# THE HARMFUL IMPACT OF DIAZINON ON THE INTESTINE OF ADULT MALE ALBINO RAT AND THE POTENTIAL PROTECTIVE ROLE OF SESAME OIL: HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

**BY**

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## ABSTRACT

**Background:** DZN is one of the most crucial organophosphorus (OPs) compounds widely utilized in agriculture and horticulture to manage pests and boost crop output. The widespread use of DZN can be harmful to several human organs. Sesame seed oil contains polyphenols that have been found to have antioxidant, antimutagenic, and anti-inflammatory properties. **Aim of the study:** Our experiment evaluated the protective and antioxidant potential of sesame oil (Ses.oil) against DZN toxicity on the jejunum of albino rats**. Material and methods:** Forty mature male albino rats weighing 180 and 200 grams were divided randomly into four groups; each group had ten rats. **Group (a) Control group**: received 1ml/kg of normal saline by oral gavage daily for 4 weeks. **Group (b) Sesame oil group:** received 4 ml/kg of body weight of sesame oil daily for 4 weeks. **Group (c) DZN treated group:** a dose of DZN (20 mg/kg body weight) was given by oral gavage daily for 4 weeks**. Group (d) DZN-Sesame oil group:** a dose of 20 mg/kg of body weight of DZN plus 4 ml/kg of sesame oil daily for 4 weeks. Rats were weighed, and the jejunal tissues were obtained for biochemical analysis to measure jejunal Malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), and nitric oxide (NO), for Histopathological examination (Hematoxylin and Eosin staining, PAS and bromophenol blue stain) and immunohistochemistry using (TNF-α and E-cadherin). **Results:** Diazinon intoxication decreased rats' body weight and intestinal oxidant/antioxidant imbalance (an increase in NO and MDA and a reduction in SOD and GSH). Also, DZN induced histopathological changes in the jejunum with strong expression of TNF-α immunoreactivity and low expression of E-cadherin immunoreactivity. Ses.oil treatment improved the previous toxic effects when administered in combination with DZN. **Conclusions: T**his study has revealed thatSes.oil supplementation alleviates DZN toxicity via its antioxidant and anti-inflammatory properties**.**

**Key words:** *small intestine, organophosphorus compounds, polyphenols, E cadherin*

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## INTRODUCTION

A high level of vegetable consumption is an essential aspect of a safe and healthy diet ***(Mason-D'Croz et al., 2019).*** Fruits and vegetables make up over 30% of the human diet, according to a World Health Organization (WHO) announcement. In addition, the expanding usage of chemical pesticides to protect crops from infestation, plant diseases, insects, fungi, and weeds is related to increased vegetable consumption ***(Basheer and Ali, 2018; Quijano et al., 2016; Parker, 2013).***

Organophosphate insecticides are one of the most used pesticides in agriculture and pest control *worldwide* ***(Ulansari and Roosdiana, 2018).*** They are quickly absorbed after eating or breathing due to their non-polar and lipophilic structure, and following absorption, they cluster in adipose tissues, kidneys, liver, and salivary glands. Organophosphates go through various metabolic processes before being eliminated as metabolites by urine, feces, and expiration ***(Anbarkeh et al., 2019).***

The widespread use of organophosphates pollutes the environment and has harmful effects on humans and animals ***(Abdel-Daim et al., 201; Abd-Eldaim and Halawa, 2014).***

Diazinon (DZN) (0, 0-diethyl-0-[2-isopropyl-6-methyl-4-pyrimidinyl] phosphorothioate) is a broad-spectrum organophosphate (OP) insecticide that is used to control insects in crops, lawns, fruits and vegetables, and as pesticide in domestic animals and agriculture ***(Handy et al., 2002).***

DNZ enters the body through the skin, lungs, and digestive system. DZN toxicity includes hematological and reproductive disorders, and toxicity to the kidneys, liver, cardiovascular system, and central nervous system

***(ElMazoudy and Attia, 2012; Razavi et al., 2013).*** The digestive system is the most crucial place associated with food poisoning since toxin absorption begins with food consumption. The detrimental effect of diazinon pesticide poisoning through the digestive tract generally occurs in the small intestine ***(Ulansari and Roosdiana, 2018).***

Acetylcholine (ACh) builds up in the synaptic cleft as a result of the cholinesterase (ChE) suppuration caused by serine residues being phosphorylated in the dynamic location of the enzyme. This accumulation results in nicotinic and muscarinic symptoms as well as signs of intoxication in the peripheral and central nervous systems, respectively ***(Čolović et al., 2010; Lukaszewicz-Hussain, 2010).***

Another mechanism was discovered to be the generation of reactive oxygen species and free radicals (ROS) by OPs, which leads to oxidative stress in a number of tissues **(*Abdou and El Mazoudy, 2010; Bhatti et al., 2010; Lukaszewicz-Hussain, 2010).***

ROS can alter cellular processes like metabolism and membrane function by interacting with biological macromolecules like nucleic acids, proteins, lipids, and carbohydrates. Cells are protected from ROS damage by enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) as well as non-enzyme antioxidants such as reduced glutathione (GSH). The gap between the rate of ROS production and the antioxidants' ability to protect cells is known as oxidative stress ***(Bhatti et al., 2010; Monteiro et al., 2009; Ojha et al., 2011).***

Numerous studies have suggested that DZN toxicity, whether acute or chronic, leads to the formation of oxidative stress ***(Danaei et al., 2019; Shah and Iqbal, 2010; Yaghubi et al., 2021).***

Because of its fewer adverse effects, ease of use, and low cost, a lot of study is now being done on the use of plant products as natural therapies. The medicinal use of plants and their extracts could be a potential treatment option for a variety of ailments. The use of herbal medicines for health prevention and treatment is becoming more popular around the world ***(Kennedy et al., 2016).***

Sesame oil is an extract from the Sesamum indicum plant, which belongs to the Pedaliaceae family. Phenolic compounds, non-protein amino acids, alkaloids, cacogenic glycosides, polyunsaturated fats and lipids, mucilage, phospholipids, thiazole, disulphide, ketones, aldehyde, vitamins B1, B2, C, and E, as well as trace elements such as calcium, magnesium, iron, copper, zinc, and phosphorus, are all found in sesame oil ***(Konan et al., 2008) (Shittu et al., 2009).***

Sesamin, sesamolin, and sesaminol lignan fractions in sesame oil are known to contribute to its oxidative stability and antioxidative activity ***(Elleuch et al., 2007).***

## AIM OF THE WORK

The current study aimed to obtain new insight into the potential ameliorative role of Ses.oil as a novel herbal medicine versus DZN-induced toxicity in the jejunum of adult albino rats.

## MATERIAL AND METHODS

- **Chemicals:**

Diazinon (DZN) has purchased from Kafr El Zayat pesticide and chemicals Co. Sesame oil was obtained from El Captin Company for herbs, cosmetics, and extracting natural oils (Cairo, Egypt).

- **Animals:**

A total of forty mature male albino rats weighing between 180 and 200 grams were collected from Zagazig University's animal house. The animals were housed in a space with a temperature control and a 12-hour light-dark cycle. There was food, water, and libitum for the rodents. The rats were randomly divided into four groups of ten each.

**Group (a) Control group**: received 1ml/kg of normal saline by oral gavage daily for 4week as a vehicle ***(Abdou and El Mazoudy, 2010)***.

**Group (b) Sesame oil group:** received 4 ml/kg of body weight of sesame oil daily for 4week ***(Salehzadeh et al., 2019).***

**Group (c) DZN treated group:** a dose of DZN (20 mg/kg body weight) was given by

oral gavage daily for 4week ***(Abdou and El Mazoudy, 2010)***.

**Group (d) DZN-Sesame oil group:** a dose of 20 mg/kg of body weight of DZN plus 4 ml/kg sesame oil daily for 4week.

By the end of experiment, the animals were weighed up (using an electronic balance, BXX 40; BOECO, Germany) and sedated with sodium thiopental (50 mg/kg) intraperitoneal injection [i.p] just before being sacrificed ***(Mohamed et al., 2020).***

- **Biochemical assays:**

The jejunums were homogenized in cold 50 mM sodium phosphate buffer (pH 7.0) adding 0.1 mM EDTA to yield a 5 percent homogenate (w/v). After that, the homogenates were centrifuged at 6000 rpm for 10 minutes at 4° C to remove nuclei and debris. Separated supernatants were aliquoted and kept at -80° C until chemical analysis.

The antioxidant defense system and lipid peroxidation and nitric oxide were calculated. MDA estimation was carried out using ***(Ohkawa et al., 1979)*** approach. NO was estimated according to ***(Bradford, 1976)***. Furthermore, SOD and CAT were determined using the previously reported procedures of ***(Nishikimi et al., 1972)*** and ***(Aebi, 1984),*** respectively. The procedures were done exactly as directed by the manufacturer's guidelines (Biodiagnostic, Cairo, Egypt).

- **Histopathological analysis:**

According to Bancroft and Layton, jejunal specimens were preserved in 10% neutral-buffered formalin and embedded in paraffin Hematoxylin and eosin (H&E), Periodic acid-Schiff reagent (PAS) ***(Bancroft and layton, 2018)*** and the mercury bromphenol blue method ***(Mazia et al., 1953)*** were used to visualize total protein in 5 m-thick sections deparaffinized in xylene was implemented. The Leica DM500 with Leica ICC50 W Camera Module light microscopy was used to examine the stained slides at Zagazig University's Image Analysis Unit of the Anatomy and Embryology Department. Two distinct pathologists performed the blinded histopathological evaluations. Using Chiu's scoring system ***(Chiu et al., 1970)***, the severity of the intestinal injury was assessed histopathologically as follows: grade 0 villus appearance is normal; grade 1 capillary congestion and subepithelial Gruenhagen's space (subepithelial spaces/lifting of surface epithelium); grade 2 separation of the epithelial layer from the lamina propria and an increased subepithelial distance; large epithelial parting and some villi tips being stripped, grade 3; grade 4 denudation of the villi and an expanded capillary; grade 5 ulceration and hemorrhage as a result of digestion and breakdown of the lamina propria

- **Immunohistochemical analysis**

For the purpose of immunostaining, sequential sections with a thickness of 5 m were cut from paraffin blocks and placed on glass slides. After that, the slides were incubated at 65° C for an entire night to ensure that the sections adhered precisely to the slides. After being deparaffinized for one to two minutes in xylene, the slide sections were rehydrated for three minutes each in ethanol (in descending grades of 100%, 95%, and 70% ethanol) and then rinsed with distilled water for five minutes. After that, these sections were submerged in an epitope retrieval solution containing 10 mM citrate buffer (pH 6.0) and subjected to heat-mediated antigen retrieval for 20 minutes in a water bath at 98° C. Endogenous peroxidase activity was reduced by a 10-minute medication containing 0.3% hydrogen peroxide at room temperature (RT, 21° C).Non-specific binding (PBS) was reduced after washing in phosphate-buffered saline for one hour at room temperature (RT) in 5% normal goat serum. After that, the sections were incubated with the primary antibodies for about an hour at room temperature: anti-TNF alpha and anti-E-cadherin. The slices were rinsed in PBS for twenty minutes prior to being treated with a secondary biotinylated antibody. After that, a solution containing an enzyme conjugate known as "Streptavidin–Horseradish peroxidase" was used to treat the slices for ten minutes. The chromogen 3, 3-diaminobenzoic acid (DAB) was diluted in PBS with H2O2 to a 0.03 percent concentration just before use to

observe secondary antibody binding. PBS was used to rinse the sections prior to each of the aforementioned procedures. The slides were counterstained with Mayer's hematoxylin before being rinsed until they turned blue, in distilled water. After mounting, dehydrating, and covering with glass, the slices. Leica light microscopy was used to analyse the stained slides.

- **Morphometrical analysis:**

Both the villus height (measured from the tip of the villus to the villus-crypt junction) and the crypt depth (measured by subtracting the villus height from the total mucosa thickness) were determined through morphometric analysis at a magnification of 100.Additionally, Goblet cell count/100 m was evaluated using PAS slides at 400x magnification (Cirilo et al.,2013), and morphometric analysis was used to quantify the positively labeled (brown-colored) areas of E-cadherin and TNF alpha immune-expression at 400 magnification. The National Institutes of Health, USA's free public-domain image-processing tool Image J" 1.49v/Java 1.6.0_244" was used to quantify the data. In the beginning, the image analyzer was calibrated automatically to translate the program's measurement units (pixels) into actual micrometres. Ten non-overlapping fields were randomly selected and analyzed from five successive sections of each specimen slide in each group.In order to determine the mean and standard deviation, the data were logged, graphed, and statistically analyzed.

**STATISTICAL ANALYSIS:**

SPSS version 16.0 was used to do statistical calculations ***(Stehlik-Barry and Babinec, 2017)***. Continuous variables were reported as mean±standard deviation (SD) because the data had a normal distribution (parametric). The Kolmogorov–Smirnov test was used to ensure that the data was normal. To find significant differences between groups, the independent T-test and one-way analysis of variance (ANOVA) were performed. Post hoc tests were used when the ANOVA test revealed a significant difference. Tukey's honestly significant difference (Tukey HSD) test was used for multiple comparisons across groups because the data was homogeneous. At a P value of 0.05, differences were regarded statistically significant (*), and at a P value of 0.001, they were considered highly significant (**)

## RESULTS

- **Body weight results**

It is a delicate judge of harmful adverse effects of Diazinone. When comparing the Diazinone treated groups to the control groups of the same age, the mean value of body weight revealed a significant decrease. There was no significant difference between Sesame oil (Ses.oil), control, and protected (DZN + Ses.oil) groups as presented in **Table (1).**

- **Biochemical results**

As antioxidant markers, the effects of diazinone on catalase (CAT) and superoxide dismutase (SOD) were found to be significantly lower in the DZN treated groups than in the two control groups for CAT.SOD was significantly lower in the DZN-treated groups than in the two control groups, even after Ses.oil was given to the protected groups; SOD levels were significantly higher than those of the DZN-treated group.

As lipid peroxidation markers, the effects of diazinone on nitric oxide (NO) and malondialdehyde (MDA) were examined. The results showed that diazinone significantly increased NO. In addition, the effects of DZN on MDA demonstrated a significant increase in the DZN-treated groups in comparison to the two control groups, as well as in the protected groups following the administration of sesame oil; MDA levels were significantly lower in the DZN-treated group than in either of the control groups **(Table.2).**

- **Histological results**

**1. Hematoxylin and eosin stain results**

There was no damage to the simple columnar epithelium of the villi in either the control or Sesame oil (Ses.oil) groups of rats' jejunums, which had normal histology. The small intestine has two muscle layers, one inner circular and one outer longitudinal, that are covered externally by the visceral peritoneum and lined by a mucous membrane in its general

microscopic structure **(Figs. 1A, B).** The epithelial membrane and the lamina propria, a supporting framework of thin connective tissue, comprise the small intestine mucosa. They rest on the mucosa of the muscularis. The latter is made up of some elastic tissue and smooth muscle fibers. The plica circularis, or crescent-shaped circular folds, are folded into the mucosa. The intestinal villi also expand the surface of absorption. Two kinds of small intestinal surface cells cover the intestinal villi. Absorbing rather than secretory simple columnar epithelial cells **(Figs.1C, D).** Goblet cells in various stages of mucus discharge can be distinguished between these straightforward columnar cells. The brush border of simple columnar epithelial cells contains a large number of microvilli, which boosts the epithelial membrane's absorptive capacity. The normal villi of jejunum were illustrated in **(Figs. 1E, F).**

Treatment with Diazinon was represented by severe histopathological changes; the villi pattern was generally ill-defined. Most of jejunal villi were short, thick, and distorted with desquamated blunt tips. The mucous layer lost natural regulation and seemed like cellular decomposition **(Fig. 1G).** Most enterocyte cells were malformed with darkly stained pyknotic nuclei and other cells were vacuolated. There were a lot of areas with lost brush borders with abundant epithelial exfoliations in jejunal lumen **(Figs. 1H, I).** There were many vacuoles and spaces together with areas of hemorrhage inside the lamina propria of the villi. Dilated congested blood vessels and even many areas of hemorrhage were seen near the crypts. The epithelial cells lining distorted crypts and distinct regions of the intestine exhibited many Vacuolations, pyknotic darkly stained nuclei, and vacuolated cells. Submucosal separation and a large number of goblet cells were seen. Some villi had excess inflammatory cell infiltrations **(Figs. 1D, J, K, L).**

In the protected (DZN + Ses.oil) group, the situation appeared to be normal. Jejunal villi regained their length but retained a blunt tip, whereas others were short and had some blunt tips. Their lining columnar epithelial cells had either vesicular or darkly stained nuclei. Some areas showed brush boundaries while others were still losing them. Most villi contained normal amount of goblet cells while others still had an excess number of them **(Figs.1, M, N, O).**

Morphometrical image analysis revealed that villi height was highly significantly reduced in the DZN. Treated group when compared to both control groups. Crypt depth also significantly decreased in DZN. Treated group, but in the protected (DZN + Ses.oil) group both height and depth improved **(Table 3).**

The impact of co-treatment of Ses.oil and Diazinon on intestinal damage was evaluated using Chiu's score. The control group experienced no histopathological alterations. In comparison to the control group, Diazinon significantly damaged the intestinal mucosa, as evidenced by ulceration and bleeding ($P>0.005$). In contrast to the Diazinon group, these damages were impressively nonexistent in the Ses.oil co-treatment group ($P>0.005$) **(Table. 4).**

**2. PAS staining results**

PAS-stained sections of jejunum of both control and Sesame oil (Ses.oil) groups showed continuous intact brush borders of enterocytes and normal average number of goblet cells **(Figs. 2A, B).** DZN Treated group showed lost brush border with apparent high increase in number of goblet cells (Figs. 2C). In the protected (DZN + Ses.oil) group, some areas showed continuous intact brush borders while others were still losing them. Most villi contained normal average number of goblet cells while others still had an excess number of them **(Fig. 2D).** Morphometrical image analysis revealed that goblet cells count/100 µm was highly significantly increased in DZN Treated group more than the control groups. In the protected (DZN + Ses.oil) group, there was a significant difference between (DZN + Ses.oil) group vs both control and DZN Treated groups **(Fig. 2E).**

**3. Bromophenol staining reactions for protein**

Bromophenol-stained sections of jejunum of both control and Sesame oil (Ses.oil) groups showed deep intensely stained cytoplasm of the epithelial cells lining villi while the cytoplasm showed weak blue bromophenol staining in DZN. Treated group and there was moderate staining in protected (DZN + Ses.oil) group **(Figs. 3A, B, C, and D).**

- **Immunohistochemical results**

**1. TNF-α analysis**

The cytoplasm of the epithelial cells lining villi of both control and Sesame oil (Ses.oil) groups showed normal weak expression of TNF-α while it showed strong reaction in DZN. Treated group. The cytoplasm showed moderate expression in protected (DZN + Ses.oil) group **(Figs. 4A, B, C, and D).**

Moreover, morphometrical image analysis for TNF in DZN treated group revealed that there were highly significant increase vs control groups, also after Ses.oil administration in protected (DZN+ Ses.oil) group; there was a high significant difference in DZN+ Ses.oil group as compared to DZN treated group and both control groups **(Fig. 4E).**

**2. E-cadherin analysis**

Immuno-stained intestinal sections for E-cadherin showed normal strong positive expression in cytoplasm of epithelial cells lining villi of both control and Sesame oil (Ses.oil) groups while it was decreased in DZN. Treated group and there was a moderate expression in the protected (DZN + Ses.oil) group **(Fig. 5A, B, C, and D).**

Moreover, morphometrical image analysis for E-cadherin in DZN treated group revealed that there were highly significant decrease vs control groups, also after Ses.oil administration in protected groups; there was a high significant difference in DZN+ Ses.oil group as compared to both control groups **(Fig.5E).**

**Table (1): Initial and final body weights of rats in different groups.**

| Group | Control | Ses. oil | DZN. Treated | DZN+Ses.oil | F | P |
|---|---|---|---|---|---|---|
| **Initial body weight** | **241.3±8.16** | **239.3±6.05** | **240.7±7.05** | **239±6.32** | **0.2469** | **0.8625 (NS)** |
| **Final body weight** | **251.8±8.3** | **253.2±9.4** | **213.0±2.8** [a,b] | **238.2±6.4** [a,b,c] | **39.68** | **< 0.0001** |

[a] *Significant versus control group* [b] *Significant versus Ses.oil group*
[c] *Significant versus DZN Treated group* *P: Significant versus control group*

**Table (2): Biochemical markers of the different studied groups.**

| Age group | Control group | Ses.oil group | DZN Treated | DZN+Ses.oil | F | P |
|---|---|---|---|---|---|---|
| **CAT (ng/mg)** | **9.62± 1.64** | **9.95± 1.63** | **6.66±1.06** [a,b] | **8.10±1.3** | **5.58** | **0.0082** |
| **SOD (U/mg)** | **95.04±15.03** | **95.72±14.67** | **54.24±9.46** [a,b] | **82.04±14.04** [c] | **10.34** | **0.0005** |
| **NO (nmol/mg)** | **28.76±4.48** | **28.32±4.17** | **39.38±6.18** [a,b] | **32.90±4.79** | **5.32** | **0.0098** |
| **MDA (nmol/mg)** | **4.41±0.76** | **4.54± 0.71** | **9.6±1.53**[a,b] | **6.63±0.75** [a,b,c] | **29.39** | **<0.0001** |

[a] *Significant versus control group* [b] *Significant versus Ses.oil group*
[c] *Significant versus DZN Treated group* *P: Significant versus control group*

**Table (3): Quantitative analysis of the villi height and crypt depth of the different studied groups.**

| Variables | Control | Ses. oil | DZN Treated | DZN + Ses.oil | F | P |
|---|---|---|---|---|---|---|
| **Villi height** | **203.1 ± 25.3** | **223.9±26.6** | **121.3±18.9 [a,b]** | **194.2±26.6 [c]** | **16.66** | **<0.0001** |
| **Crypt depth** | **165.5±20.8** | **164.6±14.2** | **102.2±20.6 [a,b]** | **140.3±39.0** | **6.81** | **0.0036** |

[a] *Significant versus control group* [b] *Significant versus Ses.oil group*
[c] *Significant versus DZN Treated group* *P: Significant versus control group*

**Table (4): Chiu's histo-pathological evaluation scoring of intestine damage in the different studied groups.**

| Chiu's Scoring | Control | Ses. oil | DZN Treated | DZN + Ses.oil | P |
|---|---|---|---|---|---|
| **Medium (IQR)** | **0.0** | **0.0** | **2.833 ± 0.7528 [a,b]** | **0.6667 ± 0.8165[c]** | **< 0.0001** |

[a] *Significant versus control group* [b] *Significant versus Ses.oil group*
[c] *Significant versus DZN Treated group* *P: Significant versus control group*

- **List of abbreviations:**

| Abbreviation | Meaning |
|---|---|
| DZN | Diazinone |
| OPs | organophosphorus compounds |
| Ses.oil | sesame oil |
| MDA | malondialdehyde |
| SOD | superoxide dismutase |
| GSH | glutathione |
| TNF-α | Tumor necrosis factor alpha |
| WHO | World Health Organization |
| ACh | acetylcholine |
| ChE | cholinesterase |
| ROS | Reactive oxygen species |
| GST | glutathione S-transferase |
| PBS | phosphate-buffered saline |
| DAB | diaminobenzoic acid |
| RT | room temperature |
| SD | standard deviation |
| ANOVA | one-way analysis of variance |
| LSD | least significant difference |

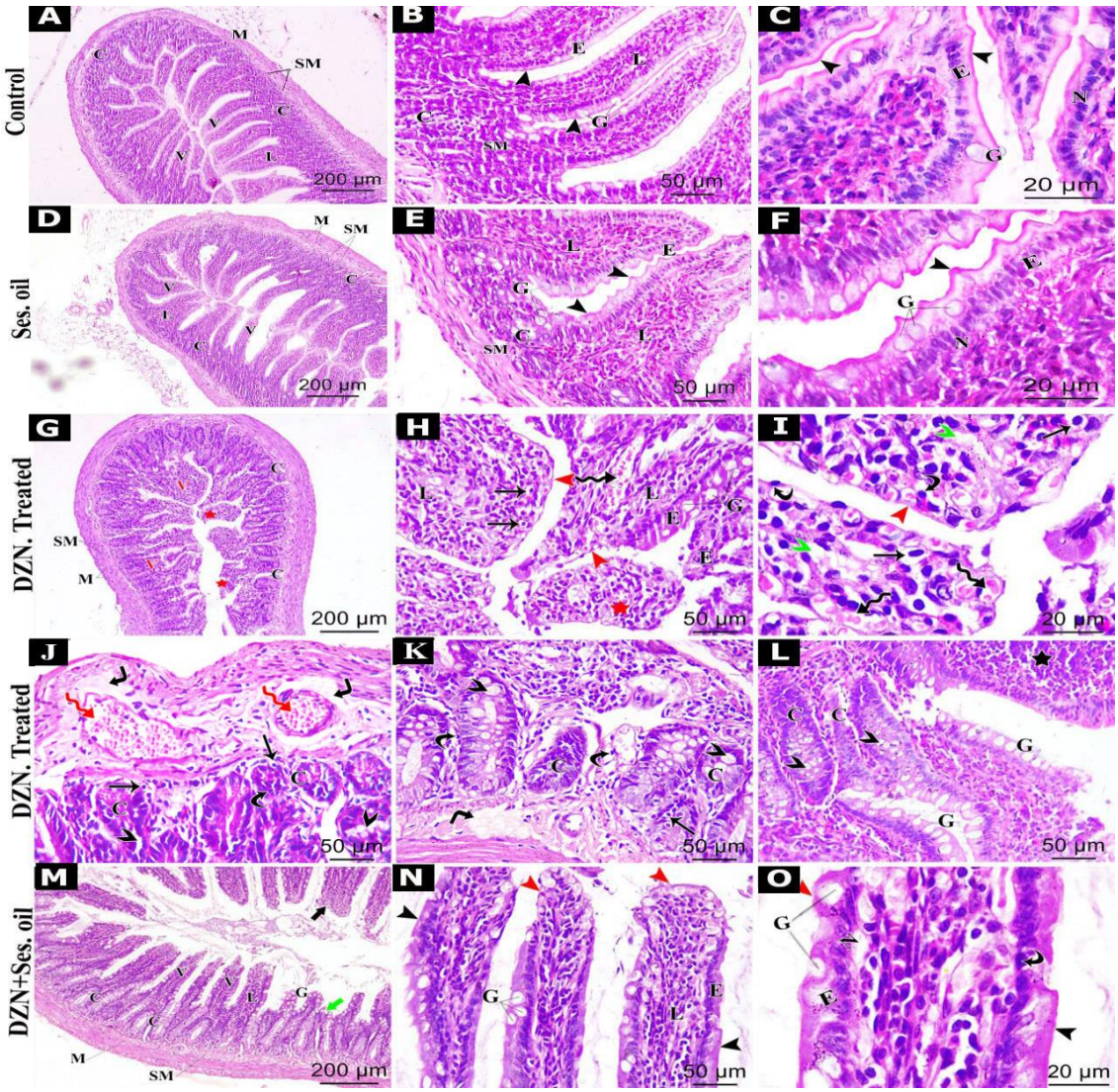


***Figure (1):*** *Photomicrographs of H&E sections of the jejunum of rats of different experimental groups. (A, B, C) Control and (D, E, F) Sesame oil (Ses. oil) groups showing normal histology of the jejunum with normal tall slender villi (V) lined with densely packed tall columnar absorptive enterocyte cells (E) with basal nuclei (N). There are intact lamina propria (L), intact basal crypts (C), intact submucosa (SM), and intact muscularisexterna (M). The mucosal enterocyte cells (E) have intact brush border (Arrow heads). Goblet cells (G) are inserted inside this continuous brush border. (G, H, I, J, K, L) in DZN-treated group, most of jejunal villi (red V) are short, thick, and distorted with desquamated blunt tips. Most enterocytes (E) are malformed with darkly stained pyknotic nuclei (black curved arrow) and other cells are vacuolated (thin arrows). There are a lot of areas with lost brush borders (red arrow heads) with abundant epithelial exfoliations (red star) in jejunal lumen. There are many vacuoles and spaces inside the lamina propria of the villi (green empty arrowhead). Dilated congested blood vessels are seen (red wavy arrow) and even there are many areas of hemorrhage (black wavy arrow). (J, K), notice the presence of many Vacuolations (black empty arrowhead), pyknotic darkly stained nuclei (black curved arrows), and vacuolated cells (thin arrows) in the lining epithelial cells of the distorted crypts (C). Submucosal separation (right angled arrow) and many goblet cells (G) are seen. Some villi have excess inflammatory cell infiltrations (black star). (M, N, O) the protected (DZN+ Ses. Oil) group, some jejunal villi (V) havenormal length but still have a blunt tip (thick black arrow), while others are short (thick green arrow). Some lining columnar epithelial enterocytes are normal (E) having vesicular nuclei (N) while other lining cells have darkly pigmented nuclei (black curved arrow), with some obtaining brush boundaries (black arrowhead) and others losing them (red arrowhead). Most villi contain normal amount of goblet cells while others still have an excess number of them (G).* ***(H&E, A, D, G, M:x 100 Scale bar= 200 µm; B, E, H, K, L, N: x 400 Scale bar= 50 µm; C, F, O: x 1000- Scale bar= 20 µm).***

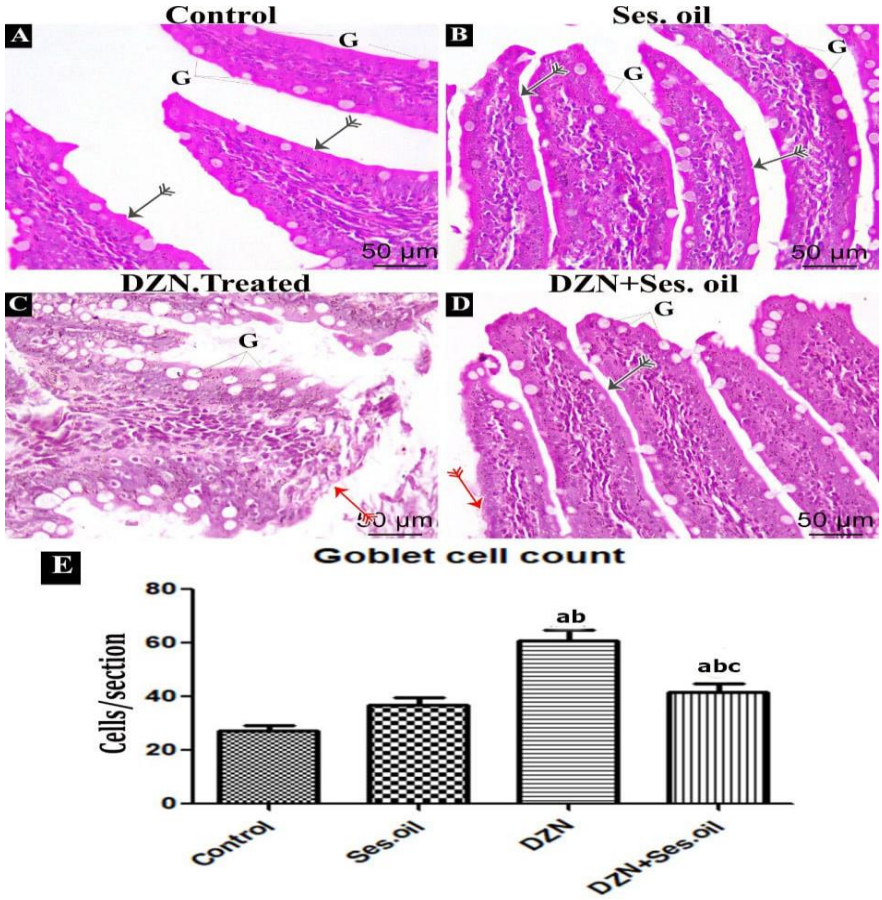


***Figure (2):*** *photomicrographs of PAS sections of the jejunum showing continuous intact brush borders (black crossed arrow) of enterocytes and goblet cells (G). There are some areas with lost brush border (red crossed arrow). A: control group; B: Ses.oil group; C: DZN-Treated group; D: DZN+ Ses.oil group.(E) Chart shows morphometric analysis of goblet cell count in different studied groups. ([a]) Significant difference vs control group. ([b]) Significant difference vsSes.oil group. ([c]) Significant difference vs DZN group. (* ***PAS stain X400, Scale bar= 50 µm)****.*

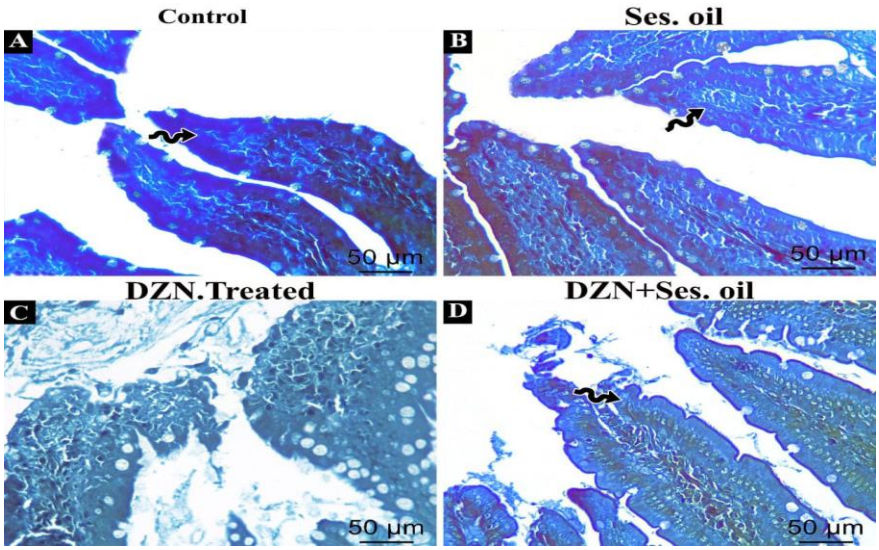


***Figure (3):*** *photomicrographs of Bromophenol sections of the jejunum showinghigh intensely stained cytoplasm (black zigzag arrow) of the epithelial cells of villi of both control and Ses.oil groups and moderate stained cytoplasm in (DZN + Ses.oil) group. A: control group; B: Ses.oil group; C: DZN-Treated group; D: DZN+Ses.oil group.* ***(Bromophenolstain X400, Scale bar= 50 µm)****.*

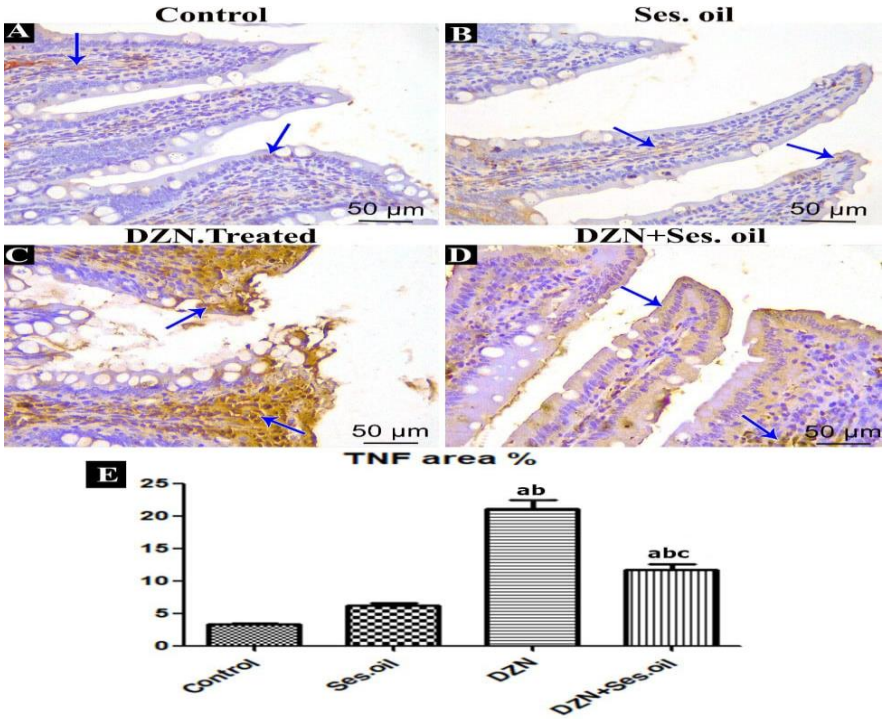


***Figure (4):*** *photomicrographs of Immunoreactivity for TNF alpha in jejunal crypts: positive stained nuclei are taking brown color (blue arrow). A: control group; B: Ses.oil group; C: DZN-Treated group; D: DZN+ Ses.oil group. (E) Chart shows morphometric analysis of area percentage of TNF alpha in different studied groups. ([a]) Significant difference vs control group. ([b]) Significant difference vsSes.oil group. ([c]) Significant difference vs DZN group.* ***(TNF alphaimmunoperoxidase stain counter stained with H.; X400, Scale bar= 50 µm)****.*

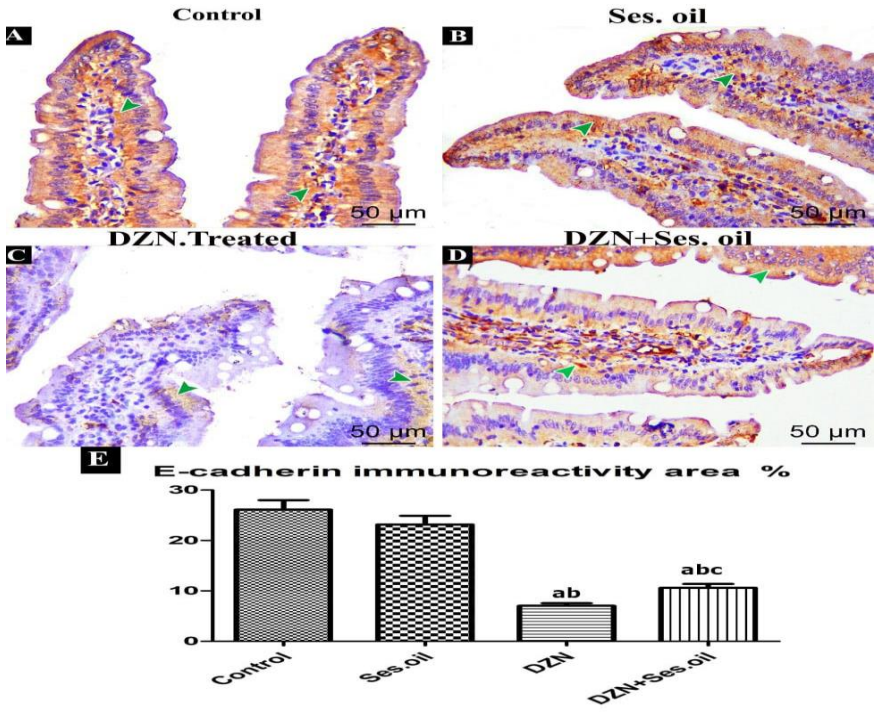


***Figure (5):*** *Photomicrographs of Immunoreactivity for E- cadherin in jejunal crypts: positive stained cytoplasm is taking brown color (green arrowhead). A: control group; B: Ses.oil group; C: DZN-Treated group; D: DZN+ Ses.oil group. (E) Chart shows morphometric analysis of area percentage of E- cadherin in different studied groups. ([a]) Significant difference vs control group. ([b]) Significant difference vsSes.oil group. ([c]) Significant difference vs DZN group.* ***(E- cadherinimmunoperoxidase stain counter stained with H.; X400, Scale bar= 50 µm)****.*

## DISCUSSION

Because pesticides are used worldwide, most individuals are exposed to low doses of these active chemicals and their residues through non-occupational exposure routes, such as nutrition and the environment. DZN is a widely used organophosphorus (OPs) insecticide that can harm various organs ***(Parrón et al., 2014).***

The gastrointestinal system is the first line of defense against pesticide contamination in meals. The intestine is a target of DZN ***(Lecoeur et al., 2006).***

The principal phenolic compounds of sesame oil are sesamin and sesaminol, which have a wide range of pharmacological actions, including antimutagenic, antioxidant, antihypertensive, anti-inflammatory, and antithrombotic properties ***(Sankar et al., 2005).***

This study set out to learn more about the potential protective effects of Ses.oil, a new herbal remedy, on DZN-induced toxicity in the jejunum of adult albino rats.

In this study, body weight was not significantly different between the Sesame oil (Ses.oil), control, or protected (DZN + Ses.oil) groups. However, the DZN treated group had a significantly lower body weight than the control group ***(Almalki, 2019; Jouhari et al., 2010)*** concur with this finding who also noticed that the organophosphorus pesticide-exposed animals were losing weight. The effect of insecticides on the gastrointestinal tract, which reduces appetite and increases absorption of nutrients from the gut, or direct toxicity, could cause the weight loss ***(Sankar et al., 2012).***

In the current study, there was a significant rise in jejunal MDA and NO levels and a significant drop in jejunal SOD and CAT levels compared to both control and Sesame oil groups. This imbalance indicates that DZN caused oxidative jejunal damage, these findings were in line with previous studies, which revealed that oxidative stress was involved in DZN-induced toxicity ***(Oksay et al., 2013; Sargazi et al., 2015).***

According to ***(Scandalios, 2005),*** oxidative stress results in tissue damage when the crucial equilibrium between oxidants and antioxidants is disrupted by either a lack of antioxidants or an excessive buildup of reactive oxygen species (ROS), or both. In addition, all essential bio compounds, including DNA, proteins, and membrane lipids, are damaged by intracellular ROS accumulation, which causes cell death ***(Yamamoto et al., 2007)***.

Lipid peroxidation (MDA) produces malondialdehyde as its final product. Because it is a primary oxidation product of polyunsaturated fatty acid peroxidation, an increase in MDA content is a key indicator of lipid peroxidation ***(Hariri et al., 2010; Mossa et al., 2014; Ogutcu et al., 2006)***.

In the current study, the level of MDA in jejunal tissues is significantly higher in the DNZ-treated group than in the control group or the Sesame oil group. This finding is in line with the ***Boussabbeh et al.,*** who demonstrated that DZN raised MDA concentrations in HCT116 cells ***(Boussabbeh et al., 2016)***. However, according to Almalki**,** who also found that Ses.oil ameliorates MDA changes in malathion-intoxicated rats, the (DZN + Ses.oil) group had a significantly lower MDA level than the DZN group ***(Almalki, 2019).***

The production of superoxide radicals by the oxidation of molecular oxygen can lead to high lipid peroxidation. In addition, this reaction generates hydrogen peroxide, which triggers the peroxidation of unsaturated fatty acids in the membrane, causing membrane dysfunction, the inactivation of membrane receptors and enzymes, and an increase in tissue permeability which contributes to organ injury ***(Karimani et al., 2018; Rahman, 2005).***

This study found that the concentration of jejunal NO was significantly higher in the DNZ-treated group than in the control group or the Sesame oil group. This finding is consistent with ***(Beydilli et al., 2015)*** *and* ***(Vahidirad et al., 2018)*** which showed that NO levels increased after DZN toxicity. When sesame oil was administered concurrently with DZN, NO levels significantly decreased in comparison to the DZN group. This finding is consistent with ***(Abdel-Daim et al., 2016)*** which demonstrated that sesame oil decreased NO levels in the liver and cardiac tissue of DZN-treated rats.

The nitric oxide synthetase (NOS) enzyme converts L-arginine into endogenous nitric oxide (NO) ***(Tutanc et al., 2012)***. NO is a functioning particle that has various physiologic and obsessive outcomes, and when it responds with superoxide anion ($O_2^-$) in a high-impact climate, it produces peroxynitrites ($ONOO^-$), which prompt harm and in the long run LPO arrangement in cells ***(Weinstein et al., 2000)*** and ***(Sayed-Ahmed et al., 2001).*** Oxidative stress and tissue damage are linked to excessive NO production ***(Peresleni et al., 1996)***.

Tissues and cells are shielded from the harmful effects of oxidative stress by antioxidant enzymes (SOD, CAT), which are essential biological defense systems. Turf and Feline are compounds that detoxify the oxygen revolutionary by switching it over completely to H2O2 and water ***(Hassani et al., 2018).***

The results a agree with ***(Farouk et al., 2021)*** who reported that basil and sesame seed oils ameliorated DZN toxicity and induced a decrease of SOD and CAT levels in the liver, kidney, testis, and epididymis tissues. In the present study, the concentrations of jejunal SOD and CAT were significantly decreased in the DNZ-treated group compared to the control and Sesame oil groups. Additionally, there was a significant increase in SOD and CAT levels when sesame oil was co-administered with DZN.

It has been hypothesized that oxidative stress is one of the primary mechanisms that contribute to the toxicity of numerous environmental pollutants like diazinon and is also involved in the activation of inflammatory processes ***(Abdel-Daim et al., 2018)***.

TNFα protein is produced by a variety of cell types in the human body, however the monocytic lineage cells, such as macrophages, are the primary producers, TNF plays an important role in steady-state and pathologic circumstances, including infections, damage, inflammation, and tumor growth ***(Parameswaran and Patial, 2010)***. As a result, TNF- overproduction plays a crucial role in the clinical consequences of numerous inflammatory disorders ***(Kowalski et al., 2001)***.

The current study's biochemical findings were supported by histopathological changes which indicate that most of the jejunal villi in the DZN group are short, thick, and distorted with desquamated blunt tips. Most enterocytes are malformed with darkly stained pyknotic nuclei and other cells are vacuolated. There are a lot of areas with lost brush borders with abundant epithelial exfoliations in the jejunal lumen. There are many vacuoles and spaces inside the lamina propria of the villi. Dilated congested blood vessels are seen and even there are many areas of hemorrhage. Submucosal separation and a large number of goblet cells are seen. Some villi have excess inflammatory cells infiltration. These results are in agreement with ***(El-fakharany and Abdel Hamid, 2017)*** who showed that the CPF-treated group's jejunum had damaged villi, with epithelial cell loss and evident vacuolation, as well as apoptotic darkly pigmented nuclei, muscular fibers were thickened and separated in the muscle layer

The intestinal epithelial tissues in the (DZN+ Ses.oil) group show a considerable improvement, becoming nearly like the normal ones. These results are consistent with those of ***(Farouk et al., 2021),*** who showed that Ses.oil and DZN together can partially protect against DZN-induced tissue damage in the liver, testis and kidney.

This was explained by **(*Rice-Evans et al., 1996)*** who revealed that Sesame's antioxidant properties prevent DZN from being activated into the reactive form, which may be the cause of its potential protective function. Moreover, antioxidants can delay or inhibit the oxidation of vulnerable cellular substrates, preventing oxidative stress.

The jejunal mucosa provides an essential barrier against harmful and toxic substances and guards a person against various antigenic and inflammatory reactions. Villi and crypts distinguish small intestinal epithelium. Villi are epithelial folds into the lumen with a lamina propria core. The jejunum has the tallest villi. The villus epithelium is composed of

enterocytes and goblet cells. Damage to the before mentioned structures is linked to impairment of intestinal barrier function and results in the entry of intraluminal substances into the bloodstream ***(Rahner et al., 2001; Saudi et al., 2009; Suzuki and Hara, 2010)***.

Our result revealed that there was a significant decrease in villous height and crypt depth in DZN treated group this result in agreement with ***(El-fakharany and Abdel Hamid, 2017)*** who showed a significant decrease in jejunal villi height in CPF (chlorpyrifos) group.

***Ueno et al., (2011)*** added that decreased villous height and crypt depth in the jejunum was related to decreased trans mucosal resistance and increased permeability which indicates intestinal barrier dysfunction.

Goblet cells are specialized cells that secrete intestinal mucus, a complex glycoprotein gel made up of bioactive compounds and secretory mucin glycoproteins ***(Kim and Ho, 2010)***. Goblet cell counts and mucin release varied in response to a variety of factors, including changes in diet, infections, altered microbiota, and surgical procedures, according to numerous previous studies ***(Sharma and Schumacher, 1995).***

Comparing the DZN-treated rats to the control groups, PAS and morphometric analysis revealed a significant increase in the number of goblet cells per villous. According to ***(Rajini, 2014),*** a similar increase in the number of goblet cells per villous was observed in the jejunum of MCP-treated rats.

Mantle et al., provided an explanation for this, claiming that as adaptive responses, goblet cell hyperplasia and increased mucin secretion were caused by insecticide residues in the intestinal lumen. Goblet cell mucus is also rapidly and massively secreted, preserving the integrity of the mucus protective layers and assisting in eliminating pathogens and insecticide residues ***(Mantle et al., 1989).***

In agreement with ***(Rady, 2009)*** which demonstrated a significant decrease in the total protein in the lung and intestine of pigs treated with DZN, this work revealed that there was weak reactivity of protein content in the jejunum of the DZN treated group in comparison to the control group ***(Sivaprasado et al., 1983)*** suggested that the release of hydrolytic enzymes from ruptured lysosomes triggered by toxic agents might cause the decrease in total protein and carbohydrate content.

An increase in protein content was noticed in DZN and Ses.oil group, we attribute this improvement to the antioxidant effect of Ses.oil according to ***(Onosaka et al., 1987)*** who stated that the antioxidant effect of vitamin C canprotect SH groups of metallothionein and other proteins against oxidation.

The immunohistochemical results of this work revealed that there was significant increase in TNF alpha expression in DZN group in comparison to control and Sesame.oil groups, this finding is consistent with ***(Birdane et al., 2021)*** who revealed that DZN was expressed to increase pro inflammatory cytokine levels like IL-1β, IL-10, and TNF-α. ***(Hariri et al., 2010)*** *and* ***(Abdel-Daim et al., 2018)*** who also reported that diazinon dramatically elevated blood TNF-alpha levels, and ***(Ouyang et al., 2009)***, who revealed that TNF-a mRNA expression increased in the mouse liver and spleen following acute organophosphorus pesticide poisoning. There was a significant decrease in TNF alpha expression in Sesame oil and DZN group compared to DZN group this result in agree with ***(Farouk et al., 2021)*** who observed that basil and sesame seed oils decrease TNF alpha immuno-expressions in n hepatorenal, testicular, and epididymal tissues in DZN treated rats.

As the integrity of the intestine's epithelial cell junction barrier is assumed to be important in the modulation of inflammation and disease development ***(Turner, 2009)***. E-cadherin is a protein that is required for the establishment of tight junctions and adherent's junctions ***(Tunggal et al., 2005)***. Changes in E-cadherin structure and production promote a pro inflammatory state by facilitating neutrophil transmigration ***(Muise et al., 2009) and (Kountouras et al., 2003).***

In agreement with ***(Luo et al., 2019)*** who observed a decrease in E-cadherin expression in the jejunum of rats exposed to the individual and combined effects of DON and Cd, the present study's immunohistochemical results revealed that there was a significant decrease in E-cadherin expression in DZN treated rats in comparison to the control and Sesame oil groups. However, there was a significant increase in E-cadherin expression in the DZN+Ses.oil group compared to the DZN group. This finding is consistent with ***(Mohammadzadeh et al., 2021)*** who stated that sesame oil raises mRNA levels of the E-cadherin gene in the testicles of elderly mice.

We credit the higher biochemical and histological outcomes in the rats given Ses. oil for being rich in sesamol and sesamin, which have antioxidant, anti-inflammatory, and antimutagenic properties ***(Sankar et al., 2005; Yadav et al., 2016).***

## CONCLUSION

The results of this study revealed that DZN induced damage in the intestinal tissue as evidenced by biochemical and histological findings. In addition, the Ses.oil administration, protected from these toxic effects.

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**Conflict of Interest:** This manuscript's research, authorship, and publication are all free of potential conflicts of interest, according to the authors

**Ethical Approval:** All experimental procedures were approved by Zagazig University's Institutional Animal Care and Use Committee (ZU-IACUC) under the approval number *ZU-IACUC/3/F/4/2022*, and they were carried out in accordance with ARRIVE guidelines.

**Data Availability:**

This published article [and its additional information files] contain all data produced or analyzed during this investigation. The corresponding author will provide the datasets used and/or analyzed during the current work upon reasonable request.

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