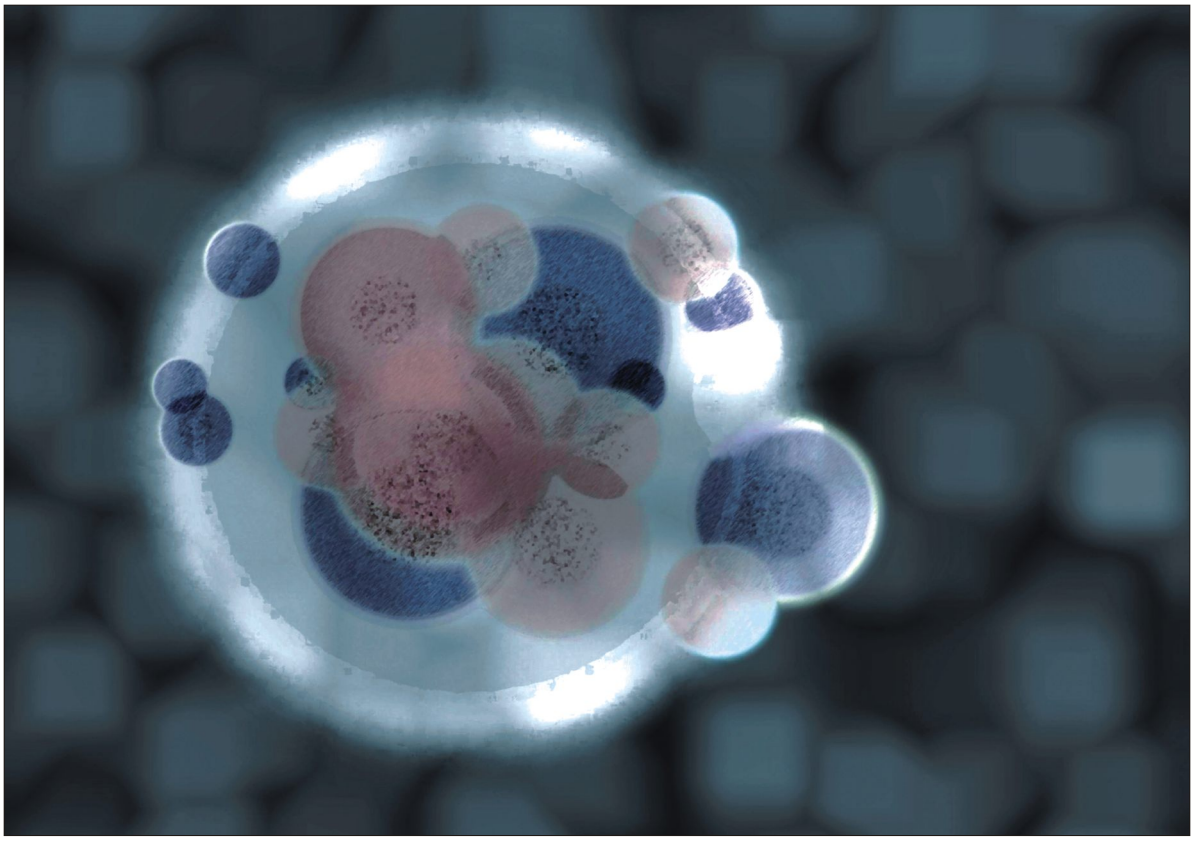

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Research Article
Hematological, Physiological, Histopathological and Immunological Effects of Pinworm (*Aspiculuris tetraptera*) Infection in Laboratory Mice

[1,2]Dalia Fouad, [1]Asma Hajla, [3]Farid S. Ataya and [1]Ebtsam Al-Olayan

[1]Department of Zoology, College Science, King Saud University, Riyadh, Saudi Arabia
[2]Department of Zoology and Entomology, Faculty of Science, Helwan University, Ain Helwan, Helwan, Egypt
[3]Department of Biochemistry, College Science, King Saud University, Riyadh, Saudi Arabia

Abstract

Background and Objective: Many animal houses in the Middle East are infected with some common parasites like *Aspiculuris tetraptera*. Some researchers consider this infection as normal although it could have unforeseen impacts on experimental assays and study outcomes. This study aimed to investigate the effects of *A. tetraptera* infection on the haematological, physiological, histopathological and immunological parameters of laboratory mice in the King Saud University's animal house. **Materials and Methods:** Twenty-five mice were used in this experiment, twenty were used as the experimental group following natural infection with *A. tetraptera*, while the other five were not infected and used as the control group. This study involved blood cell count liver and kidney function tests, lipid profile and histological and immunological examination of tissue samples. Statistical analyses were carried out using SPSS software and unpaired T-Tests. **Results:** Haematological tests revealed marked increases in the white blood cell count (WBC) of infected mice when compared with the control group, whereas no significant changes were observed in Red Blood Cells (RBC) count. Physiological analysis of liver function revealed significant increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum of infected mice indicating liver injury. The lipid profiles of infected mice showed significant increases in total cholesterol, triglycerides, HDL-c and LDL-c. In addition, histological and immune histochemical evaluations of IL-6 and TNF-α in the liver, kidney and spleen showed significant differences between the two groups. **Conclusion:** Taken together, these results support the hypothesis that *A. tetraptera* infection interferes with research results as indicated by altered blood cell count, liver function, lipid profile and abnormal tissue histology over the normal uninfected mice.

Key words: Mice, pinworm, liver, kidney, IL-6, TNF-α, lipid profile

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Corresponding Author: Dalia Fouad, Department of Zoology, College Science, King Saud University, Riyadh, Saudi Arabia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mouse colonies housed in conventional research facilities are frequently infected with helminths in the animal house or they can become infected in the laboratories where they are maintained over the course of the experiments. Undiagnosed parasitic infections in these animals, even with no clinical symptoms, may act as unknown variables in some experimental assays[1] which may bias or influence their outcomes[2,3].

The next most common internal parasites of laboratory mice after protozoa are the oxyurids or pinworms[4], which inhabit the caecum and colon. Infections caused by pinworms are generally considered mildly- or non-pathogenic in immune-competent rodents and seldom penetrate the mucosa of the gastrointestinal tract[5,6].

Even in the event of high parasite loads, clinical signs are rarely seen, older literature does suggest that very high pathogen levels may cause decreased growth rates, catarrhal enteritis, hepatic granulomas and perianal irritation, but co-infection with other agents, like *Helicobacter*, *Dentostomella translucida and Syphacia obvelata* may contribute to this more obvious symptoms[7-9].

The prevalence of pinworms in an infected rodent population depends on many factors, including environmental load, gender, age, strain and immune status. Males have been found to have higher parasite burdens than females, while young animals tend to suffer more than older animals, as a result of immune activation in the older rodents[6,10]. Resistance to re-infection and control of these parasites through the immune response were found to be hallmarks of *A. tetraptera* infection[11].

Based on these facts, this study aimed to shed light on the impacts of pinworm infection on haematological, biochemical and immunological parameters and the possible variation in mice liver and kidney histology due to such infection. This study support the recommendation to avoid using any naturally infected mice with *A. tetraptera* due to its effect on various studied parameters which may influence the research outcomes.

MATERIALS AND METHODS

Study area: Male albino mice (*Mus musculus*) were obtained from the animal house of King Saud University, Riyadh, Saudi Arabia in February to April, 2020 and the study was carried out at the laboratories of College of Science, King Saud University.

Experimental animals: A total of 25 male albino mice (*Mus musculus*), that were approximately 33 gin weight were obtained from the animal house of King Saud University, Riyadh, Saudi Arabia. The mice were housed in a room with a controlled temperature 24±2°C, lighting 12 hrs light/dark cycle and lative humidity of 40-70%. A standard diet and water ad libitum were also administered to the mice. All animals were handled according to the recommendation of the Ethics Committee at King Saud University (KSU), Riyadh, Saudi Arabia (SE-19-137).

Experimental design: The mice under study were divided into 2 groups, the first group contained 20 naturally infected mice with the pinworm *A. tetraptera* and the other group, five Specific-Pathogen-Free (SPF) and non-infected mice were used as the control.

Whole blood was collected for Complete Blood Count (CBC) analysis used to evaluate RBC and WBC. Serum was also collected for the analysis of liver and kidney functions, glucose and lipid profile tests.

In addition, liver, kidney and spleen samples were extracted for histological and immune histochemical examination.

Hematologic analysis: Whole blood was collected from the trunk and heart immediately after decapitation and then placed in either normal tube for serum collection or in EDTA tubes for CBC analysis, which used to assess Red Blood Cells (RBCs), haemoglobin (HGB), haematocrit (HCT), mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cells (WBCs), lymphocytes (LYMs), monocytes (MONs) and neutrophil (NEUs) according to standard methods[12]. All indices and haematological parameters were measured using a fully automated haematology analyzer (Beckman Coulter, Germany; Ac.T 5 diff CP) according to the manufacturer's instructions.

Biochemical analysis: Serum samples were used for colorimetric determination of the concentration and activities of AST, ALT, creatinine, urea, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), cholesterol, triglycerides and glucose in mouse serum using the Reflotron Plus Dry-Chemistry Analyzer (Roche, Germany).

Histological study: The liver, kidney and spleen were cut into small pieces and stored in 10% neutral formalin for 24-48 hrs, placed the tissue processer cassettes and embedded in paraffin wax to prepare the paraffin sections for staining with Haematoxylin and Eosin (H and E).

Immunohistochemical detection of IL-6 and TNF-α: Using the paraffin embedded blocks prepared beforehand, immune histochemical assays were performed. Sections were deparaffinised with xylene, rehydrated through a graded series of ethanol and then incubated at room temperature for 1 hr in blocking solution. After that, the sections were incubated overnight at 4°C with one of the primary antibodies, (monoclonal antibody against rat tumor necrosis factor alpha (TNF-α) dilution (1:50; R and D Systems, Minneapolis, MN) or interleukin 6 (IL-6) (1:100 dilutions; Santa Cruz Biotechnology, Santa Cruz, CA). All antibodies were then diluted using blocking solution. Immunostaining was performed with an avidin biotin-peroxidase complex kit and then counterstained with hematoxylin[13].

Statistical analysis: Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) software. Unpaired T-Tests were used in order to compare the mean values of infected and control groups and the results are expressed as Mean±SE. Statistical significance was defined at a $p \leq 0.05$.

RESULTS

In this study, haematological, biochemical analyses, histopathological and immunological examinations in liver, kidney and spleen tissues were used to study the potential effects of Pinworm (*A. tetraptera*) infection in laboratory mice.

The data presented in Table 1 showed no significant differences ($p \geq 0.05$) in the RBCs ($9.236 \times 10^{12} \pm 0.423$ and $9.652 \times 10^{12} \pm 0.858$ L^{-1}), MCH (14.540±0.890 and 15.460±0.415 pg), HGB (13.430±0.396 and 14.760±0.654 g dL^{-1}), MCHC (35.220±1.893 and 34.480±1.275 g dL^{-1}), HCT (34.040±2.553 and 35.114±4.266%) and MCV (42.600±0.547 and 43.400±0.547 fl) between control and infected groups, respectively. However, most white blood cell components are significantly increase (WBCs increase from $1.7800 \times 10^9 \pm 0.021$-$8.2000 \times 10^9 \pm 0.644$ L^{-1}, ($p \leq 0.001$), LYMs $1.4380 \times 10^9 \pm 0.242$-$6.1520 \times 10^9 \pm 0.428$ L^{-1} and NEUs ($0.3760 \times 10^9 \pm 0.133$-$1.8180 \times 10^9 \pm 0.089$ L^{-1}), while MONs showed a slightly less significant change ($0.0250 \times 10^9 \pm 0.011$-$0.1440 \times 10^9 \pm 0.058$ L^{-1}) ($p \leq 0.01$) between the control and infected groups, respectively.

Table 1: Effects of *A. tetraptera* infection on different blood parameters between control and infected mice

Parameters	Control	Infected
RBC (10^{12} L^{-1})	9.236±0.423[a]	9.652±0.858[a]
HGB (g dL^{-1})	13.430±0.396[a]	14.760±0.654[a]
HCT (%)	34.040±2.553[a]	35.114±4.266[a]
MCV (fl)	42.600±0.547[a]	43.400±0.547[a]
MCH (pg)	14.540±0.890[a]	15.460±0.415[a]
MCHC (g dL^{-1})	35.220±1.893[a]	34.480±1.275[a]
WBC (10^9 L^{-1})	1.7800±0.021[a]	8.2000±0.644[b***]
LYM (10^9 L^{-1})	1.4380±0.242[a]	6.1520±0.428[b***]
MON (10^9 L^{-1})	0.0250±0.011[a]	0.1440±0.058[b**]
NEU (10^9 L^{-1})	0.3760±0.133[a]	1.8180±0.089[b***]

Values are expressed as (Mean±SD). Similar superscripts indicate non-significant differences, different superscripts indicate significant differences. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

Table 2: Effects of infection with *A. tetraptera* on different biochemical parameters between control and infected mice

Parameters	Control	Infected
AST (U L^{-1})	57.200±9.391[a]	388.60±6.308[b***]
ALT (U L^{-1})	39.676±1.357[a]	114.62±8.741[b***]
Glucose (mmol L^{-1})	6.2760±1.008[a]	7.2040±0.535[a]
Cholesterol (mg dL^{-1})	58.380±4.124[a]	169.20±9.148[b***]
Triglyceride (mg dL^{-1})	154.80±3.834[a]	196.00±11.811[b***]
HDL (mg dL^{-1})	51.960±5.322[a]	64.040±7.355[b**]
LDL (mg dL^{-1})	45.162±6.850[a]	149.12±10.45[b***]
Creatinine (mg dL^{-1})	0.5580±0.046[a]	0.6360±0.078[a]
Urea (mg dL^{-1})	54.260±1.564[a]	57.798±5.227[a]

Values are expressed as (Mean±SD). Similar superscripts indicate non-significant differences, different superscripts indicate significant differences. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

Serum AST and ALT levels were highly significantly increased in infected groups compared with the control group ($p \leq 0.001$). The AST and ALT levels in the non-infected group were 57.200±9.391 and 39.676±1.357 and 388.60±6.308 and 114.62±8.741 U L^{-1} in the infected animals, respectively, which indicates the hepatotoxicity induced in the infected group (Table 2).

On the other hand, creatinine (0.558±0.046 and 0.636±0.078 mg dL^{-1}) and urea levels (54.26±1.564 and 57.798±5.227 mg dL^{-1}) showed no significant changes ($p \geq 0.05$) when compared to the control group (Table 2). This means that there is no noticeable effect on kidney functions in mice infected by Pinworm (*A. tetraptera*).

Glucose levels did not show significant change in both groups (6.2760±1.008 mmol L^{-1} in non-infected mice and 7.2040±0.535 mmol L^{-1} in the infected group ($p \geq 0.05$) (Table 2). While the lipid profiles from both groups were

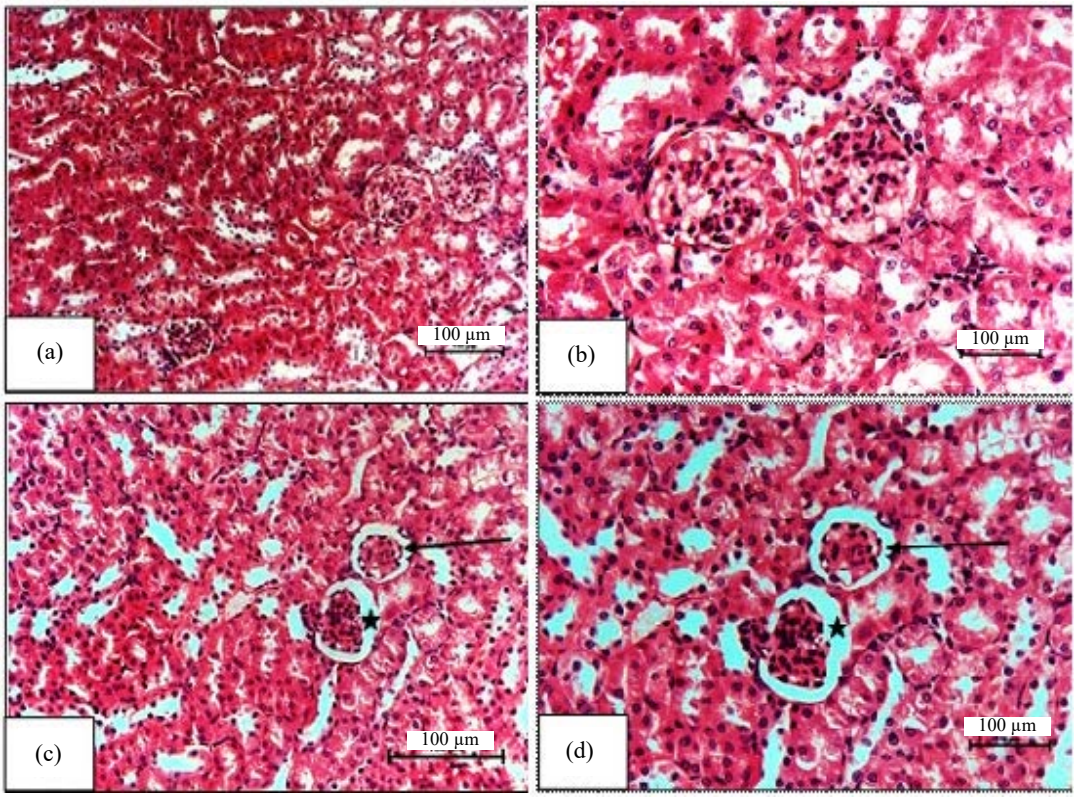

Fig. 1(a-d): Photomicrograph of kidney sections stained with H and E

(a-b) Kidney sections from a control mouse with normal Bowman' capsule architecture (left 20×, right 40×) and (c-d) Kidney sections from an infected mouse showing shrunken glomeruli (arrow) and rather wide Bowman' spaces (star) (left 20×, right 40×)

significantly different. Total serum cholesterol, triglycerides, LDL and HDL levels in the infected group were (169.20±9.148, 196.00±11.811, 64.040±7.355 and 149.12±10.45 mg dL^{-1}, respectively) compared to the control group (58.380±4.124, 154.80±3.834, 51.960±5.322 and 45.162±6.850 mg dL^{-1}, respectively) (Table 2).

The effect of pinworm infection on kidney histology of mice was studied and compared with the uninfected ones. Figure 1a and b (magnification 20× and 40×, respectively) showed normal histological kidney architecture of control group as confirmed by normal figure of both the glomeruli and the renal tubules that were lined by a single layer of cells with areas of dense nuclei (Fig. 1a-b). Whereas, histopathological changes were observed in the kidneys of infected mice including the development of necrotic glomeruli and an expansion of the Bowman's capsule space (Fig. 1c, 20X and 1D, 40X).

Similarly, the histological examination of liver sections from uninfected mice showed that the hepatocytes sections maintained regular hepatic architecture with normal hepatic strands and central vein (Fig. 2a, 20X and 2B, 40X) while the infected mice showed enlarged hepatocytes around the portal vein (Fig. 2c, 20X and 2D, 40X). Another set of sections showed enlarged hepatocytes around the Central Vein (CV) and an increased number of fat deposits between the hepatocytes (Fig. 2e, 20X and 2F 40X, respectively).

Spleen sections from control group showed normal architecture with normal white pulp (Fig. 3a-b). Increased Extra Medullary Haematopoiesis (EMH) was observed in splenic sections of infected mice with increased numbers of erythroid precursors, myeloid cells and megakaryocytes compared to control group (Fig. 3c-d).

Immunohistochemical assays was used to evaluate IL-6 in mice kidney (Fig. 4a-b), liver (Fig. 4c-d) and spleen (Fig. 4e-f) for the normal and infected mice, respectively. The control kidney and liver showed normal appearance in control mice while the infected ones demonstrated highly increased expression of IL-6. On the other hand, a mild elevation in IL-6 expression was observed in the spleen infected mice (Fig. 4f) compared to control.

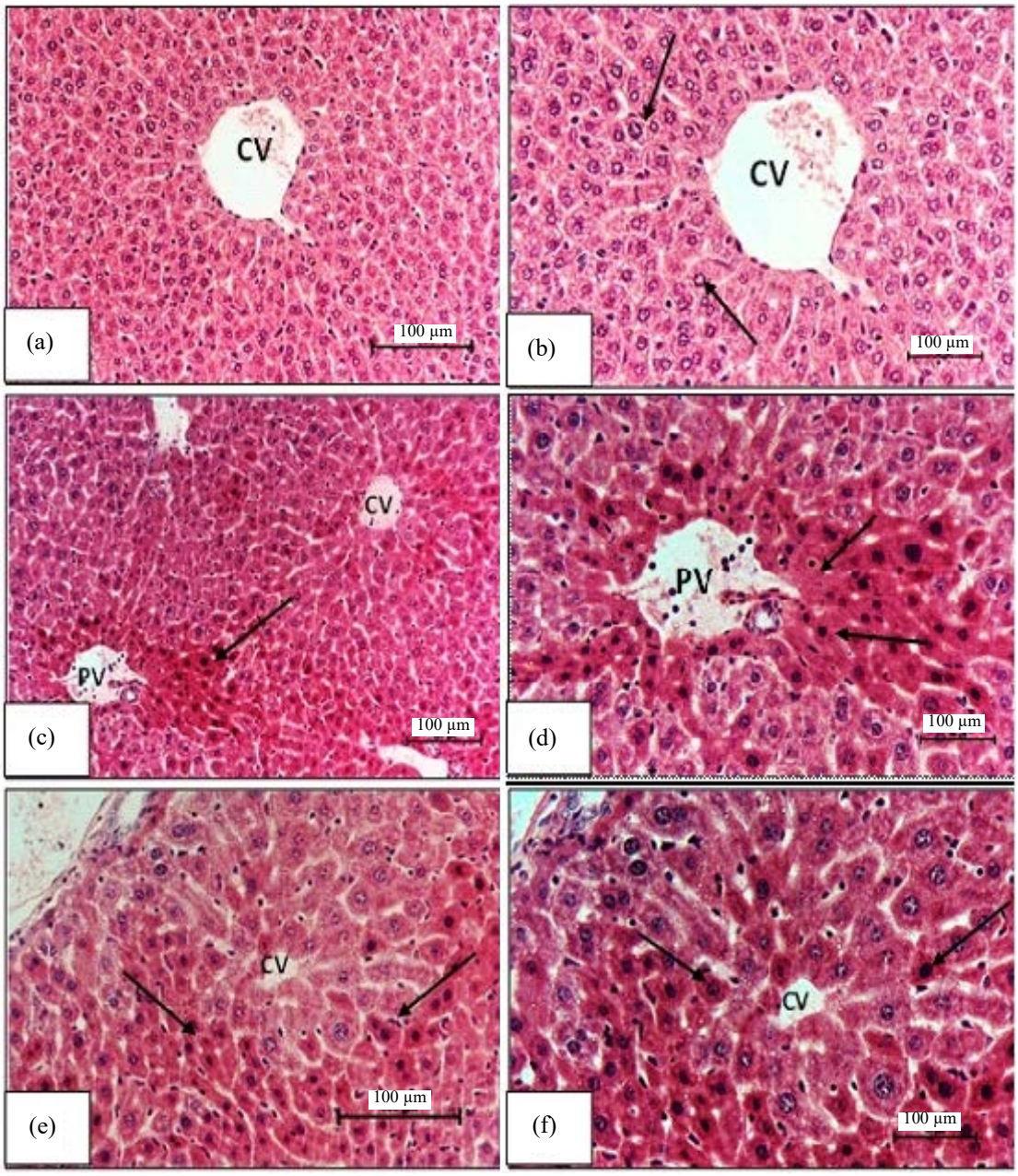

Fig. 2(a-f): Photomicrograph of liver sections stained with H and E

(a-b) Liver sections from a control mouse with normal hepatocytes (arrow) and central vein (CV) architecture (left 20×, right 40×), (c-d) Liver sections from an infected mouse showing enlarged hepatocytes (arrow) around the portal vein (PV) (left 20×, right 40×) and (e-f) Another set of sections of the liver from an infected mouse showing enlarged hepatocytes (arrow) around the central vein (CV) and fat deposits between the hepatocytes (white space between hepatocytes) (left 20×, right 40×)

Similarly, the same result was obtained in the immunohistochemical evaluation of TNF-α in kidney (Fig. 5a-b), liver (Fig. 5c-d) and spleen (Fig. 5e-f) for the normal and infected mice, respectively. Normal TNF-α expression was observed in uninfected mice kidney and liver, while the expression increased in infected groups. Moreover, a mild immunochemical reaction was observed in spleen of infected mice (Fig. 5f) compared to control mice group (Fig. 5e).

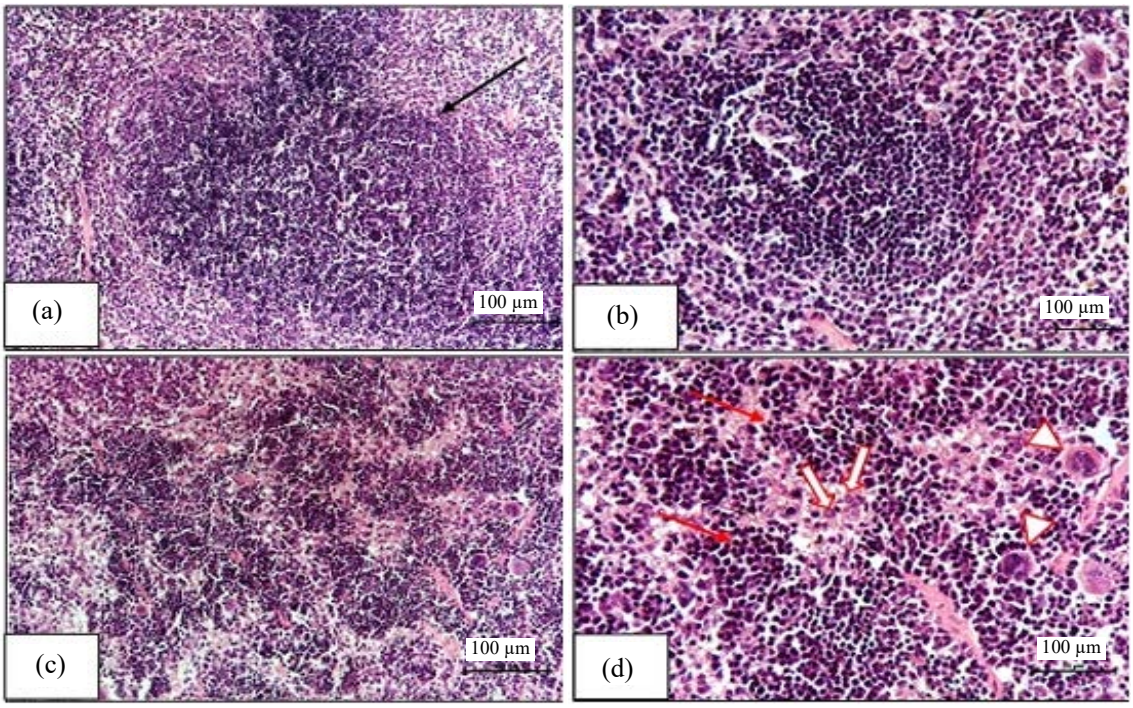

Fig. 3(a-d): Photomicrographs of spleen sections stained with H and E

(a-b) Spleen sections from a control mouse with normal white pulp (arrow) (left 20×, right 40×) and (c-d) Spleen sections from an infected mouse showing increased Extra Medullary Haematopoiesis (EMH), including increased numbers of erythroid precursors (arrow) and myeloid cells (bold arrow). The arrowhead, however, shows megakaryocytes (left 20×, right 40×)

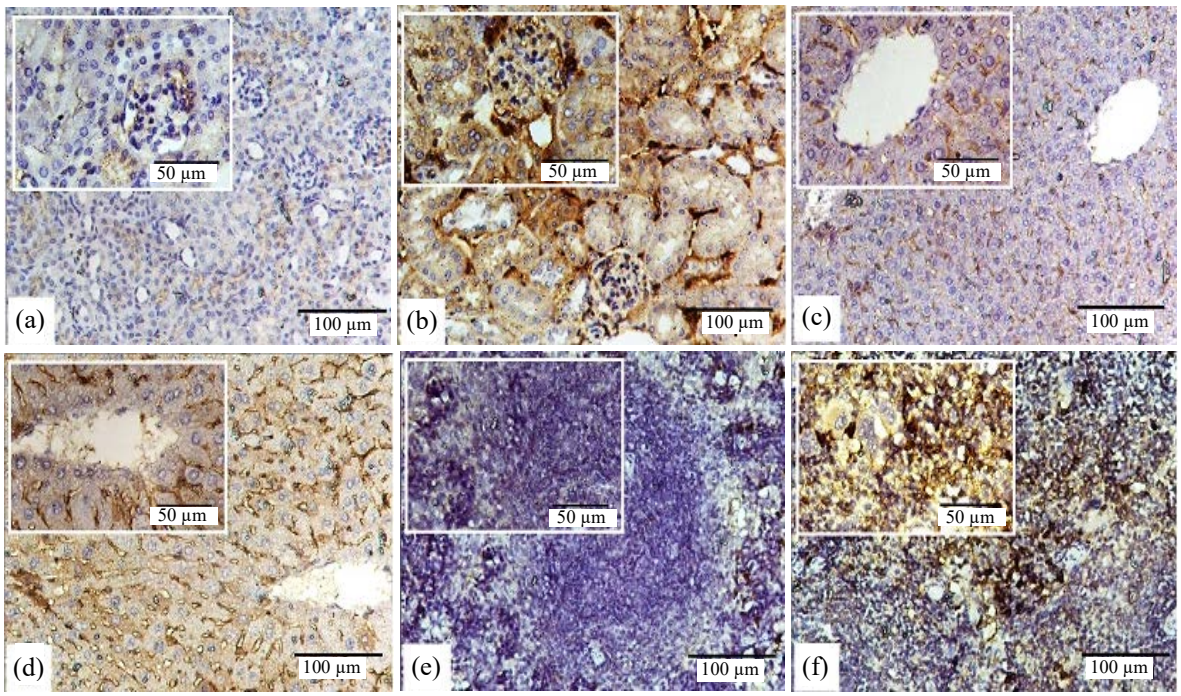

Fig. 4(a-f): Immunohistochemical staining of interleukin 6 (IL-6) in the kidney, liver and spleen of both control and infected mice

(a,c,e) Sections of different organs from control mice showing low IL-6 expression (left 40×, right 20×) and (b,d,f) Sections from infected mice showing high IL-6 expression (brown stain) (left 40×, right 20×)

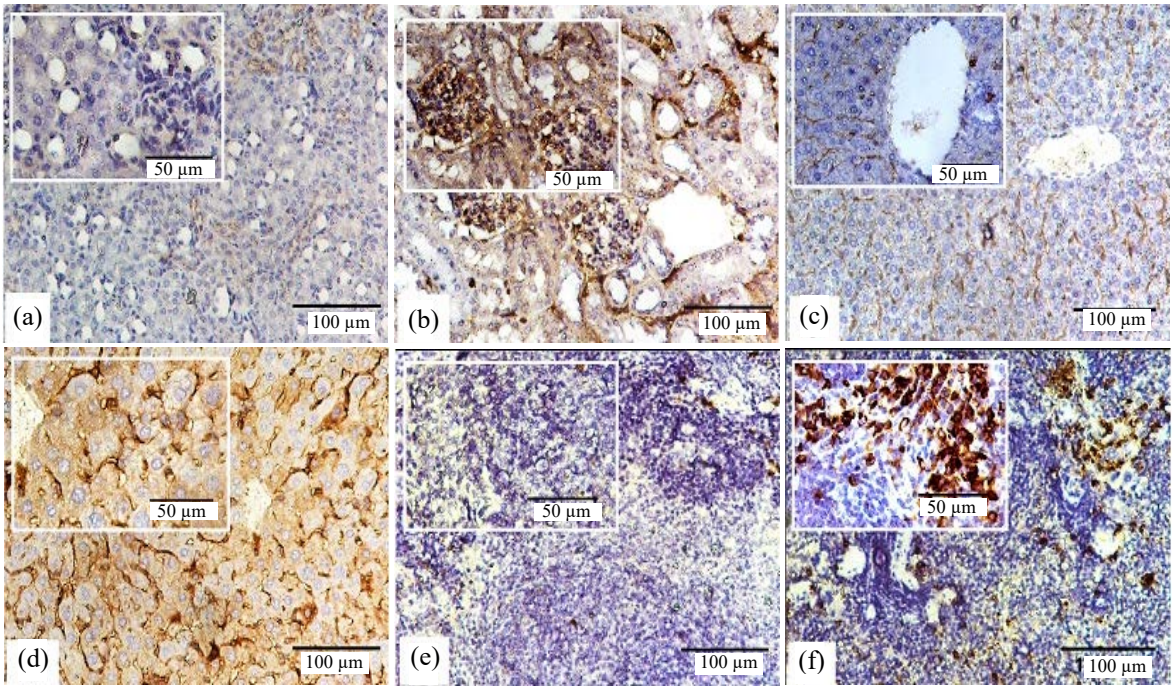

Fig. 5(a-f): Immunohistochemical staining for TNF-α in the kidney, liver and spleen of both control and infected mice
(A), (C) and (E) sections of different organs from control mice showing low TNF-α expression (left 40×, right 20×) and (B), (D) and (F) sections from infected mice showing high TNF-α expression (brown stain) (left 40×, right 20×)

DISCUSSION

Nematodes comprise the largest group of helminth parasites in laboratory animals and those from the Oxyuridae family are known to have simple life cycles. These properties make them frequent contaminants of both Specific Pathogen Free (SPF) and conventional colonies of laboratory mice[14].

It is important to note that some previous studies have found that pinworms may interfere with research through their modification of the host immune system including the induction of the Th2-associated immune response, increases in the mouse's humoral immune response to nonparasitic antigenic stimuli and also accelerate the development of the hepatic monooxygenase system[6,7].

In this study, physiological effects of the infection with *A. tetraptera* were examined using both haematological and biochemical analyses, along with histological and immunohistochemical examinations.

Some haematological tests used to evaluate a number of different blood parameters (RBCs, HGB, HCT, MCV, MCH and MCHC) showed no significant differences ($p \geq 0.05$) between control and infected groups. While the white blood cell parameters (WBCs, LYMs, MONs and NEUs) were all very highly significantly different from the control ($p \leq 0.001$) except for the monocytes which were only highly significantly different ($p \leq 0.01$) (Table 1).

Evaluating haematological parameters helps to determine the health or disease status of an organism; these factors can act as both indicators and valuable tools for assessing the harm caused by certain injuries, infections or treatments[15].

The physiological effects accompanying pinworm infections in laboratory mice were tested using liver function tests (ALT, AST), kidney function tests (Creatinine and Urea) and glucose and lipids profile tests (Cholesterol, Triglycerides, LDL and HDL).

Overall, the results of the liver function tests indicated that there are very highly significant differences ($p \leq 0.001$) in both AST and ALT between the two groups (Table 2). According to Sher and Hung[16], elevated AST and ALT levels usually result from liver injury. It is also indicative of cellular leakage and loss of cell membrane function in hepatocytes. These losses facilitate the passage of cytoplasmic enzymes into the blood stream leading to their elevated levels in the sera[17] which was confirmed by the histological results in the current study.

However, kidney function tests evaluating creatinine and urea and serum glucose levels showed no significant differences ($p \geq 0.05$) between control and infected groups (Table 2). This observation is in accordance with Otto *et al.*[18] and Coman *et al.*[19].

The lipid profile tests showed very highly significant differences ($p \leq 0.001$) between the two groups except for HDL cholesterol which showed a slightly smaller significant difference ($p \leq 0.05$). Although highly variable as a result of dietary differences and often associated with other complications like type II diabetes and metabolic syndrome; elevated plasma triglycerides levels are also often associated with low HDL-c levels as observed in this study[20].

The abnormally high levels of both cholesterol and triglycerides in serum samples from infected animals are in accordance with the increased number of fatty deposits in the liver tissues (Table 2).

Tissue sections were taken from the kidney, liver and spleen to evaluate the effects of pinworm infection using histological examination (Fig. 1-3). The kidneys were seen to experience widening of the bowman's capsules and shrinking of the glomeruli following pinworm infection. The liver exhibited abnormal and enlarged hepatocytes around the portal and central veins along with fat deposits between the strands and hepatocytes. Spleen sections showed a clear increase in Extra Medullary Haematopoiesis (EMH), which is known to increase the numbers of erythroid precursors and myeloid cells. The result about histological examination of spleen sections was in agreement with a previous study done by Baker *et al.*[21].

Immunohistochemical assays used to evaluate IL-6 and TNF-α in the liver, kidney and spleen showed increased expression for both IL-6 and TNF-α in both liver and kidneys and a mild elevation of their expression in the spleen (Fig. 4-5).

IL-6 and TNF- α are known proinflammatory cytokines, which are released in response to infections and tissue injuries. Their expression strengthens the host defence through the stimulation of acute phase responses, haematopoiesis and immune reactions[22].

It is recommended that the experimental animals should be as free from infection as possible. Moreover, infection with even a few helminth parasites, with no visible lesions, may still influence experimental results[23]. In many laboratories animal houses, various disinfectants are used to assure good animals are used. However, using disinfectants may also influence experimental results. For instance, fenbendazole which is used to eliminate pinworms and many other gastrointestinal parasites was found to occasionally act as a tumour promoter if certain initiators are given concurrently[7]. It has also been reported that if fenbendazole is combined with supplemented vitamins it can act as a growth inhibitor in human lymphoma cell lines transplanted into severe combined immune deficient mice, which means that the addition of vitamins and fenbendazole during antitumor studies may lead to unpredictable interactions with test substances and thus alter research results[24].

CONCLUSION

Heavily parasitized animals are not suitable for critical experiments as normal physiological and immunological reactions and some haematological and biochemical markers could alter and might result in confounding data. It can be concluded in the current study that *A. tetraptera* infection changes the physiological and immunological characteristics of laboratory mice, which may have unforeseen impacts on experimental assays and study outcomes. The results recommend using experimental animals immediately after receiving them and not keeping them in the animal house for long periods in order to avoid infection.

SIGNIFICANCE STATEMENT

This study discovered the impact of using naturally infected mice with pinworm *A. tetrapteraas* an animal model on haematological, physiological, histopathological and immunological parameters that can be beneficial for getting rational data. This study will help the researchers avoiding possible interference in the results of the studied parameters in infected mice that many researchers were not able to explore.

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REFERENCES

1. Bazzano, T., T.I. Restel, R.M. Pinto and D.C. Gomes, 2002. Patterns of infection with the nematodes *Syphacia obvelata* and *Aspiculuris tetraptera* in conventionally maintained laboratory mice. Mem. Inst. Oswaldo Cruz, 97: 847-853.
2. Michels, C., P. Goyal, N. Nieuwenhuizen and F. Brombacher, 2006. Infection with *Syphaciaobvelata* (Pinworm) induces protective Th2 immune responses and influences ovalbumin-induced allergic reactions. Infect. Immu., 74: 5926-5932.

3. Ito, A., 1982. *Hymenolepis nana*: Immunogenicity of a lumen phase of the direct cycle and failure of autoinfection in mice. Exp. Parasitol., 54: 113-120.
4. Chen, X.M., X. Li, R.Q. Lin, J.Y. Deng and W.Y. Fan *et al*., 2011. Pinworm infection in laboratory mice in southern China. Lab Anim. 45: 58-60.
5. Taffs, L.F., 1976. Pinworm infections in laboratory rodents: A review. Lab Anim., 10: 1-13.
6. Sharma, S., 2018. A study on occurrence of *Aspiculuris tetraptera* infection and mortality in mice. J. Anim. Res., 8: 667-671.
7. Pritchett, K. and N. Johnston, 2002. A review of treatments for the eradication of pinworm infections from laboratory rodent colonies. Contemp. Top. Lab. Anim. Sci., 41: 36-46.
8. Burr, H., L. Paluch, G. Roble and N. Lipman, 2012. Parasitic Diseases. In: The Laboratory Rabbit, Guinea Pig, Hamster and Other Rodents, Suckow, M.A., K.A. Stevens and R.P. Wilson, Academic Press, London, ISBN: 978-0-12-380920-9, pp: 839-866.
9. Pinto, R.M., D.C. Gomes and D. Noronha, 2003. Evaluation of coinfection with pinworms (*Aspiculuristetraptera*, *Dentostomellatranslucida* and *Syphaciaobvelata*) in gerbils and mice. Contemp. Top. Lab. Anim. Sci., 42: 46-48.
10. Pritchett-Corning, K.R., J. Cosentino and C.B. Clifford, 2009. Contemporary prevalence of infectious agents in laboratory mice and rats. Lab. Anim., 43: 165-173.
11. Whary, M.T., N. Baumgarth, J.G. Fox and S.W. Barthold, 2015. Biology and Diseases of Mice. In: Laboratory animalmedicine, Fox, J.G., L.C. Anderson, G. Otto, K.R. Pritchett-Corning and M.T. Whary (Eds.), Elsevier, Accedmic Press, New York, pp: 43-149.
12. Wintrobe, M.M. and J.P. Greer, 2009. Wintrobe's Clinical Heamatology. 12th Edn., Wolters Kluwer/Lippincott Williams& Wilkins Health, Philadelphia, pp: 2488-2493.
13. Giani, J., M. Muñoz, R. Pons, G. Cao, J. Toblli, D. Turyn and F. Dominici, 2011. Angiotensin-(1–7) reduces proteinuria and diminishes structural damage in renal tissue of stroke-prone spontaneously hypertensive rats. Am. J. Physiol. Renal Physiol., 300: F272-F282.
14. Pritchett-Corning, K. and C. Clifford, 2012. Parasitic Infections of Laboratory Mice. In: The Laboratory Mouse, Chapter 3.4, Hedrich, H.J. (Ed.), Elsevier Ltd., Wilmington, Massachusetts, USA, ISBN: 978-0-12-382008-2 pp: 503-518.
15. Fouad, D., H. Alobaid and A.A. Al-Jafary, 2013. Effect of melatonin on the oxidative stress induced by the food additive (C.I. Food Yellow 3) on some blood parameters and antioxidant enzymes in male rat kidney. Life Sci. J., 10: 343-350.
16. Sher, Y.P. and M.C. Hung, 2013. Blood AST, ALT and Urea/BUN Level Analysis. Bio-Protocol., Vol. 3. 10.21769/BioProtoc.931.
17. Fouad, D., H. Alhatem, R. Abdel-Gaber and F. Ataya, 2019. Hepatotoxicity and renal toxicity induced by gamma-radiation and the modulatory protective effect of *Ficus carica* in male albino rats. Res. Vet. Sci., 125: 24-35.
18. Otto, G.P., B. Rathkolb, M.A. Oestereicher, C.J. Lengger and C. Moerth *et al*., 2016. Clinical chemistry reference intervals for C57BL/6J, C57BL/6N and C3HeB/FeJ mice (*Mus musculus*). J. Am. Assoc. Lab. Anim. Sci., 55: 375-386.
19. Coman, C., E. Vlase and A. Dinu, 2018. Hematological and plasma biochemical values in four specific pathogen-free mice strains produced in the animal facility at cantacuzino institute, bucharest. Sci. Works Ser. C. Veter. Med., 44: 115-120.
20. Yin, W., E. Carballo-Jane, D.G. McLaren, V.H. Mendoza and K. Gagen *et al*., 2011. Plasma lipid profiling across species for the identification of optimal animal models of human dyslipidemia. J. Lipid Res., 53: 51-65.
21. Baker, D. and N. Lipman, 2015. Factors That Can Influence Animal Research. In: Laboratory Animal Medicine, Fox, J.G., C.L. Anderson, G.M. Otto, K.R. Pritchett-Corning and M.T. Whary, (Eds.), Elsevier, Wikipedia, pp: 441-1496.
22. Tanaka, T., M. Narazaki and T. Kishimoto, 2014. IL-6 in inflammation, immunity, and disease. Cold Spring Harbor Perspect. Biol., Vol. 6. 10.1101/cshperspect.a016295.
23. Habermann, R.T. and F.P. Williams, Jr., 1958. The identification and control of helminths in laboratory animals. J. Natl. Cancer Inst., 20: 979-1009.
24. Gao, P., C. Dang and J. Watson, 2008. Unexpected antitumorigenic effect of fenbendazole when combined with supplementary vitamins. J. Am. Assoc. Lab. Anim., 47: 37-40.