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Original Research Article

Assessment of the Effects of Broccoli Sprout Extract on Ovarian and Renal Tissues in Female Albino Rats Treated with Methotrexate

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Abstract: The aim of this study was to evaluate the protective effect of broccoli sprout extract (BSE) against ovarian and renal toxicity induced by methotrexate (MTX) in female albino rats. Methotrexate, an antimetabolite frequently utilized in cancer and autoimmune diseases, has been linked to nephrotoxicity and primary ovarian insufficiency through the induction of oxidative stress and apoptosis. Because of their rich content in glucoraphanin and sulforaphane, two powerful antioxidants, broccoli sprouts were chosen. This study comprised six groups of Thirty female albino rat (180–225 g) (n = 5): (A) Control; (B) MTX, 0.15 mg/kg/week; (C) BSE, 5 mg/kg/day; (D) BSE, 7.5 mg/kg/day; and (E) MTX + BSE, at doses of 5 and 7.5 mg/kg respectively. All treatments were administered orally. The administration of MTX induced severe ovarian histological injuries, indicated by significantly reduced number of follicles, blood vessels rupture, inflammatory cell infiltration and partly fibrosis. Administration of MTX significantly increased the levels of urea and creatinine in renal tissues and induced glomerular damage such as congestion, necrosis, and cellular vacuolation. Consistent with this, these histopathological changes were prevented by simultaneous administration of BSE which appears to restore folliculogenesis and renal function parameters. In conclusion, the findings indicate that BSE strongly protects against MTX toxicity through a mechanism involving enhancement of antioxidant homeostasis. Thus, to summarise, broccoli sprout extract is a effective naturl adjuvant for mencerpt treatment in rescinding side effects on female reproductive and renal systems.

Keywords: Methotrexate (MTX), Broccoli Sproute Extract (BSE), Ovarian Tissue, Sulforaphane, Kidney Fonctions.

INTRODUCTION

Cancer affects many women, many at the age of reproduction and causes infertility, making chemotherapy-induced infertility a global issue of concern (Zhao *et al*., 2023). Methotrexate (MTX), a folic acid antagonist, has been extensively used in treatment of autoimmune diseases as well as malignancies including leukemia and osteosarcoma (McLaren *et al.,* 2009: Bedoui *et al*., 2019). Nevertheless, its cytotoxic effect is not limited to neoplastic cells but include healthy proliferating tissue such as ovarian follicles and therefore it represents a high threat for young women fertility (Bedoui *et al*., 2019; Gol *et al.,* 2009).

All these gonadotoxic effects of methotrexate (MTX) are closely related to oxidative stress, inflammation and apoptosis. This leads to the accumulation of reactive oxygen species (ROS), loss of mitochondrial membrane permeability, and altered expression of pro- and anti-apoptotic proteins including Bax (Bcl-2-associated X protein) and B-cell lymphoma 2) (Bedoui *et al.,* 2019; Keçeci & Karaoluk, 2025). In experimen) studies, MTX exposure decreased primordial follicle count and anti-Müllerian hormone (AMH) (Gol *et al*., 2009), both of which are important markers indicating ovarian reserve. In addition, histopathological studies shows areas of connective tissue fibrosis and degeneration of the follicle consistent with a reduced capacity for reproduction (Demir *et al*., 2024; Nabih *et al*., 2025).

In addition, it represents one of the more common causes of nephrotoxicity (tubulopathy and glomerulonephritis) which can develop into AKI or CKD (Abd-Elhamid *et al*., 2018). Increased oxidative stress, infiltration of immune cells/inflammation and apoptosis all contribute to MTX-induced kidney injury (Abd El-Twab *et al*., 2019). Since these

pathways are tightly interconnected to the pathogenesis of acute kidney injury (AKI) through oxidative stress, inflammation and impaired repair mechanisms (Li *et al*., 2019), their targeting may contribute as successful therapeutic approaches. Thus, this study was designed to evaluate the protective impact of broccoli sprout extract on MTX-induced renal and ovarian damage.

Recent studies have shown that plant-based products can stop or even reverse the disease process of many diseases associated with oxidative stress, which are rich in antioxidants. When it comes to the pharmacological activity of dietary plants containing antioxidant compounds such as polyphenols, glucosinolates, vitamin C and flavonoids, most investigations focus on protective action (Bhardwaj *et al*., 2019), driving defenses (Sajeesh *et al*., 2011; Chen *et al*., 2008; Lee *et al*., 2004) and potentially serving as alternative therapy for drug-induced diseases. "Broccoli (Brassica oleracea var. italica) is a cruciferous vegetable of the Brassicaceae family. It is considered one of the most GSL-rich crops, characterized by a high glucosinolate (GSL) content, particularly glucoraphanin (GRP), alongside a significant phenolic composition, the major GSL found in broccoli, generates the bioactive isothiocyanate (ITC) sulforaphane (SFN; 1-isothiocyanato-4-methylsulfinylbutane) after hydrolysis. By upregulating multiple detoxification enzymes, SFN has been shown to slow or inhibit cancer growth in humans (Bauman *et al*., 2016; Zhang *et al*., 1994; Zhang *et al*., 1992). Moreover, it controls genes and pathways associated with various oncogenic processes, such as susceptibility to carcinogen, cell cycle control, apoptosis, invasion and metastasis (Zhang & Tang, 2007; Rausch *et al*., 2010; Li *et al*., 2010). In several animal studies, anticancer activity of SFN was assessed and results consistently show that SFN or from broccoli sprout extract inhibits cancer growth and/or progression in a variety of organs.

MATERIALS AND METHODS

Experimental Animals and Design

A total of thirty female albino rats (Rattus norvegicus) weighing 180–225 g were purchased from the animal house of Tikrit University, Iraq. During the experiment and seven days before study, rats were hosted in metal cages under best environmental and nourish conditions.

The animals were randomly divided into six groups (n = 5 per group), and all treatments were administered orally as follows:

Group a (Control): Administered distilled water only.

- Group B (MTX): Received 0.15 mg/kg of methotrexate weekly.
- Group C (BSE 5): Received 5 mg/kg of broccoli sprout extract daily.
- Group D (BSE 7.5): Received 7.5 mg/kg of broccoli sprout extract daily.
- Group E (MTX + BSE 5): Received 0.15 mg/kg MTX weekly and 5 mg/kg BSE daily.
- Group F (MTX + BSE 7.5): Received 0.15 mg/kg MTX weekly and 7.5 mg/kg BSE daily.

Dose Preparation

Methotrexate tablets 2.5 mg/kg were obtained from Medwise (UK) were crushed into powder and suspended in water. Dose of BSE selected based on (Jian *et al*., 2019) and doubled to ensure the effectiveness of the dose Route of Administration selected based on (Yoko *et al*., 2019). MTX dose for rheumatoid arthritis, route of administration and dosage calculation based on (Nair & Jacob, 2016).

Broccli Sprout Extract Powder Preparation

Seeds of broccoli (Brassica oleracea Botrytis v. Cymosa) was obtained from Sgaravatti, (Italy). Seeds sprouted for five days to get sprouts and washed to pure it of seed husks and ungerminated seed (Masuma *et al*., 2019). BSE was prepared as mentioned in (Li *et al*., 2013), by quick steaming of fresh broccoli sprout for 10 min over boiling water, frozen in liquid nitrogen, freeze- dried for 48 h by lypholizer, and then ground into fine powder.

Histopathological Examination

Tissue Preparation

Kidneys and Ovarian tissues were carefully dissected and immediately fixed in 10% neutral-buffered formalin for at least 24 h to ensure optimal preservation of cellular morphology and tissue architecture. Following fixation, samples were processed through a graded ethanol series for dehydration, cleared in xylene, and embedded in paraffin wax using standard histological techniques. Paraffin blocks were trimmed, and serial sections of 5 µm thickness were obtained using a precision rotary microtome. To prevent tissue folding and ensure uniform sectioning, the microtome blade was regularly replaced, and sections were floated on a water bath at 45 °C before mounting.

Staining and Imaging

The prepared sections were subjected to hematoxylin and eosin (H&E) staining to visualize general histological features. Hematoxylin was applied to stain the nuclei, while eosin counterstained the cytoplasm and extracellular matrix,

providing contrast and enhancing cellular detail. After staining, sections were mounted onto glass slides with a synthetic resin for long-term preservation. Microscopic evaluation was conducted using an Olympus BX51 light microscope equipped with an Olympus C-5050 high-resolution digital camera. Consistent microscope settings were maintained across all samples to ensure uniformity in image capture and subsequent analysis.

Biological Assays

Kidneys functions test in rat (S. Creatinine, B. Urea) was determined with kit (Agappe Pharmaceutical Co., Ltd, India).

Statistical Analysis

Renal functions data are presesnted as mean ± standard deviation (SD). SPSS 31.0 was used to determine statistical analysis, the largest arithmetic mean represented by (a), symbol (c) the smallest and (bc) indicates that there is no significant difference.

RESULT

Ovarian Results

Histological observation Control group (Figure 1) histopathlogical structure of ovaries was normal. Ovarian cortex was histologically normal; visible stages of ovarial follicle development, including primordial and primary follicles. The granulosa cells were arranged in an orderly fashion, with no degenerative or inflammatory changes. These results are in accordance with the reports of El-Sayyad *et al*., (2023) and Said *et al*., (2016) were found to visualize non-pathologic ovarian architecture in healthy animal models.

Conversely, the two doses of methotrexate (MTX 0.15 mg/kg) (Figure 2)+ were connected with significant pathological changes. Histologically, there was hemorrhage and infiltration of red blood cells and inflammatory cells in the ovarian tissues. In addition, these follicles had evidence of follicular atresia, degenerated follicles and focal fibrosis as well as degenerative changes occurring within the follicular structures.

Figure 3 shows developing follicles from the group treated with broccoli sprout extract (BSE 5 mg/kg), in which ovaries early stages displayed clearly visible oocytes surrounded by intact cellular layers. The ovarian stroma (the fibrous connective tissue structure of the ovarian body) with normal fiber density and spindle or round cells that were organised properly in places. The vessels had sharply delineated walls with little congestion.

On the other hand, in BSE receiving group 7.5 mg/kg (Figure 4) developing follicles with prominent central oocyte were found surrounded by clear zone pellicule. Granulosa cells were layered in multiple organized layers surrounding the oocyte. The theca surrounding the follicles were well-defined, and stroma had population with relatively normal cellular density without appropriate focal necrosis or marked congestion.

Histologically, the ovarian stroma in combination group (MTX 0.15 mg/kg + BSE 5 mg/kg) also seemed irregular with very small intercellular spaces (Figure 5). There was persistent fibrous tissue or mild edema in some areas, degenerated and necrotic follicles, and disorganized granulosa cells too. Mild vascular engorgement was noted.

Lastly, the matured follicles were increased in the MTX 0.15 mg/kg + BSE 7.5 mg/kg group (Figure 6 A,B) compared to this groups earlier upe of experimentals Granulosa cells in the (MTX + BSE 10 mg/kg) group were clearly more organized and higher density than those observed in (MTX + BSE 5 mg/kg), although there was still minor fibrosis. There was no congestion of blood vessels adjacent to the developing follicles, and the antrum was well-formed with follicular fluid as also shown in a regular pattern.

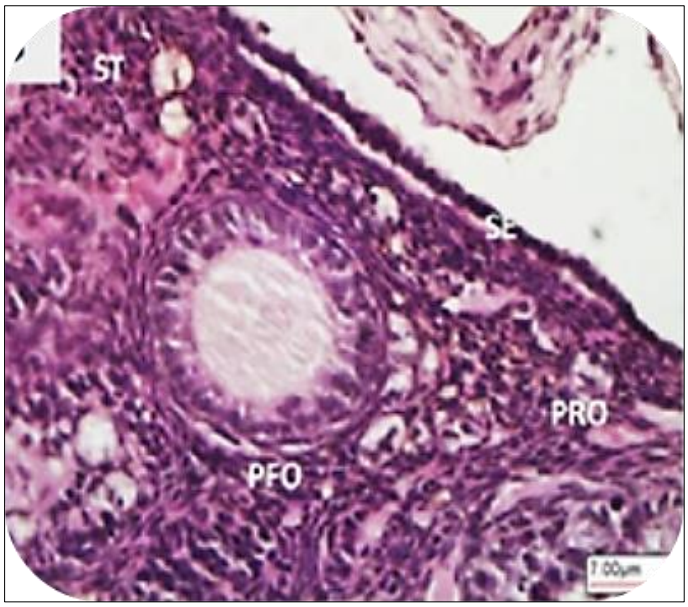

Fig. 1: Histology section of H&E-stained (400x) Samples of Ovary Normal Ovarian Cortex and Medulla the cortex consists of primordial follicle (PRO), primary follicles (PFO), and stroma (ST) GC Granulosa cells

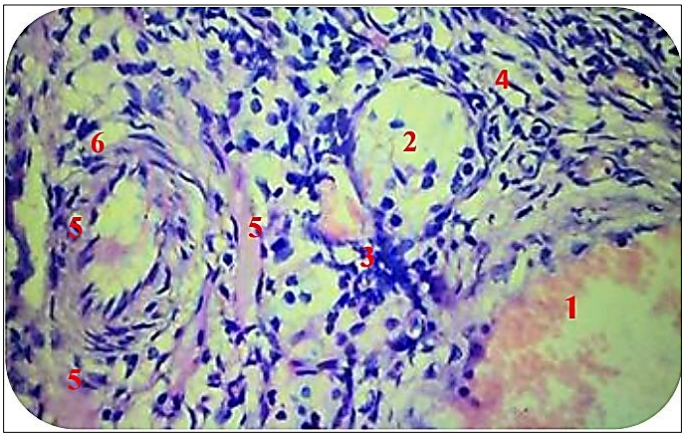

Figure 2: Histological section of a female rat ovary treated with (MTX 0.15 mg/kg): 1. Hemorrhage and red blood cell infiltration within the ovarian stroma. 2. Degenerating follicle. 3. Inflammatory cell infiltration. 4. Vacuolar degeneration. 5. Fibrosis. 6. Atretic follicle. H&E-stained (400x)

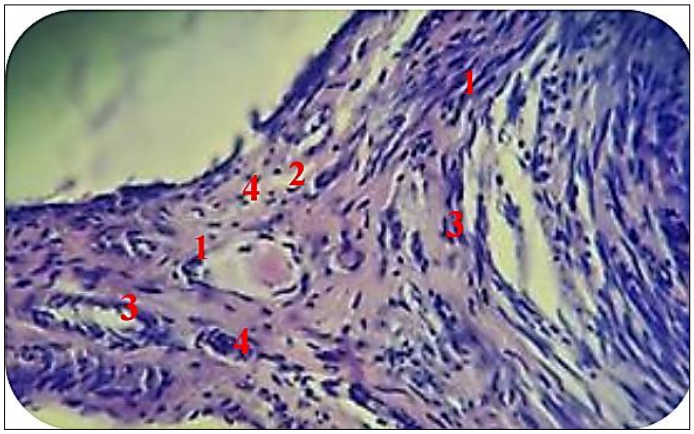

Figure 3: Histological section of a female rat ovary treated with (BSE 5 mg/kg): 1. Primordial Follicle. 2. Ovarian Stroma. 3. Blood Vessel. 4. Stromal Cells. H&E-stained (400x).

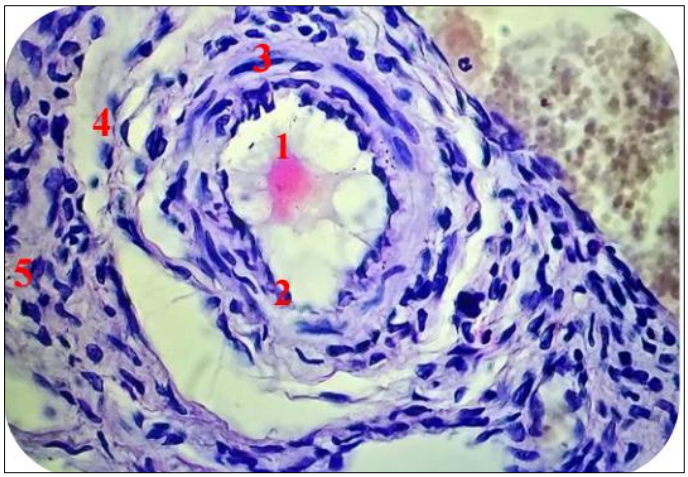

Figure 4: Histological section of a female rat ovary treated with (BSE 7.5 mg/kg): 1. Oocyte. 2. Developing Follicle. 3. Granulosa Cells. 4. Theca Layer. 5. Stroma. H&E-stained (400x)

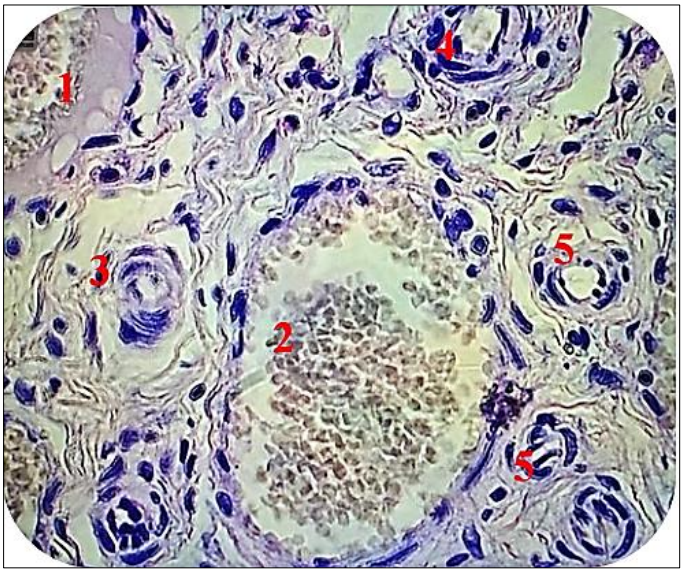

Figure 5: Histological section of a female rat ovary treated with (MTX 0.15 mg/kg + BSE 5 mg/kg): 1. Mild fibrosis. 2. Congested blood vessel. 3. Degenerated developing follicle. 4. Primary Follicle. 5. Increased number of developing follicles. (H&E-stained, 400x).

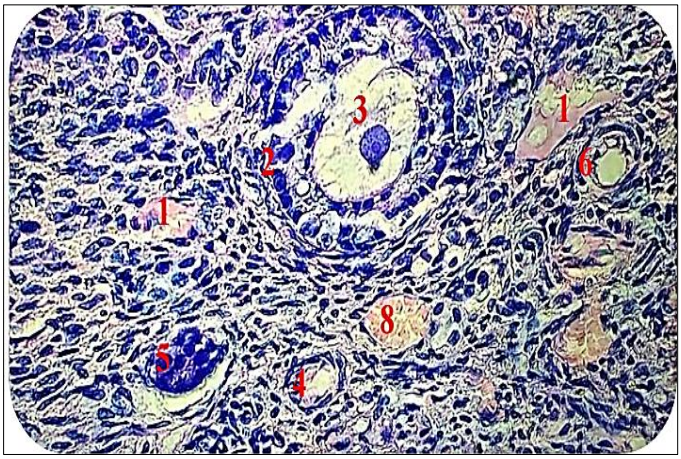

Figure (6-A): Histological section of a female rat ovary treated with (MTX 0.15 mg/kg + BSE 7.5 mg/kg): 1. Fibrosis. 2. Granulosa cells. 3. Secondary ovarian follicle containing a clear oocyte with a distinct nucleus. 4 and 5. Ovarian follicles at different stages of development. 6. Primary Follicle. 7. Blood vessel. (H&E-stained, 400x)

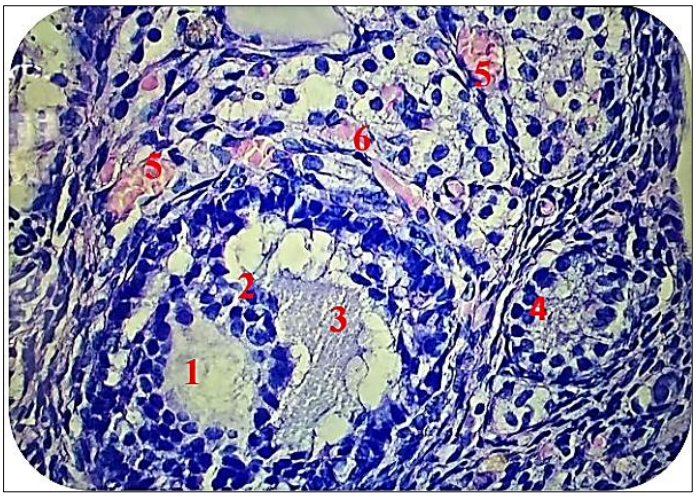

Figure (6-B): Histological section of a female rat ovary treated with (MTX 0.15 mg/kg + BSE 7.5 mg/kg): 1. Mature ovarian follicle (Graafian follicle). 2. Granulosa cells forming the Corona Radiata. 3. Cumulus Oophorus. 4. Primary ovarian follicle. 5. Blood vessels. 6. Mild fibrosis. (H&E-stained, 400x)

RENAL RESULTS

Renal urea and creatinine were significantly elevated following methotrexate (MTX) administration. Also, beside necrosis, vascular congestion and glomerular damage caused by MTX, cellular degeneration and vacuolization was observed in renal tissues.

From histological sections of the kidneys (Figure 7), control group presented normal histological architecture. Normal and well-organized appearance of both proximal convoluted tubules (PCT) and distal convoluted tubules (DCT), with no signs of degenerative or inflammatory change were noted to be in keeping with the normal histological pattern expected for healthy renal tissues.

On the other hand, notable pathological changes such as glomerular hypercellularity and reduction of Bowman's space were seen in the group receiving methotrexate (MTX 0.15 mg/kg) (Figure 8). Vacuolar degeneration, cellular swelling, and vascular congestion were also noted. There was also significant inflammatory cell infiltration and acute tubular necrosis (ATN) in the renal tubules.

BSE–treated group (BSE 5 mg/kg) (Figure 9), renal glomeruli appear dense dark spherical clusters of capillaries. These sections showed that the glomeruli remained relatively intact within their definable Bowman's space, indicating preservation of the renal capsule. In addition, the urinary tubule lumens were patent and uniformly sized, arguing against obstructive casts or voluminous proteinaceous casts that are characteristic of acute kidney injury.

Group BSE 7.5 mg/kg (Figure 10, overall normal looking renal tubules); The tubules are protected by sulforaphane from oxidative stress. The tubular lumens did not show any cellular debris or casts of the urine. They had clear central nuclei and proper viability and functionality. In addition, the interstitial space did not show inflammatory infiltration and appeared normal, consistent with the strong anti-inflammatory activities of broccoli sprout extract.

Histological Sections from Kidneys. The combination group (MTX 0.15 mg/kg + BSE 5 mg/kg) (Figure 11) had sections compatible with glomerular congestion and hypertrophy. Tubular epithelial lining the tubular epithelium showed severe vacuolar degeneration and interstitial tissue infiltration of inflammatory cells. Tubular cell death and loss of distinct cellular boundaries evidence acute tubular necrosis (ATN). There was also glomerular hypercellularity and crowding, in addition to capsule narrowing, which may reflect either the inflammatory response or the toxic impact of MTX.

Renal histological architecture was moderately improved in the group treated with MTX 0.15 mg/kg + BSE 7.5 mg/kg (Figure 12). The renal glomeruli retained their morphological appearance but showed a moderate dilation of the Bowman capsule; this indicates a partial protective effect of broccoli extract against MTX toxicity. Cytoplasmatic vacuolation and mild to moderate cellular degeneration were noted in the renal tubules indicating cell stress. In tubular lumens, sparse cellular debris and mild interstitial tissue inflammatory cell infiltration were observed. These results suggest that BSE at this dose offers partial protection from MTX-stimulated renal damage.

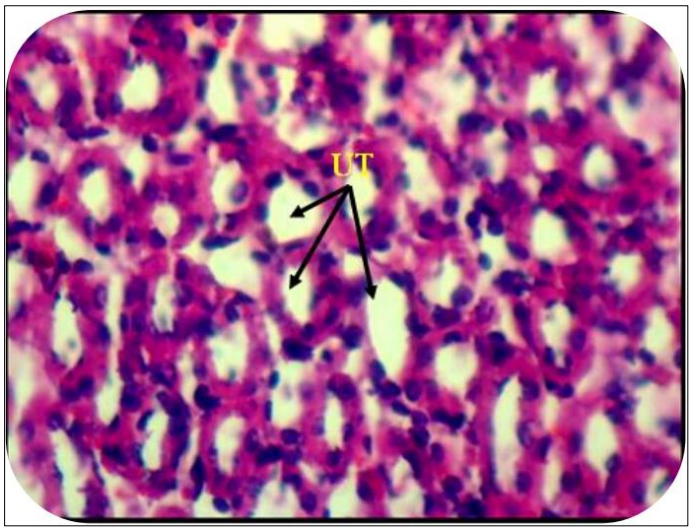

Figure 7: A histological section of a normal kidney (control) showing the urinary tubules (UT), specifically the proximal convoluted tubules (PCT) and the distal convoluted tubules (DCT). H&E stain (400x).

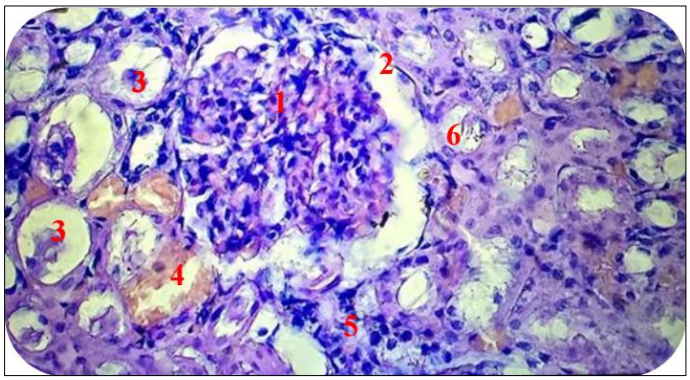

Figure 8: A histological section of a female rat kidney treated with (MTX 0.15mg/kg) showing: 1. Increased glomerular cellularity (Hypercellularity). 2. Narrowing of the urinary space (Bowman's space). 3. Vacuolar degeneration and swelling. 4. Congestion (Vascular congestion). 5. Inflammatory cell infiltration. 6. Tubular necrosis. H&E stain (400x).

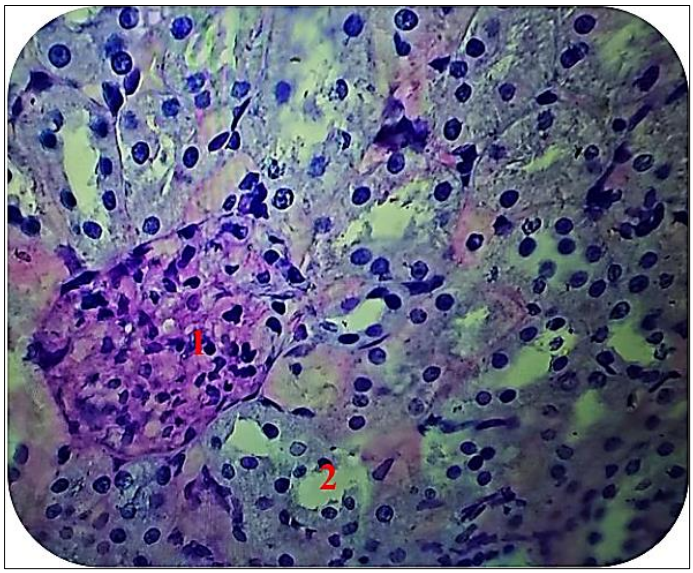

Figure 9: A histological section of a female rat kidney treated with (BSE 5mg/kg) showing: 1. The Glomerulus. 2. The lumen of the urinary tubules. H&E stain (400x)

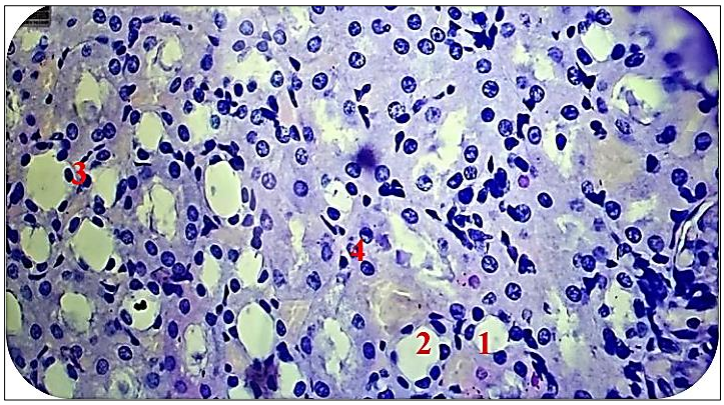

Figure 10: A histological section of a female rat kidney treated with (BSE 7.5mg/kg) showing: 1. Renal tubules. 2. Tubular lumen. 3. Interstitial space. 4. Epithelial cells. H&E stain (400x).

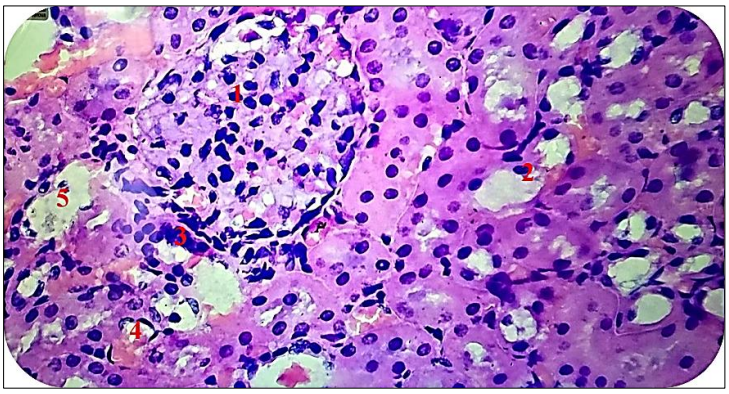

Figure 11: A histological section of a female rat kidney treated with (MTX 0.15mg/kg + BSE 5mg/kg) showing: 1. Renal glomerulus with hypercellularity. 2. Vacuolar degeneration. 3. Inflammatory infiltration. 4. Blood congestion (Vascular congestion). 5. Acute Tubular Necrosis (ATN). H&E stain (400x).

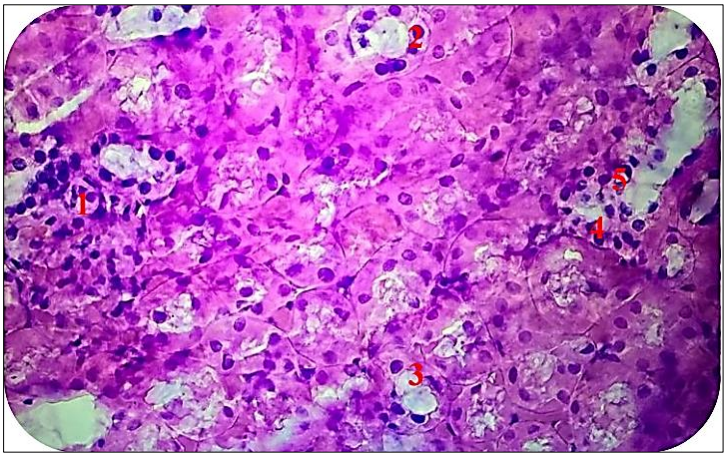

Figure 12: A histological section of a female rat kidney treated with (MTX 0.15mg/kg + BSE 7.5mg/kg) showing: 1. Renal glomerulus with narrowing of Bowman's space. 2. Degeneration in the renal tubules. 3. Tubular lumen. 4. Mild inflammatory infiltration between the tubules. H&E stain (400x).

Renal Function Parameters

Table (1), showing changes in mean serum urea concentration among the six experimental female rat groups. The results showed that Group B (MTX 0.15 mg/kg) had a significantly ($P < 0.05$) higher urea level than Control group. On the contrary, broccoli sprout extract (BSE) at both doses 5 and 7.5 mg/kg significantly reduced urea levels compared to other groups, falling below control values with Group D showing the least value of all group. Effect of Bse on The Urea Levels: In the combination groups (MTX+BSE), the extract reduced MTX-induced elevate in urea; values in Groups E and F were similar to those of control and for the BSE group (MTX-) only. This reflects a strong protective effect of the extract in protecting renal tissues from injury induced by MTX and restoring urea levels to its normal range. Statistical analysis revealed that there was a statistically significant difference ($P < 0.05$) between Control group (39.04 ± 1.83) and MTX

group (50.37 ± 4.12). On the other hand, any notable deviations in groups C, D, E and F compared to the control (C) were not noticed which made this particularly extract successful in normalizing urea (Figure 13).

The differences in mean serum creatinine levels observed in the six groups are shown in table (2). Group B (MTX 0.15 mg/kg) had a significantly higher creatinine level than the Control group, followed by Group C and then Group A among other groups in each week, with values recorded for the highest creatinine levels in Group B. On the other hand, administration of BSE alone (5 and 7.5 mg/kg) (Groups C and D) showed a creatinine concentration similar to control group demonstrating no harmful effects of extract when used alone. The reduction in creatinine relative to the MTX group (B) was also observed in the combination groups (E and F). Interestingly, Group F (MTX + BSE 7.5 mg/kg) showed the lowest creatinine level compared to the other groups that received treatment, indicating a dose-dependent protective action of broccoli sprout extract against nephrotoxicity induced by methotrexate (Figure 14).

Table 1: explain differences in Mean of Urea in (6) groups in rat female

Group	Mean ± SE of Urea
Control (A)	39.04 ± 4.10 a
MTX 0.15mg/ kg (B)	50.37 ± 9.23 b*
BSE 5 mg / kg(C)	34.14 ± 3.78 a
BSE 7.5 mg / kg (D)	30.64 ± 2.668 a
MTX0.15 mg/ kg + BSE 5 mg/ kg (E)	34.87 ± 5.729 a
MTX 0.15mg/ kg + BSE 7.5mg/kg (F)	34.13 ± 3.807 a

Means with the same letters indicate no significant difference between treatment groups at the level of ($P \leq 0.05$).

Means with different letters indicate a significant difference between treatment groups at the level of ($P \leq 0.05$).

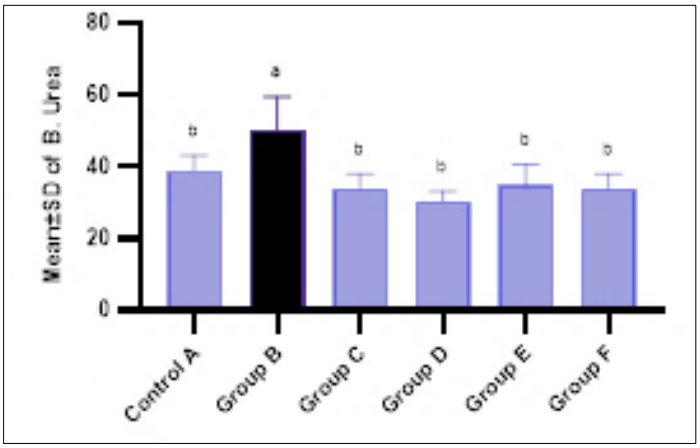

Figure 13: Mean differences of (Urea)

Table 2: explain differences in Mean of creatinine in (6) groups in rat female

Group	Mean ± SE of creatinine
Control (A)	1.020 ± 0.130 a
MTX 0.15mg/ kg (B)	1.420 ± 0.114 a
BSE 5 mg / kg (C)	1.010± 0.434 a
BSE 7.5 mg / kg (D)	1.010 ± 0.173 a
MTX0.15 mg/ kg + BSE 5 mg/ kg (E)	0.848 ± 0.392 a
MTX 0.15mg/ kg + BSE 7.5mg/kg (F)	0.760 ± 0.369 a

Means with the same letters indicate no significant difference between treatment groups at the level of ($P \leq 0.05$).

Means with different letters indicate a significant difference between treatment groups at the level of ($P \leq 0.05$).

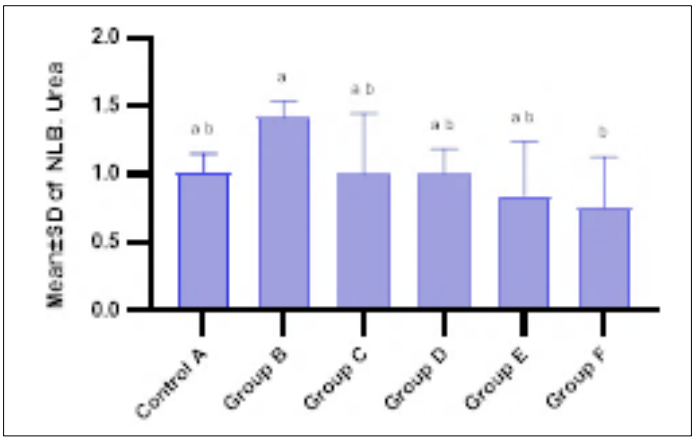

Figure 14: Mean differences of (creatinine)

DISCUSSION

Here we show that treatment with methotrexate (MTX) at a dose of 0.15 mg/kg has an adverse effect on the histological structure of ovarian tissues in rats. Frank inflammation and hemorrhaging associated with increases in oxidative stress occurred across all developmental stages of follicle development following treatment with MTX, which is associated with significant reductions in the number of follicles for all categories. Particularly follicular degeneration and early fibrosis.

These results are in agreement with past studies (Al-Moziel *et al*., 2013; Amnah & Alsuhaibani, 2013; Xiong *et al*., 2011), which verified that although MTX is an effective treatment for malignancies and auto immune diseases, Oxidative stress induced by MTX causes apoptosis of the follicle granulosa cells and breakdown of ovar ian histologic homeostasis. This damage depletes the ovarian reserve, with most affected types being primordial and early-stage follicles, potentially leading to infertility or primary ovarian insufficiency (POI) Amnah & (Alsuhaibani, 2013; Xiong *et al*., 2011).

Treatment with broccoli sprout extract (BSE) concomitantly reversed these alterations, indicating a protective effect likely involving multiple mechanisms. BSE: Compared to synthetic drugs, natural products including BSE are known for lower side effects and a better safety profile. Clinical studies have established that the antioxidant properties of broccoli are useful for some diseases, including diabetes and hepatic diseases (Ahmed *et al*., 2012; Farahmandi *et al*., 2013; Hashem *et al*., 2013; Patel & Sharma, 2014). Herein, we show that BSE had such a property and this will explain its remarkable protective effect against methotrexate-induced histopathological changes in the ovarian tissue of the rat which may be related to its strong antioxidant potency.

Microscopic examinations indicate that administration with BSE only at both doses (5 mg/kg and 7.5 mg/kg) preserved the histological integrity of the ovary as in control group. This favorable result has been attributed to the high level of organosulfur agents in broccoli sprouts, particularly sulforaphane (SFN), known for its potency to induce antioxidant enzymes and protect cells against tissue injuries. Additionally, the capacity of BSE to minimize MTX-triggered injury when simultaneously administered was apparent. While the higher combined dose (BSE 15 mg/kg + MTX) offered protection for follicles from toxicity, it did not restore organization of ovarian stroma with widespread fibrosis remaining and only mild residual congestion; suggesting attenuation of MTX-induced inflammation from BSE treatment but failure to initiate tissue repair.

Summary as similar to earlier reports of MTX-induced fibrous tissue remodeling and inflammatory-mediated degeneration in multiple organs including the ovaries (Zhang *et al.,* 2021; Hortu *et al*., 2020; Battan *et al*., 2017). A study by Liu et al. Sulforaphane, a potent inductor of detoxification enzymes, alleviated fibrosis (Liu *et al*., 2021). Level of collagen deposition was significantly reduced in the groups treated with BSE, indicating that the extract has anti-fibrotic effect along with its antioxidant and anti-inflammatory actions (Zhang *et al*., 1992a; Zhang *et al*., 1994b).

Here the academic professional translation of this part of discussion, using scientific terminology and systematic association between tissue and biochemical results:
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Figure 4 Ovarian Sections: histological analysis of ovarian sections among the group treated withBSE 7.5 mg/kg + MTX showed more mature follicles (Mature Follicles), organized granulosa cell layers and normal antrum. This suggests

that the larger dose of extract conferred significant cytoprotective effects in the ovary, allowing folliculogenesis to continue despite methotrexate-induced damage.

According to the findings, as illustrated in Group B, MTX has unequivocal deleterious effects on renal functional performance reflected by pronounced rise of serum urea and creatinine levels. MTX administration resulted in acute nephrotoxicity, which was evident by significant histological and biochemical changes (Table 2). This injury is mainly due to the ability of MTX to produce oxidative stress and lay up of free radicals causing depletion of intrinsic antioxidant profile in renal tissues. This result is consistent with the findings of Al-Motabagani (Al-Motabagani, 2006) confirmed that the histological and biochemical changes in MTX-treated rats cause a decrease in glomerular filtration rate (GFR), resulting in increased nitrogenous waste products release into the blood.

Glomerular hypercellularity, narrowed Bowman's space, vacuolization and acute tubular necrosis (ATN) were noted in the MTX-treated group on histological sections. Such observations are similar to study done by Ali *et al*., (2022), reported that MTX inhibited the enzyme dihydrofolate reductase (DHFR), and consequently hindered DNA synthesis at renal tubular cells and induces apoptosis. The inflammatory cell infiltration seen is also an indication of the immune response triggered through the drug-induced release of pro-inflammatory cytokines.

By contrast, the results showed a strong protective effect of broccoli sprout extract (BSE). The combination groups (Groups E and F) significantly lowered serum urea and creatinine concentrations to values very close to the control group. The high dose of sulforaphane present in broccoli contributes to effect with renoprotective (Sajeesh *et al*., 2011) due to a strong inducer for endogenous defense pathways. As for Guerrero-Beltrán *et al*., Sulforaphane is also directly involved in the prevention of cell death and inflammation in the kidneys via Nrf2 activation to boost endogenous antioxidants, protecting the kidneys against chemotoxicity (Guerrero-*Beltrán et al.*, 2012).

This mechanism was apparent in the results of BSE-treated groups, demonstrating a dose-dependent improvement. The 5 mg/kg dose offered partial protection, whereas the 750 µg recently appeared at high citation long seemed to offer complete restoration of the normal histological architecture of renal tubules and glomeruli. Bax et al.'s findings provide additional support for this protective capacity. Powered by Nrf2 activating sulforaphane, which is critical to upregulating antioxidant enzymes and preserving cells from chemical harm and inflammation (Guerrero-Beltrán *et al*., 2012), the NQO1 group reduced cell death ($P<0.01$) that was induced from BF-22 than an equal other gene pathway not involved with the same chemotherapy process.

In addition, the combination of MTX and the higher dose of BSE (7.5 mg/kg) not only maintained Bowman's space capacity but also reduced cell swollen in kidneys than MTX group alone. This indicates that the bioactive compounds in broccoli were effectively able to quench the free radicals generated from MTX metabolism before they could harm the renal tubular basement membrane. These observations are consistent with the results of Heber *et al*., in this work (Naguib *et al*., 2017), phytochemicals in cruciferous vegetables were confirmed as effective protective agents to reduce nephrotoxicity caused by chemotherapeutic drugs.

CONCLUSION

In conclusion, the study suggests that BSE is a powerful natural adjuvant and that it reduces MTX-induced toxicity in both ovarian and renal tissues of female rats. This protective mechanism may be due to improved antioxidant homeostasis and high sulforaphane content that induces detoxification enzymes and stimulates top-up of endogenous defense pathways such as Nrf2. BSE administration reverses oxidative stress and apoptosis in the ovaries, restoring folliculogenesis, protecting granulosa cells and maintaining ovarian reserve. For kidney health, the extract effectively decreases serum urea and creatinine levels and preserves glomeruli damage and acute tubular necrosis whilst returning these two back to reference ranges. Additionally, these results are also dose-dependent with the high-dose protection at 7.5 mg/kg being more complete in both covering needs and restoration of histological architecture than lower doses. Conclusion: In summary, broccoli sprout extract is a potential therapeutic approach to protect female reproductive health and renal function from the adverse effects of chemotherapy at cytotoxic doses.

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