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THE EFFECT OF DRUGS USED IN ANTI - HELICOBACTER THERAPY ON THE LIVER AND GUT MICROBIOME - AN EXPERIMENTAL STUDY

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Abstract. Study objective: To investigate the adverse effects of antibacterial drugs used for *Helicobacter pylori* (HP) eradication and included in anti-Helicobacter therapy regimens. The use of various antibacterial drugs led to adverse effects on the hepatobiliary system (manifesting as toxic hepatosis) and the gastrointestinal tract (manifesting as disturbances in the large intestine microbiome) in experimental rats receiving standard anti-Helicobacter therapy. **Materials and methods:** Experiments were conducted on 20 Wistar rats (males, 4–5 months old, mean body weight 220 g) divided into two groups: Group 1 (control/normal)—intact animals receiving a standard vivarium diet (complete balanced feed); and Group 2 (experimental)—animals receiving a complete diet plus a daily oral mixture of the following drugs for 8 days: *omeprazole* (Farmak, Kyiv) at 1.3 mg/kg, *amoxyl* (Kyivmedpreparat, Kyiv) at 50 mg/kg, and *clarithromycin* (Kyivmedpreparat) at 7.5 mg/kg. Euthanasia was performed on the 12th day of the study under thiopental anesthesia (20 mg/kg) via total cardiac exsanguination.

Research results: The leukocyte count and the percentages of neutrophils and lymphocytes were determined in the blood of the experimental animals; additionally, immunodeficiency indices were calculated based on the lymphocyte – to – neutrophil ratio. In the serum of the experimental rats, the following were measured: levels of "liver" markers

(alanineaminotransferase and alkaline phosphatase activity); levels of inflammation markers (malon dialdehyde content and elastase activity); and the activity of two protective enzymes—lysozyme (an indicator of nonspecific immunity) and catalase (an antioxidant enzyme). In liver homogenate (50 mg/mLin 0.05 M Tris – HCl buffer), the content of malon dialdehyde and the activity of the following enzymes were determined: elastase, alkalinephosphatase, catalase, lysozyme, and urease (an indicator of microbial load). The antioxidant – prooxidant index was calculated based on the ratio of catalase activity to malondialdehyde content, while the degree of dysbiosis was determined according to Levytskyi's method using the ratio of the relative activities of urease and lysozyme. Statistical analysis of the results was performed according to standard recommendations; data are presented as the mean value (M) and the standard error of the mean (±m). Differences were considered statistically significant at $p < 0.05$. **Conclusions:** The use of drugs included in the anti-Helicobacter therapy regimen in an experiment with rats significantly reduced serum lysozyme activity in the test animals, leading to intestinal dysbiosis and a substantial decline in non - specific immunity; concurrently, there was a significant increase in the activity of "liver" markers—alanineaminotransferase and alkaline phosphatase—indicating the development of liver damage in the form of hepatitis and cholestasis.

Key words: anti – Helicobacter therapy; hepatitis; cholestasis; dysbiosis; alanineaminotransferase; alkaline phosphatase; malon dialdehyde content.

Introduction: Comprehensive anti – Helicobacter therapy (AHT)—refined in 2015 at the Maastricht V/Florence Consensus—is currently the standard treatment for chronic gastritis and gastric ulcer disease associated with HP [1 – 4].

The AHT regimen involves the use of a proton pump inhibitor (PPI) (e. g., *omeprazole, gasek, omez, pantoprazole*) to suppress gastric acid secretion [5, 6], along side two or three antimicrobial agents (*clarithromycin, amoxicillin, metronidazole, tetracycline)* and gastroprotective agents (colloidal bismuth

subcitrate).

Adverse effects of AHT include a significant reduction in gastric acidity and the bactericidal activity of gastric juice; this leads to dysbiosis caused by the suppression of normal (probiotic) microflora by the antimicrobial drugs. Consequently, there is an increased release of toxins into the gastrointestinal tract, which primarily reach the liver, where detoxification occurs.

Furthermore, the drugs used in AHT possess hepatotoxic properties. While this is sue warrants investigation, studying the pathogenetic impact of AHT on liver damage is feasible only in experimental settings. Therefore, the aim of our study was to assess the functional state of the liver in experimental rats receiving standard AHT.

Materials and Methods: Experiments were conducted on 20 Wistar rats (males, 4–5 months old, mean body weight 220 g) divided into two groups: Group I (control/normal)—intact animals receiving a standard vivarium diet (complete balanced feed); and Group II (experimental)—animals receiving a complete diet plus a mixture of drugs used in AHT administered *per os* daily for 8 days: *omeprazole* (Farmak, Kyiv) at 1.3 mg/kg, *amoxyl* (Kyivmedpreparat, Kyiv) at 50 mg/kg, and *clarithromycin* (Kyivmedpreparat) at 7.5 mg/kg.

The animals were euthanized on the 12^{th} day the study under thiopental anesthesia (20 mg/kg) via total cardiac exsanguination.

Results

Table 1 presents the blood analysis results for the experimental animals treated with AHT, specifically the leukocyte count and the percentages of neutrophils and lymphocytes. These data indicate that the use of the drugs mentioned reduces the percentage of neutrophils (which determine the degree of the inflammatory response) by half and increases the percentage of lymphocytes (which likely reflects the degree of the specific immune response) by 1.5 times. As a result, the lymphocyte – to – neutrophil ratio increases more than three fold, which may indicate an elevated immune response to the drugs included in the AHT regimen; this, in turn, may be attributable to the side effects of the drugs, particularly regarding the liver.

Table 1

Effect of drugs included in the AHT regimen on leukocyte formula parameters in experimental animals (M ± m, n = 10)

Parameters	**AHT**	Control (norma)
Leukocytes, g/L	13.5±0.3 (p>0.05)	12.9±0.2
Neutrophils, %	20.9±0.9 (p<0.001)	43.1±0.8
Lymphocytes, %	66.1±2.7 (p<0.01)	45.3±1.5
Lymphocytes/Neutrophils	3.15±0.09 (p<0.01)	1.08±0.05

Note: p – compared to the control group.

To test our hypothesis, we conducted a biochemical analysis of liver - related blood

parameters in the experimental animals; the results are presented in Table 2. As the data show, rats receiving the drugs included in the AHT regimen exhibited a significant increase in the activity of the enzymes alanine aminotransferase (ALT) and alkaline phosphatase (ALP), indicating liver dysfunction in the form of hepatosis and cholestasis. Concurrently, there was a significant rise in levels of elastase and malon dialdehyde (MDA)—which can be considered markers of inflammation — along with an 18.5% decrease in lysozyme activity, an indicator of nonspecific immunity. These parameters suggest the presence of liver dysfunction. The latter was subsequently verified through experiments measuring liver tissue parameters.

Table 2

Effect of drugs included in the AHT regimen on biochemical parameters of rat blood serum (M ± m, n = 10)

Parameters	**AHT**	Control (normal)
ALP, μkat/L	1.46±0.11 $p<0.05$	0.93±0.08
ALT, μkat/L	0.58±0.03 $p<0.05$	0.39±0.03
Elastase, μkat/L	176.9±5.5 $p<0.05$	142.1±8.6
Lysozyme, U/L	106±3.8 $p<0.05$	133±7
MDA, mmol/L	0.68±0.02 $p<0.05$	0.55±0.03
Catalase, μkat/L	0.24±0.02 $p>0.05$	0.26±0.02

Note: p – compared to the control group.

Table 3 presents the results of biochemical analyses of experimental animals liver tissue after the use of drugs included in the AHT. These data indicate that AHT increases the levels of inflammatory markers in the liver: MDA by 63.7% and elastase by 23%. Signs of liver dysfunction are evident, most likely due to the development of inflammation. There is also a statistically significant increase — by 26.9%—in the activity of the enzyme ALP (an indicator of cholestasis development). In other words, inflammation may be accompanied by liver tissue edema, leading to increased pressure in the bile ducts and bile stasis. Urease activity, an indicator reflecting the degree of microbial colonization, increases by 52.6% following AHT, where as lysozyme activity decreases by 60.4%. Thus, increased microbial colonization of the liver impairs its immune defense capabilities.

Table 3

Effect of AHT on liver biochemical parameters in experimental rats (M ± m, n=10)

Parameters	AHT	Control (normal)
Elastase, μkat/kg	0.31±0.02 p<0.05	0.25±0.01
MDA, mmol/kg	33.9±3.1 p<0.05	20.7±3.0
Urease, μkat/kg	0.32±0.02 p<0.01	0.21±0.02
ALP, μkat/kg	6.56±0.50 p<0.05	5.16±0.42
Lysozyme, U/kg	41±8 p<0.01	104±12
Catalase, μkat/kg	6.26±0.06 p>0.3	6.33±0.08

Note: p – compared to the control group

It is important to note that the increase in MDA levels did not cause a decrease in liver antioxidant activity, indicating a primary activation of lipid peroxidation that remains uncompensated. As a consequence of this damage, the degree of dysbiosis increases 3.9 - fold in the liver (Fig. 1) and 8 - fold in blood serum (Fig. 2) following AHT; this indicates that the rising level of microorganisms in the blood and liver causes a decline in non specific immune defense, there by closing the vicious cycle associated with AHT.

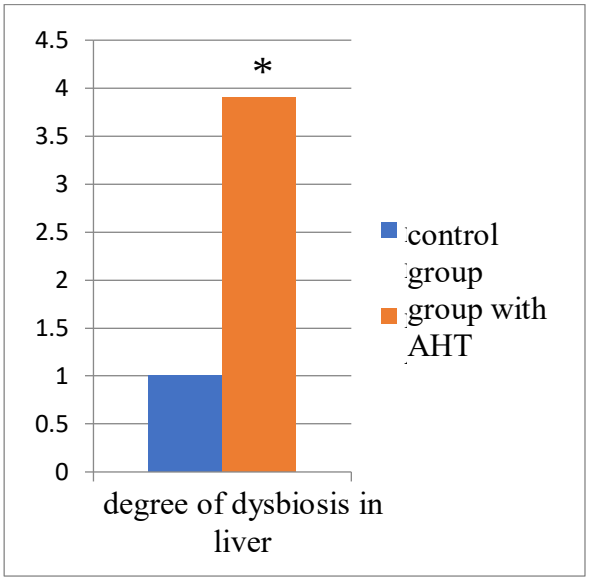

Fig. 1. Effect of drugs included in the AHT on the degree of dysbiosis in the liver of experimental animals

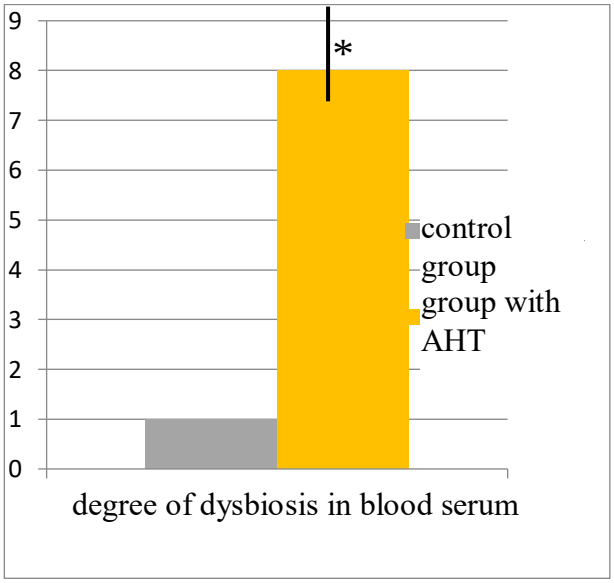

Fig. 2. Effect of drugs included in the AHT on the degree of dysbiosis in the blood serum of experimental animals

Note. * – statistically significant changes relative to the control group, p<0.05.

Given that the antioxidant – prooxidant index (API)—which reflects the balance between antioxidant and prooxidant systems in the body — decreased by 39.3% in the liver (Fig. 3) and by 23.2% in the blood serum (Fig. 4), this indicates the presence of oxidative stress that is not compensated for by adequate antioxidant defense. Oxidative stress itself may be one of the primary biochemical mechanisms underlying liver damage.

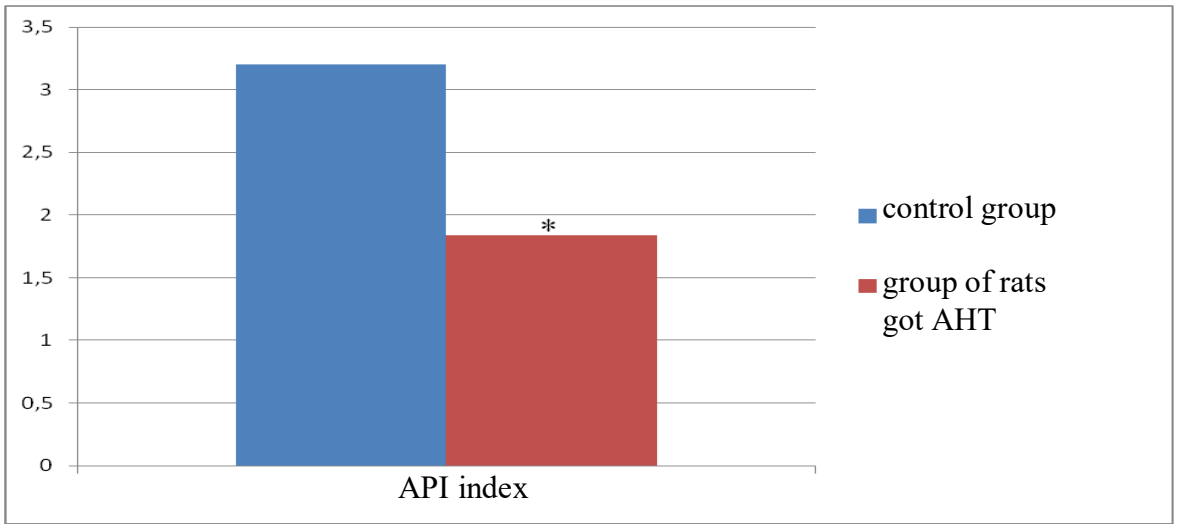

Fig. 3. Effect of drugs included in the AHT on the API in the liver of experimental animals

Note. * – statistically significant changes relative to the control group, $p<0.05$.

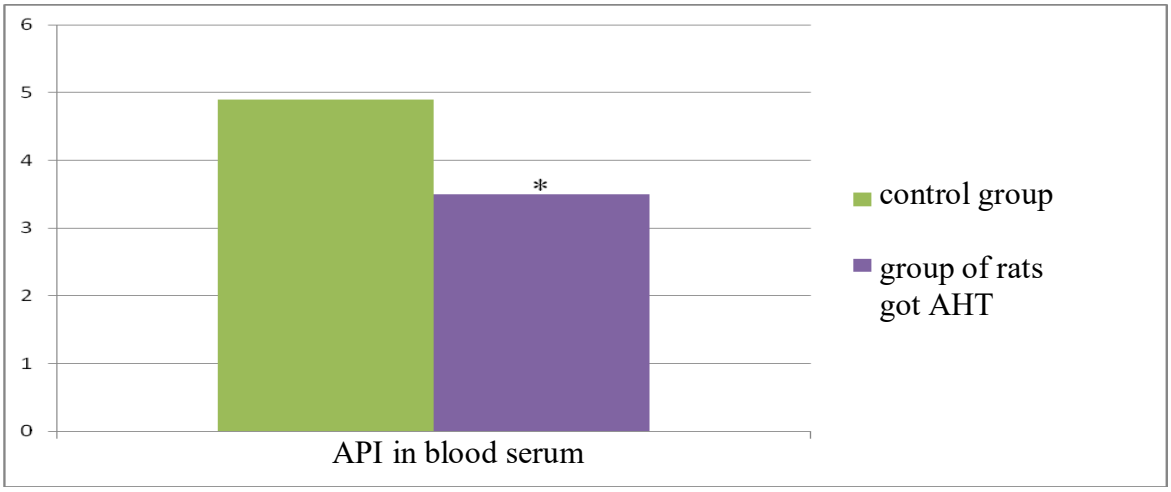

Fig. 4. Effect of drugs included in the AHT on the API index in the blood serum of experimental animals

Note: * — statistically significant changes relative to the control group, $p<0.05$.

Thus, the administration of drugs included in the complex AHT can directly induce liver damage in the experiment animals. This occurs, on the one hand, through intestinal

dysbiosis — which fosters liver inflammation leading to pathology — hepatitis and cholestasis—and, on the other hand, through systemic inflammation in the experimental animals, as evidenced by changes in the blood serum. It can be assumed with a high degree of probability that a significant decrease in nonspecific immunity — indicated by lysozyme activity—may be one of the causes of intestinal dysbiosis.

The status of specific immunity—indicated by lymphocyte count—changes following AHT; this may reflect the presence of an autoimmune component that must be considered when prescribing immunomodulatory drugs to patients with gastritis and gastric ulcers and who have undergone AHT. In other words, such an immune response is more likely indicative of the emergence of autoimmune reactions in response to primary liver injury.

Consequently, the presence of dysbiotic manifestations in patients treated with the AHT regimen necessitates anti – dysbiosis therapy using probiotics and prebiotics, as well as nonspecific immunity stimulators [9]; these agents, in combination with hepatoprotectors, can help counteract the adverse effects of anti-Helicobacter therapy.

Conclusions. The use of drugs included in the anti-Helicobacter therapy regimen under the conditions of an experiment significantly reduced serum lysozyme activity in the animals, led to the development of intestinal dysbiosis and a substantial decline in nonspecific immunity. This occurred alongside a significant increase in the activity of "liver" markers—alanine aminotransferase and alkaline phosphatase—indicating the development of liver damage in the form of hepatitis and cholestasis.

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