Histopathology Description of Chicken Liver Infected by L2 Toxocara Vitulorum

by Iwan Sahrial
Histopathology Description of Chicken Liver Infected by L2 Toxocara Vitulorum

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ABSTRACT

Transmission of Toxocara vitulorum Infection causes a decrease in livestock productivity and results in various types of diseases in humans. Chickens are one of the paratenic hosts of toxocarasis which has the potential for transmission of toxocarasis to humans. The main location affected by T. vitulorum larval infection is the liver. The current study aimed to analyze the description of histopathological changes in the liver of broiler chickens infected by L2 Toxocara vitulorum. The present study was a true experiment using a completely randomized design. A total number of 28 broiler chickens aged 14 days were selected as the sample in this study. Samples were infected using L2 Toxocara vitulorum larvae and were grouped in accordance with observations of the 1, 2, 3, 7, 14, and 21 days after the larvae were given to the samples. Toxocara vitulorum larval infection caused changes in histopathological features of broilers chickens. This infection caused hydropic inflammation and degeneration of liver cells, cholangitis, and eventually necrosis of the cells. Exposure to infection over a long period of time can worsen liver cell and other organ damages as well as increasing the potential for the transmission of Toxocara vitulorum larvae.

Keywords: Chicken, Histopathology of liver, Infection, Toxocara vitulorum

INTRODUCTION

Toxocarasis is one of the worm-originating diseases that can attack ruminants, especially calves of cows and buffaloes and the mothers (Hübner et al., 2001). Toxocara vitulorum which attacks cows at all ages can be transmitted through food boxes or placenta that can infect the fetus in the womb (Bevin et al., 1995). Toxocara vitulorum is commonly found in tropical and subtropical climates (Starke et al., 1996). This infection leads to a reduction in livestock productivity, which will be a financial burden for farmers if not controlled. In addition, T. vitulorum infection causes anorexia, stomach pain, diarrhea, constipation, dehydration, bad breath, and also a decrease in the body weight of cattle (Raza et al., 2013).

Humans or animals that consume raw or undercooked liver of paratenic hosts of Toxocara spp. are the potential to being contaminated with toxocarasis (Yoshikawa et al., 2008). Some paratenic hosts of toxocarasis are mice, rats, dogs, birds, chickens, humans, and other mammals (Azizi et al., 2007; Yoshikawa et al., 2008; Raza et al., 2013). Larvae can move to various tissues and survive for a long period of time (Azizi et al., 2007; Strube et al., 2013). The movement of larvae into the tissues (lung, liver, and kidney) or milk is thought to be a medium of transmission to humans (Kusnoto et al., 2005). The consumption amount of raw or undercooked meat increases the prevalence of toxocarasis case (Taira et al., 2011) leading to human zoonosis diseases, such as visceral larva migrans (VLM) and ocular larva migrans (OLM).

T. vitulorum larvae can cause liver and lung lesions, inflammation of lymph nodes, as well as eosinophilia during the life cycle of the parasite (Abbott et al., 2006; Khan et al., 2007). Toxocara spp. larvae migrate to the liver through the porta hepatica systems and cause hepatomegaly which is a common phenomenon (Soulby and Monnick, 1982). In humans, the human infection of Toxocara spp. leads to hepatocellular necrosis and inflammatory reactions (Hübner et al., 2001). On the other hand, histopathological examination of visceral organs using hemithiastasis has not been performed much, especially to see the histopathological picture of the liver as the site of second-stage T. vitulorum larvae migration in...
chickens as paratenic host, where parasites can live but cannot develop into adulthood (Cardillo et al., 2008). Therefore, this study was conducted to describe the histopathological changes in the liver of broiler chickens after being infected by L2 *Toxocara vitulorum*.

**MATERIALS AND METHODS**

The present study was a true experiment using a completely randomized design performed at the Helminthology Laboratory of the Parasitology Department of Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, in 2020. The liver histopathology examination was carried out in the Pathological Laboratory. Three variables have been investigated in the current study. The histopathological image of the liver (degeneration and necrosis of hepatocytes) was considered as the dependent variable, the effective dose of L2 *T. vitulorum* and liver sampling time were taken into account as independent variables. Finally, controlled variables included strain, sex, age, feed, and also environmental conditions of chickens.

**Experimental design**

Broiler infection involves eggs containing L2 *T. vitulorum*. Phosphate Buffer Saline (PBS) (Sigma Co.) was used as the media of L2 *T. vitulorum*, while the protozoa growth and other microorganisms were avoided using formalin 0.5-1%. In addition, Trypsin 1% was used for releasing L2 *T. vitulorum* from the formed liver tissue.

**Isolation and preparation of *Toxocara vitulorum* eggs**

Infective eggs of *T. vitulorum* worm inoculant material comes from the intestine of 10 cows contaminated by *toxocariasis*, during visiting the slaughter-house. Worms were washed in 0.85% saline to remove debris, and they were fixed into 70% ethanol. The worms were then cleaned with aquadest and transferred to a container containing PBS solution as a development medium. After that, the worms were incubated at 37°C for up to three days in order to lay *T. vitulorum* eggs. The *T. vitulorum* egg retrieval was performed through the worm’s reproductive tract by the surgical process. Subsequently, a gradient preparation technique was used to separate the dirty debris from the eggs.

**Toxocara vitulorum eggs fertilization**

Egg fertilization was carried out in PBS medium with the addition of five drops of formaldehyde 10%. This addition served to prevent the interference of other microbes with the growth of *T. vitulorum* eggs until the first (L1) and second larvae were obtained (L2). The development of worm eggs was observed using a dissecting microscope (Olympus upright microscope) and documented in the form of photographs on a regular basis. The process took 21-28 days at room temperature until the egg developed into L2 (Kusnoto et al., 2011).

**Calculation of *Toxocara vitulorum* eggs**

The egg calculation was carried out using a modified calculation of the worm eggs per gram excretion introduced in the Lucient Brumpt method (Kusnoto et al., 2007). An amount of 1 ml of *T. vitulorum* egg suspension from the culture media was then taken and diluted 10 times until 10 ml of suspension achieved. Then, 1 ml of suspension was taken by means of a Pasteur pipette to calculate the number of drops for every 1 ml of suspension. One drop of the suspension was put on a glass object and then examined through a light microscope with 100× magnification. Eggs that appeared through the microscopic magnification were counted using the formula which is written below (Kusnoto et al., 2007).

\[
\text{Number of eggs} = \text{Number of drops per ml} \times \text{Number of worm eggs per drop} \times \text{number of dilutions}
\]

**Treatment of experimental animals**

A total number of 28 broiler chickens with body weights of 100-200 gr were selected as experimental animals of the current study. The chickens were raised on the farm and the floor. The adaptation period was one week. There was no available information about the vaccination program since the study was performed during rearing. Broilers were required to be 14 days old for deviation to seven treatment groups. The chickens were quarantined (A week) before being randomly divided into seven treatments with four replications in each. The chickens were feeding ad-libitum every afternoon and morning with strict hygiene. Then, each broiler chicken was infected by using 3000 eggs containing *T. vitulorum* second stage larvae (L2) when it was added to the food. The broiler chicken groups were divided into six groups (Azizi et al., 2007; Taira et al., 2011). The broiler chickens in the control group (K) were not infected with *T. vitulorum*. The P1 consisted of broilers infected by L2 *T. vitulorum* with a dose of 3000 eggs per chicken, and euthanized a day after infection. The broilers in P2 group were infected by L2 *T. vitulorum* with a dose of 3000 eggs per chicken, and euthanized two days after infection. The P3 group entailed broilers infected with L2 *T. vitulorum* with a dose of 3000 eggs per chicken, and euthanized three days after infection. The P4 had broilers infected by L2 *T. vitulorum* with a dose of 3000 eggs per chicken, and euthanized seven days after infection. Moreover, P5 was
composed of broilers infected by L2 *T. vitulorum* with a
dose of 3000 eggs per chicken, and euthanized 14 days
after infection. Finally, the P6 group encompassed broilers
infected by L2 *T. vitulorum* with a dose of 3000 eggs per
chicken, and euthanized 21 days after infection.

Liver extraction
Liver extraction for histopathological preparation
was carried out 1, 2, 3, 7, 14, and 21 days after L2 *T.
vitulorum* infection. Extraction of the chicken liver was
done after euthanasia and surgery. Broiler’s liver organs
were stored in aquadest and formalin 10%. Chicken’s
livers were cleaned with physiological NaCl then put in a
plastic pot containing aquadest and formalin 10%, and
subsequently stored for 24 hours before making the
histopathological preparations.

Examination of preparations
The materials used for liver histopathological
preparation were multilevel ethanol (70%, 80%, 90%, and
absolute), formalin 10% added to the solution, ether,
physiological saline (NaCl 0.9%), paraffin, entellan
(transparent adhesive), Harris’s Haematoxylin-Eosin
double coloring, emersion oil, and xylol. Examination of
preparations was performed using a light microscope with
400x magnification of five different fields of view (LP)
for each sample. The observed changes included
degeneration, the swelling of cell size due to vacuoles in
the cytoplasm, Infiltration of inflammatory cells around
the central vein, whether porta hepatitis or sinusoid.
Subsequent examination of preparations was assessed
according to the Knodell score method (Knodell et al.,
2019).

Statistical analysis
The research data including the histopathological
score of liver cells of chickens were analyzed using
Kruskal Wallis test, then continued with the Z-test.
Differences were considered significant when *p* < 0.05.

RESULTS AND DISCUSSION
The present study obtained the results from the
observation of isolated *T. vitulorum* worm eggs from adult
worms that were fertilized and incubated for about one
month. This process also obtained a second-stage larvae
(L2) (Figure 1). The results of the treatment on broilers
microscopically demonstrated a histopathological change
in the chicken liver after being infected with L2 *T.
vitulorum*. Non-parametric Kruskal Wallis test indicated a
significant difference (*p* < 0.05) for each treatment in
broilers (Table 1). Provision of infective larval infections
(L2) *T. vitulorum* affects the histology of broiler chickens’ liver. This study found a significant difference
between the control group (K) and treatment groups which
were euthanized 1 - 21 days post-L2 infection (*p* < 0.05).
The obtained scores were then followed by a multiple
comparison test (Z test) to determine the order of the change
rate in the liver histopathological pictures among
the seven treatment groups.

Histopathological pictures of the liver tissue in the
treatment groups presented damage due to hydropic
degeneration (cloudy swelling), necrosis, inflammation,
and cholangitis. The Z test indicated significant
differences in the treatment groups P1, P3, P4, P5, and P6
with the control group (K). However, there was a change
in histopathological features in P2 which were not
significantly different from the control group. Group P6
represented the worst results, compared to other treatment
groups (Figure 2).

In Figure 3, part A, hepatocytes were normal (blue
sign) and did not appear to have inflammation and
degeneration, and ductal images were still normal (green
signs). In figure B, the cholangitis in the P6 group was
characterized by inflammatory cells (yellow marking) and
epithelial proliferation (red marks) of the bile duct. In
figure C, the black mark referred to the presence of
hydropic degeneration, and cytoplasm appeared turbid
(cloudy swelling) and the green mark referred to the
necrosis of the nucleus which appeared to be picnotic.
The yellow mark in figure D indicated inflammation around
the portal area.

Table 1. Statistical results on the extent of liver damage to
the broiler chickens infected by *T. vitulorum*

| Treatment | Liver Damage Value (Mean Rank ± SE) |
|-----------|------------------------------------|
| K         | 2.50 ± 0.289                       |
| P1        | 15.50 ± 1.472                      |
| P2        | 9.67 ± 0.816                       |
| P3        | 12.80 ± 1.443                      |
| P4        | 14.75 ± 1.323                      |
| P5        | 21.25 ± 1.190                      |
| P6        | 25.38 ± 0.645                      |

Different superscripts in the same column show significant
*differences* (*p* < 0.05) *SE*: Standard Error. K: control group, broilers
were not infected with *T. vitulorum*. P1: Broilers were infected by L2 *T.
vitulorum* at a dose of 3000 eggs per chicken, and euthanized a day after
infection. P2: Broilers were infected by L2 *T. vitulorum* at a dose of 3000
eggs per chicken, and euthanized two days after infection. P3: Broilers
were infected with L1 *T. vitulorum* at a dose of 3000 eggs per chicken,
and euthanized three days after infection. P4: Broilers were infected by
L2 *T. vitulorum* at a dose of 3000 eggs per chicken, and euthanized seven
days after infection. P5: Broilers were infected by L2 *T. vitulorum* at a
dose of 3000 eggs per chicken, and euthanized 14 days after infection.
P6: Broilers were infected by L2 *T. vitulorum* at a dose of 3000 eggs per
chicken, and euthanized 21 days after infection.
Figure 1. The results of identification of the *Toxocara vitulorum* worm eggs and their development up to L2 stage with 100x Magnification. A: worm eggs (1 cell), B: morula, C: L1, D: L2

Figure 3. Histopathological picture of changes in different groups of infected broiler chickens’ liver with L2 *Toxocara vitulorum*. Haematoxylin-Eosin coloring, 400x Zoom. A: control group; B: Chicken liver cholangitis P6, C: liver degeneration and necrosis P6, D: chicken liver inflammation P6
DISCUSSION

The oral administration of L2 T. vitulorum caused a significant change in the liver histopathology picture of broiler chickens (p < 0.05). This was due to the migration of larvae into the tissue. However, the larvae were not always found on liver histopathological examination (Ferny et al., 2001). The orally administrated 3,000 eggs presented a white spot on the hepatic surface of the chickens indicating the presence of necrotic foci, eosinophil infiltration, and some lymphocytes around the necrotic area (Azizi et al., 2007; Taira et al., 2011).

 Infective eggs of T. vitulorum hatched within 2 hours followed by penetration into the intestinal cell to reach the liver through the porta hepatica system. The life cycle of Toxocara spp. involves a phase of migration in tissue at every stage, starting from the egg, larva, and adult stages. Every stage of Toxocara spp. growth has different antigenic devices and immunogenicity in triggering the formation of antibodies. Infective larval migration can cause histopathological changes in the cells of organs (Santos et al., 2017).

The liver experienced severe damage in the first post-infection day with the occurrence of degeneration, necrosis, severe inflammation, and cholangitis. On the second day, the liver was damaged but there were no significant differences in histopathological features which were found between treatment groups and the control group. L2 Toxocara spp. was most commonly found in the liver on the first day after infection and L2 migrated to another site on the second day (Taira et al., 2011). On the second post-infection day, the L2 Toxocara spp. was mostly found on the pulmonary of the chickens. Injuries of the liver cells were reversible and the cell would return to its original stable state within a certain time limit (Kumar et al., 2013). Histopathological picture of chicken liver in P3, P4, P5, and P6 groups indicated liver damage, especially around the central port and venous regions. Toxocara spp. migrated to other tissues through the circulatory system. The route of migration through the bloodstream can subsequently cause hemorrhage and multifocal necrosis in the liver. Inflammatory cell findings and epithelial proliferation in the bile duct were also observed in all treatment groups. Infective larvae T. vitulorum can migrate through the portal vein and then enter the bile duct through enterohepatic circulation (Azizi et al., 2007).

Histopathological pattern of liver cells infected with L2 T. vitulorum experienced degeneration, swelling and was accompanied by necrosis, inflammation, and cholangitis. Toxocara spp. larvae secrete metabolic material that caused injury to liver cells. The products or secretions of infectious organisms are toxic to the metabolism or integrity of the cell membrane (Underwood, 1996). Degenerated liver cells experiencing cloudy swelling, microscopically present the granular cytoplasm and appeared to be foggy (Thomson, 1984). This change reveals that when water accumulates in the cytoplasm, cytoplasmic organelles also absorb water which causes swelling of the mitochondria and enlargement of the rough endoplasmic reticulum accompanied by the loss of ribosomes (Cotran et al., 1994).

Liver cell necrosis is characterized by three changes in the cell nucleus, including piconiosis which means the cell nucleus appears round, dark, and smaller than the normal cell nucleus, karyorrhexis is splitting the cell nucleus into several parts, and karyolysis means when the cellular nucleus chromatin disappears and leaves holes in the cell (Thomson, 1984). L2 T. vitulorum infection in experimental animals caused cell necrosis and disabled the cells to stimulate changes so that eventually cell death occurred. This death is a result of releasing several enzymes, such as ATP-ase, phospholipase, protease, and endonuclease. Great or lethal lesions lead to irreversible cell damages because the cell cannot defend itself against injury.

Toxocara spp. larvae secrete metabolic material that increases the production of eosinophils as an immune reaction. Cellular activity and pressure of infection can stimulate microbicidal secretions, effectors, and inflammatory mediators. This pressure responds to cells to protect and fight unwanted conditions by minimizing damage and maintaining the integrity of the host tissue. Endoplasmic reticulum and mitochondrial tissue are key cellular organelles which give signals to cellular pressure (Abbas and Lichtman, 2003). Cholangitis is inflammation of the bile wall due to lumen infection. This situation can originate from any lesion that blocks the bile duct.

Therefore, L2 L. vitulorum can migrate to various organs and cause damage, hence some prevention can be done by health workers such as conducting training and counseling on the importance of cleanliness and environmental management. In addition, it is also necessary to provide support and regular assistance to farmers. This aims to minimize the spread of infection by reporting the cases to health workers.
CONCLUSION

Infective larvae of (L2) *Toxocara vitulorum* orally could provide a change in the histopathological picture of broiler chicken’s liver. Liver cell damages included cell degeneration, inflammatory cell infiltration, necrosis, and cholangitis. The P6 treatment group presented the most damage, compared to the other treatment groups, since the liver cells and other organs in chickens were exposed to toxic metabolic material released from the *Toxocara vitulorum* larvae during a longer period of time.

REFERENCES

Abbas AK, and Lichtman AH (2003). Cellular and molecular immunology. 5th ed. Philadelphia: Saunders, 32 (1): 65-66. DOI: https://doi.org/10.1002/hbmb.20049803019907

Abbott NJ, Romball L, and Hanson E (2006). Astrocyte-endothelial interactions at the blood brain barrier. Nature Reviews Neuroscience, 7(1): 41-53. DOI: https://doi.org/10.1038/nrn1824

Azizi S, Oryan A, Sadjadi SM, and Ziaee M (2007). Histopathologic changes and larval recovery of *toxocara cati* in experimentally infected chickens. *Parasitology Research*, 102(1): 47-52. DOI: https://doi.org/10.1007/s00436-006-0723-5

Castillo N, Adriana R, Ribichich M, and Lopez CM (2008). Experimental infection with *toxocara cati* in BALB/c mice, migratory behavior and pathological changes. Zoonoses and Public Health, 55(4): 198-205. DOI: https://doi.org/10.1111/j.1863-2378.2008.01182.x

Cotran RS, Kumar V, and Robbins SL (1994). Robbins’ pathologic basis of disease. Philadelphia: W.B. Saunders, 12 (4): 377-377. DOI: https://doi.org/10.1002/97804704730212

Fawzy S, Ollero MD, Gilles JL, and Del AC (2001). Animal models in ocular toxocariasis. Journal of Helminthology, 75(2): 119-124. Available at: http://pubmed.ncbi.nlm.nih.gov/11520434/

Hübner J, Uhlíková M, and Leisová E (2001). Diagnosis of the early phase of larval toxocariasis using IgG avidity. Epidemiological Microbiology and Immunology, 50(2): 67-70. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11332729

Khan AZ, Khan K, Zaman G, Ullah S, and Habibullah Q (2007). Prevalence of gastritis-renal haematological parasites of economic importance in dairy buffaloes in peshawar, Sarhad Journal of Agriculture, 23 (3): 781-792. Available at: https://www.sajc.org.pk/sja_pdf

Knodell RG, Ishak KG, and Black WC (2019). Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology, 165: 431-435. Available at: http://www.ncbi.nlm.nih.gov

Kumar V (2013). NKT cell subsets: Promoters and protectors in inflammatory liver disease. Journal of Hepatology, 59(3): 618-620. DOI: https://doi.org/10.1016/j.jhep.2013.02.052

Kusnoro (2005). Prevalensi toxocariasis pada kucing liar di surabaya melalui bedah slannah Pencanaan. Media Kelodk Hewan, 21(1): 7-11. Available at: http://journal.ainar.ac.id/download-fullpapers-MKH21-1-02.pdf

Kusnoro S, Sosiarwati S, Subeki S, Koesdarto S, and Paspianawati H (2007). Peninjauan praktikum ilmu penyakit hewan Veteriner. 2nd ed. Surabaya: Departemen Pendidikan Nasional Universitas Airlangga.

Kusnoro, Subeki S, Khusir SI, and Soedarto (2011). Characterization and isolation of specific protein from Excretory-Secretory (ES) material of L2 dormant of toxocara cati for the diagnostic development of toxocariasis by ELISA Technique. Journal UIN, 13(1): 56-65. Available at: http://journal.ainar.ac.id/download-fullpapers-VOl%2013%20No%201%20Art%202011-7.pdf

Levine ND (1995). Buku Pelajaran Parasitologi Veteriner. Yogyakarta: Gajah Mada University Press. Available at: http://library.unm.ac.id/free-content/index.php/buku-lenteran-buku-pelajaran-parasitologi-veteriner-norman-d-levine-peremakan-gajat-ashadi-editor-wardiarto-288.html

Oryan A, Sadjadi SM, and Azizi S (2010). Longevity of toxocara cati larvae and pathology in tissues of experimentally infected chickens. *Korean Journal of Parasitology*, 48(1): 79. DOI: https://doi.org/10.3347/kjp.2010.07.129

Raza MA, Murtaza S, and Madar Ayaz M (2013). Toxocara vitulorum infection and associated risk factors in cattle and buffalo at multan district Pakistan. *Science International*, 25(2): 291-294. Available at: https://www.sciencedirect.com/science/article/pii/099292430180294-0

Santos SVD, Santos FYH, Lescano SAZ, Santos DMD, Tuico EDS, Fonseca GR, Ribotto MCSA, and Chiodi PP (2017). Migration pattern of *Toxocara canis* larvae in experimentally infected male and female Rattus norvegicus. Revista da Sociedade Brasileira de Medicina Tropical, 50(5): 698-700. DOI: https://doi.org/10.1590/0037-86820171271

Seoulby RJL, and Monig K (1982). Helminths, arthropods and protozoa of domesticated animals. 7th ed. London: Bailliere Tindall. Available at: https://www.worldcat.org/title/helminths-arthropods-and-protozoa-of-domesticated-animals/oclc/4191258

Sterike WA, Machado RZ, Bechara GH, and Zoccoler MC (1996). Skin hypersensitivity tests in buffaloes parasitized with *Toxocara vitulorum*. *Veterinary Parasitology*, 63(3-4): 293-300. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8966094

Sterke C, Heuer L, Janecek E, and *Toxocara SPP* (2013). Infections in paratenic hosts. *Veterinary Parasitology*, 193(1-2): 375-389. DOI: https://doi.org/10.1016/j.vetpar.2012.12.053

Taira K, Saitoh Y, and Kapel CMO (2011). Toxocara canis larvae persist and retain high infectivity in muscles of experimentally infected chickens. *Veterinary parasitology*, 180(3-4): 287-291. DOI: https://doi.org/10.1016/j.vetpar.2011.03.020

Thomson RG (1984). General Veterinary Pathology. Available at: https://books.google.co.id/books?id=TeSNz2kdKMCC

Underwood JCE (1996). General and Systematic Pathology 2nd Edition. 2nd ed. London Churchill Livingstone. Available at: https://www.amazon.com/General-Systematic-Pathology-1/- Underwood/dp/0443052824

Yoshikawa M, Nishiofuji M, and Moriya K (2008). A familial case of visceral toxocariasis due to consumption of raw bovine liver. *Parasitology Introduction*, 57(4): 525-529. DOI: https://doi.org/10.1016/j.parint.2008.08.002

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in humans: symptomatic course of toxocarosis correlates significantly with levels of IgE/anti-IgE immune complexes", Parasite Immunology, 2002

www.ncbi.nlm.nih.gov

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Hamza Avcioglu, Ibrahim Balkaya. "A comparison of the efficacy of subcutaneously administered ivermectin, doramectin, and moxidectin against naturally infected Toxocara vitulorum in calves", Tropical Animal Health and Production, 2011

Meixin Gao, Xiulan Li, Lingling He, Junru Yang, Xiaohui Ye, Fan Xiao, Hongshan Wei. "Diammonium Glycyrrhizinate Mitigates Liver Injury Via Inhibiting Proliferation Of NKT Cells And Promoting Proliferation Of Tregs", Drug Design, Development and Therapy, 2019
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