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HISTOPATHOLOGICAL RESPONSES TO EXPERIMENTAL ANCYLOSTOMIASIS IN HEALTHY AND DIABETIC MURINE MODELS

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ABSTRACT

Helminthic infections remain a significant global health concern, particularly in immunocompromised individuals where disease severity and tissue responses may be altered. *Ancylostoma caninum* infection, primarily a parasite of dogs, has widely been used as an experimental model to study host-parasite interactions due to its ability to induce systemic pathological changes. Moreover, metabolic disorders such as diabetes mellitus are known to modulate immune responses, potentially exacerbating infection outcomes. This study was aimed to elucidate the tissue-specific histopathological responses following experimental infection with *A. caninum* in both healthy and diabetic Wistar rats. A total of 108 rats were randomly allocated to six groups (n = 18 per group). Groups I and II served as healthy and diabetic controls, respectively. Experimental infection was performed using L3 larvae of *A. caninum* administered either orally (200 larvae rat^{-1}) or percutaneously (1000 larvae rat^{-1}) based on group designations. Histopathological assessments of lung, liver, skeletal muscle, and brain tissues were conducted on six randomly selected rats from each group on days 15, 30, and 45 post-infections. No significant lesions were observed in groups I, II, V, and VI. In contrast, group III and IV (orally infected healthy and diabetic rats, respectively) exhibited pronounced pathological changes, with severe manifestations in diabetic animals. These findings highlight the exacerbated tissue responses associated with hyperglycemia in helminthic infections and provide insights into the pathogenesis of ancylostomiasis in immunocompromised hosts.

Keywords: *Ancylostoma caninum*, diabetes mellitus, histopathology, parasitic infection, tissue pathology, Wistar rats

INTRODUCTION

Hookworms of genus *Ancylostoma* are among the most pathogenic gastrointestinal nematodes in canines and pose a significant zoonotic risk. Global estimates indicate that approximately 21% human population is affected by hookworm infections, resulting in cumulative blood loss equivalent to the total blood volume of approximately 1.5 million individuals (Hotez *et al.,* 2004; Bowman, 2014). The zoonotic implications of *Ancylostoma* spp., particularly their potential to induce cutaneous larva migrans in humans, further elevate their public health relevance (Hochedez and Caumes, 2007; Traub *et al.*, 2008). In natural hosts, L3 larvae penetrate the skin, traverse the dermis to enter circulatory system, and migrate through pulmonary tissues before localizing in small intestine where they mature into adults (Burke and Roberson, 1985a; Schad, 1990). This migratory trajectory elicits diverse pathological responses in multiple organ systems.

Diabetes mellitus is a chronic metabolic disorder characterized by impaired immune responses, thereby predisposing the affected individuals to more severe and frequent infectious complications (Maritim *et al.,* 2003; Casqueiro *et al.,* 2012). Several studies have studied the histopathological changes associated with helminth infections and have documented tissue damage such as mucosal erosion, inflammatory cell infiltration, and vascular alterations in organs including intestine, liver, and lungs (Prociv and Croese, 1990; Loukas *et al.,* 2005). Experimental models of hookworm infections have similarly described host-parasite interactions leading to localized and systemic pathology (Schad and Anderson, 1985). However, most of these studies have been conducted under normoglycemic conditions or have focused on single-organ pathology, with limited emphasis on systemic histopathological alterations. There is a paucity of studies examining the combined impact of metabolic disorders such as diabetes mellitus on the progression and severity of hookworm-induced tissue damage. In particular, comparative histopathological evaluations between normoglycemic and hyperglycemic states in controlled experimental models are scarce. Furthermore, earlier studies have largely overlooked the interaction between altered host immunity in diabetes and parasite migration–induced multi-organ pathology, thereby limiting a comprehensive understanding of disease dynamics (Casqueiro *et al.,* 2012).

Given this background, the present study was designed to investigate the histopathological alterations in various tissues of both normoglycemic and hyperglycaemic murine models following experimental infection with *A. caninum*. The novelty of present work lies in its integrated approach, combining comparative evaluation of diabetic and non-diabetic conditions with multi-organ histopathological assessment in a controlled experimental model. Additionally, this study builds upon previously established haemato-biochemical perturbations (Sharma *et al.,* 2024) by correlating them with detailed tissue-level changes, thereby providing a more comprehensive understanding of host-parasite interactions under altered metabolic conditions.

MATERIALS AND METHODS

The present study was carried out at Division of Veterinary Parasitology, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-J, R.S. Pura, Jammu (India). A total of 108 clinically healthy Wistar rats (*Rattus norvegicus*) of either sex, weighing 150-200 g, were maintained under standard laboratory conditions. Prior to the initiation of experiment, the animals were acclimatized for 20 days to ensure stabilization under a controlled environment comprising a 12 h light/dark cycle. Rats were provided with a standard pelleted diet and *ad libitum* access to clean drinking water. The experimental work was initiated after approval by the Institutional Animal Ethics Committee vide No 02/IAEC/2015 dated 28/01/2015, following all the ethical norms approved by the Committee. Following acclimatization, the rats were randomly allocated into six experimental groups (n = 18 per group). Group I was healthy control which received no treatment while group II served as diabetic control (streptozotocin-induced diabetic rats without parasitic challenge). Group III comprised of healthy rats infected orally with 200 L3 larvae of *A. caninum* while group IV was diabetic rats infected orally with 200 L3 larvae of *A. caninum.* Group V consisted of healthy rats infected percutaneously with 1000 L3 larvae of *A. caninum* while group VI had diabetic rats infected percutaneously with 1000 L3 larvae of *A. caninum.*

Induction of diabetes

Diabetes mellitus was induced via a single intraperitoneal injection of streptozotocin (STZ) @ 60 mg kg^{-1} b.w., freshly prepared in 0.1 M citrate buffer (pH 4.5), following an overnight fast. Blood glucose levels were measured five days post-STZ administration using a glucometer (Model: Contour Plus; Ascensia Diabetes Care Holding, Switzerland). Animals exhibiting fasting blood glucose levels >225 mg dL^{-1} were classified as diabetic and included in the relevant experimental groups (Lenzen, 2008).

Larval preparation and infection protocol

Adult *A. caninum* worms were recovered post-mortem from the intestines of naturally infected stray dogs. Eggs obtained from gravid females were subjected to standard coproculture techniques to harvest infective 3rd stage larvae (L3) (Burke and Roberson, 1985b). Infection was carried out as per group designation, either orally or via the percutaneous route, in a single administration.

Histopathological analysis

At each of three time points (days 15, 30, and 45 post-infection), six rats from each group were humanely euthanized using chloroform inhalation anaesthesia. Tissue samples from brain, lungs, liver, skeletal muscle, and skin were collected and fixed in 10% neutral-buffered formalin. After standard histological processing, the tissues were embedded in paraffin, sectioned at 4-5 µm thickness, stained with hematoxylin and eosin (H&E), and examined under a Carl Zeiss AXIO light microscope and Imager-A1 (Carl Zeiss, Germany) for istopathological evaluation following established protocols (Luna, 1968).

RESULTS AND DISCUSSION

Neuropathological alterations

Histopathological examination of brain in control group rats (group I and II) did not show any microscopic lesions of pathological importance during the entire study period. On day 15 post-infection, group III showed mild parasite remnants (Fig. 1). These findings are consistent with earlier work describing neuroinflammation following *Toxocara* spp. infection (Fabiyi and Adeleye, 1982; Schantz, 1991). Inflammatory cells were also seen. Similar but more sever changes were noticed in group IV on day 15 post-infection. Histopathological section of this group revealed the presence of remnant of parasite as well as foci of glial cells and inflammatory cells (Fig. 2). The presence of parasite remnants, including cuticular and muscular components, confirmed neurotropism of *A. caninum* in hyperglycemic hosts which are suggestive of impaired immune clearance mechanisms. Similar exacerbations of neuro-inflammatory responses under diabetic conditions have been documented in models of *T. canis* larval migrans (Sangster and Gill, 1999; Krolewiecki and Lammie, 2013). At day 45 post-infection, no lesions of pathological significance were seen in group III but in group IV mild congestion was noticed.

Skeletal muscle pathology

Histopathological examination of skeletal muscles in control group rats (group I and II) did not show

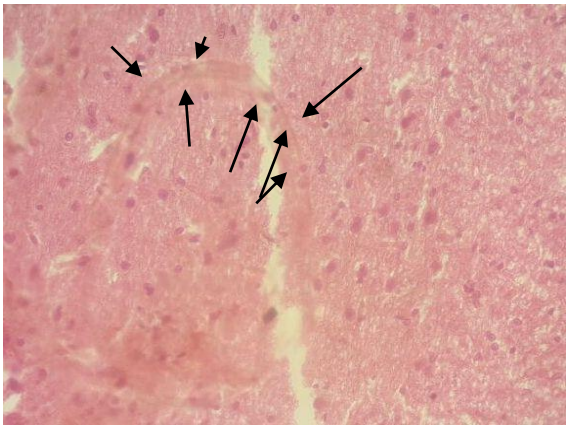

Fig. 1: Longitudinal section of whole parasite in brain (marked by arrows). (H & E, 40x)

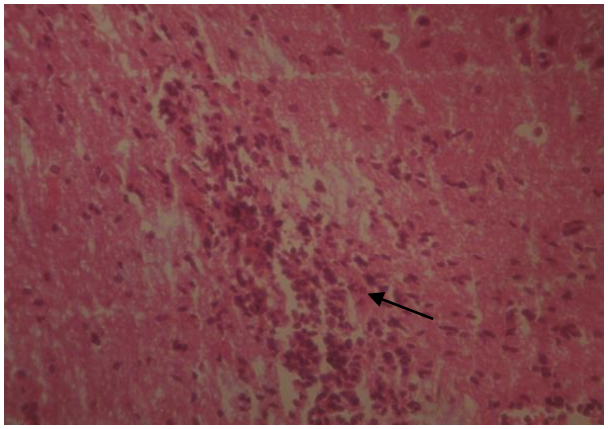

Fig. 2: Encephalitis, destruction/ disorganization of neurofibrils, infiltration of MNC's (arrow) (H & E, 40x)

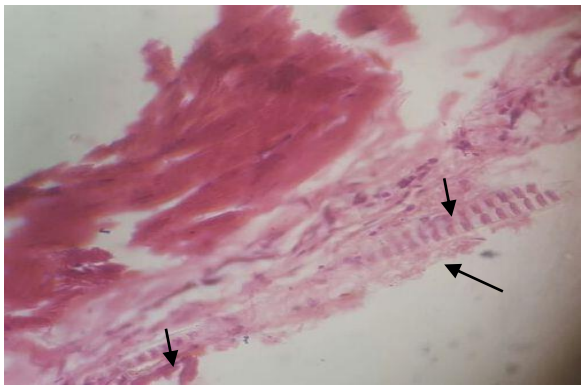

Fig. 3: Cuticle section of parasitic larvae, surrounded by fibrous tissue and invasion of migratory tract in skeletal muscle (arrows) (H&E, 10x).

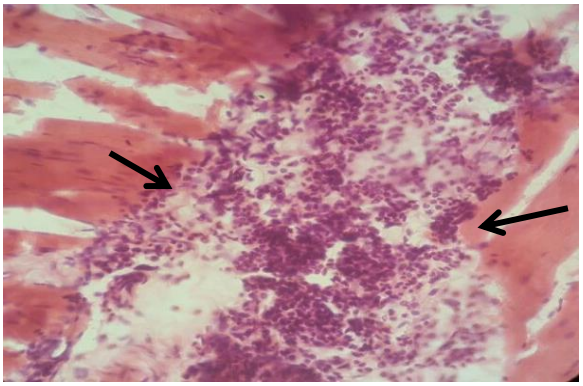

Fig. 4: Migratory tracts of parasitic larva with destruction and infiltration of MNCs (arrow) in skeletal muscles (H&E,10x) with destruction and fragmentation of muscle fibers and presence of inflammatory mast cells and eosinophil's in skeletal muscle (arrows) (H&E, 40x).

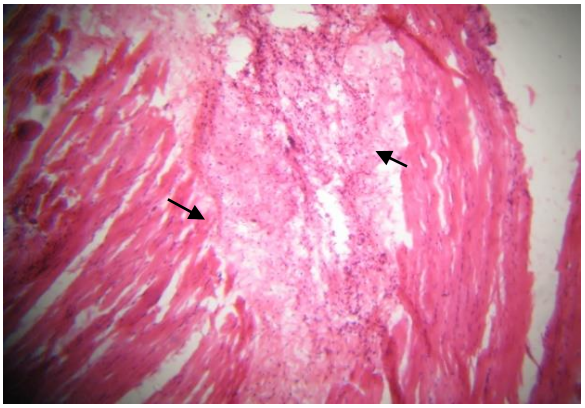

Fig. 5: Necrosis of skeletal muscle fibers replaced by eosinophilic debris (arrows) and fibrosis (H&E, 40x).

any microscopic lesions of pathological significance during the entire study period. On day 15 post-infection, group III showed parasite cuticle and mild reaction consisting of inflammatory cells mostly mono-nuclear cells, macrophages and few neutrophiles (Fig. 3). These findings parallel the earlier descriptions of *Ancylostoma*-induced sarco-cellular injury in rodent models (Craig and Faust, 1913; Maizels and Yazdanbakhsh, 2003). Disruption of musculature, fragmentation and loss of striations surrounding the inflammatory areas were significantly less compared to group IV. Similar pattern of lesions with significantly more severity were seen in IV group. There were necrotic areas in muscles and necrotic muscle was replaced by a large number of inflammatory cells mainly eosinophils, lymphocytes and macropahges (Fig. 4). Chronic granulomatous inflammation with mast cell involvement indicated a prolonged immune response, potentiated by hyperglycemia-induced immune dysregulation (Maritim *et al*., 2003; Stepek *et al*., 2006).

On day 30 post-infection, lesions in group III consisted of small areas of inflammation whereas in group IV necrotic muscle was replaced by pink fibrino-necrotic debris and fibrous connective tissue (Fig. 5). These findings are in line with those in diabetic models of trichinellosis, where delayed resolution of muscle inflammation was observed (Chatterjee, 2009) On day 45 post-infection, mild inflammatory reaction consisting mainly of mononuclear cells and presence of fibrosis was seen in both group II and IV but necrotic areas were bigger in group IV.

Pulmonary histopathology

The histopathological examination of lungs in control group rats (group I & II) did not show any microscopic lesions of pathological significance during the entire study period. On day15, in III group thickened lung septa due to inflammatory reaction consisting mainly of mononuclear cells were seen. Mild peri-bronchiolar infiltration of inflammatory cells and hyperplasia of smooth muscles along with section of parasitic larvae were also seen in group III (Fig. 6). These features reflect larval transit and alveolar-capillary interface disruption, as noted in experimental pulmonary helminthoses (Luna, 1968; Kassai, 1999). In group IV, mild thickening of alveolar septa with necrosis and infiltration of chronic inflammatory cells were

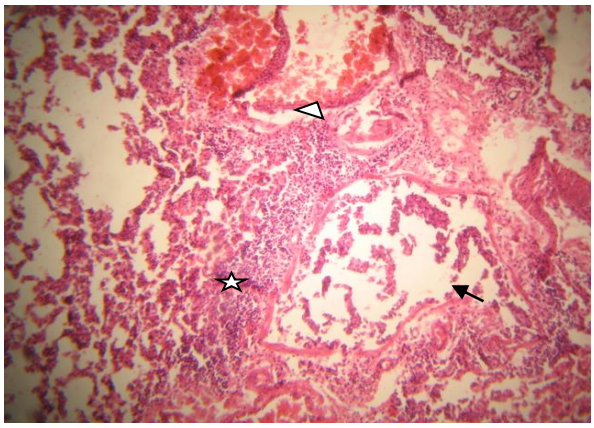

Fig. 6: Peribronchiolar lymphoid aggregates degenerated and necrotic bronchiolar epithelium (arrow), perivascular odema, invasion of MNC's (star), degenerated and necrotic blood vessel wall in lungs (arrow head) (H&E, 10x).

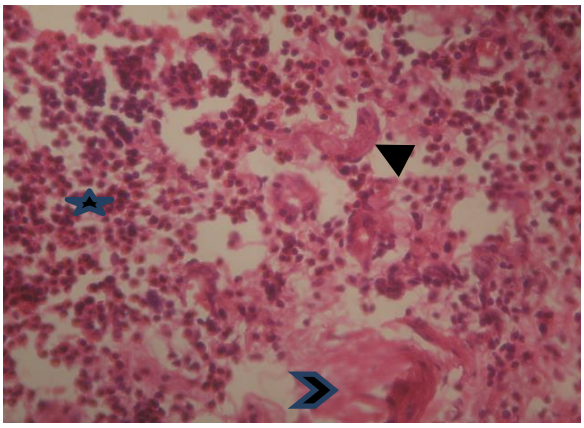

Fig. 7: Parasitic remnants (arrow), destruction of blood vessel walls and destruction of lining of bronchiolar wall (arrow head) and inflammatory cells (star) (H&E, 40x).

seen. At many places,the presence of hyaline degeneration and necrosis of blood vessels with infiltration of mononuclear cells were indicative of enhanced pulmonary vascular injury. Remnant of larva were also seen in group IV (Fig. 7). These patterns were analogous to the findings in *Strongyloides* and *Toxocara*-induced pneumonitis in immuno-compromised rodents (Coombs and Crompton, 1991; Holland and Smith, 2017) On day 45, group IV showed more severe and multiple areas of inflammatory reactions as compared to group IV.

Hepatic lesions

Histopathologically the control group rats showed the normal histological appearances of liver and revealed prominent central vein, normal hepatic lobes with cord portion and hepatocytes with vesicular nucleus and normal cytoplasm. On day 15, group III showed mild congestion and focal areas of small mononuclear cell infiltration especially replacing hepatocytes could be seen (Fig 8). These hepatic alterations are consistent with prior studies reporting hepatobiliary inflammation during larval migration of *A. caninum* and other nematodes (Brooker *et al*., 2006; Dold and Holland, 2011). Group IV also showed similar lesion but in this group multifocally in portal triads, proliferation of bile duct, inflammatory cell infiltration consisting mainly of mononuclear cells were frequently seen (Fig. 9). Diabetic animals exhibited more sustained portal tract inflammation, likely reflecting reduced antioxidant defenses and altered cytokine profiles under hyperglycemia (Hotez and Wilkins, 2009; Roberts and Janovy, 2009). On days 30 and 45, group IV showed milder lesion comprising of infiltration of MNC around portal tract area along with hyperplasia of bile duct and mild fibrosis In group III, similar lesions but mild in character has been observed on day 45 post infection.

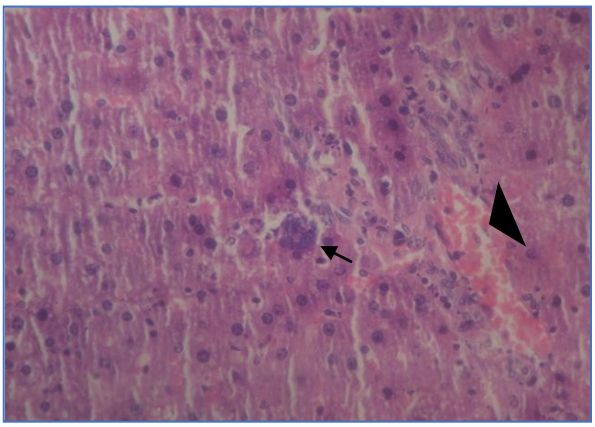

Fig. 8: Mild congestion (arrowhead), disruption of hepatic, chord, mild focal infiltration of MNCs (arrow) (H&E, 10x).

Dermatopathological observations

Histopathological examination of lung, liver, brain and skeletal muscle of rats of all percutaneously infected groups (group I, II, III,) showed no microscopic lesions of pathological significance supporting the hypothesis that

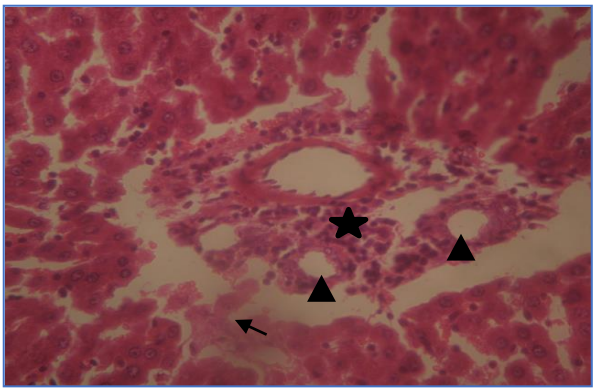

Fig. 9: Mild inflammatory reaction portal triad (star), destruction of surrounding hepatic chord and necrosis of hepatocytes (arrow), bile duct hyperplasia (arrow head) (H&E, 40x).

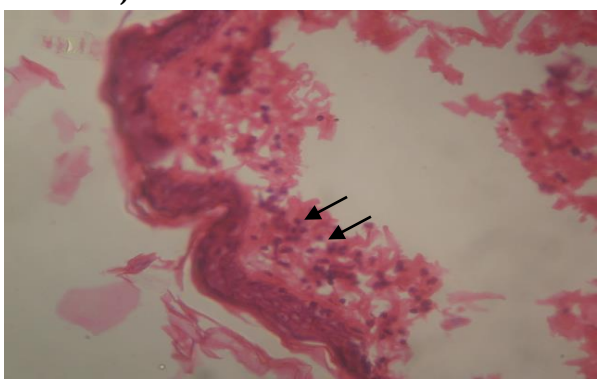

Fig. 10: Mild hyperkeratosis and hyper keratinization and MNC's infiltration (arrows) in skin dermis (H&E, 10x).

larval penetration via skin in Wistar rats is largely non-migratory. However, histo-pathological section of skin of VI group showed mild multifocal inflammatory reaction comprising mainly of polymorpho-mononuclear cells in dermis on day 15 and 30 post-infection (Fig. 10) indicating transient cutaneous immune activation at larval entry sites. These findings are in agreement with the self-limiting nature of cutaneous larva migrans (CLM), wherein inflammatory responses are largely confined to the dermis without systemic dissemination (Heukelbach and Feldmeier, 2008). Absence of visceral larva migrans (VLM) in percutaneously infected groups supports species-specific barriers to larval migration in murine models, as previously observed with *A. braziliense* (Prociv and Luke, 1985).

Conclusion: Oral *Ancylostoma caninum* infection caused multisystemic damage, intensified in diabetic rats, with severe neuro-inflammation, hepatic fibrosis, lung injury, and muscle degeneration. Diabetes heightened lesion severity and persistence. Percutaneous infection caused mild, localized skin inflammation without systemic spread, highlighting the route-dependent pathology and the critical impact of metabolic status on disease progression.

Ethical statement: The work was approved by the Institutional Animal Ethics Committee vide No 02/IAEC/2015 dated 28/01/2015, and all the ethical norms approved by the Committee were followed.

Conflict of interest: Authors declare to have conflict of interest regarding this publication.

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