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Research Article
Hesperidin Reduces Ovary Toxicity Induces by Cyclophosphamide in Female Rats via Anti-inflammatory and Antioxidant Effects

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Abstract

Background and Objective: Cyclophosphamide commonly used as a chemotherapeutic agent and used for treating various cancers, including neck, head, breast, colon, testis, bladder, cervical and renal. Cyclophosphamide induced toxic effects such as cause ovary cancer. Hesperidin showed the anti-inflammatory effect and in this current experimental study, we estimate the ovary protective effect of hesperidin against the cyclophosphamide (CP) induced ovarian cancer via an anti-inflammatory pathway. **Materials and Methods:** Swiss Wistar rat was used for experimental study and subcutaneous injection of cyclophosphamide was used to induce the ovary cancer and received the dose-dependently treatment of hesperidin. Bodyweight and ovary weight was determined at the end of the study. The antioxidant parameter in the ovary tissue and serum were estimated. Hormonal assays were estimated at the end of the study. Inflammatory, cytokines and apoptosis parameters were also estimated. **Results:** Hesperidin significantly ($p<0.001$) increased body weight as well as decreased the ovarian tumour. Hesperidin significantly altered the antioxidant levels in the ovary tissue and serum in a dose-dependently manner. It also reduced the biochemical parameters such as myeloperoxidase (MPO) and Nitric Oxide (NO). Hesperidin significantly ($p<0.001$) down-regulated the cytokines viz., interleukin-6 (IL-6), Tumour Necrosis Factor-α (TNF-α), interleukin-1β (IL-1β); inflammatory mediators such as cyclooxygenase-2 (COX-2), Nitric Oxide Synthase (iNOS) and apoptosis parameters such as caspase-3 and Bcl-2. **Conclusion:** We can say that hesperidin considerably reduced ovary cancer via an anti-inflammatory mechanism.

Key words: Hesperidin, ovary cancer, cyclophosphamide, inflammation, apoptosis, antioxidant, chemotherapy

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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

As we know that increases the new technology for detecting disease and the advancement of therapeutic regimens, the 5-year survival cancer rate has enhanced from 49-68% in the last 30 years. Increases in the cancer survival rate, moreover, a side effect is the biggest problem with the current treatment during and after chemotherapy[1,2]. Ovary toxicity is the main side effect of chemotherapeutics for women of reproductive age. The main issue for inducing ovary damage is a limited quantity of oocytes observed in pre-birth ovarian follicles[3,4]. The total ovarian reserve of a woman is measured by this population of primordial follicles and a woman's reproductive life ends when this number of follicles drops to less than a thousand. To date, various chemotherapeutic agents have been reported to damage the ovarian follicles and enhance premature ovarian failure risk, infertility and premature menopause[5,6]. The induction of ovary damage decreases the quality of patient life and boosted the medical expenses[7].

An antineoplastic drug such as Cyclophosphamide (CP) is successfully used for treating cancer, but antineoplastic drugs having side effects. The main serious side effects of antineoplastic agents are reproductive dysfunction[3,4]. Cyclophosphamide commonly used for treating cancer, but it has an immunosuppressive effect, few investigators have exhibited a protective effect against the autoimmune disease[3]. Moreover, destructive morphological alterations to the testis, uterus and ovaries, CP also induces serious side effects such as reduction gonadal function, oligospermia and azoospermia. Few researchers suggest that CP reduces blood testosterone level and dysfunction gonadotropin secretion. Research suggests that infertility and pre-mature ovarian failure are the most common side effect of CP treatment. CP treatment induces the dysfunction of the ovary in the rat and finally causes infertility[2-4]. The breast cancer patients treated with CP showed dysfunction of ovarian damage, which further causes early menopause. Gonadotropins commonly used with CP, to treat cancer. Gonadotropins preserve ovarian function. However, some researchers have used antioxidant therapy along with adjuvant antineoplastic drugs to minimize side effects[3,4].

CP reduces ovarian follicular reserve by causing irreversible damage to ovarian germ cells, which supports stromal cells and eventually contributes to POF[3,4]. Toxic and protective effects of CP are associated with its active metabolites such as phosphoramide mustard and acrolein. Both metabolites bind with DNA lead the cell death and DNA synthesis disturbance. The development of Reactive Oxygen Species (ROS) by conjugation with glutathione, resulting in interference with the ovary's antioxidant protection system, resulting in mitochondrial damage, inflammation and lipid peroxidation induced via various inflammatory mediators, leading to caspase family activation and ovarian follicle activation[3,4].

Erythroid-related factor 2 (Nrf2) is a crucial signalling transcription factor that provides defence against cellular oxidative stress caused by endogenous or exogenous chemicals. During the physiological conditions, when a cell is subjected to oxidative stress, ROS can activate Nrf2 and cause it to translocate into the nucleus, where it can then trigger the expression of target genes like Heme Oxygenase-1 (HO-1)[8-10].

It is well documented that HO-1 caused various cellular stresses such as ischemia/reperfusion damage, hypoxia, Nitric Oxide (NO) and bacterial endotoxins. The HO pathway has recently stimulated interest in numerous disciplines, including the CNS, cardiovascular physiology, renal and hepatic systems[8-10]. The HO pathway has recently sparked interest in various disciplines. HO-1 boosted the inflammatory reaction and oxidative stress. In this protocol, we scrutinized the ovary protective effect of hesperidin against the cyclophosphamide-induced ovary damage.

MATERIALS AND METHODS

Study area: The experimental study was carried out in the Jiangjin Centra Hospital from February-March, 2020.

Experimental animal: Swiss Wistar rat (100 rats) weight 140-170 g aged 9-10 weeks, female in sex was used. All the rat used in the experimental study was procured from the Institutional Animal house and kept in the controlled condition. During the acclimation, the rat was kept at the controlled temperature of 20±3°C and 50-70% relative humidity. The rat was received food and water (*ad libitum*) in a complete experimental study. The rat was acclimatized for 7 days before the experimental protocol to adjust the laboratory conditions. All experimental protocol was performed using the Institutional Animal Care and Use Committee (IACUC).

Preparation of drug: Intraperitoneal injection of CP (150 mg kg^{-1}) was used for induction the ovary cancer. Hesperidin suspension was prepared by preparing the suspension using 1% Tween 80[10].

Preclinical study: After the acclimation period of rats, the rat was divided into the following groups and each group contains 20-rat:

- Group I: Control rat treated the only saline
- Group II: CP
- Group III: CP+hesperidin (10 mg kg^{-1})
- Group IV: CP+hesperidin (20 mg kg^{-1})
- Group V: CP+hesperidin (40 mg kg^{-1}), respectively

The rat has received treatment for 42 days and received one treatment in a day. Bodyweight and ovary tissue weight calculated at the end of the experimental study. At the end of the experimental study, blood samples were collected and centrifuged at 15 k rpm for 10 min at 4°C and collected the clear supernatant for biochemical parameter determination. After that, the rats were sacrificed via cervical dislocation and collected ovary tissue for the estimation of different parameters.

Antioxidant parameters: For estimating antioxidant parameters in the ovary tissue, the ovary tissue was homogenized in 150 mM (dilution 1:10 w/v) using the Teflon glass homogenizer. The prepared homogenates were centrifuged at 18 k rpm for 30 min at 4°C.

CAT, MDA, GPx, SOD and GSH were estimated in serum and ovarian tissue using Enzyme-Linked Immunosorbent Assay (ELISA) (Nanjing Jiancheng Co., Nanjing, China) using the manufacture protocol.

Hormonal assay: Serum E2, anti-Müllerian hormone (AMH), FSH and LH were determined using the ELISA kits (Sunlong Biotech Co., Ltd. Zhejiang, China) following the manufacture protocol.

Estimation of cytokines: The cytokines (pro-inflammatory) include interleukin-6 (IL-6), Tumour Necrosis Factor-α (TNF-α), Interleukin-1β (IL-1β) and Nuclear Factor Kappa B (NF-κB) were scrutinized using the ELISA kits (Yehua, Shanghai, China) following the manufacture protocol.

Inflammatory parameter: Inflammatory parameters such as inducible Nitric Oxide Synthase (iNOS) and cyclooxygenase-2 (COX-2) activities in ovarian tissue were estimated by using the ELISA kit (Yehua, Shanghai, China).

Caspase-3 activity: Caspase-3 activity was determined using the ELISA kit (Yehua, Shangai, China) following the manufacture protocol.

Bcl-2 level: Bcl-2 level in the ovary tissue was estimated using the ELISA kit (Yehua, Shangai, China) following the manufacture protocol.

Statistical analysis: In the current experimental study, all biochemical values were analysed using one-way ANOVA using the GraphPad Prism (version 5, USA). Dennett's comparison test was followed to compare the groups and all values are given as Mean±standard error (SEM). The $p<0.05$ was considered as statistically significant.

RESULTS

Effect on ovary tissue: CP induced rats exhibited increased ovary weight at the end of the experimental study. CP group rats boosted the ovary weight by almost double compared to vehicle control group rats. Hesperidin treatment considerably ($p<0.001$) dose-dependently suppressed the ovary weight (Fig. 1).

Effect on hormone level: The data of Fig. 2(a-d) showed the effect of hesperidin on hormone levels such as E2 (Fig. 2a), FSH (Fig. 2b), LH (Fig. 2c) and AMH (Fig. 2d). During the ovarian toxicity reduced the level of E2, AMH and boosted the level of FSH, LH. The CP control group rats exhibited similar results and confirm the ovarian toxicity in the experimental rats. Vehicle control group rats did not show any changes in the hormone level. CP induced ovary toxicity rats treated with the hesperidin notably boosted the level of FSH, LH and reduced the levels of E2, AMH.

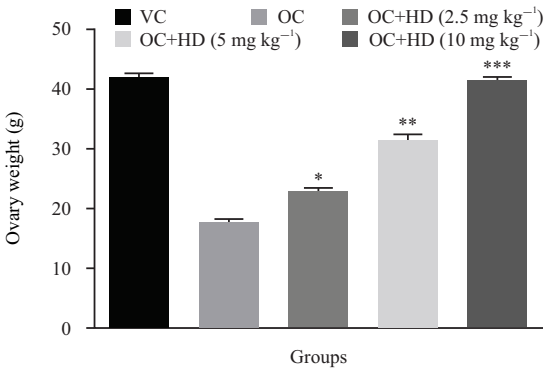

Fig. 1: Exhibited the effect of hesperidin on the ovary weight of cyclophosphamide induced ovary toxicity in rats

All the data showed in the ±SEM. Significance was set at $p<0.05$, Hesperidin treated rats compared vs. cyclophosphamide-treated group rats. where *$p<0.05$ was considered as significant, **$p<0.01$ was considered as more significant and ***$p<0.001$ was considered as extremely significant

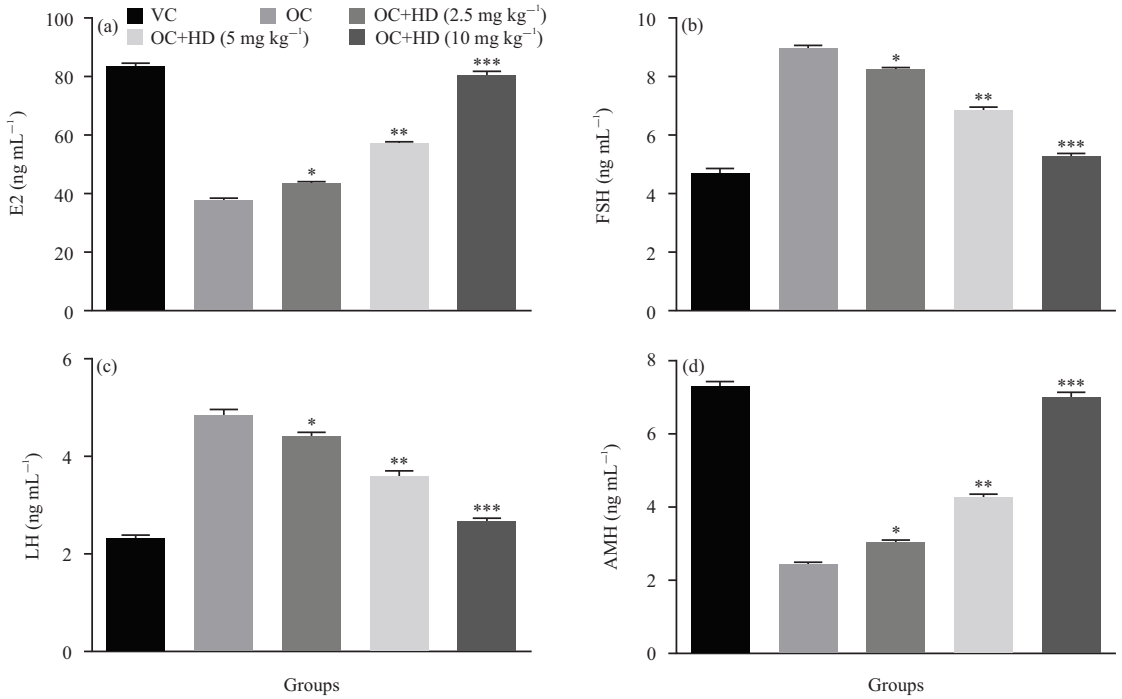

Fig. 2(a-d): Exhibited the effect of hesperidin on the hormone level of cyclophosphamide induced ovary toxicity in rats

(a) E2, (b) FSH, (c) LH and (d) AMH. All the data showed in the ±SEM. Significance was set at p<0.05, Hesperidin treated rats compared vs. cyclophosphamide-treated group rats. where *p<0.05 was considered as significant, **p<0.01 was considered as more significant and *** p<0.001 was considered as extremely significant

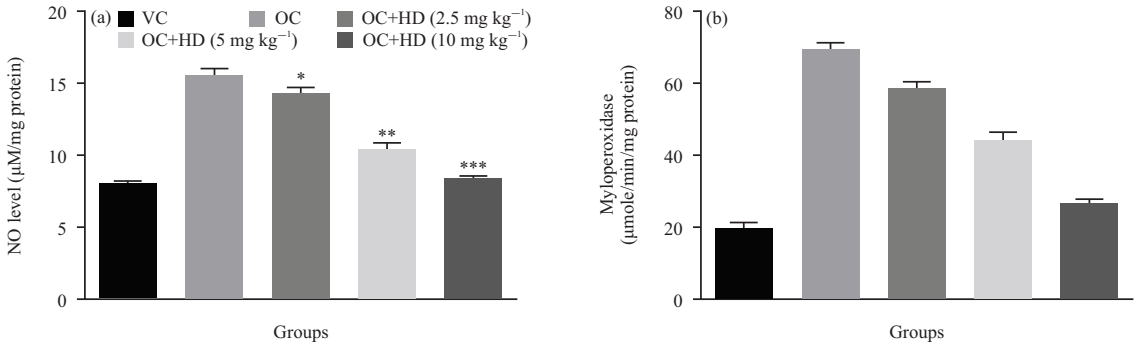

Fig. 3(a-b): Exhibited the effect of hesperidin on the biochemical parameters of cyclophosphamide induced ovary toxicity in rats

(a) NO and (b) MPO. All the data showed in the ±SEM. Significance was set at p<0.05, Hesperidin treated rats compared vs. cyclophosphamide-treated group rats. where *p<0.05 was considered as significant, **p<0.01 was considered as more significant and ***p<0.001 was considered as extremely significant

Effect on NO and MPO level: The data in Fig. 3(a-b) showed the level of NO and MPO of the treated group rats. Vehicle group rats did not alter the level of NO and MPO. In the country, CP induced group rats showed a boosted level of NO and MPO compared to other treated or non-treated rats. Hesperidin treated rats considerably (p<0.001) reduced the level of NO and MPO.

Effect on antioxidant parameters: Antioxidant parameters such as MDA (Fig. 4a), GSH (Fig. 4b), SOD (Fig. 4c), GPx (Fig. 4d) and CAT (Fig. 4e) were estimated in the all-groups rats. CP induced ovary toxicity rats showed increased levels of MDA and reduced levels of GSH, SOD, GPx, CAT compared to other groups. Hesperidin treatment considerably (p<0.001) suppressed the level of MDA and boosted the level of GSH, SOD, GPx and CAT.

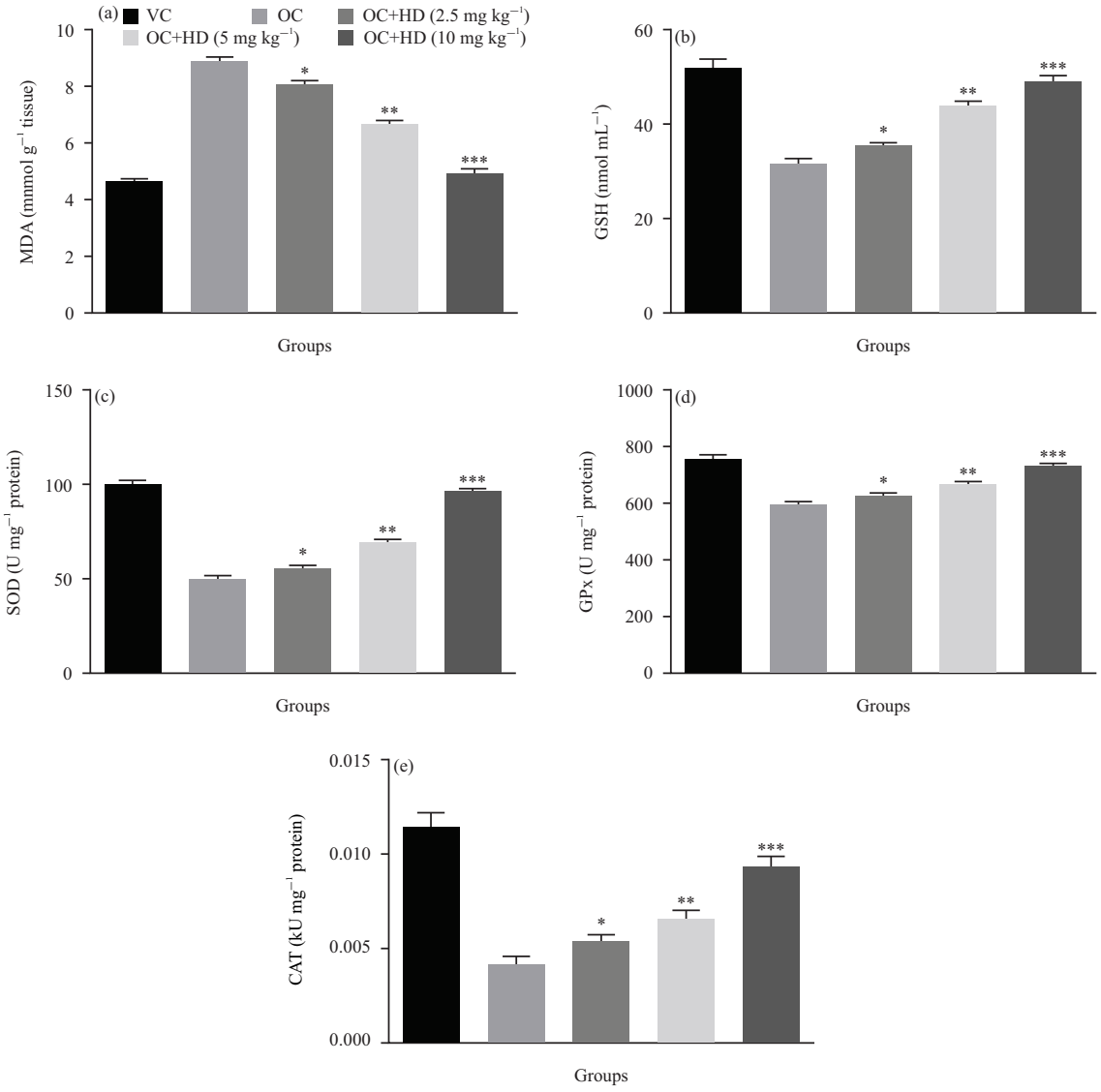

Fig. 4(a-e): Exhibited the effect of hesperidin on antioxidant parameters of cyclophosphamide induced ovary toxicity in rats
(a) MDA, (b) GSH, (c) SOD, (d) GPx and (e) CAT. All the data showed in the ±SEM. Significance was set at p<0.05, Hesperidin treated rats compared vs. cyclophosphamide-treated group rats. where *p<0.05 was considered as significant, **p<0.01 was considered as more significant and ***p<0.001 was considered as extremely significant

Effect on pro-inflammatory cytokines: An inflammatory reaction plays a crucial role in the expansion of ovarian toxicity. The inflammatory reaction was estimated in terms of pro-inflammatory cytokines. A similar result was observed in the CP group rats. CP induced ovary toxicity in rats demonstrated the increased level of cytokines such as TNF-α (Fig. 5a), IL-6 (Fig. 5b) and IL-1β (Fig. 5c) and hesperidin treatment considerably ($p<0.001$) reduced the level of cytokines. Hesperidin (10 mg kg^{-1}) treated group rats reduced the level of pro-inflammatory cytokines almost near the vehicle control group rats.

In this experimental study, we estimated the pro-inflammatory cytokine level in the ovary tissue and we have observed a similar result as found in the serum. CP induced ovary toxicity group rats showed increased levels of pro-inflammatory cytokines and hesperidin-treated group rats showed a reduction in the level of pro-inflammatory cytokines such as TNF-α (Fig. 6a), IL-6 (Fig. 6b) and IL-1β (Fig. 6c).

Effect on the inflammatory parameters: An inflammatory reaction expands the various organ toxicity inside the body. It is well proofed that inflammatory reaction induces ovary

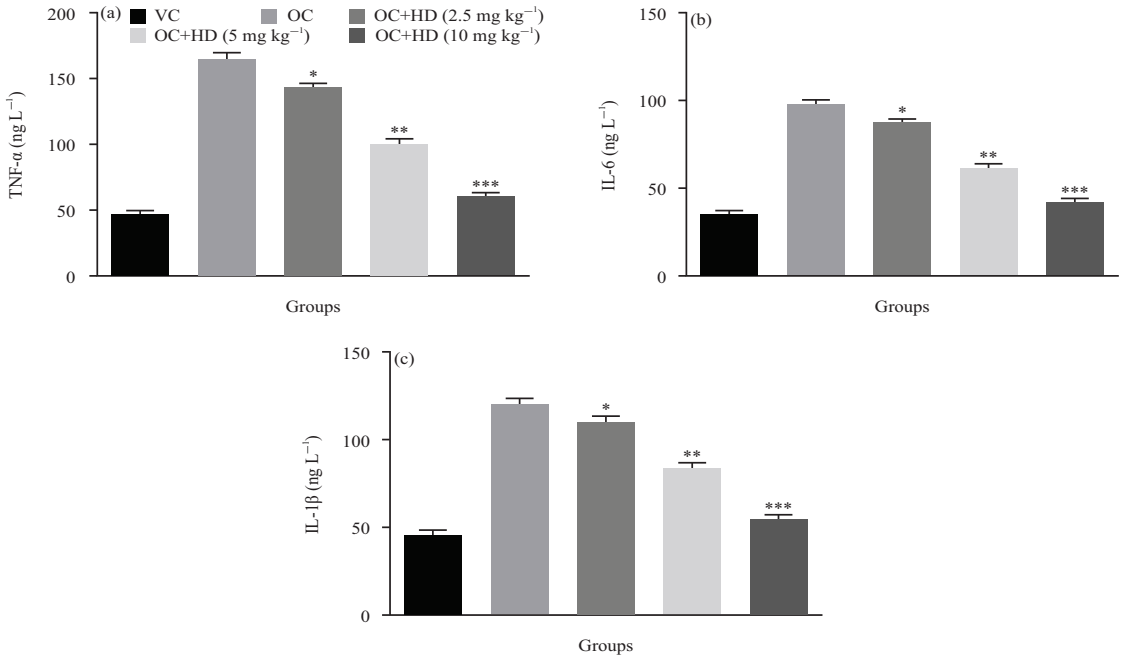

Fig. 5(a-c): Exhibited the effect of hesperidin on the pro-inflammatory cytokine level (serum) of cyclophosphamide induced ovary toxicity in rats

(a) TNF-α, (b) IL-6 and (c) IL-1β. All the data showed in the ±SEM. Significance was set at p<0.05, hesperidin treated rats compared vs. cyclophosphamide-treated group rats. where *p<0.05 was considered as significant, **p<0.01 was considered as more significant and ***p<0.001 was considered as extremely significant

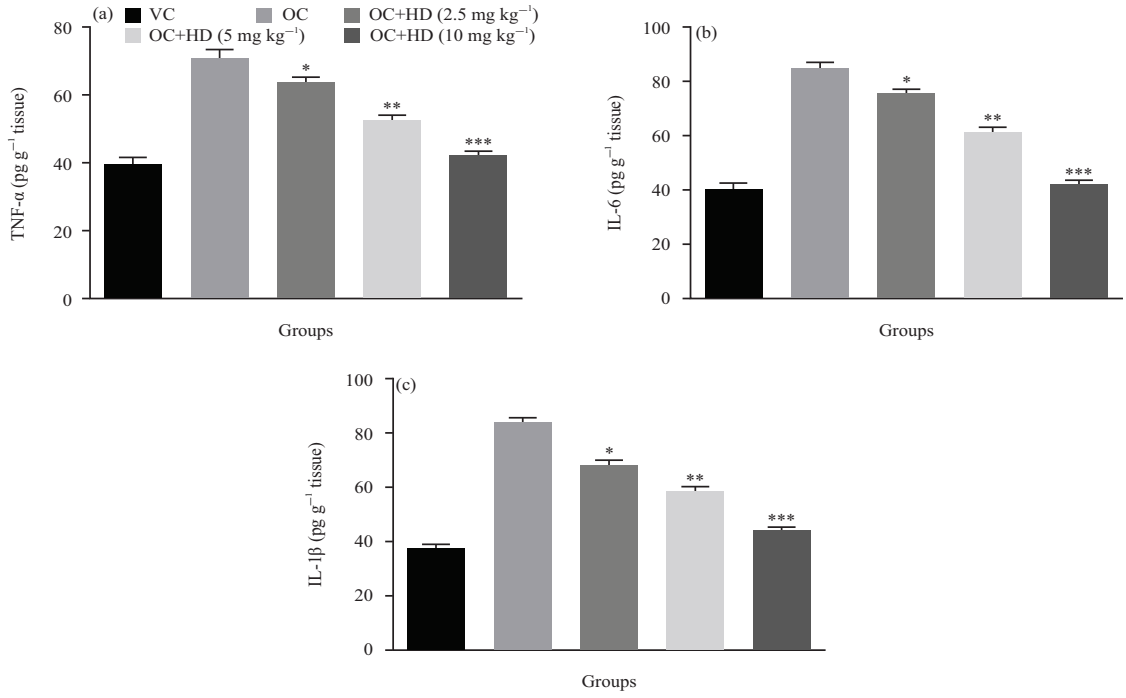

Fig. 6(a-c): Exhibited the effect of hesperidin on the pro-inflammatory cytokine level (ovarian tissue) of cyclophosphamide induced ovary toxicity in rats

(a) TNF-α, (b) IL-6 and (c) IL-1β. All the data showed in the ±SEM. Significance was set at p<0.05, hesperidin treated rats compared vs. cyclophosphamide-treated group rats. where *p<0.05 was considered as significant, **p<0.01 was considered as more significant and ***p<0.001 was considered as extremely significant

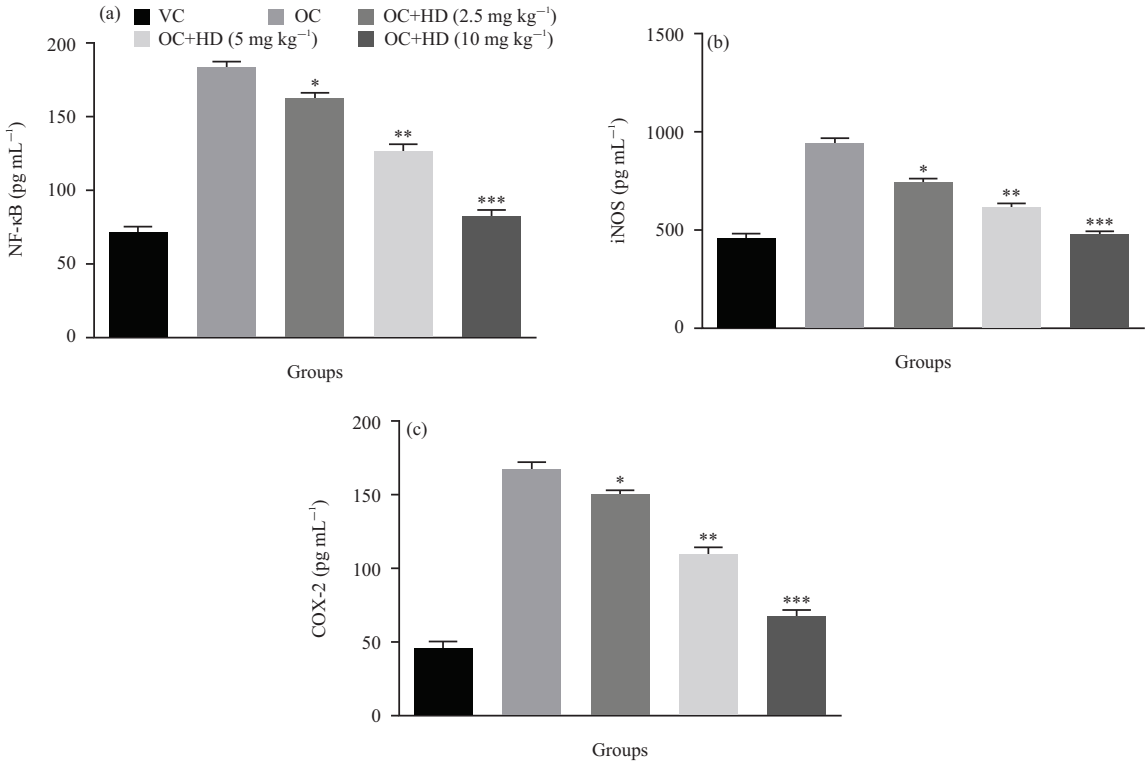

Fig. 7(a-c): Exhibited the effect of hesperidin on the inflammatory parameter level (serum) of cyclophosphamide induced ovary toxicity in rats

(a) NF-κB, (b) iNOS and (c) COX-2. All the data showed in the ±SEM. Significance was set at $p<0.05$, hesperidin treated rats compared vs. cyclophosphamide treated group rats. where *$p<0.05$ was considered as significant, **$p<0.01$ was considered as more significant and ***$p<0.001$ was considered as extremely significant

toxicity. CP induced ovarian toxicity group rats showed an increased level of inflammatory parameters such as NF-κB (Fig. 7a), iNOS (Fig. 7b) and COX-2 (Fig. 7c) and hesperidin treatment significantly ($p<0.001$) reduced the level of NF-κB (Fig. 7a), iNOS (Fig. 7b) and COX-2 (Fig. 7c) at dose-dependent manner.

In this experimental study, we have estimated the inflammatory mediator NF-κB (Fig. 8a), iNOS (Fig. 8b) and COX-2 (Fig. 8c) in the ovary tissue and CP group rats showed a similar result. Hesperidin treatment considerably ($p<0.001$) reduced the level of NF-κB (Fig. 8a), iNOS (Fig. 8b) and COX-2 (Fig. 8c) at dose-dependent manner.

Effect on caspase-3 and Bcl-2: The data of Fig. 9 shows the effect of hesperidin on the level of Bcl-2 (Fig. 9a) and caspase-3 (Fig. 9b). CP induced ovarian toxicity rats showed an increased level of caspase-3 and reduced level of Bcl-2. Hesperidin treatment considerably reduced the level of caspase-3 and increased the level of Bcl-2.

DISCUSSION

Traditional medicine has a mixed reputation, but there is optimism about its efficacy. The various researcher has been suggested that the hesperidin having a long history of use in Asia, especially China. In the current investigation, we investigated the protective effect of hesperidin on CP induced ovarian damage. CP proofed their antineoplastic effects on various malignancies such as lung cancer, testicular cancer, solid tumours and metastatic ovarian tumours. Clinical research suggests that CP is ganodotoxic and induces ovarian dysfunction in female patients (40% cases) and it induces hormonal imbalance, both of which lead to permanent or temporary infertility.

It is well documented that high doses of CP exhibit reduced follicles and boosted follicular atresia as well as induces the apoptotic alteration in the granulosa cells of medium follicles[9,10]. A previous report suggests that CP induces ovarian dysfunction characterized by enhanced follicular apoptosis, a reduction in the secretion of AMH,

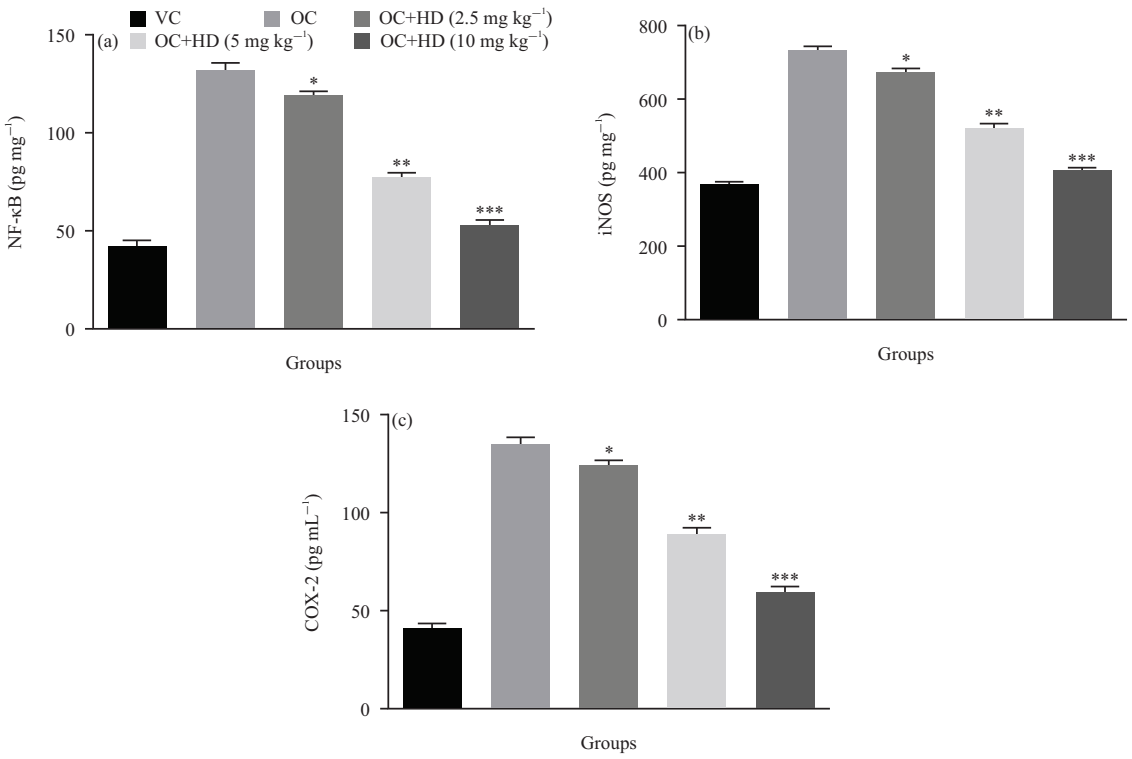

Fig. 8(a-c): Exhibited the effect of hesperidin on the inflammatory parameter level (ovarian tissue) of cyclophosphamide induced ovary toxicity in rats

(a) NF-κB, (b) iNOS and (c) COX-2. All the data showed in the ±SEM. Significance was set at $p<0.05$, hesperidin treated rats compared vs. cyclophosphamide treated group rats. where *$p<0.05$ was considered as significant, **$p<0.01$ was considered as more significant and ***$p<0.001$ was considered as extremely significant

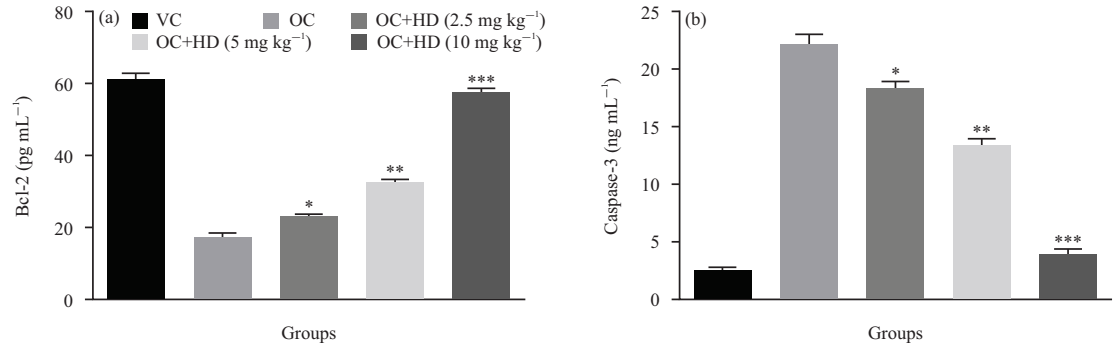

Fig. 9(a-b): Exhibited the effect of hesperidin on the Bcl-2 and caspase-3 parameters of cyclophosphamide induced ovary toxicity in rats

(a) Bcl-2 and (b) Caspase-3. All the data showed in the ±SEM. Significance was set at $p<0.05$, hesperidin treated rats compared vs. cyclophosphamide treated group rats. where *$p<0.05$ was considered as significant, **$p<0.01$ was considered as more significant and ***$p<0.001$ was considered as extremely significant

suppression in the production of follicles, alter in the cycle of oestrous[11,12]. Hormone such as AHM, FSH, E2 and inhibin B is considered significant markers for estimating chemotherapy-induced ovary dysfunction[13,14]. Hormone such as FSH is considering circulating hormones that boost expansion in post-pubertal mammals and follicular development. Inhibin B and E2 are generated via granulosa cells that take part in the loop of the pituitary ovarian axis to reduce FSH secretion[9,15]. Moreover, after the CP treatment, boosted the FSH hormone level due to the reduction of E2 hormone level due to start the secretion from granulosa cells. In the current experimental protocol, we have found a reduction in the level of E2

hormone and boosted level of FSH hormones in the CP control group and confirm the induction of toxicity to follicular function and ovary[9,11,15,16]. Hesperidin treatment considerably increases the level of E2 hormone and reduced the level of FSH hormone and suggesting the protective effect against ovary toxicity.

The study suggests that CP induces ovary toxicity by targeting DNA in the body[16]. CP attack DNA and start the DNA replication and generates free radicals. The continuous generation of free radicals booted the level of free radicals, which resultant induction of oxidative stress and finally induces tissue toxicity. Oxidative stress boosted the level of ROS[11,12,16]. ROS have a high affinity for cellular components, including carbohydrates, lipids, proteins and DNA[13,14]. CP treatment induces ROS formation, such as hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and superoxide anion (O_2). ROS formation can boost the level of lipid peroxidation by changing the permeability and fluidity of biomembranes, as well as induces polymerization and cross-linking of macromolecules including nucleic acids and proteins[9,15,17]. The continuous formation of ROS attaches to the lipid and start the boosted level of lipid peroxidation. LPO is a significant marker of oxidative stress and a variety of research exhibits a considerably boosted level of MDA (an LPO marker), after the CP treatment[11,13]. Previous studies suggest that the endogenous antioxidant system plays an important role in the elimination of free radicals from biological systems. Endogenous antioxidants such as enzymatic and non-enzymatic play a significant role in the removal of free radicals[9,14,16]. First-line antioxidant enzymes such as SOD and CAT play an imperative role in the expansion of free radicals. Both enzymes break O_2 and decompose H_2O_2, resultant decrease the oxidative stress, which is an effective way to protect the cell from damage[11,13]. Both the enzymes work together to eliminate the ROS and minor changes in physiological concentrations can affect lipid, DNA resistance and cellular proteins to oxidative damage. The activity of both enzymes is necessary to eliminate free radicals and protect the organ and tissues against oxidative stress[6,7]. Non-enzymatic antioxidants such as GSH protect the tissue and organ from the undesirable effect of ROS and protect against peroxidation[18,19]. GSH protects the tissue from lipid peroxidation by conjugating with the electrophile includes 4-hydroxy-3-nonenal (HNE), generated during the LPO. In the GSH redox cycle, decreased GSH together with GPx converts lipid peroxides to nontoxic products, thus guard the integrity of mitochondria and cell membranes[6,7,20]. In this study, we have found that CP induces a reduction in the activity of antioxidant enzymes, including GPx, CAT, SOD (enzymatic) and GSH (non-enzymatic) and hesperidin treatment considerably improved the level of enzymatic and non-enzymatic antioxidants. The result suggests that hesperidin is a powerful antioxidant drug that protects the rodent from ovary toxicity.

In this experimental study, we have observed an increased level of pro-inflammatory cytokines, suggesting the inflammatory role in the CP induced ovary toxicity, imitating that pro-inflammatory cytokine production may result from each of the biochemical alterations in cellular metabolism and environment or injury in the DNA with a reduction in cell cycle progression induced via the interaction of chemotherapy with a target cell[2,3,15]. Our results exhibited similar results. Immune cells formed the pro-inflammatory cytokines (TNF-α, IL-1β and IL-6), causing host damage in various inflammatory diseases. Pro-inflammatory cytokines play a crucial role in the pathogenesis of CP induced ovary toxicity. They affect the inflammatory response's systemic development to end-organ dysfunction. CP induced ovary toxicity group rats exhibited an increased level of cytokines and hesperidin treatment noticeably suppressed the level of pro-inflammatory cytokines, which is in agreement with various previous investigations[2,8,9].

Pro-inflammatory cytokines are low molecular weight proteins that attribute the cell-cell interaction. Pro-inflammatory cytokines also control cell survival, cell migration, proliferation, immune cell activation, cell death and cell differentiation. Dysregulation of NF-κB is related to Crohn disease and ulcerative colitis and other inflammatory diseases such as cancer, diabetes, asthma and rheumatoid arthritis. Consequently, the reduction of NF-κB may help suppress ovary toxicity. For example, the suppression of NF-κB activity in hepatic cancer is targeted in the previous study. Additionally, the suppression of tumorigenicity in ovarian cancer cells after blocking the NF-κB[2,8,9].

Inflammatory cells create ROS that interrupts the membrane through protein oxidation and lipid peroxidation and finally triggers the inflammatory reaction[3,16]. Moreover, oxidative stress can boost the inflammatory reaction in CP ovary toxicity via the release and recruitment of pro-inflammatory mediators, leading to a systemic inflammatory response. An inflammatory reaction plays an important role in the expansion of organ toxicity. Pro-inflammatory cytokines, oxidative stress and inflammatory mediators boost the inflammatory reaction[5,6,19]. Inflammatory parameters such as iNOS used to generate nitric oxide in the body. Excess amounts of nitric oxide react with the free radical such as

superoxide anion to nitrate and oxidize macromolecules include DNA, lipids and proteins from the peroxynitrite radical, which is responsible for induction cell injury[10,15]. Additionally, an excess amount of NO absorbs by intracellular GSH, thereby boosting the sensitivity to oxidative stress. Various studies showed the adverse effects of NO and NF-κB, after the CP treatment. NF-κB is the most significant transcription factor known to be sensitive to oxidative stress. NF-κB pathway commonly targeted because it plays a crucial role in the regulation and activation of various transcriptional factors includes COX-2, TNF-α, iNOS, IL-6, IL-1β and iNOS[9-11]. In this study, we have found that the increased levels of pro-inflammatory cytokines and inflammatory mediators in the CP group and hesperidin treatment significantly suppressed the level of pro-inflammatory cytokines and inflammatory mediators.

Pro-inflammatory cytokines include TNF-α that activates the various apoptotic proteins that boosting the many phases of apoptotic degradation[8,13]. Caspase-3, which is estimated in the serum and tissue in this experimental study, is considered as the effector enzyme is starting the death cascade and it consider as a significant marker for cells undergoing an apoptotic signal pathway. During the CP-induced ovary toxicity, increased the level of caspase-3 in the serum and tissue, treatment with hesperidin considerably reduced the level of caspase-3, which was the agreement of the previous study.

CONCLUSION

Even though more research is required, based on the current findings, we can say that hesperidin administration protects the ovary tissue. In this experimental protocol, we have found that hesperidin considerably reduced ovary toxicity via multiple mechanisms such as altered the hormone level, improved antioxidant levels (enzymatic and non-enzymatic), reduced the level of pro-inflammatory cytokines, suppressed the level of inflammatory mediators and down-regulated the level of apoptosis parameters.

SIGNIFICANCE STATEMENT

This study discovered that the ovarian protective effect of hesperidin against the cyclophosphamide-induced ovarian toxicity in female rats via antioxidant and anti-inflammatory effects. Our findings showed the use of hesperidin against cyclophosphamide-induced ovarian toxicity. This study will help the researcher to uncover the critical areas of ovary toxicity that many researchers were not able to explore. Thus a new theory on hesperidin (Phyto-constituents) may be arrived at.

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