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Role of infection with *Hymenolepis nana* in alteration of some Biochemical, Immnunological and Histological parameters in Mice/Balb-C

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ABSTRACT. The current study was conducted from November 2023 to February 2024 on 20 male laboratory mice. The current study evaluated the effect of infection with the dwarf worm on some biochemical and immunological parameters and some histological changes of the liver and intestine of laboratory mice. During the current study, liver enzymes, some cellular motility and immunoglobulin E were measured using ELISA technology by providing a set of work from well-known international companies. Histological sections of the liver and intestine of mice were also prepared for both the control and dwarf worm-infected groups. The current study showed significant differences between the levels of enzymes, cellular motility and immunoglobulin E levels by comparing the results of the infected group with the control group. In addition, the current study also showed changes in the liver and intestine tissues by comparing with the control group as well. It appears from the results of the current study that infection with the dwarf worm causes changes in liver functions and stimulates the immune response in addition to histological changes in both the liver and intestine.

Keywords: tape worm, biochemical and Immunological parameters; histopathological changes.

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Introduction

Rodents are commonly utilised in experimental research, and a significant portion of our comprehension about the efficacy of several anti-helminthic medications against H. nana is based on investigations using Rodents infections (Al-Olayan et al., 2023). Prior to initiating an experiment, it is imperative that animals are in a state of good health and completely devoid of any infections, including parasites, since their existence can significantly influence the results of the experimental parameters under investigation. Rodents are recognised as reservoirs for numerous zoonotic infections, such as Hymenolepiasis (Khan et al., 2022), and Hymenolepiasis is diagnosed through the identification of cestode eggs using stool microscopy (Pérez-Chacón et al., 2017). This infection can result in symptoms including vomiting, nausea, abdominal pain, diarrhea, and itching in the anal or nasal regions. This virus is prevalent globally (Roberts et al., 2013).

The family Hymenolepididae is comprised of a large number of genera that have species found in both birds and mammals (Roberts et al., 2013). Humans can only be infected by *Hymenolepis nana* and *H. diminuta* (Mehlhorn, 2022), also can the life cycle of these parasites involve humans, rats and mice as definitive hosts (Coello-Peralta et al., 2020). The structure of the family is rather uncomplicated when compared to Pseudophyllidea, for instance. Additionally, the majority of species within this family are diminutive, translucent, and amenable to scientific examination. The group is distinguished by its most prominent morphological trait, which is the limited number of testes, often ranging from one to four. The presence of a small number of testes, often seen on one side of the body, along with a prominent external seminal vesicle, allows for straightforward identification of the family. Every species except *H. nana* requires arthropod intermediate hosts (Roberts et al., 2013).

H. nana, also known as the dwarf tapeworm, is globally most prevalent common parasite among cestodes (Al-Olayan et al., 2023), particularly affecting youngsters (Karim et al., 2024). The adult *H. nana* measures 15–40 mm in length and 0.5–1.0 mm in width. The scolex is spherical, refractile, and comprises four suckers. The suckers possess a rostellum that enables the adult worm to adhere to the ileal mucosa. The rostellum aligns approximately 20 to 30 hooklets in a linear formation. The neck is slender, unsegmented, and of intermediate length, giving rise to 200 proglottids that are immature, mature, and gravid (Bhosel, 2022). *H*

nana exhibits a distinct life cycle in contrast to other tapeworms as it does not necessitate an intermediate host. After being consumed by a human or a rodent, the eggs hatch in the duodenum and emit oncospheres. Subsequently, these oncospheres penetrate Intestinal lining. Then it is developed into a cysticercoid, the latter settles in the intestine and develops into an adult worm (Roberts et al., 2013).

Material and methods

Blood samples were obtained in anticoagulant-free test tubes and allowed them to clot at room temperature for 30 minutes, followed by centrifugation at 2000 rpm for 10 minutes to separate the serum using a micropipette. The serum was preserved in a sterile test tube and stored at -20 °C until testing is conducted (Bain et al., 2017).

Microscopic investigations

The microscopic exams included the wet mount smear of Mice feces to determine the infection by *H. nana* (World Heath Organization [WHO], 1991; Fitte et al., 2018).

Measurement each AST, ALT and ALP concentrations

Concentrations of AST, ALT and ALP were determined using a commercially available kit and equipment provided by Randox England Co.

Cytokines and Immunoglobulin-E (IgE) examinations

The levels of interleukins (IL-4, IL-5, and TNF-α) and IgE in the blood serum of laboratory mice were measured using a specialised analysis kit developed by the Chinese company SUNLONG.

Histopathological study

The sections of tissues were produced using the Bancroft and Stevens (1987) approach to identify histological alterations in the liver and small intestine.

Statistical analysis

Analyzed the data by using the t-test, with the means are tested at a significance level of P value ⩽ 0.05, the SPSS program Version 22 used (Cleophas & Zwinderman, 2016).

Results and discussion

Microscopic investigations

The investigations by microscope were included wet mount smear of mice feces by using Logal´s Iodin stain for detecting of infection with *H. nana*, look Figure1.

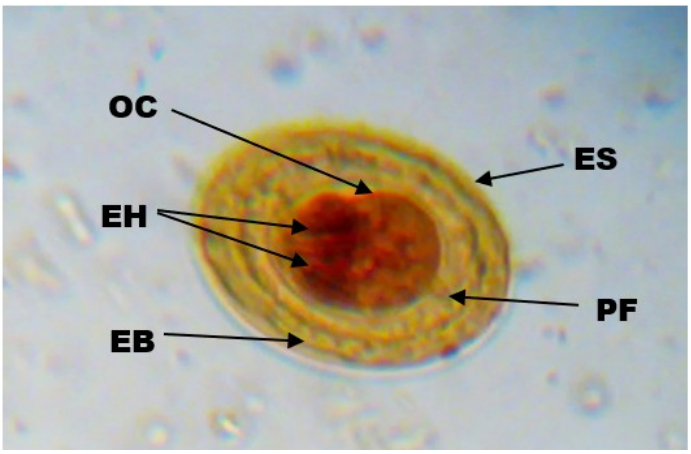

Figure 1. Egg of *H. nana* sp. ×400 Stained by lugal's Iodin.

Eggs (EG) are surrounded by an egg shell (ES) that contains an embryophore (EB) and three polar filaments (PF) and oncosphere (OC) with six hooks of embryo (EH).

The current results concurrent with the results of Bimi et al. (2021), who detected that some of the rats and mice under study were infected with *Hymenolepis* sp. by using microscopic examination.

Biochemical parameters

The present results showed (as in Table 1) significant elevation in concentration of each AST, ALT and ALP of infected group with *H. nana* compared to normal group, it reached (88.33, 67.44 and 87.10) IU L^{-1} of infected group, consecutively, while it was IU L^{-1} of normal group (62.25, 49.30 and 70.47), consecutively.

Table 1. The concentrations of biochemical parameters of each mice infected with *H. nana* and normal group.

Groups / Parameters	Normal Mice	Infected Mice
	Mean± SD (IU L^{-1})	
AST	62.25 ± 3.40	88.33* ± 4.70
ALT	49.30 ± 3.20	67.44* ± 5.40
ALP	70.47 ± 5.88	87.10* ± 7.93

* significant differences at the $p \leq 0.05$.

The current findings align with the study conducted by Kapczuk et al. (2018), which demonstrated a significant rise in the levels each ALT, AST, and ALP in rats infected with H. diminuta compared to uninfected rats.

The present results were not concurrent with Bayoumy et al. (2020), which indicated a non-significant decrease in the levels of both AST and ALT in infected groups with the *H. nana* worm in contrast with normal group, where reached (41.67 and 36.67) IU L^{-1} of infected group, consecutively, while it was (45 and 40) IU L^{-1} of normal group, consecutively. Also Sarrafan et al. (2021) indicated that AST was 67.60 IU L^{-1} in normal mice.

H. nana possesses the capability to engage not only at the infection site but also in distant organs, including the liver. Elevated levels of liver enzymes, including alkaline phosphatase and aminotransferases, indicate compromised liver function in mice infected with *H. nana* (Kapczuk et al., 2018).

Immunity parameters

The results from Table 2 indicate that the levels of IgE, IL-5, and tumour necrosis factor (TNF-α) in infected mice were significantly higher compared to uninfected mice. Specifically, the IgE levels were 91.34 and 52.25 pg mL^{-1}, the IL-5 levels were 26.12 and 10.41 pg mL^{-1}, and the TNF-α levels were 53.15 and 22.56 pg mL^{-1}, respectively. On the other hand, the IL-4 level in infected mice was significantly lower than in uninfected mice, measuring 34.53 and 14.30 pg mL^{-1}, respectively.

Table (2). The concentrations of Immunity parameters of each mice infected with *H. nana* and normal group.

Groups / Parameters	Normal Mice	Infected Mice
	Mean± SD (pg mL^{-1})	
IgE	52.25 ± 3.40	91.34* ± 8.60
IL-4	34.53 ± 4.62	14.30* ± 2.20
IL-5	10.41 ± 3.31	26.12* ± 6.90
TNF-α	22.56 ± 4.77	53.15* ± 7.83

* significant differences at the $p \leq 0.05$.

The current findings align with those of Abdel-Latif et al. (2017), who indicated that the mRNA expression of TNF-a was markedly elevated in the group infected with the H. nana parasite relative to the non-infected group. In contrast, the mRNA expression of IL-4 was markedly diminished in the infected group relative to the non-infected group.

Helminthic infections provoke an immune response governed by Th2 cells, with eosinophils significantly contributing to the host's defensive mechanisms. Intestinal parasites in the host organism result in increased synthesis of immunoglobulin E (IgE). Pro-inflammatory mediators are produced by basophils, mast cells, and eosinophils in response to IgE interacting with antigens on the parasite's surface, resulting in inflammatory reactions. Thereafter, the activated Th2 cells can secrete interleukins, namely IL-4, IL-5, and IL-13. IL-5 stimulates increased eosinophil production, which adhere to the parasite while concurrently releasing major basic protein (MBP) and eosinophilic cationic protein (ECP). Thus, this mechanism damages the parasite, resulting in its expulsion from the host organism (Kapczuk et al., 2018; Chaudhury, 2022).

Ahmed et al. (2015) reported that expression of the gene of TNF-α was considerably increased in rats infected with the *H. diminuta* worm compared to rats that were non-infected. According to their study, TNF-

α is a potent mediator in the inflammation that happens in the mucosal layer of intestine because of parasite infections, such as the infection with *H. diminuta*. However, the exact role of TNF-α is not apparent.

Pérez-Chacón *et al.,* (2017) proposed that an imbalance among the Th1, Th2, and Th17 immune responses to Hymenolepis sp. leads to compromised absorptive activities of the small intestine, resulting in chronic diarrhoea, dehydration, and weight loss in the current patient as secondary effects.

The histopathological study

The liver

The current results showed congested in central vein (Figure 2), infiltiration of inflammatory cells, degeneration of hepatocyte, Nucleus chromatin condensation of degenerated cell, Apoptotic body and Necrotic pyknosis compared to normal group (Figure 3).

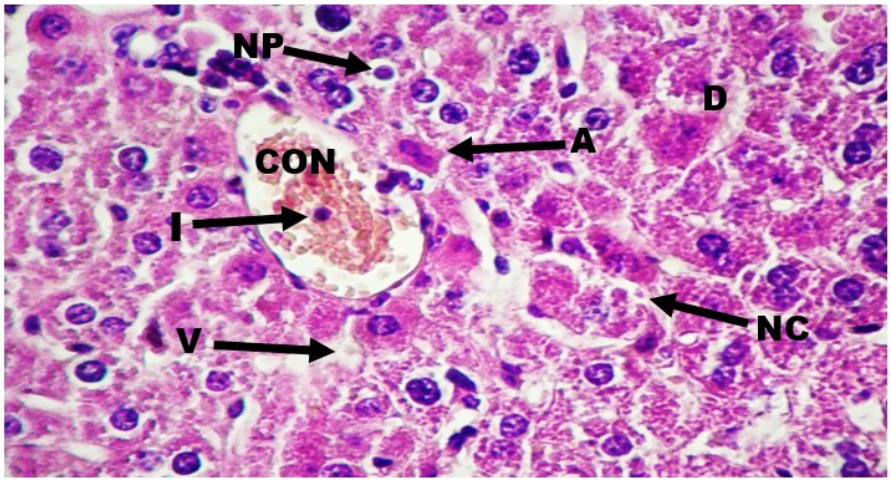

Figure 2. Liver section of infected group shows congested central vein (CON), Infiltration of inflammatory cells (I), Degeneration of hepatocyte (D), Cytoplasmic vacuolation (V), Nucleus chromatin condensation of degenerated cell (NC), Apoptotic body (AB) & Necrotic pyknosis (NP). H & E. 400X.

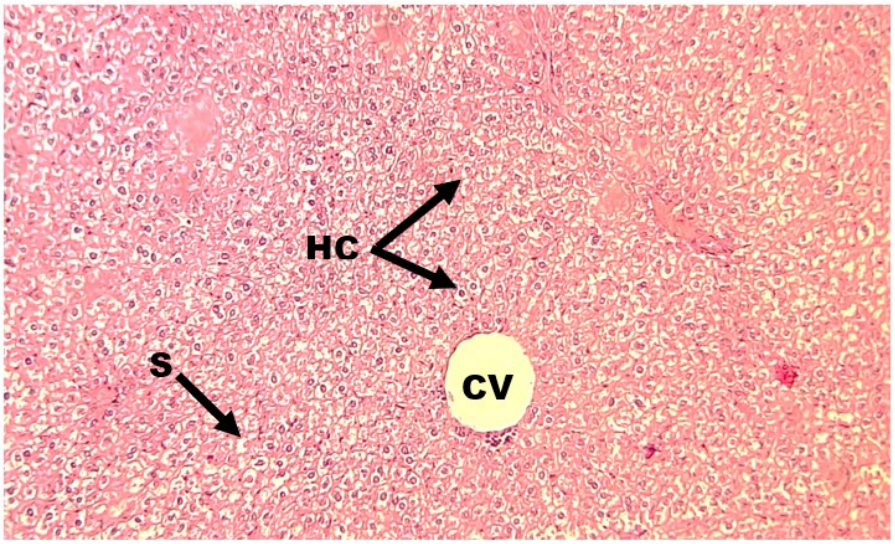

Figure 3. Liver section of normal group shows central vein (CV), Normal hepatocyte (HC), and Sinusoids (S), H & E. 100X.

Bayoumy et al. (2020) showed the microscopic examination of liver section in infected group showed the presence of inflammatory cell accumulation around the central vein, hepatic artery, bil duct, and hepatic sinusoidal tissue. Most of the hepatocytes represented a spongy structure and others lost their nuclei.

The intestine

The current study revealed necrosis of sterio villi, serous gland (Figure 4), degeneration of Lieberkühn crypt and collapse of mucus gland compared to normal group (Figure 5).

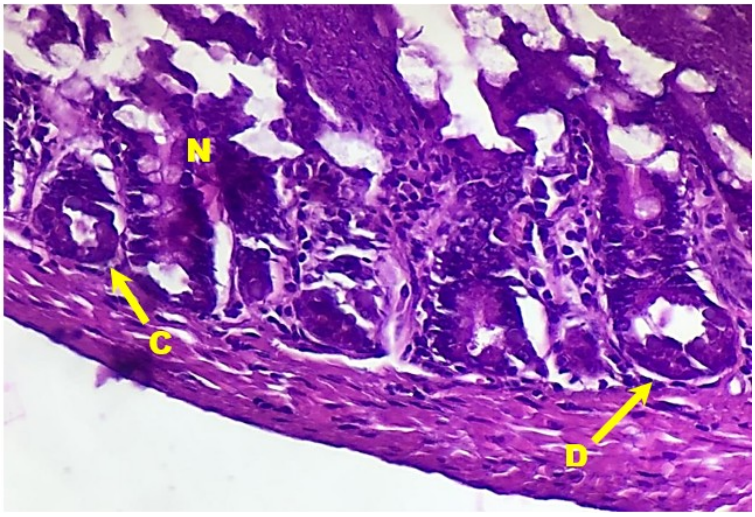

Figure 4. Small intestine section of infected group shows Necrosis of sterio villi (N), Serous gland (Sg), Degeneration of Lieberkühn crypt (D), Collapse of mucus gland (C), H & E. 400X.

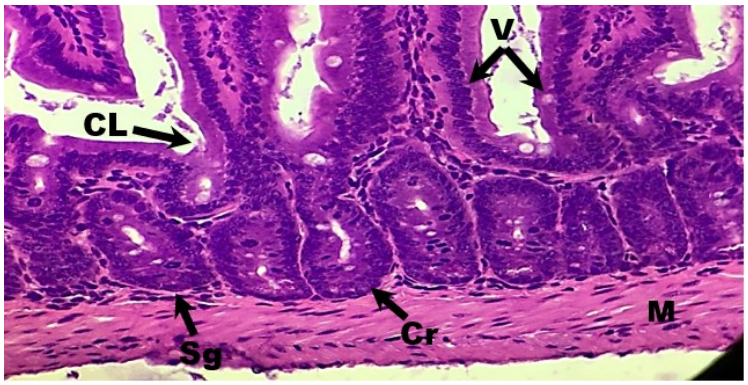

Figure 5. Small intestine section of control group shows normal configure of sterio villi (V), Columnar epithelial lined villi (CL), Serous gland (Sg), Crypt of Lieberkühn (Cr), Muscularis layer of smooth muscles (M) and a serosa layer, H & E. 400X.

A significant number of research on hymenolepidosis is dedicated to the morphometric assignment of the digestive duct mucosa. There is study examined rats type Buffalo that were infected by *H. diminuta*, they found that most notable alterations occurred in the ileum, whereas the duodenum showed the least significant changes, and other study reported that rats infected with *H. diminuta* showed infiltrate inflammatory cells and occure erosions that extended to the muscle layer by day 40 from infection. The researchers observed a decrease in the intestinal villi size and an increase in crypts depth in the gastrointestinal tract of rats. The scientists observed partial removal and the flattening of intestinal villi, as well as an increase in mucus secretion into the lumen of the rats' intestines (Kapczuke et al., 2018).

Abdel-Latif et al. (2017) found that infection with *H. nana* caused the destruction of normal villous architecture in mice. Morphometric analysis of the villi showed significant reductions in both length and width, as well as increased depth of the crypts, in the infected group compared to the non-infected group. The number of goblet cells reduced (hypoplasia) in infected individuals compared to non-infected individuals.

Yadav et al. (2017) demonstrated significant atrophy of the mucosal villi and blunting of the intestinal villi, accompanied with the presence of infiltrating cells in both the mucosa and submucosa. Additionally, there was an observed thickening of the intestinal wall and goblet cell hyperplasia.

Minor inconsistencies may arise due to variations in the experimental settings, such as the specific animal laboratory or strain used (Kapczuke et al., 2018).

Al-Olayan et al. (2023) showed by microscopic examinations that the small intestine of the control group showed normal intestinal structure with long villi covered by the columnar epithelia. Severe patho-logical alterations were observed in the intestinal tissue of the infected mice group with *H. nana* manifested by a change in the shape of columnar epithelia that changed to small cuboidal cells with hyperplasia besides wide degeneration of the lamina propria, on the other hand, peptic ulcers were seen accompanied with the splitting of the muscularis layer.

Conclusion

The occurrence of an increase in the levels of ALP, ALT and AST enzymes in Infected mice with *H. nana* compared to the uninfected mice. The increase in the levels of Cytokines (IL-4, IL-5 and TNF-α) in Infected mice compared to the uninfected mice. It appears from the results of the current study that infection with the dwarf worm causes changes in liver functions and stimulates the immune response in addition to histological changes in both the liver and intestine.

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Data availability

The data underlying the results presented in the study are available within the article.

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