Official Dental Journal of Suez Canal University
DENTAL SCIENCE Updates
Print ISSN 2636-3836
Online ISSN 2636-3844
www.dsujournals.ekb.eg
DSU — Vol. 6, No. 2, September (2025) — PP. 403:416

ASSESSMENT OF THE PROTECTIVE EFFECTS OF VIRGIN COCONUT OIL AND PROPOLIS AGAINST METHOTREXATE-INDUCED TOXICITY IN SUBLINGUAL SALIVARY GLANDS OF ALBINO RATS

Nada Tarek Ali Zaki[1], Enas Mahmoud Hegazy[2], Reham Magdy Amin[3], Elham Fathy Mahmoud[4]

DOI: 10.21608/dsu.2025.375817.1297
Manuscript ID: DSU-2504-1297

KEYWORDS

Apoptosis, Methotrexate, propolis, sublingual salivary glands damage, virgin coconut oil.

- E-mail address: nada.tarek@bue.edu.eg

1. Assistant Lecturer of Oral Biology, Faulty of Dentistry, The British University in Egypt.
2. Professor of Oral Biology, Faulty of Dentistry, Suez Canal University, Egypt
3. Professor of Oral Biology, Faulty of Dentistry, Ain Shams University, Egypt
4. Professor of Oral Biology, Faulty of Dentistry, Suez Canal University, Egypt

ABSTRACT

Introduction: Methotrexate is an antimetabolite drug that is used as anti-cancerous drug which can cause cell damage by weakening the body's antioxidant defenses. Antioxidants like propolis and virgin coconut oil may help protect against this damage by reducing oxidative stress caused by MTX administration. **Aim:** This study aimed to evaluate the protective effect of propolis and virgin coconut oil (VCO) against toxicity induced by methotrexate (MTX) on sublingual salivary glands. **Materials and Methods:** Fifty rats were randomly and equally divided into five groups (n=10), Group 1 (Control): Rats received saline by oral gavage for 17 days. Group 2 (MTX): Rats were injected intraperitoneally (Ip) with MTX (20 mg/kg) on day 14 only. Group 3 (Propolis and MTX): Rats were administered propolis by oral gavage at a dose of 100 mg/kg/day for 17 days and injected (Ip) with MTX (20 mg/kg) on day 14. Group 4 (VCO and MTX): Rats were administered VCO by oral gavage at a dose of 5 ml/kg body weight for 17 days, with MTX (20 mg/kg) injected (Ip) on day 14. Group 5 (VCO, Propolis, and MTX): Rats received propolis (100 mg/kg/day) and VCO (5 ml/kg) for 17 days, with MTX (20 mg/kg) injected (Ip) on day 14. **Results:** This study revealed that both propolis and VCO effectively reduce MTX-induced cytotoxicity on sublingual glands. Propolis preserved gland structure and reduced caspase-3 immunoexpression, while VCO maintained gland architecture and blood vessel integrity leading to a decrease in caspase-3 levels. Combined, they provided protective effects similar to those observed in the control group. **Conclusion:** These findings suggest propolis and VCO could serve as complementary therapies during chemotherapy to reduce salivary gland damage and improve patient quality of life.

INTRODUCTION

Cancer is a terrifying disease that has impacted multicellular organisms for over 200 million years, originating from the transformation of normal human cells into pathological ones. There were only few alternatives of cancer treatment as radiation therapy, surgery, and chemotherapy which is a term introduced by German chemist Paul Ehrlich, seeks to halt cell growth and tumor spread, thereby preventing invasion and metastasis of cancer. However, it can cause severe side effects, including fatigue, nausea, hair loss, vomiting, and, in extreme cases, death [(1)].

Methotrexate (MTX) is a folate antimetabolite and cytotoxic chemotherapeutic agent which targets rapidly dividing cells, especially in the S-phase of the cell cycle, by inhibiting dihydrofolate reductase (DHR) leading to decreases in tetrahydrofolic acid (THF) levels, which are crucial for DNA replication leading eventually to DNA damage and cell death[2].

As the cell membrane of many cancer cells has folate receptors on, its potent anti-inflammatory and immunomodulatory activities, MTX is effective in treating patients with ulcerative colitis, breast carcinoma, small-cell lung carcinoma, head and neck tumors and non-metastatic osteosarcoma [3,4].

Therapy with MTX leads to increase apoptosis in transformed T cells depending on the dose and duration of treatment and this effect may possibly be due to reactive oxygen species (ROS) production. Some common side effects of MTX include nephrotoxicity, hepatotoxicity, gastrointestinal mucosal damage which can sometimes prevent the continuation of treatment[5]. Oral mucositis and ulcerative lesions were noted in patients treated with MTX for leukemia, lymphoma, and osteosarcoma[6]. Meanwhile MTX-induced effects on parotid lesions showed degeneration of acinar and ductal cells on histological and ultrastructural levels [7].

Living organisms have an antioxidant defense system both exogenous and endogenous to counterbalance oxidative stress from ROS. An imbalance between ROS and antioxidant defenses can cause oxidative stress, which can disrupt various cellular functions, resulting in injury to tissues and accelerated cell death by damaging DNA, RNA, proteins, and lipids, contributing to the development of many diseases as cancer. Antioxidants can directly scavenge ROS, enhance antioxidant defenses, or inhibit ROS production, offering protection against diseases like cancer [8-10].

Recently, there has been growing interest in safe, natural antioxidant sources, especially from plants, as dietary methods to reduce the toxicity of MTX which are often preferred over pharmacological interventions. These dietary approaches are considered safer, more affordable, and easier to incorporate into daily diets [11]. The main bioactive compounds in natural antioxidant sources are phenolics and flavonoids. Phenolic compounds, like caffeic acid in virgin coconut oil and ferulic acid in propolis, serve as natural antioxidants. Their protective effects stem from their antioxidants, antimutagenic, anticarcinogenic, antimicrobial, and anti-inflammatory properties [12].

Egyptian propolis, as a model of natural antioxidant, is a resinous substance produced by bees from tree resins and saps mixed with their enzyme-rich saliva. Its chemo-preventive and direct anti-tumor effects are due to its ability to inhibit tumor cell proliferation and induce apoptosis. Additionally, propolis' indirect antitumor effects are linked to its antioxidative properties, which include scavenging free radicals and enhancing antioxidant enzyme activities [13, 14]. several studies demonstrated that propolis exhibited cytotoxic effects on cancer cell lines and reduced tumor volume, viable tumor cell counts and improved the lifespan of mice models [15].

Virgin coconut oil (VCO), as another model of natural antioxidant, is rich in nutrients, such as vitamin E, and contains high levels of bioactive compounds like polyphenols. It is well-established that VCO anti-tumor effects are achieved by alleviating oxidative stress and inflammation through its remarkable antioxidant activities making it a promising natural oil for controlling MTX-induced toxicity in cancer treated patients [16, 17]. Also it has been found that pre-treatment with VCO followed by MTX administration led to diminishing

the MTX-induced alterations in the activities of oxidative stress markers and reduced the levels of pro-inflammatory markers interleukin-6 (IL-6) and nitric oxide (NO) [18].

In the previous review, it was noted that there were few reliable studies that demonstrated the protective effects of propolis and VCO against the toxic effects caused by MTX on salivary glands. Consequently, the primary aim of this study was to provide more information about both the individual and combined effects of propolis and VCO in alleviating the toxicity induced by MTX specifically on the sublingual salivary glands.

MATERIALS AND METHODS

Ethical consideration

The present research was conducted after the approval of the Research Ethics Committee (REC) of the Faculty of Dentistry, Suez Canal University in approval number (473/2022). The study was conducted on 50 adult male albino rats (according to sample size calculation). Ethical considerations involving experimental animals care guidelines were followed.

Materials

Materials used in this study were Methotrexate in a concentration of 50 mg/2ml dissolved in 0.9% saline in form of vials, Hikma Pharmaceuticals, Giza Governorate, Egypt. Propolis in the proportion of 10 g propolis to 100 ml of solvent (ethanol 80%) prepared in the Beekeeping Research Section, Plant Protection Research Institute, Agriculture Research Centre at Giza governorate, Egypt. Natural Virgin Coconut Oil, Imtenan Company, Cairo Governorate, Egypt.

Methods

Study design: The study was carried out on fifty adult male albino rats with an average of 150-180 grams body weight. Rats were acclimated 7 days before the experimentation. They were housed in rat cages, labeled with numerical numbers and kept in well-ventilated animal house of the faculty of dentistry, Suez Canal University under the supervision of specialized veterinarians. They were kept at a temperature of 27-30°C, 12 hours natural light and 12 hours darkness. The animals were fed with dry rat pellets and allowed drinking water *adlibitum*.

Animals:

Sample Size Calculations

The sample size for this study was calculated according to Charan and Biswas [19] using the following equation:

$$N = \frac{(Z_\alpha)^2 * (S)^2}{(d)^2}$$

N = Total sample size

Zα= Is Standard normal variate and its equal 1.96 at P< 0.05

SD = Standard deviation of variable

d = Absolute error or precision

Zα	SD	d
1.96	7.21	2

Total sample size N =

$$\frac{(1.96)^2 X (7.21)^2}{(2)^2} = 49.926 \approx 50 \text{ rats}$$

The total sample size calculations revealed that a sample size should be **50**, and they were divided equally into five groups.

Animal grouping:

Fifty rats were randomly and equally divided into 5 groups (n=10) as follows:

- **Group 1** (control): rats were received saline by oral gavage for 17 days.
- **Group 2** (MTX): rats were injected (Ip) MTX (20 mg/kg) on day 14 only [17].
- **Group 3** (propolis and MTX): rats were administrated propolis by oral gavage with a dose of 100 mg/kg/day body weight for 17 days and were injected (Ip) MTX (20 mg/kg) on day 14 only [20].
- **Group 4** (VCO and MTX): rats were administrated VCO by oral gavage with a dose of (5 ml/ kg body weight of rat) for 17 days. MTX (20 mg/kg) was injected (Ip) on day 14 only [17].
- **Group 5** (VCO, propolis and MTX): rats were administrated propolis by oral gavage with a dose of 100mg/kg/day body weight for 17 days, VCO by oral gavage with dose of (5 ml/ kg body weight of rat) for 17 days and MTX (20 mg/kg) was (Ip) injected on day 14 only.

After the experiment period (17 days), rats were euthanized by extra dose of anesthesia. Their sublingual salivary glands of the right and left sides were dissected out and separated. Sublingual glands of the *right side* were processed for histological and immunohistochemical evaluation while the left side were processed for the ultrastructural evaluation. The sacrificed rat bodies were executed in the Faculty of Medicine incinerator of Suez Canal University.

Histological procedure

Preparation and staining of specimens

Sublingual glands on the *right side* were preserved in 10% buffered formalin for fixation. The specimens were washed properly under running water, dehydrated by transferring through increasing concentrations of alcohol (50%, 60%, 80%, 90%, 96% and absolute alcohol), then transferred to xylol to clear the specimen from alcohol. The glands were then infiltrated in paraffin wax and embedded in the center of paraffin wax block. Tissue sections of 5 microns thickness were cut and prepared for:

1. Hematoxylin & eosin stain to study the general histological changes.
2. Immunohistochemical staining using caspase -3 to evaluate the apoptotic changes.

Immunohistochemical evaluation

Caspase-3 expression appeared as brownish cytoplasmic staining in the acinar and ductal cells of the salivary gland. After staining procedures, each section was mounted and examined with ZEISS primo star light microscopy photographed by Tucsen IS 1000 10.0 MP camera. For evaluation of caspase-3 immunoreactivity, the area fraction of immunopositivity was measured automatically. The area fraction represented the percentage of immunopositively area to the total area of the microscopic field. The mean area fraction for each case was calculated.

Ultrastructure procedure

The specimens of the left sides of sublingual glands of different experimental groups were preserved in 3% phosphate-buffered glutaraldehyde (pH 7.2) and then processed to be used for ultrastructural examination.

Small specimens (1mm^3) from the sublingual salivary glands were fixed in 3% phosphate-buffered glutaraldehyde (pH 7.2) for 1-2 hours, followed by three washes in phosphate buffer. They were then post-fixed in 1% buffered osmium tetroxide at 4°C for 1-2 hours and thoroughly washed again. The specimens underwent dehydration through ascending grades of ethanol, with final dehydration in propylene oxide for 30 minutes. Embedding occurred in oven-dried gelatin capsules using fresh epoxy resin, which polymerized at 60°C for 24-36 hours. Semi-thin sections (1-2 microns thick) were cut, stained with toluidine blue, and examined under a light microscope. Ultrathin sections (0.06 microns) were then prepared, mounted on copper grids, and stained with uranyl acetate and lead citrate.

Ultrastructural evaluation

Ultra-thin sections prepared at approximately 75-90 μm thickness were examined and photographed by (JEOL JEM 1010 Transmission Electron Microscope; Jeol Ltd, Tokyo, Japan. Images were captured by CCD camera model AMT, optronics camera with 1632 x 1632-pixel format as side mount configuration. This camera uses a 1394 fire wire boarded for acquision.

Statistical analysis

Obtained data was collected and tabulated. The numerical data were analyzed for normality by checking the distribution of data, calculating the mean and median values, evaluating histograms and normality curves by using Social Science software computer program version 23 (SPSS, Inc., Chicago, IL, USA). One-way Analysis of variance (ANOVA) and Tukey tests were used for comparing data regarding the area percentage of positive reaction to caspase-3. P value less than 0.05 was considered statistically significant.

RESULTS

1. Histological results: Figure 1 (A-J)

Group 1: (Control Group)

A: The mucous acini lined by pyramidal mucous cells with basely located flattened nuclei The striated ducts were lined with columnar cells displaying prominent basal striations (yellow arrow) and basely located spherical nuclei (red arrow), occasionally accompanied by blood vessels. **B:** The excretory ducts were surrounded by fibrous connective tissue and lined with pseudostratified columnar epithelium containing goblet cells, displaying lumens of varying diameters.

Group 2: (MTX Group)

C: The mucous acini exhibited less defined cell boundaries (red arrow). Acinar cell nuclei were deeply stained and irregular. Striated ducts displayed ill-defined basal striations, with some lining cells degenerating (blue arrow) and stagnated mucous secretion in their lumens (black arrow). **D:** Excretory ducts showed some areas of degeneration (yellow arrow), an ill-defined pseudostratified appearance, the ductal lumen showed some stagnated secretions together with some desquamated epithelial cells (red arrow).

Group 3: (Propolis and MTX Group)

E: The mucous acini were faintly stained with ill-defined cell boundaries. Mucous cell nuclei were pressed against the cell bases. The striated duct displayed ill-defined cell outlines, with some degeneration (yellow arrow). Neighboring blood vessels were congested with RBCs. **F:** The excretory duct showed loss of pseudo-stratification and cytoplasmic vacuolization (yellow arrow) in some lining cells. The duct lumens appeared empty.

Group 4: (VCO and MTX Group)

G: The mucous acini had thickened cell boundaries and nuclei compressed against the cell bases. Striated ducts had columnar lining cells with basal striations and basally located rounded nuclei. Neighboring blood vessels were congested with RBCs. **H:** Some excretory duct lining cells showed a pseudostratified appearance, while others did not. The ductal lumen contained stagnated secretion, with normal-sized blood vessels nearby.

Group 5: (VCO, Propolis and MTX Group)

I: The mucous acini exhibited a histological appearance similar to the control Group. The Mucous cells had nearly normal cytoplasmic density, and their nuclei were compressed against the cell bases. Striated ducts were lined by tall columnar cells with basal striations and rounded nuclei. **J:** The excretory ducts had thin pseudostratified columnar epithelium and displayed empty lumens.

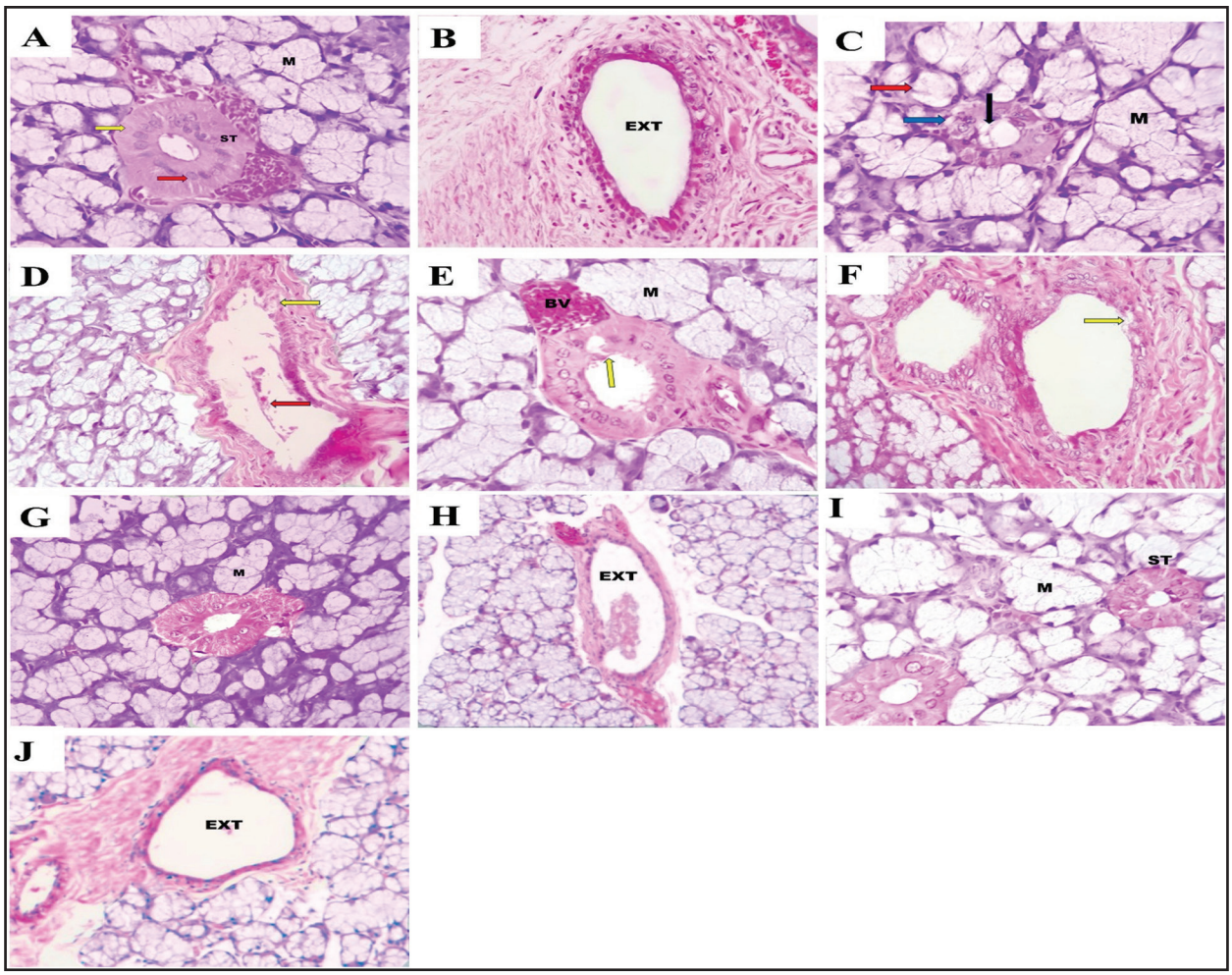

Fig. (2) **A:** The mucous acini (M) and the striated ducts (ST) of control Group. **B**: The excretory ducts (EXT) of control Group. **C:** the mucous acini (M) and the straited duct of Group 2 **D:** The excretory duct of Group 2 **E:** The mucous acini (M), the straited duct and congested blood vessels (BV) of Group 3 **F:** The excretory duct (EXT) of Group 3. **G:** the mucous acini (M) and the straited duct of Group 4 **H:** The excretory duct of Group 4. **I:** The mucous acini (M) and the striated ducts of Group 5. **J:** The excretory duct (EXT) of Group 5.

2. Immunohistochemical results: Figure 2 (A-E)

A: the sublingual glands of Group 1 (control) showed no cytoplasmic staining reaction of caspase-3 in the acini cells and mild reaction in the duct cells. **B:** For Group 2, immunohistochemical localization of caspase-3 showed moderate cytoplasmic staining reaction of the acini cells and strong cytoplasmic reaction of the duct cells. **C:** For Group 3, immunohistochemical localization of caspase-3 showed mild to moderate cytoplasmic staining reaction of the acini cells and moderate to strong cytoplasmic reaction of the duct cells. **D:** For Group 4, immunohistochemical localization of caspase-3 showed mild cytoplasmic reaction of acini cells and moderate cytoplasmic reaction of duct cells. **E:** For Group 5, immunohistochemical localization of caspase-3 showed no cytoplasmic reaction in the acini cells and mild cytoplasmic reaction in the duct cells.

3. Transmission Electron microscopic results: Figure 3 (A-J)

Group 1: (Control Group)

A: The mucous acini were spherical, lined by pyramidal cells around a narrow lumen. Apical secretory granules (yellow arrows) with poor electron density were detected. Each cell contained a rounded, basally located nucleus with an electron-dense nucleolus. **B:** Numerous parallel rough endoplasmic reticulum (RER) arrays (red arrow), randomly distributed mitochondria (white arrows) with their characteristic cisternae, and basally and laterally located Golgi bodies.

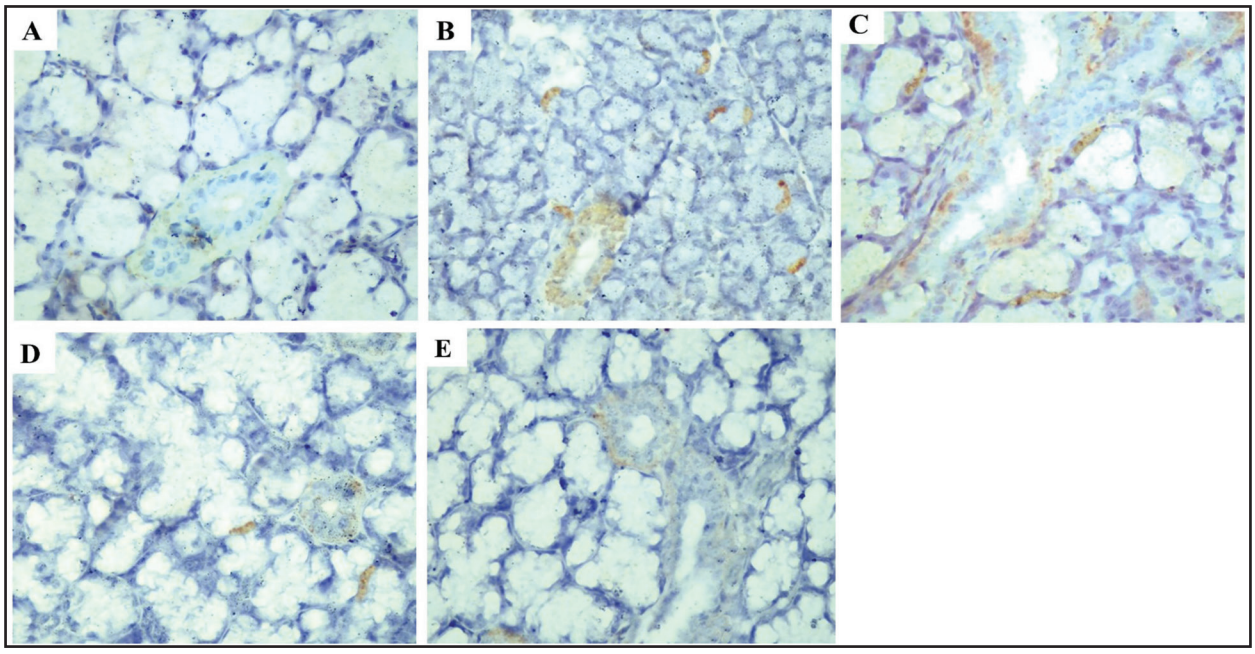

Fig. (2) **A**: Group 1 showing no cytoplasmic staining reaction of caspase-3 in the acini cells and mild cytoplasmic staining reaction in the duct cells **B:** Group 2 showing moderate cytoplasmic staining reaction of caspase-3 in the acini cells and strong cytoplasmic staining reaction in the duct cells. **C:** Group 3 showing mild to moderate cytoplasmic staining reaction of caspase-3 in the acini cells and moderate to strong cytoplasmic staining reaction in the duct cells. **D:** Group 4 showing mild cytoplasmic staining reaction of caspase-3 in the acini cells and moderate cytoplasmic staining reaction in the duct cells. **E:** Group 5 showing no cytoplasmic staining reaction of caspase-3 in the acini cells and mild cytoplasmic staining reaction in the duct cells.

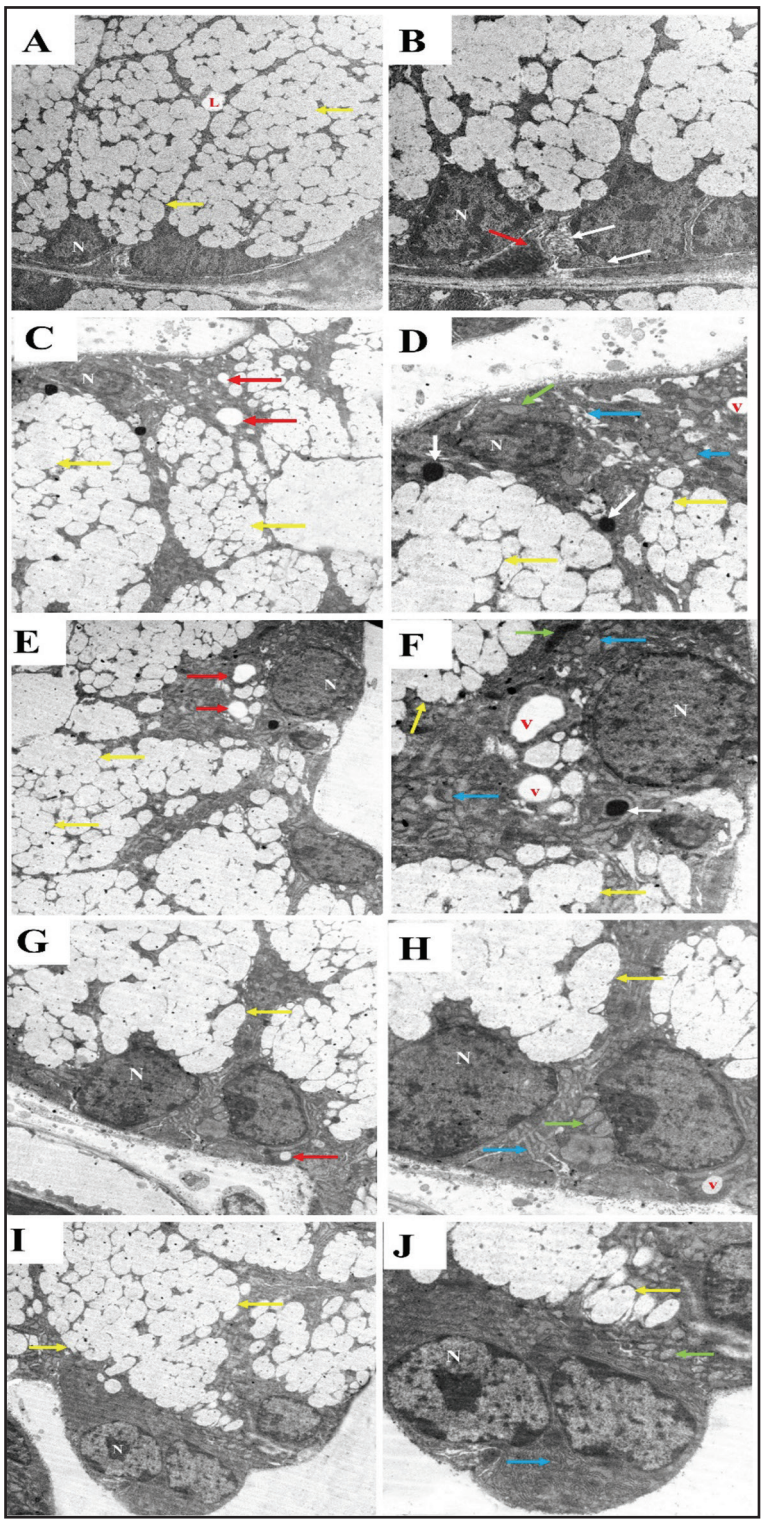

Fig. (2) **A**: Mucous acinar cells with narrow lumen (L), rounded basally located nuclei (N) of Group 1. Higher magnification of figure A showings RER (red arrow) and mitochondria (white arrows) of Group 1. **C:** Mucous acinar cells, irregular basally located nuclei (N) of Group 2 **D:** Higher magnification of figure C demonstrating acinar cells, irregular basally located nuclei (N) and cytoplasmic vacuolization (V) of Group 2. **E:** Mucous acinar cells and irregular basally located nuclei (N) of Group 3. **F:** Higher magnification of figure E demonstrating acinar cells with irregular basally located nuclei (N) and cytoplasmic vacuolization (V) of Group 3. **G:** Mucous acinar cells with rounded basally located nuclei (N) of Group 4 **H:** Higher magnification of figure G demonstrating acinar cells with basally located nuclei (N) and little cytoplasmic vacuolization (V)of Group 4. **I:** Mucous acinar cells with rounded basally located nuclei (N) of Group 5. **J:** Higher magnification of figure I demonstrate acinar cells with rounded basally located nuclei (N) of Group 5.

Group 2: (MTX Group)

C: The acinar cells appeared pyramidal in shape with different sizes and irregular basally located nuclei, accumulation of electron-lucent secretory granules (yellow arrows), and different sized cytoplasmic vacuoles (red arrows) were observed.

D: Higher magnification of figure C showing the secretory cells pyramidal in shape with different sizes and irregular basally located nuclei with narrow lumen. Accumulation of ill-defined secretory granules (yellow arrows) and cytoplasmic vacuolization was encountered in most of the parenchymal elements. Marked dilation of the rough endoplasmic reticulum (RER) and lysosomes filled with electron-dense particles (white arrows) were detected. Dilated strands of RER (blue arrows) and swollen mitochondria with damaged cristae (green arrow) were noticed.

Group 3: (Propolis and MTX Group)

E: The acinar cells appeared pyramidal in shape with different sizes and irregular basally located nuclei. Accumulation of ill-defined secretory granules (yellow arrows) and cytoplasmic vacuolization (red arrows) were observed. **F:** The secretory cells were arranged in irregular acini with irregular basally located nuclei. Accumulation of ill-defined secretory granules (yellow arrows) and cytoplasmic vacuolization were observed. Lysosomes filled with electron-dense particles (white arrows), dilated RER cisternae (blue arrows) and swollen mitochondria with damaged cristae (green arrow) were noticed.

Group 4: (VCO and MTX Group)

G: The acinar cells demonstrated pyramidal shape, more or less uniform in size with relatively rounded basally located nuclei. There was accumulation of electron-lucent secretory granules (yellow arrow) and little cytoplasmic vacuolization (red arrow). **H:** The acinar cells appeared with basally located nuclei with accumulation of secretory granules (yellow arrows), almost normal radially arranged mitochondria (green arrow) and RER cisternae (blue arrows) were noticed.

Group 5: (VCO, Propolis and MTX Group)

I: The acinar cells appeared pyramidal in shape apparently uniform in size and with rounded basally located nuclei. There was accumulation of secretory granules (yellow arrows) with no obvious cytoplasmic vacuolization noticed in the acinar cells. **J:** The pyramidal shaped acinar cells demonstrated apparent uniform size, rounded basally located nuclei and packed with secretory granules (yellow arrow). Almost normal radially arranged mitochondria (green arrow) and RER cisternae (blue arrows) were noticed.

4. Statistical results (Figure 4)

The MTX group exhibited the highest mean area percentage of Caspase-3 immunoexpression (10.86 ± 0.2), which was significantly greater than all other groups ($p < 0.05$). The Propolis-treated group showed a moderate reduction in Caspase-3 expression compared to the MTX group, with a significant difference ($p < 0.05$). The VCO group had a similar but slightly lower mean area percentage than the Propolis group and was statistically insignificant from both the Propolis and mixed groups. The VCO group had a similar but slightly lower mean area percentage than the Propolis group and was statistically insignificant from both the Propolis and mixed groups.

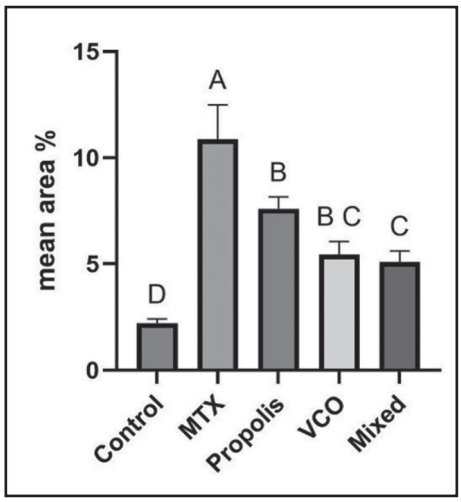

Fig. (4) Bar chart showing mean, standard deviation, and multiple comparison test of Caspase-3 mean area % immunoexpression of the sublingual gland. Different letters indicate significance.

DISCUSSION

Natural antioxidants such as zinc, calcium, and vitamins C, D, E, and B as well as propolis and VCO are all studied as they help in cancer prevention and treatment through their antioxidant activity by protecting against free radicals as they inhibit cancer cells' proliferation and reducing chemotherapy drugs' toxicity [21]. The aim of this study was to evaluate the possible counteracting effect of virgin coconut oil and propolis against the induced toxic effect of MTX on sublingual salivary glands of albino rats. Rat models were chosen as they are commonly used in toxicity testing, justified by their shared anatomical and physiological characteristics with humans, extensive databases which are vital for comparative studies. Also, rats are easy to breed and maintain at low cost [22]. Chemotherapy-induced salivary dysfunction can cause oral mucositis and dry mouth, leading to issues like cavities, chewing and swallowing difficulties, loss of taste, and oral inflammation, all of which reduce quality of life [23].

MTX toxicity is known to be the reason for salivary gland dysfunction and cytotoxicity, reduced salivary secretion, manifested by xerostomia, which can adversely impact the patient's quality of life and nutritional status during the course of cancer treatment [23]. In the present study, MTX dose (20 mg/kg) was selected according to previous studies that investigated MTX-induced oxidative stress-mediated cerebral neurotoxicity and inflammation in rats [17, 24].

In the present study the histological results of Group 1 (control) which didn't receive any treatment, showed normal sublingual gland architecture which formed mainly of mucous secretory end piece. The mucous acini were lined by mucous cells while some of the mucous acini caped by serous demilunes (seromucous demilunes). The duct system formed of striated ducts and apparently large excretory ducts. These findings were in accordance with [25, 26] who stated that like human, rat sublingual salivary glands are almost purely mucous where the mucous unit is dominant and the serous caped as demilune on mucous acini or containing serous acini.

The histological findings of the mucous acini of Group 2 receiving MTX showed apparent shrinkage and degeneration of acinar cells. These findings were agreed with previous study who observed similar signs of degeneration and apoptosis in the glandular tissue on the histopathological as well as ultrastructural scales in the form of shrunken nuclei, cytoplasmic vacuoles and degenerated cell organelles, denoting MTX-induced cytotoxicity [27,28]. Also, these findings were explained by further studies suggesting that MTX administration induce these histological changes by halting protein synthesis through folate cofactors depletion, leading to the formation of cyto-lysosomes, a sign of apoptosis [29, 30].

The histological examination of striated ducts of Group 2 showed loss of basal striations, and the ultrastructural investigation showed distorted mitochondria with distorted cristae with loss of basal infoldings. These findings coincided with another study who explained that MTX administration caused ballooned, distorted mitochondria and cristae with loss of basal infoldings [31]. These findings could be attributed to MTX-induced degeneration of these cells as a consequence of free radicals and ROS generation [7].

The histological examination of the excretory ducts showed dilation and retained secretions in their lumen. These observations align with previous studies, which attributed these findings to MTX inhibition of protein synthesis through folate cofactor depletion, ultimately leading to glandular dysfunction and impaired salivary secretion. Additionally, the dilation of the ducts may result from MTX's pathological effects on myoepithelial cells surrounding the acini, hindering the expulsion of secretions into the oral cavity and contributing to xerostomia [23, 29].

A lot of cytoplasmic vacuolization was encountered in most of the parenchymal elements of the sublingual salivary glands of Group 2. These results were in accordance with previous studies who explained these swellings as a result of fatty degeneration and accumulation of lipid droplets within the cytoplasm. These fat droplets might unit together forming a large vacuoles which eventually lead to lipid degeneration and cell death [32, 33]. All the previous results were supported by immunohistochemical investigation which showed significant caspase-3 overexpression in MTX-treated groups supported the proposed assumption that the cytotoxicity caused by MTX might be as a results of oxidative stress and ROS production [34, 35].

In the ongoing study, propolis intake led to decrease the cytotoxic effect of MTX administration as demonstrated histologically, immunohistochemically and by ultrastructural examination. Propolis, a natural resin, is recognized for its potent antioxidant properties, which can neutralize free radicals and decrease oxidative stress [13, 14].

Histological and ultrastructural examination of Group 3 receiving propolis revealed an improvement in both acinar and ductal cells. Little cytoplasmic vacuolization was seen in all ductal cells with mild stagnant secretions in straited lumens were detected. Blood vessels still appeared dilated and engorged with RBCs. These results came in agreement with a study that demonstrated oral administration of propolis decreased oxidative stress induced by MTX and its potent antioxidant effect [20]. These findings were in line with studies that reported the ability of propolis to modulate the activities of antioxidant enzymes and suppresses the oxidative damage induced by MTX in liver and brain tissues, in addition to its ability to decrease number of apoptotic cells and improved kidney morphology in rats received MTX [36, 37]. Immunohistochemical examination of the propolis-treated group showed a marked reduction in caspase-3 immuno-expression compared to the MTX group, confirming its cytoprotective effect [38].

In the present study, VCO intake significantly ameliorates the MTX-induced cytotoxic effect in sublingual glands as demonstrated histologically, immunohistochemically and by ultrastructural examination. VCO has been identified for its many beneficial effects as it can alleviate oxidative stress and inflammation through its remarkable antioxidant activities [39]. More improvement in the histological and ultrastructural features in both acinar and ductal cells of Group 4 was detected. No obvious cytoplasmic vacuoles nor stagnant secretion

in ductal lumen were observed. Blood vessels with RBCs engorgement were markedly improved. These results agree with another study where VCO significantly decreased cerebral neurotoxicity induced by MTX through inhibition of oxidative stress and anti-inflammatory mechanisms [40]. Another study explained the hepatoprotective and antioxidant effects of VCO supplementation against MTX-induced oxidative damage by enhancing the antioxidant defense system [41]. Furthermore, caspase-3 immuno-expression was significantly reduced in the VCO-treated group, indicating the cytoprotective, anti-apoptotic, anticancer, and antioxidant properties of VCO [42].

The combined administration of both propolis and VCO in Group 5 had a superior effect compared to individual administration. The histological and ultrastructural examination of Group 5 showed almost the same features as the control group in both acinar and ductal cells. The serous acini demonstrated normal size with no obvious cytoplasmic vacuoles. Blood vessels appeared normal sized with no obvious RBCs engorged. Numerous data demonstrated that combined antioxidant agents administration is effective in reducing the ROS and even on cancer progression [43].

The ability of combined antioxidant therapy to provide superior protection against chemotherapy-induced toxicity has been extensively documented. Results reported by [44] evaluated the protective effects of both antioxidants, VCO and lauric acid (LA) where both had a hepatoprotective effect by decreasing liver injury and oxidative stress, findings that align with the results of the present study Another study investigated the protecting effect of propolis and ginger in improving the MTX-induced ileum injury and ameliorate mucosal destruction and these protective actions may be due to a combination of both free radical scavenging and interaction with enzyme functions [45].

This study is among the initial investigations into the combined protective effects of propolis and VCO against MTX-induced toxicity in the sublingual gland. The results suggest that administering them together may be an effective strategy for alleviating chemotherapy-induced cytotoxicity. Further research is needed to explore their clinical applications and the specific molecular pathways through which they provide protection. Additionally, future studies should prioritize long-term evaluations and dose optimization to maximize therapeutic effects while reducing side effects.

CONCLUSION

This study emphasizes the potential of VCO and propolis as effective protective agents against MTX-induced cytotoxicity in the sublingual salivary glands. Their ability to reduce oxidative stress, prevent apoptosis, and maintain tissue integrity indicates they could be valuable adjunctive therapies in chemotherapy treatments. With their natural origins and strong safety profiles, integrating these antioxidants into clinical practice may offer a promising approach to mitigating chemotherapy-induced salivary gland dysfunction and improving the quality of life for patients.

REFERENCES

1. Hausman DM. What is cancer? Perspect Biol Med 2019;62(4):778-784.
2. Chan ES, Cronstein BN. Methotrexate—how does it really work? Nat Rev Rheumatol 2010;6(3):175-178.
3. Cronstein BN, Aune TM. Methotrexate and its mechanisms of action in inflammatory arthritis. Nat Rev Rheumatol 2020;16(3):145-154.
4. Hannoodee M, Mittal M. Methotrexate. StatPearls [Internet]. StatPearls Publishing; 2022.

5. Brown PM, Pratt AG, Isaacs JD. Mechanism of action of methotrexate in rheumatoid arthritis, and the search for biomarkers. Nat Rev Rheumatol 2016;12(12):731-742.
6. Valer JB, Curra M, Gabriel AdF, Schmidt TR, Ferreira MBC, Roesler R, et al. Oral mucositis in childhood cancer patients receiving high-dose methotrexate: prevalence, relationship with other toxicities and methotrexate elimination. Int J Paediatr Dent 2021;31(2):238-246.
7. Omar AI, Yousry MM, Farag EA. Therapeutic mechanisms of granulocyte-colony stimulating factor in methotrexate-induced parotid lesion in adult rats and possible role of telocytes: a histological study. Egypt J Histol 2018;41(1):93-107.
8. Bulut N, Kocyigit UM, Gecibesler IH, Dastan T, Karci H, Taslimi P, Durna Dastan S, Gulcin I, Cetin A. Synthesis of some novel pyridine compounds containing bis-1, 2, 4-triazole/thiosemicarbazide moiety and investigation of their antioxidant properties, carbonic anhydrase, and acetylcholinesterase enzymes inhibition profiles. J Biochem Mol Toxicol 2018;32(1):e22006.
9. Krawczyk H. The stilbene derivatives, nucleosides, and nucleosides modified by stilbene derivatives. Bioorg Chem 2019;90:103073.
10. Anraku M, Gebicki JM, Iohara D, Tomida H, Uekama K, Maruyama T, Hirayama F, Otagiri M. Antioxidant activities of chitosans and its derivatives in in vitro and in vivo studies. Carbohydr Polym 2018;199:141-149.
11. Mnafgui K, Kchaou M, Ben Salah H, Hajji R, Khabbabi G, Elfeki A, Allouche N, Gharsallah N. Essential oil of Zygophyllum album inhibits key digestive enzymes related to diabetes and hypertension and attenuates symptoms of diarrhea in alloxan-induced diabetic rats. Pharm Biol 2016;54(8):1326-1333.
12. Gulcin İ. Antioxidants and antioxidant methods: an updated overview. Arch Toxicol 2020;94(3):651-715.
13. Patel S. Emerging adjuvant therapy for cancer: propolis and its constituents. J Diet Suppl 2016;13(3):245-268.
14. Airen B, Sarkar PA, Tomar U, Bishen KA. Antibacterial effect of propolis derived from tribal region on Streptococcus mutans and Lactobacillus acidophilus: an in vitro study. J Indian Soc Pedod Prev Dent 2018;36(1):48-52.
15. Salem MM, Donia T, Abu-Khudir R, Ramadan H, Ali EM, Mohamed TM. Propolis potentiates methotrexate anticancer mechanism and reduces its toxic effects. Nutr Cancer 2020;72(3):460-480.
16. Wallace TC. Health effects of coconut oil—A narrative review of current evidence. J Am Coll Nutr 2019;38(2):97-107.
17. Famurewa AC, Aja PM, Nwankwo OE, Awoke JN, Maduagwuna EK, Aloke C. Moringa oleifera seed oil or virgin coconut oil supplementation abrogates cerebral neurotoxicity induced by antineoplastic agent methotrexate by suppression of oxidative stress and neuro-inflammation in rats. J Food Biochem 2019;43(3):e12748.
18. Famurewa AC, Maduagwuna EK, Folawiyo AM, Besong EE, Eteudo AN, Famurewa OA, Ejezie FE. Antioxidant, anti-inflammatory, and antiapoptotic effects of virgin coconut oil against antibiotic drug gentamicin-induced nephrotoxicity via the suppression of oxidative stress and modulation of iNOS/NF-ϰB/caspase-3 signaling pathway in Wistar rats. J Food Biochem 2020;44(1):e13100.
19. Charan J, Biswas T. How to calculate sample size for different study designs in medical research? Indian J Psychol Med 2013;35(2):121-126.
20. Çetin A, Kaynar L, Eser B, Karadağ C, Sarayman B, Öztürk A, Koçyiğit İ, Hacıoğlu SK, Çiçek B, Silici S. Beneficial effects of propolis on methotrexate-induced liver injury in rats. Acta Oncol Turc 2011;44(1):18-23.
21. Marino P, Pepe G, Basilicata MG, Vestuto V, Marzocco S, Autore G, Procino A, Gomez-Monterrey IM, Manfra M, Campiglia P. Potential role of natural antioxidant products in oncological diseases. Antioxidants 2023;12(3):704.
22. Weber K. Differences in types and incidence of neoplasms in Wistar Han and Sprague-Dawley rats. Toxicol Pathol 2017;45(1):64-75.
23. El Emam HF, Abd El Salam NN, Ghazy SE. Impact of bone marrow stem cells application on the methotrexate induced submandibular salivary gland degenerative changes in albino rats (histological and immuno-histochemical study). Egy Dent J 2019;65(2):1751-1762.
24. Abdel-Raheem IT, Khedr NF. Renoprotective effects of montelukast, a cysteinyl leukotriene receptor antagonist, against methotrexate-induced kidney damage in rats. Naunyn Schmiedebergs Arch Pharmacol 2014;387:341-353.

25. AL-Hashemi WH, Mansur ME, Homady MH. Intracomparative and immunocytochemistry study of parotid, submandibular and sublingual gland in domestic rabbit of Iraq. Biochem Cell Arch 2019;19(1).

26. Yasser S, Shon A. Histomorphometric and immunohistochemical study comparing the effect of diabetes mellitus on the acini of the sublingual and submandibular salivary glands of albino rats. Open Access Maced J Med Sci 2020;8(A):49-54.

27. AbdelFattah S, El-din ME, Abdelfattah N, Nady G. Propolis versus Nigella sativa Oil challenging the apoptotic pathway in Propylthiouracil-induced Hypothyroidism on parotid gland. Res squ plat 2023.

28. Taha RM, Abdel-Latif GA, Said RH. The prospective effect of green tea versus pomegranate peels extracts on submandibular salivary glands of albino rats after methotrexate administration (histological and immunohistochemical study). Int J Dent 2024;2024(1):3290187.

29. Mahmoud EF, Mahmoud MF, Abd Al Haleem M. Royal Jelly ameliorates oxidative stress and tissue injury in submandibular salivary gland of methotrexate treated rabbits: Immunohistochemical study. J Am Sci 2012;8:501-508.

30. Xu M, Wu S, Wang Y, Zhao Y, Wang X, Wei C, Liu X, Hao F, Hu C. Association between high-dose methotrexate-induced toxicity and polymorphisms within methotrexate pathway genes in acute lymphoblastic leukemia. Front Pharmacol 2022;13:1003812.

31. Isaac MR, Abdelhady GT, Ahmed EH. Protective role of L-carnitine against methotrexate-induced side effects on submandibular gland in adult male albino rats. Ain Shams Med J 2024;75(2).

32. Mubarak R. Effect of red bull energy drink on rats submandibular salivary glands (light and electron microscopic study). J Am Sci 2012;8(1):366-372.

33. El-Agamy AA, Afifi OK, Sheta AA. Protective role of Panax ginseng on fluvoxamine maleate induced structural changes in the submandibular salivary gland of rats. Nat Sci 2014;12(4):21-29.

34. Natarajan K, Abraham P, Kota R. Activation of the mitochondrial apoptotic pathway contributes to methotrexate-induced small intestinal injury in rats. Cell Biochem Funct 2017;35(7):378-391.

35. Takahashi S, Nakamura S, Domon T, Yamamoto T, Wakita M. Active participation of apoptosis and mitosis in sublingual gland regeneration of the rat following release from duct ligation. J Mol Histol 2005;36:199-205.

36. Khalil FA, EL-Kirsh AA, Kamel EA, EL-Rahmany NG. Beneficial effect of propolis extract (bee glue) against methotrexate-induced stress in liver and brain of albino rats. Indian J Med Res Pharm Sci 2016;3:24-35.

37. Ulusoy HB, Öztürk İ, Sönmez MF. Protective effect of propolis on methotrexate-induced kidney injury in the rat. Ren Fail 2016;38(5):744-750.

38. Mohsen ROM, El-Messiry HM. The possible ameliorating role of platelet rich plasma versus propolis on submandibular salivary glands damage induced by methotrexate in adult male albino rats (light and transmission electron microscopic study). Egypt J Histol 2023;46(2):993-1006.

39. Hamsi MA, Othman F, Das S, Kamisah Y, Thent ZC, Qodriyah HM, Zakaria Z, Emran A, Subermaniam K, Jaarin K. Effect of consumption of fresh and heated virgin coconut oil on the blood pressure and inflammatory biomarkers: An experimental study in Sprague Dawley rats. Alex J Med 2015;51(1):53-63.

40. Famurewa AC, Folawiyo AM, Enohnyaket EB, Azubuike-Osu SO, Abi I, Obaje SG, Famurewa OA. Beneficial role of virgin coconut oil supplementation against acute methotrexate chemotherapy-induced oxidative toxicity and inflammation in rats. Integr Med Res 2018;7(3):257-263.

41. amurewa AC, Ufebe OG, Egedigwe CA, Nwankwo OE, Obaje GS. Virgin coconut oil supplementation attenuates acute chemotherapy hepatotoxicity induced by anticancer drug methotrexate via inhibition of oxidative stress in rats. Biomed Pharmacother 2017;87:437-442.

42. Abdel Moneim A, Ezzat A, Salem FEH, Kassab R, El-Yamany NA. Protective effect of virgin coconut oil against doxorubicin-mediated hepatotoxicity in rats. Adv Basic Appl Sci 2023;1(1):46-54.

43. Mantovani G, Macciò A, Madeddu C, Mura L, Gramignano G, Lusso MR, Murgia V, Camboni P, Ferreli L, Mocci M, Massa E. The impact of different antioxidant agents alone or in combination on reactive oxygen species, antioxidant enzymes and cytokines in a series of advanced cancer patients at different sites: correlation with disease progression. Free Radic Res 2003;37(2):213-223.

44. El-Bagoury AA, El-Tantawi HG, El-Naggar SA, Kwilla AM, Khalaf AM. Effects of virgin coconut oil and lauric acid "with or without 5-fluorouracil" on dimethylhydrazine-induced hepatotoxicity in male rats. Egypt J Zool 2019;71:56-69.

45. Abdul-Hamid M, Salah M. Intervention of ginger or propolis ameliorates methotrexate-induced ileum toxicity. Toxicol Ind Health 2016;32:313-322.