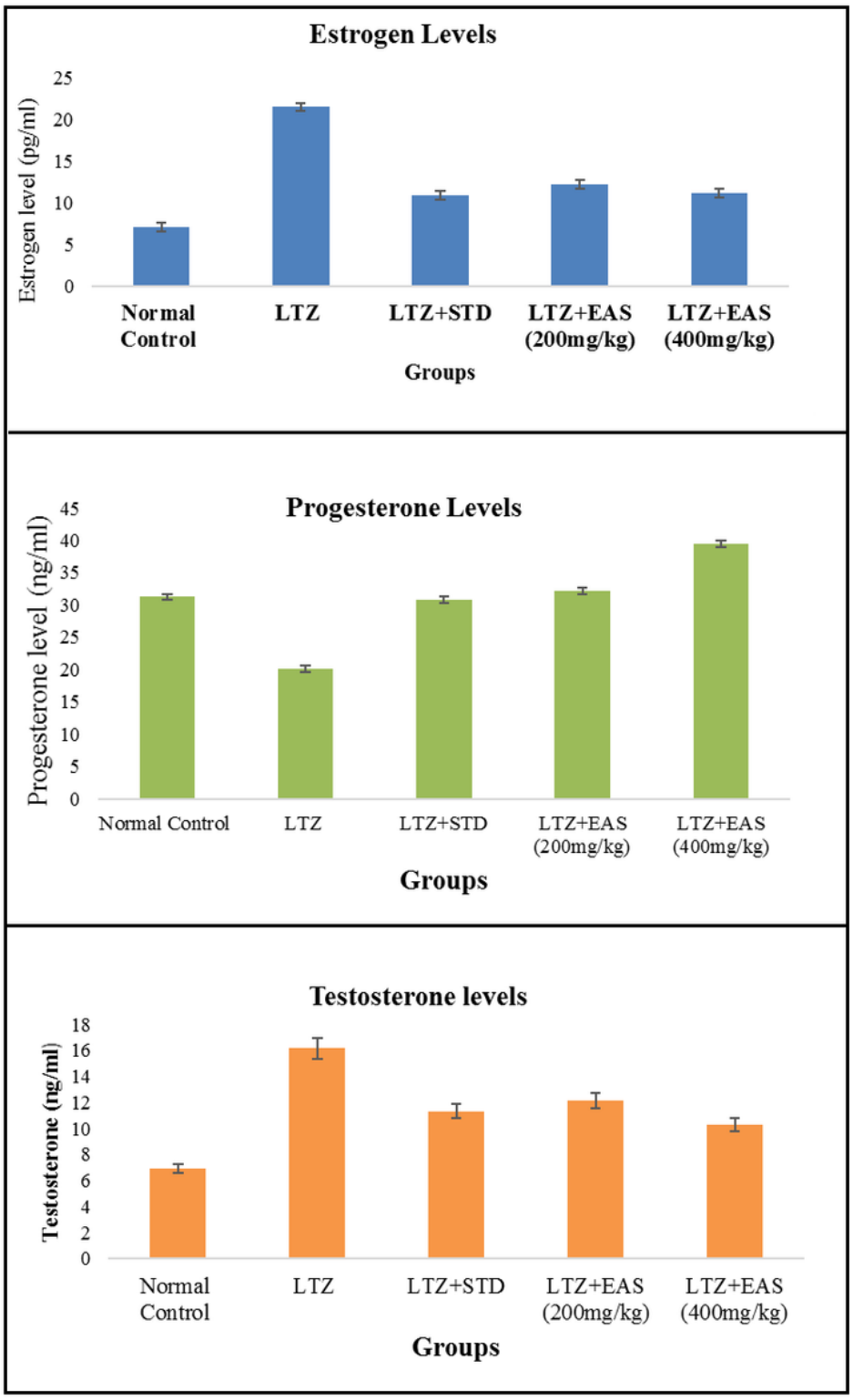
Natural recovery group animal cells in the estrus cycle under a microscope;

a) Proestrous b) Estrus c) Metaestrus d) Diestrus



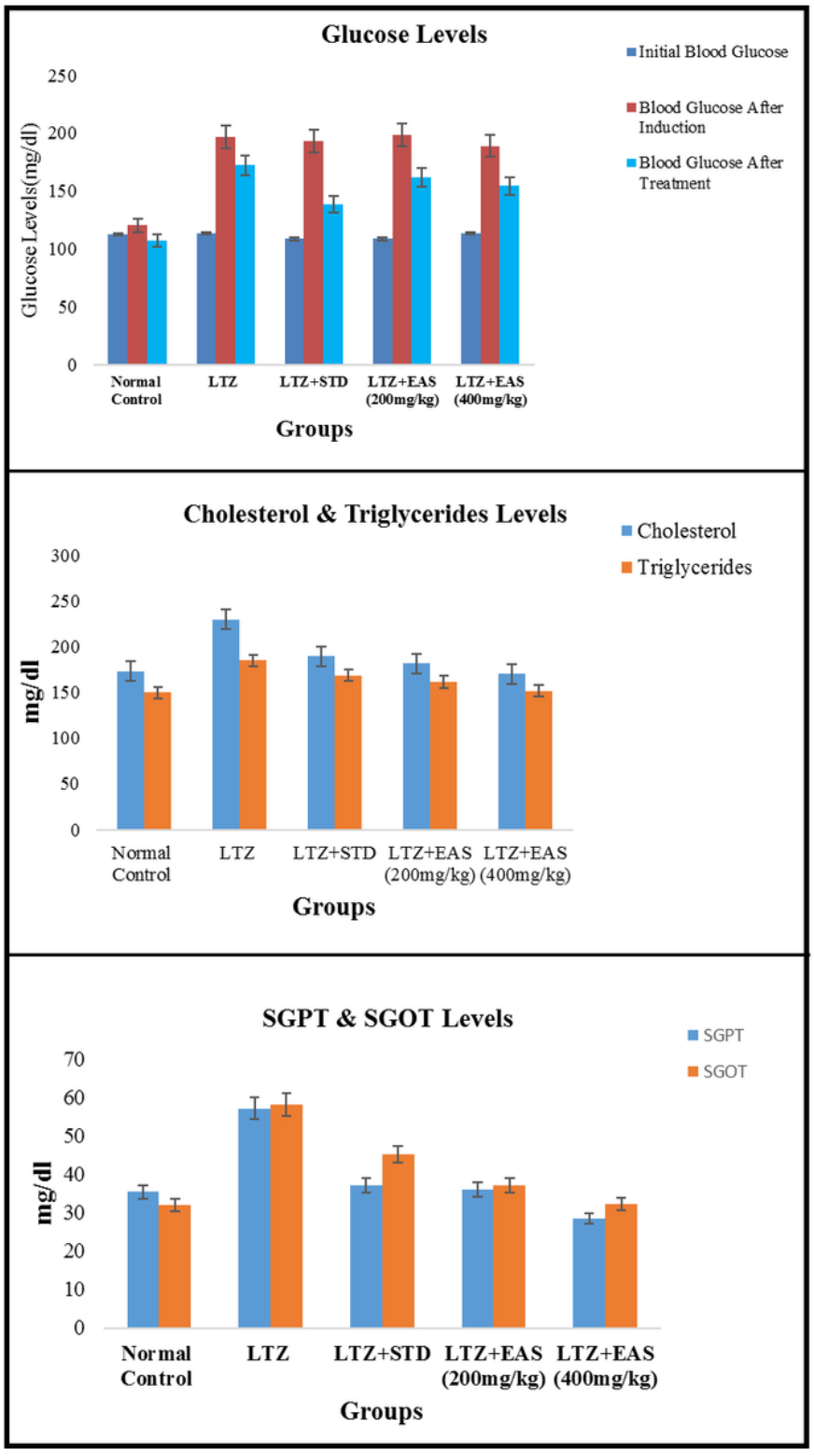

## Figure 6

Effect of *Agaricus Subrufescens* on body weights and feed intake in PCOS induced rats




## Figure 7

Effect of *Agaricus Subrufescens* on Estrogen, Progesterone and testosterone levels in PCOS induced rat




## Figure 8

Effect of *Agaricus Subrufescens* on blood glucose level; Cholesterol & Triglycerides; SGPT & SGOT levels in PCOS induced rats

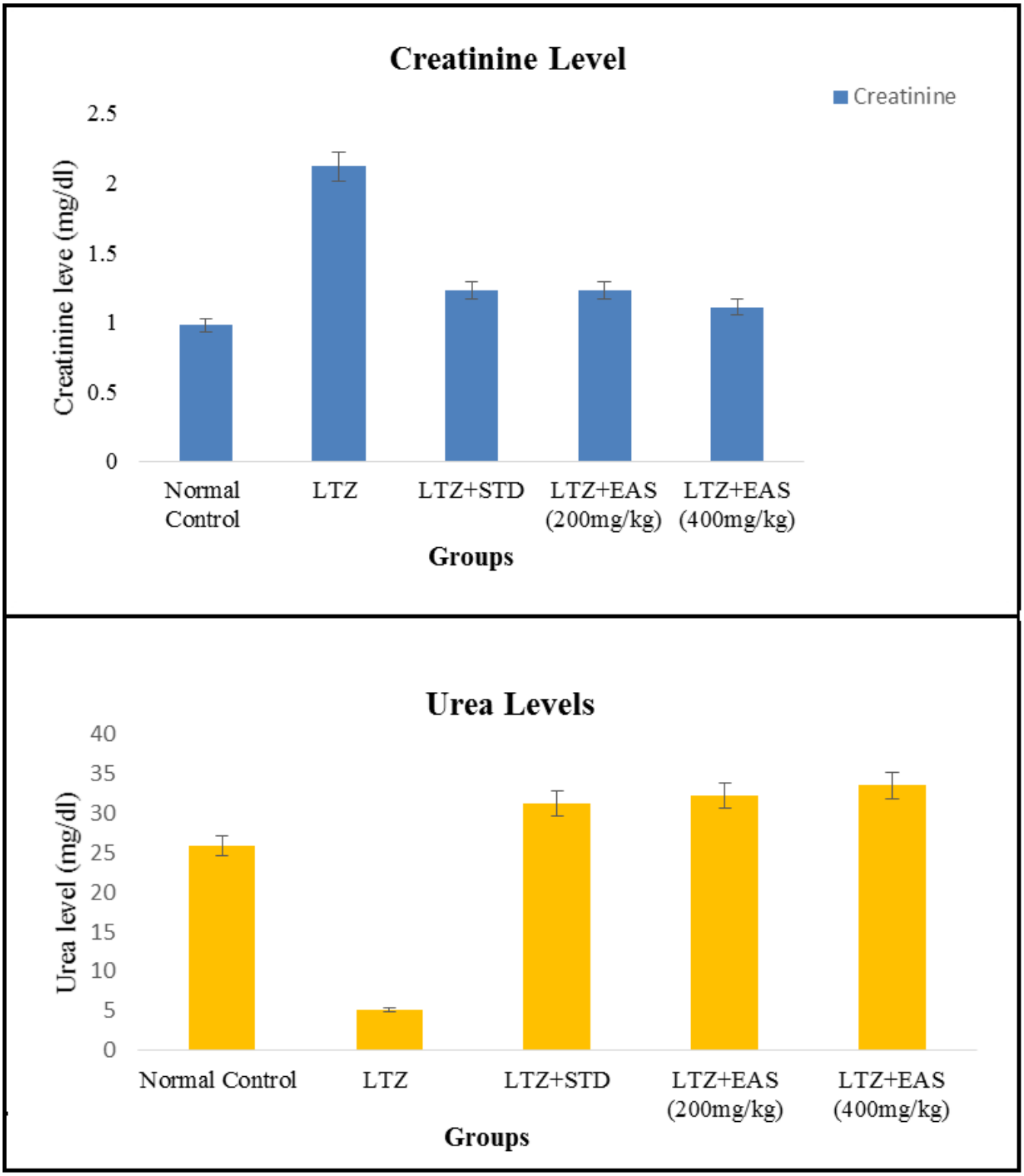



**Figure 9**

Effect of *Agaricus Subrufescens* on Creatinine and Urea levels in PCOS induced rats

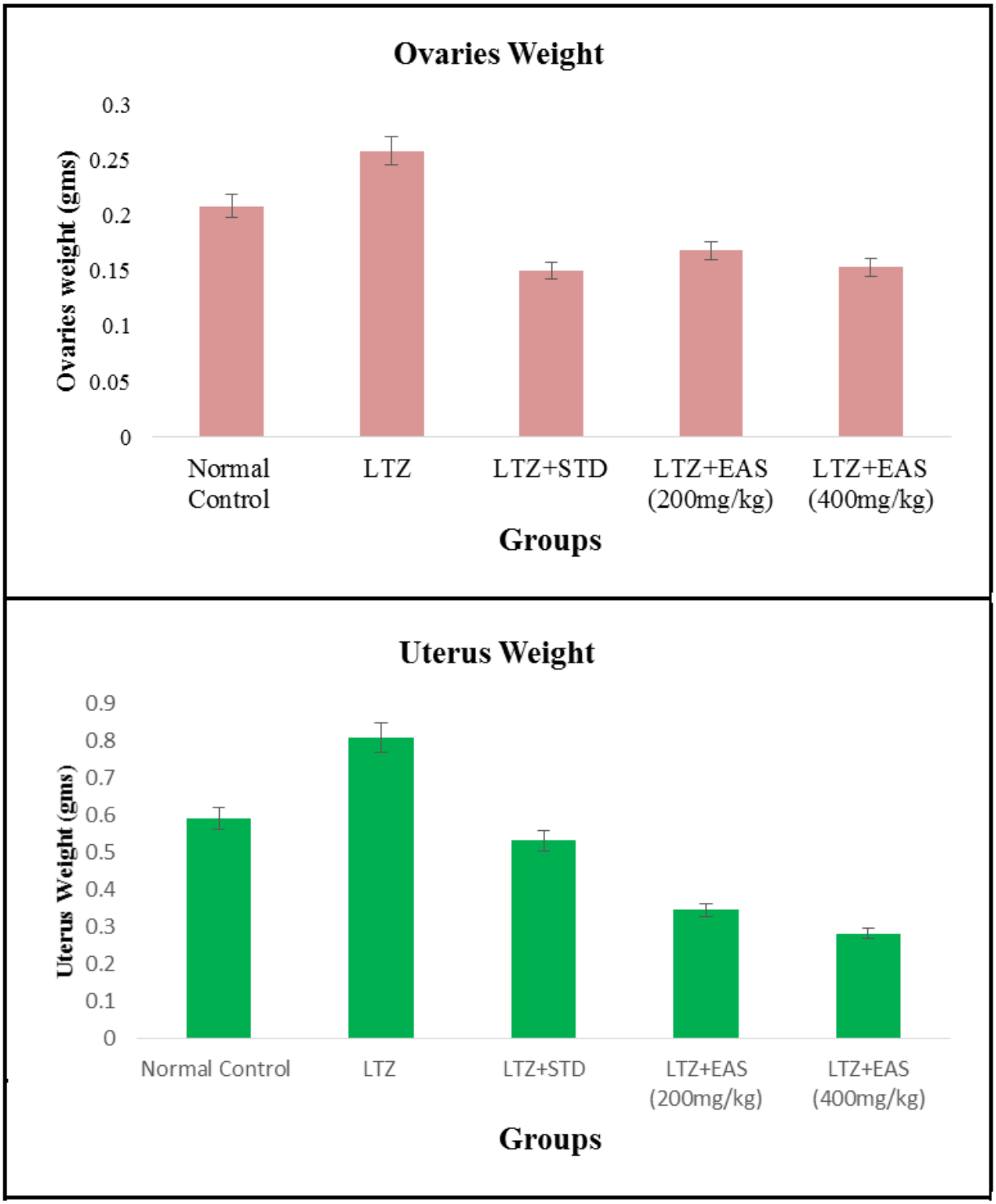



**Figure 10**

Effect of *Agaricus Subrufescens* on Ovaries and Uterine weights in PCOS induced rats

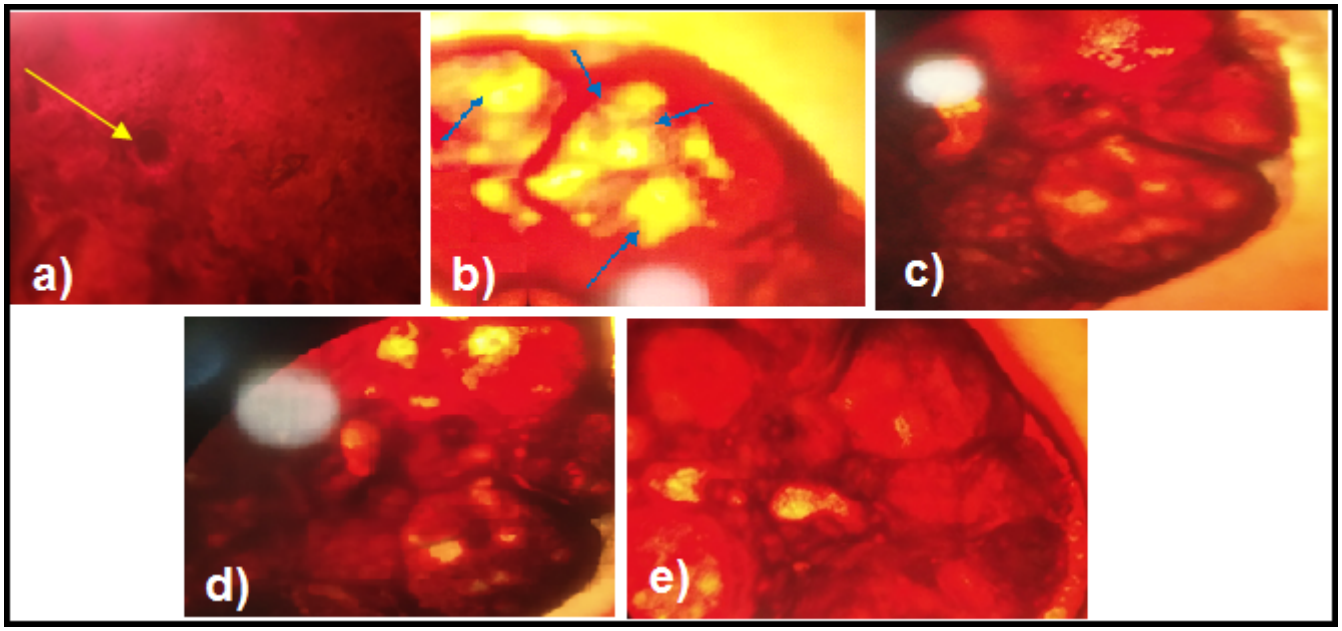



**Figure 11**

Histopathology observation a) Normal control b) Letrozole induced animals c) Letrozole+clomiphene citrate (standard) d) EAS 200mg/kg e) EAS 400mg/kg