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Clinico-Pathological Studies on *Helicobacter pylori* Infection in Albino Rats

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Abstract: *Helicobacter pylori* (*H. pylori*) is known to be the most common agent in peptic ulcers and chronic gastritis. The goal of this study was to investigate how *H. pylori* infection affect several hematological and biochemical parameters. To achieve this goal, thirty albino male rats were divided into three groups for challenge with *H. pylori* Ag. Two groups were daily fed on *H. pylori* suspensions in food and water, whereas the third group was kept as a control group. Serum iron, ferritin, copper (Cu), zinc (Zn), magnesium (Mg), lipid profile, SGPT (ALT), SGOT (AST), and full blood picture were all measured at day 90 (the end of the experiment). The findings demonstrated that *H. pylori* stool Ag was present in the feces of infected rat groups and was elevated throughout the trial. Antibodies against *H. pylori* have increased in serum. Hematological and biochemical markers, particularly iron and ferritin, as well as hemoglobin and leukocytes, were all negatively affected. In addition, all signs of gastric inflammation were demonstrated among the infected groups. Overall, after exposure to *H. pylori* infected food and water, numerous haematological parameters were disturbed and seriously impacted various organs.

Keywords: *Helicobacter Pylori*; Iron; Ferritin, Copper, Zinc, magnesium; ALT; AST

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1. Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium with a high urease activity that can grow and thrive in the stomach's acidic environment (Kusters et al., 2006). *H. pylori* has been linked to various digestive problems, including chronic active gastritis, peptic ulcer disease, gastric cancer, and gastric lymphoma (Makola et al., 2007). Infection with *H. pylori* is the most common bacterial infection in humans, affecting almost half of the world's population. However, the prevalence of the disease varies across developed and undeveloped countries (Brown, 2000). In Egypt, the prevalence of *H. pylori* has risen to almost 50% (Alboraie et al., 2019).

The most prevalent transmission mode in the general population is from person to person, either via the oral-oral channel (by vomitus or may be saliva) or the fecal-oral route (Brown, 2000). Although the most widely recognized theory is that infection occurs via the fecal-oral route, contaminated water and foods may play a key role in the microorganism's transfer to humans. Drinking water, seawater, vegetables and foods of animal origin have all been reported to contain *H. pylori*. Moreover, *H. pylori* can be also found in milk, vegetables, and ready-to-eat foods (Quaglia and Dambrosio, 2018). *H. pylori* has been isolated from nonhuman primates, domestic cats (but not dogs - Neiger and Simpson, 2000), and sheep despite of the fact that the zoonotic risk of transmission is negligible. Domestic flies have also been implicated as a vector for the spread of this disease (Brown, 2000).

Several studies have linked *H. pylori* infection to changes in serum and hematological parameters, such as an increase in the total number of peripheral blood leukocytes, an increase in platelet count, and an increase in serum transaminase (SGOT) values, and to iron deficiency anemia and other metabolic disturbances in patients (Graham et al., 1998; Cardenas et al., 2006; Qu et al., 2010; Monzón et al., 2013; Buzás, 2014; Guclu and Agan, 2017; Haile and Timerga, 2021).

Transmission of *Helicobacter* spp. depends on the species involved and environmental conditions. For example, feces–oral transmission is the most common natural infection pathway in rodents. Thus, frequent screening and eradication of *Helicobacter* spp. from laboratory animal facilities should be top priorities for researchers (Chichlowski and Hale, 2009). It is also necessary to have a thorough understanding of the epidemiology and mechanism of transmission of *H. pylori*, particularly in endemic locations. Therefore, this study was conducted to assess the impact of *H. pylori* transmission on albino rats fed contaminated drinking water and food containing infected human stool, focusing on hematological, biochemical, and pathological effects.

2. Material and Methods

2.1. Animals and sampling

Thirty adult male albino rats (160-200 g/bwt) were divided into three groups (10 rats each) and housed in separate cages with a temperature of 22-25 °C and a 12-hour light/dark cycle. They were fed a chew diet of regular pellets and free access to drinking water. This experiment was done according to the ethical codes of experimental animals approved by the Animal Ethical Committee, Damanhur University. Before beginning the experiment, the animals were maintained for 14 days to acclimate.

About 10 g of fresh stool from *H. pylori*-infected persons that had tested positive for *H. pylori* Ag by ELISA was mixed with water and food given to rats as a source of infection. The experimental design was assigned as follows:

Group I (G1, normal control group): ten rats received water and food free from infection for 3 months (the experiment duration).

Group II (G2, given infected water): ten rats received water containing stool with *H. pylori* antigen daily for 3 months while the food was free from infection.

Group III (G3, given infected food): ten rats received a chew diet mixed with stool infected with *H. pylori* antigen daily for 3 months, while the water was free from any infection.

2.2. *ELISA assay*

H. pylori Ag was detected using an ELISA test in feces samples collected from rats in all three groups weekly. The ELISA kit (Perkin Elmer Health Sciences, USA) was utilized for *H. pylori* Ag concentration.

H. pylori antibodies (IgM, IgG, and IgA) were detected in rats' serum using ELISA kit (Perkin Elmer Health Sciences, USA).

2.3. *Hematological investigations*

Blood samples were taken from the medial canthus of the eyes after 90 days of infection and kept in EDTA-coated tubes for hematological analysis. Hematological parameters including total erythrocytes count (RBCs), hemoglobin (Hb), packed cell volume (PCV), total leukocytes count (WBCs), leukocyte differential percentages (Neutrophils, lymphocytes, monocytes, eosinophils, and basophils**)**, and platelets count (PC) were determined using the Cell-Dyn 3700 Hematology Analyzer (Abbott Laboratories, Illinois).

2.4. *Biochemical analysis*

Some blood samples were obtained in plain tubes and subsequently centrifuged at 1845 g for 15 minutes to separate serum for biochemical analysis. Biochemical parameters include iron, ferritin, copper, zinc, magnesium, lipid profile (cholesterol, triglycerides, HDL, LDL, and VLDL), and liver enzyme activity (SGPT and SGOT) were determined by Spekol 1500 spectrophotometric device.

2.5. *Necropsy and histopathological examination*

A complete necropsy was immediately done after euthanasia by intraperitoneal administration of pentobarbital sodium (100 mg/kg body weight). Tissue samples from the stomach, liver, intestine, and lung were obtained from experimented animals at the end of the experiment. Tissues were kept in neutral buffered formalin 10% for 24-48 hours for fixation. Following fixation, tissue samples were dehydrated and embedded in paraffin wax using routine methods, sectioned at 5 µm, stained with hematoxylin and eosin (HE) and examined by light microscopy (Bancroft and Gamble, 2008).

2.6. *Urease Test*

Urease testing was performed on gastric swabs collected from the rats after scarification. The test was performed by streaking the surface of a urea agar slant tube with gastric swabs collected from rats' stomachs; place the tube in an incubator at 35°-37°C in ambient air for 48 hours to 7 days. Positive Reaction: Pink coloration in 15 minutes to 24 hours (for as long as 7 days). Negative Reaction: No change in color (Tille, 2015; Leber, 2020).

2.7. *Statistical analysis*

The IBM SPSS software program version 20.0 (Armonk, NY: IBM Corp) was used to analyze the data. The tests used were the Chi-square test for categorical variables, Fisher's Exact or Monte Carlo correction for chi-square when more than 20% of the cells have an expected count less than 5, F-test (ANOVA) for normally distributed quantitative variables, and Post Hoc test (Tukey) for pairwise comparisons.

3. Results

3.1.The level of H. pylori stool Ag estimated in experimentally infected rats

The levels of *H. pylori* Ag in groups II and III infected rats increased marginally to 15.1 and 13.9 ng/ml, respectively, during the first week following infection. From the second week, it rose to 30.9 and 25.6 ng/ml in G II and III, respectively, and became substantially upregulated. The level of *H. pylori* Ag in G II and G III feces grew dramatically during the 12th week after infection, reaching 125.2 and 162.5, respectively, which are considered highly infected levels (> 100 ng/ml). Throughout the investigation, the amount of *H. pylori* Ag in the control group (G1) did not exceed 13.0 ng/ml, as shown in **Figure1**.

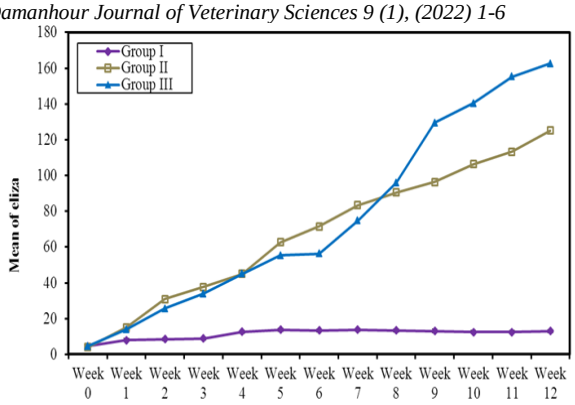

Figure 1. Distribution of the studied cases according to H. pylori stool Ag ELISA in each group

3.2. H. pylori antibodies detected in serum in experimentally infected rats

Using an ELISA technique, the serum levels of immunoglobulins against *H. pylori* infection were compared between the study groups. When compared with the control group (GI), serum levels of IgM, IgG, and IgA were considerably higher in groups II and III, as shown in **Figure 2**.

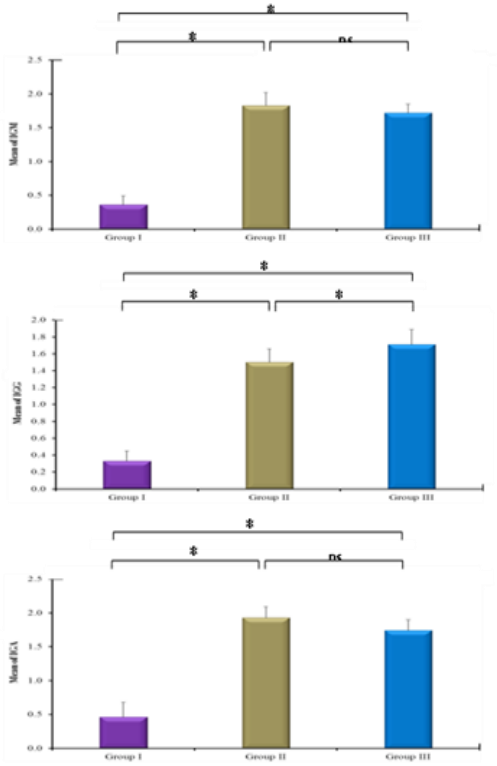

Figure 2. Comparison between the three studied groups according to H. pylori IgM, IgG, and IgA tested in serum among experimentally infected rats.

3.3.Variations in hematological parameters between the three studied groups

As shown in **Table 1**, when compared to the control group (GI), erythrocytic parameters such as Hb, RBCs, and PCV values were considerably lower in groups II and III. The total leukocytic counts (WBCs) in the infected groups II and III were considerably higher than in the control group (GI). The percentages of Neutrophil cells increased to 77.40% and 85.10% in the blood of infected groups II and III, respectively, compared with 56.50% in the control group (GI), while lymphocyte percentages decreased from 33.20% in group I to 14.10% and 11.60% in groups II and III, respectively. Monocytes showed a significant decline in the blood of infected group III. However, a slight non-significant decrease was observed in group II compared with the control group. Eosinophils and basophils exhibited minor to no differences in proportion between all the groups studied. Surprisingly, the platelet level sharply decreased in infected rats (GII & GIII) than in non-infected rats (GI).

3.4.*Variations in biochemical parameters between the three studied groups*

As indicated in **Table 2**, when compared with the control group (GI), the infected groups (GII & GIII) had significantly lower levels of iron, ferritin, and magnesium but significantly higher levels of copper and zinc. Infected rats' serum levels of cholesterol and triglycerides were substantially higher than non-infected rats', but HDL levels were significantly lower. Compared with the control group (GI), LDL and VLDL levels were significantly higher in the infected groups (GII & GIII). Compared with the control group (GI), the infected groups (GII & GIII) had significantly higher liver enzymes SGPT and SGOT. Notably, the previously investigated biochemical parameters levels differed significantly between groups II and III.

3.5.Urease activity

As shown in **Table 3**, all gastric swabs from rats in groups II and III showed positive for urease. On the other hand, the urease test showed a negative reaction in group I.

3.6.Histopathological findings

All 20 mice either administrated water containing *H. pylori* antigen (GII) or challenged with a diet mixed with *H. pylori* antigen-infected stool (GIII) for 3 months developed moderate to severe gastric mucosal inflammation (**Figure 3A-C**). Two rats developed macroscopic ulceration of the gastric mucosa. Eight of the 20 rats showed gastric mucosal cell proliferation and squamous transformation (**Figure 4 A, B**). No lymphoma was observed in these areas. Five rats inoculated with *H. pylori* (2 rats from G11 AND 3 rats from G111) showed mild to moderate hydropic degenerations in parenchymal, periportal areas (**Figure 3D**), in addition, coagulative necrosis of hepatocytes with inflammatory cell infiltration was seen in 2 rats and the inflammatory cells were mainly lymphocytes and neutrophils (**Figure 3D**). Mild to moderate enteritis (Fig.4.B) was detected in 3 and 6 rats in G11 and G111, respectively. Various degrees of pneumonia were detected in 4 rats in both infected groups (GII and GIII), (**Figure 4C**). No lesions were observed in the examined tissues of the control group rats (GI).

Table 1. Comparison between the three studied groups according to CBC

CBC	GroupI (n = 10)	GroupII (n = 10)	GroupIII (n = 10)	p
HB *(g/dl)*	13.37 ± 1.0	9.88 ± 0.54 [a]	10.26 ± 0.82 [a]	<0.001*
RBCs *(10^6/µl)*	5.25 ± 0.27	3.04 ± 0.33 [a]	3.47 ± 0.51 [a b]	<0.001*
PCV *(%)*	45.98 ± 1.92	32.61 ± 1.76 [a]	33.88 ± 2.72 [a]	<0.001*
WBCs *(10^3/µl)*	8.45 ± 1.25	39.56 ± 4.72 [a]	18.53 ± 0.84 [a b]	<0.001*
Neutrophil *(%)*	56.50 ± 3.98	77.40 ± 5.08 [a]	85.10 ± 4.79 [a b]	<0.001*
Lymphocyte *(%)*	33.20 ± 4.29	14.10 ± 4.12 [a]	11.60 ± 5.13 [a]	<0.001*
Monocyte *(%)*	8.0 ± 0.82	6.50 ± 2.07	2.0 ± 1.33 [a b]	<0.001*
Eosinophil *(%)*	1.50 ± 0.53	1.40 ± 0.52	1.10 ± 0.99	0.439
Basophil *(%)*	0.50 ± 0.53	0.60 ± 0.52	0.20 ± 0.42	0.185
Platelets *(10^3/µl)*	347.30 ± 53.65	199.70 ± 31.64	173.50 ± 22.17 [a]	<0.001*

p: p value for comparing between the studied groups. a: Statistically significant with **Group I**, b: Statistically significant with **Group II**

*: Statistically significant at $p \leq 0.05$

Table 2. Comparison between the three studied groups according to different parameters

Tested parameter	GroupI (n = 10)	GroupII (n = 10)	GroupIII (n = 10)	p
Iron *(µg/ml)*	10.73 ± 0.19	9.34 ± 0.36 [a]	8.16 ± 0.63 [a b]	<0.001*
Ferritin *(ng/ml)*	35.05 ± 1.47	31.65 ± 0.88 [a]	29.20 ± 1.40 [a b]	<0.001*
Copper *(µg/dl)*	128.99 ± 0.86	133.53 ± 1.70 [a]	136.71 ± 0.92 [a b]	<0.001*
Zinc *(µg/dl)*	137.26 ± 0.86	140.49 ± 0.31 [a]	143.01 ± 0.73 [a b]	<0.001*
Magnesium *(mg/dl)*	2.47 ± 0.29	1.85 ± 0.12 [a]	1.44 ± 0.13 [a b]	<0.001*
Cholesterol *(mg/dl)*	156.44 ± 3.33	165.17 ± 2.34 [a]	172.64 ± 2.04 [a b]	<0.001*
Triglycerides *(mg/dl)*	103.19 ± 1.28	135.99 ± 2.34 [a]	155.39 ± 6.44 [a b]	<0.001*
HDL *(mg/dl)*	44.27 ± 0.61	36.80 ± 1.33 [a]	31.54 ± 1.08 [a b]	<0.001*
LDL *(mg/dl)*)	91.53 ± 3.38	101.2 ± 3.23[a]	110.0 ± 2.16[a b]	<0.001*
VLDL *(mg/dl)*)	20.64 ± 0.26	27.20 ± 0.47 [a]	31.08 ± 1.29 [a b]	<0.001*
SGPT *(U/L)*	18.69 ± 0.41	21.77 ± 0.56 [a]	23.73 ± 0.32 [a b]	<0.001*
SGOT *(U/L)*	17.59 ± 0.43	22.23 ± 0.53 [a]	24.11 ± 0.69 [a b]	<0.001*

p: p value for comparing between the studied groups, a: Statistically significant with **Group I**. b: Statistically significant with **Group II**

*: Statistically significant at $p \leq 0.05$

Table 3. Comparison between the three studied groups according to urease test.

Urease test	Group I (n = 10)		Group II (n = 10)		Group III (n = 10)		χ^2	^{MC}p
	No.	%	No.	%	No.	%		
Negative	10	100.0	0	0.0	0	0.0	30.262*	<0.001*
Positive	0	0.0	10	100.0	10	100.0		

χ^2: **Chi square test**

MC: **Monte Carlo**

p: p value for comparing between the studied groups

*: Statistically significant at $p \leq 0.05$, and IgA tested in serum among experimentally infected rats.

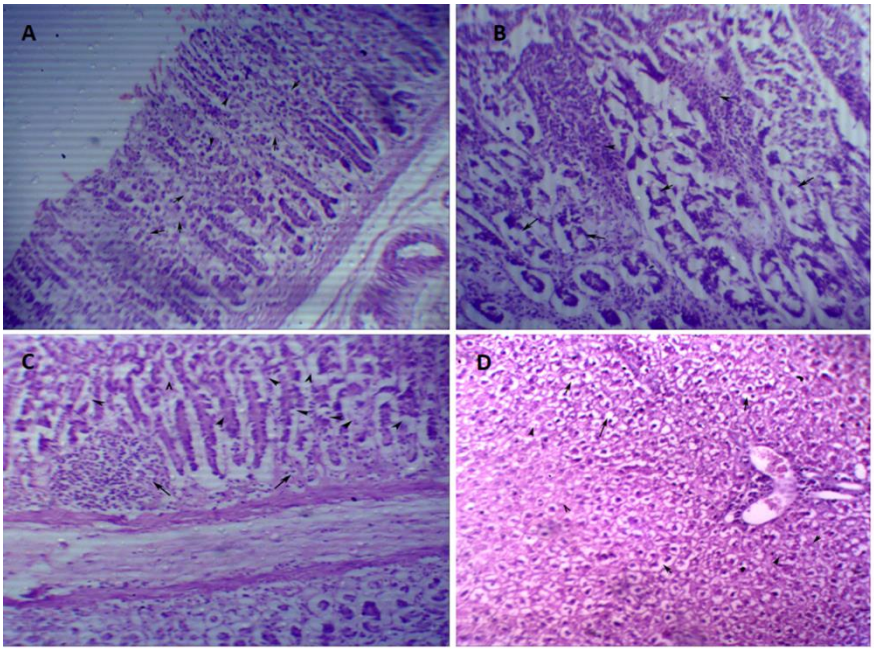

Figure 3. (A–B) Widespread significant lymphocytic gastritis of the entire mucosa of stomach of infected rats by *H. pylori* Ag, represented by severe necrosis of epithelial mucosal cells (arrows) with lymphocytic cell infiltrations (arrowheads), H&E X 200 and X 400 respectively. (C). Gastritis with severe necrosis of mucosa epithelium (arrowheads) which extended to the submucosal layers and aggregations of lymphocytic cells (arrows), H&E X 200. (D). Hydropic degeneration (arrows) with coagulative necrosis (arrowheads)of liver of infected mice by *H. pylori* Ag, H&E X 400.

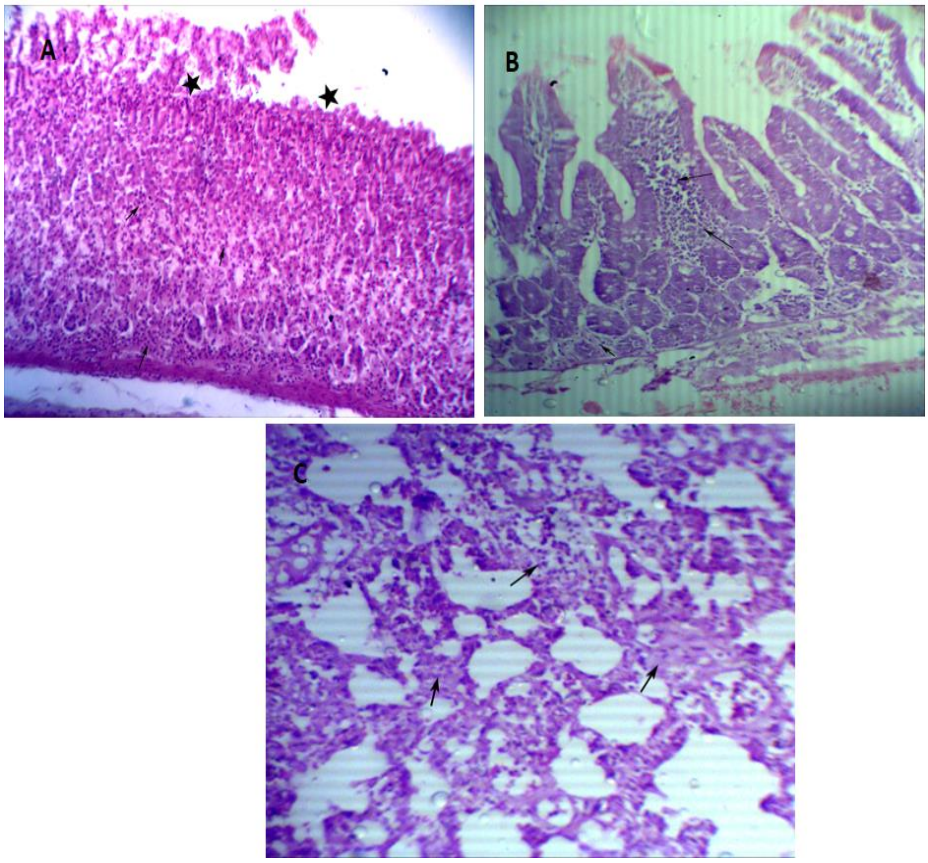

Figure 4. (A) Gastric mucosal cell proliferation with superficial epithelial desquamation (black star) and leukocytic cell infiltrations (arrows), H&E X 100. (B) Enteritis of intestine of infected rat with H. pylori Ag, infiltrated by lymphocytic cells (arrows), H&E X 400. (C) Serolymhocytic pneumonia (arrows) of lung of infected rat by *H. pylori* Ag, H&E X 400.

4. Discussion

H. pylori prevalence varies dramatically across the globe. Even at young ages, about 80% of the population in several underdeveloped nations is *H. pylori* positive. According to available data as of 2018, the prevalence of *H. pylori* in Egypt ranges from 13% to 72% in children and 26% to 90% in adults (Alboraie et al., 2019).

Disease outcome depends on many factors, including bacterial genotype, host physiology and genetics, and environmental factors such as diet (Makola et al., 2007). The most common transmission in the general population is from person to person, either through the oral-oral channel (via vomitus or saliva) or the fecal-oral route. Understanding the path of *H. pylori* transmission is critical for public health interventions to prevent the disease from spreading. Waterborne transmission, most likely due to fecal contamination, might be a significant cause of illness, particularly in areas where untreated water is prevalent (Brown, 2000). In the current study, we investigated what kind of hematological, biochemical and pathological effects on the regular consumption of *H. pylori* Ag-infected water or feed in albino rats for three months.

In this study, induction of *H. pylori* infection was carried out in two groups of rats, group II which drank contaminated water daily (water + *H. pylori* stool positive) and group III, which fed on contaminated feed daily (feed + *H. pylori* stool positive) while ten rats were kept as control named group I consumed normal water and normal feed, for 12 weeks as a total period for the experiment. Herein, the detection of *H. pylori* infection among the experimental groups of rats was estimated via various diagnostic tools comprising stool antigen, serological, and urease tests.

The stool antigen test for *H. pylori* is a useful tool for the primary diagnosis of infection (Shimoyama, 2013) and is more applicable to diagnosing *H. pylori* infection in a mass survey (Shimoyama et al., 2009). Our findings showed that, during the experiment for 12 weeks, the level of *H. pylori* Ag was estimated in the fecal matter of experimentally infected rats' groups weekly. There was a dramatic rise of *H. pylori* Ag level throughout the experiment, reaching a higher positive level (> 100 ng/ml) from the 10^{th} week and 9^{th} week 12^{th} week in groups II and III, respectively.

Serological assays represent an effective diagnostic value because *H. pylori* infection evokes systemic and local antibody responses. The systemic immune response typically shows a transient rise in *H. pylori* IgM antibodies, followed by a rise in IgG and IgA antibodies, that persists during infection (Herbrink and Doorn, 2000). In this study, the serum level of IgM, IgG, and IgA were significantly increased in groups II and III compared with the control group (GI). IgM is non-specific immunoglobulin that is elevated during primary infection. Since IgM antibodies against *H. pylori* are detected only transiently, they have little value for the serological diagnosis of *H. pylori* infection (Herbrink and Doorn, 2000). However, the combined IgG and IgA antibody determinations for serodiagnosis of *H. pylori* infection are very useful (Martín-de-Argila et al., 1997). Significant associations were found between serum anti-*H. pylori* IgG and IgA antibody titers and the development of atrophic gastritis (Yamamoto et al., 1995). Moreover, IgA antibodies showed an effective clinical value in *H. pylori* -related gastritis (Jaskowski et al., 1997) being in harmony with our findings IgA level was superior to IgM and IgG levels in the serum of infected rat groups.

There is a fact that gastric urease allows the organism to colonize the acidic stomach and serves as a biomarker for the presence of *H. pylori* (Graham and Miftahussurur, 2018). Based on our results for the urease test, all the rats in both infected groups (GII and GIII) showed a positive reaction to the urease test, as shown in Table 3. This might be a good indicator of the presence of *H. pylori* in the stomach of infected rats. Similarly, Cătoi et al. (2006) detected positive results for the urease test in all experimental rats, not in the control.

The results revealed a significant decrease in iron and ferritin levels in serum among infected rats' groups (GII and GIII) compared with the control group. Similarly (Maksoud et al., 2016) investigated a significant decline in iron and ferritin in *H. pylori*-positive patients. *H. pylori* may impair iron metabolism. *H. pylori* may directly compete with the host for available iron by impairing its uptake (Ciacci et al., 2004; Qujeq, 2011). Seriously, *H. pylori* infection was found to be associated with iron deficiency/IDA regardless of the presence or absence of peptic ulcer disease (Cardenas et al., 2006; Monzón et al., 2013). In this study, copper and zinc levels showed a significant increase in the serum of infected rats compared with the control uninfected ones. Likely, Ranjbar and Azimzadeh, (2015) detected elevated plasma levels of both Cu and Zn as a biochemical effect of oral administration of *H. pylori* in rats. The serum level was higher among infected children; however, the Cu level declined (Öztürk et al., 2015). On the other hand, Wu et al. (2014) reported that serum zinc and copper levels had no significant difference between before and after *H. pylori* eradication therapies compared with uninfected individuals. Magnesium levels showed a significant decrease among the infected rats. These findings followed that reported by Öztürk et al. (2015). Regarding Metabolic consequences of *H. pylori*, upregulation of the total and low-density lipoprotein-cholesterol (LDL-C) and decrease of high-density lipoprotein (HDL-C), may be associated with infection, creating an atherogenic lipid profile and promoting atherosclerosis (Buzás, 2014). These data were similar to that reported in infected elder Egyptian individuals with H pylori (Maksoud et al., 2016). According to our data, total cholesterol and triglycerides were higher in both infected groups II and III than in the control group. LDL and VLDL levels increased in groups II and III after infection, whereas HDL levels declined.

Evidence shows an association between *H. pylori* infection and liver dysfunction and hepatitis. It was shown that patients' serum levels of liver enzymes (alanine transaminase (ALT) and aspartate transaminase (AST) decreased after receiving an eradication regimen of *H. pylori* (Salehi et al., 2014). Specific *H. pylori* genes were also detected in liver of experimentally infected mice with mild-to-moderate multifocal hepatitis (Huang et al., 2009). Herein, serum levels of liver enzymes ALT and AST were significantly higher in both infected groups II and III than in the control. The overall tested biochemical parameters infected group III showed a significantly more variation in either decline or elevation than group II, indicating that the effect of H pylori administered in feed might be more prominent.

Parsonnet and Haggerty (1999) have detected the fecal shedding of *H. pylori* in the stool of healthy *H. pylori*-infected patients. All gastric swabs from rats in groups II and III tested positive for urease, indicating that *H. pylori* suspension from a patient's stool can cause infection in rats when given orally.

Gastritis, peptic ulcers, and gastric cancer are all caused by *H. pylori*, which lives in the mucus layer over the gastric epithelium (Ernst and Gold, 2000). Studies have shown that experimental animals inoculated with *H. pylori* developed mild to moderate or severe gastric inflammation (Huang et al., 2009; Werawatganon, 2014). Similarly, in this experiment, rats in groups GII and GIII developed moderate to severe gastric mucosal inflammation; some had gastric ulcers. In the examined liver sections among the infected groups, hydropic degenerations in parenchymal, and periportal areas and coagulative necrosis of hepatocytes with inflammatory cell infiltration were seen. They were suggesting that *H. pylori* organisms could reach the hepatobiliary system and cause inflammation as an independent etiological factor, as previously reported in mice (Huang et al., 2009).

5. Conclusion

In conclusion, exposure to *H. pylori* contaminated food and water rats; many hematological parameters were disturbed. By modifying distinct biochemical profiles, *H. pylori* significantly impacted the different organ function assessments. These changes may, in part, reflect the severity of inflammation of the gastric mucosa. Additionally, *H. pylori* have a minimal impact on rats' liver and lungs and are linked to enteritis.

Conflict of Interest

The author(s) confirm that this article's content has no conflict of interest.

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