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TOXICOPATHOLOGICAL AND IMMUNOTOXICITY INDUCE BYCYGON IN GASTROINTESTINAL TRACT OF ALBINO MICE

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ABSTRACT

The purpose of the current study was to examine the harmful effects of cygon in mice, as well as the impact of immunological stimulation with Aftovaxpur DOE vaccine and histopathological abnormalities. Eighty-four-week-old adult Swiss Albino mice were split up into four groups. The first group received 4.5 mg/kg of Cygon orally every day for four weeks. The second group was given 0.1 ml of the Aftovaxpur DOE vaccine intraperitoneally (I/P) twice at two-week intervals while also receiving cygon orally every day for four weeks. I/P administered 0.1 ml of the Aftovaxpur DOE vaccine to the third group. The negative control was assigned to the fourth group. Samples of blood were drawn from the first, second, third, and fourth groups in order to measure serum IL8, IL10 levels using an ELISA kit. The results of ELISA tests for detecting IL8 and IL10 titers in group three($P \leq 0.05$) demonstrated a substantial increase ($P \leq 0.05$) when compared to group two, group one, and group four. Clinicalsigns of treated mice showed depression and decreased appetite throughout the trial period, the anorexia and weight loss were brought on by pathological lesions that resulted in intestinal malabsorption and stomach indigestion. Pathological lesions of the intestine showed desquamation in epithelial cells and mucus accumulation in addition to necrotizing enteritis also degeneration of mucous glands and inflammatory cells infiltration between mucous glands, as well as hyperplasia of goblet cells, in stomsch congestion of blood vessels and moderate hyperkeratosis in epithelial cellsEven so, the groups that received Cygon treatment and the Aftovaxpur DOE vaccination showed improvement against the negative effects of Cygon.

KEYWORDS: cygon Histopathological changes, Immunotoxicity, intestine, Cygon**,** IL8, IL10

INTRODUCTION

Cygon is a common organophosphate pesticide and acaricide. American Cyanamid patented and marketed it in the 1950s. Cygon, like other organophosphates, is an acetylcholinesterase inhibitor, meaning it inhibits cholinesterase, an enzyme required for central nervous system function. It works through both touch and ingestion. It is easily absorbed and disseminated throughout tissues, and it degrades quickly (Hulse *et al*., 2019). Cygon is a organophosphate that is insoluble in alcohol and acetone but soluble in water. It also has a high lipophilicity. Crystals that are color-less, white, or light beige can be found (WHO).

Few studies on the histopathological effects of Cygon(Persis, 2001) in animals have been conducted. Furthermore, Cygon causes hyperglycemia and a variety of harmful effects on the rat pancreas after acute, subchronic, and chronic exposure (Kamath *et al*., 2008). In experimental animals, pesticides have harmful effects. It has the ability to affect biochemical, hormonal, reproductive, and oxidative stress indicators. In experimental animals, it also has cytotoxic, autogenetic, genotoxic, and carcinogenic properties (Mossa *et al*, 2017). Oxidative stress, and the subsequent destruction to critical cell components induced by oxygen-free radicals, is often regarded as a significant process (Livingstone, 2001).

Hydrogen peroxide (H2O2)-induced oxidative stress is known to cause a wide spectrum of physiological, biochemical, and immunological dysfunctions in experimental animals and people. Furthermore, it triggers cell death and induces apoptosis as a result of DNA damage (Ramond *et al*., 2011; Luma*et al*., 2013)

Previous research suggested that some pesticide-related effects could be caused by changes in membrane fluidity (Mansour and Mossa, 2009), lipid composition and inhibition of enzyme activities (Mansour and Mossa,

2010). IL-8 was initially identified as a neutrophil-specific chemotactic factor before being categorized as, a member of the chemokine family. Following interleukin-1a, 1b, 17, tumor necrotic factor alpha, or TLR activation, a variety of cells, including macrophages, neutrophils, lymphocytes, and endothelial and epithelial cells, produce IL-8 (Coelho *et al* 2005). The activation of neutrophils and their migration to the site of infection or injury are the main effector activities of IL-8. In addition to neutrophils, NK cells, T cells, basophils, and eosinophils primed with GM-CSF or IL-3 are also drawn to IL-8. (Burke 2008).

Huda (2020) showed that in convalescent patients, the modulation of innate immune response coupled with the establishment of adaptive immunity neutralizing antibodies, memory T and B cells provides optimism for active immunization. Examining the immunotoxic and histopathological effects of Cygon on the digestive system was the aim of this study.

MATERIAL AND METHODS

Chemicals

Cygon, is an organophosphorous pesticide with achemical formula: SCH3NHCOCH2SP (OCH3)2

Trade name: cekuthoatedimetferkethion, roxion, rego, afidox, rogodial, rogodan, trimetion and sevigordimethoate as moderatelyhazardous.

Preparation of cygon solution:
To prepare the cygon solution the following procedure was followed. About 90 milligrams of cygon(powder) was dissolved in 200 ml of distilled water and the final concentration was 90 mg/200ml. For each 10 g of body weight of mice, 0.1 mL of cygon solution was administrated orally to reach 4.5 mg/kg.BW.(Sabina 2001).

Aftovaxpur DOE preparation

Animals were immunized with this antigen made in accordance with House *etal*. (1989).

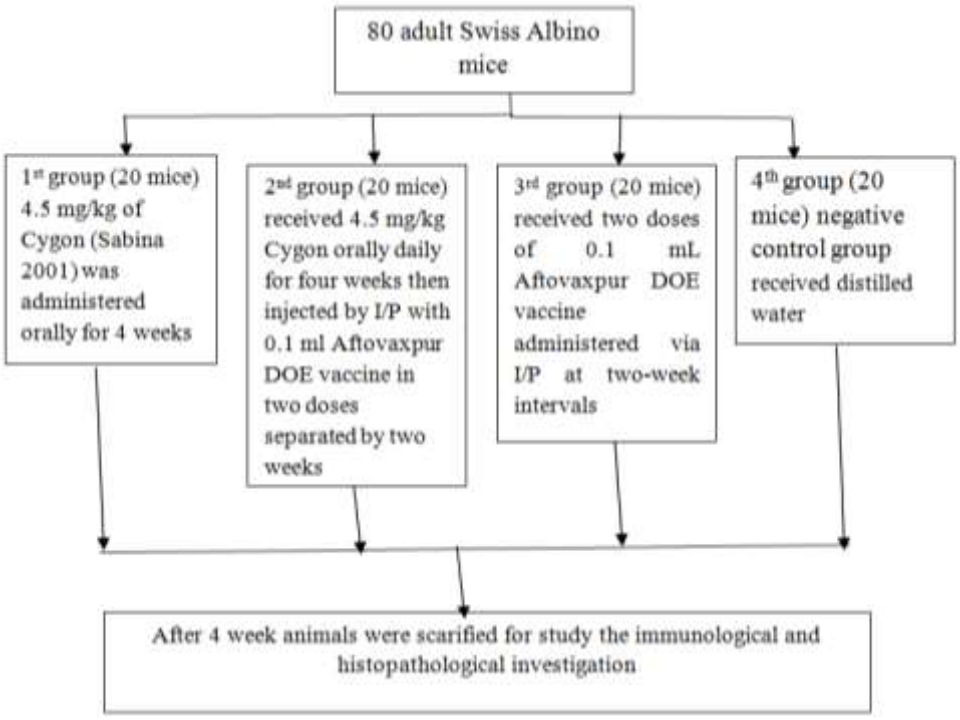
Experimental Animals

For adaptation, 80 adult Swiss Albino mice weighing between 25 and 30 grams at 4 weeks of age were acquired from the animal house of the College Vet. Med. University of Baghdad. The animals were kept in plastic cages in a room with air conditioning that was kept at 25±2˚C. The bedding in the cages was made of hard wood chips, and it was changed frequently to maintain a hygienic environment. Mice were provided with food pellets and unlimited water.

Immunological tests

Blood was drawn directly from the mice hearts; for **Immunological** testing, placed in coagulant test tube, blood samples were centrifuged for five minutes at 3000 rounds per minute (rpm), and serum was extracted. It was then frozen at -20˚C until it was needed for **Immunological** test (serum level of IL8, IL10). An enzyme-linked immunosorbent assay (ELISA) kit from Elabscience (U.S.A.) was used to measure the concentrations of IL8 and IL10 in mouse serum, this sandwich kit is for the accurate quantitative detection of Mouse Interleukin 8,10(also known asIL-8, IL10) in serum, plasma, cell culture supernates, Ascites, tissue homogenates or other biological fluids. this test was carried out in accordance with the manufacturer's procedure.

Experimental Design



Histopathological examination

At the conclusion of the four-week experiment, all animals were killed, and stomach and intestine samples were taken. After fixing the tissues in a 10% formaldehyde solution, we used a histokite to process them as usual. Hematoxylin and eosin staining and microtome were used to section paraffin blocks containing implanted tissue slices, which were subsequently examined under a light microscope (Luna, 1968).

Statistical analysis

Statistical analysis was conducted using a two-way ANOVA, and the statistical package for social sciences (SPSS) revealed that the mean difference was significant at the (P≤0.05) level.

RESULTS

ELISA test for detection levels of IL8 and IL10 titer

The final result of ELISA test showedthat the mean values of serum IL8 and IL10(mg/dl), at 28dayGroup 3's post-immunization rate was noticeably significantly increase (266.84±1.04,143.30±0.20), then those values in the G1, G2 (5.80±1.12,7.71±0.09), (44.55±1.05,58.00±0.22) and negative control groups G4(11.45±0.13,8.45±1.01) respectively, as shown in Table1.

Table (1): The mean value of serum IL8 and IL10 in immunized groups and control negative group at 28day post immunization by ELISA test.

Groups	IL8 (mg/dl) (Mean ± SE) at 4 weeks	IL10 (mg/dl) (Mean ± SE) at 4 weeks
G1	5.80±1.12 D	7.71±0.09 D
G2	44.55±1.05 B	58.00±0.22 B
G3	266.84±1.04 A	143.30±0.20 A
G4	11.45±0.13 C	8.45±1.01 C

Means with distinct capital letters differ significantly in the same column are significantly differ (P≤0.05).

Clinical signs of treated mice

All of the treated mice showed signs of depression and decreased appetite throughout the trial period. The anorexia and weight loss were brought on by pathological lesions that resulted in intestinal malabsorption and stomach indigestion, whereas the control group consumed normally.

Histopathological Examination

The histopathological changes in mice of (1st group)

Intestine

The microscopic section of the intestine showeddesquamation in epithelial cells and mucus accumulation (Figure,1) in addition to necrotizing enteritis (Figure2), alsodegeneration of mucous glands and inflammatory cells infiltration between mucous glands (Figure3), as well as hyperplasia of goblet cells (Figure4)

Stomach

The microscopic section of the stomach showed congestion of blood vessels (Figure5), as well as moderate hyperkeratosis in epithelial cells (Figure6)

The histopathological changes in mice of 2nd group

Intestine

The microscopic section of the intestine showed necrosis in epithelial cells and inflammatory cells infiltration in lamina propria) (Figure7), in other section showed mucus accumulation in lumen (Figure8).

The histopathological changes in mice of 3rd group

Intestine

The microscopic section of the intestine showed hyperplasia of lymphoid tissue (Figure9)in other section showedproliferation of goblet cells in lamina propria (Figure10).

Figure (1): Histopathological section of intestine at 4 weeks in 1st group shows desquamation in epithelial cells (black arrow), mucus accumulation (red arrow) (black arrow) (H and E stain 100X)

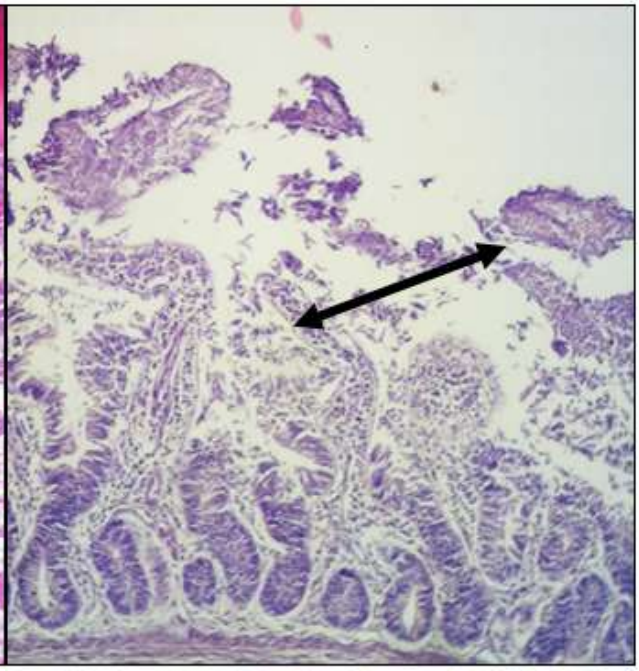


Figure (2): Histopathological section of intestine at 4 weeks in 1st group shows necrotizing enteritis (black arrow (H and E stain 100X)

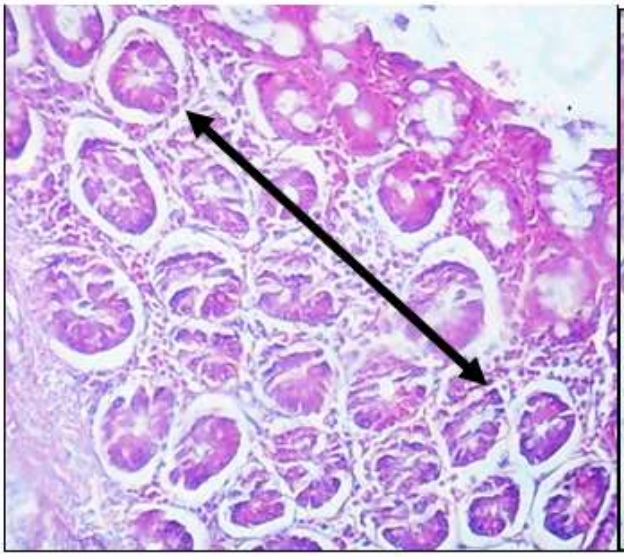


Figure (3): Histopathological section of intestine at 4 weeks in 1st group show degeneration of mucous glands and inflammatory cells infiltration between mucous glands (black arrow) (H and E stain 400X)

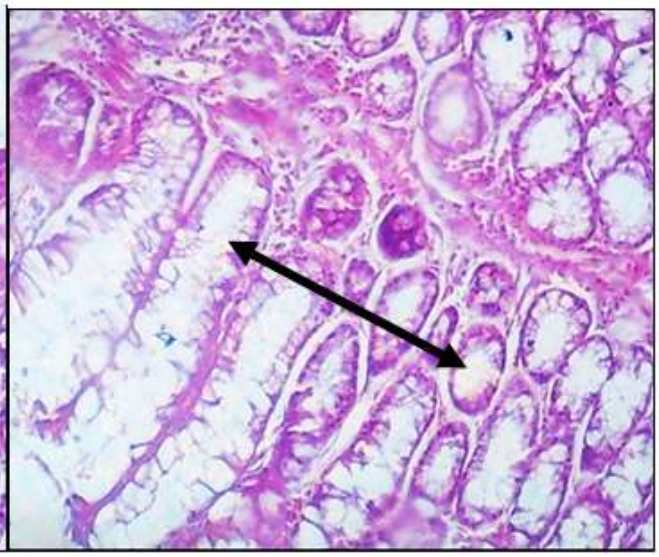


Figure (4): Histopathological section of intestine at 4 weeks in 1st group show hyperplasia of goblet cells (black arrow) (H and E stain 400X)

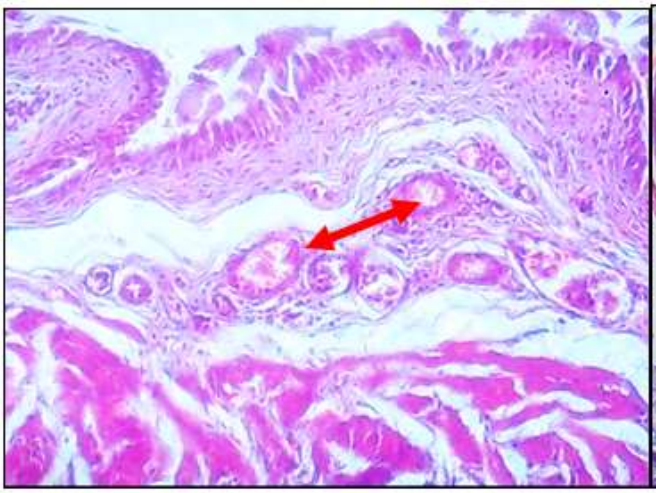


Figure (5): Histopathological section of stomach at 4 weeks in 1st group shows, congestion of blood vessels (red arrow) (H and E stain 100X)

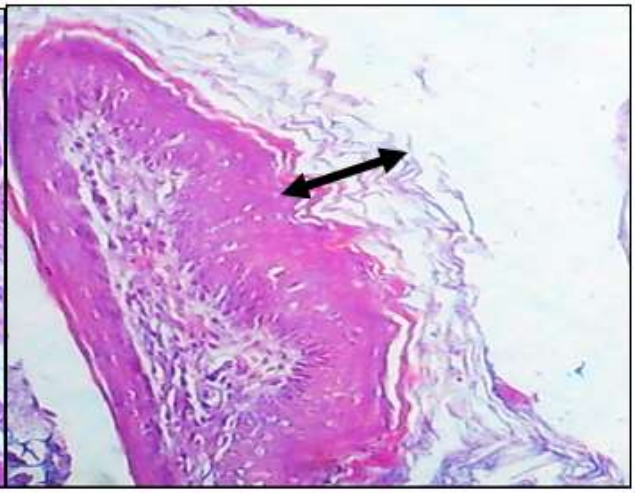


Figure (6): Histopathological section of stomach at 4 weeks in 1st group shows, moderate hyperkeratosis in epithelial cells (black arrow) (H&E stain, 100×).

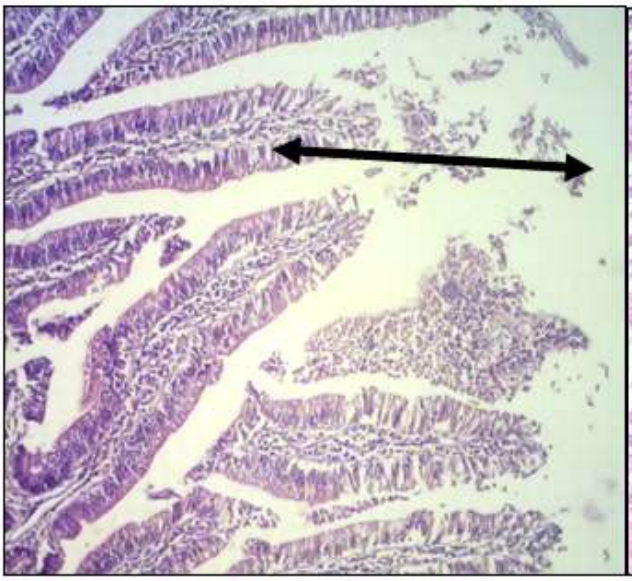


Figure (7): Histopathological section of intestine at 4 weeks in 2nd group shows necrosis in epithelial cells and inflammatory cells infiltration in lamina propria) (black arrow) (H&E stain, 100×).

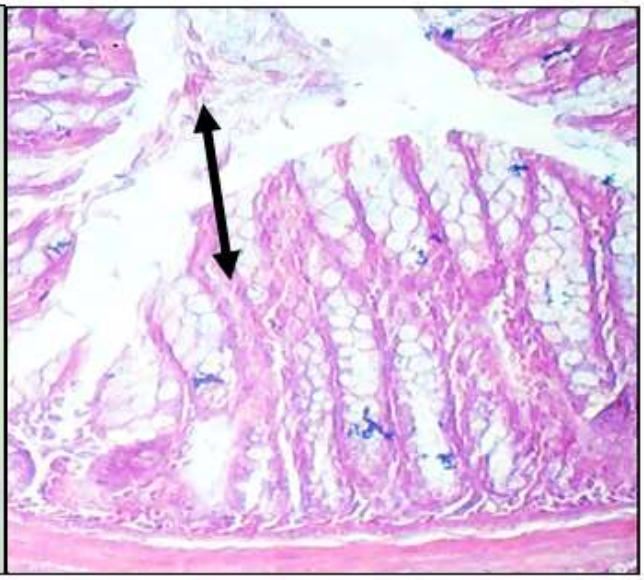


Figure (8): Histopathological section of intestine at 4 weeks in 2nd group shows mucus accumulation in lumen (black arrow) (H and E stain 100X)

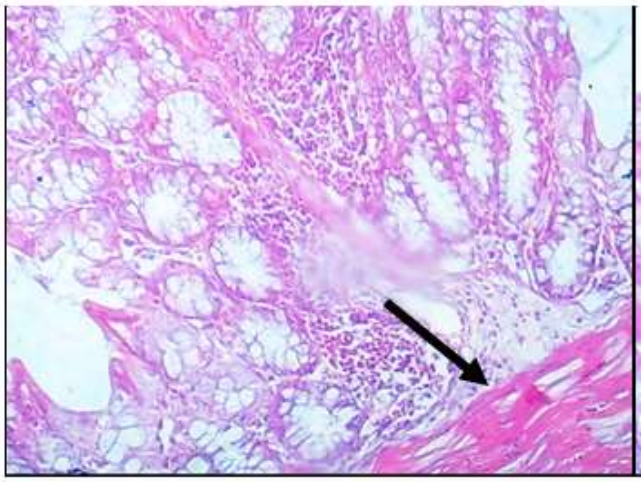


Figure (9): Histopathological section of intestine at 4 weeks in 3rd group shows hyperplasia of lymphoid tissue (black arrow) (H&E stain, 100×).

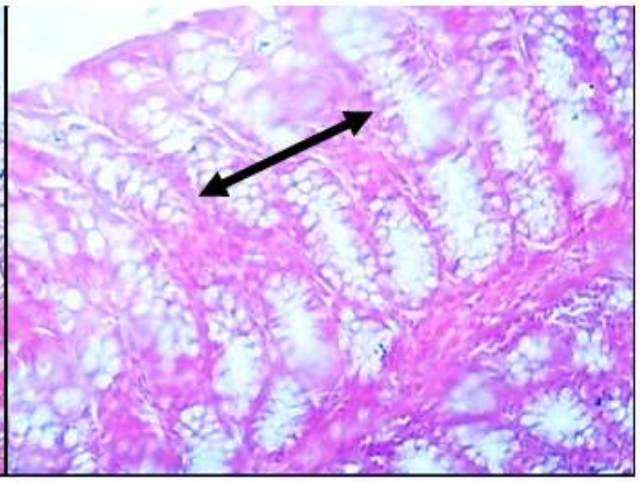


Figure (10): Histopathological section of intestine at 4 weeks in 3rd group shows proliferation of goblet cells in lamina propria (black arrow) (H&E stain, 100×).

DISCUSSION

As previously mentioned, the results may indicate that antigens boosted the immune response while Cygon decreased the Ag's immunological response. These data may also suggest that pesticide contamination in the environment is linked to lower immunization program activity against Aftovaxpur DOE agricultural animals. Furthermore, Cygon lowered immunity and alterations within the suppressor gene, indicating that immunological problems, gene mutations, and oxidative stress can all have an impact on functioning cells. (El Okda *et al*, 2017) discovered differences in the amounts of the antioxidant-protective enzymes SOD, CAT, GPx, and GSH. Occur during pyrethroid exposure. The immunotoxicity of Cygon was studied in 30 employees (El Okda *et al*, 2017).

Permethrin also suppressed splenic T-lymphocyte growth. Permethrin inhibited spleen cells growth by inhibiting thymic cell proliferation. Furthermore, higher apoptosis was seen in CD48 and CD48+ thymocytes, although the CD4+8+ thymocyte subpopulation was dramatically decreased (WHO). Permethrin increases oxygen and neutrophil activity. After one hour of incubation with permethrin or its metabolites, neutrophil apoptosis was also observed (Gabbianelli *et al* 2009). It increases superoxide dismutase and alanine aminotransferase (AAT) in mice (Kumar and Sharma, 2015). By activating caspases, Cygon causes oxidative stress and triggers the mitochondrial death pathway (Kumar and Sasmal, 2018). Caspase-3 activation promotes apoptosis in spleen cells by activating a death protease (Kumar and Sharma, 2015). Cox-2 expression has been investigated for its capacity to induce inflammation, and bcl-2 and p53 proteins have been investigated for their capacity to induce apoptosis (Maalej et al, 2017). Decreases in bcl-2 protein levels and increases in cox-2 and p53 protein levels cause apoptosis.

According to Ma et al. (2023), our earlier study demonstrated that prolonged exposure to cygon may cause mitochondrial damage and increased oxidative stress in the colonic epithelial cells of mice, resulting in colonic inflammation.

One of the pathological mechanisms of numerous diseases is oxidative stress (Zheng et al., 2020). Research has indicated that oxidative stress plays a role in the onset of fibrosis (Masarone et al., 2018). The expression of several antioxidant enzymes is regulated by the crucial transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) (Chen et al., 2020).

A significant inflammatory response will result from the excess ROS (Su et al., 2019). ROS triggers the classic NF-κB inflammatory signaling pathway in pathological conditions. Numerous proinflammatory cytokines, including interleukin-1β, interleukin-6, and TNF-α, are then released, exacerbating the inflammatory damage in the liver (Wei et al., 2018). TNF-α causes tissue damage and persistent inflammation by inducing the production of chemokines by fibroblasts, endothelial cells, and macrophages. Nrf2 is an upstream regulator that controls the oxidative stress response and, by controlling cytokine production, reduces inflammation (Baiyun et al., 2018). Accordingly, the addition of Res decreased DLM-induced inflammation in this trial. Thus, exposure to cygon causes the quail's liver to become inflamed by activating the NF-κB/TNF-α signaling pathway.

On the other hand, the vaccine by itself causes cytokines IL8 and IL10 to increase. According to Mahajan et al. (2005), Brucella melitensis surface proteins can elicit humoral immune responses as well as cell-mediated immunity. The Th2 response was triggered by spleen cells in mice that were immunized with soluble Brucella antigens. The proteins that were secreted by Brucella were effective in promoting cell-mediated immunity.

Oxidative stress may be linked to the pathological abnormalities in the examined organs in the group that received Cygon treatment., which is important in the pathophysiology of many intestine and stomach illnesses produced byCygon therapy. may generate oxidative stress, leading in free radical generation and lipid peroxidation, and may be the underlying molecular mechanism causing pesticide-induced toxicity (Ismail and Mohamed, 2012). By oxidizing lipids, proteins, and DNA, excessive oxidant production can cause oxidative damage in cells. There is emerging evidence that oxidative stress, namely stress caused by reactive oxygen and reactive nitrogen species, can lead to a wide range of inflammatory and degenerative diseases (MacDonald-Wicks et al., 2006). The gut is a critical site of hazardous chemical absorption. Because the gut is thought to be the primary route for pesticide absorption, it is not surprising that the duodenum showed goblet hypertrophy. The pathological changes observed in the intestine and stomach in the current study are consistent with the findings of Velmurugan et al (2007), Manna et al (2005), and the present histopathological study showed pyknotic nuclei and vacuolated. Furthermore, the key components of the histopathologic change include congestion and bleeding. Cygon direct cytotoxic effects on endothelial cells might explain these findings (Alejandro et al., 2012). Both severe congestion and hemorrhaging by damaging vascular walls and resulting in dilatation and congestion, oxidative stress and lipid peroxidation can cause blood artery congestion (Shady and Noor El-Deen, 2010). Cygon-induced oxidative stress may also result in degeneration.

ROS in various organs causes tissue damage (tissue atrophy, necrosis, exfoliation, and inflammation). This outcome is consistent with past toxicological investigations (Jeon *et al*., 2013; Eman and Ghusoon, 2019). In vitro and in vivo, it might also help prevent or reduce oxidative damage to tissues and cells (Velmurugan et al., 2002, Reifen et al., 2004). Furthermore, degenerative alterations in the serosa, mucosa, and submucosal layers were seen, as well as localized necrosis, proliferation, and desquamation of the superficial villi (Eddleston and Clark 2011). Endosulfan exposure, causes alterations in the epithelial lining of the colon, indicating a disruption in intestinal absorption. Cengiz *et al*. (2001) discovered endosulfaninduced edema, degeneration, lymphocyte buildup in the lamina propria, pycnotic nuclei, and necrosis in the stomach of Gambusia affinis.After exposure to deltamethrin, the gut of fish showed infiltration of mononuclear leucocytes and eosinophils towards the lamina propria, as well as necrosis (Cengiz and Unlu, 2006). (Anwar, et al., 2020) observed that organophosphorus insecticides such as Endosulfan increased pathogenicity in the gut and stomach due to the fact that organochloride generates organochloride acid in the presence of HCl released in the stomach, which has extremely corrosive qualities. This acid damages the mucous secreting cells of the gut lining, resulting in the abnormalities observed.

Endosulfan has also been related to pathological gastrointestinal symptoms such as mucosal lining damage, microvilli loss, cracked clay appearance of duodenal mucosa, and gastric mucosa with desquamated epithelial cells. As a result, it irritates and damages the intestinal mucous membrane, reducing absorption. Lead was thought to induce gastric ulcers by interfering with oxidative metabolism in the stomach, which increased the occurrence of gastric ulcers (Olaleye *etal*., 2007).

The histopathological lesions in the intestine and stomach of animals receiving Aftovaxpur DOEvaccine were characterized by moderate lesions caused by the Aftovaxpur DOE vaccine innate immune response and provided partially protective immune responses against the Cygon effect on organs by a significant increase in IL-1 and IFN- by DC (Petrovsky and Aguilar 2004). Other potential effectors include CD4+ T-helper (Th) lymphocytes and cytotoxic CD8+ T lymphocytes, which can stop the spread of infectious diseases by identifying and killing infected cells or by releasing particular antiviral cytokines. These Th cells may contribute to the body's defense by generating cytokines and supporting the development and upkeep of CD8+ and B T-cell responses. Their primary cytokine production (interleukin [IL]-4 or interferon) led to the classification of effector CD4+ T cells as either T-helper 1 (Th1) or T-helper 2 (Th2). This distinction was rendered obsolete upon the discovery that Th cells are classified into multiple subgroups, each possessing distinct cytokine-producing and homing characteristics. (Geginat *et al* 2014).

the possible long-term consequences of exposure to Cygon. causes irreversible histopathological alterations that result in long-term diseases like cancer or fibrosis; this finding is consistent with earlier research that demonstrated exposure to varying cygon dosages can cause cancer and liver fibrosis (Li et al., 2021b). Notably, our earlier study showed that mice can develop colitis and colonic damage after 8 weeks of exposure to low-dose DLM (0.2 mg/kg/day) through the promotion of oxidative stress and disruption of intestinal flora (Ma et al., 2023).

Conclusions and Recommendation:

Based on the experimental findings of this study, we demonstrated that Cygon has a harmful toxic effect on mice, manifested in changes to the digestive system's histology and immunology. Based on the current study, we recommend that Cygon use be limited to a particular program.

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NOVELTY STATEMENT

The novelty of the study is focus on the immunotoxicity of Cygon as well as the histological status of stomach and intestine in mice.

AUTHOR'S CONTRIBUTION

Sabrin Ibraheem Mohsin, Salema Lafta Hassan, Taghred Jabbar Humadai, Designed and Performed the experiments, analyzed the data, contributed reagents, materials, analysis tools and wrote the paper.

Conflict of interest

The authors have declared no conflict of interest.

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