ORIGINAL ARTICLE

Zebrafish (*Danio Rerio*) Histopathology to Determine the Toxicity of Triple Antibiotic Paste, Nano-Silica, and Nano-Silica Triple Antibiotic Paste

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Abstract

Introduction: This paper is an exploratory research on the histopathological impact of various nanoformulations on zebrafish tissues, in comparison with the therapeutic potential of triple antibiotic paste (TAP), nano-silica (NS), and nano-silica triple antibiotic paste (NSTAP). Although TAP and NS formulations have been demonstrated to be effective in antimicrobial applications, they have some tissue compatibility limitations. This study examines the issue of NSTAP, which is a combination of NS and antibiotics, as a more balanced and biocompatible treatment option with fewer side effects. **Materials and Methods:** The adult male and female zebrafish were separated into three treatment groups: Control, TAP, NS, and NSTAP, where each treatment was evaluated at Day 1 and Day 7 after treatment. Gill, liver, intestine, and kidney tissues were removed and stained using hematoxylin and eosin to view the morphological changes. The histopathological analysis was on structural integrity, cellular degeneration, inflammatory reaction, and evidence of tissue adaptation or toughness in the various treatments. Qualitative determination of the findings was done according to the presence of cellular atrophy, vacuolization, inflammatory infiltrate, and tissue-specific adaptations. **Results:** Both the gills and kidneys showed signs of high cellular stress in the TAP-treated group, which was accompanied by distinct lamellar atrophy, hepatocyte vacuolization, and degeneration of glomeruli. Tissue stress was exhibited in the NS treatment with inflammatory infiltrates and vacuolar changes of the liver and degeneration of intestinal and renal cells. Conversely, NSTAP showed an equalized histopathological pattern of slight hyperplasia in gill epithelial cells, less hepatic and renal degeneration, and indicators of goblet cell hyperplasia in the intestines, indicating a protective mucosal reaction. All in all, the structural degradation and adaptive changes were reduced in NSTAP-treated zebrafish as compared to TAP and NS groups. **Discussion:** The comparative analysis states the potential of NSTAP in facilitating the adaptability of tissues, which means that there is a synergistic effect between NS and antibiotics. It was demonstrated that NSTAP demonstrated improved biocompatibility with zebrafish tissues, which was presumably caused by the controlled drug release and enhanced bioavailability. The altered structural responses in NSTAP-treated tissues are controlled, which implies the activation of protective pathways, potentially related to the immune modulation and oxidative stress alleviation. TAP and NS, in turn, exhibited increased cellular stress levels, which implies that standalone formulations would need adjustments to be biocompatible. **Conclusion:** NSTAP is proposed to be a potential nanoformulation as it exhibits lower tissue stress and improved biocompatibility in zebrafish than TAP and NS. The observed balanced tissue response in gills, liver, intestines, and kidneys puts NSTAP in the spotlight of future research in biomedical practices, especially where there are long-term drug delivery and minimal tissue damage requirements. These studies have highlighted the significance of NS as a stabilizing agent, which could be used to lead to safer and more effective nano-based therapeutic solutions. The

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long-term effects, as well as mechanistic research, should be performed in the future to identify the therapeutic benefits of NSTAP.

Key words: Histopathology, innovative, nanoformulations, nano-silica triple antibiotic paste, product innovation, tissue adaptation

INTRODUCTION

Triple antibiotic paste (TAP) finds extensive usage in the medical and dental practice, particularly in the field of endodontics, as it has a high degree of antibacterial property and is effective in fighting infections.[1] TAP, a combination of ciprofloxacin, metronidazole, and minocycline, is a broad-spectrum antimicrobial agent with a specific effect in treating more complicated conditions such as periapical lesions, inflammatory root resorption, and even fractures of the root.[2] They play an important role in the process of regenerative endodontics because, in this case, TAP will help to remove pathogens and support the healing of tissues. Nevertheless, inasmuch as TAP remains successful in fighting the infections, this has been accompanied by high biocompatibility challenges that cast doubts on its safety in its clinical application.[3]

Overall, the cytotoxic potential of TAP is a salient fact whereby increased rates of TAP have been found to lower cell viability, particularly in human stem cells and fibroblasts.[4] Such cytotoxicity is of special interest considering that TAP is used directly in root canals, where it can be in contact with the surrounding tissues and stem cells in the periapical area. Furthermore, tooth discoloration has been linked with the use of minocycline in TAP, and this may impact the esthetic results of dentistry.[5] Furthermore, the current application of antibiotic combinations such as TAP is associated with the threat of the selection of antibiotic-resistant strains of bacteria that, in turn, will compromise the effectiveness of their analogous antibacterial therapies. These restrictions have also influenced the quest to find ways of changing the TAP formulation to become more biocompatible without affecting its bactericidal activity.[6]

The introduction of nano-silica (NS) in TAP to form a new product, TAP, which is NS-enhanced TAP, is one of the potential modifications as these modifications are meant to preserve the antibacterial capabilities in TAP but may help mitigate its cytotoxic behavior.[7] The capability of NS to work as a stabilizer and a carrier has attracted interest in drug delivery and biomedical applications, where it can enhance the bioavailability of drugs and allow controlled release.[8] Such precise delivery would be beneficial in the context of reducing the toxicity of a high dose of antibiotics since the NS application as a carrier can be utilized to create therapeutic concentrations at low doses.[9] Besides, the ability of NS to improve the stability of TAP may lower the tendency of degradation of the formulation and the possibility of generating reactive by-products, which in turn would lead to a safer usage.[10] However, the addition of NS in TAP adds other variables, and the long-term and systemic biocompatibility impacts of nano-silica triple antibiotic paste (NSTAP) before it can be suggested to be used in clinical practice are essential.[11]

Animal models provide a priceless insight into determining the biocompatibility and possible toxicity of both TAP and NS-enhanced TAP. The zebrafish (*Danio rerio*) model is one of them that is gaining more and more recognition as a useful tool in toxicological research because of the unique genetic and physiological properties of the zebrafish.[12] Zebrafish have about 70% of the protein-coding genes that humans have, and about 84% of human disease-related genes have functional parallels in zebrafish. This has made it a strong model in predicting human responses to various compounds.[13] Moreover, zebrafish embryos are clear and develop very quickly, which means that the responses of the embryo to hazardous substances can be monitored in real-time, and the high-throughput screening is also efficient. All these benefits have cemented the use of the zebrafish model as an essential instrument in the evaluation of the toxicity and biocompatibility of recent formulations, including those containing nanomaterials such as NS.[14]

The toxicity of drugs and formulations is especially worth studying using the zebrafish due to the ability of histopathological analysis to study specific tissues and organs, including the liver, kidneys, heart, and gills, in more detail.[15] All these organs are vital parts of the metabolic and physiological processes, and any histological alteration of them can give valuable information about the toxicity of the tested substances. Namely, hepatotoxicity may be indicated by hepatocyte degeneration or necrosis in the liver, and respiratory stress during exposure to toxic compounds may be indicated by changes in gill structure.[16] In like manner, kidney disease manifestation, including tubular necrosis, may indicate impaired kidney function, and heart defects may indicate possible cardiotoxic outcomes.[14] Researchers can use these histopathological pointers to describe the cellular and systemic effects of TAP and NS-TAP on biological health and give a complete picture of their safety profiles.[17]

Considering these issues and the prospective value of NS improvement, the research will address the main knowledge gaps of comparative toxicity of TAP, NS, and NS-TAP by emphasizing histopathology results of zebrafish.[18] Certain aims of the research are to determine organ-specific reactions, the level of cytotoxicity in the cellular range, and whether the NS modification truly increases the safety of TAP without

reducing its bacterial qualities.[19] This research will attempt to draw a clearer picture of the degree to which NS interacts with TAP with regard to its biocompatibility and toxicity on the basis of a comparison of the histopathological effects of these formulations.[17] The findings will form the necessary information to explore the feasibility of NS-TAP as a safer substitute for therapeutic use, which will eventually help in the formulation of better antibacterial therapeutic agents that balance both its efficacy and biocompatibility.[12]

MATERIALS AND METHODS

Synthesis of NS

A conical flask was used to mix 1.57 mL of ammonia, 37 mL of ethanol, and 5 mL of water in a sterile environment, followed by stirring for 5 min. Then, 3 mL of tetraethyl orthosilicate was added, and the solution was stirred. Subsequently, the silica nanoparticles were centrifuged at 10,000 rpm for 30 min. The resulting pellet was then dried in a hot air oven at 60°C to obtain the final NS powder.

Preparation of silica nanoparticle-based antibiotic combinations

For each antibiotic combination, 100 mg of the synthesized silica nanoparticles was mixed with 100 mg of the respective antibiotic (doxycycline, Flagyl, or ciprofloxacin) in a 1:1 weight ratio. These mixtures were dissolved in 1 mL of distilled water and subjected to thorough mixing on a vortex mixer for 10–15 min to ensure uniform distribution of the antibiotic onto the NS particles.

Zebrafish maintenance and treatment protocol

Adult zebrafish (*Danio rerio*) were maintained under standard laboratory conditions with a 14:10 light–dark cycle at a temperature of 28 ± 1°C. The fish were fed twice daily with commercial zebrafish feed. Before the experiment, zebrafish were acclimatized for a week in laboratory conditions. Four treatment groups were established: Control, TAP, NS, and NSTAP, with each group containing both male and female zebrafish. Each treatment group had 10 fish (5 males and 5 females).

Exposure regimen

Fish were exposed to subethal concentrations of TAP, NS, and NSTAP dissolved in tank water. The control group was maintained in tank water without any additives. Treatments were refreshed every 24 h. The exposure period lasted for 7 days, after which the fish were euthanized for tissue collection.

Tissue collection and histological processing

After the completion of the treatment period, fish were euthanized by overloading tricainemethanesulfonate (MS-222). Tissues were carefully removed (gills, liver, intestines, and kidneys). The tissues were absorbed in 10% buffered formalin for 24 h at room temperature, dried using a series of ethanol, cleared with xylene, and embedded in paraffin. Slides of 5 µm were cut and placed on glass.

Hematoxylin and eosin (H&E) staining

To study it under the microscope, the sections were stained using H&E. Cell nuclei were stained blue with hematoxylin, and the cytoplasm and the extracellular matrix were stained pink with eosin. The staining was used to examine the morphological abnormalities and the cell integrity of the tissues. Histopathological analysis: A pathologist who never saw the treatment groups viewed stained slides using a light microscope. The analysis was conducted on the pathological changes, including atrophy of the cell, vacuoles, inflammatory infiltrates, and tissue-specific changes. The histological features were graded on the severity of change on a scale of zero (no change) to three (severe change).

Statistical analysis

The results were reported as mean and standard deviation. One-way analysis of variance and *post hoc* test of Tukey to compare the mean values were used to statistically compare the groups. The *P*-value below 0.05 was found to be significant.

RESULTS

Histopathological analysis

Control group

Control male and female zebrafish histopathology showed different cellular and structural characteristics in the gills, liver, intestine, and kidney, and certain gender differences. Both genders of zebrafish had hyperplasia of epithelial cells in the gills, in addition to erythrocytes, pillar cells, and chloride cells, which are the normal respiratory and ionic regulatory cell types. Gill structural integrity was found to be maintained by cartilage that supported venous sinuoids in both sexes. Nevertheless, female gills were observed to have an aneurysm, which was not observed in males, and it was a potential unique response to vascularity or stress in females (Figure 1).

Hepatocyte vacuolization, which is an indication of metabolic stress, was observed in both sexes of the liver. It showed cellular degeneration, hyperchromatic nuclei, and nuclear atrophy in both genders, with the presence of inflammatory

cells in the blood vessels and sinuoidal spaces suggesting a mild inflammatory reaction. Interestingly, the female liver exhibited a greater cellular atrophy, implying that there may be an increased level of cellular stress in females as opposed to males.

Atrophic enterocytes and degeneration of villi were identified in the intestine of both male and female zebrafish, indicating that the intestinal function is impaired. Hyperplasia of goblet cells in males was also evident, perhaps as a compensatory process to maximize mucosal protection, but the effect was not very prominent in females. Other indicators of cellular destruction, such as vacuolization of enterocytes, lamina propria displacement, and epithelial detachment, were present in both sexes, which showed a breach of the structural integrity of the intestinal barrier.

Both male and female zebrafish exhibited glomerular atrophy and tubular degeneration of the kidneys and had evidence of cytoplasmic degeneration of tubular cells, indicating decreased renal function. In males, vessel rupture and hyperemia were seen, which suggests the possibility of vascular stress, whereas in the female kidney, there were signs of tubular atrophy and degeneration. In general, there were typical structural traits in both the male and female control zebrafish in the evaluated body organs, with differences between genders present. Gender-based variation in the cellular responses under baseline conditions is indicated by the presence of an aneurysm in female gills, hyperplasia of the goblet cells in male intestines, and the higher degree of liver atrophy in females. These results form a control baseline, which is the basic component of the comparison of the treatment groups in the future to determine particular pathological effects.

Group I-TAP

Histopathological analysis of Day 1 and Day 7 male and female zebrafish treated with TAP showed that the gills, liver, intestine, and kidney were greatly affected with significant differences in the level and kind of tissue reactions across days.

Day 1 observations

On Day 1, the gills of the male zebrafish were found to have lost their normal structure with epithelial cell and chloride cell atrophy, aneurysms, and primary lamellae fusion. Hepaticocyte cells showed vascular degeneration, shrunken spaces that are sinuoidal in character, and a high number of inflammatory cells, which is a pointer to an acute inflammatory response. There was also dilatation of the central vein, which is an indication of circulatory stress. Villi degeneration and extensive vascular degeneration of epithelial cells were seen in the intestine; the lamina propria became detached, and lymphocytic infiltrations occurred. The kidney showed poor organization of tubular cells, necrosis, rupture of vessels, and a hyperchromatic nucleus, which showed a lot of renal stress and cellular death (Figure 2).

On Day 1, the gill structure of the female zebrafish was characterized by the widening of primary lamellae and focal fusion in secondary lamellae, the presence of epithelial cells and pillar cells. There was vascular degeneration, hyperchromatic nuclei, and cellular degeneration of hepatic tissue with foci of hyperplastic nuclei. There were signs of intestinal villi degeneration, epithelial atrophy, cytoplasmic atrophy, epithelial cell hyperplasia, and lamina propria detachment. The KD presented with atrophied renal tubules with hyperchromatic nuclei, necrosis, vessel rupture, and chronic inflammatory infiltrates with cellular damage and inflammatory reaction of a similar nature but with minor structural differences.

Day 7 observations

On Day 7, progressive alterations were observed in the gills of TAP-treated male zebrafish, with hyperplasia of the epithelial cells and secondary lamellae fused locally. The hepatocyte atrophy, increase in sinuoidal spaces, and atrophy of the nucleus became prominent in the liver, which showed that the cellular degeneration continued. The intestine exhibited a high rate of degeneration of epithelial cells, atrophy of goblet cells, and lamina propria, and it also demonstrated dysfunction of structural integrity. Renal tubules in the kidney showed continued degeneration, glomerular atrophy, necrosis, and nuclear and cytoplasmic detail loss, which indicated an increasing renal damage over time (Figure 3).

In the zebrafish female on Day 7, both primary and secondary lamellae were degenerative, nuclei of epithelial cells atrophied, and inflammatory infiltrates existed, which suggests sustained inflammatory reactivity and structural disintegration. The liver had extensive vascular degeneration, and much nuclear and cytoplasmic detail loss was observed. The intestine showed widespread injury of villi and degeneration of goblet cells, and shedding of the lamina propria. Tubular degeneration, hyalinization, and chronic inflammation infiltrates were evident in the kidney, and this is a sign of severe renal impairment.

Comparative Analysis When the male and the female TAP-treated zebrafish are compared on Day 1 and Day 7, there is progressive degeneration of the tissues and a cellular reaction to the process of stress encountered by both sexes. Males and females had similar acute responses on Day 1, with the liver, intestine, and kidney having vascular degeneration, inflammatory infiltrates, and cellular atrophy. Nonetheless, structural breakdown was stronger in the gills of males, with females experiencing more nuclear hypertrophy in the liver.

At Day 7, the tissue damage was more widespread in both genders, and the cellular degeneration as well as inflammation were more pronounced in all the organs observed. The gills of the males were hyperplastic, and those of the females degenerated more severely and were inflammatory. Both genders had hepatic and renal degeneration, which was severe, with females having a little more vascular degeneration in

hepatocytes and focal hyalinization in renal tubules. All in all, the histopathological changes in zebrafish were progressive and severe, with gender differences in response in the tissue and structural degeneration over time in TAP treatment.

Group II-NS

The comparative histological observation of the male and female zebrafish samples on both Day 1 and Day 7 in Group II (treated with NS) shows different trends of tissue damage and degeneration in different organs.

Day 1 observations

In male zebrafish, the gills also had hypertrophied primary lamellae (black arrow) and atrophied secondary lamellae (green arrow) with evidence of nuclear atrophy, cytoplasmic detail loss, and secondary lamellae fusion. There were inflammatory infiltrates in these structures. There was vascular degeneration in the cytoplasmic hepatocytes (red arrow) with an atrophied nucleus, and contraction of the sinuoidal spaces (green arrow) was noticed, with the presence of chronic inflammatory cells. Enterocyte nuclei and cytoplasm degeneration (red arrow), hyperplasia of the goblet cells, and the lamina propria displacement were observed in the intestine. Renal tissue showed signs of degeneration of tubular cells, including hyperchromatic nuclei (green arrow) and chronic inflammatory infiltrates (red arrow) (Figure 4).

Day 1 female zebrafish were also characterized by atrophied primary and secondary lamellae, fusion in secondary lamellae (black arrow), and cartilage anchoring venous sinuoids. There was hyperplasia of chloride cells (green arrow) and displacement of the epithelial cells. The hepatocytes were characterized by vascular degeneration (red arrow), hyperchromatic nuclei (black arrow), and sinuosity of the space. The intestines underwent degeneration of enterocytes, which did not contain nuclear and cytoplasmic characteristics (yellow arrow). The presence of hyperchromatic nuclei and a high presence of chronic inflammatory infiltrate were observed in renal tubular cells (yellow arrow).

Day 7 observations

Hyperplasia of epithelial cells of gills and localized gill secondary lamellae fusion were found in male zebrafish. There was extensive vascular degeneration of hepatocytes, atrophied nuclei, and a decrease in cytoplasmic content. The sinuous spaces of the liver were significantly slender. There was evidence of degenerated epithelial cells in the intestinal tissue, loss of goblet cells in the intestinal tissue, and the detachment of lamina propria. Renal samples demonstrated tubular degeneration, absence of cytoplasmic detail, and areas of necrosis (Figure 5).

Day 7 female zebrafish developed degeneration of primary and secondary lamellae as well as epithelial atrophy, secondary lamellae fusion, and chronic inflammatory infiltrates. There was severe vascular hepatic tissue degeneration, cytoplasmic

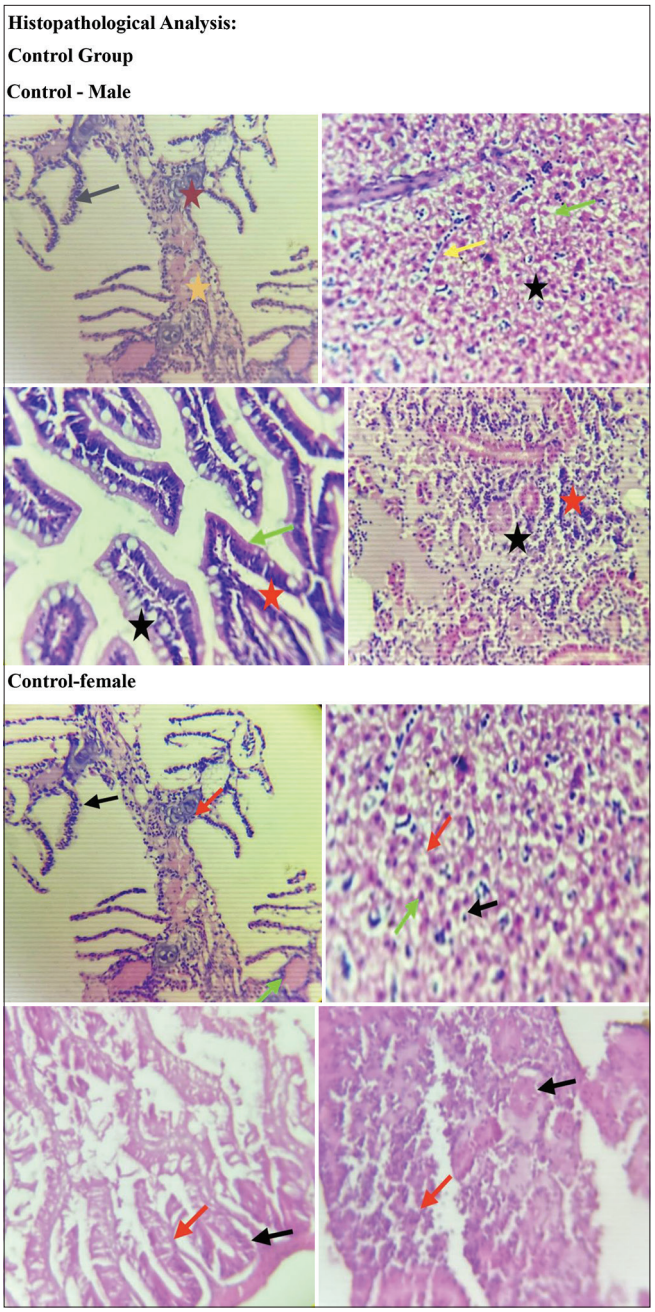

Figure 1: Histopathological examination of gill tissues from control zebrafish, showing normal epithelial and lamellar structures without signs of stress or damage

and nuclear loss of cytoplasmic details, and sinuoidal space expansion. Enterocytes degeneration and lamina propria detachment were observed in the intestinal section. There were hyperchromatic nuclei in the renal tubules with foci of hyalinization and inflammation.

Comparison

There was a similarity between male and female zebrafish in the case of tissue degeneration and long-term chronic inflammatory responses. Nonetheless, the degree of injury, e.g., epithelial and hepatic degeneration, appeared a bit more severe in the female samples at Day 7. Male gill cell

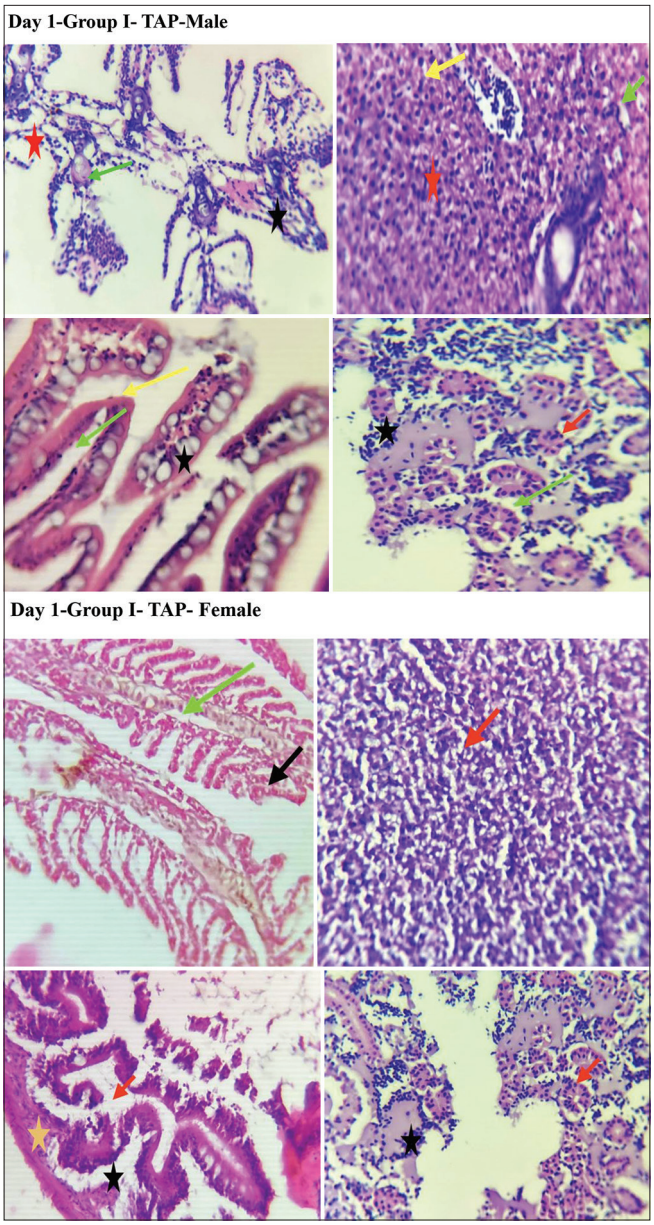

Figure 2: Histopathological examination of gill tissues from Group I, Day 1 triple antibiotic

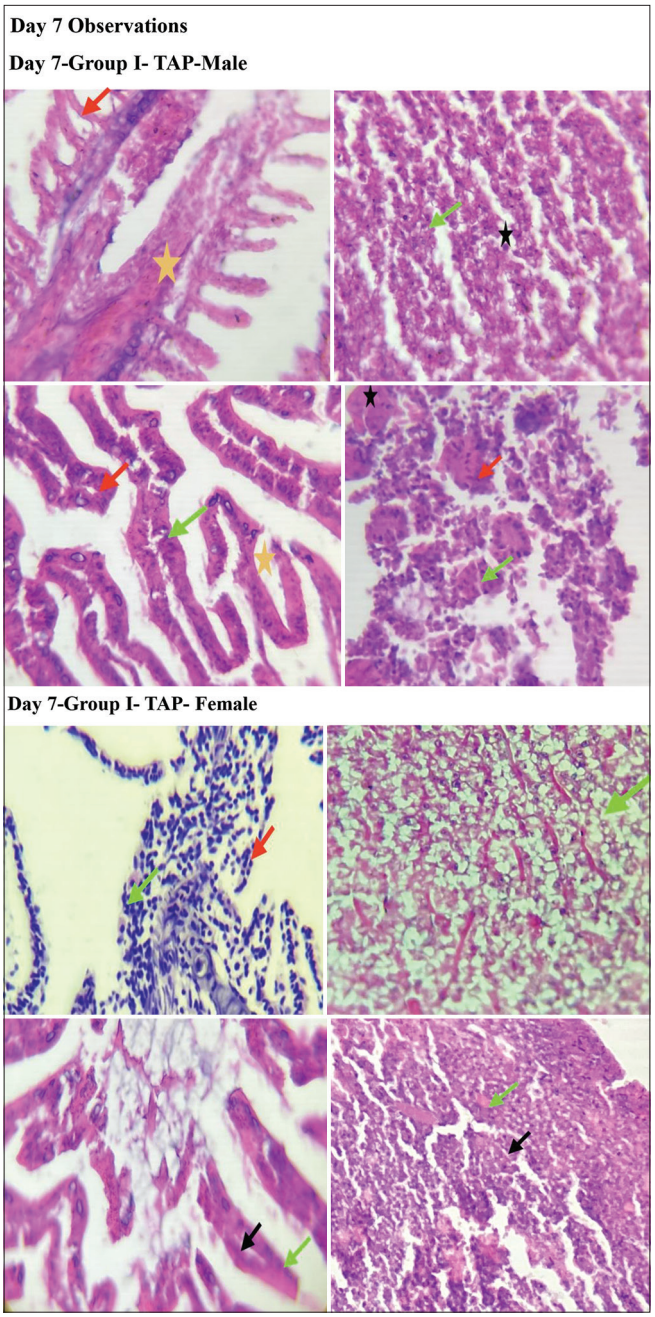

Figure 3: Histopathological examination of gill tissues from Group I Day 7 triple antibiotic

hyperplasia and severe intestinal enterocyte degeneration in females were remarkable. In general, the chronic inflammation and structural atrophy were also consistent between genders and time points, and show that NS treatment has a persistent degenerative effect.

Group III-NSTAP

The histopathological analysis of the male and female zebrafish under NSTAP treatment on Day 1 and Day 7 indicated that there were significant changes in the gills, liver, intestine, and kidney, which indicated how the treatment affected the cellular and structural integrity. On Day 1, the gills of male zebrafish had atrophic epithelial cells in primary and secondary lamellae (black arrow), cartilage between venous sinuoids (red asterisk), epithelial cell detachment, and absence of nuclear details. Gills in females showed hyperplasia of the epithelial cells (red arrow), hyperplastic chondrocytes that hold sinuoidal cells, pillar cells, and chloride cells, and aneurysms, and pushed aside the epithelial cells (green arrow). At Day 7, male gills remained hyperplastic with epithelial cells (black arrow), and atrophic with secondary lamellae (green arrow) and other degeneration (red arrow) still present. Female gills had an epithelial cell that was hyperplastic (black arrow), cartilage that supported a venous sinusoid (green arrow), and hyalinization spots (Figure 6).

Observations on Day 1, males were characterized by hepatocyte degeneration without details of the cytoplasm,

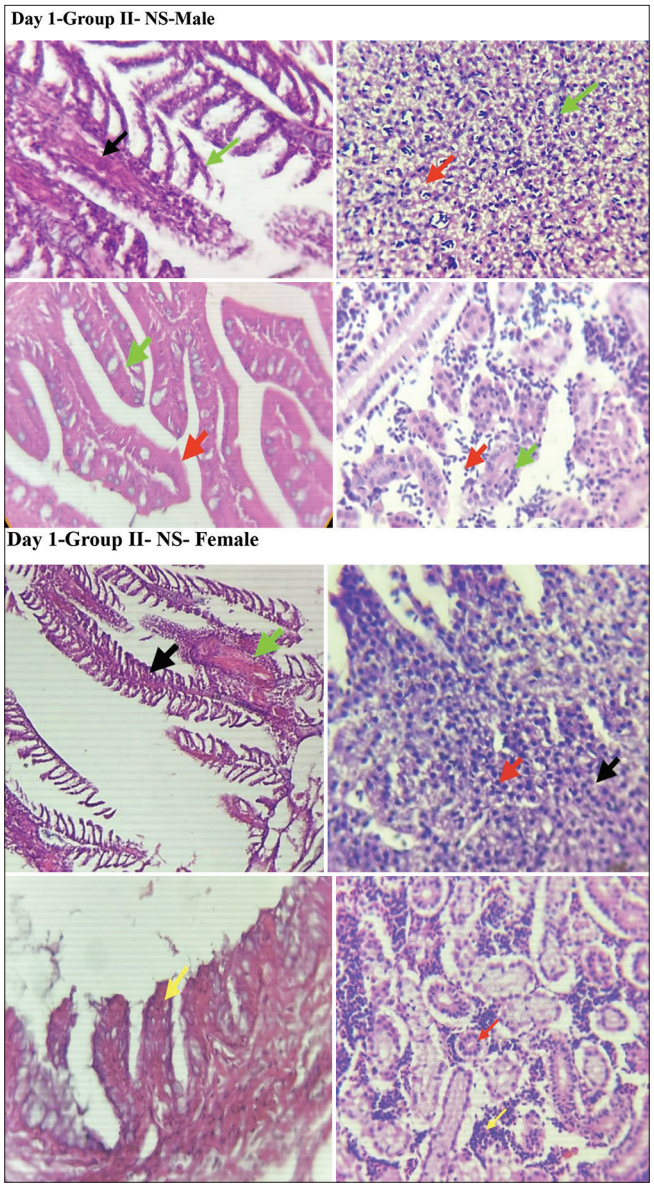

Figure 4: Histopathological examination of gill tissues from Group II – nano-silica Day 1 zebrafish

hyperchromatic nuclei, and constricting sinuoidal spaces (red arrow). The livers of female zebrafish showed atrophic nuclei, a lack of cytoplasmic features, and expanded sinuoid spaces with inflammatory cell infiltration (black arrow). At Day 7, vascular degeneration was widely seen in both sexes, cytoplasmic details were lost, nuclei atrophic, and there were chronic inflammatory filaments. Narrowing of the sperm (green arrow) was observed in males, and females exhibited further sinusoidal expansion.

Male zebrafish intestines had villus degeneration, epithelial cell atrophy, and lamina propria detachment on Day 1 (red asterisk). There were atrophic intestinal enterocytes, lamina propria detachment (red arrow), and degeneration of epithelial cells (black arrow) in female intestines. On Day 7, degeneration was still visible in both groups. Goblet cell hyperplasia (black arrow) and lamina propria detachment (red

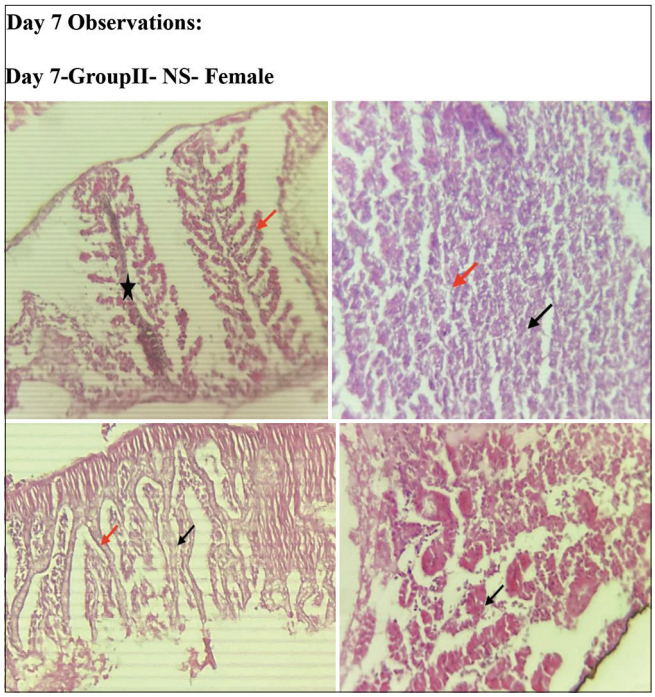

Figure 5: Histopathological examination of gill tissues from Group II – nano-silica Day 7 zebrafish

arrow) were found in males, and widespread cytoplasmic and nuclear degeneration in enterocytes (green arrow) and more extensive goblet cell hyperplasia in females (Figure 7).

On Day 1, maladjusted renal tubular cells, necrosis (black asterisk), and rupture of vessels were observed in male kidneys, and atrophic renal tubular cells, local degeneration, and chronic inflammatory foci were observed in females (black arrow). At Day 7, kidneys in both sexes had shown further degeneration, loss of cytoplasmic details, hyperchromatic nuclei, and augmented necrotic regions.

Zebrafish kidneys of male fish were ruptured, and female fish were more inflamed and hyalinized (green arrow). Both male and female zebrafish that were treated by NSTAP showed progressive histopathological alterations across time. Gills presented with hyperplasia and degeneration. There was vascular and cytoplasmic degeneration and inflammation in the liver, villi atrophy and goblet cell hyperplasia in the intestines, and extensive tubular degeneration and necrosis of the kidneys. The same findings indicate that the NSTAP treatment, with time, results in significant structural and cellular perturbation of zebrafish.

DISCUSSION

The paper presents detailed information about the histopathology of TAP, NS, and NSTAP preparations on zebrafish tissues and underlines the individual responses to each of the treatment groups. Although TAP and NS formulations revealed certain limitations caused by tissue

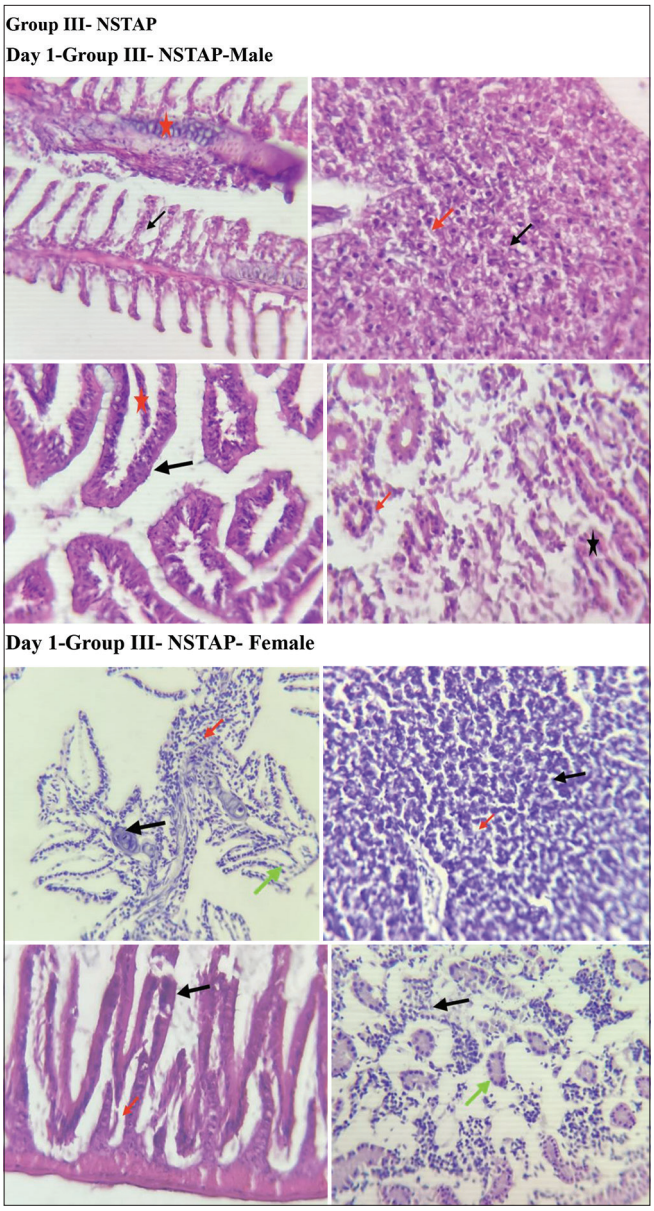

Figure 6: Histopathological examination of gill tissues from Group III – nano-silica triple antibiotic

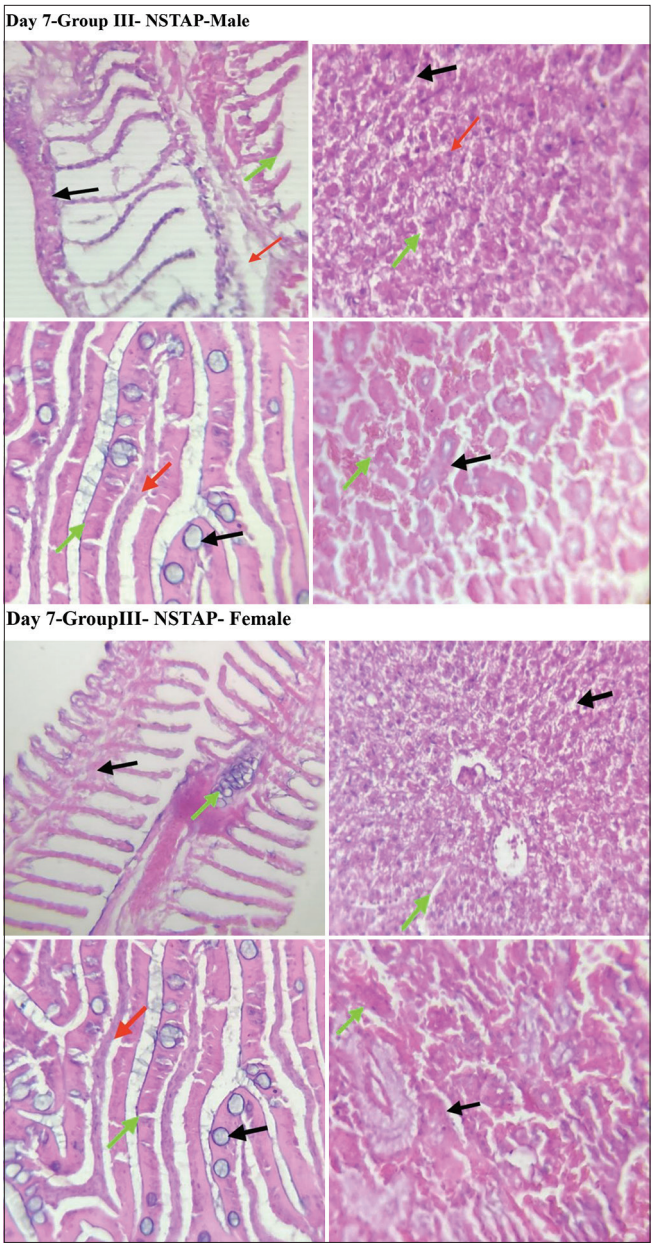

Figure 7: Histopathological examination of gill tissues from Group III – nano-silica triple antibiotic

stress and degeneration, NSTAP always presented a more balanced tissue response, and it is important to highlight that it has the potential to become an effective therapeutic formulation. The adaptability of the observed tissues of the NSTAP group, as well as the relative reaction of TAP and NS groups, can provide useful information that can guide the creation of safer and more efficient nano-based therapies.

In the three formulations, the gills showed different levels of adaptive and stress responses. TAP treatment led to a decrease in the gill lamellae atrophy and fusion, which could represent a sign of structural stress.[20] Equally, NS exposure resulted in hypertrophic primary lamellae and atrophic secondary lamellae, and inflammatory infiltrates. Conversely, the gills of NSTAP-treated fishes showed hyperplasia of the epithelial cells with local fusion of the secondary lamellae, indicating a tropic adaptive response and not damage *per se*.[21] The adaptability of the gills toward NSTAP underscores the fact that it would be useful in the development of the resilience of the respiratory surface, which is critical in the sustainability of respiratory efficiency. Such disparity in the reaction suggests that the model of NSTAP could offer a better-fitting variant to respiratory tissues than TAP and NS.[22,23]

TAP treatment in the liver was also linked to vascular degeneration and sinuoidal narrowing, and NS treatment was linked to a more pronounced vascular change accompanied by a greater nuclear atrophy and chronic inflammatory infiltrates.[24] The response of the liver to NSTAP, however, was balanced with vascular degeneration and sinusoidal widening, which can indicate increased detoxification and tolerance of

the same.[25] The hepatocytes of NSTAP-treated zebrafish showed fewer signs of cellular stress than either TAP-treated or NS-treated, but their sinuosity was better able to support good blood flow and clearance.[26,27] This observation suggests that NSTAP might be better applied in instances where the liver's dominance to detoxify is critical, and this will provide a safer profile than the other formulations.[4,28]

The intestinal tissues showed different reactions in the three groups. Exposure to TAP resulted in atrophic enterocytes and vacuolization, and displacement of the lamina propria, indicating possible stress in the gut lining.[29] Enterocyte and epithelial cell degeneration and scanty evidence of protective adaptations were also seen in NS treatment. Conversely, NSTAP showed hyperplasia of goblet cells, which is an indicator of heightened mucus secretion, an important protective response to gut integrity.[30] The NSTAP-induced hyperplasia of goblet cells in the intestines implies that NSTAP would be beneficial to retain intestinal mucosal integrity to allow oral or injected preparations that need to be compatible in the gut. The responses to NSTAP exhibited by the intestines are more protective and adaptive in comparison to those of TAP and NS, which support its possible superiority as a safer nano-biomimicked therapeutic agent.[31]

TAP and NS both led to significant tubular degeneration and cellular atrophy in the kidneys, indicating stress exposure in the kidney with a low adaptation response.[32] The kidneys of TAP-treated cases were characterized by glomerular atrophy and cytoplasmic degeneration, whereas the kidneys in NS-treated cases had inflammatory infiltrates and hyperchromatic nuclei.[33] Controlled tubular adjustments with fewer signs of extensive cellular damage formed the response to NSTAP. The kidneys of NSTAP exhibited glomerular and tubular strength, and this indicates effective filtration without causing extensive degeneration.[34] This controlled adaptation indicates that NSTAP has a potential for formulations that necessitate renal clearance, since it does not seem to place strain on the renal tissues as compared to TAP and NS.[35]

The relative outcomes of TAP, NS, and NSTAP highlight the merit of using NS along with antibiotics, which was observed in the balanced responses of the tissues in NSTAP. The NS part probably also leads to controlled release to enhance bioavailability of the antibiotic with a low incidence of adverse tissue responses.[36] Such synergy might increase the therapeutic efficacy of NSTAP, and subsequently, they would be more desirable compared to TAP or NS individually. Adaptive reactions in the NSTAP group are indicative that the group might stimulate protective cellular events associated with oxidative stress responses and immune regulation that suppress the potential risks of tissue degeneration in the course of time.[37] The knowledge of these mechanisms might result in the further optimization of NSTAP and make the latter a multifunctional nanoformulation applicable in different biomedical settings.

Although TAP and NS demonstrated potential in some ways, the tissue responses show the possibility of improvements. TAP has impacts on gills and kidneys that indicate that an increase in concentrations or a longer duration of exposure can lead to an increase in tissue stress.[38] Nevertheless, TAP still has a chance to be potentially useful with changes in formulation or dosage, particularly in situations where the speed of antibiotic activity is necessary. NS, however, showed moderated vacuolar hepatic alterations and moderate goblet cell hyperplasia, implying partial similarity.

Future studies are possible to consider modified formulations of NS to include stabilizing agents to reduce adverse effects and maintain their antimicrobial effect.[39] Recent studies show that the changing scene with intracanal medicaments includes green-synthesized nanoparticles, herb-based agents, and novel nanoformulations.[40-42]

Future directions

This paper presents an overall comparative study of TAP, NS, and NSTAP, which will give the background knowledge on tissue-specific responses. The clear benefit of NSTAP to stimulate adaptive over degenerative responses indicates that it has great potential to be used in clinical settings. Long-term exposure studies should be carried out in the future to examine chronic effects and the potential of recovering in such formulations. Further, molecular tests and gene expression analyses may give information on certain pathways triggered by each formulation, which can help in the formulation of safer and targeted nanoformulations.

CONCLUSION

NSTAP turns out to be the most promising of the three tested and has better biocompatibility and flexibility across zebrafish tissues. The results of the balanced responses to gills, liver, intestines, and kidneys show that NSTAP should also be able to cause protective adaptations, decreasing the chances of tissue damage. This observation places NSTAP as one of the strong candidates in the development and application of drug delivery and therapy. The paper provides the significance of nanoscale modification in the improvement of therapeutic safety and efficacy as a basis for advancing nano-based biomedical formulations in the future.

AUTHOR CONTRIBUTION

Conceptualization: MSH, DPA, and RS. Data curation: MSH. Formal analysis: MSH, DPA, and RS. Funding acquisition: MSH. Investigation and Methodology: MSH and DPA. Project administration: MSH, DPA, and RS. Resources: MSH and RS. Supervision: MSH, DPA, and RS. Validation: MSH, DPA, and RS. Visualization: MSH, DPA, and RS.

Writing-original draft: MSH, DPA, and RS. Writing - review and editing: MSH, DPA, and RS.

DATA AVAILABILITY STATEMENT

The data are accessible from the corresponding author upon reasonable request.

ETHICAL APPROVAL

The study has been approved by the scientific review board with the reference number SRB/SDC/PhD/ENDO-1901/23/242.

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