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Cytokines, Chemokines, Insulin and Haematological Indices in Type 2 Diabetic Male Sprague Dawley Rats Infected with *Trichinella zimbabwensis*

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Abstract: Diabetes mellitus is a chronic metabolic disease induced by the inability to control high blood glucose level. Helminth-induced immunomodulation has been reported to prevent or delay the onset of type 2 diabetes mellitus (T2DM), which, in turn, ameliorates insulin sensitivity. Therefore, there is a need to understand the underlying mechanisms utilized by helminths in metabolism and the induction of immuno-inflammatory responses during helminthic infection and T2DM comorbidity. This study aimed at using a laboratory animal model to determine the cytokines, chemokines and haematological indices in diabetic (T2DM) male Sprague Dawley (SD) rats infected with *Trichinella zimbabwensis*. One hundred and two male SD rats (160–180 g) were randomly selected into three experimental groups (i. T2DM-induced group (D) ii. *T. zimbabwensis* infected + T2DM group (TzD) and iii. *T. zimbabwensis*-infected group (Tz)). Rats selected for the D group and TzD group were injected with 40 mg/kg live weight of streptozotocin (STZ) intraperitoneally to induce T2DM, while animals in the Tz and TzD group were infected with *T. zimbabwensis*. Results showed that adult *T. zimbabwensis* worm loads and mean *T. zimbabwensis* larvae per gram (lpg) of rat muscle were significantly higher ($p < 0.001$) in the Tz group when compared to the TzD group. Blood glucose levels in the D group were significantly higher ($p < 0.001$) compared to the TzD group. An increase in insulin concentration was observed among the TzD group when compared to the D group. Liver and muscle glycogen decreased in the D when compared to the TzD group. A significant increase ($p < 0.05$) in red blood cells (RBCs) was observed in the D group when compared to the TzD and Tz groups. An increase in haematocrit, haemoglobin, white blood cells (WBCs), platelet, neutrophils and monocyte were observed in the D group when compared to the TzD group. TNF-α, IFN-γ, IL-4, IL-10 and IL-13 concentrations were elevated in the TzD group when compared to the D and Tz groups, while IL-6 concentration showed a significant reduction in the Tz when compared to the D and the TzD groups. A significant increase in CCL5 in the D and TzD groups was observed in comparison to the Tz group. CXCL10 and CCL11 concentration also showed an increase in the TzD group in comparison to the Tz and the D groups. Overall, our results confirm that *T. zimbabwensis*, a parasite which produces tissue-dwelling larvae in the host, regulates T2DM driven inflammation to mediate a positive protective effect against T2DM outcomes.

Keywords: comorbidity; type 2 diabetes mellitus; haematological indices; insulin; cytokines; chemokines; helminths; immune response; *Trichinella zimbabwensis*

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1. Introduction

Type 2 diabetes mellitus (T2DM) is considered an inflammatory disease associated with the development of insulin resistance [1]. Globally, the prevalence of diabetes in

adults is expected to rise from 8.8% (424.9 million people) to 9.9% (628.6 million people) by 2045 [2]. This rising trend in T2DM can be attributed to ageing, a rapid increase in urbanization, as well as an increase in obesogenic environments [3].

Haematological parameters such as red blood cells (RBC), white blood cells (WBC), platelets (PLT), and other variables such as blood glucose and insulin levels are commonly used as clinical indicators of health and disease [4]. Changes detected in these parameters could provide an insight into the onset and spread of degenerative impediments in diabetes mellitus (DM) [5]. White blood cells are known to be fixed biomarkers of inflammation in metabolic diseases and in T2DM and its complications [6]. Studies have shown an increase in peripheral WBC (e.g., neutrophils, eosinophils and basophils) with no difference in monocytes counts in T2DM patients [7]. In addition, peripheral increased WBC count is known to be associated with insulin resistance, stroke, T2DM, coronary artery disease (CAD), diabetes micro and macrovascular complications [8]. Therefore, haematological markers may play a complementary role for the assessment of changes in form, number, size and maturity of various blood cells and the evaluation and management of T2DM patients [9].

Studies have shown that T2DM is linked with inflammation and the induction of T helper 1 (Th1) immune responses, M1 macrophages (MACs) activation and the production of pro-inflammatory cytokines such as interleukin IL-1 β, interferon gamma (IFN-γ), Tumour Necrosis Factor alpha (TNF-α), and Interleukin-6 (IL-6), which regulate the destruction of pancreatic Beta (β) cells, as well as insulin resistance in glucoregulatory tissues [10]. One of the numerous pathophysiological mechanisms underlying the progression of T2DM is the development of chronic, low-level inflammation, which is characterized by a pronounced exaggerated pro-inflammatory cytokines and chemokines [11].

The outbreak of inflammatory metabolic diseases such as T2DM has increased, especially in high-income countries and the absence of exposure to helminth infections has been hypothesized to be one mechanism to explain this markedly high prevalence [12,13]. Helminth infections affect approximately one-quarter of the world's total population and are widespread in lower- to middle-income countries [14,15]. Previous studies have reported that the prevalence of helminth infections was remarkably lower in patients with T2DM compared to non-diabetic individuals [16,17], hence confirming the protective effect of helminths against T2DM.

Helminth infections are known for their ability to regulate harmful inflammatory responses and support local and systemic metabolic homeostasis [18]. Previous studies indicated that helminth infections are usually characterized by the induction of Type 2 immune responses, with an increase in Type 2 and regulatory cytokines [11]. Moreover, it has been reported that enlistment and growth of regulatory T cells (Tregs), as well as modulation of inflammation, is enhanced by helminth infections [19]. Furthermore, it has been established that infections of mice with different species of parasitic helminths is associated with remarkably high type 2 innate lymphoid cells (ILC2s), eosinophils, alternatively activated macrophages (M2 MACs), and T helper 2 (Th2) cytokines, which result in the restoration of glucose levels and ameliorate insulin sensitivity [20]. Several studies have shown the immunomodulatory effect of other helminth species on T2DM [11,17,18]. Tissue dwelling larvae of nematodes such as *Trichinella* spp. are known to induce hypoglycaemia [21]; however, there is paucity of information on the effect of this nematode species and the pathophysiology of a host with T2DM.

In the intestinal phase of *Trichinella zimbabwensis* infection, the immune response has been reported to comprise both T helper type 1 (Th1) and T helper type 2 (Th2) responses [22]. In the early stages, the parasite induces a Th1 responses, followed by a dominant Th2 type of response. This later type of immune response is marked by an increased production of cytokines IL-4, IL-5, IL-9, IL-10, IL-13, immunoglobulin E (IgE), as well as the mobilization of basophils, eosinophils and mast cells [22]. *Trichinella* antigens also function in the stimulation of dendritic cells and macrophages to interrelate with T cells [23]. Helminth infection also influences the production of the anti-inflammatory cytokines and may result in the protective effect against diabetes onset [24]. Considering

the increase in the prevalence of helminths infection and the increasing number of T2DM in developing countries, it is important to understand the immunological and physiopathological mechanisms that are induced during comorbidity of *T. zimbabwensis* (a tissue dwelling helminth species) and T2DM. Therefore, this study aimed at using a laboratory animal model to determine the cytokines, chemokines and haematological indices in T2DM male Sprague Dawley (SD) rats infected with *Trichinella zimbabwensis*.

2. Materials and Methods

2.1. Experimental Animals

One hundred and two (102) male Sprague Dawley rats with body weight ranging from 160 to 180 g were randomly selected from a colony bred and maintained at the Biomedical Resource Unit (BRU), University of KwaZulu-Natal (Westville Campus) in Durban, South Africa. The experimental rats were maintained and subjected to standard laboratory conditions of constant temperature (22 ± 2 °C), Carbon dioxide (CO_2) content of <5000 ppm, illumination (12 h light/dark cycles) and relative humidity of 55 ± 5%. They were allowed access to feed freely (standard rat chow diet from Meadows, Pietermaritzburg, South Africa), and water was given ad libitum throughout the experimental period. All experimental procedures and protocols were reviewed and approved by the animal research ethics committee, University of KwaZulu-Natal, Durban, South Africa, under the ethical protocol reference number AREC/028/018D.

2.2. Study Design

The experimental rats were acclimatized for a period of one week in their new cages, followed by random allocation of the 102 animals into three groups as follows: Tz = *Trichinella zimbabwensis*-infected group (*n* = 36), D = T2DM-induced group (*n* = 36), and TzD = *T. zimbabwensis* infected + T2DM group (*n* = 30) (Table 1).

Table 1. Experimental design.

Group	*n*	Induced D	Infected with Tz	Days of Sacrifice Post Tz Infection
Tz	36	−	+	0, 7, 14, 21, 28, and 35
D	36	+	−	0, 7, 14, 21, 28, and 35
TzD	30	+	+	7, 14, 21, 28, and 35

Tz = *Trichinella zimbabwensis*; D = Type 2 diabetes; TzD = *T. zimbabwensis* and type 2 diabetes mellitus comorbidity.

2.3. Type 2 Diabetes Induction

Animals in the D and TzD groups were given 10% fructose solution ad libitum for two weeks before administration of streptozotocin to induce insulin resistance [25]. Streptozotocin (STZ) was dissolved in freshly prepared citrate buffer (pH 4.5). A sterilized 0.45 millipore filter was used to filter the solution. Experimental animals in the D and TzD groups were fasted overnight and given a single low dose of streptozotocin (STZ; 40 mg/kg), STZ, Sigma-Aldrich, St. Louis, MO, USA). The non-fasting blood glucose (NFBG) level of the experimental animals were measured using a glucometer (Glucoplus Inc., Saint-Laurent, QC, Canada), one week after STZ injection by collecting a drop of blood from the tail vein. Animals which had a NFBG level of >18 mmol/L were considered diabetic, and animals that did not reach this blood glucose level were considered nondiabetic and were excluded from the study. NFBG levels were measured at a two-day interval throughout the experimental period.

2.4. Trichinella zimbabwensis Infection

Animals were infected with *Trichinella zimbabwensis* using a crocodile-derived *T. zimbabwensis* (ISS1209) strain which is maintained in Sprague Dawley rats at the BRU, University of KwaZulu-Natal. Muscle larvae (ML) were obtained from digesting the muscle of infected stock rats following the method previously described by Pozio et al. [26]. Seven days after diabetes induction, the experimental rats in the Tz and TzD groups were infected with

T. zimbabwensis larvae through oral gavage at a dose of 3 mL/g of rat body weight using an 18 G curved oral dosing needle.

2.5. Food, Water Intake, Body Weight and Glucose Measurement

Food, water intake, glucose and animals' body weight were measured at a two-day interval for all groups for the duration of the experimental period. Measurement of food and water intake were carried out by subtracting the leftover food and water measurements from the initial measurements. The live body weight was measured by using a calibrated digital balance (Zeiss West, Germany). Blood glucose was measured from a drop of blood taken from the animal tail vein using a glucometer (Glucoplus Inc., Saint-Laurent, QC, Canada). Levels of blood glucose were compared with the Sprague Dawley established reference values [27], which served as the control for the study.

2.6. Recovery of Trichinella zimbabwensis Worms

Trichinella zimbabwensis infection was considered to have successfully reached the host muscle at day 28 post-infection (pi). Detection and confirmation of larvae was conducted by digesting the muscle tissue using the modified artificial digestion protocol as described by Pozio et al. [26] at day 28 and 35 pi. Adult worm recovery from the intestines was conducted using a protocol as described by Mukaratirwa et al. [28] at day 7, 14 and 21 pi as follows: saline solution (0.85%) was used to immerse the small intestines after splitting them open longitudinally using scissors, then incubated for a period of 12 h at 37 °C and washed through a 212 µm aperture sieve. A Zeiss Stemi DV4 stereo microscope (Germany) was used to view and count *T. zimbabwensis* adult parasites at 20× objective.

2.7. Serum Insulin Measurement

To measure the levels of insulin, blood samples from the experimental animals were collected through cardiac puncture and centrifuged to separate the plasma and the serum. A Mercodia Ultrasensitive Rat Insulin ELISA Kit was used to determine the serum insulin concentrations according to the manufacturer's instructions. The samples and standard optical densities were derived by reading the optical density at 450 nm using a spectrophotometer microplate reader (BMG Labtech, Ortenberg, Germany). Serum insulin concentrations were determined by using a 4-parameter logistic equation in GraphPad PRISM version 5.04 for windows (Graph Pad Software, San Diego, CA, USA).

2.8. Liver and Muscle Glycogen Measurement

Liver and muscle glycogen measurements were determined according to the method of Lo et al. [29] with slight modification. Liver and muscle glycogen content were estimated by sectioning 0.3 g of liver and muscle tissue samples and these were homogenized with 1.5 mL of 30% potassium hydroxide saturated with sodium sulphate (Na_2SO_4). The contents were placed in a test tube and allowed to completely submerge in ice and boiled at 100 °C for 30 min until a homogeneous solution was obtained. The resulting solution was taken from the boiling bath and immediately cooled in ice. Thereafter, 670 µL of 95% ethanol was added into the sample and allowed to stand in ice for 30 min to precipitate the glycogen. Content was then centrifuged twice at 840× *g* for 30 min. Afterwards, the supernatant was carefully aspirated, and glycogen precipitate was then dissolved using 3 mL of distilled water. The glycogen precipitate and the glycogen standards (10, 20, 30, 40, 50, 60, 70, 80, 90 µg/mL) were further prepared by the addition of 1 mL of 5% phenol, followed by 1 mL of concentrated sulphuric acid (96–98%). The content was mixed thoroughly and subjected to heat in a boiling water bath for 10 min and cooled for 10 min. Absorbance was read using a 96-well plate reader at 490 nm with a Spectrostar Nano microplate reader (BMG Labtech, Ortenberg, Germany). Glycogen content was extrapolated from a standard curve and results recorded as mg/g liver and muscle tissue.

2.9. Determination of Haematological Parameters

Blood samples collected for haematological parameters were stored in sample bottles with ethylene diamine–tetra-acetic acid (EDTA). The following haematological parameters were measured using a Roche Sysmex XN1000 analyser: erythrocytes (RBC) ($10^6/\mu L$), haematocrit (HCT) (%), haemoglobin concentration (HGB) (%), and the number of platelets (PLT) ($10^9/L$), as well as white blood cells which included leukocytes (WBC) ($10^3/\mu L$), basophils (BA) (%), neutrophils (NE) (%), monocytes (MO) (%), lymphocytes (LY) (%) and eosinophils (EO) (%). All parameters obtained from the experimental groups were compared with the reference values for Sprague Dawley rats, as reported by He et al. [27], which served as a control.

2.10. Terminal Studies

The three experimental groups: Tz, D and TzD, were terminated by euthanizing six (n = 6) animals from each group at day 0, 7, 14, 21, 28, and 35 pi (Table 1). The procedure was performed by subjecting the experimental animals to isoform inhalation in an anaesthetic chamber for three minutes. Blood samples were collected from the experimental animals on the days of euthanasia by cardiac puncture into heparin tubes containing clotting activator gel (dvac gel and clot activator tubes). The blood samples were centrifuged using a Thermo scientific Heraeus Labofuge 200 microprocessor-controlled table-top centrifuge at 132× g for 10–15 min at 4 °C. Serum was collected into Eppendorf micro-centrifuge tubes and stored in a Bio Ultra freezer (Snijers Scientific, Tilburg, The Netherlands) at −80 °C until assayed.

2.11. Serum Cytokines and Chemokines Measurement

Cytokine and chemokine concentrations were measured in serum samples collected at day 0, 14 and 28 pi using ELISA. To determine the levels of cytokines and chemokines in the experimental rats, Procartalplex Mix & Match Rat 9-plex was used to measure TNF-α, IFN-γ, IL-4, IL-6, IL-10, IL-13, IP-10 (CXCL10), RANTES (CCL5) and Eotaxin (CCL11) levels in serum according to manufacturer's instructions.

2.12. Statistical Analysis

Data were presented as means ± standard error of means (SEM) and data were analysed using a two-way analysis of variance (ANOVA) and followed by Turkey–Kramer multiple comparison tests to establish statistical comparison between the Tz, D and TzD groups. Significance levels were determined by Bonferroni post hoc test analyses. Statistical comparison of means of the experimental groups with the 95% upper and lower confidence intervals, and/or box plots with the median and the 25% and 75% quartiles for haematology parameters, were performed using Graphpad PRISM version 5.04 for Windows (Graphpad Software, San Diego, CA, USA) and the differences between groups were considered statistically significant when p-value was less than 0.05.

3. Results

3.1. Trichinella zimbabwensis Adult Worms and Muscle Larvae Establishment

Adult worm loads, as shown in Figure 1, were significantly higher ($p < 0.001$) in the Tz group when compared to the TzD experimental group at day 7 pi, with a mean of 22.10 ± 4.81 in the Tz group and 16.96 ± 3.69 in the TzD group. At day 14 pi, a mean of 5.56 ± 2.92 of adults were recovered in the Tz and 2.70 ± 0.79 in the TzD group, respectively. There were no muscle larvae recovered at day 21 pi, but rather a few adult worms were recovered with a mean of 1.60 ± 0.47 in muscles in the Tz and 0.56 ± 0.32 in the comorbidity group (TzD). The mean larvae per gram (lpg) of muscle (22.40 ± 2.07 lpg) at day 28 pi was significantly higher ($p < 0.001$) in the Tz group when compared to the TzD group (16.56 ± 3.58 lpg) and this followed the same trend at day 35 pi, with the Tz group having a mean of 31.20 ± 1.30 lpg, and the TzD group a mean of 19.20 ± 4.21 lpg ($p < 0.001$) (Figure 1).

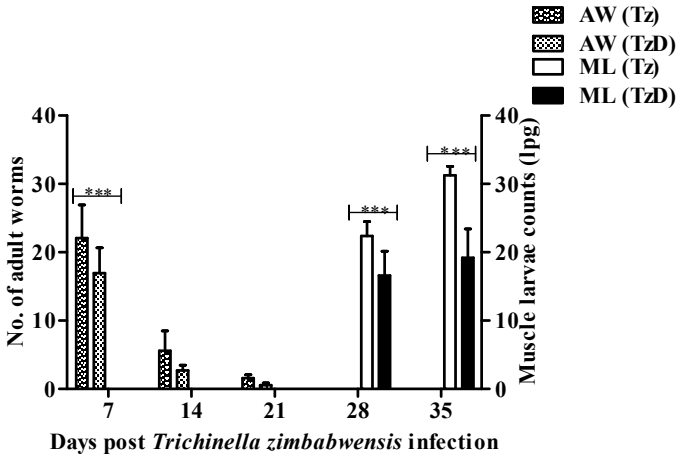

Figure 1. Mean number (±SEM) of *Trichinella zimbabwensis* adult worms (AW) and larvae per gram (lpg) of muscle larvae (ML) recovered from the intestines and muscles of male Sprague Dawley rats infected with *T. zimbabwensis* (Tz) and male diabetic rats infected with Tz (TzD). Values are presented as means and vertical bars indicate SEM (n = 6 in each group) and asterisks represent significant differences between the Tz and the TzD groups (*** $p < 0.001$).

3.2. Water and Food Intake

Water and food intake of Tz, D and TzD are shown in Table 2. There was a significant increase ($p < 0.001$) in the water intake by the D group from day 0 through to 28 pi when compared to the Tz group and a significant increase ($p < 0.01$) at day 35 pi when compared to the Tz group. A significant increase ($p < 0.001$) in water intake was also observed in TzD group at day 0 to 14 pi and significant increase ($p < 0.01$) at day 21 and 28 when compared to the Tz group. The trend in the food intake by the D group increased exponentially throughout the experimental period in comparison with the TzD, although, the food intake of the D and TzD group showed no significant difference compared to the Tz group.

Table 2. Mean water and food intake in male Sprague Dawley rats comorbid with type 2 diabetes mellitus and *Trichinella zimbabwensis*. Sprague Dawley rats were infected with *T. zimbabwensis* (Tz); with induced type 2 diabetes (D) and co-morbid with *T. zimbabwensis* and type 2 diabetes (TzD). Day 0 represents the day of Tz infection to diabetic rats with L3 from muscles. n = 6 for each group. Values with asterisks represent significant differences between the experimental groups, (** $p < 0.01$, *** $p < 0.001$).

Water and Food Intake	Days Post *T. zimbabwensis* Infection	Experimental Groups		
		Tz	D	TzD
Water intake (mL)	Day 0	243.3 ± 13.33	703.3 ± 72.99 ***	620.0 ± 68.37 ***
	Day 7	155.6 ± 20.34	640.0 ± 69.19 ***	490.0 ± 47.17 ***
	Day 14	206.3 ± 18.75	793.8 ± 85.62 ***	631.3 ± 51.41 ***
	Day 21	258.0 ± 22.11	808.3 ± 88.19 ***	641.7 ± 58.33 **
	Day 28	300.0 ± 8.00	937.5 ± 12.50 **	772.5 ± 122.5 **
	Day 35	360.0 ± 10.00	962.5 ± 12.50 **	762.5 ± 12.50
Food intake (g)	Day 0	176.2 ± 14.49	248.5 ± 19.47	201.2 ± 26.68
	Day 7	124.0 ± 11.62	208.8 ± 27.17	156.4 ± 39.11
	Day 14	140.0 ± 22.43	248.3 ± 25.37	213.0 ± 63.32
	Day 21	173.3 ± 10.68	259.3 ± 38.28	228.3 ± 25.78
	Day 28	193.5 ± 5.50	330.0 ± 22.00	293.5 ± 19.50
	Day 35	213.0 ± 5.00	341.0 ± 1.00	303.0 ± 2.08

3.3. Body Weight and Blood Glucose Level

The mean weekly body weight changes observed throughout the period of experiment for all experimental groups are shown in Figure 2a. There was no significant difference

observed among the three groups at day 7 pi. However, there was a significant decrease ($p < 0.05$) in the body weight of the TzD group at day 14 pi and a significant increase ($p < 0.05$) at day 35 pi, respectively, when compared to the Tz and D groups. A reduction in body weight in the D group was observed at day 21 pi and a significant decrease ($p < 0.001$) at day 35 pi was also observed when compared to the Tz and TzD groups. Interestingly, there was a steady body weight increase in the Tz group throughout the experimental period (Figure 2a).

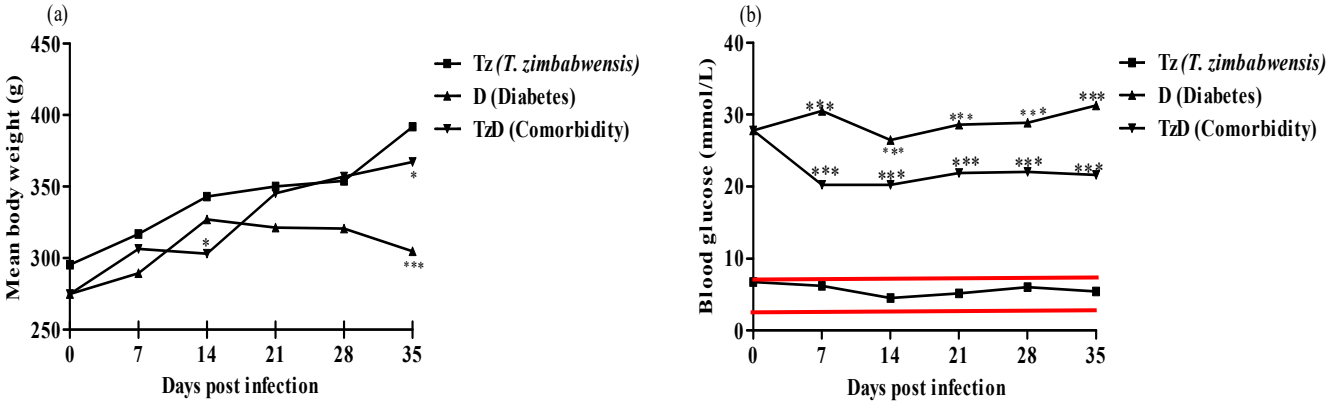

Figure 2. Mean body weight (**a**) and blood glucose concentration (**b**) in male Sprague Dawley rats that were; comorbid with type 2 diabetes mellitus (TzD), diabetic (D) and infected with *Trichinella zimbabwensis* (Tz). Horizontal redlines depicts the range of reference values for blood glucose (4.70–6.70) in male Sprague Dawley rats He et al. [27]. Values are presented as means ($n = 6$ in each group) and asterisk represent significant differences between the groups (* $p < 0.05$, *** $p < 0.001$).

Blood glucose results from this study were compared to the reference values by He et al. [27]. Blood glucose were within the reference value range during the study (4.53–6.70) for the Tz group when compared to the reference values (4.46–7.24), except for the D and TzD groups, which were diabetic (Figure 2b). Blood glucose concentrations in the D group was significantly higher ($p < 0.001$) at day 7, 14, 28 and 35 pi compared to the TzD group (Figure 2b).

3.4. Serum Insulin Concentration

The insulin concentration was decreased in the D and in the TzD when compared to the Tz at day 0, and at day 7 pi (Figure 3). There was an increase in the TzD (0.21 pmol/L), while the D and the Tz groups had 0.12 pmol/L and 0.15 pmol/L, respectively. An increase was also observed in the TzD (0.31 pmol/L) on day 14 pi in comparison to the D (0.24 pmol/L) and the Tz (0.25 pmol/L); however, the D group showed a decrease on day 21 pi, but the decrease was not statistically significant ($p > 0.05$) when compared to the Tz and TzD. On day 28 pi, the TzD insulin level was reduced compared to the D and the Tz, but increased again on day 35 in comparison to the D group (Figure 3).

3.5. Liver and Muscle Glycogen Concentration

Liver and muscle glycogen concentrations are shown in Figure 4a,b, respectively. No significant difference ($p > 0.05$) in liver glycogen was observed when compared among the experimental groups. A non-significant increase was, however, observed at day 28 pi in the TzD (6.25 mg/g tissue) when compared to the D (4.83 mg/g tissue) and the Tz (4.89 mg/g tissue) groups, and at day 35 pi. A drastic reduction in glycogen concentration was observed in the D (4.60 mg/g tissue) group when compared to the Tz (5.40 mg/g tissue) and the TzD (4.78 mg/g tissue) groups, although it was not significant ($p > 0.05$). Muscle glycogen concentration showed no significant difference ($p > 0.05$) when compared among the experimental groups, although a decrease in the glycogen concentration was observed in the D (2.16 mg/g tissue) group on day 28 pi when compared to the TzD (3.28 mg/g tissue) and the Tz (2.33 mg/g tissue) groups.

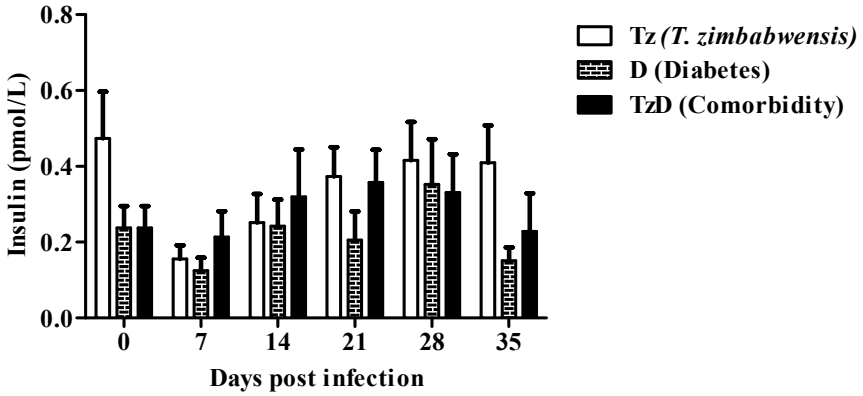

Figure 3. Insulin concentration in groups of male Sprague Dawley rats that were; comorbid with type 2 diabetes mellitus (TzD), diabetic (D) and infected with *Trichinella zimbabwensis* (Tz). Values are presented as mean ± SEM (n = 6 for each group).

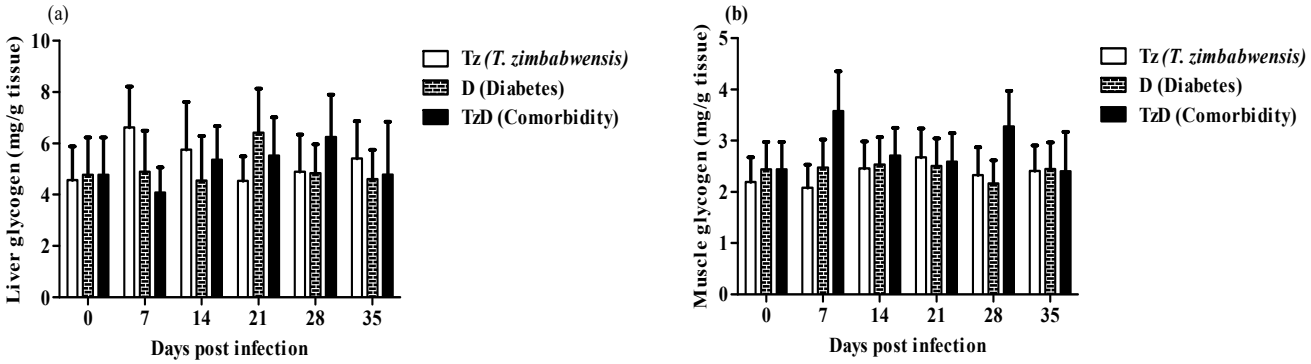

Figure 4. Liver glycogen concentrations (**a**) and muscle glycogen concentrations (**b**) in male Sprague Dawley rats that were; comorbid with type 2 diabetes mellitus (TzD), diabetic (D) and infected with *Trichinella zimbabwensis* (Tz). Values are presented as mean ± SEM (n = 6 in each group).

3.6. Haematological Parameters

3.6.1. Red Blood Cells

Haematological parameters from the experimental groups were compared to reference values as described by He et al. [27]. Changes in RBC concentration and haematocrit percentage of the experimental groups at different days post Tz infection are shown in Figure 5a,b, respectively. The RBC concentration was within the reference values range while some values were higher at day 7 in the Tz (7.71–9.62), D (7.30–8.44) and TzD groups (7.80–9.23) when compared to the reference values (6.39–8.01). At day 14 pi, the RBC levels were higher in some individual animals, while some were within the reference value in the Tz (8.17–9.19), D (8.21–9.20) and TzD (7.57–8.81) when compared to the reference value (6.39–8.01). Additionally, at day 21 pi, an increase was observed in the Tz (7.49–8.92), D (8.83–10.09) and TzD (8.94–9.49) when compared to the reference range (6.39–8.01). The RBC concentration were higher than the reference value range for the present study in all the experimental groups at day 28 pi in the Tz (7.29–8.18), D (8.96–9.99) and TzD (7.70–8.76), although some values were within the reference range. At day 35 pi, the Tz (8.50–9.18), D (7.73–8.99) and TzD (7.79–9.03) had higher concentrations when compared to the reference value (6.39–8.01), although some values in the D and TzD were within the reference range, while some were high. The D group showed a significant increase ($p < 0.05$) in RBC at day 28 pi (Figure 5a). Haematocrit percentages were higher in all experimental groups, except on day 28 in the Tz (43.86–48.10) group, where the values were within the reference value range (42–49). Groups D and TzD had a significantly higher percentage of haematocrit ($p < 0.05$) at day 21 pi and a significantly higher percentage of haematocrit ($p < 0.05$) in the D at day 28 pi in comparison to Tz and TzD groups. However,

at day 35 pi, a decrease was observed in both the D and TzD (Figure 5b). The haemoglobin concentration was within the reference value range, while some values were higher at day 7 pi in the Tz (13.53–17.13) and TzD (14.58–17.23) groups when compared to the reference values (13.50–15.90). At day 14 pi, an increase in the Tz (15.23–17.30) and D (15.25–16.95) was observed while in the TzD (13.90–16.15), some values were high when compared to the reference value (13.50–15.90). There was a significantly higher percentage ($p < 0.05$) of haemoglobin concentration observed at day 21 pi in the D group. Significantly higher ($p < 0.01$) levels of haemoglobin were also observed in the TzD group on day 21. However, there was a slight increase in the D (14.65–16.85) group on day 28 and T (15.23–16.40) group at day 35, although some values were still within the reference range when compared to the reference values (13.50–15.90) (Table 3).

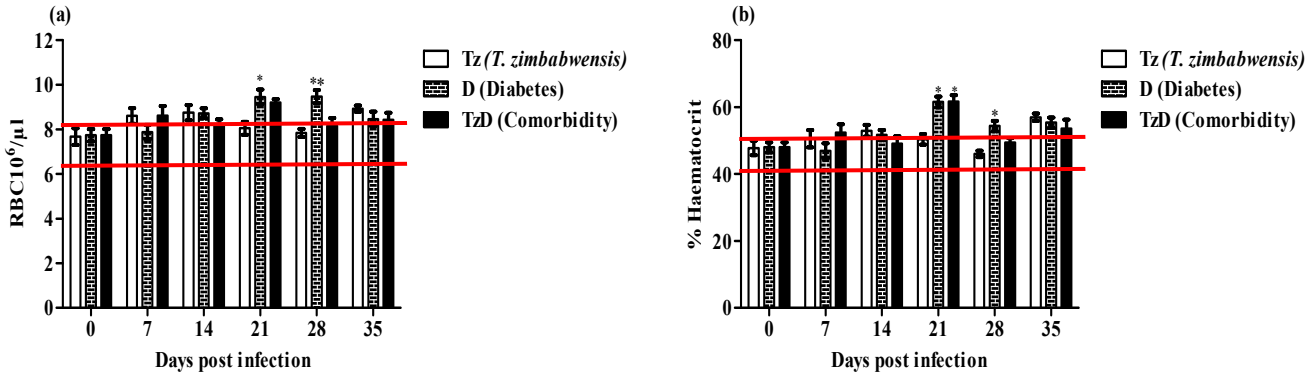

Figure 5. Red Blood Count (RBC) concentration (**a**) (± SEM) and haematocrit (**b**) (± SEM) in male Sprague Dawley rats comorbid with type 2 diabetes mellitus and *Trichinella zimbabwensis*. Sprague Dawley rats were infected with *Trichinella zimbabwensis* (Tz); with induced type 2 diabetes (D) and co-morbid with *Trichinella zimbabwensis* and type 2 diabetes (TzD). Horizontal red lines depict the range of reference values for RBC (6.39–8.01) and haematocrit (42–49) in male Sprague Dawley rats (He et al.) [27]. Values are presented as means and vertical bars indicate SEM ($n = 6$ in each group) and asterisks represent significant differences between the groups (* $p < 0.05$, ** $p < 0.01$).

3.6.2. White Blood Cells

The WBC concentration was within the reference value range in the present study in all experimental groups Tz, D and TzD at day 0, 14 and 35 when compared to the reference value range (3.60–9.22). At day 7 pi, the Tz (3.8–7.74) and TzD (3.57–6.96) groups were within the reference value range (3.60–9.22), while in the D group (2.68–5.64) some values were below, while some values were within the reference value range. Additionally, at day 21 pi, the WBC concentration in Tz (5.21–7.76) and TzD (5.29–7.23) were within the reference value (3.60–9.22), while in the D (6.37–10.36), some values were higher when compared to the reference values (3.60–9.22). At day 28, the values of the present study were below the reference range in the Tz (2.46–3.68), D (3.38–5.76) and the TzD (2.44–4.84) groups, although some values were within the reference values (3.60–9.22). On day 35 pi, the Tz (5.49–7.82) and TzD (4.09–5.56) were within the reference value, although some animals in the D (3.24–4.20) were below the reference value (3.60–9.22) (Table 3).

Table 3. Median values (25–75% quartiles) of white blood cells concentration and differentials in Sprague Dawley rats infected with *Trichinella zimbabwensis* (Tz), with induced type 2 diabetes (D) and co-morbid with *T. zimbabwensis* and type 2 diabetes (TzD). Day 0 represents the day of Tz infection to diabetic rats with Tz 1st stage larvae from muscles. $n = 6$ for each group. Values with asterisks represent significant differences between the experimental groups are significantly different (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Haematological Parameter	Days Post *T. zimbabwensis* Infection	Experimental Groups			
		Tz	D	TzD	Range of Reference Values (He et al., 2017) [27]
WBC ($10^3/\mu L$)	Day 0	3.94 (3.44–5.51)	6.03 (3.45–8.11)	6.03 (3.45–8.11)	3.00–9.22
	Day 7	5.52 (3.8–7.74)	4.87 (2.68–5.64)	5.66 (3.57–6.96)	
	Day 14	6.99 (4.43–8.70)	5.87 (5.43–7.18)	4.88 (4.14–6.83)	
	Day 21	6.97 (5.21–7.76)	8.45 (6.37–10.36)	6.65 (5.29–7.23)	
	Day 28	3.37 (2.46–3.68)	4.56 (3.38–5.76)	2.57 (2.44–4.84)	
	Day 35	6.19 (5.49–7.82)	4.02 (3.24–4.20) *	4.70 (4.09–5.56)	
Neutrophils (%)	Day 0	15.65 (11.45–18.78)	16.60 (13.90–22.20)	16.60 (13.90–22.20)	6.14–22.9
	Day 7	12.00 (11.10–16.28)	18.15 (14.98–19.85)	8.75 (7.70–16.08)	
	Day 14	9.10 (8.17–13.25)	12.80 (10.85–13.25)	15.60 (13.35–18.95)	
	Day 21	15.45 (13.43–21.00)	20.80 (16.10–24.00)	19.40 (16.25–21.95)	
	Day 28	14.85 (12.28–18.08)	12.90 (12.05–18.10)	16.90 (10.70–19.65)	
	Day 35	19.80 (16.93–22.68)	34.40 (31.93–38.98) ***	28.30 (24.95–35.70) **	
Lymphocytes (%)	Day 0	77.40 (73.23–78.00)	73.25 (69.50–77.43)	73.25 (69.50–77.43)	69.68–86.89
	Day 7	79.75 (78.52–82.50)	77.35 (73.38–79.45)	84.10 (75.80–86.35)	
	Day 14	77.80 (73.90–81.13)	76.40 (73.90–77.40)	70.20 (67.10–71.75)	
	Day 21	70.45 (66.90–75.13)	66.30 (63.35–69.90)	63.00 (60.10–67.55)	
	Day 28	76.65 (70.83–79.23)	74.10 (63.90–79.23)	69.20 (64.85–75.80)	
	Day 35	65.75 (65.23–75.75)	50.55 (43.78–54.20) ***	56.40 (41.00–57.45) ***	
Monocytes (%)	Day 0	8.35 (5.22–9.00)	9.30 (3.87–11.15)	9.30 (3.87–11.15)	3.77–10.82
	Day 7	2.60 (1.65–8.025)	3.10 (2.32–5.22)	3.85 (2.37–10.20)	
	Day 14	9.55 (6.25–12.68)	9.20 (5.3–12.85)	9.00 (7.15–12.30)	
	Day 21	8.05 (5.62–8.85)	11.60 (10.10–12.00)	10.60 (6.50–12.00)	
	Day 28	4.10 (2.65–9.50)	13.80 (2.80–21.95) *	11.50 (11.20–12.30)	
	Day 35	8.55 (4.90–11.53)	11.55 (10.53–20.75)	11.70 (8.80–13.80)	
Basophils (%)	Day 0	0.40 (0.30–0.60)	0.20 (0.15–0.32)	0.20 (0.15–0.32)	0.04–0.44
	Day 7	0.30 (0.20–0.42)	0.25 (0.15–0.47)	0.45 (0.30–0.62)	
	Day 14	0.25 (0.15–0.42)	0.20 (0.10–0.35)	0.20 (0.10–0.35)	
	Day 21	0.25 (0.07–0.37)	0.30 (0.20–0.40)	0.50 (0.05–0.50)	
	Day 28	0.30 (0.15–0.32)	0.50 (0.35–0.60)	0.40 (0.10–0.40)	

Table 3. *Cont.*

Haematological Parameter	Days Post *T. zimbabwensis* Infection	Experimental Groups			
		Tz	**D**	**TzD**	**Range of Reference Values (He et al., 2017) [27]**
	Day 35	0.25 (0.20–0.42)	0.20 (0.0–0.22)	0.20 (0.10–0.35)	
Eosinophils (%)	Day 0	0.65 (0.55–1.40)	1.00 (0.77–1.12)	1.00 (0.77–1.12)	0.54–3.39
	Day 7	2.05 (1.65–2.45)	2.40 (1.12–3.17)	0.60 (0.17–0.82)	
	Day 14	2.45 (1.92–3.10)	1.50 (1.30–2.90)	4.40 (2.95–6.35)	
	Day 21	4.70 (1.55–6.47)	1.90 (0.85–6.47) **	6.60 (5.70–9.35) *	
	Day 28	3.60 (2.82–4.95)	1.00 (0.35–1.40) **	2.20 (1.65–3.25)	
	Day 35	2.05 (1.40–3.12)	1.20 (0.97–3.00)	7.80 (6.35–9.30) ***	
Haemoglobin (%)	Day 0	14.80 (12.75–15.83)	14.35 (13.40–15.35)	14.35 (13.40–15.35)	13.50–15.90
	Day 7	15.45 (13.53–17.13)	13.95 (13.33–15.70)	16.00 (14.58–17.23)	
	Day 14	16.55 (15.23–17.30)	15.60 (15.25–16.95)	15.10 (13.90–16.15)	
	Day 21	14.00 (13.58–15.08)	16.60 (15.95–17.10) *	16.60 (15.75–17.75) **	
	Day 28	13.60 (13.43–14.60)	15.40 (14.65–16.85)	14.30 (14.10–15.30)	
	Day 35	15.70 (15.23–16.40)	14.60 (13.95–15.25)	14.40 (13.50–15.85)	
Platelets (10^9/L)	Day 0	1006 (925.0–1086)	1167 (1123–1207)	1167 (1123–1207)	923–1580
	Day 7	1037 (1007–1128)	1024 (943.0–1082)	1027 (939.5–1206)	
	Day 14	1031 (947.8–1071)	985.0 (933.5–996.5)	756.0 (738.0–943.5)	
	Day 21	801 (791.0–912.0)	938.0 (871.5–1049)	1065 (1004–1087) **	
	Day 28	920.5 (860.3–1005)	1111 (998.0–1156)	894.0 (828.5–956.5)	
	Day 35	1264 (1032–1376)	964.5 (825.3–1083) ***	1072 (983.0–1191)	

Percentage of lymphocytes was within the reference value range in all experimental groups on day 0, 7 and 14 pi when compared with reference values (69.68–86.89). At day 21 pi, the percentage lymphocytes were low in some animals, while some were within the reference value in Tz (66.90–75.13), the D (63.35–69.90) and TzD (60.10–67.55) were below the reference value when compared to (69.68–86.89). Additionally, at day 28 pi, a decrease was observed in the D (63.90–79.23) and TzD (64.85–75.80) groups, although some values were within the reference value (69.68–86.89). Percentage lymphocytes were below the reference value range in all the experimental groups, Tz (65.23–75.75), D (43.78–54.20) and TzD (41.00–57.45) at day 35 pi when compared to the reference value (69.68–86.89), although some values were within the reference range in the Tz. Lymphocytes were significantly lower ($p < 0.001$) in both the D and TzD at day 35 pi compared to the Tz group, and a reduced percentage of lymphocytes was also observed at day 21 and 28 pi; however, this was not significant (Table 3). Basophil concentration was within the reference value range in the Tz group on day 7 pi, and slightly higher in the D (0.15–0.47) and TzD (0.30–0.62) groups, respectively, when compared to the reference values (0.04–0.44). There was an elevation of basophils (%) in the TzD group at day 21 pi (0.05–0.50) when compared to (0.04–0.44). Additionally, there was an increase at day 28 pi in the D (0.35–0.60) group when compared to (0.04–0.44), although some values were still within the reference range. All differences observed among experimental groups were not significant (Table 3).

Eosinophil levels were lower than the reference value range in the TzD group on day 7 pi (0.17–0.82), while on day 14 pi (2.95–6.35), the eosinophils levels were within the reference values in some animals, while some were higher when compared with the reference values (0.54–3.39). At day 21 pi, the Tz (1.55–6.47) and D (0.85–6.47) had some values that were within the reference value range, while some were higher; the TzD (5.70–9.35) group was higher when compared to the reference value range (0.54–3.39), the Tz group (2.82–4.95) was slightly higher, although some values were within the reference range, and D (0.35–1.40) were lower, while some values were within the reference range (0.54–3.39) and at day 35 pi the TzD (6.35–9.30) was higher than the reference value. Eosinophil levels were significantly decreased ($p < 0.01$) in D group at day 21 and 28 pi and significantly higher at day 21 ($p < 0.05$) and 35 ($p < 0.001$) pi, respectively, in the TzD (Table 3). The percentage neutrophils were within the reference value range for all experimental groups on days 0, 7 and 14. Some values were higher, while some were within the reference range in the D (16.10–24.00) group on day 21 when compared to the reference values (6.14–22.9). Neutrophil concentration was within the reference value range on day 28 pi in all the experimental groups but higher on day 35 in the D (31.93–38.98) and TzD (24.95–35.70) groups, respectively, when compared to (6.14–22.9). A significant elevation ($p < 0.001$) of neutrophils was observed in the D group at day 35 pi, and also in the TzD ($p < 0.01$) in comparison to the Tz group. No significant differences were seen on other days in all experimental groups (Table 3).

Percentage monocytes were below the reference value range in all experimental groups Tz (1.65–8.025), D (2.32–5.22) and TzD (2.37–10.20) at day 7, although some values were higher, while some were within the reference range (3.77–10.82). At day 21 pi, the D (10.10–12.00) group had higher ranges whilst the TzD (6.50–12.00) groups had values that were within the reference range, although some values were higher when compared to the reference value (3.77–10.82). Monocyte concentration showed a significant increase ($p < 0.05$) in the D group at day 28 pi in comparison to the Tz group, and a slight increase was observed among the Tz (4.90–11.53) and the TzD (8.80–13.80), though some values were still within the reference range. The D (10.53–20.75) group had higher monocytes at day 35 pi, when compared to the reference values (3.77–10.82) (Table 3). Platelet concentration was within the reference value range on days 0 and 7 pi, but below the reference on day 14 pi for TzD (738.0–943.5), although some values were still within the range when compared to the reference value (923–1580). At day 21 pi, platelets concentration for Tz (791.0–912.0) was below the reference value, and D (871.5–1049) also had values below, while some values were within the reference value range. Additionally, at day 28 pi, in Tz

(860.3–1005) and TzD (828.5–956.5), some values were below, while some were within the reference value range and at day 35 in the D (825.3–1083) group when compared to the reference value range (923–1580). Platelets were significantly higher ($p < 0.01$) in the TzD group at day 21 pi compared to the Tz group, and significant reduced ($p < 0.001$) platelets were also observed at day 35 pi in the D group (Table 3).

3.7. Effect of T. zimbabwensis Infection on Serum Cytokine Concentration

3.7.1. TNF-α Concentration

There was a significant reduction ($p < 0.05$) in TNF-α concentration in the Tz group (3.17–32.76) compared to the D (3.67–63.43) and TzD (3.67–63.43) groups at day 0 post infection (pi). At day 14 pi, an increase was observed in the TNF-α concentration in the TzD (5.40–77.71) group when compared to the Tz (3.17–14.21) and D (3.17–10.80) groups. At day 28 pi, TNF-α levels showed a decrease in the D group (3.50–27.73) when compared to the TzD (9.92–28.92) and Tz (3.17–37.78) groups, although these differences were not significant (Figure 6a)

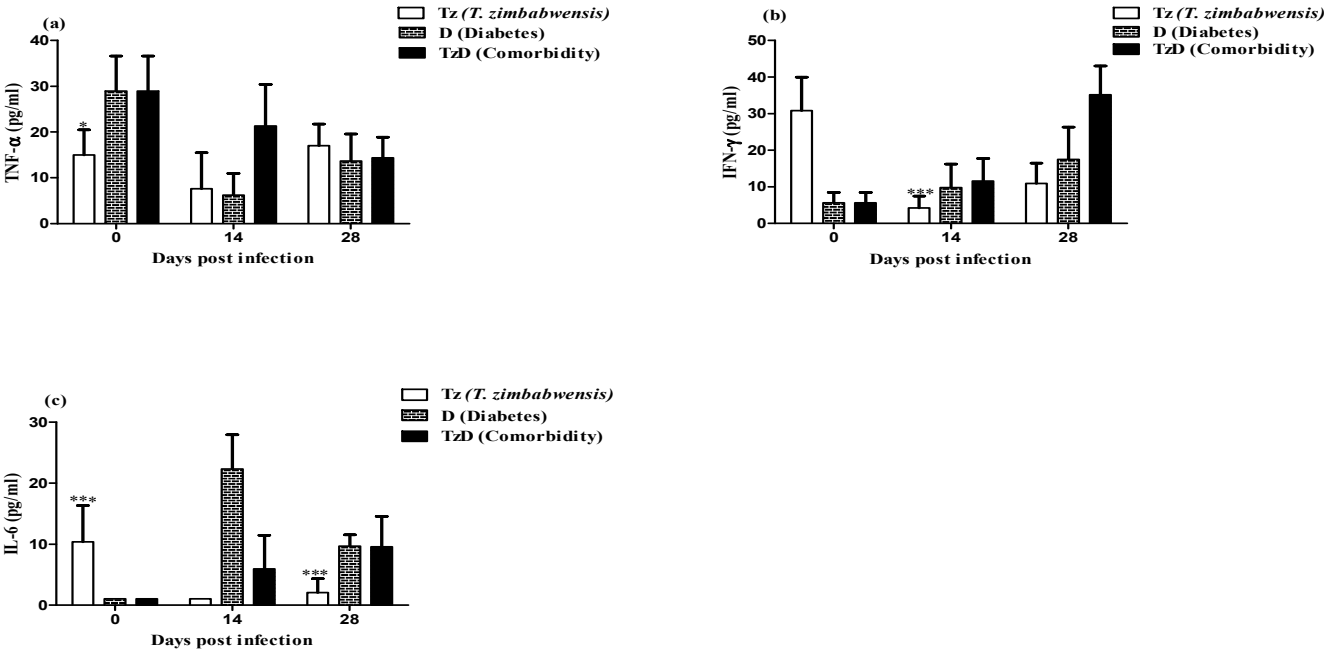

Figure 6. TNF-α concentrations (**a**) IFN-γ concentrations (**b**) and IL-6 concentrations significance asterisk in the Tz is compared to D and TzD (**c**) in male Sprague Dawley rats that were comorbid with type 2 diabetes mellitus (TzD), diabetic (D) and infected with *Trichinella zimbabwensis* (Tz). Values are presented as mean ± SEM ($n = 6$ in each group) and asterisks represent significant differences between the groups (* $p < 0.05$, *** $p < 0.001$).

3.7.2. IFN-γ Concentration

IFN-γ showed an increase in the Tz (10.27–48.24) group in comparison to the D (1.03–11.92) and the TzD (1.03–11.92) groups at day 0 pi. At day 14 pi, there was a significant reduction ($p < 0.001$) in IFN-γ concentration (1.03–9.16) in the Tz group when compared to D (1.03–22.96) and TzD groups (0.85–26.08). There was an increase in the TzD (23.05–91.15) when compared to the Tz (1.03–24.15) and D (1.03–81.45), although no significant differences for IFN-γ concentration at day 28 pi were observed among all groups (Figure 6b).

3.7.3. IL-6 Concentration

There was a significant elevation ($p < 0.001$) of IL-6 concentration in Tz group (1.03–24.40) compared to the D (1.03–1.03) and TzD (1.03–1.03) groups at day 0 pi. At day 14 pi, an increase was observed in the D (1.03–38.90) group, when compared to the Tz

(1.03–1.03) and TzD (1.03–13.33) groups, although it was not significant. At day 28 pi, the IL-6 concentration showed a significant reduction ($p < 0.001$) in the Tz (1.03–3.60) group when compared to the D (1.03–22.67) and the TzD (0.77–22.71) groups (Figure 6c).

3.7.4. IL-4 Concentration

IL-4 concentration was higher in the Tz group (1.27–21.36) when compared to the D (0.30–3.10) and TzD (0.30–3.10) groups at day 0 pi, although the increase was not significant ($p > 0.05$). At day 14 the concentration was reduced in the D group (0.36–5.93) when compared to the Tz (0.36–8.72) and TzD (0.78–8.52). At day 28 pi, there was a significant elevation of IL-4 concentration in the TzD (2.68–31.94) group ($p < 0.01$) when compared to D (0.36–4.70) and Tz (1.27–6.93) groups (Figure 7a).

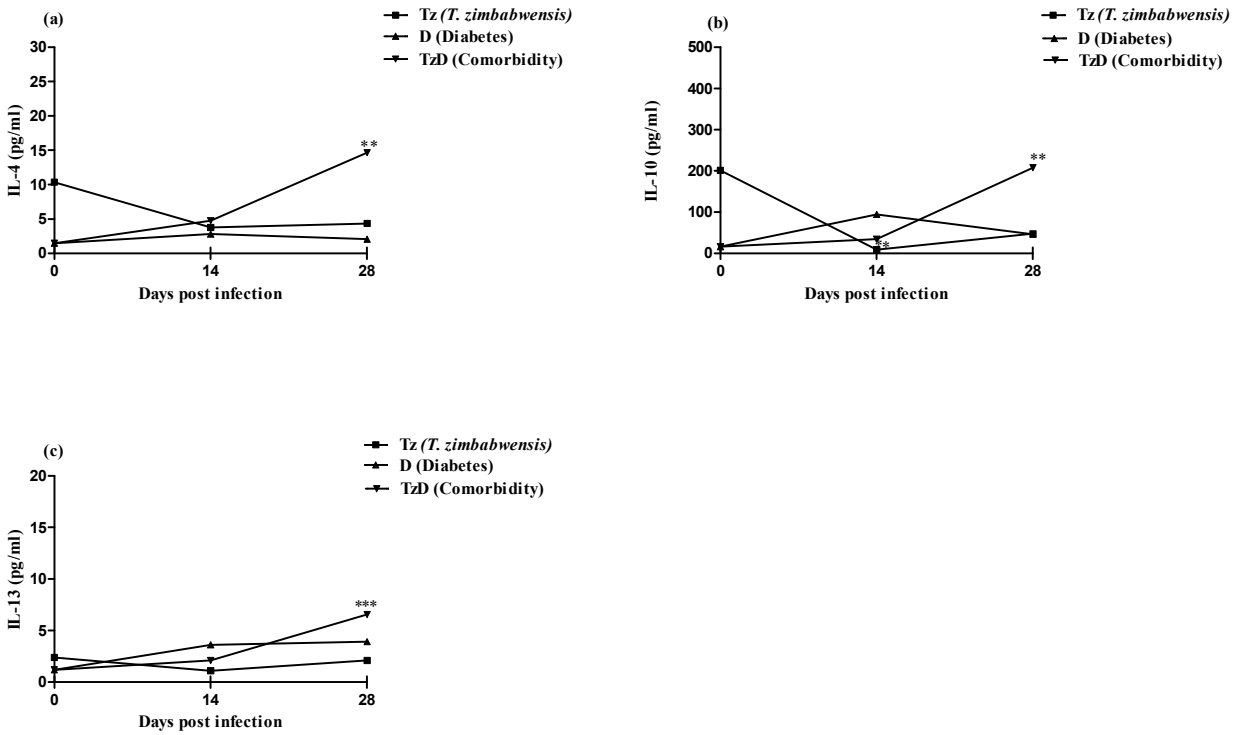

Figure 7. IL-4 concentrations (**a**) IL-10 concentrations (**b**) and IL-13 concentrations (**c**) in male Sprague Dawley rats that were comorbid with type 2 diabetes mellitus (TzD), diabetic (D) and infected with *Trichinella zimbabwensis* (Tz). Values are presented as mean ± SEM ($n = 6$ in each group) and asterisks represent significant differences between the groups (** $p < 0.01$, *** $p < 0.001$).

3.7.5. IL-10 Concentration

A significant reduction ($p < 0.01$) in IL-10 concentration was observed in the D (4.53–34.03) and TzD (4.53–34.03) groups in comparison to the Tz (132.70–287.10) group at day 0 pi. At day 14 pi, IL-10 concentration showed an increase in the D (59.37–128.70) and TzD (4.53–72.71) group, while a significant reduction ($p < 0.01$) was observed in the Tz (2.56–18.30) group. There was a significant increase ($p < 0.01$) in IL-10 concentration in the TzD (4.53–51.39) group in comparison to the D (4.53–98.01) and the Tz (4.53–94.72) groups at day 28 pi (Figure 7b).

3.7.6. IL-13 Concentration

IL-13 concentration was higher in the Tz (1.26–4.07) when compared to the D (1.07–1.26) and TzD (1.07–1.26) at day 0 pi. At day 14 pi, there was an increase in the D (1.26–7.13) group when compared to the Tz (0.74–1.40) and the TzD (1.26–3.18) groups, although the increase was not significant. A significant increase ($p < 0.001$) was observed in the TzD

(1.38–14.06) in comparison to the Tz (0.67–3.98) and the D (1.26–7.92) groups at day 28 pi (Figure 7c).

3.8. Effect of Trichinella zimbabwensis Infection on Serum Chemokine Concentration

3.8.1. CCL5 Concentration

CCL5 showed an increase in the Tz (537.3–779.3) when compared to the D (99.04–587.3) and the TzD (99.04–587.3) groups at day 0 pi, although the increase was not significant. Additionally, the concentration was significantly reduced in the Tz group ($p < 0.001$) in comparison to the D (276.4–643.9) and TzD (159.4–584.8) group, which had a significantly higher CCL5 concentration ($p < 0.01$) at day 14 pi. CCL5 showed a significant elevation in the D (439.2–765.5) and TzD (543.6–859.4) groups in comparison to the Tz (254.1–678.6) group at day 28 pi (Figure 8a).

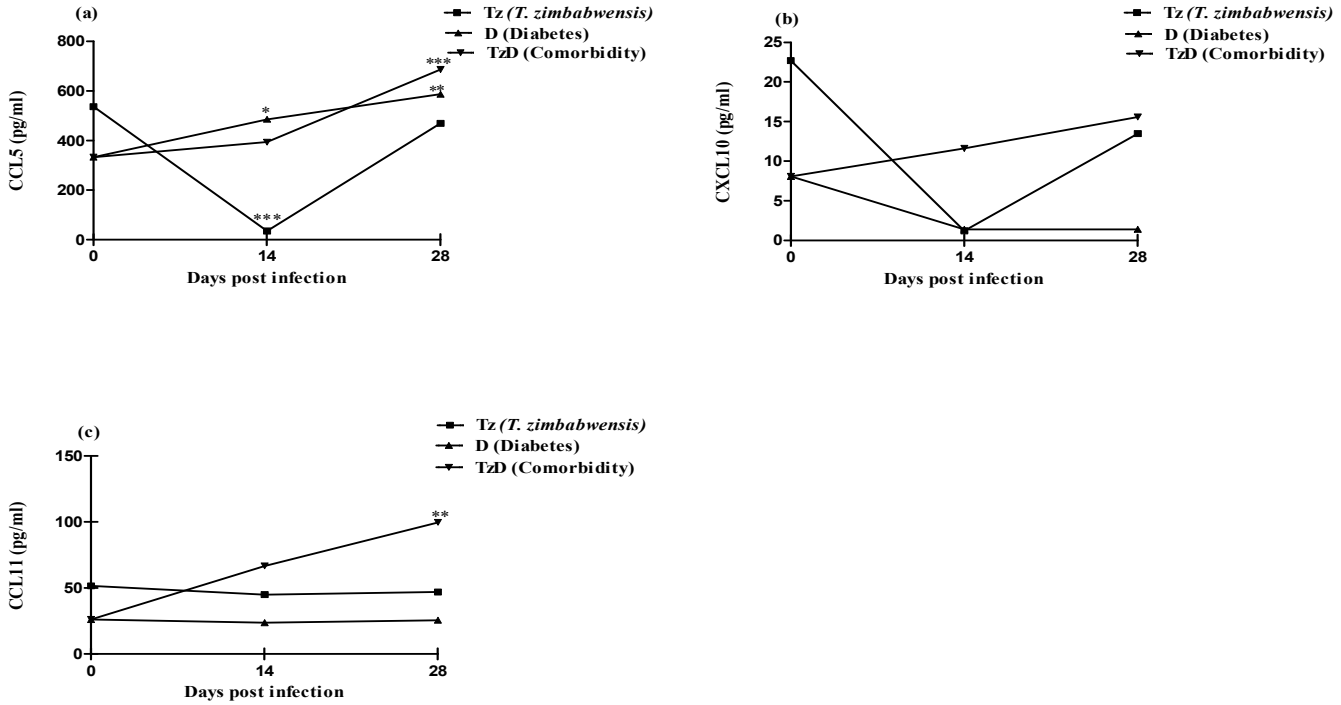

Figure 8. CCL5 concentrations (**a**) CXCL10 concentrations (**b**) and CCL11 concentrations (**c**) in male Sprague Dawley rats that were; comorbid with type 2 diabetes mellitus (TzD), diabetic (D) and infected with *Trichinella zimbabwensis* (Tz). Values are presented as mean ± SEM ($n = 6$ in each group) and asterisks represent significant differences between the groups (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3.8.2. CXCL10 Concentration

CXCL10 concentration was significantly reduced in the D (1.41–18.07) and TzD (1.41–18.07) group at day 0 pi in comparison to the Tz (8.97–36.09) group. There was an increase in the TzD (4.93–18.38) in comparison to the D (1.41–1.41) and Tz (0.91–1.41) at day14 pi. However, the increase was not significant. Reduced CXCL10 concentration was observed in the D (1.41–1.41) group, in comparison to the Tz (7.05–20.09) and TzD (1.41–29.31) groups at day 28 pi (Figure 8b).

3.8.3. CCL11 Concentration

CCL11 concentration was higher in the Tz (17.14–77.09) group in comparison to the D (9.43–43.71) and TzD (9.43–43.71) groups at day 0 pi. At day 14 pi, a reduction in concentration was observed in the D (0.90–53.08) group when compared to the Tz (0.90–110.8) and the TzD (51.82–76.91) groups. There was a significant increase ($p < 0.01$) in the TzD (56.16–159.0) group in comparison to the Tz (30.90–63.32) and the D (0.90–50.48) groups at day 28 pi (Figure 8c).

4. Discussion

The aim of the study was to investigate the changes in the haematological, cytokines and chemokines indices in induced T2DM male Sprague Dawley rats (SD) comorbid with *T. zimbabwensis*. The number of *T. zimbabwensis* AW were significantly higher in Tz group when compared to the TzD group and this suggests that T2DM had an effect in the establishment of the parasite in the small intestines in the TzD group. A low number of larvae were detected in the muscle tissue at day 21 pi and significantly increased at day 21 and 28 pi in both groups. According to Kapel et al. [30], the number of larvae in muscle is usually proportional to the larvae number ingested, and the presence of larvae in the muscles of susceptible mammals, except primates, is without any negative physiological effects, which suggests a mutual relationship between the host and parasite. Studies have also shown that in the intestinal phases of *T. zimbabwensis* infection, *T. zimbabwensis*-specific antibodies are rapidly produced [31], activating a protective innate immune response against new-born larvae (NBL) and AW [32].

A lower blood glucose level in the Tz and TzD groups indicate that *T. zimbabwensis* ML influences glucose metabolism, as supported by Wu et al. [21], which reported that Trichinella infection causes hypoglycaemia in humans and in animals. Our study further shows an increase in insulin on day 35 pi in the TzD when compared to the D group, which is in agreement with a study conducted by Hussaart et al. [33] who demonstrated that infection with *Schistosoma mansoni* exhibited higher insulin levels than uninfected controls. According to a previous study, filarial nematode *Litomosoides sigmodontis* infection resulted in glucose tolerance improvement in obese mice after infection [34]. The reduction in liver and muscle glycogen content in the D group at day 28 and 35 pi could be due to decrease in insulin signalling [35]. A study conducted previously showed that patients who have insulin resistance and obese people had elevated concentrations of adipose tissue glycogen content, and provided proof that excess accumulation could be pathological due to high inflammation [36].

A decrease in the body weight in the D group rats in this study agrees with the findings of Oyedemi et al. [37], where body weight loss was reported in diabetic animals induced with streptozotocin. This decrease in the body weight has been linked to deterioration of structural proteins and muscle wastage, which are commonly observed as symptoms of T2DM [25]. Increased water intake in the D group and the subsequent polydipsia in the D and TzD groups are well known markers of T2DM in animal models, which are a direct consequence of insulin deficiency [37–40].

A significant increase in RBC at day 28 pi in the D group was observed when compared to the TzD and the Tz. This finding agrees with previous studies [41,42] and is related to indirect traits of insulin resistance (IR) syndrome [43]. In contrast to our findings, a recent study conducted by Mokgalaboni et al. [44] demonstrated a decrease in the RBC count, which could result in anaemia and further prompts T2DM patients to develop microvascular (neuropathy, nephropathy, and retinopathy), as well as macrovascular complications (coronary artery disease (CAD), peripheral arterial disease and stroke) [45].

A significantly higher percentage of haematocrit was observed in the D group at day 28 pi in comparison to the TzD and Tz groups. Increased levels of haematocrit are associated with resistance to insulin and are independent predictors of T2DM [46]. Significantly higher levels of haemoglobin were observed in our present study in the D group at day 28 pi in comparison to the TzD and Tz groups. Our result agrees with a previous study that reported elevated levels of haemoglobin in type 1 diabetes, which is a risk factor in proliferative retinopathy in male subjects [47].

We observed that the WBC and platelet concentrations were higher at day 28 pi among the D group when compared with the Tz and TzD. Our finding agrees with previous studies which reported that platelet counts are higher and contribute to vascular events in insulin-resistant patients [48]. An increase in WBC and platelets coupled with high blood glucose in patients with diabetes mellitus may be a result of a stress response, inflammation and infectious diseases [49].

A significant decrease in lymphocyte counts observed in the D and TzD may signify high antigenic stimulation, which leads to the accelerated transformation of lymphocytes to plasma cells, leading to anti-Tz antibody production, resulting in lymphopaenia, following chronic Tz infection [50]. The observation in our present study is also consistent with findings from a previous study which showed lymphopenia following co-infection of *Trypanosoma brucei* and *Plasmodium berghei* (Pb) in mice [51].

Significantly increased levels of eosinophils were observed at day 35 pi in the TzD group when compared to the D and Tz groups, although we expected to observe the same results with the Tz group as eosinophilia is normally associated with parasitic infections. This result in the TzD group is in partial agreement with a previous study that demonstrated increased peripheral blood and tissue eosinophilia, which characterizes trichinellosis in humans [52]. An increase in neutrophils ($p < 0.001$) was observed in the D group at day 35 pi, and also in the TzD ($p < 0.01$) in comparison to the Tz group. Neutrophils play a vital role during host inflammatory response against infection [53], and it has been documented that the chemotactic activity of neutrophils from patients with diabetes is significantly reduced when compared to cells from healthy controls [54]. An elevated monocyte concentration was observed in the D group at day 28 pi when compared to the TzD and Tz. Monocytes are secreted by the bone marrow and their function in blood is not different from the role of macrophages in tissues [50,55].

TNF-α and IFN-γ concentration were elevated in the TzD when compared to the D group at day 14 and 28 pi. Our study is in contrast with the previous study by Rajamanickam et al. [56] which showed low IFN-γ and TNF-α, concentrations in T2DM patients infected with *Strongyloides stercoralis* than those with T2DM only. Increased pro-inflammatory cytokines production such as IFN-γ and TNF-α is pivotal in pancreatic β-cells obliteration and insulin resistance in the muscle, liver and adipose tissue [10]. IL-6 concentration showed a significant reduction in the Tz group when compared to the D and the TzD groups, and this is consistent with the study of Rajamanickam et al. [11], where similar results were found in patients comorbid with helminth infection and T2DM. However, reported elevated levels of IL-1β and IL-6 are predictive of T2DM [57,58], which are indicative of innate immune cells activation in response to high nutrient concentration [11]. This response in T2DM contributes to glucotoxicity, lipotoxicity, oxidative and endoplasmic reticulum stress (ER) of pancreatic islets [59].

IL-4 concentration showed a significant elevation in the TzD group when compared to D and Tz groups. Our finding agrees with a previous report from Hubner et al. [60] which demonstrated that diabetic mice with infected *Litomosoides sigmodontis* showed an increase in the production of type 2 cytokines IL-4 when compared to the uninfected mice. Several molecules are involved type 2 immune responses, including the type 2 cytokines IL-4, IL-5, and IL-13, which are influenced by chronic helminth infections [61].

There was a significant increase in IL-10 concentration in the TzD group in comparison to the D and the Tz groups on day 28 pi. A previous study by Onkoba et al. [31] reported the elevation of regulatory cytokine IL-10 concentrations during the establishment of the larvae in the striated muscle cells. This could be due to inflammation caused by *T. zimbabwensis* larvae migrating and encysting in the muscle tissue. Helminth infections enhance the production of the regulatory cytokines IL-10 and TGF-β [62,63], leading to the promotion of insulin sensitivity via alternatively activated macrophages development, promoting eosinophilic adipose tissue infiltration and the activation of innate lymphoid cells [64,65]. IL-13 concentration showed a significant elevation in the TzD in comparison to the Tz and the D groups at day 28 pi. Our study agrees with a previous study by Morimoto et al. [20], which showed a significant increase in IL-13 in KK-Ay/TaJcl mice and a large accumulation of alternatively activated macrophages (AAMacs) found in the submucosa of the small intestine in the *Heligmosomoides polygyrus* infected group.

A significant elevation of CCL5 in the D and TzD groups was observed in comparison to the Tz group at day 28 pi, in addition to increased CXCL10 and CCL11 concentration in the TzD group, in comparison to the Tz and the D groups on day 28 pi. Our study is in

contrast with a previous study by Rajamanickam et al. [11], which reported that individuals with *S. stercoralis* and T2DM had significantly decreased concentrations of chemokines when compared to individuals with *S. stercoralis* only. Chemokine released from cells also have the ability to trigger the destruction of immune cells into essential tissues or organs, as observed in both type 1 and type 2 diabetes where glucotoxicity is activated by pancreatic β-cells [66].

5. Conclusions

To the best of our knowledge, this is the first laboratory animal study that has investigated changes in the cytokines, chemokines insulin and haematological parameters indices of T2DM-induced SD rats infected with *T. zimbabwensis* (a tissue-dwelling helminth parasite). This study showed differences in the establishment of adult worms and larvae of *T. zimbabwensis* with significantly low burdens in the TzD group when compared with the Tz group. Additionally, glucose levels were influenced by *T. zimbabwensis* infection, with the TzD group recording low levels of blood glucose compared to the D group. The increase in insulin levels recorded in the TzD in our study is an indication that *T. zimbabwensis* ML influences glucose metabolism and ML migration may favour muscle glucose uptake by inhibiting glucose metabolism and glycogenolysis. The increase in RBC, haematocrit, haemoglobin, WBC, platelet, neutrophils, and monocyte were observed in the D in comparison to the TzD group. TNF-α, IFN-γ, IL-4 and IL-13 concentrations were elevated in the TzD when compared to the D ad Tz groups. IL-10 showed a significant increase in the TzD group in comparison to the Tz group, while IL-6 also showed a reduction in the Tz group in comparison to the D and the TzD groups. Additionally, CXCL10 and CCL11 concentration showed an increase in the TzD group in comparison to the Tz and the D groups. Elevated CCL5 in the D and TzD groups was observed in comparison to the Tz group.

A comparison of the results of haematological, cytokine, chemokine, insulin, and glucose indices from our study groups strongly support that the *T. zimbabwensis* parasite has the capability to regulate T2DM-driven inflammation to mediate a positive protective effect against T2DM outcomes. The production of high pro-inflammatory cytokines such as TNF-α, IFN-γ, and IL-6 play a vital role in the destruction of pancreatic β-cells, and insulin resistance in adipose tissue (AT), as well as liver and muscle. Additionally, elevated levels of Th2 cytokines such as IL-4 and IL-13 play a crucial role in the regulation of gastrointestinal homeostasis, which leads to whole body metabolic homeostasis, and it has also been shown that IL-4 can directly reduce ongoing Th1-driven autoimmune inflammation. Helminth infection influences the production of the anti-inflammatory cytokines such as IL-10 and this cytokine plays an important role in protecting against T2DM as well as promoting the survival of the parasite and reduced the risk of inflammatory injury in the host. Chemokines such as CCL5 is involved in the destruction of immune cells into essential tissues, and CXCL10 and CCL11 plays a role in islet inflammation Rajamanickam et al. [11]. Our data also strongly support recommendations made from similar studies using different helminth species that helminth infection could offer novel therapeutic approaches to treating inflammatory metabolic diseases. The authors, therefore, recommend further studies to unravel the various mechanisms underlying the immunoregulation during the process of comorbidity, and *T. zimbabwensis* is an ideal parasite for use in animal model studies.

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