DJS Vol. 51(1)(2025) –pp.46-63 ISSN: 1012-5965

Delta Journal of Science

Available online at

https://djs.journals.ekb.eg/

Research Article

ZOOLOGY

Immunomodulatory Effects of *Trichinella spiralis* on Streptozotocin-Induced Diabetes in Mice: Prophylactic and Therapeutic Implications

Kareem E. Bakr, Nahla A. Radwan, Mohamed N. Mohamed, Mona M. Elwan*

Zoology Department, Faculty of Science, Tanta University, Tanta 31527, Egypt

***Corresponding author:** Dr. Mona Mohamed Elwan **email:** mona.elwan@science.tanta.edu.eg

Received: 20/7/2025 **Accepted:** 7/8/2025

KEY WORDS

Trichinella spiralis, immunomodulation, type I diabetes mellitus, streptozotocin, histopathology

ABSTRACT

The growing burden of diabetes mellitus type 1 (T1-DM), stresses innovative therapeutic strategies. Helminth infections, such as *Trichinella spiralis* (*T. spiralis*), show powerful immunomodulatory effects that may mitigate autoimmune conditions. The current study explores the prophylactic and therapeutic potential of *T. spiralis* infection and its larvae- derived antigens in T1-DM mice. 120 male Swiss albino mice were divided into prophylactic and therapeutic groups, each comprising six groups (N=20); normal control (G1), control T1-DM mice (G2) injected with STZ as 0.04 mg/g, low inoculum (200 larvae/mouse) of *T. spiralis* infection (G3), high inoculum (500 larvae/mouse) of *T. spiralis* (G4), low dose (100 µl / mouse of 500 µg/ml) larvae antigen intra-peritoneally administration (G5), and high-dose (100µl / mouse of 1000µg/ml) larva antigen (G6). Blood glucose levels, body weight changes were assessed, and histopathological investigation of liver and pancreas were performed using H&E staining. Statistical significance was assessed by ANOVA. Prophylactic management with *T. spiralis* antigens (G5 and G6) significantly decreased hyperglycemia and restored the body weight compared to T1-DM group (G2). High dose antigen treatment (G6) exhibited almost normal glucose levels, preserved hepatic architecture and reduced pancreatic β-cell degeneration. Larvae infection in G3 and G4 showed limited efficacy compared to antigen-specific immunomodulation. Therapeutic protocol confirmed the reduction of hepatic necrosis, regularization of pancreatic islets, and minimal inflammatory infiltration in high-dose antigen groups. *T. spiralis* antigens, particularly at high doses, exhibit strong prophylactic and therapeutic potential against T1-DM. Results magnify the promise of helminth-derived immunomodulators as novel therapy for autoimmune diabetes.

Introduction

The extraordinary prevalence of helminth infections undoubtedly reflects their ability to develop a variety of complex strategies to control the host immune system; they seem to successfully reset immunological reactivity thresholds and neutralize immune pathways. They also potentially help the host by reducing reactions to irrelevant bystander specificities like allergens and autoantigens **(Fogang et al., 2025)**. The incompletely understood mechanism is applied through secretion of immunomodulatory molecules that suppress host immune responses and enable them to evade immunological attacks and establish chronic infections **(McSorley & Maizels, 2012)**. Helminth-mediated immunomodulation by inducing a non-inflammatory immune environment characterized by production of interleukin 4 (IL-4), IL-10, and IL-13, as well as the proliferation of regulatory T cells (T-reg) that suppress cytotoxic T cell activity. By shifting immune responses from Th1 to Th2 dominance, helminths may provide protection against autoimmune conditions, including diabetes **(Bashi et al., 2014).**

Diabetes mellitus (DM) remains one of the world's most pressing health challenges, currently affecting approximately 537 million people globally. Type 1 diabetes mellitus (T1-DM), characterized by autoimmune destruction of insulin-secreting pancreatic β-cells and hyperglycemia, with complications including severe hypoglycemia, ketoacidosis, blindness, renal failure, and cardiovascular disease if not properly managed **(Bashi et al., 2014; Huang et al., 2022)**. Experimental models demonstrate that various helminthes infections, including *Trichinella spiralis, Taenia crassiceps, Heligmosomoides polygyrus, Schistosoma mansoni, and Strongyloides venezuelensis*, can prevent or suppress T1-DM development in non-obese diabetic (NOD) mice **(Ajendra et al., 2016)**.

Belal et al. (2016) stated that rats with diabetes brought on by Alloxan (ALX) or streptozotocin (STZ) have pathophysiological changes in their livers as well as biochemical changes in their blood. Similar to the alterations seen in the human liver, these alterations can range from steatosis to steatohepatitis and liver fibrosis. The histological analysis of pancreatic tissue from diabetic mice revealed cellular damage and pronounced insulitis, characterized by increased lymphocyte infiltration and inflammation **(Amdare et al., 2015)**. Proteins generated from helminths may be beneficial in measures to mitigate disease by enhancing the Th2 response and upregulating T-regs during the inflammatory tissue damage associated with T1-DM **(Yan et al., 2020)**.

Trichinellosis, a significant global health concern and economic burden disease, is caused by the largest known intracellular parasite, Trichinella. The infection exhibits remarkable immunomodulatory capabilities that impact both innate and adaptive immunity and maintains a delicate immunological balance with its host, enabling remarkable longevity within muscle tissue and making it an important model for studying chronic infection and immune modulation **(Bruschi et al., 2020)**. This is characterized by distinct Th1 and Th2

response phases corresponding to different infection stages. The intestinal phase predominantly elicits Th1 responses, while the muscular phase triggers Th2 immunity, with IL-4 playing an important role in suppressing Th1 activity while promoting Th2 amplification **(Ding et al., 2017)**. Recent studies using animal models of allergies and autoimmune diseases, like experimental autoimmune encephalomyelitis and TIDM, have greatly improved our understanding of how Trichinella avoids the immune system.

The current study aims to clarify the potential implications of *T. spiralis*'s infection and Trichinella larvae-derived antigens against induced diabetes mellitus in mice by throwing light on the histological alterations in the liver and pancreas of both control and experimental groups applied to the prophylactic and therapeutic protocol.

Materials and Methods

The study's experimental plan is illustrated in Fig. (1).

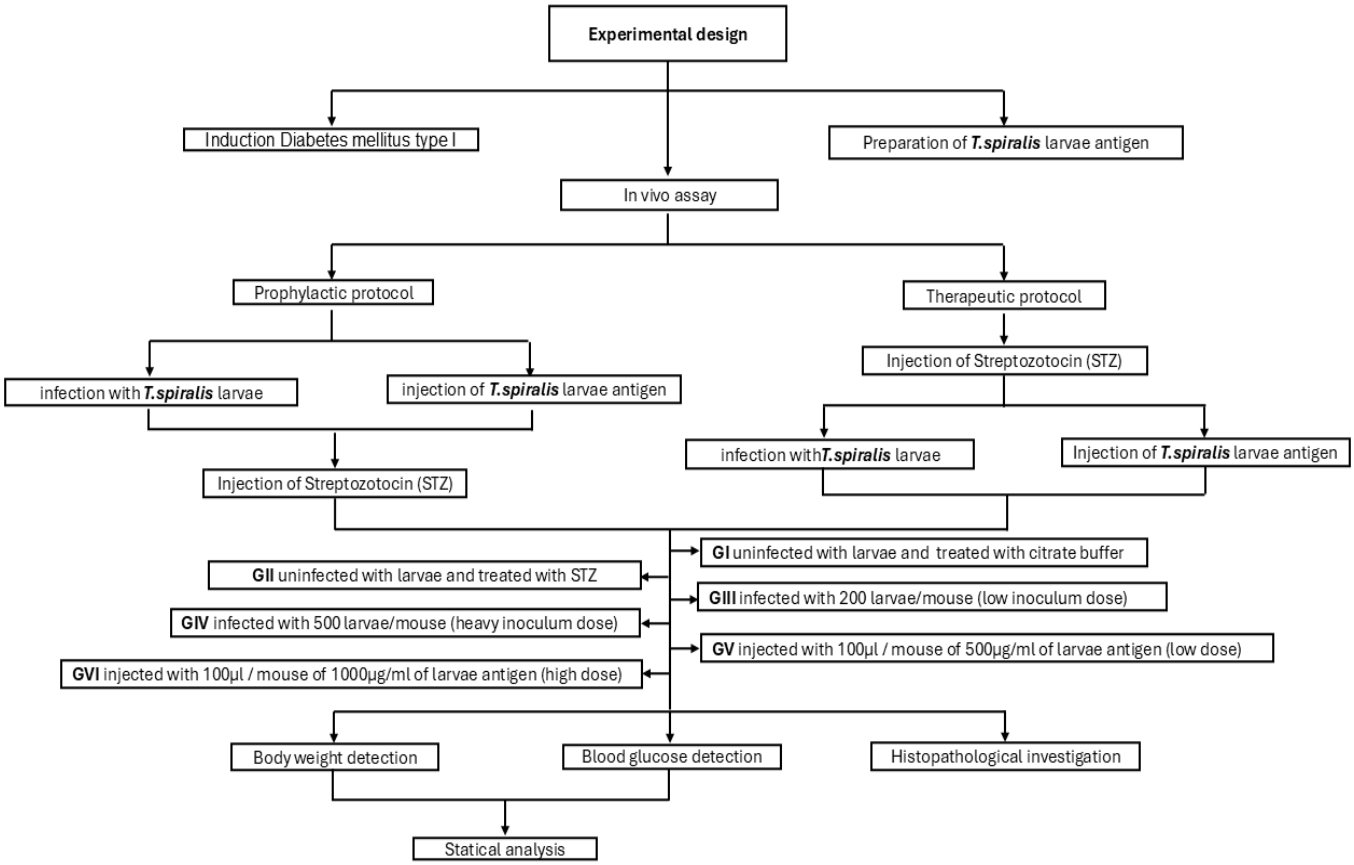

Fig. (1): A flow chart illustrating the experimental study procedures.

Trichinella spiralis

Infected pig muscles with *T. spiralis*'s larvae were collected from Cairo pig abattoir. The artificial standard pepsin hydrochloride digestion method was applied to acquire first muscle larvae (L1) **(Dvorožňáková et al., 2011)**. The experimental maintenance of the muscle larvae applied by successive passages through donor albino rats. Each rat was infected orally by 300 larvae using a stomach gavage after starvation of twenty-four hours. 35 days post-infection, the infected donor rats were eviscerated killed, their skins were removed, and the muscle larvae were retrieved by digestion.

Male Swiss albino mice, *Mus musculus*

One hundred and fifty male Swiss albino mice (6-8 week-old and 25-30 g weighted) were obtained from the Egyptian Company to produce Serums, Vaccines, and Medicines, Cairo Governorate, Egypt, kept under optimal conditions in the animal facility of Faculty of Science, Tanta University.

Streptozotocin

Streptozotocin (STZ) ($C_8H_{15}N_3O_7$), Cat no.100557, MP Biomedicals, LLC pharmacy California, USA.

Induction of diabetes mellitus type I

Type I diabetes mellitus was induced to mice using STZ in sodium acetate buffer. To detect the effective dose induces the disease without mortality; three doses were applied as follows: 40mg/kg, 100 mg/kg and 200 mg/kg, of STZ for three successive days to thirty mice by inter-peritoneal injection.

***T. spiralis* larvae antigen preparation**

The larvae antigen was prepared according to **Bien et al., (2013)**. Briefly, after thoroughly washing the larval pellet three times in phosphate buffer saline (PBS), the protein components were dissolved by mixing it with lysis solution (4% CHAPS, 8 M urea, 40 mM Tris base, supplemented with protease inhibitor cocktail). The mixture was sonicated to break it up and further homogenized in a glass Potter homogenizer. Centrifugation at 14,000 × g for 10 minutes at 4 °C in an Eppendorf microcentrifuge was used to clarify the lysis solution.

In vivo assay

One hundred and twenty mice were applied in two main subcategories; prophylactic and therapeutic protocols. Each protocol consists of six groups with ten mice of each. The groups were classified as follows: Negative control group (G1); uninfected with larvae and treated with citrate buffer pH 4.5 (100 µl/mouse). Positive control group (G2); uninfected with larvae and treated with 100 µl (0.04 mg/g) of STZ, Group 3 (G3); infected with 200 larvae/mouse (low inoculum dose), Group 4 (G4); infected with 500 larvae/mouse (heavy inoculum dose),Group 5 (G5) mice were inter-peritoneally (i.p) injected with 100 µl / mouse of 500 µg/ml of larvae antigen (low dose), and Group 6 (G6) mice were i.p injected with 100µl / mouse of 1000µg/ml of larvae antigen (high dose). In prophylactic groups, mice were received larvae antigen injection once weekly for three successive weeks. Mice were obtained STZ definite dose injection, one week after larvae antigen injection and twenty-eight days after larvae infection. In therapeutic groups, mice were received STZ definite dose injection, one week before larvae infection or larvae antigen injection twice weekly for six successive weeks and blood glucose levels were detected

daily for one week after experiments in all groups, then mice were sacrificed.

Detection of body weight

The body weight of mice from all treated groups will be recorded to the nearest grams on a digital balance at the beginning of the experiment. The percentage of the change in the total body weight (% T.B.W.) was determined at the end of the experiment as:

Final b.wt – Initial b.wt / Initial b.wt × 100

Determination of blood glycose levels

Mice from every group had their blood glycose levels measured using Accu-Chek Active device man_07135980001_02_EN-ROW.indb2 (Roche Diabetes Care Company). The top third of the mouse's tail was coated with a thin layer of lidocaine gel (2–5%) for 30 minutes, and then was covered with a little piece of Parafilm. Following superficial anesthesia, the cover was removed, and a tiny and sterile needle enclosed with the device was used to puncture the tail vein. Mouse's blood glucose level was measured after one drop of fresh blood was placed on the device strip and inserted within the apparatus.

Histopathological examination:

Small specimens of the liver and pancreas of animal groups were collected and fixed in 10% of neutral formalin for 24 hrs. Sections were dehydrated through sequences of ethyl alcohol. The sections were cleared through xylol. Impregnated by paraffin wax, molten at (58-60˚C). Embedded in a mold containing a molten paraffin wax. Paraffin-embedded sections were cut at 5 µm by microtome and stained with eosin and hematoxylin (H&E). Mounted by Canada Balsam for histopathological studies **(Bancroft and Gamble, 2008).**

Statistical analysis

Statistical analyses were performed using SPSS software. Data is represented as Mean ± SE, of three replicates. The One-Way Analysis of Variance (ANOVA) was used to evaluate the effects of two independent factors; diabetes infection and *T. spiralis* immunomodulation on the dependent variable, which was b.wt and blood glucose level separately.

Ethical approval

All animal experiments and protocols are approved by the ethical committee of Faculty of Science, Tanta University, Tanta, Egypt (Code No. IACUC-SCI-TU-0205).

Ethical guidelines

In accordance with the principles of the Basel Declaration, the authors affirm their adherence to the highest standards of scientific integrity, reproducibility, and responsibility in this study.

Results

Effect of inoculation of *T. spiralis* larvae and administration of *T. spiralis* larvae antigen on the mice body weight change

Prophylactic implication

Body weight dramatically drops (G2). However, there was no discernible difference between the treated group with a low inoculum dose of *T. spiralis* (G3) and G2, but the treated group with a high inoculum dose of *T. spiralis* (G4) exhibited an increase in weight outcomes. Larvae antigen (G5 & G6) treatments, especially at high dosages (G6), aids in restoring body weight to levels that are almost normal (G1) Fig. (2).

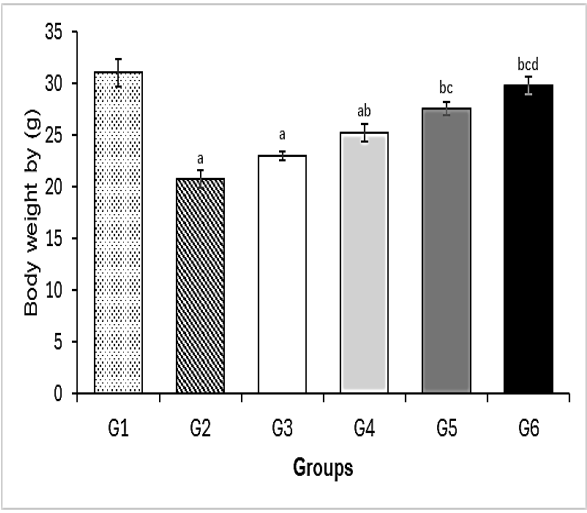

Fig. (2): Body weight of mice in the prophylactic protocol. G1: normal mice; G2: T1-DM non-infected mice; G3; T1-DM mice pre-infected with a low inoculum dose of *T. spiralis*; G4: T1-DM mice pre-infected with a high inoculum dose of *T. spiralis*; G5: diabetic mice pre-injected with low dose of *T. spiralis* larvae antigen; G6: T1-DM mice pre-injected with high dose of *T. spiralis* antigen. Letters (a, b, c, d) denote statistical groupings. Groups sharing the same letter are not significantly different (p> 0.05), while different letters indicate significant differences (p< 0.05).

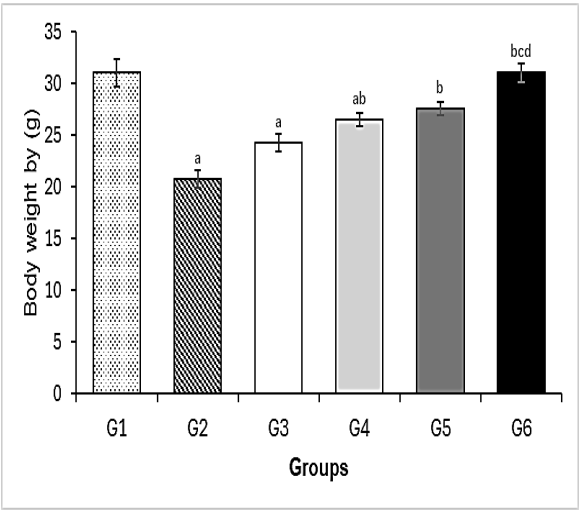

Fig. (3): Body weight of mice in the therapeutic protocol. G1: normal mice; G2: T1-DM non-infected mice; G3; T1-DM mice post-infected with a low inoculum dose of *T. spiralis*; G4: T1-DM mice post-infected with a high inoculum dose of *T. spiralis*; G5: T1-DM mice post-injected with low dose of *T. spiralis* larvae antigen; G6: T1-DM mice post-injected with high dose of *T. spiralis* antigen. Letters (a, b, c, d) denote statistical groupings. Groups sharing the same letter are not significantly different (p> 0.05), while different letters indicate significant differences (p< 0.05).

Therapeutic effect

The body weight of G2 was significantly lower than that of control. There has been modest improvement in the experimental infection with larvae (G3, G4). Antigen treatment (G5, G6), especially high dose (G6), leads to nearly complete recovery of body weight which was significantly different from G2, G3, and G4 exhibited the best treatment outcome Fig. (3).

Effect of inoculation of *T. spiralis* larvae and administration of *T. spiralis* larvae antigen on blood glucose level

Prophylactic impact

The G2 has the highest glucose concentration, where both G1 and G2 have significantly different intermediate glucose levels than do G3, G4, and G5. Mice in G6 showed the lowest glucose level and significant difference than both G2 and G3 Fig. (4).

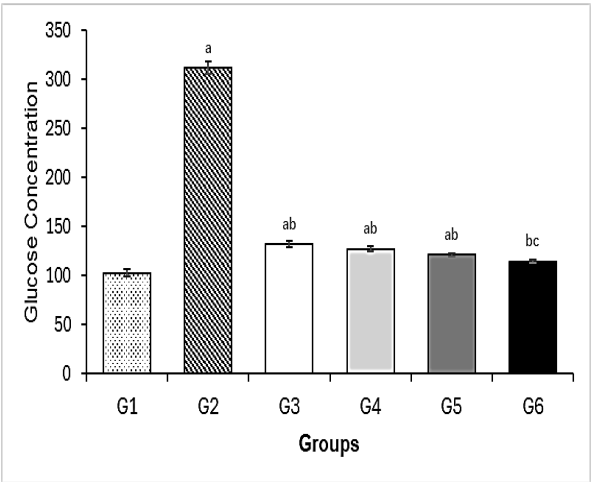

Fig. (4): Blood glucose concentration of mice in prophylactic protocol. G1: normal mice; G2: T1-DM non-infected mice; G3; T1-DM mice pre-infected with a low inoculum dose of *T. spiralis*; G4: T1-DM mice pre-infected with a high inoculum dose of *T. spiralis*; G5: T1-DM mice pre-injected with low dose of *T. spiralis* larvae antigen; G6: T1-DM mice pre-injected with high dose of *T. spiralis* antigen. Letters (a, b, c, d) denote statistical groupings. Groups sharing the same letter are not significantly different (p> 0.05), while different letters indicate significant differences (p< 0.05).

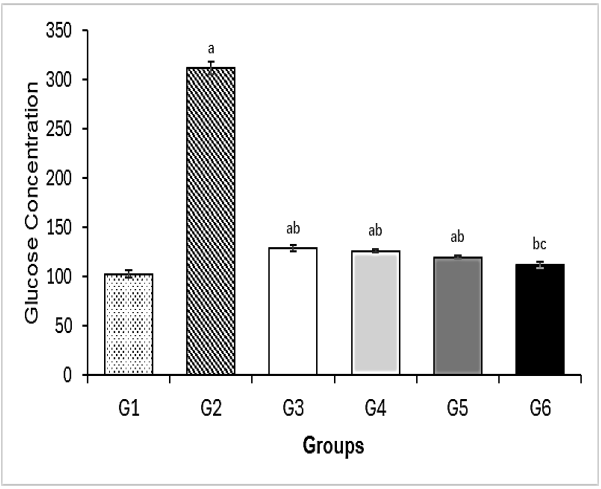

Fig. (5): Blood glucose concentration of mice in therapeutic protocol. G1: normal mice; G2: T1-DM non-infected mice; G3; diabetic mice post-infected with a low inoculum dose of *T. spiralis*; G4: T1-DM mice post-infected with a high inoculum dose of *T. spiralis*; G5: T1-DM mice post-injected with low dose of *T. spiralis* larvae antigen; G6: T1-DM mice post-injected with high dose of *T. spiralis* antigen. Letters (a, b, c, d) denote statistical groupings. Groups sharing the same letter are not significantly different (p> 0.05), while different letters indicate significant differences (p< 0.05).

Therapeutic Influence

T1-DM mice exhibit a substantial increase in blood glucose when compared to the control group. T1-DM mice treated with low and high inoculum dose *T. spiralis* larvae infection or low-dose larvae antigen are not significantly different from the control group and exhibit lower glucose levels than T1-DM mice. Mice treated with high doses of larvae antigen (G6) exhibit a further decrease in glucose concentration Fig. (5).

Histopathological observations liver

Prophylactic study

Light microscope examination of the liver sections of control group (G1) display normal hepatic construction, as evidenced by a normal central vein, normal radiating hepatocytes with centrally located nuclei and normal chromatin distribution, and a normal portal region that includes a bile ductule and portal vein. Normal Kupffer cells and a proper blood sinusoid are also shown Fig. (6a). Liver sections of T1-DM (G2) exhibited that the hepatic structure is clearly disorganized, the hepatic cords are disturbed, necrotic regions emerge, and the major veins widens and becomes congested.

Histopathological alterations of the nuclei include pyknotic and vacuolated nuclei, as well as irregular blood sinusoids were observed Fig. (6b). Figure (6c) shows liver sections with low inoculum doses of *T. spiralis* larvae. The hepatic structure is mildly improved, as evidenced by a normal central vein, a few hepatocytes with normal stainability, primarily vacuolated hepatocytes, others with pyknotic and karyolitic nuclei, and irregular and widening blood sinusoids. However, the hepatic architecture, which is characterized by dilated and congested central veins, does not improve with a high inoculum dose of *T. spiralis* larvae. Hepatocytes that are disorganized are seen to have irregular blood sinusoids, pyknotic, karyolitic, and vacuolated nuclei, as well as vacuolated cytoplasm Fig. (6d). Hepatic structures are enhanced in liver sections of low dosage larval antigen group (G5). Normal radiating hepatocytes with centrally localized nuclei and their stainability are present, as normal central veins; however, blood sinusoids with activated Kupffer cells are shown Fig. (6e). Additionally, liver sections from mice treated with high dose of larval antigen (G6) show a slight improvement in the hepatic structure; some hepatocytes have vacuolated cytoplasm, while others have megakaryocyte nuclei, a regular central vein, and a blood sinusoid with typical Kupffer cells Fig. (6f).

Therapeutic implication

The hepatic architecture is shown to improve with a low inoculum dose of larval infection; the central vein is regular, the hepatocytes are normal, the central nuclei have normal dispersed chromatin, only a few numbers are degenerated, and the blood sinusoids have normal Kupffer cells Fig. (7a). In addition, liver sections containing a high inoculum dose of larvae show a slight improvement in the hepatic structure; the central vein is uneven and expanding, and only a small percentage of hepatocytes are normal, primarily those with vacuolated cytoplasm. Histopathological alterations in nuclei include pyknotic and karyolitic nuclei were observed Fig. (7b). The hepatic structure is clearly improved in liver sections with a low concentration of larvae antigen, as evidenced by normal hepatocyte cords with normal stainability and nuclei, a small number of hepatocytes with megakaryocytic nuclei, a regular central vein, and regular blood sinusoids with activated Kupffer cells Fig. (7c). Nevertheless, liver sections treated with high dose of larvae antigen showed a slight improvement in the hepatic structure; there were irregular blood sinusoids with normal Kupffer cells, a normal central vein, normal radiating hepatic cords, some hepatocytes with vacuolated cytoplasm, and the appearance of necrotic areas Fig. (7d).

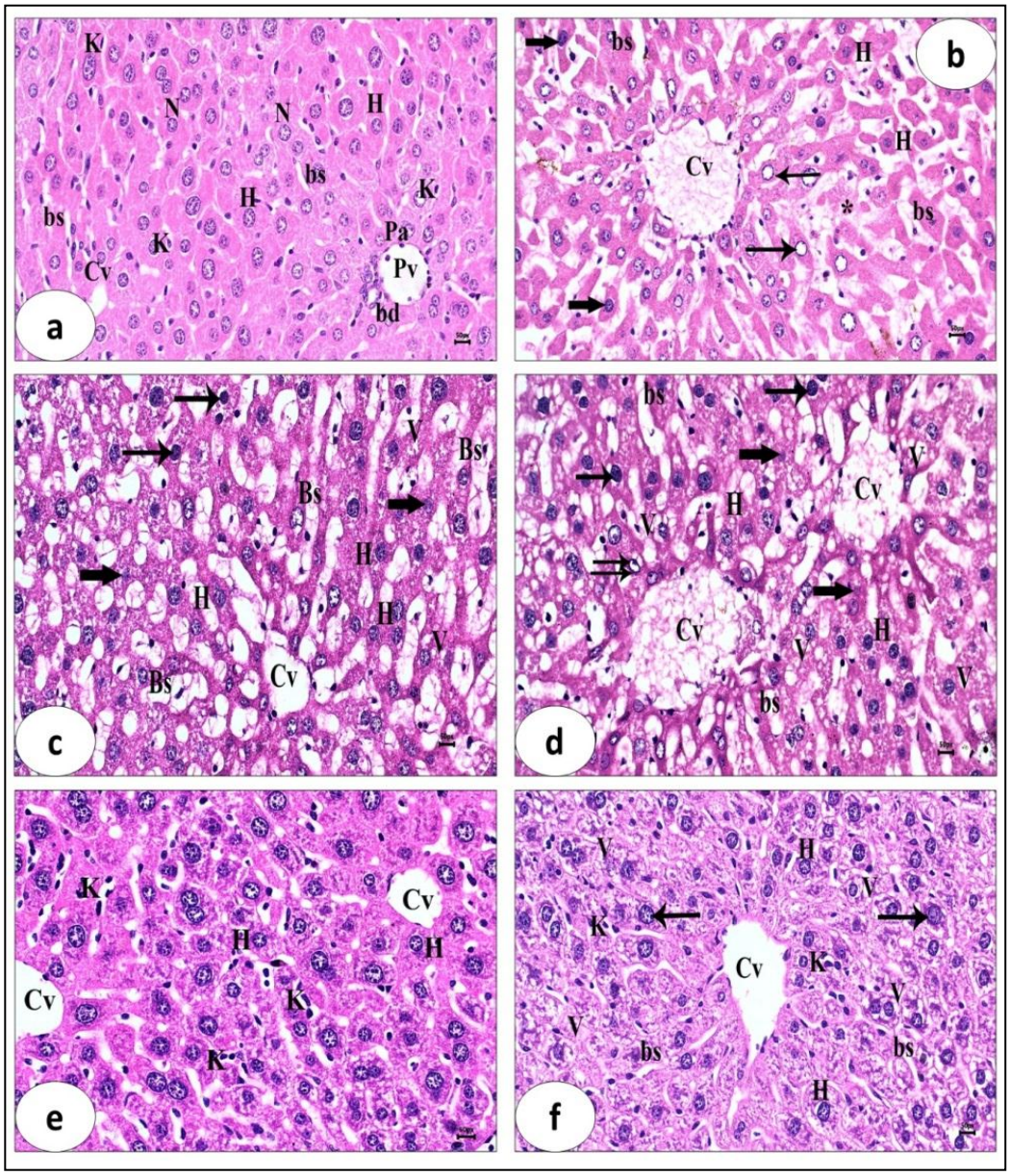

Fig.(6): Photomicrographs of liver sections of different groups in prophylactic study stained with H&E (X 400): a) Liver sections of control group showing normal hepatic structure represented by a normal central vein (Cv), normal radiating hepatocyte (H) with centrally localized nuclei (N), and normal portal area (Pa) which contain portal vein (Pv) and bile ductule (bd). Also, regular blood sinusoid (bs) with normal Kupffer cells (K). **b)** Liver sections of T1-DM mice (+ve control) illustrate marked disorganization of the hepatic structure, disrupted hepatic cords (H), appearance of necrotic areas (*), widening and congested central vein (Cv), vacuolated nuclei (arrows) and pyknotic ones (thick arrows), also irregular blood sinusoids (bs) were observed. **c**) Liver sections of a prophylactic low inoculum doses of *T. spiralis* larvae showing mild improvement of the hepatic structure; normal central vein (Cv), few ones of hepatocytes are normal (H) with normal stainability, mostly ones with vacuolated cytoplasm (V), Others with pyknotic nuclei (arrows) and karyolitic ones (thick arrows). Blood sinusoids are irregular and widening (bs). **d**) Liver section of a prophylactic high inoculum doses of *T.spiralis* larvae displays no improvement in the hepatic construction; dilated and congested central veins (Cvs), disorganized hepatocytes (H), mostly hepatocytes with vacuolated cytoplasm (V) and their nuclei are pyknotic (arrows), karyolitic (thick arrows) and vacuolated ones (double arrows). Also, blood sinusoids are irregular (bs). e) Liver sections of a prophylactic low dose larval antigen showing enhancement of the hepatic structure; normal radiating hepatocytes (H) with centrally localized nuclei and retain their stainability, the central veins (Cvs) are normal, but blood sinusoids with activated Kupffer cells (K). f) Liver sections of a Prophylactic high dose larval antigen explain mild improvement in the hepatic construction; few no of hepatocytes (H) are degenerated (V), others with megakaryocyte nuclei (arrows), regular central vein (Cv). and blood sinusoid (bs) with normal Kupffer cells (K).

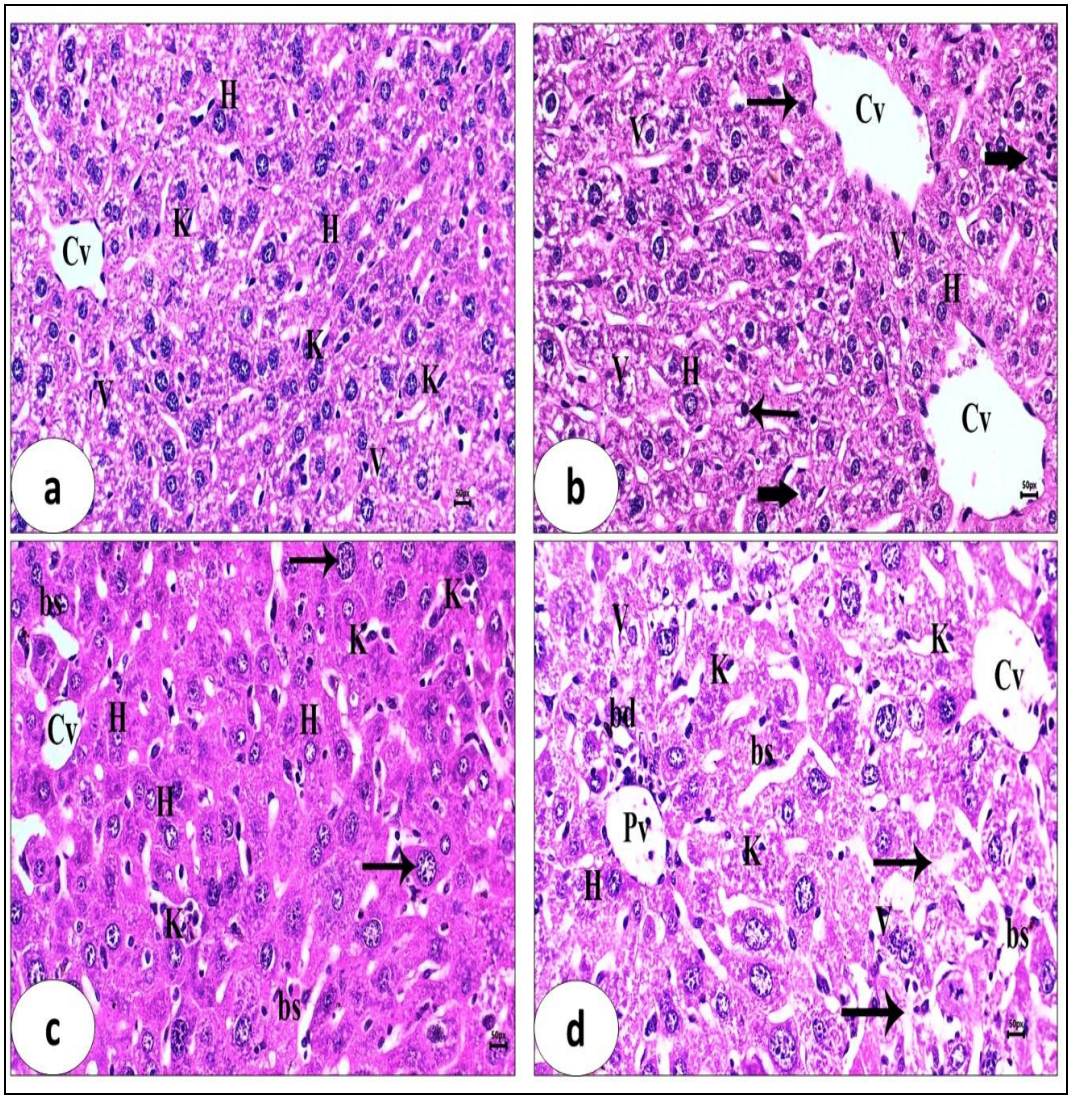

Fig. (7): Photomicrographs of liver sections of different groups in therapeutic study stained with H&E (X 400): a) Liver section of a therapeutic low inoculum doses of *T.spiralis* larvae explains noticed improvement of the hepatic architecture; regular central vein (Cv), normal hepatocytes (H) with normal central nuclei that have normal distributed chromatin, few ones are degenerated (V), regular blood sinusoids with normal Kupffer cells (K). **b**) Liver section of a therapeutic high inoculum doses of *T.spiralis* larvae exhibits mild improvement of the hepatic construction; irregular and widening central vein (Cv), few numbers of hepatocytes are normal (H), mostly ones with vacuolated cytoplasm (V). Nuclei show some histopathological changes, such as pyknotic nuclei (arrows) and karyolitic ones (thick arrows) (X400). c) Liver sections of a Therapeutic low dose larval antigen exhibit obvious enhancement of the hepatic structure, normal cords of hepatocytes (H) with normal stainability and normal nuclei, few numbers of hepatocytes with megakaryocytic nuclei (arrows), regular central vein (Cv), regular blood sinusoids with activated Kupffer cells (K). d) Liver sections of a therapeutic high dose larval antigen showing mild improvement of the hepatic structure; normal central vein (Cv), normal radiating hepatic cords, some hepatocytes with vacuolated cytoplasm (V), appearance of necrotic areas (arrows) and irregular blood sinusoids (bs) with normal Kupffer cells (K).

Pancreas

Prophylactic study

Figure (8a) illustrated pancreatic sections of control mice that revealed typical architecture. The exocrine component forms are firmly packed by acinar cells. Interlobular connective tissue septa were intact and separated the pancreatic lobules. Interspersed among the acinar cells were islets of Langerhans. The intralobular duct was visible, and the islets were less stained than the surrounding acinar cells**.** Many histopathological changes in pancreatic sections of a diabetic mice (G2) were observed including atrophy and cytoplasmic degenerative changes in the majority of islet cells, particularly in the middle of the islet (notice the uneven outlining of the islet). Additionally, disrupted acinar cells with degenerated lining epithelia (some of them with pyknotic nuclei), Intralobular duct with degenerated lining epithelia, and necrotic area and congested blood vessel were noticed Fig. (8b). While pancreatic sections of low inoculum dose of larvae infected mice (G3) show mild improvement of the pancreatic architecture with nearly regular outline of islets, normal appearance of acinar cells and regular size of interlobular septa. Irregular intralobular ducts and few hemorrhages were observed Fig. (8c). Diabetic mice pre-infected with high inoculum dose of larvae (G4) show no improvement of the pancreatic structure; irregular islets of Langerhans, few numbers of acinar cells are normal, intralobular ducts are proliferated and congested blood vessels are noticed Fig. (8d).

Furthermore, there is a noticeable enhancement of the pancreatic architecture including normal appearance of most acinar cells and regularity of the interlobular septa Fig. (8e) in all diabetic mice pre-injected with low dose of larval antigen. The improvement kept limited in those pre-injected with the high dose of larval antigen, where most of acinar cells are normal and few enclose pyknotic nuclei, interlobular septa appear regular and few leukocytic infiltration was detected surrounding islet of Langerhans Fig. (8f).

Therapeutic study

Administration of a low inoculum dose of larvae (G3) preserved near-normal pancreatic architecture, characterized by regular islets of Langerhans, intact acinar cells with homogeneous cytoplasm, and normal nuclei Fig. (9a). In contrast, a high inoculum larval dose (G4) induced mild improvement, with mostly normal acinar cells but occasional vascular congestion Fig. (9b). Treatment with a low concentration of larvae antigen demonstrated significant tissue recovery, featuring well-organized islets, predominantly normal acinar cells, minor leukocytic infiltration Fig. (9c), and slight interlobular septal widening. Conversely, a high-concentration antigen elicited only modest improvement, with regular islets, scattered acinar cell with pyknotic nuclei, and edema-induced occlusion of intralobular ducts Fig. (9d). These findings suggest dose-dependent therapeutic effects, with low-concentration antigen offering the most pronounced histological restoration.

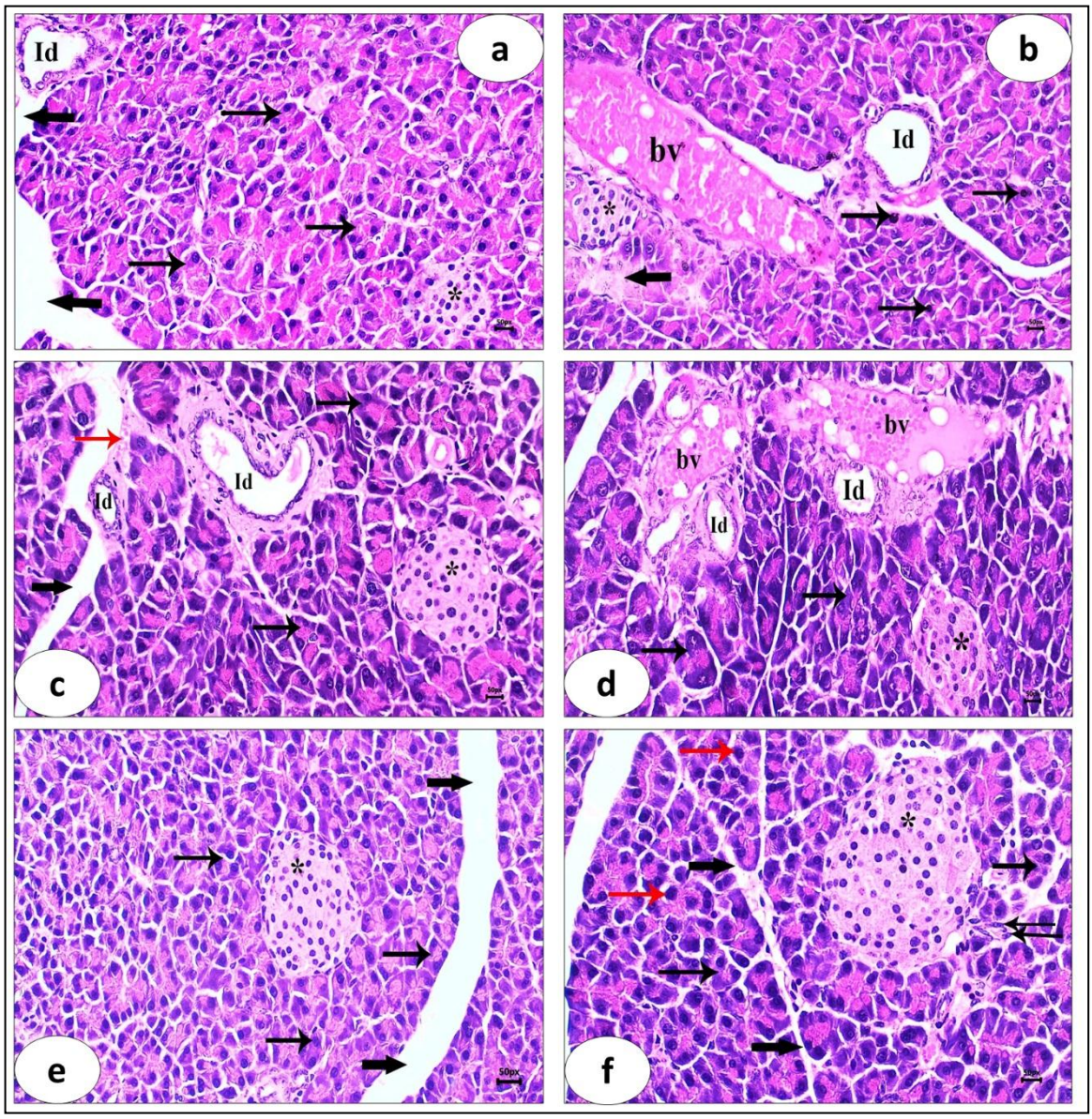

Fig. (8): Photomicrographs of pancreatic sections of different groups in prophylactic study stained with H&E (X 400). a) Pancreatic sections of control mice revealed normal architecture. The exocrine component forms are tightly packed by acinar cells (arrows). Interlobular connective tissue septa (thick arrows) were intact and separated the pancreatic lobules, islets of Langerhans (*). The intralobular duct was visible (Id)**. b**) Pancreatic sections of T1-DM mice (group II) showing some histopathological alterations; atrophy and cytoplasmic degenerative changes in most islet cells, especially in center of the islet (*). Notice the irregular outlining of the islet. Disrupted acinar cells with degenerated lining epithelia (arrows), some of them with pyknotic nuclei (arrows), congested blood vessel (bv). Intralobular duct (Id) with degenerated lining epithelia and necrotic area were noticed (thick arrow). **c**) Pancreatic sections of a prophylactic low inoculum doses of *T. spiralis* larvae showing mild improvement of the pancreatic architecture showing the nearly regular outline of an islet (*) with apparently normal appearance of most acinar cells (arrows) and regular interlobular septa (thick arrow). Irregular intralobular ducts (Id) and few hemorrhage (red arrow) were observed (Id)**. d**) Pancreatic sections of a prophylactic high inoculum doses *T. spiralis* larvae showing no improvement of the pancreatic structure; irregular islets of langerhan's (*), few numbers of acinar cells are normal (arrows), intralobular ducts are proliferated (Id) and congested blood vessels are noticed (bv). e) Pancreatic sections of a Prophylactic low dose larval antigen showing improvement of the pancreatic architecture showing normal and nearly regular outline of an islet cells (*) with apparently normal appearance of most acinar cells (arrows) and regular interlobular septa (thick arrows). f) Pancreatic sections of a Prophylactic high dose larval antigen exhibit mild improvement of the pancreatic tissue; normal islet of Langerhans (*), mostly acinar cells are normal (arrows), others with pyknotic nuclei (red arrows), regular interlobular septa (thick arrows) and few leukocytic infiltration was noticed surrounding islet (double arrows).

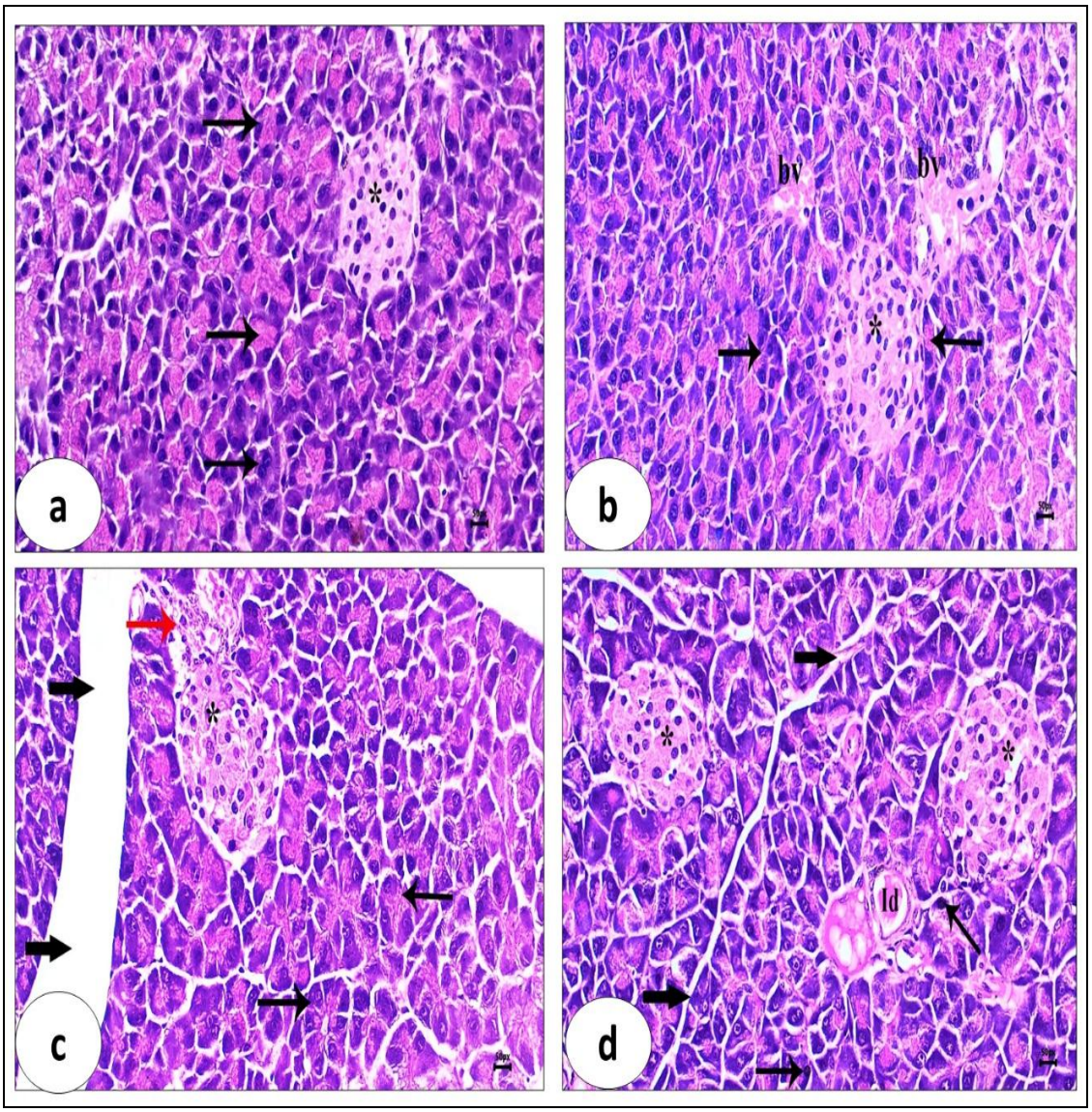

Fig. (9): Photomicrographs of pancreatic sections of different groups in therapeutic study stained with H&E (X 400). a) Pancreatic sections of a therapeutic low inoculum doses of T. spiralis larvae showing normal like structure of the pancreatic tissue; normal islet of langerhan's with regular outline (*), normal acinar cells with homogenous cytoplasm and normal nuclei (arrows). b) Pancreatic sections of a therapeutic high inoculum doses of T. spiralis larvae exhibits mild improvement that is represented by normal islet cells (*), mostly acinar cells are normal (arrows) but few numbers of blood vessels are congested (bv). C) Pancreatic sections of a therapeutic low dose larval antigen reveal improvement of the pancreatic architecture; regular islets of langerhan's (*), mostly acinar cells are normal (arrows), few ones are damaged; few leukocytic infiltration (red arrow) and slight widening of interlobular septa (thick arrows) were observed. d) Pancreatic sections of a therapeutic high dose larval antigen showing mild improvement; normal and regular islet cells (*), few numbers of acinar cells with pyknotic nuclei (arrows), regular inter-lobular septa (thick arrows) and intralobular duct with degenerated lining epithelia and occluded with edema (Id).

Discussion

Helminths and their derivatives have been proposed as a potent weapon against various inflammatory conditions, including allergic disorders, colitis, arthritis, T1-DM Crohn's disease, asthma and encephalomyelitis **(Kuijk & van Die, 2010; McSorley et al., 2013)**; such effects are mainly based on the ability of helminths and their antigens to induce strong Th2-biased responses, with increases in cytokines such as IL-4, IL-10, IL-13, and TGF-β **(Reyes & Terrazas, 2007; Maizels & McSorley,**

2016). Furthermore, they promote the activation of regulatory cells, including alternatively activated macrophages (AAMϕs), T-reg, and MDSCs, which are associated with reduced inflammatory responses, diminished tissue damage, and enhanced wound healing processes. Recently, various immunological down-regulatory substances derived from parasites have been identified and considered as safe alternatives to live parasite infections **(Du et al., 2011; Jang et al., 2011)** . Consequently, supplementary strategies are essential for the advancement of an alternative immunotherapy for T1-DM.

TI-DM is an autoimmune disorder characterized by the destruction of insulin-producing β-cells by T cells, CD4+, CD8+ and classically activated macrophages CAMϕs **(Denis et al., 2004; Jahromi & Eisenbarth, 2007)**. In this study, we tried to elucidate the potential effects of *T. spiralis* infection and larvae-derived antigens on induced T1- DM in mice.

In the present work, T1-DM mice (G2) had a significant increase in the total % body weight. While groups infected with low dose and high dose of *T. spiralis'* larvae or injected with larvae-derived antigen, in both prophylactic and therapeutic protocols, achieved raising in weight, particularly at high dosages (G6). This is alien with the findings of **Yan et al. (2020)**, who detected that the body weights of T1-DM mice decreased faster than those of the mice in the recombinant fructose-1,6-bisphosphate aldolase (rSjFBPA) and *S. japonicum* cystatin (rSjcystatin) treated groups.

The T1-DM mice differs considerably from normal mice in control group (G1), where it exhibits the highest glucose concentration. The prophylactic infection of *T. spiralis* larvae or injection with its antigen decrease the blood glucose levels. The treatment with high dose larvae antigen (G6), brings glucose levels closer to normal. Previous study's findings showed that helminth-mediated defense against autoimmune disease could support filaria **(Hübner et al., 2009)** and *T. crassicep*s **(Espinoza-Jiménez et al., 2017)** infection to shield NOD mice from developing hyperglycemia, insulitis and T1-DM. The latest study assessed the potential positive effects of *T. crassiceps*-derived products, specifically soluble (TcS) and excreted/secreted (TcES) antigens, on the progression of experimental T1-DM. Administration of varying dosages prior to or subsequent to the onset of T1-DM.

The therapeutic infection of *T. spiralis* larvae (G3, G4) and its antigen injection (G5, G6) significantly reduces elevated blood glucose levels in the diabetic mice, restoring them to virtually normal physiological ranges. These results were comparable with **Amdare et al. (2015)**, who showed that the recombinant *Brugia malayi* abundant larval transcript 2 (rBmALT-2) and *Wuchereria bancrofti* L2 (rWbL2) proteins, provided either separately or in combination, resulted in decreased blood glucose levels and a reduction in the incidence of T1-DM in mice and recorded that the therapeutic effect was linked to the inhibition of tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) release, with an augmented synthesis of IL-4, IL-5, and IL-10 by the splenocytes of diabetic mice.

Histopathological analyses further support these findings, demonstrating that *T. spiralis* intervention ameliorates diabetes-induced hepatic and pancreatic damage. The hepatic tissues of the T1-

DM mice revealed disrupted hepatic cords, appearance of necrotic patches, a widened and congested central vein, aberrant hepatocyte nuclei (vacuolated and pyknotic), and irregular blood sinusoids. These results were in consistent with **Belal et al. (2016)**, who elucidated that diabetes induced by ALX or STZ results in biochemical changes in the blood and pathophysiological alterations in the liver of rats, characterized by diffuse macrovesicular fatty degeneration, occasionally accompanied by mild periportal fibrosis and interstitial mononuclear inflammatory infiltrates (steatohepatitis). Although there is a slight improvement in the hepatic tissue of diabetic mice prophylactically infected with low inoculum dose of larvae, the hepatocytes were primarily vacuolated, and the blood sinusoids were irregular. Therapeutically, low inoculum larval dose showed observable improvement with normal hepatocytes and regular central veins. Slight improvement is seen in the T1-DM mice post infected with high larvae dose, where number of hepatocytes exhibiting vacuolated or pyknotic nuclei and aberrant central veins were detected. Unexpectedly, low dose derived – antigen injection clearly improves the condition of normal hepatocytes and regular central veins, while the high dose showed only a slight enhancement. However, it was the first report to clarify the potential effects of *T. spiralis* infection and Trichinella-derived antigens or helminth derivatives on the hepatic tissue in the diabetic mice and presented a more advanced approach.

The pancreatic sections of diabetic mice displayed a significant damage, including damaged acinar cells, enlarged blood vessels, atrophy, and degeneration of islet cells. Also, irregular islet outlines. These results were in consistent with **Amdare et al. (2017),** who mentioned that histopathological examination of the pancreatic tissue of the mice in the control groups (STZ and STZ-Alum groups) showed cell destruction and severe insulitis with a higher degree of lymphocyte infiltration and inflammation. Prophylactic infection of high inoculum dose of larvae did not demonstrate any improvement, further more low dose provided a slight improvement. While, pancreatic sections of a prophylactic low and high doses larval antigen exhibit improvement of the pancreatic tissue, normal islets of Langerhans and mostly acinar cells are normal, and regular interlobular septa are present. These results were in accordance with **Yan et al. (2020)**, who reported that prophylactic administration of the two proteins of *S. japonicum* (rSjFBPA and rSjcystatin) remarkably reduced the incidence of diabetes and effectively attenuated islet inflammation and the degree of damage in the experimental animals.

Therapeutic treatment with both low inoculum doses of larvae infection or larvae-derived antigen produced notable benefits that were represented by normal acinar cells and islets of Langerhans with regular outlines. Only slight benefits were seen with high inoculum dose of larvae and its antigen; mostly acinar cells are normal, with a few with pyknotic nuclei and widening of the interlobular septa, as previously reported by **(Amdare et al., 2017)**, who stated that two filarial proteins (rBmALT-2) and (rWbL2) have led to reduced lymphocytic infiltration and decreased islet damage as well as inflammation.

Also**, Singh et al., (2024)** reported that *Wuchereria bancrofti* Macrophage migration inhibitory factor-2 (Wba-MIF2) therapy may effectively inhibit activated macrophage infiltration, which would otherwise contribute to the advancement of T1-DM. It could be concluded that *T. spiralis* infection and its larval-derived antigens have a promising immunomodulatory effect of in mitigating Type 1 Diabetes Mellitus (T1DM) in mice. Both prophylactic and therapeutic administration of *T. spiralis* larvae or their antigens significantly improved key diabetic markers, including body weight stabilization, reduced hyperglycemia, and restoration hepatic and pancreatic tissues damage induced by diabetes.

References

Ajendra, J., Min, L., Yao, S., Hou, Y., Zhao, X., Wang, H., & Shen, J. (2016). Helminth infections protect non-obese diabetic mice from autoimmune diabetes by inducing a shift from Th1 to Th2 immune responses. *J. Helminthol*. *90*(5): 582–590. https://doi.org/10.4049/jimmunol.1100335

Amdare, M. D., Pathan, M. M., & Pagar, M. P. (2015). Antidiabetic activity and histopathological study of *Bauhinia racemosa* Lam. in alloxan-induced diabetic mice. *Asian Pac. J. Trop. Biomed.* *5*(1): 11–15. https://doi.org/10.4103/0976-500X.92518

Amdare, N., Khatri, V., Yadav, R., Tarnekar, A., Goswami, K., & Reddy, M. (2017). Therapeutic potential of the immunomodulatory proteins Wuchereria bancrofti L2 and Brugia malayi abundant larval transcript 2 against streptozotocin-induced type 1 diabetes in mice. *J. Helminthol.*, 91(5): 539-548. https://doi.org/10.1017/s0022149x1600064x

Bashi, T. ; Bizzaro, G. ; Shor, D.; Blank, M.; Shoenfeld, Y. (2014). The mechanisms behind helminth's immunomodulation in autoimmunity. *Autoimmun. Rev.*, 14. 10.1016/j.autrev.2014.10.004.

Bancroft, J. D.; Gamble, M. (2002). Theory and Practice of Histo logical and Histochemical techniques 5 th ed. Churchill Livingstone, London, New York and Philadelphia, Cancer communications, 3(7): 207-212.

Belal, A. S., El-Mahdy, A. R., & El-Sayed, H. H. (2016). Hepatic and renal histopathological alterations associated with experimentally induced diabetes mellitus in rats. *IJARBS, 3*(6): 45–54.

Bien, J., Cabaj, W., & Moskwa, B. (2013). Recognition of antigens of three different stages of the Trichinella spiralis by antibodies from pigs infected with T. spiralis. *Exp. Parasitol.*, 134(2): 129-137. https://doi.org/10.1016/j.exppara.2013.02.007

Bruschi, F. ; Ashour, D. ; Othman, A. (2022). Trichinella-induced immunomodulation: Another tale of helminth success. *IAFWP*, 27. e00164. 10.1016/j.fawpar.2022.e00164.

Denis, M. C., Mahmood, U., Benoist, C., Mathis, D., & Weissleder, R. (2004). Imaging inflammation of the pancreatic islets in type 1 diabetes. *PNAS.* 101(34): 12634-12639. https://doi.org/10.1073/pnas.0404307101

Ding J, Bai X, Wang X, Shi H, Cai X, Luo X, Liu M, Liu X. (2017). Immune Cell Responses and Cytokine Profile in

Intestines of Mice Infected with *Trichinella spiralis*. Front. Microbiol. 2017 Oct 31;8:2069. https://doi: 10.3389/fmicb.2017.02069

Du, L., Tang, H., Ma, Z., Xu, J., Gao, W., Chen, J., Gan, W., Zhang, Z., Yu, X., & Zhou, X. (2011). The protective effect of the recombinant 53-kDa protein of Trichinella spiralis on experimental colitis in mice. *Dig. Dis. Sci.,* 56(10): 2810-2817. https://doi.org/10.1007/s10620-011-1689-8

Dvorožňáková, E., Hurníková, Z., & Kołodziej-Sobocińska, M. (2011). Development of cellular immune response of mice to infection with low doses of Trichinella spiralis, Trichinella britovi and Trichinella pseudospiralis larvae. *Parasitol. Res.,* 108: 169-176. https://doi.org/10.1007/s00436-010-2049-x

Espinoza-Jiménez, A., De Haro, R., & Terrazas, L. I. (2017). Taenia crassiceps antigens control experimental type 1 diabetes by inducing alternatively activated macrophages. Mediators Inflamm. *2017*(1), 8074329. https://doi.org/10.1155/2017/8074329

Fogang, B.A.; Debrah, L.B.; Owusu, M.; Agyei, G.; Meyer, J.; Gmanyami, J.M.; Ritter, M.; Arndts, K.; Adu Mensah, D.; Adjobimey, T.; et al. Helminth Coinfections Modulate Disease Dynamics and VaccinatLiion Success in the Era of Emerging Infectious Diseases. *Vaccines* 2025, 13: 436. https://doi.org/10.3390/vaccines13050436

Huang, H., Hu, D., Chen, Z., Xu, J., Xu, R., Gong, Y., Fang, Z., Wang, T., & Chen, W. (2022). Immunotherapy for type 1 diabetes mellitus by adjuvant-free Schistosoma japonicum-egg tip-loaded asymmetric microneedle patch (STAMP). *JNB. 20*(1): 377. https://doi.org/10.1186/s12951-022-01581-9

Hübner, M. P., Thomas Stocker, J., & Mitre, E. (2009). Inhibition of type 1 diabetes in filaria-infected non-obese diabetic mice is associated with a T helper type 2 shift and induction of FoxP3+ regulatory T cells. *J. Immunol. 127*(4): 512-522. https://doi.org/10.1111/j.1365-2567.2008.02958.x

Jahromi, M. M., & Eisenbarth, G. S. (2007). Cellular and molecular pathogenesis of type 1A diabetes. *CMLS.* 64(7): 865. https://doi.org/10.1007/s00018-007-6469-4

Jang, S. W., Cho, M. K., Park, M. K., Kang, S. A., Na, B.-K., Ahn, S. C., Kim, D.-H., & Yu, H. S. (2011). Parasitic helminth cystatin inhibits DSS-induced intestinal inflammation via IL-10+ F4/80+ macrophage recruitment. *KJP*. 49(3): 245. https://doi.org/10.3347/kjp.2011.49.3.245

Khueangchiangkhwang, S., Wu, Z., Nagano, I., & Maekawa, Y. (2023). Trichinella pseudospiralis-secreted 53 kDa protein ameliorates imiquimod-induced psoriasis by inhibiting the IL-23/IL-17 axis in mice. *Biochem. Biophys. Rep.* , 33: 101415. https://doi.org/10.1016/j.bbrep.2022.101415

Kuijk, L. M., & van Die, I. (2010). Worms to the rescue: can worm glycans protect from autoimmune diseases? *IUBMB life*, 62(4): 303-312. https://doi.org/10.1002/iub.304

Maizels, R. M., & McSorley, H. J. (2016). Regulation of the host immune system by helminth parasites. *J. Allergy Clin. Immunol.*, 138(3), 666-675. https://doi.org/10.1016/j.jaci.2016.07.007

McSorley, H. J., Hewitson, J. P., & Maizels, R. M. (2013). Immunomodulation by helminth parasites: defining mechanisms and mediators. *Int. J. Parasitol.*, 43(3-4): 301-310. https://doi.org/10.1016/j.ijpara.2012.11.011

McSorley, H. J., & Maizels, R. M. (2012). Helminth infections and host immune regulation. *Clin Microbiol Rev.*, 25(4), 585-608. https://doi.org/10.1128/cmr.05040-11

Reyes, J., & Terrazas, L. (2007). The divergent roles of alternatively activated macrophages in helminthic infections. *Parasite Immunol.*, 29(12): 609-619. https://doi.org/10.1111/j.1365-3024.2007.00973.x

Singh, I., Hoti, S., Chauhan, N., Joshi, R., Prasad, T. K., Sarikhani, M., Kaushik, M., Unger, B. S., Jadhav, P., & Modi, P. K. (2024). Immunomodulation of streptozotocin induced Type 1 diabetes mellitus in mouse model by Macrophage migration inhibitory factor-2 (MIF-2) homologue of human lymphatic filarial parasite, *WBAT*. 252: 107142. https://doi.org/10.1016/j.actatropica.2024.107142

Yan, C., Wang, W., Sun, G. G., Liu, R. D., Song, Y. Y., Jiang, P., Zhang, X., Wang, Z. Q., & Cui, J. (2020). Trichinella spiralis-derived proteins promote regulatory T cell responses and ameliorate autoimmune diabetes in NOD mice. *Front. Immunol.*, 11: 1802. https://doi.org/10.3389/fimmu.2025.1637569

التأثيرات المُعدِّلة للمناعة لطفيلي "Trichinella spiralis" على مرض السكري المُستَحث بواسطة الستربتوزوتوسين في الفئران: الدلالات الوقائية والعلاجية.

كريم بكر هلال، أ.د/ نهله عبد العليم رضوان، أ.د/ محمد ناصف محمد، د/ منى محمد علوان
قسم علم الحيوان - كلية العلوم- جامعة طنطا - طنطا- مصر

العبء العالمي المتزايد لمرض السكري، ولا سيما داء السكري من النوع الأول (T1-DM)، يبرز الحاجة إلى استراتيجيات علاجية مبتكرة. تُظهر العدوى بالديدان الطفيلية، مثل T. spiralis (Trichinella spiralis) ، تأثيرات قوية في تعديل الاستجابة المناعية، مما قد يساهم في التخفيف من الحالات المناعية الذاتية. تستكشف الدراسة الحالية الإمكانات الوقائية والعلاجية للعدوى بـ *T. spiralis* والـأنتيجينات المستخلصة من يرقاتها في فئران مصابة بداء السكري من النوع الأول، مع التركيز على التجديد النسيجي لأنسجة البنكرياس والكبد. تم تقسيم مئة وعشرين (١٢٠) فأرًا من ذكور فئران Swiss albino إلى مجموعتين: وقائية وعلاجية، كل منهما تضم ست مجموعات (عدد الأفراد في كل مجموعة = ٢٠) على النحو التالي: المجموعة الأولى (G1): مجموعة ضابطة سلبية، المجموعة الثانية (G2): فئران مصابة بداء السكري من النوع الأول تم حقنها بستربتوزوتوسين (STZ) بجرعة ٠,٠٤ ملغم/غم، المجموعة الثالثة (G3): فئران مصابة بعدد منخفض من يرقات *T. spiralis* (200 يرقة/فأر) ، المجموعة الرابعة (G4): فئران مصابة بعدد مرتفع من يرقات T. spiralis (500 يرقة/فأر)، المجموعة الخامسة (G5): فئران حُقنت داخل الصفاق بجرعة منخفضة (١٠٠ ميكرولتر/فأر من محلول بتركيز ٥٠٠ ميكروغرام/مل) من أنتيجينات اليرقات، المجموعة السادسة (G6): فئران حُقنت داخل الصفاق بجرعة مرتفعة (١٠٠ ميكرولتر/فأر من محلول بتركيز ١٠٠٠ ميكروغرام/مل) من أنتيجينات اليرقات. تم تقييم مستويات سكر الدم، وتغيرات الوزن، كما أُجري فحص نسيجي للكبد والبنكرياس باستخدام صبغة الهيماتوكسيلين والإيوسين (H\&E). وقد تم تقييم الدلالة الإحصائية باستخدام تحليل التباين (ANOVA). أظهرت المعالجة الوقائية باستخدام أنتيجينات T. spiralis (G5, G6) انخفاضًا ملحوظًا في فرط سكر الدم واستعادة في وزن الجسم مقارنة بمجموعة السكري (G2). كما أظهرت المعالجة بجرعة مرتفعة من الأنتيجينات (G6) مستويات جلوكوز قريبة من الطبيعي، مع الحفاظ على البنية النسيجية للكبد وتقليل تدهوروتدميرخلايا بيتا في البنكرياس. أما العدوى المباشرة بـ *T. spiralis* في المجموعتين (G3 وG4) فقد أظهرت فاعلية محدودة مقارنة بالتعديل المناعي الموجه باستخدام الأنتيجينات. وقد أكّد البروتوكول العلاجي انخفاضًا في درجة نخر الكبد، وانتظامًا في شكل جزر البنكرياس، مع حد أدنى من االارتشاح الالتهابي في مجموعة الأنتيجينات ذات الجرعة المرتفعة. تُظهر أنتيجينات *T. spiralis* ، وخاصة عند الجرعات العالية، إمكانات قوية من الناحيتين الوقائية والعلاجية ضد داء السكري من النوع الأول. وتُعزز هذه النتائج الآمال في استخدام معدلات المناعة المستخلصة من الديدان كعلاج جديد لمرض السكري المناعي الذاتي.