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ASSOCIATION OF AZITHROMYCIN'S BOUNDLESS EXPOSURE DURING COVID ERA WITH DYSMOTILITY OF SMALL INTESTINE AND DEPLETION OF TOTAL BACTERIAL COLONY OF GUT

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ABSTRACT

Background: Azithromycin, a drug of macrolide groups, gives clinical benefits to the several pathogenic bacterial infected patients. Beyond a lot of good clinical insights, it possesses some catastrophic impact on human health. Specially, during the period of COVID this drug was used in a boundless manner which might have some impact on gut flora. **Aim:** Thus, our study is aimed to find out those severity after the exposure of azithromycin. **Methodology**: Ten, eight weeks old male albino rats were obtained having 150-160g body weight. They were grouped into two (n=5), viz. control and treated. Treated group received Azithromycin at a dose of 33mg/kg/day for 14 consecutive days. Then fecal parameters, gut motility and total bacterial colony count were measured from both groups, followed by statistical analysis of the previously mentioned parameters. **Results and conclusion:** Decreased fecal frequency and increased fecal water content were found after the Azithromycin

exposure. The irregularity of gut motility and increased transit time were seen in the treated rats. Decreased total bacterial colony count was also found in both fecal and small intestinal tissue samples after the treatment. Impairment of gastrointestinal system and related gut microbial dysbiosis after azithromycin exposure can be concluded after this entire study.

Keywords: Gastrointestinal system; gut bacteria, gut motility; Gastrointestinal system; GI transit; colonic transit

INTRODUCTION

Gut commensal microbial composition plays an important role in human health. Disruption of this composition might induce several diseases as well as increases some pathogens in gut. Medications have a great role in opposing the foreign pathogen that enters the body but it might also be linked with downplay of commensal microbiome of gut. Previous study conveys that after administration of different medicines may lead to decreased gut microbiota as well as increased pathogenic bacteria in its place. A short-term course of clindamycin (7 days) resulted in significant disturbances in the bacterial community such as a sharp decline in *Bacteroides* that remained for up to 2 years post-treatment and was accompanied by increased levels of ARGs and its strains **[1-3]**. The composition, function and metabolic activity of the gut microbiota are interlinked with diet, lifestyles, environmental factors, physical stressors, and drug abuse **[4]**. In this field, it is emphasized that antibiotic drugs are no longer considered only beneficial, but also potentiate harmful and undesirable impairments in gut microbiota and intestinal microbial diversity **[5]**.

Azithromycin (AZT), a drug of macrolide group is mainly used in India to treat a variety of illnesses, such as enteric fever, bacterial dysentery, and acute respiratory tract infections **[6]**. The mode of action of this drug is compiled by binding of 50S ribosomal subunits in bacterial cells and the process of bacterial peptide transfer gets defected as a result protein synthesis is inhibited, thus, it achieves the antibacterial effects **[7]**. A study demonstrated that administration of azithromycin to allergic asthmatic patients could alter gut microbial composition and showed that Lactobacillus disappeared in the control group after azithromycin administration **[8]**.

In recent years of pandemic, the clinical trial manifested the use of AZT along with Hydroxychloroquine might be related with the treatment of corona virus though no significant results or mechanisms has been established yet. But it has been used in a very high dose as well as for the long period of time. A meta-analysis study on randomized controlled trial has found that neither

hydroxychloroquine nor azithromycin are associated with the decreasing COVID related mortality **[9]**.

The unnecessary use of this drug might have the effect on our gastrointestinal system and its microbial composition and study is required to find out the occurring side effects of the enormous use of this drug. Thus, our study is aimed to investigate the impact of the huge use of this drug on gut bacteria by evaluating several faecal parameters and intestinal contractility.

MATERIALS AND METHODS

2.1) Ethics statement

All animal experiments are done after the approval of animals by IAEC of Raja N.L Khan Women's College (Autonomous), Midnapore, West Bengal, India. Experiments were done in accordance to CPCSEA guidelines. Ethical approval no :04/IAEC(1)/S/RNLKWC/2023 of Raja N.L Khan Women's College (Autonomous) Midnapore.

2.2) Animal Treatment and housing

Ten male eight weeks old albino rats weighing about 150-160 g were obtained and they were acclimatized for seven days. Animals were grouped into two (n=5 animals each), viz. control and treated. The first group, considered as control, got food and water and the treated or test groups received Azithromycin including food and water. Animals were housed in plastic cages under the facility with a regular day–night cycle at room temperature.

2.3) Dose selection

Azithromycin dihydrate was purchased from Sigma Aldrich, and it was given 33mg/kg/day orally through gavage for fourteen consecutive days. The dose was adjusted by the body weight of each rat day by day. This dose has been prepared according to the doses used in COVID 19 for human **[10]** and then it was converted human to rat doses as per FDA dose conversion guidelines **[11]** by considering its lethal dose >2000 mg/kg body weight of rat **[7]**.

2.4) Faecal parameter measurement

2.4.1 Faecal pellet frequency

After the completion of dose tenure all rats were observed for 8 h, and the number of pellets was counted every 2 h.

2.4.2 Faecal water content

Faecal water content percentage was measured by comparing the wet weight of the pellets with dry weight after drying 24 h at 60 °C **[12]**.

2.5) Gastrointestinal & Colonic transit

After fasting overnight with free access to water, rats were administrated by gavage with a semiliquid solution (0.1 ml) containing Evans blue and methylcellulose **[13]**. Then time was recorded at the presence of the first blue pellet and colonic transit of rats was measured with a bead expulsion test **[14]**.

2.6) Smooth muscle contractility and amplitude measurement

After sacrificing the rats by cervical dislocation, duodenum, ileum, and jejunum portions of the intestine of rats for both control and treated groups were excised. Smooth muscle contractility test was done by the Dale's apparatus **[15]**. Kymographic recording were taken from previously mentioned segments of each animal. Ten consecutive amplitudes were calculated from each curve of both control and treated groups by considering a fictional baseline at the end.

2.7) Total count of anaerobic bacteria from faecal and segments of small intestine by plate culture technique

Fecal solution was prepared at a dilution factor 10 with autoclaved distilled water, then dilution was done up to 10^{-7}. The first prepared solution was considered as 10^{0}. Then nutrient agar media was prepared and microbes were cultured from different dilution at anaerobic condition by using anaerobic chamber and total colony count was measured. Same way, the tissue homogenates were prepared from duodenal, jejunal and ileal segments, then microbes were counted.

2.8) Total count of aerobic/facultative anaerobic from faecal and segments of small intestine by plate culture technique

From the previously prepared fecal solution and tissue homogenates microbes were cultured in nutrient agar media from different dilution at aerobic condition and total colony count was measured. Same way, the tissue homogenates were prepared from duodenal, jejunal and ileal segments, then microbes were counted.

2.9) Statistical analysis

Data were measured and expressed as mean ± SD in each bar diagram. Statistical analysis was done by using two tail t-test method and the significant difference was calculated by considering $p<0.05$ as significant.

3. RESULTS AND DISCUSSION

We have studied the effect of AZT treatment on intestinal movements and microbial composition by analyzing the following parameters

3.1 Evaluation of faecal parameters

After analyzing the fecal pellet count, we found control rats had fecal pellet count of 21 ± 2.45 per 8 hours in contrast AZT-treated rats had significantly lower pellet count of 10.4±1.49 per 8 hours. Fecal water content was found significantly higher in treated rats than the control rats. Fecal water content percentage was 34.23± 2.78 in control rats and treated rats had higher fecal water content percentage i.e.,74.81± 2.84 (**Figure 3.1.1, Figure 3.1.2**).

3.2 Evaluation of transit time (minutes)

After evaluating the transit time, colonic transit time (mins) (85.2±2.31 vs 154.8 ±2.63) was found significantly higher in the AZT-treated rats than the control and similarly GI transit time (mins) was found significantly higher i.e. 325±8.98 in the treated rats than that in control group i.e. 194.2±3.6 (**Figure 3.2.1**), (**Figure 3.2.2**).

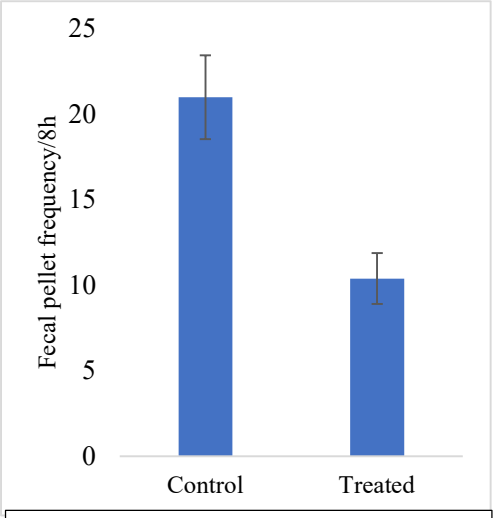

Figure 3.1.1: Bar diagram represents the difference in fecal pellet count/8h between Control and AZT-treated rats p<0.05

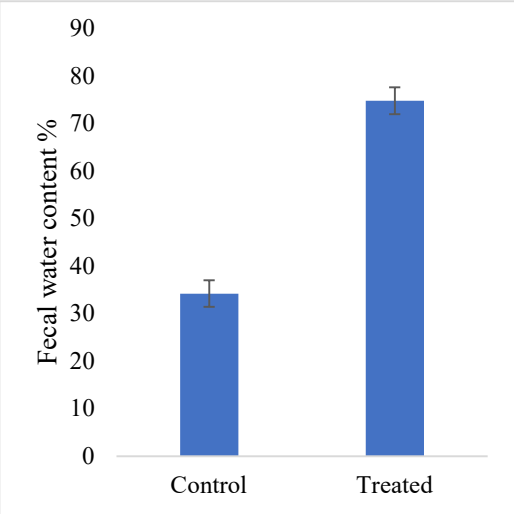

Figure 3.1.2: Bar diagram represents the difference in fecal water content % between Control and AZT-treated rats p<0.05

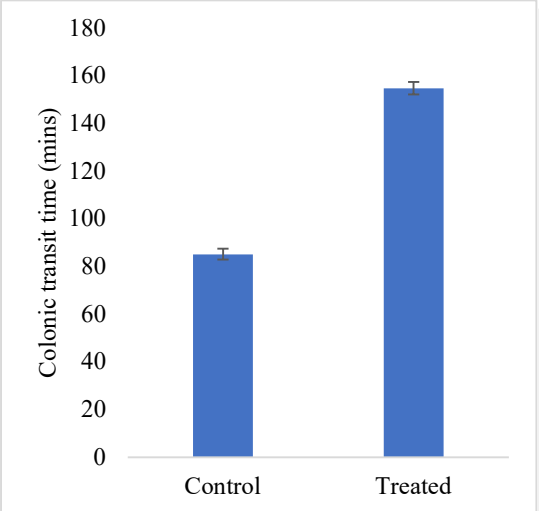

Figure 3.2.1: Bar diagram represents the difference in colonic transit time (mins) between Control and AZT-treated rats p<0.05

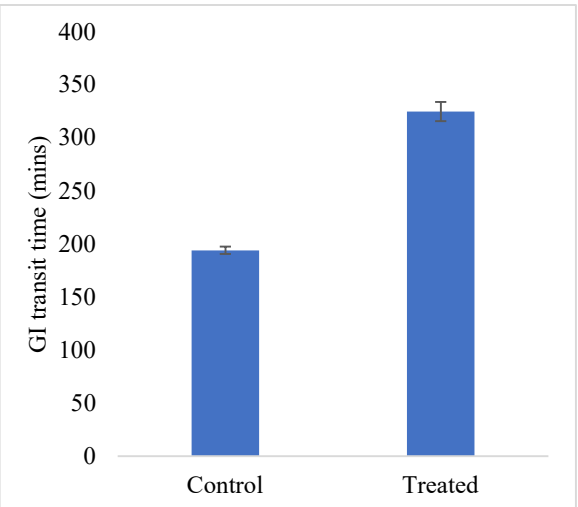

Figure 3.2.2: Bar diagram represents the difference in GI transit time (mins) between Control and AZT-treated rats p<0.05

3.3 Analysis of Smooth muscle contractility

In-vitro smooth muscle contractility was measured from the three segments i.e. duodenum, jejunum, ileum of small intestine. The amplitudes were found significantly lower in all segments of the small intestine in case of treated rats in contrast to that of the control rats. As compared to the control group, treated rats have abnormal duodenal and jejunal movements, sometimes the curves touched the baseline when the movements get stopped or becomes irregular. Furthermore, ileal segments of treated rats didn't show any irregular movements **(Figure 3.3.1)**.

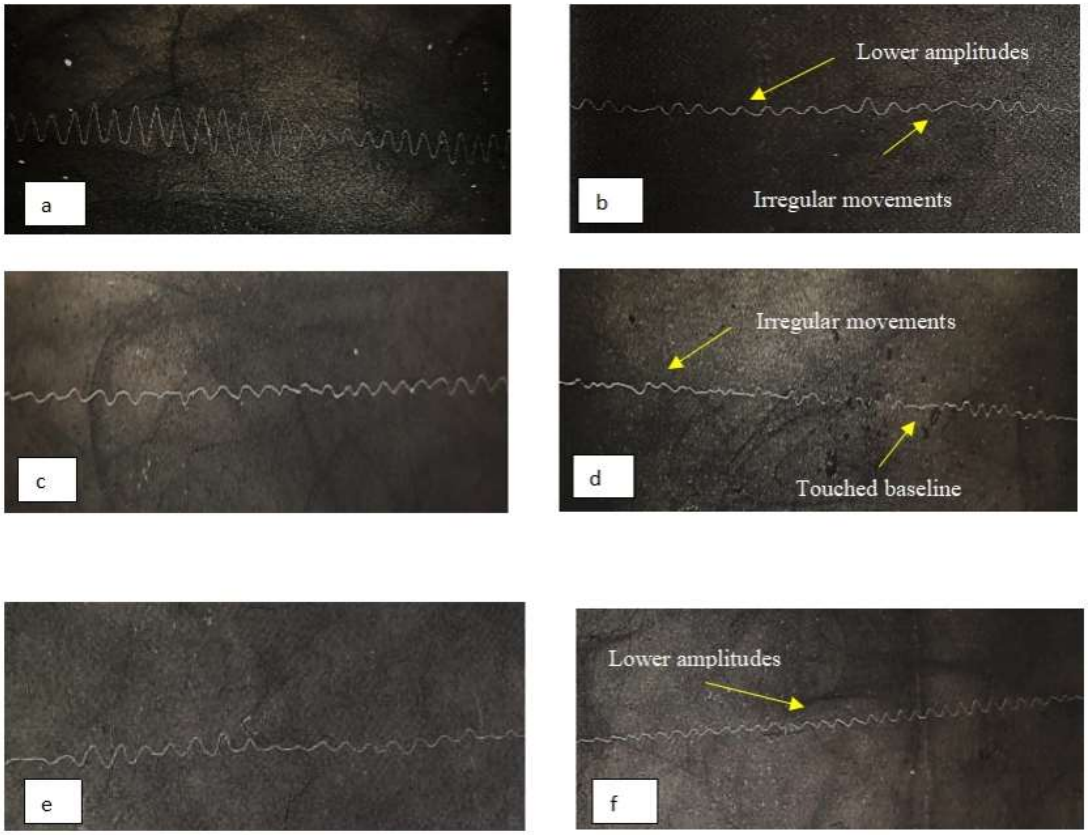

Figure 3.3.1: Kymographic recordings of duodenal movements of control (a) and AZT-treated (b) groups at a drum speed 2.5mm/sec with the calculated amplitude of 0.57±0.06 cm for control and 0.14± 0.04 cm for treated,jejunal movements of control (c) and AZT-treated (d) groups at a drum speed 2.5mm/sec with the calculated amplitude of 0.21±0.03 cm for control and 0.15 ± 0.05 cm for treated,ileal movements of control (e) and AZT-treated (f) groups at a drum speed 2.5mm/sec with the calculated amplitude of 0.26±0.05 cm for control and 0.16± 0.06 cm for treated.

3.4 Analysis of total anaerobic bacterial count

Aerobic faecal bacterial total count was significantly lower after the AZT exposure i.e.,9.58±0.45 log CFU/g than that in control i.e. 6.46±0.46 log CFU/g of faeces. Small intestinal segment also showed the significantly decreased colony count after AZT exposure, duodenum of control rats possessed bacteria 5.54±0.42 log CFU/g of

tissue in contrast treated rats had lower bacterial colony count i.e.,4.29±0.27. Jejunum and ileum also had lower bacterial count i.e.,7.36±0.47 (control) vs 5.71±0.33 (treated) log CFU/g of tissue and 9.44±0.47 (control) vs 5.39±0.38 (treated) log CFU/g of tissue respectively (**Figure 3.4.1**) (**Figure 3.4.2**).

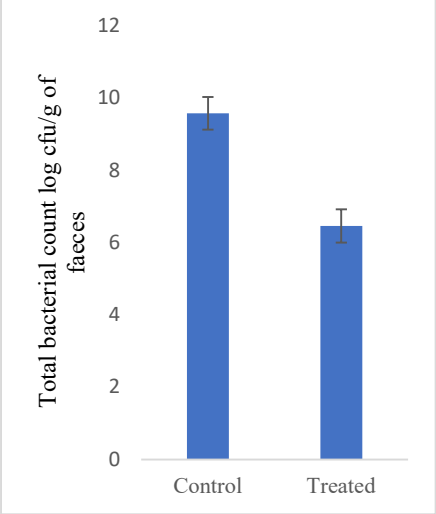

Figure 3.4.1: Bar diagram represents the difference in fecal total anaerobic bacterial count between Control and AZT-treated rats p<0.05

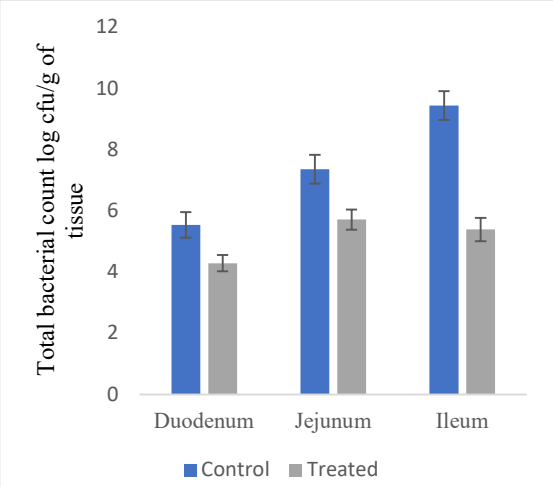

Figure 3.4.2: Bar diagram represents the difference in total anaerobic bacterial count of small intestinal segments in between Control and AZT-treated rats p<0.05

3.5 Analysis of total aerobic bacterial count

After the statistical analysis of total aerobic bacterial count, it was found significantly lower in case of treated rats than that of control rats (**Figure 3.5.1**) (**Figure 3.5.2**).

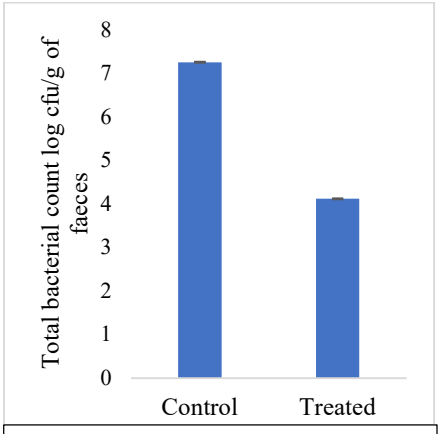

Figure 3.5.1. Bar diagram represents the difference in fecal total aerobic bacterial count between Control and AZT-treated rats p<0.05

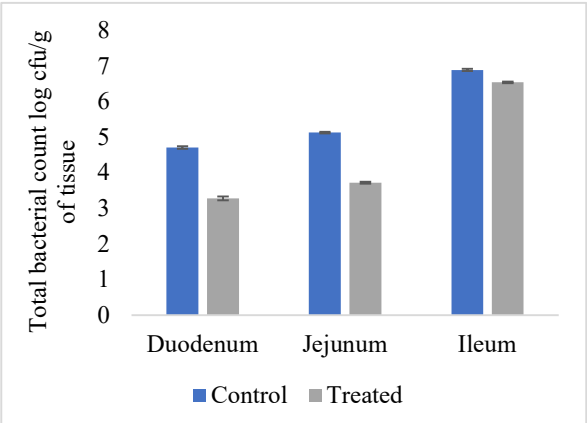

Figure 3.5.2 Bar diagram represents the difference in total aerobic bacterial count of small intestinal segments in between Control and AZT-treated rats p<0.05

Aerobic faecal bacterial total count was significantly lower after the AZT exposure i.e.,4.12±0.010 log CFU/g than that in control i.e. 7.26±0.014 log CFU/g of faeces.In the three segments of small intestines duodenum, jejunum, ileum we found significant decrease in total colony count of bacteria in the treated rats. Duodenal bacterial count was 4.71±0.036 log CFU in control and in contrast AZT-treated rats had lower total bacterial count i.e.,3.28 ±0.058 log CFU. Control and treated jejunal bacterial count (5.13±0.020 vs 3.72 ±0.026) and ileal bacterial count (6.89±0.029 vs 6.54 ± 0.022) showed the similar result (**Figure 3.5.3**).

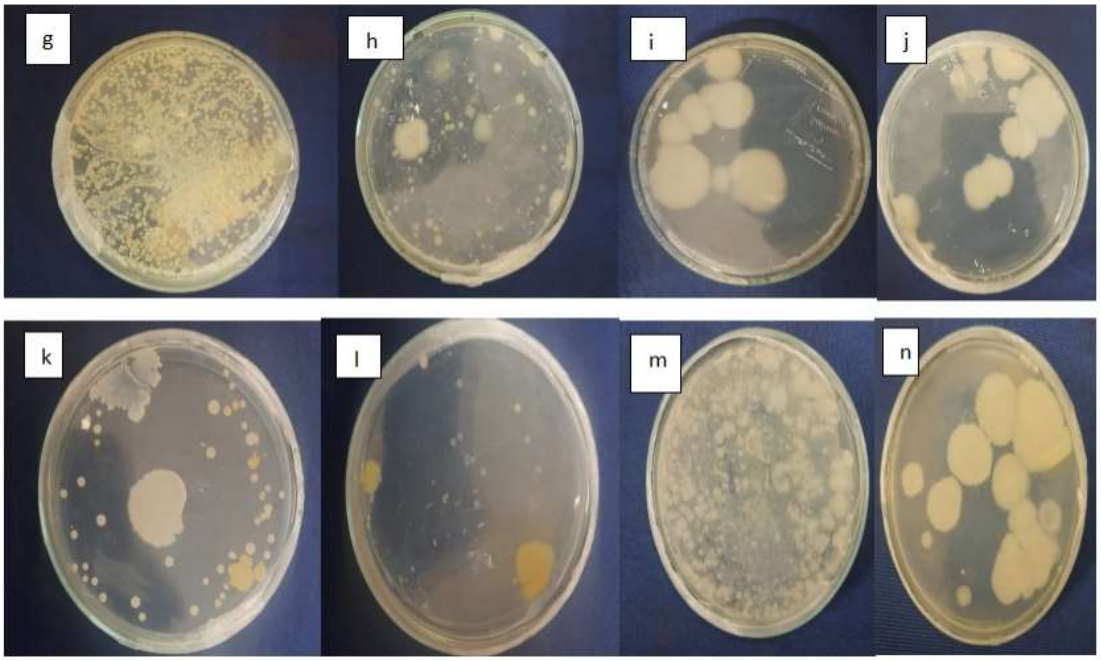

Figure 3.5.3: Photographs of some of the cultured aerobic bacterial plates of fecal and tissue samples at different dilution level (g) Control fecal bacteria at 10^-3 dilution (h) Treated fecal bacteria at 10^0 dilution (i) Control duodenal bacteria in 10^-1 dilution (j) Treated duodenal bacteria in 10^0 dilution (k) Control jejunal bacteria in 10^-1 dilutions (l) Treated jejunal bacteria in 10^0 dilutions (m) Control ileal bacteria in 10^-3 dilution (n) Treated ileal bacteria in 10^-3 dilution, showing the decreased total bacterial colony in case of treated rats

Damages occur due to uncontrolled use of medicines, Xiaolong *et.al.*, 2017 in their study found that due to the exposure of several well-known antibiotics viz. ampicillin, neomycin, and vancomycin, when administered in animals they had suffered with decreased gut motility and as a result GI transit time and colonic transit time were increased. Their study also found that gut motility is associated with the microbial composition of the gut. Exposure of antibiotics, depleted the gut microbes and gut motility decreased for which decreased water absorption might have occurred in the gut, that caused higher fecal water content **[13]**. Our study on AZT exposure also

indicates the same, fecal pellet frequency is significantly lowered in the case of treated rats but the water content percentage is much higher in feces in the treated rats which signifies the lower absorption of water in gut.GI transit time and colonic transit time were significantly higher in the AZT-treated rats which denotes the slower movement of the gut in treated rats than the control. We have seen that irregular movement occurs in the treated rats' duodenum and jejunum portions. Kymographic recording with low amplitudes showed the decreased gut motility in the treated rats. Lower amplitude and inhibition of transit time might be associated with the overgrowth of *E. coli* according to a study, done by Wan-Chun Wu *et al.,* 2007, on non-alcoholic steatohepatitis (NASH) rats **[16]**. Thus, it can be said that all strains of *E. coli* are not beneficial, some might have related with severe pathogenesis. Delungahawatta T *et al.,*2017, in their study showed that decreased gut motility due to antibiotic exposure might be linked with modulation of enteric nervous system **[17]**. Decreased gut motility might be associated with increasing pathogenic bacteria, Monica vera *et.al*., 2015 found that due to antibiotics exposure gut microbiota demodulates and some pathogenic bacteria like *C. botulinum, C. perfringes*, etc. can grow [18]. In this study, we found significantly decreased total aerobic and anaerobic bacterial colony count in fecal and small intestinal segments in the AZT-treated rats than that of control. Previous study on a renowned journal, The Lancet, showed the same that Azithromycin treatment induced a perturbation in the gut microbiota on 14 days exposure to the children **[19]**.16srRNA analysis is required to find out which taxa is affected mainly due to AZT exposure, along with this further study is needed on some protective effect to treat this microbial dysbiosis due to AZT exposure.

CONCLUSION

Hence, from our study, we can conclude that AZT exposure might lead to a complete impairment of the gastrointestinal system. This might be associated with the abnormalities in the small intestinal motility which might be related to the changes of gut bacterial composition.

Acknowledgement

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Conflict of interest

The authors declare that there are no conflicts of interest

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Ethics approval

Declaration: Above study was done after the approval of institutional animal ethical committee (IAEC) of Raja N.L Khan Women's College (Autonomous) Midnapore and accordance with the national guidelines i.e., The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical approval no :04/IAEC(1)/S/RNLKWC/2023 of Raja N.L Khan Women's College (Autonomous) Midnapore.

Abbreviations

AZT: Azithromycin

GI: Gastro-intestinal

CFU: colony forming unit

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