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Role of Liver and Cardiac Enzymes as Markers of Parasite Load and Recovery after Treatment in Experimental Murine Toxocariasis

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ABSTRACT

Background: *Toxocara* is mainly a parasite of animals. The disease is caused mainly due to accidental ingestion of *Toxocara canis* embryonated eggs and to a lesser extent *T.cati* eggs. Till now, there is no marker of severity of infection or treatment in toxocariasis.

Aim of the study: The current work aimed at studying the role of liver enzyme aspartate transaminase (AST) and cardiac enzyme creatine kinase-MB isoenzyme (CK-MB) as markers of severity infection and treatment of toxocariasis in correspondence to serum immunoglobulin with study of histological cardiac effects of toxocariasis.

Materials and Methods: Laboratory mice were divided into groups infected with different doses of *Toxocara* eggs. Sera were collected from each group before and after treatment for measurement of AST and CK-MB, as well as ELISA for anti-*Toxocara* immunoglobulin IgG. T test, ANOVA test and Pearson correlation tests were used to assess the results.

Results: CK-MB was elevated significantly with infection and reduced significantly after treatment. The serum level of CK-MB also correlated significantly and positively with parasite load.

Conclusion: CK-MB can be used together with anti-*Toxocara* IgG in diagnosis and CK-MB can be a good markers of treatment and parasite load in toxocariasis.

Key words: *Toxocara*- IgG- Liver enzymes-cardiac enzymes.

Running Title: Monitoring therapy of toxocariasis by liver and cardiac enzymes.

Conflict of interest: the author declares no conflict of interest.

INTRODUCTION

Toxocara is mainly a parasite of animals. The zoonotic infection with *Toxocara* is called toxocariasis [1]. The disease is caused mainly due to accidental ingestion of *Toxocara canis* embryonated eggs and to a lesser extent *T.cati* eggs [2]. Larvae are liberated in upper part of small intestine and penetrate intestinal wall reaching blood vessels and settle in different body organs [3]. *T. canis* sero-prevalence was 12.1%, by ELISA and this sero-prevalence was confirmed by Western Blot (14.5%) in the Estonian Population [4]. Out of 238 patients with uveitis of unknown etiology, 71 (29.8%) were diagnosed with ocular toxocariasis, and 80 (33.6%) had positive ELISA results for serum anti-*Toxocara* IgG in Korea[5]. Twenty-two percent of pregnant women were found to have anti-*Toxocara* IgG antibodies in pregnant women in Brazil [6]. *T. canis* prevalence was 14.5 % in Caribbean countries [7]. The sero-prevalence of anti-*T.canis* IgG antibodies was 14.9% in the research laboratories workers in Brazil [8]. Sero-prevalence of antibodies against *Toxocara spp*. is high in rural population in Gabon with prevalence for *Toxocara* spp. 59.9% [9]. Sero- prevalence of toxocariasis was 45.2 % in all samples of Medical Center Laboratory, Ho Chi Minh City, Vietnam in 2012 [10]. The recorded prevalence in rural areas in Zagazig district, Sharkyia Governorate, Egypt, was 2.2 % for *Toxocara* and it was the most prevalent helminthes among school children [11].

Clinically, patient complains of fever; larvae in tissues cause fever, hepatomegaly, respiratory, cardiac or nervous disorders termed as visceral larva migrans (VLM). When the disease is confined to the eye it is called ocular larva migrans [3]. Overt toxocariasis may go undiagnosed as diagnostic tests may be expensive, difficult and cannot be carried out in health centers [12]. The cardiac manifestations of the disease may be myocarditis, Loeffler's endocarditis or even cardiac temponades [13].The importance of cardiac manifestation and its relevance had been increasing recently [14].

The disease is usually treated with anti-parasitic drugs as albendazole, thiabendazole [15], mebendazole and nitazoxanide [16].

Liver disease is often reflected by biochemical abnormalities of 1 of the 2 different hepatic systems or of liver function. Although tests that

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measure the level of serum liver enzymes and commonly referred to as liver function tests, they usually reflect hepatocyte integrity or cholestasis rather than liver function. Liver function tests may be arranged to help diagnose or monitor liver problems. Alanine transaminase (ALT) and aspartate aminotransferase (AST) higher readings may suggest inflammation of liver cells or the death of some cells due to liver damage. Alkaline phosphatase (ALP) higher readings suggest liver disease or bile duct blockages [17].

Creatine kinase enzyme (CK) is found with 3 isoenzymes of creatine kinase (CK)-BB, -MM, and -MB. The primary source of CK-MB is myocardium, CK-MB level increases with myocardial damage [18]. The CK-MB test is a cardiac marker used to assist diagnosis of acute myocardial infarction [19]. The blood level of CK-MB, refers to the bound combination of two variants (isoenzymes CK-M and CK-B) of the enzyme phosphocreatine kinase. The newer test detects different isoforms of the B subunit specific to the myocardium whereas the older test detected the presence of cardiac-related isoenzyme dimmers [20].

Till now, there is no marker of severity of infection or treatment in toxocariasis[21]. The current work aimed at studying the role of liver enzyme AST and cardiac enzyme CK-MB as markers of severity infection and treatment of toxocariasis in correspondence to serum immunoglobulin with study of histological cardiac effects of toxocariasis.

MATERIALS AND METHODS

For the described aim 100 albino mice were used. The experiments were carried out on 6-weeks old laboratory bred mice from Theodor Bilharz Research Institute. Mice were housed in polycarbonate cages, fed *Ad libitum* and kept in animal house under standard requirements (25 ± 2°C, 60-65% relative humidity). They were classified to 5 groups (20 mice/each);

Group 1: Twenty infected mice with 250 *T.canis* eggs by oral route.

Group 2: Twenty infected mice with 500 *T.canis* eggs by oral route.

Group 3: Twenty infected mice with 750 *T.canis* eggs by oral route.

Group 4: Twenty infected mice with 1000 *T.canis* eggs by oral route.

Group 5: Control group.

1. Preparation of infecting inoculum and infection of mice: *T.canis* eggs were obtained from adult female worms from naturally infected dogs. The eggs were incubated in 0.5% formalin at 26 ˚c for 4-6 weeks. At time of infection eggs were washed and counted and number was adjusted per ml for experimental infection[16]. Mice in groups 1,2,3 and 4 were infected through oral intubation using 250, 500, 750 and 1000 *T.canis* eggs/250µl of water, respectively. Control group was given distilled water only.

2. Assessment of level of liver enzyme (AST) and cardiac enzyme (CK-MB), and anti *Toxocara* IgG antibodies before treatment: At 4 weeks post infection 10 mice from each group were sacrificed and sera were collected for ELISA for anti-*Toxocara* IgG, AST and CK-MB. Tissues were examined for presence of larvae.

3. Assessment of level of liver enzyme (AST) and cardiac enzyme (CK-MB), and anti *Toxocara* IgG antibodies after treatment: To evaluate this aim, mice in all groups except control were treated with mebendazole at a dose of 15mg/kg/day for 5 days, orally and sera were collected from different groups 4 weeks after treatment to evaluate the effect of treatment on tested parameters [16].

4. ELISA test for anti-*Toxocara* IgG immunoglobulin: Serum samples collected from mice in different groups before and after treatment, were examined by ELISA to detect IgG class anti-*Toxocara* antibodies using goat anti-mouse IgG HRP (ABD Serotec, USA) [22]. ELISA was carried out according to manufacturer's instructions (Nova Lisa *Toxocaracanis*IgG ELISA, Dietzenbach, Germany. cat no TOGG0450).

5. Assessment of liver enzyme (AST): Samples were processed in single batch for determination of aspartate transaminase (AST) level using commercial kits for Beckman Unicell Analyzer [23].

6. Assessement of CK-MB enzyme: Standard assay commercial kits were used to determine the serum levels of CK-MB using PD-3035 secrophotometer (APEL, Japan) [24]. Cardiac muscles of mice from each group were examined histologically after being sacrificed by Hematoxylin and Eosin stain(H&E).

7. Statistical analysis: Results were collected, tabulated, and statistically analyzed using the SPSS version 16 computer software statistical package (SPSS Inc., Chicago, USA). Two types of

statistics were performed. Descriptive statistics included the mean (χ) and SD. Analytic statistics, the *F*-test or ANOVA,T test and Pearson correlation test were used to compare means and their SD from three or more deviations. *P* value of less than 0.05 was considered statistically significant[25].

8. Ethical considerations: The experimental animal studies were conducted in accordance with international valid guidelines and they were maintained under convenient conditions.

RESULTS

Assessment of the mean level of IgG, AST and CK-MB in each group before and after treatment:

From the current study, It was found that cardiac enzyme CK-MB level were raised during *Toxocara* infection in G1 and reduced after treatment significantly. Anti-*Toxocara* IgG level and AST were either raised after treatment or non-significant (Table 1). Meanwhile in G2; AST and CK-MB were significantly reduced after treatment (Table 2). All the tested enzymes were significantly decreased after treatment in G3 as well as anti-*Toxocara* IgG level (Table 3). In group 4, serum CK-MB level was significantly reduced after treatment (Table 4).

Assessment of the mean levels of IgG, AST and CK-MB in all groups before and after treatment:

On comparison between groups before treatment regarding the tested enzymes the differences between groups were statistically significant. This was not recorded in serum anti-*Toxocara* IgG level. (Table 5). Meanwhile, on comparison between groups after treatment regarding the tested enzymes, only CK-MB level showed statistically significant difference (Table 6).

Correlation between the mean level of IgG, AST and CK-MB in groups before and after treatment and parasite load:

It was observed that there were positive correlations between parasite load and serum enzyme levels of AST and CK-MB during infection (Table, 7). Also, there were positive correlation between parasite load and serum enzyme levels of CK-MB only after treatment (Table, 8).

Detection of *Toxocara* larva in cardiac tissues of mice from different groups:

Toxocara larvae were very rare or may not be found in cardiac tissues of infected mice. While cardiac tissue appeared normal in control group (Figure 1), no larvae were detected in cardiac tissues in mice of G1. It was found that cardiac tissues showed congestion and inflammation as reported in G2 with minimal number or no larvae in its tissue (Figure 2) and G3 (figure 3). More number of migrating *Toxocara* larvae were detected in cardiac tissues of G4 (Figure 4) accompanied with muscle inflammation and degeneration.

DISCUSSION

Toxocariasis is usually diagnosed by detection of anti-*Toxocara* immunogloblins in serum. However from the current study, It was found that ELISA for anti-*Toxocara* IgG can be used in diagnosis of toxocariasis but cannot differentiate between active and treated infection and the level of antibodies does not correlate with parasite load. Clinical diagnosis of toxocariasis depends mainly on detection of anti-*Toxocara* immunoglobulin done by ELISA, however, cross reaction with other parasites as *Ascaris*, *Anisakis* and *Strongyloides* were reported by Yamasaki *et al.* [26]. False positive and false negative results were observed after comparison with Western Blot method [27] and by using secretory/excretory antigen [28]. Also ELISA test using serum of infected patient however is positive, cannot differentiate between old and recent infection as reported by Magnaval *et al.*, [29] and Lee, [30]. To combat this; clinical data and other serological data as elevated seum IgE and eosinophilia together with chest, liver or brain CT as well as elevated liver enzymes should be carried out [31]. Serum level of anti-*Toxocara* antibodies in the current work was not corresponding to parasite burden as also observed by Fenoy *et al.* (32) and Lapsoy *et al.*, [33]. Serodiagnosis of human toxocariasis is based on the detection of specific IgG antibodies by the enzyme-linked immunosorbent assay (ELISA) using *Toxocara* larvae excretory-secretory (TES) antigens, but its production is a laborious and time-consuming process being also limited by the availability of adult females of *T. canis* as source for ova to obtain larvae [34]. The serodiagnosis of human toxocariasis may be difficult in interrpretation. Specific IgGs detected routinely with ELISA based on *Toxocara* excretory-

secretory (TES) antigens often persist for years at an elevated level, which does not allow either the differentiation between an active and persistent infection or monitoring the effect of treatment. These results showed the necessity of obligatory verification of all ELISA IgG positive and questionable results by Western Blot [4]. The previous factors may illustrate the need for other tests to help diagnosis and treatment efficacy. The index of IgG avidity may be helpful in excluding recent infection, but its usefulness in detecting an active phase of invasion requires further research [35]. The sensitivity and specificity of the ELISA test were 91.5% (65 / 71) and 91.0% (152 / 167), respectively [5]. Also, No significant correlation was found among clinical features and IgG production in other studies [36].

Liver enlargement with elevated liver enzymes are common clinical finding in toxocariasis. *T. canis* antibodies were positive in 6% of children with liver enlargement[37]. The tested liver enzymes were variable in their value determining the previous parameters. They are not reliable in specific diagnosis, also cannot correlate with parasite load and effective treatment at all states of treatment. These results are in harmony with previous study showed moderate disturbance of liver enzymes and hypereosinophilia in human toxocariasis [38].

Cardiac inflammation due to toxocariasis raised CK-MB levels in the current study and that was significant. Also it was reduced significantly after treatment in all groups and its level was correlated with parasite load. So serum CK-MB can be good marker of parasite load and efficacy of treatment.

In the current study minimal number or no larvae were detected in cardiac tissues in different tested groups. Similar results were observed by Park *et al.* [14], the authors found that cardiac involvement with *Toxocara* larva is rare however eosinophilic myocarditis can be recorded in patient with toxocariasis. Cookston *et al.* [39] found that myocarditis due to toxocariasis in mice was observed with low or even no larvae in heart muscles and eosinophilic infiltrate. They explained this as the heart may be a temporary route of migrating larvae leading finally to cardiac inflammation.

Data in the current study showed that CK-MB not AST can be used as marker of parasite load and treatment in toxocariasis. This can be explained as larvae of *Toxocara* do not settle in cardiac tissue and only migrate through it, this was observed histologically which was by Cookston *et al.* [39] and Park *et al.* [14] with continuous migration and passage in cardiac tissue causing sustained injury and release of CK-MB which does not occur in liver tissue with larval settlement and short half-life of the enzyme.

Conclusion: From the current study, it was found that CK-MB can be a good marker of treatment of toxocariasis and their level can be indication of parasite load. Also CK-MB can help in diagnosis of toxocariasis together with anti-*Toxocara*IgG in diagnosis and its importance increase in children as rising level in children is suggestive.

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REFERRENCES

1. **Pinelli E, Brands S, Dormans J, Gremmer E, van Loveren H (2007).** Infection with roundworm Toxocaracanis leads to exacerbation of experimental allergic airway inflammation. Clin. Exp. Allergy, 38:649-658.
2. **Zaia M, de Oliveira S, Castro C, Soares E, Monnazzi L *et al.* (2015).** *Toxocara canis* and allergic process. Medm. Inst. Oswaldo Cruz, 110 (6):720-731.
3. **Despommier D (2003).** Toxocariasis: Clinical aspects, epidemiology and molecular aspects. Clin. Microbiol. Rev., 16(2): 165-172.
4. **Lassen B, Janson M, Viltrop A, Neare K, Hütt P *et al.* (2016).** Serological Evidence of Exposure to Globally Relevant Zoonotic Parasites in the Estonian Population. PLoS One, 11(10):e0164142.
5. **Bae KW, Ahn SJ, Park KH, Woo SJ (2016).** Diagnostic Value of the Serum Anti-*Toxocara* IgG Titer for Ocular Toxocariasis in Patients with Uveitis at a Tertiary Hospital in Korea. Korean J. Ophthalmol., 30(4):258-64.
6. **Pereira LC, Elefant GR, Nóbrega YM, Vital T, Nitz N *et al.* (2016).** *Toxocara* spp. sero-prevalence in pregnant women in Brasília, Brazil. Rev. Soc. Bras. Med. Trop., 49(5):641-643.
7. **Guo F, Forde MS, Were SR, Krecek RC, Zhu G (2017).** Sero-prevalence of five parasitic pathogens in pregnant women in ten Caribbean countries. Parasitol. Res., 116(1):347-358
8. **Mattos GT, Dos Santos PC, Telmo PL, Berne ME, Scaini CJ (2016).** Human Toxocariasis: Prevalence and Factors Associated with Biosafety in Research Laboratories. Am, J. Trop. Med. Hyg., Am J Trop Med Hyg. , 95(6):1428-1431.
9. **Lötsch F, Obermüller M, Mischlinger J, Mombo-Ngoma G, Groger M *et al.* (2016).** Seroprevalence of *Toxocara* spp. in a rural population in Central African Gabon. Parasitol. Int., 65(6 Pt A):632-634.

10. **Nguyen T, Cheong FW, Liew JW, Lau YL (2016).** Sero-prevalence of fascioliasis, toxocariasis, strongyloidiasis and cysticercosis in blood samples diagnosed in Medic Medical Center Laboratory, Ho Chi Minh City, Vietnam in 2012.Parasit. Vectors, 9(1):486.
11. **Farghly AM, Mohamed SM, Abdel-Rahman SA, Mohammed FE, El-Bahaie ES *et al.* (2016).** The relation between the prevalence of soil transmitted parasites in the soil and among school children in Zagazig district, Sharkyia Governorate, Egypt. J. Parasit. Dis., 40(3):1021-1029.
12. **Lemaire A, Trouillier S, Samou F, Delevaux I, Aumaitre O(2014).** Visceral larva migrans with cardiac manifestations: a case report and literature review. Rev. Med. Interne., 35(12): 831-837.
13. **Bolivar-Mejia A, Rodriguez-Morales AJ, Paniz-Mondolfi AE, Delgado O (2013).** Cardiovascular manifestations of human toxocariasis. Arch. Cardiol. Mewx., 83(2): 120-129.
14. **Park H, You G, Cho K, Yaug J, Kim S *et al.* (2014).** Cardiac involvement due to eosinophilia associated with toxocariasis. J. Cardiovasc. Ultrasound, 22(4): 224-227.
15. **Dorin J, Filippeti L, Debourgogne A, Volliot D, Huttin O *et al.* (2015).** Cardiac manifestations of toxocariasis: A case report of Loffler endocarditis and a literature review. J. Bacteriol. Parasitol., 6(3):1000226.
16. **Lescand SA, Santos SV, Assis JM, Chieffi PP (2015).** Efficacy of nitazoxanide against *Toxocara canis* larval recovery and humeral immune response in experimentally infe3cted mice. Rev. Inst. Med. Trop. Sao Paulo, 57(4):337-341.
17. **Edoardo G, Roberto T, Vincenzo S (2005).** Liver enzyme alteration: a guide for clinicians. C.M.A.J., 172(3): 367–379.
18. **Mair J, Artner-Dworzak E, Dienstl A, Lechleitner P, Morass B *et al.* (1991).** Early detection of acute kinase-MB. Am. J. Cardiol., 68:1545-1550
19. **Guzy PM (1977).** Creatine phosphokinase-MB (CPK-MB) and the diagnosis of myocardial infarction. West. J. Med., 127 (6): 455–460.
20. **Foreback CCand Chu JW(1981).** Creatine kinase isoenzymes: electrophoretic and quantitative measurements. CRC Crit. Rev. Clin. Lab. Sci., 15:187–230
21. **Lee TH and Goldman L (1986).** Serum enzyme assays in the diagnosis of acute myocardial infarction. Recommendations based on a quantitative analysis. Ann. Int. Med., 105:221–233.
22. **Havasiová-Reiterová K, Tomasovicová O, Dubinský P (1995).** Effect of various doses of infective *Toxocara canis* and *Toxocara cati* eggs on the humoral response and distribution of larvae in mice. Parasitol. Res.,81(1):13-17.
23. **Haque A, Best S, Amanate A, Ammerdroffer A, Labustida F *et al.* (2011).** High parasite burdens cause liver damage in mice following Plasmodium berghei ANKA infection independently of CD8+ T cell-mediated immune pathology. Infection and Immunity, 79(5): 1882-1888.
24. **Collinson P, Stubbs P, Kessler A(2003).** Multicenter evaluation of the diagnostic value of cardiac troponin T, CK-MB and myoglobin for assessing patients with suspected acute coronary syndromes in routine clinical practice. Heart, 89(3): 280-286.
25. **Addelman S (1977).** Variability of treatments and experimental units in the design and analysis of experiments. J. Am. Statist. Assoc., 65:1095–1098.
26. **Yamasaki H, Araki K, Lim PK, Zasmy N, Mak JW *et al.* (2000).** Development of a high specific recombinant *Toxocara canis* second stage larva excretory-secretory antigen for immunodiagnosis of human toxocariasis. J. Clin. Microbiol., 38(4): 1409-1413.
27. **Ozkoe S, Bayram S, Akisu C (2012).** Toksokariyaziss eolojiktanisida Trichinella caprazreaksiyonlarinindegerlendirilmesi. Mikrobiyol.Bul., 46(3): 456-473.
28. **Selek MB, Baylan O, Kutlu A, Ozyurt M (2015).** *Toxocara canis* IgG sero-positivity in patients with chronic urticaria. Iran J. Allergy Asthma Immunol., 14(4): 150-156.
29. **Magnaval JF, Glickman LT, Dorchies P, Morassin B (2001).** Highlights of human toxocariasis. Korean J. Parasitol., 39: 1-11
30. **Lee SP (2013).** Highlights and diagnostic dilemma of toxocariasis. Korean J. Med., 84:200-202.
31. **Lee JY, Yang MH, Hwang JH, Kang M, Paeng JW *et al.* (2015).** The prevalence of toxocariasis and diagnostic value of serologic tests in asymptomatic Korean adults. Allergy Asthma Immunol. Res., 7(5): 467-475.
32. **Fenoy S, Rodero M, Pons E, Aguila C, Cuellar C (2008).** Follow up of antibody avidity in BALB/c mice infected with *Toxocara canis*. Parasitol., 135(6):725-733.
33. **Laposy CB, Freitas SZ, Louzada AN, Elefant GR, Giuffrida R *et al.* (2012).** Cardiac markers: profile in rats experimentally infected with *Toxocara canis*. Rev. Bras. Parasitol. Vet., 21(3):1-4.
34. **Elefant GR, Roldán WH, Seeböck A, Kosma P (2016).** Evaluation of a di-O-methylated glycan as a potential antigenic target for the sero-diagnosis of human toxocariasis. Parasite Immunol., 38(4):236-243.
35. **Rudzińska M, Kowalewska B, Sikorska K (2016).** Clinical usefulness of Western blotting and ELISA avidity for the diagnosis of human toxocariasis. Parasite Immunol., [Epub ahead of print]
36. **Wang ZJ, Zhou M, Cao WJ, Ji J, Bi YW *et al.* (2016).** Evaluation of the Goldmann-Witmer coefficient in the immunological diagnosis of ocular toxocariasis. Acta Trop.,158:20-23.

37. **Hassan MM, Farghaly AM, Gaber NS, Nageeb HF, Hegab MH *et al.* (1996).** Parasitic causes of hepatomegaly in children. J. Egypt Soc. Parasitol., 26(1):177-189.
38. **Rey P, Bredin C, Carrere C, Froment N, Casassus-Builhe D (2005).** Toxocariasis mimicking liver tumor. Press. Med., 34(22 Pt 1):1715-1716.
39. **Cookston M, Stober SM, Kayes SG (1990).** Eosinophilic myocarditis in CBA/J mice infected with *Toxocara canis.* Am. J. Pathol., 136(5):1137-1145.

Table 1: Comparison between the mean of, IgG level, AST and CK-MB in G1 before and after treatment

	G1			
	Before ttt Mean±SD	After ttt Mean±SD	t-test	*P*-value
Serum IgG Level (mg/dl)	11.2±2.5	15.5±1.9	- 2.418	0.052
Serum AST level (IU/L)	40.5±2.1	45.5±2.2	- 2.357	0.143
Serum CK-MB level (IU/L)	168±6.6	52±1.6	37.835	<0.0001*

Table 2: Comparison between the mean of IgG level, AST and CK-MB in G2 before and after treatment

	G2		t-test	*P*-value
	Before ttt Mean±SD	After ttt Mean±SD		
Serum IgG Level(mg/dl)	13.5±2.8	17.9±1.2	-1.8	0.123
Serum AST level (IU/L)	46.3±3.2	27±2.8	8.4	0.014*
Serum CKMB level(IU/L)	237±8.9	105±3.8	30.363	<0.0001*

Table 3: Comparison between the mean of IgG level, AST and CK-MB in G3 before and after treatment

	G3		t-test	*P*-value
	Before ttt Mean±SD	After ttt Mean±SD		
Serum IgGLevel(mg/dl)	11.6±2.5	19.2±0.05	- 6.1	0.009*
Serum AST level (IU/L)	73±15.4	27.5±6.3	3.8	0.032*
Serum CKMB level(IU/L)	309.8±7.9	135±7.9	34.863	<0.0001*

Table 4: Comparison between the mean of IgG level, AST and CK-MB in G4 before and after treatment

	G4		t-test	*P*-value
	Before ttt Mean±SD	After ttt Mean±SD		
Serum IgG level (mg/dl)	13.4±0.5	14.1±3.2	- 0.208	0.854
Serum AST level (IU/L)	68.6±10	57.3±8.7	1.477	0.214
Serum CKMB level (IU/L)	411±7.6	176±5.7	55.237	<0.0001*

Table 5: Comparison between the mean of IgG level, AST and CKMB in all groups before treatment

	Before ttt				ANOVA-te	*P*-value
	G1	G2	G3	G4		
IgG Level(mg/dl)	11.2±2.58	13.5±2.8	11.57±2.5	13.4±0.5	0.578	0.644
AST(IU/L)	40.5±2.1	46.3±3.2	73±15.3	68.6±10	7.8	0.012*
CKMB(IU/L)	168±6.67	237±8.9	309.8±7.9	411±7.6	880.03	<0.0001*

Table 6: Comparison between the mean of IgG level, AST and CKMB in all groups after treatment

	After ttt				ANOVA te	*P*-value
	G1	G2	G3	G4		
IgG Level(mg/dl)	15.49±1.9	17.8±1.15	16.67±4.4	19.9±10.4	0.387	0.765
AST(IU/L)	45.5±2.1	27±2.8	27.5±6.3	57.3±8.7	12.9	0.09
CKMB(IU/L)	52±1.5	105±3.8	135±7.9	176±5.7	486.44	<0.0001*

Table 7: Correlation between the IgG level, CKMB, and AST and parasite load before treatment

	Before treatment					
	Parasite load		IgGLevel		CK-MB	
	R	P	R	P	r	P
Parasite load			.211	.342	.969	<0.0001*
IgGLevel	. .211	.342			.104	.713
CK-MB	.969	<0.0001*	.068	826		
AST	.867	<0.0001*	.484	.224	.751	.003*

Table 8: Correlation between the IgG level, CKMB and AST and parasite load after treatment

	Parasite load		IgGLevel		CK-MB	
	r	P	R	P	r	P
Parasite load			.002	.995	.969	<0.0001*
IgGLevel	.002	.995	.		-.036	.915
CK-MB	.969	<0.0001*	-.036	. 915	.	
AST	.408	.276	-.43	.335	.341	.369

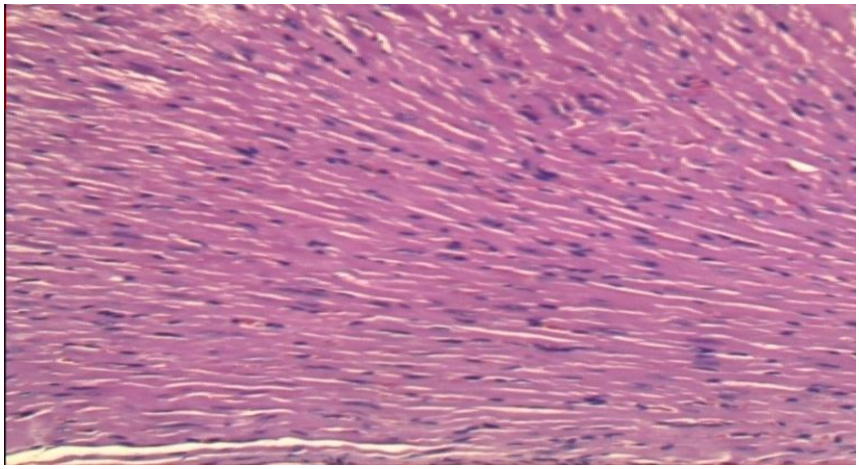

Figure 1: Showing cardiac muscle of mice in control group (H&E X100)

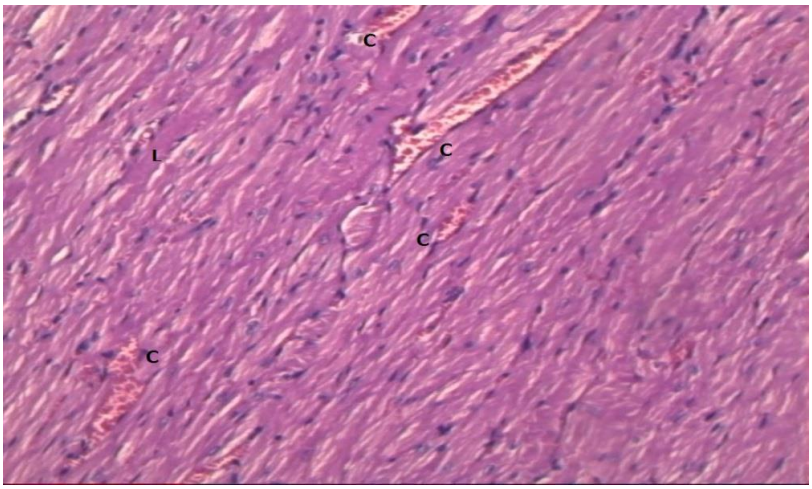

Figure 2: showing congested (c) cardiac muscles due to *Toxocara* larva(L) in tissues G2(H&E X100)

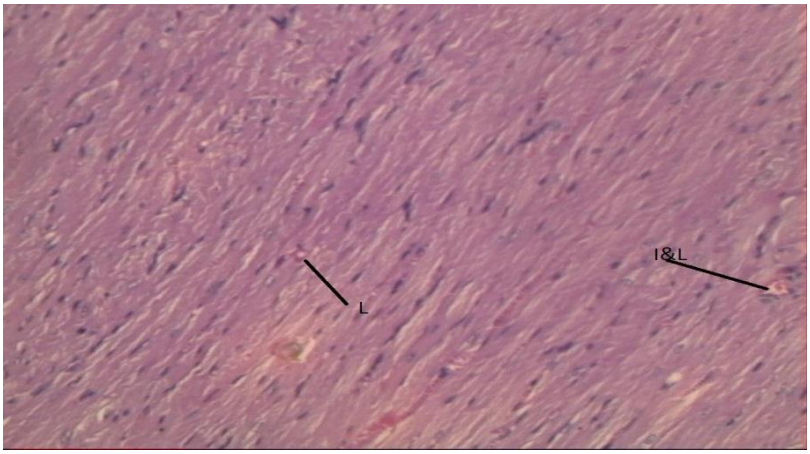

Figure 3: Showing *Toxocara* larvae in cardiac muscle (L) in G 3 group (H&E X100)

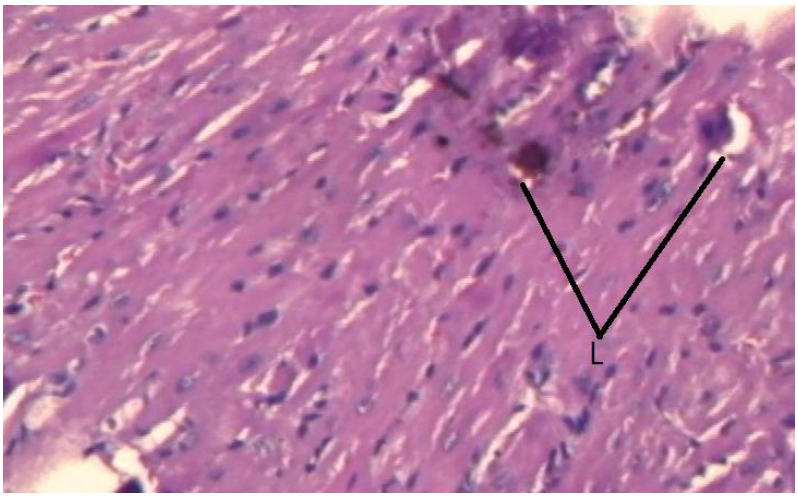

Figure 4: Showing *Toxocara* larva in cardiac muscle(L) of mice in G4 with muscle inflammation and degeneration (H&E X200)