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Research Article
Evaluation of Matcha (*Camellia sinensis*) and Ashwagandha (*Withania somnifera*) Efficacy Against Utero-Ovarian Injury in Rats

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Abstract

Background and Objective: Female infertility and reproductive problems have increased worldwide. Medical treatment of such conditions has high costs with various side effects. Alternative medicine, essentially herbal plants, has been projecting to improve female infertility and reproductive health. This study was aimed to evaluate the efficacy of single or combined administration of matcha and ashwagandha teas against H_2O_2-induced Utero-ovarian oxidative injury and cell death in female rats. **Materials and Methods:** Fifty adult female rats were used. Ten rats were kept healthy while in others Utero-ovarian oxidative injury was induced by drinking 1% H_2O_2 water *ad libitum*. Injured rats were divided into 4 groups (10 rats/each), one group set as injured control and the other 3 groups the doses of supplemented teas were 200 mg kg^{-1} b.wt. and 100 mg kg^{-1} b.wt. from each or both teas, respectively. **Results:** The results displayed that both teas contain active components including flavonoids, polyphenols and possess antioxidant activity. Drinking 1% H_2O_2 water significantly ($p \leq 0.01$)decreased the estrous cycle time, body, ovary and uterus weights, serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone and estrogen (E2) levels, uterine and ovarian superoxide dismutase (SOD) activity and reduced glutathione (GSH) level while caused a substantial increase ($p \leq 0.01$) in uterine and ovarian malondialdehyde (MDA) level, DNA fragmentation percent, caspase-3 (Casp-3), 8-hydroxydeoxyguanosine (8-OHdG), tumour necrosis factor-α (TNF-α), prostaglandin E2 (PGE2) levels as well as cyclooxygenase-2 (COX-2) activity. Moreover, microscopic observations of uterine and ovarian tissues were consistent with the biochemical results. **Conclusion:** Oral administration of tested teas improved and ameliorated all the biochemical and microscopic observations by restricting cellular DNA damage and protecting uterine and ovarian tissues from oxidative injury and cell death. The best improvement was observed in the matcha administered group.

Key words: Matcha, ashwagandha, hydrogen peroxide, oxidative injury, antioxidants, utero-ovarian tissues, reactive oxygen species, utero-ovarian malondialdehyde

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In developing countries, millions of people suffer from infertility and reproductive problems with increasing incidence worldwide. Modern lifestyle patterns, disorders and exposure to endocrine disruptor pollutants are the main infertility causes. Oxidative Stress (OS) due to excessive production of Reactive Oxygen Species (ROS) and/or insufficient cellular antioxidant capacity is the common cause of infertility. Excessive ROS production interrupts cell signalling networks and promotes oxidation resulting in cellular dysfunction[1].

ROS is any molecule or compound capable of oxidizing biological substrates and may come in the form of free radicals such as superoxide anion [$^{\cdot}O^{-2}$] or powerful oxidants such as hydrogen peroxide [H_2O_2]. Free radicals initiate a series of cellular effects including lipid peroxidation, protein denaturation, enzymatic inactivation, mitochondrial dysfunction, cytoskeletal and DNA injury. Moderate oxidation gives rise to apoptosis while severe oxidative stress results in cell death through necrosis[2].

Hydrogen peroxide is an oxidizing and bleaching factor that is used, in sewage and water treatment, as a disinfectant, antiseptic, cleansing agent and laboratory reagent and in the manufacture of paper, foam rubber, many chemicals and chemical products. Other uses for hydrogen peroxide are in cosmetics (e.g., hair bleaches, dyes and mouthwashes), in food processing and treatment of package liners in aseptic packaging. H_2O_2 is the source of the hydroxyl radical ($^{\cdot}OH$) that readily crosses the cell membrane and may cause degenerative changes[3].

Current medical treatments for female infertility, have high costs with variable successful rates and side effects. Alternative medicine, essentially herbal plants, has been projecting to improve female infertility and female reproductive system health.

Matcha is a powdered *Tencha* type of Japanese green tea (*Camellia sinensis*). Matcha is particularly rich in antioxidant compounds depending on its traditional cultivation. During matcha growth, it is shaded by natural bamboo fabric. Shading allows the plant to form a unique taste and high amounts of bioactive constituents, including l-theanine and chlorophyll[4].

High theanine and caffeine content in association with low catechin content results in the formation of higher "umami" taste ingredients in comparison to other tea types cultivated normally in the sun. This makes matcha the most aromatic green tea and a product of the highest quality[5].

Matcha green tea health benefits originate from the presence of antioxidants, amino acids and caffeine. Antioxidants like polyphenols constitute up to 30% of matcha dry mass including flavandiols, flavonols and phenolic acids. Match consumption is linked with a reduced risk of different diseases due to its immunological and detoxification ability[6].

Ashwagandha (*Withania somnifera*) is a plant from the *Solanaceae* family known as "Indian Ginseng" or "Indian Winter cherry". Ashwagandha root extract has many biological applications due to its various phytochemicals. Ashwagandha extract enhances endothelial and mitochondrial functions as well as its beneficial effects against various disorders[7].

Ashwagandha safety and edibility was tested. Ashwagandha contains bioactive constituents like alkaloids, steroidal lactones, sitoindosides VII-X, saponins and withaferin A. These components antioxidant activities help protect against cellular damage caused by free radicals[8].

Therefore the current study aimed to evaluate the efficacy of matcha and ashwagandha teas administration against H_2O_2-induced Utero-ovarian oxidative injury and cell death in female rats.

MATERIALS AND METHODS

Materials

Plants: Matcha leaves and ashwagandha root powders were purchased from Imtenan Company, Cairo, Egypt.

Chemicals: H_2O_2 was purchased from Sigma Aldrich Chemical Company (St. Louis, Missouri, USA). Other chemicals were all of the analytical reagent grade purchased from El-Gomhouria Company for Chemicals, Cairo, Egypt.

Animals: A total of 50 (Sprague Dawley-strain) virgin female rats weighing 150±10 g were supplied from Breading Unit of Animal Reproduction Research Institute, Giza, Egypt.

Methods

Preparation of plant extract: About 10 g of a plant powder was added to 50 mL of distilled water at 100°C in a conical flask. The flask was closed and rotated (Brunswick model EXCELLA E24) at a speed of 180 rpm for 10 min. The resulting mixture was filtered. The obtained filtrate was cooled up to room temperature. The final extract concentration was (200 mg mL^{-1}). Extracts were given freshly to rats[4,9]. Rats were supplemented with tea extracts at dose level of 200 or 100 mg kg^{-1} b.wt.[5,9].

Determination of total polyphenols, total flavonoids and total anti-oxidants in matcha and ashwagandha teas: The total phenolic and flavonoids contents in teas were determined according to the Folin-Ciocalteu procedure[10] while total anti-oxidants were determined using the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)[11]. The experiments were repeated in triplicate.

Study site: The study was carried out at the animal house of the Department of Biochemistry and Nutrition, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt, from January-February, 2021.

Experimental design: Female rats were acclimatized to the laboratory conditions for 1 week before the start of the experiment. They were kept individually in standard laboratory conditions. The food and water were introduced in special cups and bottles, respectively *ad libitum*. All rats were maintained on a standard lab diet prepared according to the American Institute of Nutrition (AIN-93M) and adjusted by *Reeves et al.*[12].

The rats were randomly divided into five groups composed of ten female rats and treated for 30 days as follows:

- **Group 1:** Healthy control group (HCG), rats were fed on a standard diet and drank distilled water *ad libitum*. Rats were given distilled water intragastrically daily
- **Group 2:** Injured control group (ICG), rats were fed on a standard diet and drank 1% H_2O_2 water *ad libitum*. Rats were given distilled water intragastrically daily
- **Group 3:** Injured rats supplemented with matcha tea (IMG), rats were fed on a standard diet and drank 1% H_2O_2 water *ad libitum*. Rats were supplemented with matcha tea (200 mg kg^{-1} b.wt.) intragastrically daily
- **Group 4:** Injured rats supplemented with ashwagandha tea (IAG), rats were fed on a standard diet and drank 1% H_2O_2 water *ad libitum*. Rats were supplemented with ashwagandha tea (200 mg kg^{-1} b.wt.) intragastrically daily
- **Group 5:** Injured rats supplemented with a mixture of matcha and ashwagandha teas mixture (IMAG), rats were fed on a standard diet and drank 1% H_2O_2 water *ad libitum*. Rats were supplemented with a mixture of matcha tea (100 mg kg^{-1} b.wt.) and ashwagandha tea (100 mg kg^{-1} b.wt.) intragastrically daily

Detection of estrous cycle: Estrous cycle was determined using a vaginal smear. Ten days before the experiment end, vaginal smears were collected at 8-9 am daily, stained with Giemsa solution and examined under a light microscope (Olympus, Japan). The pro-estrus smear consisted of nucleated cornified cells, the estrus smear consisted of enucleated cornified cells, the met-estrus smear consisted of an equal proportion of leukocytes, nucleated epithelial cells and cornified epithelial cells and the di-estrus smear primarily consisted of leukocytes[13].

Determination of body weight change, uterine and ovarian weights: Rats were weighed weekly to monitor body weight changes. Uterine and ovarian tissues were removed after rat scarification, washed using 0.9% saline, dried then weighed.

Collection of blood and tissue samples: After the end of the experimental period (one month), rats fasted overnight, then all rats were sacrificed under deep anaesthesia. Blood samples were collected from the hepatic portal vein for serum separation during the di-estrus phase (determined by vaginal smear). Serum was stored at -20°C until used for biochemical analyses. Random uteruses and ovaries samples were fixed in 10% neutral buffered formalin for histological study and the remaining samples were prepared to form tissue homogenate for other analysis.

Biochemical analyses: Quantitative determination of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen (E2) and progesterone levels were done by using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (Calbiotech Co. USA). Moreover, uterine and ovarian tissues superoxide dismutase (SOD) activity, Malondialdehyde (MDA) and reduced glutathione (GSH) concentrations were determined according to the methods described formerly[14-16], respectively. While uterine and ovarian tissues caspase 3 (Casp-3) activity, 8-hydroxydeoxyguanosine (8-OHdG), tumour necrosis factor-α (TNF-α), prostaglandin E2 (PGE2) levels as well as cyclooxygenase-2 (COX-2) activity were determined using ELISA kits of (MyBio source, USA), (koma biotech, Korea), (lifespan Biosciences, USA) and (Cusabio, USA), respectively.

Determination of DNA fragmentation in uterine and ovarian tissues: The percentage of DNA fragmentation was assessed by the method proposed by Boraschi and Maurizi[17]. The procedure depends on the notion that extensively fragmented double-stranded DNA can be separated from chromosomic

DNA upon centrifugal sedimentation. Includes the lyses of cells and the release of nuclear DNA centrifugation step with the generation of two fractions (corresponding to intact and fragmented DNA respectively), precipitation of DNA, hydrolysis and colorimetric quantitation after diphenylamine (DPA) staining, that binds to deoxyribose.

Microscopic examination of uterine and ovarian tissues: Uterine and ovarian specimens were collected from all rats/groups and then fixed in 10% neutral buffered formalin. Paraffin sections of 5 µm thickness were prepared and stained with hematoxylin and eosin (H and E) and then examined by a light microscope (Olympus B×50, Japan)[18]. Histopathological damage in the ovarian and uterine tissues was graded from (0-4) as follow: (0) indicated no changes, (1) indicated percentage area affected (<10%), (2) indicated percentage area affected (20-30%), (3) indicated percentage area affected (40-60%) and (4) indicated percentage area affected (>60%)[19].

Statistical analysis: Data were statistically analyzed by Statistical Package for Social Science (Version 20). Statistical Differences between groups were performed using one-way ANOVA, the mean difference was significant at ($p \leq 0.01$) level[20].

RESULTS

Total polyphenols, total flavonoids contents and total antioxidant activity of matcha and ashwagandha teas: The result in Table 1 illustrated that both matcha and ashwagandha teas contain valuable bioactive components as each 1 g of the tested matcha leaves tea contains 48.16 mg as the gallic acid equivalent of total polyphenols, 4.08 mg as catechin equivalent of total flavonoids and 83.24 mg as Trolox equivalent of total anti-oxidant capacity while each 1 g of the tested ashwagandha roots tea contains 31.05 mg as the gallic acid equivalent of total polyphenols, 2.96 mg as catechin equivalent of total flavonoids and 67.84 mg as Trolox equivalent of total anti-oxidant capacity, from these results it is found that matcha tea contains higher phenolic, flavonoids and antioxidant activities than ashwagandha tea.

Estrous cycle length in experimental rats: Results illustrated in the Table 2 showed that the estrous cycle of the healthy control group lasted on average 4.76 days, while administration of hydrogen peroxide shortened the estrous cycles significantly ($p \leq 0.01$) in comparison with the healthy control group. On the other hand supplementation with matcha tea alone, ashwagandha tea alone or in combination with injured rats restored the estrous cycle length significantly ($p \leq 0.01$) in comparison with the injured control group. The most significant improvements were recorded in the injured group that supplemented with matcha tea alone.

Body, uterus and ovaries weights in experimental rats: Results for body, uterus and ovaries weights were illustrated in Fig. 1(a-c). Regarding body weight, the rat body final weight decreased significantly ($p \leq 0.01$) in the hydrogen peroxide administered group in comparison with the healthy control group. Supplementation with tested teas improved and controlled body weight decrement significantly ($p \leq 0.01$) in comparison with an injured control group. Uterus and ovaries weights of the injured control group were significantly ($p \leq 0.01$) decreased in comparison with the healthy control group while supplementation with each tea alone or in combination improved these results significantly ($p \leq 0.01$). The most significant improvements were found in the third group that supplemented with matcha tea.

Serum sex hormones (FSH, LH, progesterone and estrogen (E2)) levels in experimental rats: Results tabulated in the Table 3 illustrated that hydrogen peroxide administration to female rats caused a significant reduction ($p \leq 0.01$) in serum FSH, LH, progesterone and estrogen levels in hydrogen peroxide administered female rats in comparison with a healthy control group. Supplementation with matcha and/or ashwagandha teas significantly ($p \leq 0.01$) improved these results. The most significant amelioration was found in the matcha tea supplemented group.

Uterine and ovarian oxidative status in experimental rats: Data in Fig. 2(a-c) and Fig. 3(a-c) illustrated that hydrogen peroxide administration to female rats caused a state of oxidative stress resulted in a significant ($p \leq 0.01$) decrease in

Table 1: Total polyphenols, total flavonoids contents and antioxidant activity of matcha and ashwagandha teas

Water extract	Total polyphenols content (mg GAE g^{-1})	Total flavonoids content (mg CE g^{-1})	Antioxidant activity (mg TE g^{-1})
Match leaves	48.16	4.08	83.24
Ashwagandha roots	31.05	2.96	67.84

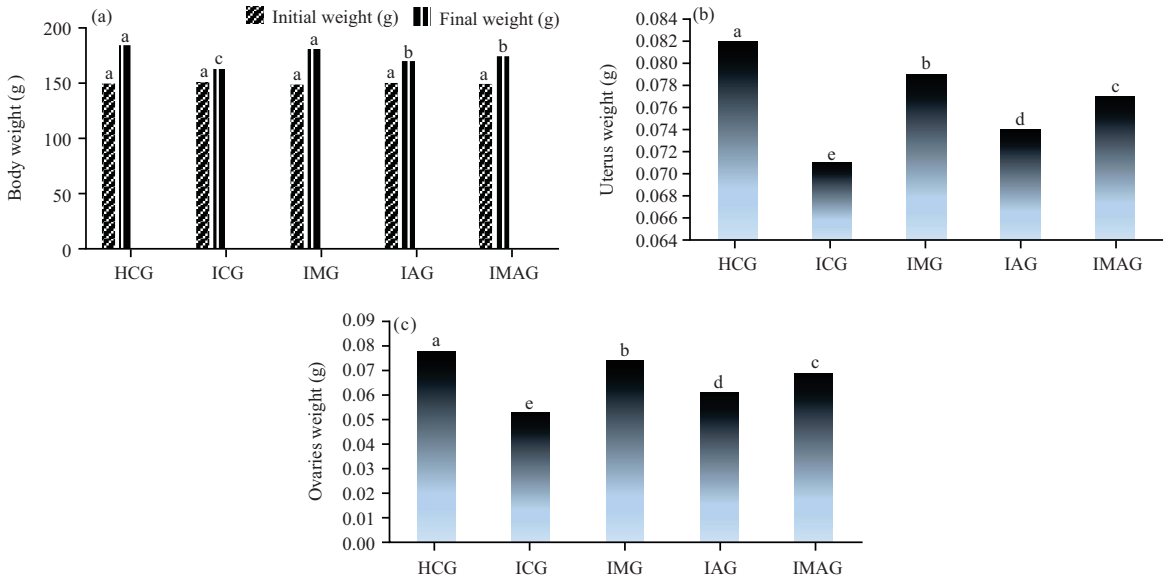

Fig. 1(a-c): Effect of matcha and/or ashwagandha teas supplementation on body, uterus and ovaries weights in experimental rats, (a) Body weight, (b) Uterus weight and (c) Ovaries weight

Values are expressed as Mean±standard deviation, n = 10. The column of the histogram with the different letters is significantly different at ($p \leq 0.01$). HCG: Healthy control group, ICG: Injured control group, IMG: Injured rats supplemented with matcha tea, IAG: Injured rats supplemented with ashwagandha tea and IMAG: Injured rats supplemented with matcha and ashwagandha teas

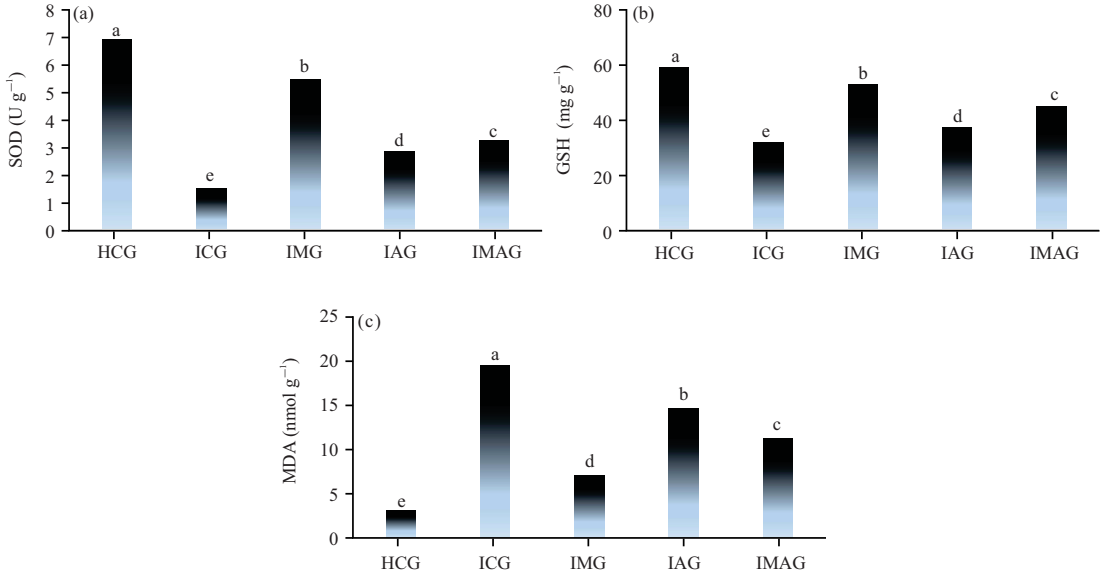

Fig. 2(a-c): Impact of matcha and/or ashwagandha teas supplementation on uterine oxidative status in experimental rats, (a) Uterine SOD activity, (b) Uterine GSH level and (c) Uterine MDA level

Values are expressed as Mean±standard deviation, n = 10. The column of the histogram with the different letters is significantly different at ($p \leq 0.01$). HCG: Healthy control group, ICG: Injured control group, IMG: Injured rats supplemented with matcha tea, IAG: Injured rats supplemented with ashwagandha tea and IMAG: Injured rats supplemented with matcha and ashwagandha teas

uterine and ovarian SOD activity and GSH level with significant ($p \leq 0.01$) increase in uterine and ovarian MDA level in the injured control group in comparison with a healthy control group. On the contrary matcha and/or ashwagandha teas supplementation counteracted oxidative status induced by hydrogen peroxide in the uterus and ovaries causing a significant ($p \leq 0.01$) increase in SOD activity and GSH level and also caused a significant ($p \leq 0.01$) decrease in MDA level in

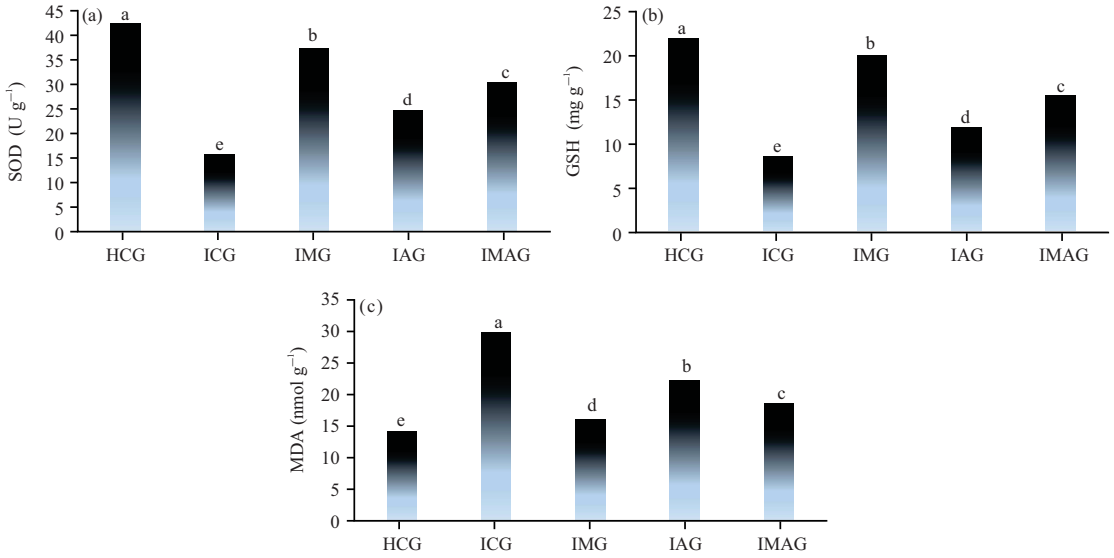

Fig. 3(a-c): Impact of matcha and/or ashwagandha teas supplementation on ovarian oxidative status in experimental rats, (a) Ovarian SOD activity, (b) Ovarian GSH level and (c) Ovarian MDA level

Values are expressed as Mean±standard deviation, n = 10. The column of the histogram with the different letters is significantly different at ($p \leq 0.01$). HCG: Healthy control group, ICG: Injured control group, IMG: Injured rats supplemented with matcha tea, IAG: Injured rats supplemented with ashwagandha tea and IMAG: Injured rats supplemented with matcha and ashwagandha teas

Table 2: Effect of matcha and/or ashwagandha teas supplementation on estrous cycle length in experimental rats

Parameter/groups	Estrous cycle (days)
HCG	4.76 ± 0.03^{a}
ICG	2.93 ± 0.04^{e}
IMG	4.39 ± 0.011^{b}
IAG	3.48 ± 0.017^{d}
IMAG	3.81 ± 0.05^{c}

There is no significant difference between means that have the same letters in the same column, n = 10 rats, ($p \leq 0.01$). HCG: Healthy control group, ICG: Injured control group, IMG: Injured rats supplemented with matcha tea, IAG: Injured rats supplemented with ashwagandha tea, IMAG: Injured rats supplemented with matcha and ashwagandha teas

supplemented groups in comparison with an injured control group. The most significant improvements were recorded in the matcha tea supplemented group followed by the group supplemented with both teas and finally, the ashwagandha tea supplemented group.

Uterine and ovarian DNA fragmentation, apoptosis and oxidative DNA damage in experimental rats: Analyzed data tabulated in the Table 4 and 5 showed that injuring effect of hydrogen peroxide on both uterine and ovarian tissues causing significant increase ($p \leq 0.01$) in DNA fragmentation percent, apoptotic marker (caspase-3) and oxidative DNA damage indicator (8-OHdG) levels in the injured control group in comparison with the healthy control group. Matcha and ashwagandha teas supplementation to the injured group preserved DNA structure and decreased caspase-3 and 8-OHdG levels significantly ($p \leq 0.01$) in comparison with the injured control group.

Uterine and ovarian inflammatory markers in experimental rats: Results presented in Fig. 4(a-c) and 5(a-c) illustrated that hydrogen peroxide administration to female rats initiated inflammatory pathways causing a significant increase ($p \leq 0.01$) in uterine and ovarian TNF-α, PGE2 levels and COX-2 activity in comparison with the healthy control group while matcha and ashwagandha teas active constituents prohibited inflammatory cascades in supplemented groups leading to a significant reduction ($p \leq 0.01$) in uterine and ovarian inflammatory markers in comparison with the injured control group.

Uterine and ovarian tissues microscopic examination in experimental rats: The results of microscopic examination of uterine and ovarian tissues in all groups (Fig. 6 and 7) showed the degenerative changes caused by H_2O_2 and the corrective role of matcha, ashwagandha and their mixture provided supportive evidence for biochemical analysis. Microscopic examination of the uterine tissues of the healthy control group (Fig. 6a) revealed normal histological

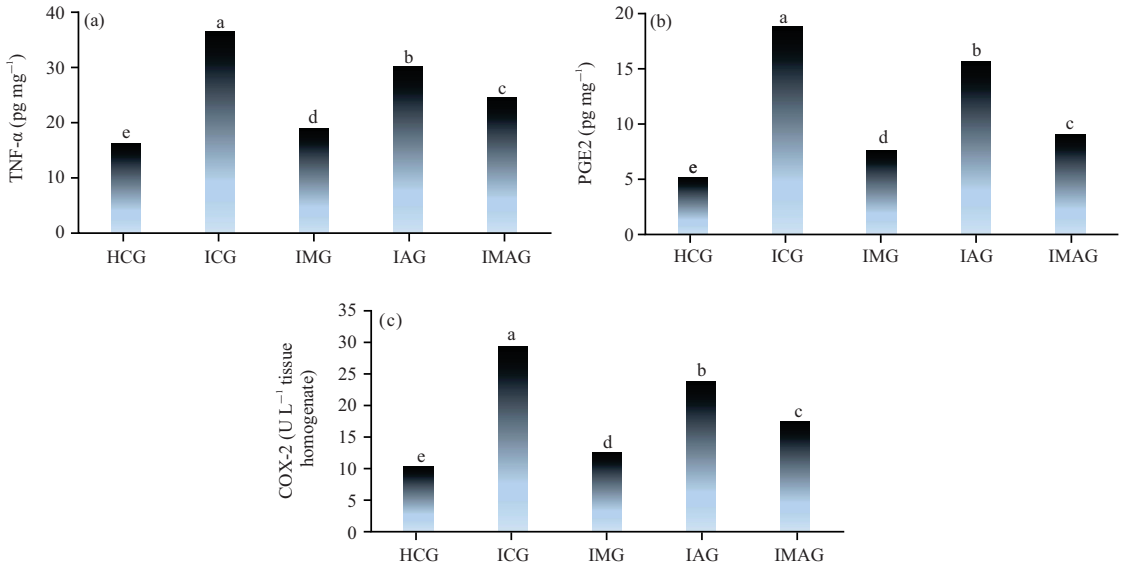

Fig. 4(a-c): Effect of matcha and/or ashwagandha teas supplementation on uterine inflammatory markers in experimental rats, (a) Uterine TNF-α level, (b) Uterine PGE2 level and (c) Uterine COX-2 activity

Values are expressed as Mean±standard deviation, n = 10. The column of the histogram with the different letters is significantly different at ($p \leq 0.01$). HCG: Healthy control group, ICG: Injured control group, IMG: Injured rats supplemented with matcha tea, IAG: Injured rats supplemented with ashwagandha tea and IMAG: Injured rats supplemented with matcha and ashwagandha teas

Table 3: Impact of matcha and/or ashwagandha teas supplementation on serum FSH, LH, progesterone and estrogen (E2) levels in experimental rats

Parameter/groups	FSH (mLU mL^{-1})	LH (mLU mL^{-1})	Progesterone (ng mL^{-1})	Estrogen (E2) (Pg mL^{-1})
HCG	1.82±0.028^{a}	0.44±0.004^{a}	34.18±0.29^{a}	87.54±0.83^{a}
ICG	0.89±0.009^{e}	0.23±0.016^{e}	16.56±0.16^{e}	64.95±0.17^{e}
IMG	1.60±0.017^{b}	0.39±0.005^{b}	29.90±0.24^{b}	79.10±0.65^{b}
IAG	1.18±0.011^{d}	0.29±0.010^{d}	20.35±0.13^{d}	68.54±0.33^{d}
IMAG	1.34±0.021^{c}	0.33±0.008^{c}	24.46±0.19^{c}	72.39±0.41^{c}

There is no significant difference between means that have the same letters in the same column, n = 10 rats, ($p \leq 0.01$). HCG: Healthy control group, ICG: Injured control group, IMG: Injured rats supplemented with matcha tea, IAG: Injured rats supplemented with ashwagandha tea, IMAG: Injured rats supplemented with matcha and ashwagandha teas

Table 4: Effect of matcha and/or ashwagandha teas supplementation on uterine DNA fragmentation, apoptosis and oxidative DNA damage in experimental rats

Parameter/groups	DNA fragmentation (%)	Caspase-3 (ng mg $^{-1}$)	8-OHdG (ng mg $^{-1}$)
HCG	0.94±0.024^{e}	1.18±0.014^{e}	12.08±0.53^{e}
ICG	38.40±0.31^{a}	10.26±0.18^{a}	41.59±0.86^{a}
IMG	3.12±0.04^{d}	2.87±0.09^{d}	18.26±0.16^{d}
IAG	16.19±0.10^{b}	8.49±0.11^{b}	30.07±0.31^{b}
IMAG	9.35±0.081^{c}	5.09±0.07^{c}	21.11±0.22^{c}

There is no significant difference between means that have the same letters in the same column, n = 10 rats, ($p \leq 0.01$). HCG: Healthy control group, ICG: Injured control group, IMG: Injured rats supplemented with matcha tea, IAG: Injured rats supplemented with ashwagandha tea and IMAG: Injured rats supplemented with matcha and ashwagandha teas

Table 5: Effect of matcha and/or ashwagandha teas supplementation on ovarian DNA fragmentation, apoptosis and oxidative DNA damage in experimental rats

Parameter/groups	DNA fragmentation (%)	Caspase-3 (ng mg^{-1})	8-OHdG (ng mg^{-1})
HCG	2.26±0.015^{e}	4.34±0.046^{e}	1.34±0.016^{e}
ICG	53.78±0.82^{a}	18.33±0.39^{a}	23.16±0.21^{a}
IMG	8.18±0.067^{d}	7.41±0.071^{d}	4.05±0.05^{d}
IAG	25.62±0.33^{b}	12.26±0.20^{b}	15.82±0.29^{b}
IMAG	16.30±0.20^{c}	9.83±0.18^{c}	11.39±0.14^{c}

There is no significant difference between means that have the same letters in the same column, n = 10 rats, ($p \leq 0.01$). HCG: Healthy control group, ICG: Injured control group, IMG: Injured rats supplemented with matcha tea, IAG: Injured rats supplemented with ashwagandha tea and IMAG: Injured rats supplemented with matcha and ashwagandha teas

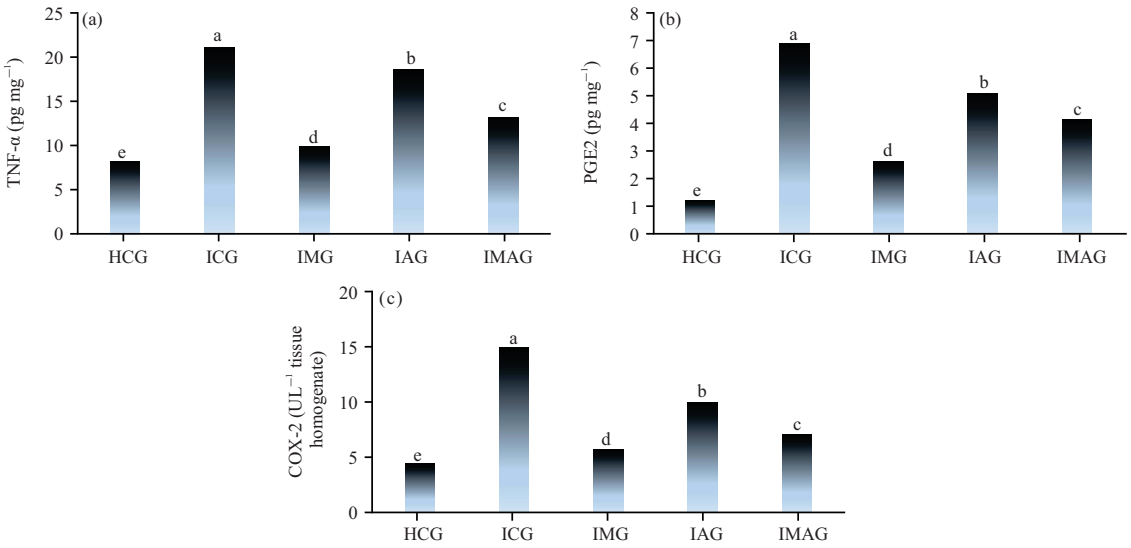

Fig. 5(a-c): Effect of matcha and/or ashwagandha teas supplementation on ovarian inflammatory markers in experimental rats, (a) Ovarian TNF-α level, (b) Ovarian PGE2 level and (c) Ovarian COX-2 activity

Values are expressed as Mean±standard deviation, n = 10. The column of the histogram with the different letters is significantly different at ($p \leq 0.01$). HCG: Healthy control group, ICG: Injured control group, IMG: Injured rats supplemented with matcha tea, IAG: Injured rats supplemented with ashwagandha tea and IMAG: Injured rats supplemented with matcha and ashwagandha teas

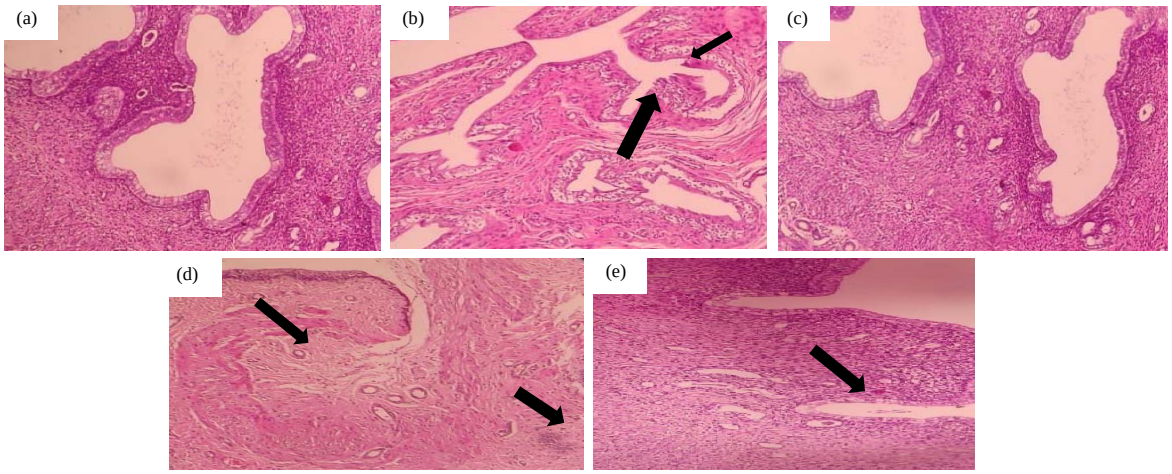

Fig. 6(a-e): Microscopic examination of uterine tissues (H and E×400), (a) Photomicrograph of HCG uterine tissue showing normal histological features of uterine layers lined by a thick mucosa (score 0), (b) Photomicrograph of ICG uterine tissue showing interstitial inflammatory cells infiltration (small arrow) and interstitial edema (large arrow) (score 4, ++++), (c) Photomicrograph of IMG uterine tissue showing semi-normal histological structures (score 1, +), (d) Photomicrograph of IAG uterine tissue showing few interstitial inflammatory cells infiltration (score 3, +++) and (e) Photomicrograph of IMAG uterine tissue showing very few interstitial inflammatory cells infiltration (score 2, ++)

features of uterine layers lined by a thick mucosa. The endometrium, the myometrium and the stroma were completely normal. In the injured control group (Fig. 6b), the uterine specimens revealed interstitial inflammatory cells infiltration and interstitial oedema recording the highest lesion score. The supplemented group with matcha (Fig. 6c) showed semi-normal histological structures with minimal lesion scores. While the ashwagandha supplemented group (Fig. 6d) showed few interstitial inflammatory cells infiltration and the group that supplemented with both teas (Fig. 6e) showed very few interstitial inflammatory cells.

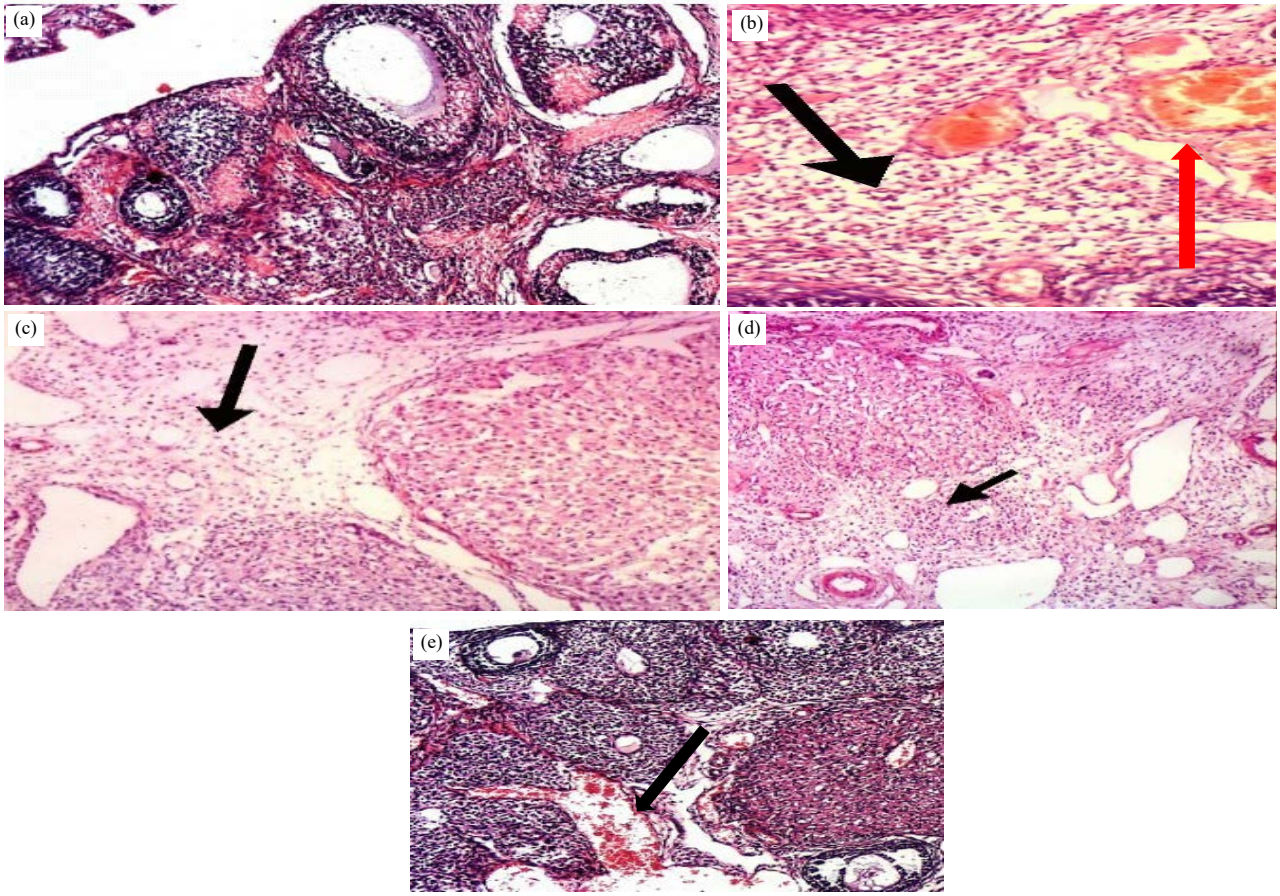

Fig. 7(a-e): Microscopic examination of ovarian tissues (H and E×400), (a) Photomicrograph of HCG ovarian tissue showing normal parenchyma (score 0), (b) Photomicrograph of ICG ovarian tissue showing congested interstitial blood vessel (black arrow) and interstitial inflammatory cells infiltration (red arrow) (score 4, ++++), (c) Photomicrograph of IMG ovarian tissue showing normal histological structure with slight edema (score 1, +), (d) Photomicrograph of IAG ovarian tissue showing interstitial inflammatory cells infiltration (score 3, +++) and (e) Photomicrograph of IMAG ovarian tissue showing slightly congested interstitial blood vessel (score 2, ++)

Microscopic examination of the ovarian tissues of the healthy control group (Fig. 7a) revealed normal histological features of ovarian parenchyma with intact ovarian cortex including many ovarian follicles in different stages of development and maturation, intact ovarian medullary connective tissue and vasculatures. In the injured control group (Fig. 7b), the ovarian specimens showed congested interstitial blood vessel and interstitial inflammatory cells infiltration with the highest lesion score. The supplemented group with matcha (Fig. 7c) showed normal histological structure with slight oedema recording the minimal lesion score. While the ashwagandha supplemented group (Fig. 7d) showed few interstitial inflammatory cells infiltration and the group that supplemented with both teas (Fig. 7e) showed slightly congested interstitial blood vessels.

DISCUSSION

Matcha green and ashwagandha teas samples analysis revealed that both teas contain significant amounts of polyphenols, flavonoids and possess strong antioxidant power these results go hand in hand with previous studies[21,22]. These bioactive components in addition to nutritional constituents encourage both people to consume these teas and researchers to examine their beneficial effects. These constituents helped in the amelioration and correction of H_2O_2 negative effects on the female reproductive system.

H_2O_2 has been shown to induce oxidative stress in animal models, leading to the generation of potent ROS. Most of the deleterious effects of H_2O_2 on tissues depend on its conversion

into $OH^{\cdot}$ radical. Cells exposed to H_2O_2 may suffer degeneration of lipids, proteins and DNA leading to various pathological conditions[23].

The research results indicated shortness of the estrous cycles time in H_2O_2 intoxicated rats this may be due to the resulted oxidative stress that causes a decrease in the number of the ovum as well as the adverse effects on the sexual hormones as documented in the results. Studies on both animals and humans have demonstrated the presence of ROS in the female reproductive system parts including the ovaries. ROS are involved in the modulation of entire physiological reproductive functions[24]. Hydrogen peroxide is constantly generated in cells and has been involved in many ovulation-related events including steroid hormone production. On the other hand, increased H_2O_2 content results in the reduction of both progesterone and estradiol production[25]. These two hormones are important in the normal functioning of the female reproductive system. The balance in the hormonal interplay between estrogen and progesterone is responsible for a normal regular cycle and this explains the shortness of cycles in tested rats[26]. Estrous cycle defect is a major sign of alterations in reproductive tasks in female rats induced by oxidative stress. On the other hand, herbal teas corrected the cycle time by opposing the oxidative stress and correcting hormones levels in supplemented groups.

The decline in weight gain during acute H_2O_2 exposure might be related to decreased appetite, reduced food intake, altered energy metabolism and increased intestinal permeability. The weight of the injured uterus and ovaries decreased than that in the healthy control group. The uterine and ovarian functions of the female injured rats were damaged. The possible reason was that a series of complex immune-modulatory responses that occurred after stress stimulation in reproductive female cells in both uterus and ovary ended with their death and so decreased weights and functions[27].

The current results showed that serum estrogen and progesterone were down-regulated. In addition, FSH and LH were dropped in the injured control group, indicating ovarian failure. The synergistic influence of LH and FSH on ovarian granulosa cells and follicles led up to an increase of estradiol in the blood, which is urgent for the manufacture of estrogen and progesterone. In a normal physiological state, the body can preserve FSH/LH in the ordinary range through self-organization. While, under stress, the tasks of the Hypothalamic-Pituitary-Gonadal (HPG) axis is influenced. This disrupts the feedback arrangement of the Hypothalamus-Pituitary-Ovary (HPO) axis, which in turn, cause a set down in the ovarian reserve task[28]. The present outcome of serum sex hormones indicated that H_2O_2 stress leads to the dysfunction of HPO and the damage of ovarian function. On contrary, hormonal analysis of rats supplemented with match and ashwagandha extracts alleviate the stress condition and maintain normal HPO function.

The result of Fig. 2 and 3 revealed that MDA contents in uterus and ovaries tissue homogenates significantly increased in H_2O_2 receiving group with a concomitant decrease in SOD activity and GSH level. On the contrary, these parameters in extracts treated rats were preserved in comparison with H_2O_2 injured group. This ameliorative effect in extracts treated groups represent antioxidant augmentation and prove that extracts have excellent anti-oxidative activity.

Our results agree with previous findings[23] reporting that H_2O_2 decreased total antioxidant capacity and increased the levels of lipid peroxidation. Lipid peroxidation is a free radicals auto-catalytic mediated process resulting in MDA formation. Biological effects of ROS are controlled by a variety of enzymatic and non-enzymatic defense mechanisms, in particular SOD, which catalyzes the dismutation of superoxide anions to hydrogen peroxide. Superoxide dismutase represents the front line of defense against oxidative damage. GSH is a non-enzymatic antioxidant that has a potential role against free radicals production. The depletion of GSH represents an early hallmark for progressive cell death that results from apoptotic stimuli in different cell types[29]. Therefore, the present decrease in GSH level may be due to its exhaustion to get rid of the evolved ROS and failure to replenish its content due to decreased activity of glutathione synthetase enzyme after H_2O_2 exposure.

Moreover, our results are following previous results which suggested a variety of phytochemicals from the natural product have been reported to have antioxidant activity. Former results showed that supplementation with matcha tea extract improve the harmful effects of H_2O_2 in rats liver[30] and also, ashwagandha extract possesses antioxidant and anti-inflammatory effects[29]. Our results suggest that treatment with match and ashwagandha extracts containing large amounts of phenolic compounds acting as natural antioxidants reduce the harmful effects of H_2O_2.

Inflammation is an organized immune-protective response to protect the body from any chemical injury. Inflammation initiation and maintenance are carried out by pro-inflammatory mediators and antagonized by the anti-

inflammatory mediators. However, oxidative stress and other environmental factors can disturb this balance and lead to excessive production of prostaglandin E2 (PGE2), via up-regulating the COX-2 activity, which consequently leads to inflammatory mediated disorders[31]. Cyclooxygenase (COX) is the key regulatory enzyme of the prostaglandin/eicosanoid synthetic pathway. Pro-inflammatory cytokines as TNF-alpha highly induced COX-2. In this study, we investigated the involvement of TNF-alpha-mediated prostaglandin E2 (PGE2) release and COX-2 activation in uterine and ovarian tissues. Many natural products with medicinal properties have been used to treat inflammatory conditions[32]. Natural products with anti-inflammatory activity have advantageous structural diversity compared with synthetic compounds[33]. Our results in Fig. 4 and 5 confirm the medicinal properties and anti-inflammatory activities of phenolic compounds in matcha and ashwagandha teas. Whereas matcha tea showed the most significant antioxidant and anti-inflammatory activities followed by the mixture of (match and ashwagandha) compared with ashwagandha tea alone. These results were following former trials that considered green tea and ashwagandha root powder as anti-inflammatory and antioxidant agents as they act as antioxidants to scavenge reactive oxygen species, leading to inflammation attenuation[34,35].

H_2O_2 injured rats showed a significant increase in uterine and ovarian tissues DNA fragmentation percent, caspase-3 and 8-OHdG levels. These results came following previous data stating that high ROS concentrations contribute to apoptotic cell death. 8-OHdG is a specific marker of ROS-mediated DNA damage. Additionally, the increased caspase-3 was implicated in the apoptotic pathway by activating cytoplasmic DNase, which subsequently emigrates to the nucleus and fragments the DNA. Therefore, DNA fragmentation-particularly when it is inter-nucleosomal is one of the best apoptosis indicators[36]. The obtained results concluded that matcha and ashwagandha teas have antioxidant activity and anti-apoptotic properties that help to protect the uterus and ovaries from oxidative injury and DNA damage induced by H_2O_2.

The results of the microscopic examination in Fig. 6 and 7 showed that healthy control groups have normal uterine and ovarian tissue structure layers and composition. Pathological features were observed in the injured control group with degeneration in tissue layers and inflammation. On the other hand, our results showed that these pathological changes were reduced in groups supplemented with matcha and ashwagandha teas alone or in combination. This is probably due to their active constituents that protected the tissues as well as nutritional components that help in correcting the possible degeneration.

CONCLUSION

Based on the present study, it could be concluded that matcha, ashwagandha and their mixture have a significant protecting and preserving role on the uterus and ovaries of the female rats. They also showed a good hormone-regulating effect, antioxidant and anti-inflammatory properties as well as DNA protecting activity. The strongest ameliorative effects have been shown by matcha tea, which can be due to its high content of varied active components. It is advised to consume matcha and ashwagandha teas as an alternative medicine to control and regulate normal body functions especially in females to compete with various toxicants and preserve their normal reproductive health.

SIGNIFICANCE STATEMENT

This study discovers the efficacy of matcha and ashwagandha teas supplementation against the induced Utero-ovarian oxidative injury and cell death in female rats and its related biochemical and microscopical alterations. This study will help the researchers to uncover the critical areas of female reproductive system disorders that many researchers were not able to explore. As a consequence, this study will open a new approach for the researcher to discover and apply a safer and more efficient treatment for female reproductive system disorders.

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