Iraqi Journal of Biotechnology, 2025, Vol. 24, Special Issue 2, 277-289

The Effect of bft2 and bft3 Toxins which have Been Extracted and Refined from Clinical Isolates of Enterotoxigenic *Bacteroides fragilis* on Mice

[1]Hussein Ali Khaleefah , [2]Ashwak Basim jasim , [3]Bushra Ibrahim Mustafa

[1,2] Institute of Genetic Engineering & Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq

[3] college of veterinary medicine ,University of Baghdad, Baghdad, Iraq

Received: February 20, 2025 / Accepted: June 29, 2025 / Published: November 16, 2025

Abstract: This study involves isolating Enterotoxigenic *Bacteroides fragilis* from 94 stool samples collected from multiple hospitals in Baghdad city from March 2020 to April 2021. Stool samples were streaked onto BBE media under anaerobic conditions for 48 hours. *Bacteroides fragilis* was identified by analyzing its morphological characteristics on BBE media, which included the presence of gray convex tiny rounded colonies surrounded by a black zone. A molecular method was also employed, specifically targeting genes such as *16S rRNA*, *bft* gene, *bft-1*, *bft-2*, and *bft-3*. A total of 34 isolates of *B.fragilis* tested positive for the *16S rRNA* gene. Among these, 5 isolates of *B.fragilis* were positive for the *bft* gene, indicating their classification as Enterotoxigenic *B.fragilis* (ETBF). Furthermore, 3 isolates of ETBF were found to be positive for both *bft-2* and *bft-3* genes, whereas 2 isolates of ETBF were negative for all three genes (*bft-1*, *bft-2*, and *bft-3*). Two isolates of Enterotoxigenic *Bacteroides fragilis* (ETBF), which tested positive for the *bft-2* and *bft-3* genes, were purified using the Van Tassel technique. A total of 40 male mice were divided into four groups, with 10 mice in each group. The first group served as the control, while the second group received daily administration of 2% dextran sulfate sodium for 30 days via a stomach tube, serving as the positive control. The third group of mice also received administration via a stomach tube. After being treated with 2%DSS for 10 days, the mice were then given 20 µg of bft2 toxin through a stomach tube for 30 days. In the fourth group, the mice were also supplied through a stomach tube. The mice were treated with a 2% dextran sulfate sodium (DSS) solution for 10 days. After this period, the animals were given 20 µg of bft3 toxin through a stomach tube for 30 days. After the trial, all groups of mice were euthanized by ethical guidelines. Based on the histological abnormalities observed, it can be concluded that the combination of DSS and bft2 toxin had the most significant impact on the liver, spleen, and intestine of mice in the third group.

Keywords:. dextran sulfate sodium , Van Tassel method ,bft2 toxin, stomach tube,16S RNA gene

Corresponding author: (Email: husen.hu@yahoo.com)

1. Introduction

Enterotoxigenic *Bacteroides fragilis* (ETBF) is a type of bacteria that is commonly found in the human colon. It is linked to cases of diarrhoea in young individuals and is also connected with the development of advanced colorectal cancer (1) (2). The toxin produced by enterotoxigenic *B.fragilis* (ETBF), known as fragilysin or BFT, is frequently expressed (3) (4). BFT is a thermolabile metalloprotease with a molecular weight of 21 kDa, which is zinc-dependent. BFT produces proenzymes with a molecular weight of 44.4 kilodaltons (kDa). (5). these proenzymes are characterised by a flexible linker, an N-terminal pro-domain, and a C-terminal catalytic domain. Three isoforms of BFT have been identified: BFT1, BFT2, and BFT3. Among these isoforms, BFT1 is the most prevalent (6) (7). BFT is a bioactive molecule that exhibits several activities, such as causing changes in the structure of intestinal epithelial cells, stimulating the release of chloride, and increasing the permeability of the human gut mucosa and epithelial cell layers (8). C57BL/6 mice with ETBF that have been present for 18 months display persistent colitis but do not demonstrate any indications of polyps (9). Animal models have shown that BFT is both essential and sufficient to cause colitis in mice and gerbils (10) (11).Mongolian gerbils have been successfully induced with colitis using dextran sulphate sodium(12).

Aim of study

This study aimed to investigate the effect of bft2 and bft3 toxin on liver, spleen and intestine of mice.

Material and method

Collection of samples

A total of 94 stool samples were collected, consisting of 50 samples from individuals with diarrhoea and 44 samples from healthy individuals serving as controls. These samples were gathered from various hospitals in Baghdad city between March 2020 and April 2021.

Isolating and identifying *B. fragilis*

A minute proportion of 94 stool samples were gathered by employing a sterile swab that had been immersed in thioglycolate broth (TB) to serve as a transport medium. The samples were subsequently streaked on Bacteroides Bile Esculin Agar Base and subjected to incubation for 48 hours at a temperature of 37 ºC in an anaerobic environment.The bacterial culture was further examined to confirm the presence of *B.fragilis* isolates and to identify Enterotoxigenic *B. fragilis* from Non ETB using specific primers. This analysis entailed the examination of physical features, doing biochemical tests, and analyzing the *16S rRNA* gene and *bft* gene.

Primers

The primers utilized in this investigation are documented in Table (1). The 16S rRNA molecule is employed to identify the presence of *B.fragilis*. The *bft* gene and its subtypes *bft-1*, *bft-2*, and *bft-3* are utilized specifically to detect enterotoxigenic *B.fragilis* (ETBF).

Table(1):Primer used in study

Target gene		5--3		
16S rRNA	F	TCRGGAAGAAAGCTTGCT	162	(13)
	R	CATCCTTTACCGGAATCCT		
bft	F	GACGGTATGTGATTTGTCTGAGAGA	294	(14)
	R	ATCCCTAAGATTTTATCCCAAGTA		
Bft-1	F	GGGATGTCCTGGT TCA		(15)
	R	AATTATCCGTATGCTCAGCG	142	
Bft-2	F	CTTAGGCATATCTTGGCTTG	219	
	R	GCGATTCTATACATGTTCTC		
Bft-3	F	TTTGGGCATATCTTGGCTCA	145	
	R	ATCATCCGCATGGTTAGCA		

The genomic DNA was isolated from bacterial growth using the QIAamp DNA Mini Kit procedure. A fluorometer was utilised to quantify the concentration of the isolated DNA. The presence of DNA bands was identified through the utilisation of the agarose gel electrophoresis technique, utilising a 1.5% agarose gel.

Table (2): The PCR cycling program is designed to amplify the *16S rRNA, bft* genes, and *bft-1, bft-2,* and *bft-3*.

<table>
<tr><th>NO</th><th>steps</th><th>Temperature (^{0}C)</th><th>Time</th><th>Number of cycle</th></tr>
<tr><td>1</td><td>Initial denaturation</td><td>95</td><td>5 min</td><td>1</td></tr>
<tr><td>2</td><td>Denaturation</td><td>95</td><td>30sec</td><td rowspan="3">30</td></tr>
<tr><td>3</td><td>Annealing</td><td>a-56
b-52
c-58</td><td>30sec
30sec
30sec
30sec</td></tr>
<tr><td>4</td><td>Extension</td><td>72</td><td>30sec</td></tr>
<tr><td>5</td><td>Final extension</td><td>72</td><td>7min</td><td>1</td></tr>
</table>

The variable A denotes the annealing temperature for the *16S rRNA* gene, whereas the variable b represents the annealing temperature for the *bft* gene. The variable c, on the other hand, represents the annealing temperature for the *bft-1*, *bft-2*, and *bft-3* genes.

Purification of bft2 and bft3 toxin from ETBF isolates:

1- In our study, we utilised two isolates of ETBF that possessed the *bft2* and *bft3* genes, as determined using PCR analysis. The purification process of metalloproteases toxins bft2 and bft3 was conducted following the Van Tassel technique (16) .

2- The protein content was determined (17).

3- The dextran sulphate sodium with a molecular weight of 40kDa was acquired from Sigma. It was made by dissolving 20 grams of the substance in one liter of distilled water.

Mice as an experimental subject

1-Animal classification

A group of forty male juvenile albinos, of the Mus musculus BALB /C strain, were measured at 20-25 g at three to five weeks of age. For two weeks, the animals were placed in plastic cages measuring 30 x 10 x 10 cm^3 and left in the room to adjust. Water and commercial feed pellets, the standard diet for rodents, were provided on a regular basis. The temperature in the house was kept at 22± 4°C, and the air was constantly changed using a ventilation vacuum and a light/dark cycle of 14/10 hours per day. Approximately once every seven days, the cages would have a new litter. This study's trial was place in the animal house of AL-Nahrain University in Baghdad, Iraq.

The first group, also known as the control group, consisted of mice that were provided with drinking water for duration of 30 days.

The second group (positive control) of mice were administered a daily dose of 2% Dextran sulphate sodium, freshly mixed in drinking water, for a duration of 30 days via a stomach tube.

Third cohort: mice given by gastric intubation Mice were orally fed 2% DSS for 10 days, along with 20μg of bft2 toxin for 30 days (18).

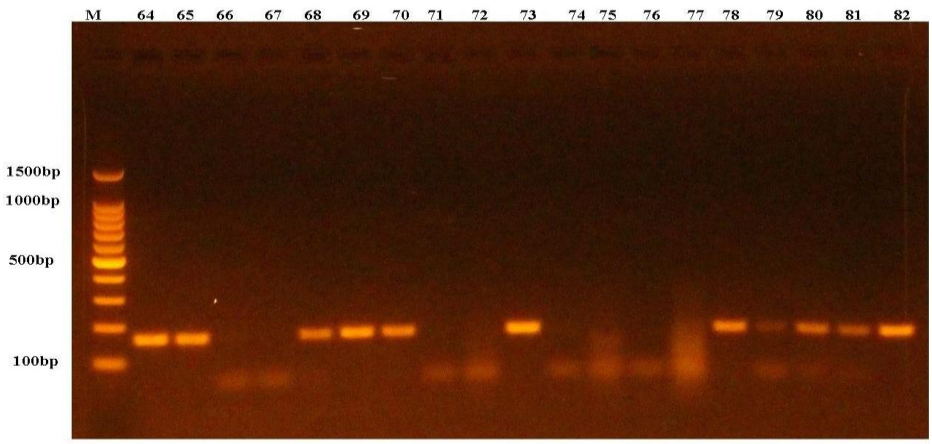
The fourth group of mice were orally treated 2% DSS for 10 days and orally delivered 20μg of bft3 toxin for 30 days. (18).

2- Study of tissue pathology

At the conclusion of the trial, all groups of mice were euthanized in accordance with ethical guidelines. Tissue samples (liver, gut, and spleen) were extracted from mice. The organs were immersed in a solution of 10% neutral buffered formalin and subsequently prepared for paraffin embedding. The histological sections, measuring 5 μm in thickness, were stained with hematoxylin-eosin using the procedure described by (19).

Result

1-The process of isolating and identifying *B.fragilis* bacteria from stool samples involved analysing 94 samples. Out of these, a total of 34 bacteria suspected to be *B.fragilis* were isolated from the BBE agar. The isolation was based on the morphological characteristics of the colonies, which were observed to be grey, convex, small, and rounded. Additionally, these colonies exhibited a black zone surrounding them, indicating esculin hydrolysis.*Bacteroides fragilis* synthesised esculetin and dextrose by the process of esculin hydrolysis. Additional examination and analysis Based on the results of biochemical tests, it was determined that all 34 isolates were *B.fragilis*.The 34 isolates of *B.fragilis* bacteria were subjected to conventional PCR procedures for additional confirmation of diagnosis. This involved using particular primers that target the *16S rRNA* gene, which are specifically designed for diagnostic purposes.All isolates yielded positive findings, and the amplified fragments (162bp) were separated via electrophoresis, stained with ethidium bromide, and captured using a gel imaging system (Fig. 1). In addition, the analysis revealed that 14.7% (5 out of 34) of *B.fragilis* samples included the *bft* gene, as indicated by the *16S rRNA* gene figure (2). These 5 isolates of *B.fragilis*, which tested positive for the *bft* gene, were classified as Enterotoxigenic *B.fragilis* .Five isolates of enterotoxigenic *B.fragilis* tested negative for bft-1, tested positive for bft-2, and tested positive for bft-3, as illustrated in figure 3 and 4.



16S rRNA

Figure (1): displays the gel electrophoresis results of the amplified 16S rRNA housekeeping gene (162bp) using standard PCR. The agarose gel was prepared at a concentration of 1.5% and subjected to an electric field of 100 volts for a duration of 60 minutes. The gel was then treated with ethidium bromide dye and observed using a UV transilluminator. Lane M has a 100 base pair DNA ladder.

.

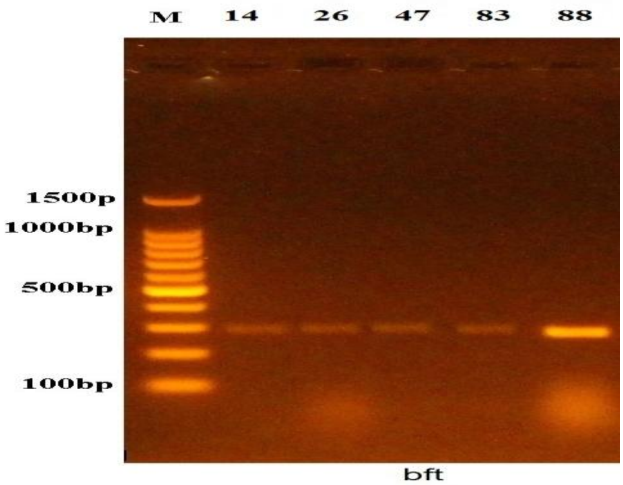


Figure (2): shows the gel electrophoresis results of the amplified *bft* gene, which has a length of 294 base pairs. The amplification was done using standard PCR. The agarose gel was prepared with a concentration of 1.5% and subjected to an electric field strength of 100 volts per centimetre for a duration of 60 minutes. Afterward, the gel was treated with ethidium bromide dye and seen using a UV transilluminator. Lane M has a 100 base pair DNA ladder.All lanes exhibit a good outcome for the *bft* gene.

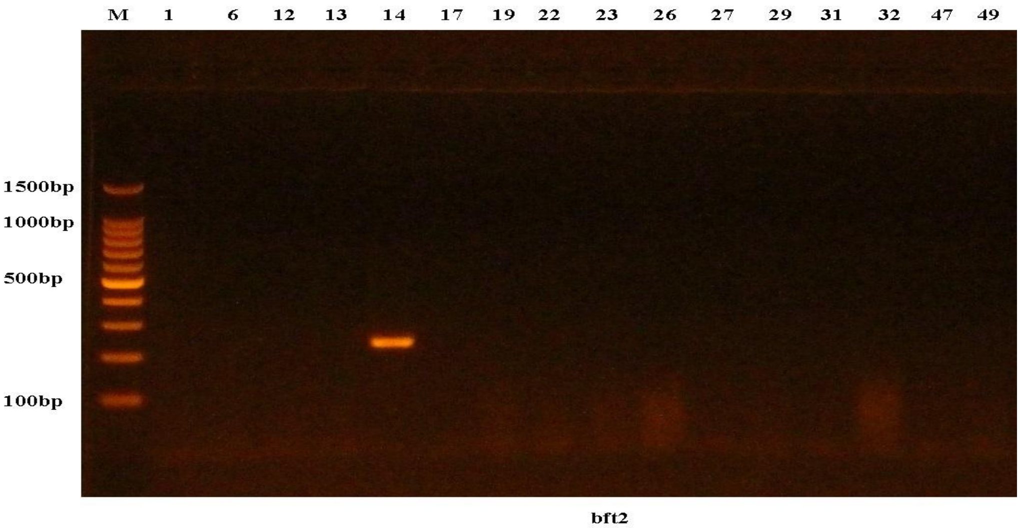


Figure (3): shows the gel electrophoresis results of the amplified *bft2* gene (219bp) from ETBF using standard PCR. The agarose gel was prepared with a concentration of 1.5% and subjected to an electric field strength of 100 volts per centimetre for a duration of 60 minutes. Afterward, the gel was treated with ethidium bromide dye and seen using a UV transilluminator. Lane M has a 100 base pair DNA ladder. Sample 14 contains amplicons of the bft2 gene for Enterotoxigenic *Bacteroides fragilis* (ETBF) in Lane 14.

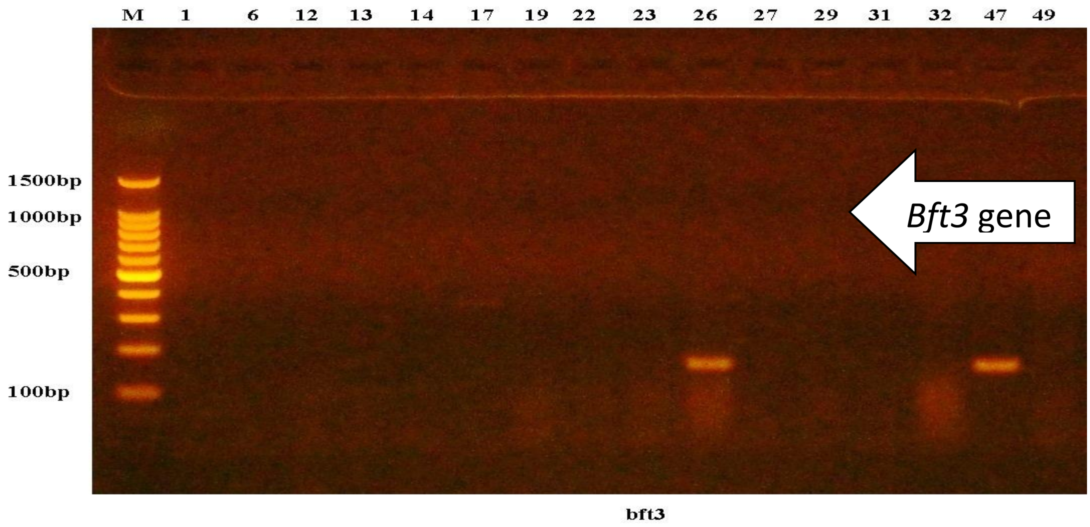


Figure (4): shows the gel electrophoresis results of the amplified bft3 gene (145bp) from ETBF using standard PCR. The agarose gel was prepared with a concentration of 1.5% and subjected to an electric field strength of 100 volts per centimeter for 60 minutes. After that, the gel was treated with ethidium bromide dye and seen under a UV transilluminator. Lane M has a 100-base pair DNA ladder.Except lanes 26 and 47, all other lanes display negative results for bft3.

2- Histopathological analysis

This study focuses on investigating the impact of isolated bft2 and bft3 toxins from ETBF on the histological sections of several organs (liver, gut, and spleen) in mice, which were stained using Hematoxylin and Eosin (HE) staining. This study is the inaugural investigation in Iraq that specifically examines the purification of bft2 and bft3 toxins, as well as their impact on the liver, gut, and spleen of mice. Metalloprotease toxins, specifically the bft variant, can function as virulence factors in bacteria.Occasionally, they directly harm the tissue during the infection or deactivate natural components that typically play a role in regulating the host's response to infections(22).The control group exhibited normal histological structure of the liver, gut, and spleen, as indicated by outcomes 5, 6, and 7, respectively. The current findings of the positive control in the intestinal region align with a previous investigation. According to (23), colon sections of mice treated with DSS had extensive damage to the mucosa, changes in the structure of the epithelial cells, significant infiltration of neutrophils and lymphocytes into the mucosal and submucosal regions, and loss of crypts. Several crucial factors, including the origin of the DSS, its molecular weight, concentration, duration of exposure, as well as the strain, source, age, gender, and body weight of the mice, along with environmental factors such as the hygienic conditions of the vivarium, are essential for effectively and consistently inducing DSS-induced colitis (24) . The histological analysis of the liver segment from the second group (positive control group) of mice, who were given only 2% dextran sulfate sodium for 30 days, revealed several modifications. These changes included a congested central vein, deteriorated hepatocytes, vacuolated hepatocytes, and narrow sinusoids. These findings are depicted in Figure 8. The histological examination of the spleen portion, as depicted in Figure 9, revealed significant red pulp bleeding, widespread lymphocyte pyknosis, and depletion of lymphoid follicles. The histopathological examination of the intestine slice in the second group, as depicted in Figure 10, revealed the following changes: an elevation in the mucin content of goblet cells in the villi, congestion and bleeding in the submucosa, and edema in the submucosa. The histological analysis of the liver segment from the third group revealed significant infiltration of mononuclear cells, predominantly macrophages, and lymphocytes. Additionally, there were dilated bile ducts and necrotic hepatocytes observed. Please refer to Figure 11 for visual representation. The histopathological analysis of the spleen slice in the third group revealed the presence of amyloid infiltration in both the red and white pulp, along with the accumulation of acidophilic protein extracellular material. Additionally, there were signs of splenic bleeding and the depletion of necrotic lymphoid follicles. The histopathological examination of the gut, as depicted in Figure 13, revealed hyperplasia of the enterocytes. The fourth group had histopathological alterations in the liver segment, as depicted in Figure 14. These changes included an enlarged and congested central vein, apoptotic hepatocytes, degraded hepatocytes with acute cellular swelling, and mild lymphocytic cuffing. The histopathological examination of the spleen portion, as seen in Figure 15, revealed the presence of amyloid infiltration and complete depletion of lymphoid follicles with necrotic tissue. The histopathological examination of the intestine slice from the third group, as depicted in Figure (16), revealed elongation and hyperplasia of the villi, together with infiltration of mononuclear cells, specifically lymphocytes, in the submucosa, accompanied by edema.

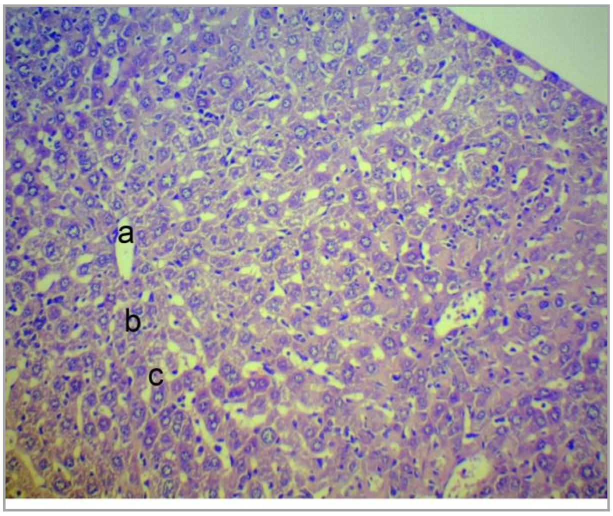


Figure (5): The normal liver portion of the control mouse exhibited the following structures: a) Central vein, b) Hepatocytes, c) Sinusoids.

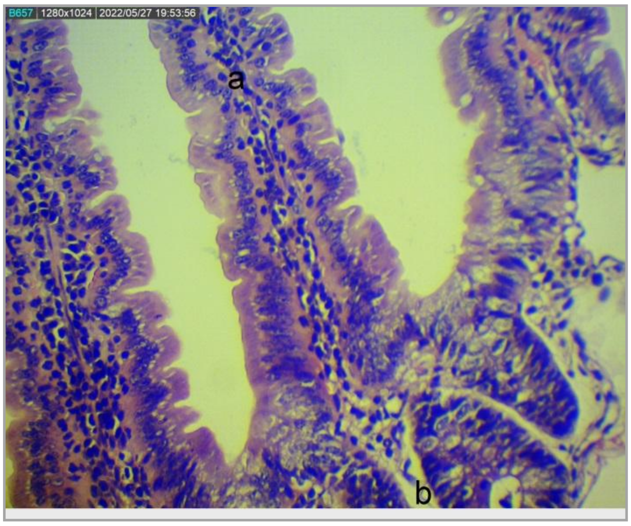


Figure (6): The control mouse exhibited a typical segment of the intestine: a) The mucosa layer b) the submucosa layer

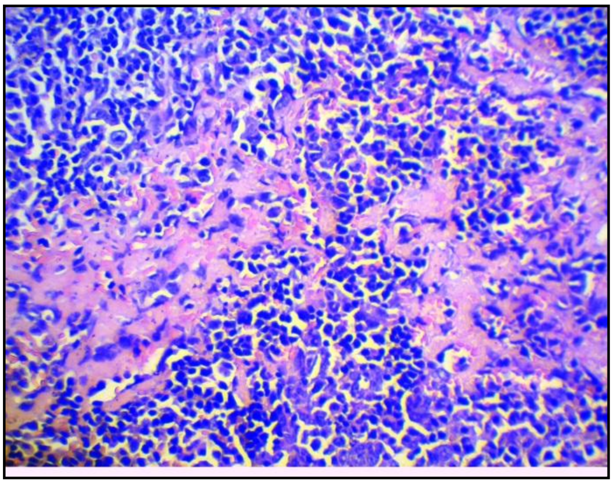


Figure (7): The following were observed in the normal spleen of the control mouse: a) an H&E-stained lymphocytic follicular

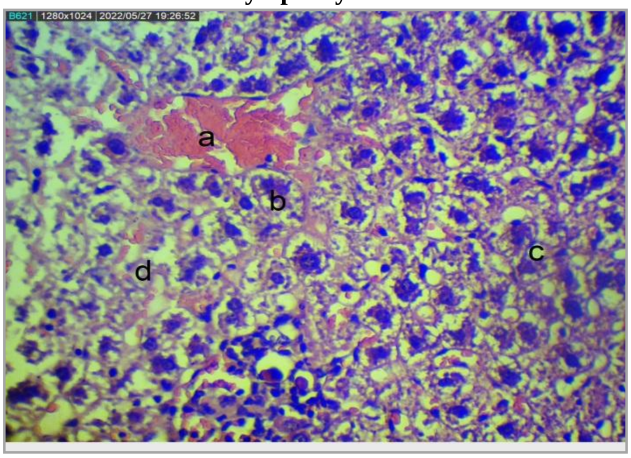


Figure (8): Histopathological examination of the liver segment from the second group of mice revealed the following alterations: a) the central vein was congested, b) all hepatocytes were deteriorated, c) hepatocytes displayed vacuolation, and d) the sinusoids were narrowed. These changes were observed using the H&E stain at a magnification of 40x.

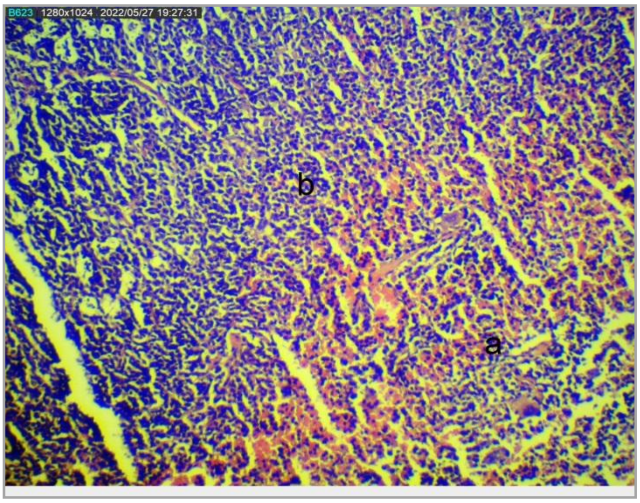


Figure (9) : The histopathological analysis of the spleen portion from the second group of mice revealed the following findings: a) significant hemorrhaging in the red pulp, and b) a high number of lymphocytes showing pyknotic alterations and depletion of lymphoid tissue.

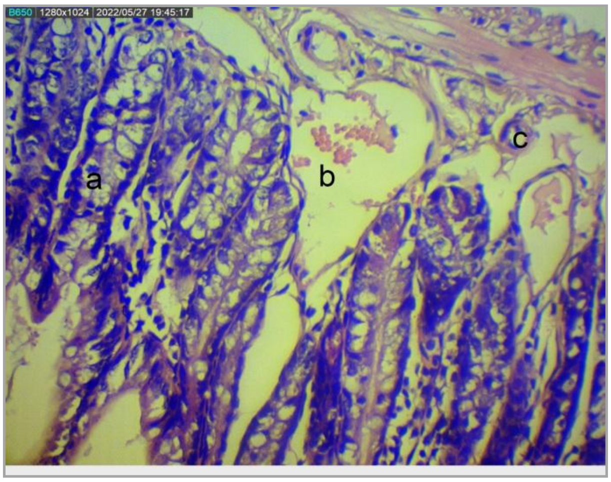


Figure (10) : Histopathological changes in intestine section of second group showed :a)increase mucin of goblet cells in villi b)congestion and hemorrhage in submucosa c)oedema in submucosa (H&E stain, 20x).

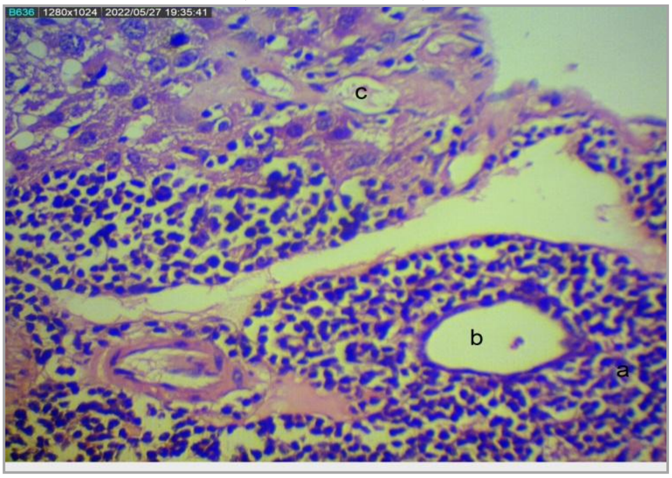


Figure (11) :Histopathological changes in liver section of third group: a) all liver tissue infiltrated with sever mononuclear cells mostly macrophage and l lymphocytes b) dilated bile duct c)necrotic hepatocytes (H&E stain, 40x).

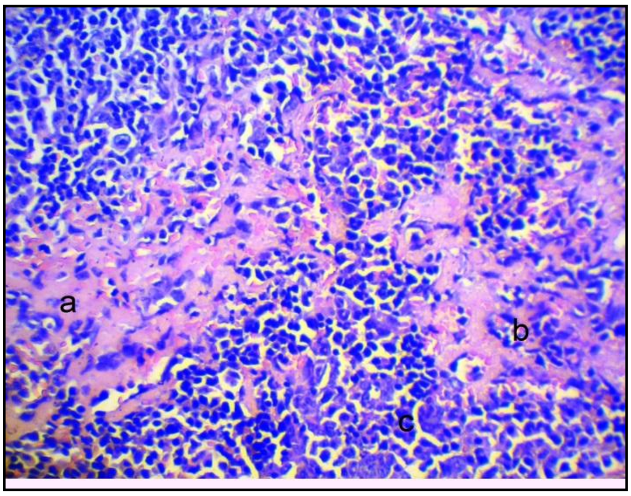


Figure (12) : Histopathological changes in spleen section of third group of mice: a)amyloid infiltration in the spleen red and white pulp acidophilic protein extracellular material, b)splenic hemorrhage)all lymphoid follicles depleted necrotic (H&E stain, 40x).

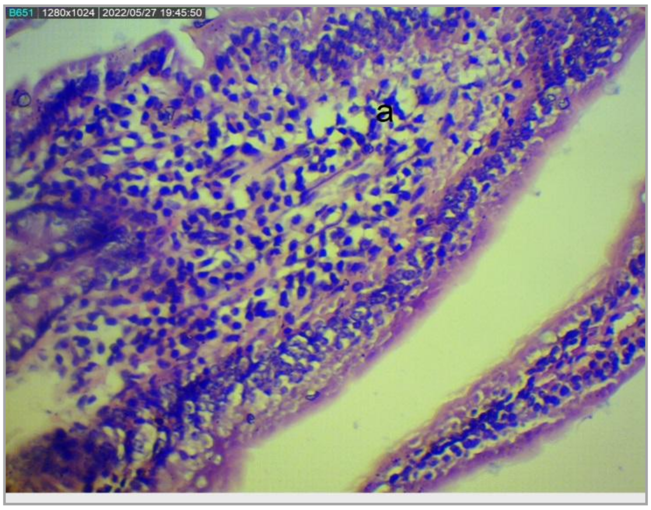


Figure (13) : Histopathological changes in intestine section of third group showed : a)Hyperplasia of enterocyte (H&E stain, 20x).

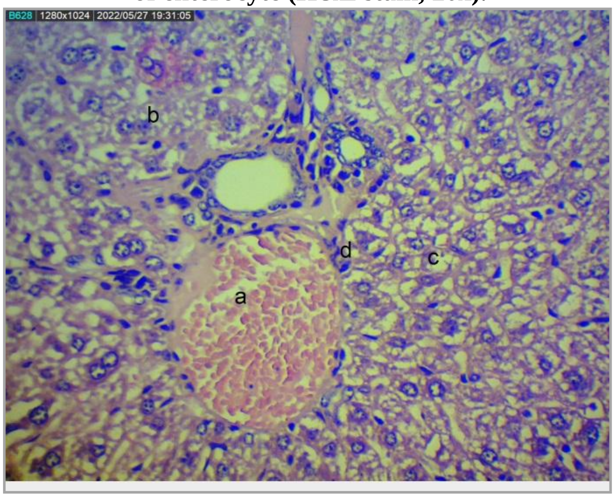


Figure (14) : Histopathoogical changes in liver section of fourth group of mice: a-enlargement and congested central vein b-apoptotic hepatocyte c-degenerated(acute cellular swelling hepatocytes d-mild lymphocytic cuffing (H&E stain, 20x).

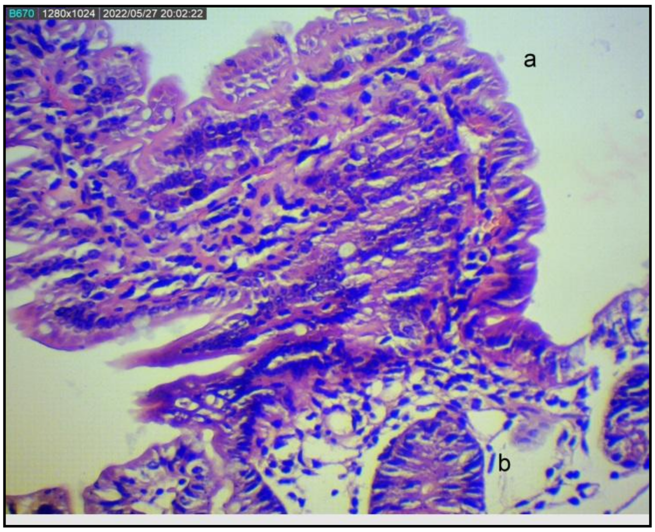


Figure (15): Histopathological changes in intestine section of fourth group of mice showed: a) elongation and hyperplasia of villi b)mononuclear cells infiltration of lymphocytes in submucosa with odema.

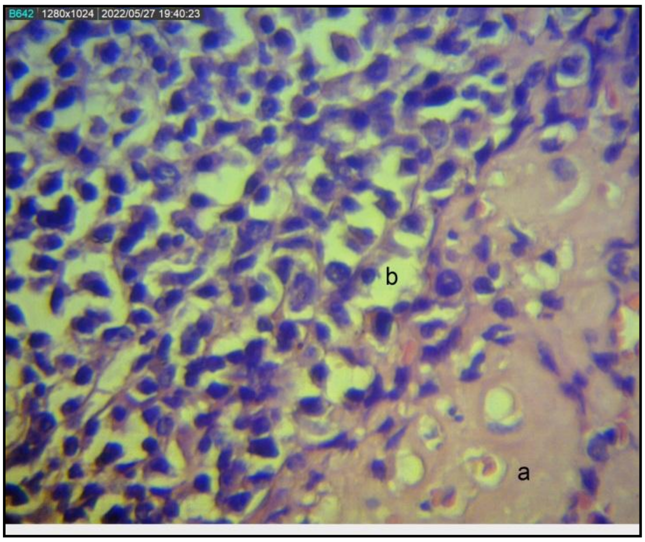


Figure (16) : Histopathological changes in spleen section of fourth groupshowed: a)severe amyloid infiltration b)depleted and necrotic lymphocytic follicles.

Discussion

The findings of the current investigation revealed that 34 out of the isolated samples were identified as *B.fragilis* based on the presence of the *16S rRNA* gene. These results are consistent with a previous study conducted by (20), which also found that 19 samples of *B.fragilis* tested positive for the *16S rRNA* gene. The results indicate that 5 isolates of *B.fragilis* possess the *bft* gene (ETBF), while the remaining isolates do not possess this gene (NTBF). The results observed in this study align with the findings reported in Baghdad (21). This work represents the first known investigation in Iraq that specifically examines the purification of bft2 and bft3 toxins, as well as their impact on the liver, gut, and spleen of mice. Metalloprotease toxins, specifically the bft toxin, can function as virulence agents in microbes. They may cause direct tissue damage during the infection or disable natural processes that typically regulate the host's response to infections. The user's text is enclosed in tags (22). The current findings of the positive control in the intestinal region align with a previous investigation (23), according to the paper, colon sections of mice treated with DSS exhibited extensive damage to the mucosa, changes in the structure of the epithelium, significant infiltration of neutrophils and lymphocytes into the mucosal and submucosal regions, and loss of crypts. Several crucial factors, including the source of DSS, molecular weight, concentration, duration of exposure, mouse strain, source, age, gender, and body weight, as well as environmental factors such as the hygienic conditions of the vivarium, are essential for the successful and replicable induction of DSS-induced colitis (24).

Conclusion

In our study, we reached that bft3 toxin was higher effect on mice organs than bft3 toxin .We need six month to one year to observe more effects of bf3 toxin, also there is need additional studies on bft2 and bft3 toxin effect on other animal laboratory type.

Reference

1. Boleij, A.; Hechenbleikner, E. M.; Goodwin, A. C.; Badani, R.; Stein, E. M.; Lazarev, M. G., *et al.* (2015). The Bacteroides fragilis toxin gene is prevalent in the colon mucosa of colorectal cancer patients. *Clinical Infectious Diseases*, 60(2): 208-215.
2. Sears, C. L.; Islam, S.; Saha, A.; Arjumand, M.; Alam, N. H.; Faruque, A. S. G., *et al.* (2008). Association of enterotoxigenic Bacteroides fragilis infection with inflammatory diarrhea. *Clinical Infectious Diseases*, 47(6): 797-803.
3. Franco, A. A.; Buckwold, S. L.; Shin, J. W.; Ascon, M. & Sears, C. L. (2005). Mutation of the zinc-binding metalloprotease motif affects Bacteroides fragilis toxin activity but does not affect propeptide processing. *Infection and immunity*, 73(8): 5273-5277.
4. Chen, L. A.; Van Meerbeke, S.; Albesiano, E.; Goodwin, A.; Wu, S.; Yu, H., *et al.* (2015). Fecal detection of enterotoxigenic Bacteroides fragilis. *European Journal of Clinical Microbiology & Infectious Diseases*, 34(9): 1871-1877.
5. Goulas, T.; Arolas, J. L. & Gomis-Rüth, F. X. (2011). Structure, function and latency regulation of a bacterial enterotoxin potentially derived from a mammalian adamalysin/ADAM xenolog. *Proceedings of the National Academy of Sciences*, 108(5): 1856-1861.
6. Rhee, K. J.; Wu, S.; Wu, X.; Huso, D. L.; Karim, B.; Franco, A. A., *et al.* (2009). Induction of persistent colitis by a human commensal, enterotoxigenic Bacteroides fragilis, in wild-type C57BL/6 mice. *Infection and immunity*, 77(4): 1708-1718.
7. d'Abusco, A. S.; Del Grosso, M.; Censini, S.; Covacci, A. & Pantosti, A. (2000). The alleles of the bft gene are distributed differently among enterotoxigenic Bacteroides fragilis strains from human sources and can be present in double copies. *Journal of clinical microbiology*, 38(2): 607-612.
8. Ulger, N.; Rajendram, D., Yagci, A., Gharbia, S., Shah, H. N., Gulluoglu, B. M., ... & Soyletir, G. (2006). The distribution of the bft alleles among enterotoxigenic Bacteroides fragilis strains from stool specimens and extraintestinal sites. *Anaerobe*, 12(2): 71-74.
9. Wu, S., Morin, P. J., Maouyo, D., & Sears, C. L. (2003). Bacteroides fragilis enterotoxin induces c-Myc expression and cellular proliferation. *Gastroenterology*, 124(2): 392-400.
10. Rhee, K. J.; Wu, S.; Wu, X.; Huso, D. L.; Karim, B.; Franco, A. A., *et al.* (2009). Induction of persistent colitis by a human commensal, enterotoxigenic Bacteroides fragilis, in wild-type C57BL/6 mice. *Infection and immunity*, 77(4): 1708-1718.
11. Yim, S., Gwon, S. Y.; Hwang, S.; Kim, N. H.; Jung, B. D. & Rhee, K. J. (2013). Enterotoxigenic Bacteroides fragilis causes lethal colitis in Mongolian gerbils. *Anaerobe*, 21: 64-66.
12. Bleich, E. M.; Martin, M.; Bleich, A. & Klos, A. (2010). The Mongolian gerbil as a model for inflammatory bowel disease. *International journal of experimental pathology*, 91(3): 281-287.
13. Tong, J.; Liu, C.; Summanen, P. Xu, H. and Finegold SM(2011). Application of quantitative real-time PCR for rapid identification of Bacteroides fragilisgroup

and related organisms in human wound samples. *Anaerobe*; 17(2): 64-8.

14. Prindiville, T. P.; Sheikh, R. A.; Cohen, S. H.; Tang, Y. J.; Cantrell, M. C. & Silva Jr, J. (2000). Bacteroides fragilis enterotoxin gene sequences in patients with inflammatory bowel disease. *Emerging infectious diseases*, 6(2): 171.
15. Merino, V. R. C.; Nakano, V., Liu C. ;Song, Y.; Finegold, S. M. & Avila-Campos, M. J. (2011). Quantitative detection of enterotoxigenic Bacteroides fragilis subtypes isolated from children with and without diarrhea. *Journal of clinical microbiology*, 49(1): 416-418.
16. Van Tassell, R. L.; Lyerly, D. M. & Wilkins, T. D. (1992). Purification and characterization of an enterotoxin from Bacteroides fragilis. *Infection and immunity*, 60(4): 1343-1350
17. Classics Lowry, O.; Rosebrough, N.; Farr, A. & Randall, R. (1951). Protein measurement with the Folin phenol reagent. *J biol Chem*, 193(1): 265-75.
18. Choi, V. M.; Herrou, J.; Hecht, A. L.; Teoh, W. P.; Turner, J. R.; Crosson, S. & Wardenburg, J. B. (2016). Activation of Bacteroides fragilis toxin by a novel bacterial protease contributes to anaerobic sepsis in mice. *Nature medicine*, 22(5): 563-567.
19. Bancroft, J. D. & Gamble, M. (Eds.). (2008). Theory and practice of histological techniques. *Elsevier health sciences*.
20. Ignacio,A.;Fernandes,MR.;Avila-Campos,MJ.;Nakano,V.(2015). Enterotoxigenic and non-enterotoxigenic Bacteroides fragilis from fecal microbiota of children. *Brazilian Journal of Microbiology*. 46(4): 1141-1145.
21. Jasim,D.A.and Melconian,A.K(2020). Prevalence of Enterotoxigenic Bacteroides Fragilis in stool specimens collected from children less than 5 years of age in Iraq. *Iraqi Journal of Science*. 61(12): 3179-3186.
22. Nakano, V.; Gomes, D. A.; Arantes, R. M.; Nicoli, J. R. & Avila-Campos, M. J. (2006). Evaluation of the pathogenicity of the Bacteroides fragilis toxin gene subtypes in gnotobiotic mice. *Current microbiology*, 53(2): 113-117.
23. Laroui, H.; Ingersoll, S. A.; Liu, H. C.; Baker, M. T.; Ayyadurai, S.; Charania, M. A., *et al.* (2012). Dextran sodium sulfate (DSS) induces colitis in mice by forming nano-lipocomplexes with medium-chain-length fatty acids in the colon. *PloS one*, 7(3): e32084.
24. Nell, S.; Suerbaum, S. & Josenhans, C. (2010). The impact of the microbiota on the pathogenesis of IBD: lessons from mouse infection models. *Nature Reviews Microbiology*, 8(8): 564-577.